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# MALACOLOGIA

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THE SYSTEMATIC RELATIONSHIP OF *POMATIOPSIS LAPIDARIA*  
AND *ONCOMELANIA HUPENSIS FORMOSANA*  
(PROSOBRANCHIA: HYDROBIIDAE)<sup>1,2</sup>

George Morgan Davis<sup>3</sup>

ABSTRACT

The North American *Pomatiopsis lapidaria* (Say) and the Oriental *Oncomelania hupensis formosana* (Pilsbry & Hirase) were chosen as representatives for 2 related hydrobiid genera. Their comparative anatomy, potential for hybridization, electrophoretic properties and laboratory ecology were studied to determine to what extent differences of value to systematics could be found.

On the basis of their anatomy *Pomatiopsis* and *Oncomelania* are judged to be distinct genera within the same subfamily, the Pomatiopsinae.

In the genus *Oncomelania* (considered to have 1 species with 4 subspecies) the shell is smooth (except in the ribbed form of *O. hupensis hupensis*), with moderately deep sutures and moderately convex whorls. The outer lip of the snell has a tendency to form a varix which is usually quite pronounced. The umbilicus is narrow, as is the apical whorl. The parietal callus is elongate. There are at least 35 gill filaments, usually 45 or more. The verge is muscular, the tip has short strips of actively beating cilia and a distinct protrudable papilla. The pleuro-supraesophageal connective is comparatively short; in consequence the osphradio-mantle nerve arising from the tip of the supraesophageal ganglion is relatively long; it usually does not bifurcate until within the cephalic wall. The supravisceral connective also arises from the tip of the ganglion. The sperm duct and spermathecal duct arise in a common sheath from the right, anterolateral surface of the bursa copulatrix. The female gonad is multibranched and the collecting duct relatively slender. The oviduct encircles the seminal receptacle in a characteristic manner. The seminal vesicle is a characteristically knotted slender tube. The verge has a single glandular type (studied in *O. h. formosana* and *O. h. quadrasi*). The cerebral commissure is short. The tentacles are elongate compared to the length of the rostrum.

Compared with *Oncomelania*, the shell of *Pomatiopsis* has a roughened microsculpture, the lip is sharp and there is no tendency to form a varix. In all 4 species the apical whorls are wide. The umbilicus is wide and pronounced, sutures are deeply impressed, and the whorls very convex (except in *P. binneyi*). In *P. lapidaria* and *P. cincinnatiensis*, there are less than 30 gill filaments. The verge does not have a pronounced musculature or papilla in the 4 species; penial cilia are lacking in 2 species (*P. lapidaria* and *P. cincinnatiensis*); when cilia occur they are bushy and generally inactive (*P. californica* and *P. binneyi*). Two species (*P. cincinnatiensis* and *P. californica*) have penial filaments, a condition not found in *Oncomelania*. The verge has 3

<sup>1</sup>Adapted from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Michigan, May, 1965.

<sup>2</sup>This investigation was sponsored (in part) by the Commission on Parasitic Diseases of the Armed Forces Epidemiological Board and was supported (in part) by the U. S. Army Medical Research and Development Command, and (in part) by a research grant (5 T1 AI 41) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

<sup>3</sup>Current address: 406 Medical Laboratory, U. S. Army Medical Command, Japan, APO San Francisco, California 96343.

glandular types (known for *P. lapidaria*). The pleuro-supraesophageal connective is elongate, the supraesophageal ganglion lies close to the lateral cephalic wall and the osphradial and mantle nerves, which usually bifurcate right after leaving the tip of the ganglion, are correspondingly quite short. The supravisceral connective, in *P. lapidaria*, arises from the lateral, posterior border of the supraesophageal ganglion, not from the tip. The oviduct does not encircle the seminal receptacle. The spermathecal duct arises from the anterior end of the bursa copulatrix (*P. lapidaria* and *P. cincinnatiensis*), and the sperm duct from the spermathecal duct. The female gonad is little branched, the collecting duct is quite wide. The seminal vesicle is a thick, regularly coiled tube. The tentacles are short, relative to the length of the rostrum.

Hybridization does not occur between *Pomatiopsis lapidaria* and *Oncomelania*.

Disc electrophoretic studies on fresh foot muscle protein of the 2 representative taxa showed that each taxon has a specific pattern. All subspecies of *Oncomelania* have 1 or more characteristic dense protein components with Rf values (ratio of the distance from the origin to the center of each band and from the origin to the front) greater than 0.75. *Pomatiopsis lapidaria* lacks dense, fast moving proteins beyond an Rf of 0.75.

All 4 subspecies of *Oncomelania* are characterized by adaptability to the laboratory culture conditions provided. In 12 months, under conditions less than optimal, the finite rate of mortality (field snails about 1 year old) was 12% per month. Young grew at about 0.65 mm per week with low mortality. Young were produced at rates as high as 2.12 per female per month continuously for more than 2 years.

The 4 species of *Pomatiopsis* investigated did not adapt well to laboratory conditions. *Pomatiopsis californica* and *P. binneyi* died rapidly without producing young. *P. lapidaria* and *P. cincinnatiensis* (field snails about 1 year old) had a finite rate of mortality of 16% per month in "optimal" conditions over a 10-month period for the former and a 3-month period for the latter, after which rates of mortality increased rapidly, in part because of the shorter life span of these snails. Young grew at less than 0.14 mm per week with mortalities exceeding 30% in 2 months. Young were produced at less than 0.51 per female per month for very short periods of time.

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## INTRODUCTION

This paper is concerned with the systematic relationship of the American genus *Pomatiopsis* (Say, 1817) and the Oriental genus *Oncomelania* Gredler, 1881. Interest in determining to what extent these prosobranch snails were related to each other was first generated when Stunkard (1946), Ward, Travis & Rue (1947) and Berry & Rue (1948) demonstrated that *Pomatiopsis lapidaria* was capable of serving as an intermediate host of the human blood fluke, *Schistosoma japonicum*. According to Abbott (1948a), malacological studies showed *P. lapidaria* to be "strikingly similar" to species of *Oncomelania*. Dundee (1957) stated that anatomically *P. lapidaria* was "quite similar" to *Oncomelania* and that differences appeared "to be minor." Van der Schalie, Getz & Dazo (1962) reported success in hybridizing 2 species of *Oncomelania* with *P. lapidaria* which "strengthened" their "contention that these genera are rather closely related." Burch (1960a) considered the genera to be synonymous but later, (Burch, 1964), after a detailed cytological study, separated them as closely related genera.

In this paper *Pomatiopsis lapidaria* and *Oncomelania formosana* were chosen

as representatives of the 2 genera. Their comparative anatomy, potential for hybridization, electrophoretic properties and laboratory ecology were studied. The investigation was undertaken to determine to what extent differences coupled with existing knowledge of the other species of each genus would serve to establish the degree of relationship between *Pomatiopsis* and *Oncomelania*, i.e., whether or not *Pomatiopsis* and *Oncomelania* are congeneric, closely related but distinct genera, or genera more widely separated than has previously been considered.

Dundee (1957) discussed the major papers pertaining to *Pomatiopsis*, i.e., ecology, systematics, general distribution and anatomy. Aside from a few anatomical details, including a discussion of variability in the radula presented by Abbott (1948a), Dundee published the only anatomical study on *P. lapidaria* giving details which can be used in a general comparative manner on the generic level. Van der Schalie & Dundee (1956) presented the basic anatomy of *P. cincinnatiensis* making possible certain specific comparisons with *P. lapidaria*, and with the so-called species of *Oncomelania*.

*Oncomelania formosana* was chosen as a representative of its genus because it appeared to be a form intermediate in the *Oncomelania* species complex. Burch (1964) studied cytological aspects of the 4 currently recognized species of *Oncomelania* [*Oncomelania hupensis* Gredler, 1881; *O. quadrasi* (Möllendorff) 1895; *O. nosophora* (Robson) 1915; *O. formosana* (Pilsbry & Hirase) 1905] and their "various hybrids" and, in the light of his findings, coupled with the fact that there was no reduced viability in the F<sub>1</sub> and F<sub>2</sub> hybrids, he stated that the 4 species were no more than geographic populations or races of the same species. Davis et al. (1965) were of the same opinion, due to successful hybridization: only 1 abnormal snail was found among thousands of hybrids of all 4 "species" of *Oncome-*

TABLE 1. The family and subfamily status of *Pomatiopsis* within the Rissoacea as indicated by various authors

Authors	Amnicolidae			Hydrobiidae		Pomatiopsidae	Rissoidae	Truncatellidae
	Pomatiopsinae	Hydrobiinae	*	Pomatiopsinae	Truncatellinae	Pomatiopsinae	Pomatiopsinae	Pomatiopsinae
Tryon 1862			x					
Gill 1863			x					
Stimpson 1865							x	
Binney 1865							x	
Gill 1871						x		
Fischer 1885				x				
Tryon 1883							x	
Call 1900	x							
Baker 1902	x							
Pilsbry & Ferris 1906						x		
Hannibal 1912				x				
Walker 1918	x							
Annandale 1924							x	
Baker 1926						x		
Baker 1928						x		
Thiele 1928					x			
Thiele 1931					x			
Wenz 1938								x
Berry 1943						x		
Abbott 1948		x						
Dundee 1957					x			
Davis this paper				x				

\* = no subfamily mentioned.

*lania* observed. They felt that the genetic compatibility involved was of a conspecific or sub-specific nature. In shell shape, there is a north-south cline, with *O. nosophora* from Japan having a long, slender shell; *O. quadrasi* from the Philippines having a relatively more short and broad shell; and *O. formosana* from Taiwan being intermediate. This intergradation was noted by Abbott (1948b), who stated that 5% of the Formosan specimens could not be distinguished from *O. quadrasi* (Philippines) while 10% had a shape and size similar to many *O. nosophora* from Japan and China. Studies by Kuo & Mao (1957) indicated that *O. hupensis* and *O. nosophora* from China are all *O. hupensis* and that there was "no clear-cut line of demarcation between them," i.e., between the smooth shelled *O. nosophora* type and ribbed *O. hupensis* type. In all following discussions in this thesis, the so-called *Oncomelania* "species" of most pre-

vious authors will be considered subspecies of *O. hupensis*, the first named of the "species."

There are a number of anatomical papers describing various features of the subspecies of *Oncomelania*. Heude (1880), Li (1934) and Kuo & Mao (1957) discussed various aspects of the anatomy of *O. hupensis hupensis*; Robson (1921), Nakamoto (1923), Itagaki (1955), Williams (Fide Ritchie, 1955, in referring to the 1954 and 1955 Professional Reports of the 406 General Medical Laboratory) and Roth & Wagner (1957) presented material on *O. hupensis nosophora*. As far as is known, no anatomical studies have been published on *O. hupensis formosana* aside from those of Roth (1960), who described the female reproductive anatomy and of Davis (1964) who depicted the structure of the female gonad. Abbott (1945) provided some anatomical notes on *O. hupensis quadrasi*.

## SYSTEMATIC DISCUSSION

*Pomatiopsis* and *Oncomelania* are representatives of the mesogastropod, rissoacean family Hydrobiidae (Troschel, 1857) subfamily Pomatiopsinae (Stimpson, 1865). As shown in Table 1, there has been a considerable difference of opinion concerning the proper family and subfamily designation for *Pomatiopsis* since 1862. *Oncomelania* was not named until 1881; this genus generally has been placed in the same subfamily with *Pomatiopsis*.

*Pomatiopsis lapidaria* was described by Say (1817) as a species of *Cyclostoma*, a genus of the Pomatiasidae currently split into several genera (Wenz, 1938-1944). Tryon (1862) recognized the basic differences between the viviparid and hydrobiid types of snails and subsequently created the family Amnicolidae. In the same paper he separated *Pomatiopsis* as a subgenus of *Amnicola* because he felt that "*A. lapidaria*" differed from the small globose shells of *Amnicola* by "shell elongate, spire (of about 6 whorls) much exceeding the length of the aperture. . .". Tryon, however, did not give a family diagnosis for the Amnicolidae. Gill (1863), in a noteworthy paper, defined the family, but stated that *Pomatiopsis* (i.e., *P. lapidaria*) was possibly an aciculid snail<sup>4</sup>. He asserted that the "validity" of *Pomatiopsis* as defined by Tryon was doubtful and that the Amnicolidae contained 3 genera: *Amnicola*, *Chilocyclus* and *Somatogyrus*. *Chilocyclus*, proposed by Gill (1863), is a synonym for *Pomatiopsis*, as the genus is currently understood, and was used to separate *Cyclostoma cincinnatiensis* (now *P. cincinnatiensis*) from species of *Amnicola*.

Stimpson (1865) rejected Gill's family

definition for the following reasons: the definition was almost an exact translation of Moquin-Tandon's (1855) definition of "*Bythinia*", and the definition did not apply to the American forms of the group, founded, at that time, on the genus *Amnicola*, e.g., the verge is not bifid in all species, the tenacles of *Amnicola* proper are not setaceous, etc. Aside from the fact that the family was poorly diagnosed, laws of priority exclude the name Amnicolidae from being used in place of the Hydrobiidae as the latter are currently understood. H. B. Baker (1960) reviewed this situation and pointed out that if one accepts Bithyniidae and Truncatellidae as separate rissoacean families, Hydrobiidae is the legal name for the rissoacean family under discussion. Bithyniidae are considered distinct for several reasons. They possess a calcareous operculum which is not found in the Hydrobiidae. Members of the Bithyniidae have a verge with a characteristic prong on the concave side. Within the prong and running back into the body is a "flagellum" described (and figured) by Baker (1928) as "very long, a blind diverticulum, . . . semi-independent, having no internal connection with the vas deferens." While some hydrobiids have penial appendages, they are structurally different from those found in the Bithyniidae and the "flagellum" is not present. Animals of the Bithyniidae are characterized by the yellow or orange pigment spots (Baker, 1928; Abbott, 1948b; Taylor, personal communication, 1965) which are not found in the Hydrobiidae. A number of features described for *Truncatella* indicate separate family status. The great reduction in the ctenidium contrasts with the well-developed ctenidium of the Hydrobiidae; concentrated cerebral, pleural and parietal ganglia in *Truncatella* are in contrast to the widely separated cerebral and parietal ganglia of the Hydrobiidae; shortened tentacles with eyes at their bases on the midline, or displaced medially, in the former, are in contrast with eyes at the

<sup>4</sup>Wenz (1938-1944) used Acmeidae for Aciculidae, but according to Opinion 344 of the International Commission on Zoological Nomenclature (1955), the correct designation is Aciculidae.

outer base of generally elongate tentacles in the latter. The elongated gonopericardial duct and the connection of the bursa copulatrix with the left kidney are unknown in the Hydrobiidae. The modified, small, pedestal-like foot of *Truncatella* differs from the elongate, broad foot of the Hydrobiidae. In the Truncatellidae the corneous operculum is sub-spiral and has characteristically, though not infallibly, a thick, outer, calcareous layer. The central tooth of the radula is triangular and supports a single, anterior, triangular cusp (drawings in Fischer, 1880-1887; Binney, 1865; Clench & Turner, 1948). The hydrobiid operculum is also corneous, usually paucispiral, sometimes multi-spiral, but not subspiral to the degree shown in *Truncatella*. The central tooth of the hydrobiid radula is trapezoidal or rectangular, the anterior edge supporting more than 1 cusp. As discussed below, the mode of progression found in the Truncatellidae is distinctly different from that in the Pomatiopsinae.

Stimpson (1865), in an outstanding paper, presented a broad diagnosis for the family Rissoidae so that it included the currently recognized families Rissoidae, Hydrobiidae and Bithyniidae. The Truncatellidae were excluded on the basis of radula, eye position and the nature of the "breathing organ." The Rissoidae are currently characterized as follows: marine, a filament arising from the operculigerous lobe and/or the presence of a pallial tentacle; foot more narrow and agile than that of the Hydrobiidae; the shell may be smooth but is more characteristically ribbed, with spiral cords, or cancellate. The aperture below may be bent outwards (Fretter & Graham, 1962).

The Hydrobiidae are a separate family of freshwater snails with a few marine, brackish water, amphibious and terrestrial forms. The shell is characteristically smooth but not infallibly so; the aperture is not bent out below. No filament arises from the operculigerous lobe and a pallial tentacle is known

only in *Hydrobia ulvae*. The difference in the foot has already been mentioned. Fretter & Graham (1962) state that the pleuroparietal connectives of the nervous system are long in the Hydrobiidae and comparatively shortened in the Rissoidae.

In summary, the family Amnicolidae is a synonym of the Hydrobiidae as the latter are currently understood. For the anatomical reasons given above, *Pomatiopsis* cannot be included in the distinct rissocean families Rissoidae, Truncatellidae, or Bithyniidae.

The question arises: Does *Pomatiopsis* deserve to be separated from the Hydrobiidae in a separate family, Pomatiopsidae? Stimpson (1865) gave excellent reasons for establishing a separate subfamily Pomatiopsinae within the wide group he considered to be Rissoidae and equal to other subfamilies such as the Hydrobiinae, "Bithyniinae", Rissoinae, Rissoininae, and Skeneinae. The principal character used was what he called "lateral sinuses" which separated the foot into anterior and posterior parts. Other distinguishing characteristics were: (1) the terrestrial habitat, (2) the peculiar mode of progression, (3) the central tooth of the radula, with basal denticles, not lateral or basolateral cusps as in the Hydrobiinae. Due to Stimpson's influence, most authors have considered *Pomatiopsis* distinctly separate from other hydrobiid snails and have placed it either in a separate family or in a distinct subfamily (Table 1).

Gill (1871) raised *Pomatiopsis* to family rank without a diagnosis; Pilsbry & Ferris (1906) did likewise. F. C. Baker (1902) recognized the subfamily Pomatiopsinae, but later (1926, 1928) used Stimpson's subfamily characteristics to justify family status. Berry (1943) considered family status justified, especially on radular characteristics.

A reanalysis of Stimpson's subfamily characteristics in the light of current studies on morphology and mode of progression is necessary in order to de-

termine whether or not Stimpson was correct in establishing the subfamily, and, if so, to determine whether or not full family status is warranted for *Pomatiopsis*.

1) Lateral Sinuses of the Foot. The following discussion pertains to Plate 1, Figs. 2 and 5, in which the right lateral view of the head-foot region of *P. lapidaria* is shown with the animal expanding its foot in preparation for advancing (Fig. 2) and with the completion of a "step" or forward advancement (Fig. 5). The head-foot region is characterized by a number of folds, grooves and creases. The ventral edge of the operculigerous lobe (Op) continues anteriorly as the suprapedal fold (P) and sweeps upwards towards the posterior part of the rostrum (R). Anteriorly the fold is interrupted by the anterior termination of the omniphoric groove (Om). As shown, the groove arises under the mantle on the right side of the "neck" and runs obliquely down towards the anterior border of the suprapedal fold. The groove is highly ciliated and serves to move fecal pellets along its path as well as particles and mucoid strings from the mantle cavity. The terminal end of the groove juts out over the antero-lateral portion of the foot onto which the fecal pellets and particles fall or are swept by ciliary currents. The groove undoubtedly serves to transport eggs to the anterior foot prior to their final encasement in a soil capsule. An identical groove is found on the left side and from time to time a particle may be seen moving anteroventrally in it. More dynamic, however, are the ciliary currents sweeping into the left side of the mantle cavity. These currents are created, in part, by cilia on the lateral surfaces of the head.

As the animal moves about it is evident that the omniphoric groove is plastic and, due to stretching of the lateral skin, may disappear. Certain movements accentuate the groove and the anterior edges of the groove appear lobed, the suprapedal lobe (Su) ventrally and the subocular lobe (S) dorsally.

Another groove, the subocular groove (So), is evident below the eye, arising from the suprapedal fold and terminating just posterior to the eye. With the exception of the suprapedal fold, which is always distinct, the other structures discussed are pliable; they appear and disappear as the animal moves about, stretching its head and body in various positions. In relaxed or preserved specimens only the suprapedal fold is evident. Dundee's figure (1957, Pl. 3) is of a narcotized specimen of *P. lapidaria*, as evidenced by the swollen and disproportionate head. The suprapedal fold is directly continuous with the rostrum, with no sign of the omniphoric or subocular grooves.

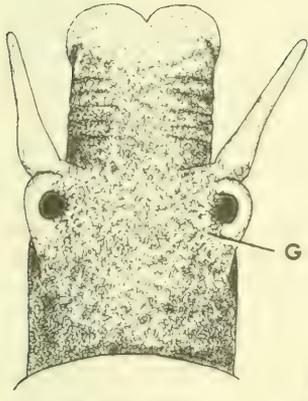
The pedal crease (Pc) shown in Fig. 5 is equivalent to the lateral sinus of the foot described by Stimpson (1865) and the vertical fold mentioned by Baker (1928). This crease is evident only upon completion of a "step" (Fig. 5) where the full weight of the animal presses down on the fully contracted foot. With reduced contraction the pedal fold becomes less evident or non-existent. Muscular contraction bringing the posterior foot forward causes a bunching of muscles which in turn creates the crease or fold. Pigmented epithelium concentrated by this contraction accentuates the crease. Stimpson (1865) was correct in stating that the pedal crease was dependent upon "the peculiar mode of movement." Abbott (1948a) stated that both *Oncomelania* and *Pomatiopsis* have the same mode of progression and produce "folds in the flesh of the foot due to the weight of shell and body."

The folds and grooves described above were illustrated by Stimpson (1865: 33, Fig. 25; 34, Fig. 26; 31, Fig. 22). Stimpson's figures were partially correct but failed to consider the plasticity of the organism. His Fig. 26 is quite misleading, as current studies show that the pedal crease is not evident when the fore-foot is expanding or expanded. Table 2 shows the extent to which Stimpson's figures were copied

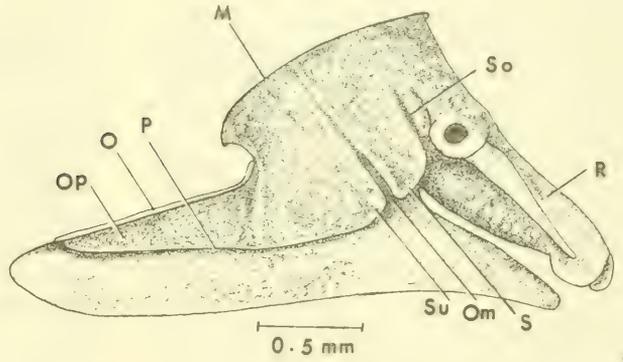
PLATE 1. *Pomatiopsis lapidaria*. Head, foot and locomotion.

- FIG. 1. Dorsal view of the head.
- FIG. 2. Right lateral view of the head-foot region; the fore-foot is extended in the first movement of a "step."
- FIG. 3. Sole of the foot without pronounced lateral indentation; see text, p 7.
- FIG. 4. Sole of the foot showing pronounced lateral indentations.
- FIG. 5. Right lateral view; the foot is contracted showing completion of a "step."
- FIG. 6. Sole of the foot when the fore-foot is beginning to expand forward.
- FIG. 7. The sole of the foot in various stages of "stepping." A. The foot is contracted; B, the fore-foot expands while the hind-foot remains firmly attached to the substrate; C, the hind-foot is drawn up to the contracted state.
- FIGS. 8,9. Variations found in the shape of the flexible foot.

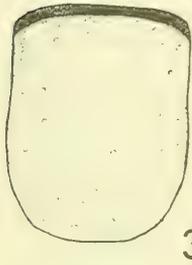
At	anterior foot	Pc	pedal crease
G	glandular units	Ps	posterior foot
L	lateral indentation	R	rostrum
M	point where the mantle covers the "neck"	S	subocular lobe
Ms	mucous slit	So	subocular groove
O	operculum	Su	suprapedal lobe
Om	omniphoric groove	x	mid-region of the foot under the pedal haemocoel
Op	operculigerous lobe		
P	suprapedal fold		



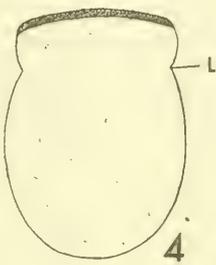
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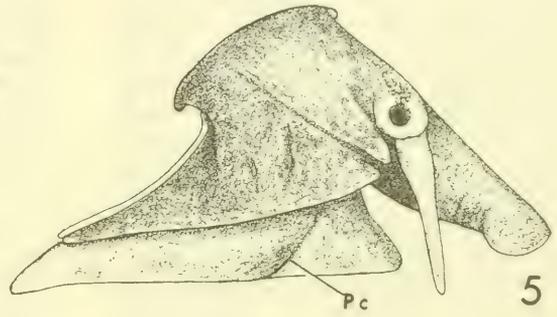
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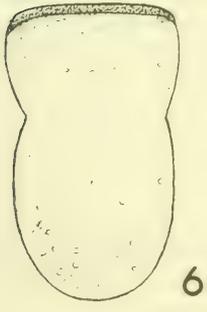
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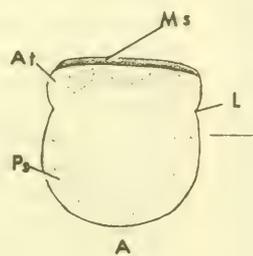
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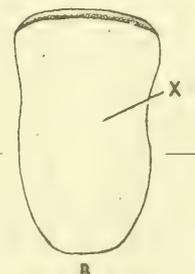
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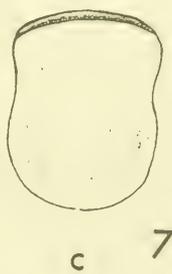
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A

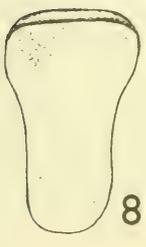


B

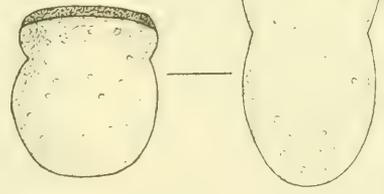


C

7



8



9

TABLE 2. References to reproductions of Stimpson's (1865) figures of *Pomatiopsis lapidaria*

Author	Page	Plate	Figure
Binney, 1865	93		187
Call, 1900		8	16
Baker, 1902	345		127
Walker, 1918	34		120
Annandale, 1924	273		1
Baker, 1928	166,167		77,78

and his influence felt. The figures depicted the crease as a most prominent and rigid structure, which in his words was a truly "distinct fold separating the foot into an anterior and posterior part..." Abbott (1948a) correctly stated that "previous accounts of the divided foot... are misleading." Pelseneer (1906) was led to assert that a transverse furrow which crosses the anterior half of the foot was found in *Pomatiopsis*, a statement which is unfounded, as the sole of the foot is simple and undivided. Annandale (1924) felt that the external anatomy of *Pomatiopsis* might differ from that of *Blanfordia*, Stimpson's drawing of the subocular and supra-pedal lobes leading him to state that the former genus had a "triangular process behind the true tentacle" which did not apply to the latter genus. Actually, the same lobes are found in both genera with about the same degree of development. Further influenced by Stimpson's drawings and description of the lateral sinus, Annandale separated *Oncomelania*, creating a new subfamily, *Triculinae*, that did not have a divided foot, as distinguished from the *Pomatiopsinae*, where the foot was divided by a "transverse furrow."

2) *Mode of Progression* (Pl. 1). The underside of the foot was examined by placing specimens on a glass slide in a drop of water, waiting until the animal began moving over the slide, inverting the slide, and supporting it above the stage of a dissecting scope. Mucoid

secretions of the foot and supportive action of the surface film of water served to keep the snail from dropping from the slide. The snails moved freely across the slide allowing a study of foot shape, structure and mode of progression.

When the animal is at rest the foot is slightly contracted and a lateral indentation is noted on either side of the foot (L, Pl. 1, Fig. 7). This indentation represents the ventrolateral end of the pedal crease (Pc, Fig. 5) and corresponds to an area separating anterior and posterior portions of the foot. The latter, as Stimpson (1865) observed, is about twice the length of the former. The degree of indentation varies considerably from individual to individual and depends a great deal upon the amount of muscular contraction. In Fig. 3, for instance, only a slight bend of the lateral margins indicated the position of the indentation. At this point the foot is capable of greater contraction. In Fig. 7, the foot at position A is fully contracted. To advance, the anterior portion of the foot (At) expands forward, during which process the lateral indentation becomes progressively less evident, as this area also expands forward. When the fore-foot is fully extended (position B) the point corresponding to the lateral indentation (L, position A) has moved forward from 0.36 to 0.42 mm. In this expansion of the fore-foot, the posterior portion (Ps) remains firmly attached to the substrate (position B). The extended fore-foot then becomes attached to the substrate and the foot musculature contracts in the area marked x (Fig. 7), drawing the posterior foot forward until the foot again assumes the shape shown in position A. These movements constitute a "step." While the snail is engaged in a series of "steps" the foot does not become fully contracted (position C, Fig. 7).

Stimpson (1865) stated that in progression, and as part of the stepping process, the "snout" was thrust forward and its "disc-like extremity affixed to

the ground as far ahead as possible." With the "snout" and the posterior portion of the foot solidly in place, "the anterior part of the foot becomes free and is thrust forward to the disk of the rostrum where it is again planted." He considered 3 points of support to be used in progression, the 2 parts of the foot and the rostral tip. In studying numerous specimens of this species it is evident that the rostrum is not used for support nor does it ever become "affixed" to the substrate. The rostrum is only used for feeding as is described more fully below. The animal is supported only by the foot with the points of greatest support associated with the mid- and posterior foot. The snail is quite capable of stretching the anterior foot forward and lifting it off the substrate while solidly supported by the mid- and hind foot. The various movements of *Pomatiopsis lapidaria* over rough and smooth substrates are classified and described below.

a. Movement and feeding on moist filter paper. Filter paper serves as a roughened but unyielding surface on which the movement of the snail is normal and unstrained. When the animal is not actually feeding, the basic movements of the foot described above operate smoothly. Here the extending fore-foot expands beneath the tip of the rostrum and beyond it often as far as 0.6-0.8 mm. The contraction drawing up the hind-foot is most often followed by a contraction of the columellar muscle which tends to raise the spire of the shell as well as pull it forward. On a smooth level substrate the last movement may not be pronounced but its occurrence gives the snail the appearance of hunching forward. With completion of the hunching movement the rostral tip is again brought before the front edge of the foot. Often the rostrum sweeps from one side to the other without touching the substrate.

While feeding, the animal may rasp the substrate within an arc limited by the extensibility of the rostrum. In

initiating a step while the animal is browsing, the fore-foot is extended to the tip of the rostrum but not beneath it. Completion of a "step" automatically causes an advance of the rostrum. With the advance, the rostrum may remain near the substrate, the radula rasping here and there, or, with the hunching motion, it may stretch straight out and, subsequently, be drawn straight back to the body while rasping the substrate. Occasionally, with the hunching movement, the rostrum is raised and the whole head stretched upward at an angle of about 60°. The rostrum is fully extended and then lowered to the substrate as far ahead of the body as possible. In all these movements the foot is coordinated with the actively probing rostrum but the animal is not dependent upon the rostrum for support.

b. Movements on soil. On loose soil, especially on a sharp incline, the step-like movement may appear less evident due to back sliding and the crumbling of soil beneath the foot. Where greater exertion is necessary the hunching movement becomes quite pronounced.

c. Movement in water. While moving across glass and submerged under water, this species does not simply glide as stated by Abbott (1948a). Water greatly reduces resistance to movement caused by the weight of shell and visceral mass. Glass affords so smooth a substrate that ciliary activity facilitates a slight glide. The main movement is achieved, however, by what appears to be an almost effortless short extension of the fore-foot and subsequent contraction at mid-foot as described above. The "step" is not at all pronounced due to the reduced pressures on the foot. Under water the hunching movement is greatly reduced while the ciliary action, as well as the undulations of the margin of the fore-foot are more pronounced.

*Pomatiopsis lapidaria* was observed moving upside down, adhering to the surface film of water. The fore-foot was extended and cupped, thus forming a deep concavity. Ciliary currents swept

into the concavity and the animal glided over the surface of the water. Only occasionally did the mid-foot contract or the snail make a hunching movement.

The point to be made here is that the foot of *P. lapidaria* is like that of the Hydrobiidae while the mode of progression is evidently an adaptive change enabling movement on land where the buoyant effect of water is lost and the increased weight of the foot makes ciliary movement impossible. The pedal crease is a result of accentuation of certain muscles enabling the step-like mode of progression and is accentuated in *Pomatiopsis* by pigmentation on the lateral foot surface below the suprapedal fold. Weight of body and shell also serve to accentuate the crease.

The basic difference between the mode

of progression of members of the Pomatiopsinae and that of members of the Truncatellidae was pointed out by Stimpson (1865) who stated that in *Truncatella* there were only 2 points of support and that progression in *Truncatella* should be called "looping" as opposed to "stepping" in *Pomatiopsis*. In the former the rostrum and the whole foot were described by Stimpson as the 2 points of support while in the later the 2 "sections" of the foot and the rostrum were considered as 3 points of support. In reality, however, as discussed above, *Pomatiopsis* does not so use the rostrum. Fretter & Graham (1962) gave the following description for the mode of progression in *Truncatella*. It "extends the snout, which is very extensible, and grips the substratum

TABLE 3. Cusp formulae for the teeth of *Pomatiopsis lapidaria* previously presented or discussed in the literature\*

Author		Central Anter. cusps Basal cusps	Lateral	Inner Marginal	Outer Marginal
1.	Stimpson, 1865	$\frac{1-1-1}{2-2}$	1-1-2	5	5
2.	Binney, 1865	copied from Stimpson			
3.	Baker, 1902	copied from Stimpson			
4.	Walker, 1918	copied from Stimpson			
5.	Annandale, 1924	$\frac{2-1-2}{2-2}$	2-1-3	10	10
6.	Thiele, 1928	$\frac{1-1-1}{2-2}$	2-1-3	6	5
7.	Baker, 1928	$\frac{1-1-1}{1-1}$	1-1-3	6	9
8.	Thiele, 1931**	$\frac{1-1-1}{2-2}$	2-1-3	4-6	3-5
9.	Abbott, 1948a	$\frac{1-1-1}{2(3)-(3)2}$	2-1-2 (3-4)	7(6)	5
10.	Dundee, 1957	$\frac{1-1-1}{2-2}$	2-1-3	-	-

\* Compare with Table 8 and Plate 19.

\*\* Description given for the genus *Pomatiopsis*.

with its tip (p 598, Fig. 315); it then pulls the foot up to grasp the ground just behind the snout, dragging the shell in its rear, releases the snout and starts the process once again. Sometimes the foot slides along the surface of the ground as it is drawn forward, sometimes it is lifted clear." They state that the small rounded foot and the expanded tip of the snout are related to this movement.

3. Radula. Stimpson (1965) figured the radula of *Pomatiopsis lapidaria*. As shown in Table 3, this drawing was extensively copied. Additional figures were prepared by Annandale (1924), Baker (1928), Thiele (1928) and Abbott (1948a). The radula of *P. cincinnatiensis* was figured by Troschel (1863) under the synonym of *Amnicola sayana* and subsequently copied by Stimpson (1865) and Binney (1865). Baker (1928) and Berry (1943) provided new figures, those of the last being the best.

Stimpson (1865) states that the radula of the Pomatiopsinae is distinct from that of the Hydrobiinae in that, in the former, the basal cusps (denticles) of the central tooth are placed at or near the base. In this view Stimpson is correct. In the Hydrobiidae the basal cusps are often attached to a thickened ridge along the lateral angle of the central tooth (La, Pl. 19, indicates that lateral angle, unthickened in the Pomatiopsinae). This arrangement is particularly noted in genera such as *Amnicola* and *Hydrobia*. In the Pomatiopsinae the supports for the cutting edge of the basal cusps do not arise from a thickened ridge along the lateral angle but from moulded thickenings of the tooth running posteriorly from points situated anteriorly on the lateral angle (Slb, central 6, Pl. 19). By changing focus on the lateral angle it is evident that each thickened support causes a slight undulation to the lateral angle where it arises. The most prominent of the supports arises just lateral to the outer anterior cusp (or cusps) flanking the large central cusp (Slb, central 6, Pl. 19). Baker (1926) in the

first diagnosis of the Pomatiopsidae used the following radula characteristics as having major importance: "radula with its few cusps of large size and the large denticles on the base of the central tooth." Baker (1928) stated in the family diagnosis: "central tooth of the radula with but one large basal denticle; denticles of the lateral and marginal teeth very large and few in number, proportionally much larger than in the Amnicolidae." Berry (1943) states that the radula is "very different from the Amnicolidae. The central tooth has the basal wing terminating as a cusp and a single basal denticle down from the lateral ridge. The few large cusps on the central, lateral and marginal teeth are distinct characters of *Pomatiopsis*, and not Amnicolidae."

The radulae of *Pomatiopsis* and *Oncomelania* are described in detail in the section on anatomy. A few comments must be made here, however, as they relate radular structure to the family or subfamily taxon characters. Baker (1926, 1928) was mistaken in using a single pair of basal cusps as part of the criterion for defining the family Pomatiopsidae. *P. lapidaria* has 2 or 3 pairs of basal denticles (Thiele, 1928; Abbott, 1948a). *P. cincinnatiensis* has 2 pairs of basal denticles (Berry, 1943), as do the radulae of *P. californica* and *P. binneyi* which I have observed. In studying the radula collection of *P. cincinnatiensis* at the University of Michigan Museum of Zoology (UMMZ), it was evident that the basal portion of the lateral angle did not terminate as a cusp, although the outer basal cusp had a definitely more external position than that of *P. lapidaria*. It was possible to discern, basolaterally to the outer basal cusp, a well-defined termination of the lateral angle. The support for the basal cusp arose quite anteriorly, on the edge of the lateral angle.

The radulae found in the Pomatiopsinae are clearly included in the range of types found in the Hydrobiidae throughout the world. They are similar in that

the central tooth is wider than long, and has basal cusps, that its anterior edge is narrower than the posterior (basal) edge, and in that there is more than one anterior cusp. The shape and denticulation of the laterals and marginals are compatible with those found in the family Hydrobiidae.

The pomatiopsid radula is distinctive in that the cusps of the laterals and marginals are generally fewer and larger than in most hydrobiids. *P. cincinnatiensis* is extreme in having only 3-4 large cusps on the marginals. *P. lapidaria* has up to 9 cusps on the inner marginal while *P. binneyi* has up to 11. In the last species, the cusps of the marginals are small and needle-like, as in many hydrobiid species. The central is distinctive in having only 1 or 2 cusps on either side of the central cusp on the anterior edge of the tooth. Other hydrobiids generally have 3 or more cusps on either side of the central cusp. The distinctive nature of the supports for the basal cusps has been mentioned.

When the radulae of other taenioglossid prosobranch families are compared with those of the Hydrobiidae, it is evident that the radula of *Pomatiopsis* is a hydrobiid type. While members of the Bithyniidae, e.g., *Bithynia tentaculata*, have a radula seemingly more similar to some hydrobiids than that of *Pomatiopsis*, *Bithynia* does show numerous other major morphological differences which clearly separate the group from the Hydrobiidae. In the Truncatellidae the unusual triangular central tooth with the single anterior cusp is distinctive. In the assimineid radulae the central and outer marginals have shapes which are distinctly different from those found in the Hydrobiidae. The length of the central is greater than its width. The central is without the lateral angle characteristic of the hydrobiid radula and is often without basal cusps. The outer

marginal is exceptionally wide compared with the relatively slender type found in the Hydrobiidae. In the littorinacean Littorinidae and the cerithiacean Pleuroceridae the central lacks distinctive basal cusps. Radular characteristics are clearly not sufficient to separate *Pomatiopsis* from members of the family Hydrobiidae.

In conclusion, the Pomatiopsinae represent a subfamily including several genera outside the United States; e.g., *Tomichia* from South Africa, *Blanfordia* from Japan, as well as *Oncomelania* from the Western Pacific area. In these genera the shell is elongated and turreted in contrast to the globose type of *Amnicola*, the bulimoid shape of *Littoridina*, or the planispiral shell of *Hovatia*. The tendency in the group is towards an amphibious to terrestrial habitat. Correlated with the amphibious habitat is a step-like mode of progression. A crease develops in the anterolateral foot upon full contraction of the foot. Eggs, laid singly, are covered with a mud capsule (not known for *Tomichia*). The radula has fewer and larger cusps on the marginal teeth than other hydrobiid snails. The anterior cusps on either side of the central cusp of the central tooth are 1 or 2 in number. The basal cusps of the central teeth have a distinctive type of support and are generally 2 or 3 in number. The eye is in a pronounced swelling at the base of the tentacle, differing from the species of the genus *Hydrobia*, where the eye is in a slight swelling of the tentacular base.

Characters in common with other hydrobiids are the simple verge (hydrobiids have verges either simple or with various appendages), a paucispiral corneous operculum, a broad simple foot which is truncate in front and gently rounded behind. The foot has an anterior transverse mucous slit (See Ms, Pl. 1, Fig. 7A). The rostral shape and tip, and basic internal anatomical features are hydrobiid.

## MATERIALS AND METHODS

The body of this paper is divided into 4 major sections: anatomy, hybridization studies, electrophoretic studies and laboratory ecology. Methods pertaining to each of these sections will be discussed under those 4 headings. The snails used throughout these studies were all fully mature adults as indicated by shell size in *Pomatiopsis* and varix formation in *Oncomelania*. The only exceptions were the newly hatched young used in the growth experiments.

Field collected *Pomatiopsis lapidaria* were utilized for anatomical studies and electrophoretic experiments. These were obtained from the Barton and Hog Back stations described by Dundee (1957) as well as the Parker Mill Station discussed by van der Schalie & Dundee (1959). These stations are within 5 miles of Ann Arbor, Michigan, U. S. A.

*Oncomelania* subspecies used were F<sub>1</sub> or F<sub>2</sub> laboratory reared snails of field collected parental stock. *O. hupensis formosana* came from Pu Yen village, a small farming community a few miles south of the city of Changhua, Taiwan (Formosa). *O. hupensis nosophora* were sent from the Kofu Valley in the Yamanashi Prefecture of Japan. *O. hupensis quadrasi* were sent from Palo, Leyte, in the Philippines.

## COMPARATIVE ANATOMY

### A. Introduction

One encounters several major problems in attempting to make detailed comparisons from published anatomical material. The material is often stylized and portrays organ systems in a general manner omitting exact contours, dimensions, variations and positional relationships with other organs. Homologous organs are presented in different views by the various authors, making comparisons difficult or impossible.

In this study the gross anatomy of the muscular, nervous, reproductive systems, parts of the alimentary sys-

tem and the external morphology are discussed. The systems and organs of both species are presented in the same manner and orientation, thereby facilitating comparisons. The systems and organs were studied in order to determine in a comparative manner (1) the presence or absence of a structure, (2) qualitative differences in the structure of homologous organs and (3) quantitative differences or similarities in organs or structure.

This is not a complete anatomical description, as details of the excretory and circulatory systems are not covered. The systems investigated were chosen because of their potential in providing characters which could be readily used in a systematic discussion.

*Pomatiopsis lapidaria* will be discussed first, followed by a similar treatment of *Oncomelania hupensis formosana*. Comparisons between the 2 species will be discussed with each anatomical section presented for *O. hupensis formosana*.

### B. Materials and Methods

Dissections were carried out under magnifications of 40X and 60X using a Nippon Kogaku dissecting microscope. Measurements of all structures were made using a standard ocular micrometer. Proportions and structural dimensions in all drawings were checked against the specimen, using this micrometer. A 9 cm Petri dish filled with paraffin and blackened with norite served as the container and substrate for all dissections.

Tools used for dissections were "Minutien-Nadeln" (insect pins) embedded in solid glass rods, iridectome scissors of the finest grade, jeweler's forceps with extra fine points, and pliers for cracking the shell.

Well over 200 snails were used for the anatomical studies of each species. The snails were studied while living or just freshly preserved. Living animals were most suitable in studying the organs and ducts of the reproductive systems.

Aqueous neutral red was very useful in accentuating the tubes of the reproductive systems as well as nerves and ganglia. Aqueous methylene blue aided in staining the visceral ganglion and associated nerves in the living snail.

In studying freshly killed snails the animals were removed from the shells, pinned out in the desired position under water, the water was poured off, and Bouin's fixative was added full strength. Structures in the head were more readily studied in the freshly killed snail, as mucoid secretions were a hindrance in the living animals. Studies on nerves were facilitated by dissecting under Bouin's solution as minute nerves stood out prominently in that fluid under direct illumination. Muscles were studied in the contracted state where the smaller muscles were more prominent and where a fairly stable configuration of muscles was assured when numbers of specimens were studied.

Radula. Radulae were dissected from the buccal mass and placed in a 10% solution of KOH for varying amounts of time (about 24 hours). Upon dissolution of attendant membranes the cleaned radula was placed on a slide with a drop of 4% acetic acid. The acid loosened the lingual membrane (radular shield) within an hour, so that the radula could be readily flattened out; it also facilitated removal of separate teeth or groups of teeth from the membrane. With the radula flattened out, measurements were made of the length and width of the radula and the number of rows of teeth were counted. Measurements and counting were carried out under a magnification of 150X, using a Nippon Kogaku compound microscope. At this point, the radular ribbon was mounted whole or teeth were stripped from the membrane to facilitate a study of each tooth. The acid was allowed to dry on the slide and then a drop of CMC-10, a non-resinous mounting medium, was added and a coverslip applied. In 24 hours the CMC-10 dried and the edge of the coverslip was ringed

with clear fingernail polish assuring the permanency of the slide. Detailed studies of the teeth were readily made without need for stain as the smallest cusp readily stood out. Drawings of the radular teeth were made using oil immersion (1000X) and camera lucida.

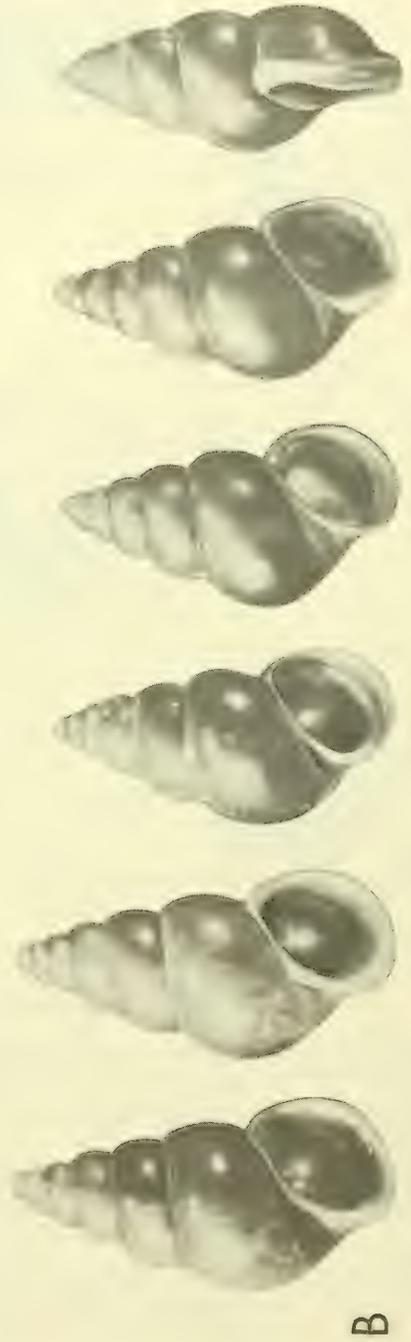
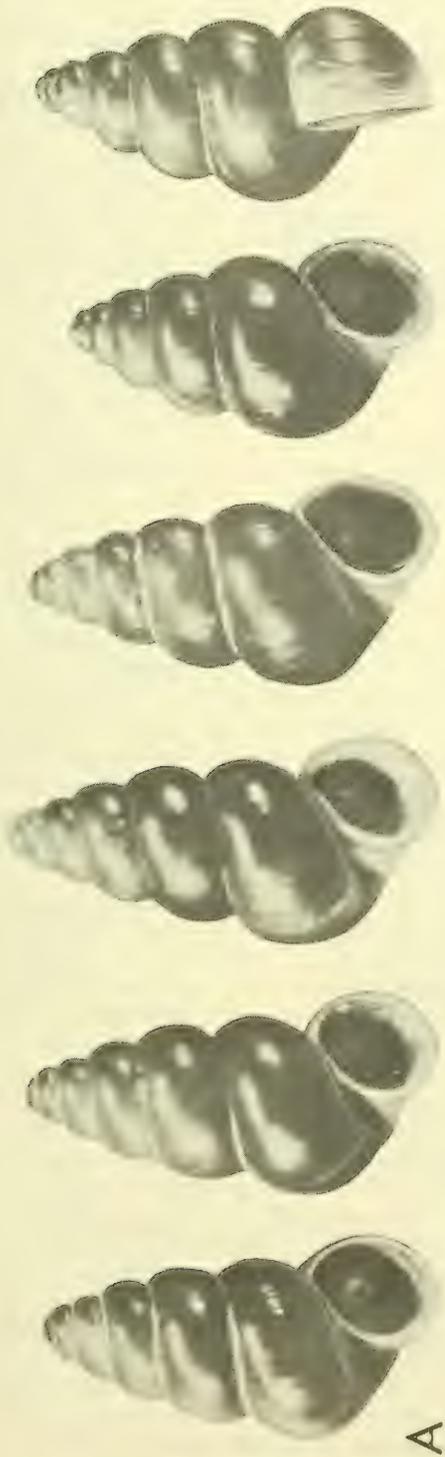
Jaws. The buccal mass was removed and the area of the outer lips cut free with iridectome scissors. This separated anterior end of the buccal mass was placed on a slide and opened from the dorsal surface, thus exposing the oral tube and the anterior part of the buccal cavity. A drop of CMC-10 was placed on the tissue, the tube was opened, and a coverslip was applied. The jaws, fully exposed, were studied under the compound microscope. They were drawn using a camera lucida.

Shell. Shells were boiled in sodium hypochlorite (5.25%) (commercial Clorox) to remove the periostracum and all occluding matter such as algae, dirt, etc. Cleaned in this manner, the sculpture, shell surface, apical whorls, and sutures were readily studied.

Anatomical orientation. There is no problem in discussing features of the head as the foot is ventral and the rostrum points anteriorly. However, the remainder of the organism is coiled within the shell and orientation becomes a problem when discussing anatomical features. As Fretter & Graham (1962) state for *Littorina*, "as the animal lies in its shell the outer part of each whorl corresponds to the dorsal surface of the body, and the inner to the ventral." The inside of the coil or ventral surface is that which is appressed to the columella.

All visceral anatomy presented here was described from the uncoiled snail, with the head lying to the right, the body surface presented in the illustrations being the columellar (ventral) side. Left lateral is towards the bottom of the drawings wherever the term is used, and right lateral is towards the top of any given figure; "posterior" means towards the apex, "anterior" towards the head.

PLATE 2



Shells of *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana*. A. *P. lapidaria*. B. *O. hupensis formosana*. Comparative sizes are given in Tables 4 (p 19) and 9 (p 81).

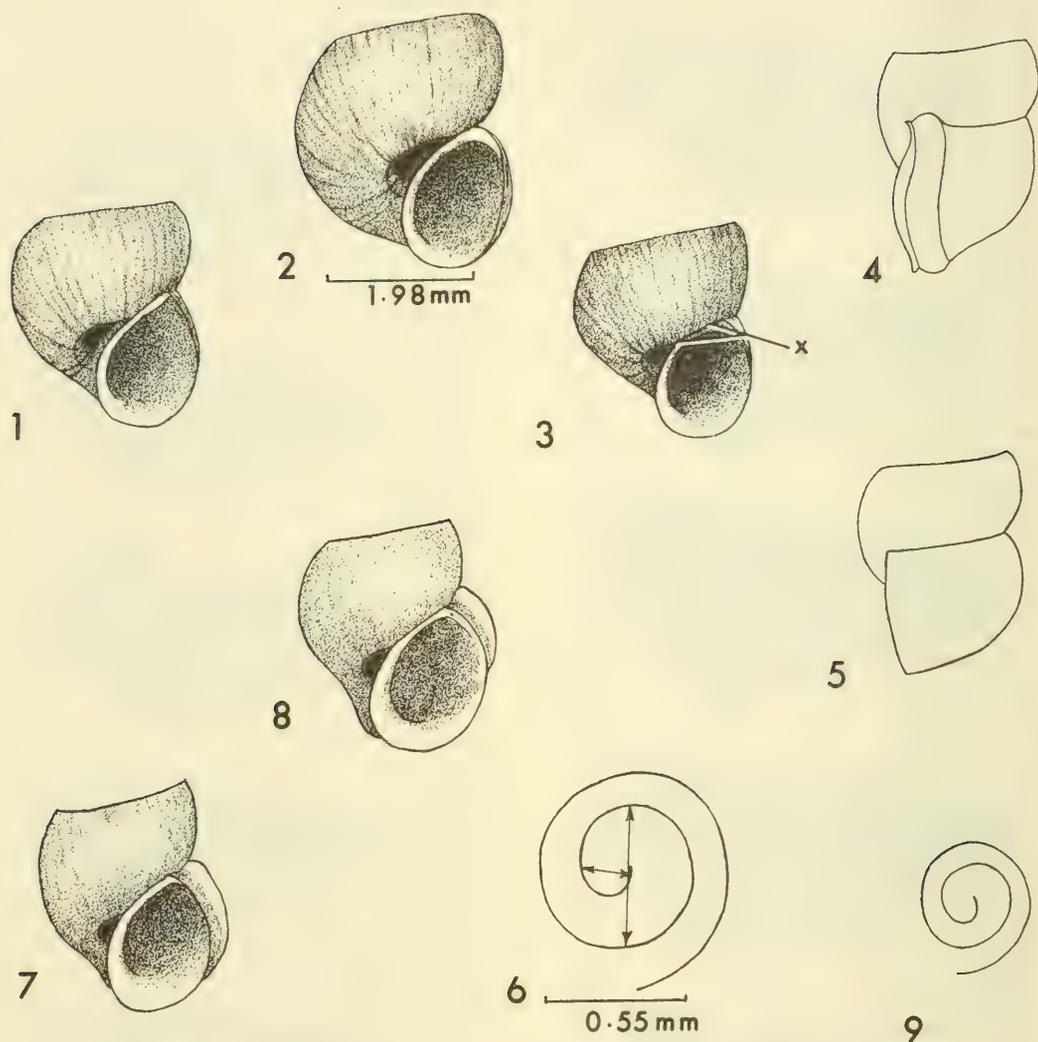


PLATE 3. Shell features of *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana*.

FIGS. 1, 2, 3. Apertural view of the shell of *P. lapidaria* showing the wide umbilicus and the shortened parietal callus (x).

FIG. 4. Shell of *O. hupensis formosana* showing the sinuate outer lip and the varix.

FIG. 5. Shell of *P. lapidaria* showing the straight outer lip without the varix.

FIG. 6. Apical whorls of *P. lapidaria*. The short arrow points out the width of the tip of the apical whorl; the long arrow points out the width of the first whorl.

FIGS. 7, 8. Apertural view of *O. hupensis formosana* showing the relatively narrow umbilicus and elongate parietal callus.

FIG. 9. Apical whorls of *O. hupensis formosana*. The scale is the same as in Fig. 6.

TABLE 4. Conchological measurements of *Pomatiopsis lapidaria* from Ann Arbor, Michigan

Structures Measured	Number of Snails	Length in mm			Number of Snails	Width in mm		
		$\bar{X}$	S	Se		$\bar{X}$	S	Se
Shell 6.0 whorls	24	5.5	0.32	0.07	24	2.9	0.20	0.04
Shell 6.5 whorls	30	6.2	0.27	0.01	30	3.1	0.11	0.02
Shell 7.0 whorls	8	6.7	0.36	0.04	8	3.2	0.18	0.06
Aperture	62	2.1	0.10	0.01	-	-	-	-
Apical Whorl	-	-	-	-	26	0.53	0.04	0.008
Tip of apical whorl	-	-	-	-	26	0.19	0.03	0.006

$\bar{X}$  = The mean

S = Standard deviation

Se = Standard error of the mean

All illustrations were made by the author.

### C. *Pomatiopsis lapidaria*

#### 1. Shell

Say (1817) described the shell in the type description as "turreted, subumbilicate, with 6 volutions, which are obsoletely wrinkled across. Suture impressed. Aperture longitudinally ovate-orbicular, rather more than 1/3 of length of shell. Length about 1/5 of an inch."

Although later authors, in particular F. C. Baker (1928), have elaborated on Say's original description, more detail and discussion of the shell is necessary. The shell (Pl. 2, A) is indeed elongate and turreted. Adult shells have 6.5-7.0 whorls. Shells of 7.5 whorls are rare in non-fossil material (see p 20). The nuclear whorls are 2.0-2.75 in number, glassy, and in cleaned material may appear amber, thereby set off from the brownish or yellow-brown horn color of the remainder of the shell. In uncleaned material the nuclear whorls may appear glistening or dull white. The first nuclear whorl, as Baker pointed out, is usually not emergent and is partially "embraced by the second whorl." This often gives the apex a flattened appearance.

The sutures of the whorls are deeply impressed and the whorls correspond-

ingly very convex. The aperture is "elongate, ovate, somewhat narrowed and angled above" (Baker, 1928). The inner lip is connected with the outer lip by a parietal callus. In some specimens the callus is so thickened that it looks as if the inner lip continued into the outer lip. Occasionally a specimen is found where the inner lip is not adnate to the parietal wall. The inner lip is slightly reflected over the umbilicus. The parietal callus varies in length, the greater the length, the more occluded the umbilicus. The outer lip is sharp, strong and does not form a varix. Observing the edge of the outer lip with the aperture rotated 90° to the left of apertural view, one observes that it is straight, not sinuate (Pl. 3, Fig. 5). The apical part of the outer lip may have a slight sinuation in some cases but this is not nearly as plain as the sinuation found in *Oncomelania lupensis formosana* (Pl. 3, Fig. 4).

The umbilicus is very pronounced and deep (Pl. 3, Figs. 1-3). As shown in these same figures, the base of the shell is rounded. Without magnification the whorls of the cleaned shells appear smooth and glistening. Under 6-16 magnifications it is evident that the surface of the whorls are wrinkled by growth lines which are irregular in diameter, vary in prominence, and are closely packed. The overall effect is to

give the shell a roughened micro-sculpture. The coarse growth lines start immediately after the nuclear whorls.

A series of measurements, which are felt to be of use in specific comparisons, are discussed below. Others could be made, but those presented are adequate for the comparisons intended. A series of 62 specimens in all, collected from the Barton, Parker Mill and Hog Back stations were studied conchologically without reference to sexual dimorphism. Length, width, aperture length, parietal callus length, width of the first nuclear whorl and width of the tip of the nuclear whorl (Pl. 3, Fig. 6) were measured. These were recorded with the corresponding whorl count. The results are shown in Table 4. Shells of 6 whorls had an average length of 5.5 mm and an average width of 2.9 mm; the corresponding measurements for shells of 6.5 whorls were 6.2 mm and 3.1 mm, and for shells of 7.0 whorls 6.7 and 3.2 mm, respectively. No shells with 7.5 whorls were found among several hundred additional snails observed (see below). The average length of the aperture for snails of 6.0-7.0 whorls was 2.1 mm. These dimensions were compared with those gathered from several lots of this species housed at the UMMZ (Table 5). These lots represented populations scattered over the extensive range of this species (distribution map in Abbott, 1948a). No significant difference was found for the parameters of shell length, width, or aperture length among these populations.

The first nuclear whorl (Pl. 3, Fig. 6) varied but little (Table 4) with a width of 0.53 mm. The tip of the first whorl averaged 0.19 mm and this width likewise was extremely constant. These features did not deviate significantly among the populations studied. The length of the parietal callus, however, did vary quite a bit in length and significantly so between populations (Table 5). The Ann Arbor snails of the current studies had an average callus length of

0.6 mm, the shortest of all the populations studied. Correlated with this feature was an unusually pronounced umbilicus. As shown in Table 5, the average callus length of various populations ranged from 0.66 mm to 0.96 mm. These differences are possibly related to growth patterns which deviate under different environmental conditions. Variation in length of parietal callus is shown in Plate 3, Figs. 1-3.

Hubricht (1960) studied the shells of *Pomatiopsis lapidaria* from a number of localities and stated that the species appeared modified by different ecological conditions; "slender, thick shells occur in dry habitats, obese thinner shells in wet ones. This is especially true in the South." In that paper Hubricht considers *P. praelonga* Brooks & Mac-Millan and *P. hinkleyi* Pilsbry synonymous of *P. lapidaria*. I agree with Hubricht in synonymizing these forms. Certainly *P. hinkleyi* (Table 5) showed no shell characteristics significantly different from *P. lapidaria* from many localities, except that in length of parietal callus, a character which is shown to be variable.

A lot of fossils (UMMZ) from the same locality as *Pomatiopsis scalaris* (Baker, 1927) a Pleistocene fossil, was also studied (Table 5). *P. scalaris* has been described as "strikingly" different from *P. lapidaria*, being longer, having 1 more whorl (8 whorls) and very deep sutures. The lot of fossils here studied formed a series which graded from the *P. lapidaria* observed in Ann Arbor to typical *P. scalaris*, i.e., the present fossil material had shells of 7.0-7.5 whorls. (*P. scalaris* was described as having 8 whorls). Many of the recent specimens of *P. lapidaria* had the very deeply impressed sutures and convex whorls attributed to *P. scalaris*. Specimens of recent *P. lapidaria*, from various parts of the country, with 7.5 whorls and the characteristics of *P. scalaris*, can occasionally be found, although they are comparatively rare. The umbilicus of *P. scalaris* as well as of the fossil series

TABLE 5. The average callus length for lots of *Pomatiopsis lapidaria* from various widespread localities in the U. S. A.

Locality	Number of specimens	Average callus length in mm
Alabama, Florence; Lauderdale Co. Lot UMMZ 69912* topotypes of <i>P. hinkleyi</i>	16	0.72
Florence; Bolder Falls; Lauderdale Co. Lot UMMZ 91487 paratypes of <i>P. hinkleyi</i>	4	0.96
Indiana, New Harmony; Posey Co. Lot UMMZ 69915 Fossil Material	11	0.72
Iowa, Marion; Linn Co. Lot UMMZ 132464	11	0.84
Michigan, Ann Arbor; Washtenaw Co. Lot UMMZ 91559 Barton Station	9	0.90
Lot UMMZ 183219 Barton Station, 1952	12	0.66
Lot UMMZ 183217 Hog Back Station, 1952	16	0.84
North Carolina; Broad River, Point Rock Lot UMMZ 91495	6	0.72
Ohio; Miami Co. Lot UMMZ 59325 Drift off Big Miami River	8	0.84
Wisconsin; Baraboo; Sauk Co. Lot UMMZ 143721	9	0.84

\*University of Michigan, Museum of Zoology catalog numbers.

from the UMMZ appeared more rounded, wider, and deeper than that met in recent *P. lapidaria* from many localities, a feature that is correlated with a pronounced tendency for a short parietal callus and an inner lip which is barely reflected. However, these same characters are quite pronounced in current populations of *P. lapidaria* from the Ann Arbor area. The width of the apical whorl in the fossil material was the same

as that found in recent *P. lapidaria*. As a result of these studies, *P. scalaris* is considered an early extreme of *P. lapidaria* and synonymous with it.

## 2. External morphology and topography

The folds and grooves of the head have been mentioned.

Pigmentation. The head, dorsally and laterally, is black to grey-black due to

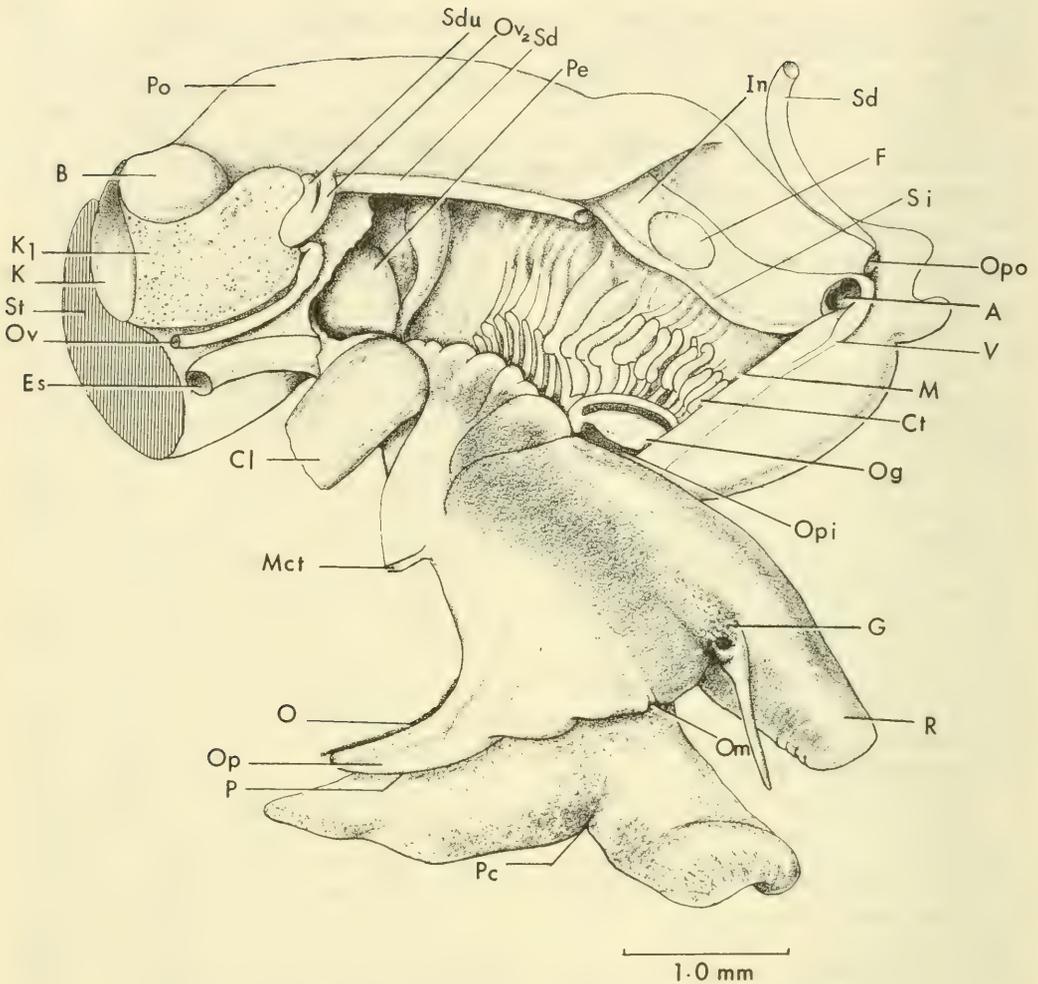


PLATE 4. Head, foot, and mantle region of *Pomatiopsis lapidaria*.

A	anus	Opi	osphradial pit
B	bursa copulatrix	Opo	opening of the pallial oviduct
Cl	columellar muscle	Ov	oviduct
Ct	ctenidium	Ov <sub>2</sub>	portion of oviduct entering pallial ovi- duct
Es	esophagus	P	suprapedal fold
F	fecal pellet	Pc	pedal crease
G	glandular units	Pe	pericardium
In	intestine	Po	pallial oviduct
K	cut edge of the kidney	R	rostrum
K <sub>1</sub>	ventral surface of the kidney	Sd	spermathecal duct
M	edge of the mantle	Sdu	sperm duct
Mct	cut edge of the mantle	Si	subintestinal sinus
O	operculum	St	stomach
Og	osphradial ganglion	V	blood vessel
Om	omniphoric groove		
Op	operculerous lobe		

heavy pigmentation (Pl. 1, Figs. 1, 2, 5). The edges of the foot below the suprapedal fold are dusted with pigment, although more lightly than the dorsal surface. Pigmentation continues along the neck into the mantle cavity but fades out towards the base of the "neck." On the anterodorsal edge of the foot pigmented patterns appear to outline channels in the foot (Pl. 4) which probably coincide with the mucous ducts figured by Abbott (1948a).

The sole of the foot (Pl. 1, Figs. 3, 4, 6-9) appeared to have 2 color patterns when studied under direct illumination. The periphery of the sole was a light slate grey to blue-grey while the central area appeared opaque white to yellow-white. The central area, on the average, was 0.50 mm from the front edge of the foot, 0.96 mm from the posterior end, and about 0.40 mm in from each side. This area corresponds to a position over the pedal haemocoel.

White granular units of about  $25\mu$  diameter were especially crowded in the posterior part of the foot, becoming sparse along the sides. Granules were sparse in the central area. In addition to the relatively large granules, the sole was covered with small whitish bodies appearing as tiny rods all perpendicular to the sole (observed at 40X). These tiny rods were closely packed at the anterior edge of the foot, and less dense posteriorly.

Viewing the living animal through the shell (apertural view with snail retracted) the pattern of pigmentation on the outer or dorsal side of the body tube is readily observed. The intestine filled with fecal pellets is clearly discerned crossing the body whorl, underlined by a thin band of pigment 0.24-0.40 mm wide. This band continues along the dorsal surface, widening above the body whorl (0.48-0.72 mm). In the apical whorls this band of pigment becomes more slender again (0.25-0.40 mm). Dundee (1957, Pl. 6) shows this pattern in the apical whorls. The band is generally positioned on the periphery of the coil

or displaced towards the aperture on each whorl. The band is not neatly delineated with parallel sides but irregularly scalloped, flammulate at the edges, especially the edge towards the apex. This pattern is observable in both males and females. From the ventral aspect some of the pigment is observed curling over from the dorsal surface (Pls. 5, 6). The exterior epithelium covering the ctenidial area (see Pl. 4) is pigmented and the pigmentation tends to outline the position of each gill filament.

Abbott (1948a) states that in *Pomatiopsis lapidaria* "the most distinguishing color markings are the bright splotching of yellow or yellowish-white granular dots over each eye forming false 'eyebrows'." These glandular units (Pl. 1, Fig. 1, 2, 5; Pl. 4; Pl. 11, Fig. 1) partially surround the eye and occlude the medial, posterior edge of the eye. Collectively they are a mass about 0.36 mm long with the greatest width of 0.17 mm. Coloration varies from white to yellow-white.

Tentacles and Eyes. The eyes are in large, distinct swellings at the outer base of each tentacle (Pl. 1, Fig. 1). Viewed ventrally these swellings appeared continuous with the tentacles but from the dorsal surface they appear as units fused with the tentacles and set off from them by a slight crease. In any event, the ocular units are not the simple swellings in the outer tentacular bases seen in *Hydrobia* and others of the Hydrobiinae and Rissoidae. The characteristic shape of the tentacles is shown in Plate 1, Fig. 1. They are highly contractible and pliable but can most often be seen with a swelling at their base anterior to the ocular units.

General Topography. In the uncoiled snail with the columellar side exposed, one can observe, in addition to the head, 3 general areas: (1) the region of the mantle cavity (Pl. 4) which extends from the mantle edge (M) where it encircles the "neck" (Pls. 5, 6) back to a point just posterior to the tip of the visceral

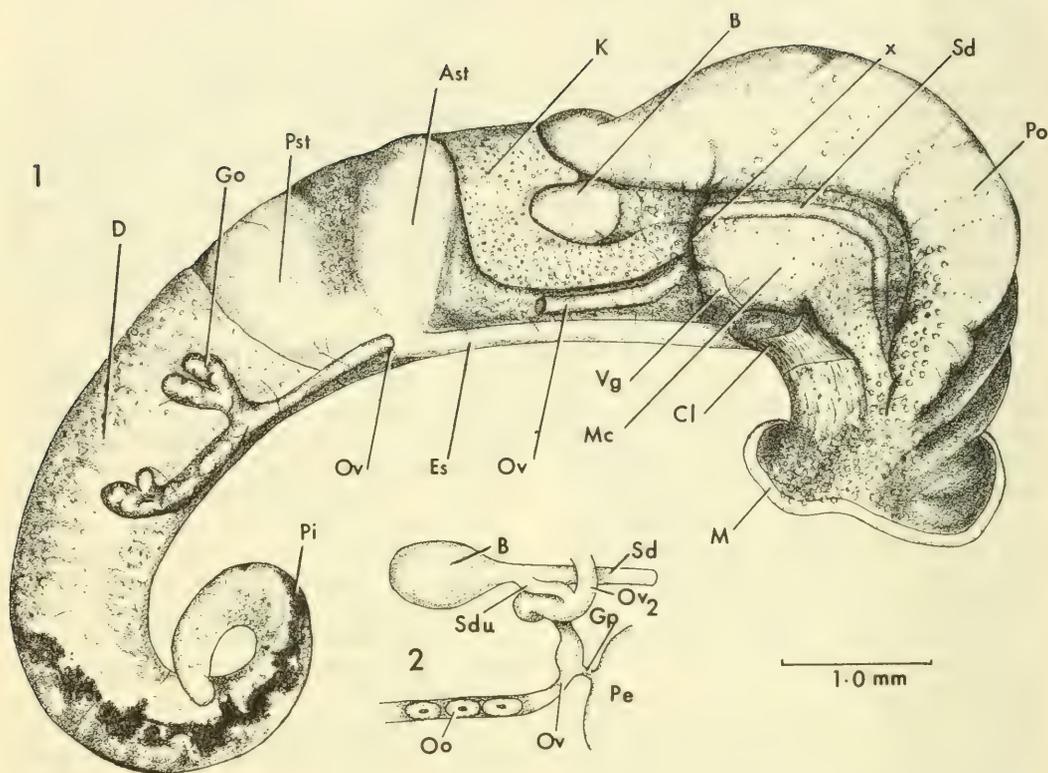


PLATE 5. Uncoiled female *Pomatiopsis lapidaria* showing parts of the female reproductive system.

FIG. 1. Uncoiled female *P. lapidaria*. The oviduct (Ov) is broken due to the stress of uncoiling the snail.

FIG. 2. The portion of the reproductive system uncovered by peeling away the connective tissue and kidney tissue between the bursa copulatrix (B) and the edge of the mantle cavity (x).

Ast	anterior chamber of the stomach	Ov <sub>2</sub>	portion of the oviduct passing ventral to the spermathecal duct to enter the pallial oviduct
B	bursa copulatrix	Pe	pericardium
Cl	columellar muscle	Pi	pigment band showing the flammulate pattern at the edge
D	digestive gland	Po	pallial oviduct
Es	esophagus	Pst	posterior chamber of the stomach
Go	gonad	Sd	spermathecal duct
Gp	gonopericardial duct	Sdu	sperm duct
K <sub>1</sub>	ventral surface of the kidney	Vg	visceral ganglion
M	edge of the mantle	x	the posterior end of the mantle cavity
Mc	ventral wall of the mantle cavity		
Oo	oocyte		
Ov	oviduct		

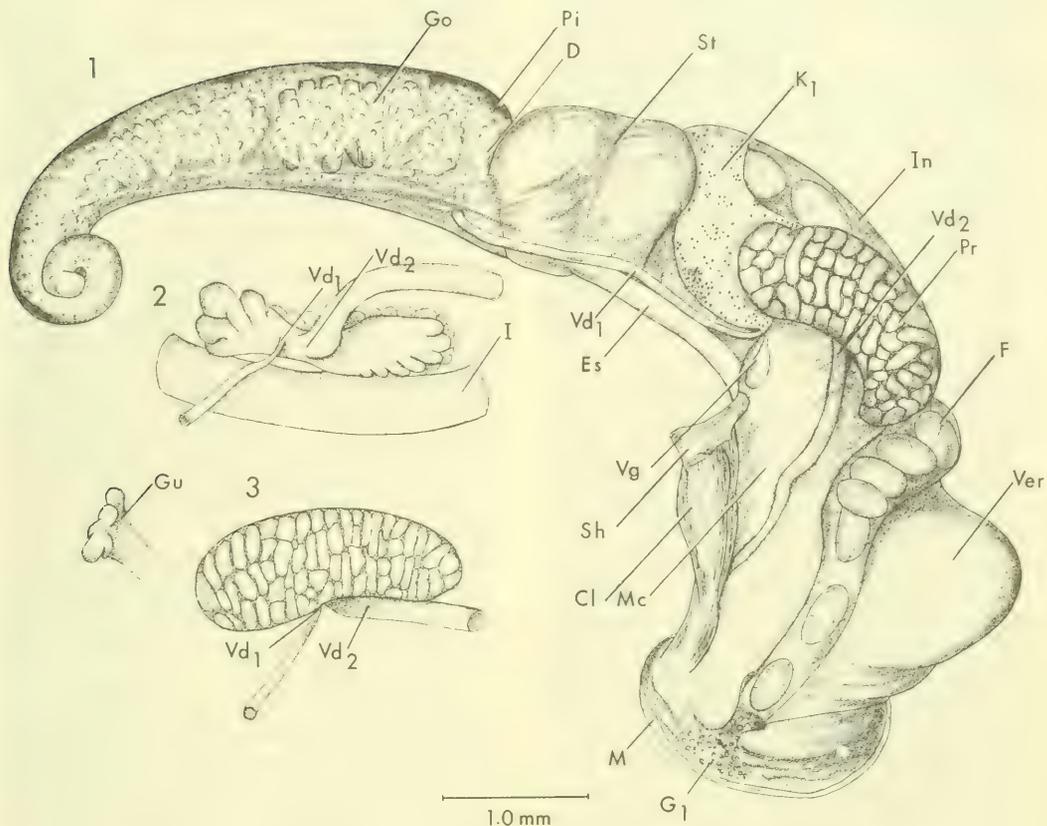


PLATE 6. Uncoiled male *Pomatiopsis lapidaria* showing parts of the male reproductive system.

FIG. 1. The uncoiled snail.

FIG. 2. The prostate pressed against the intestine and turned over to expose the point of entrance of the posterior portion of the vas deferens in relationship to the point of exit of the anterior portion of the vas deferens.

FIG. 3. The prostate as viewed in Fig. 1; the connective tissue was cleared away to show the posterior vas deferens ( $Vd_1$ ) passing under the edge of the prostate.

- |       |   |        |                                       |
|-------|---|--------|---------------------------------------|
| F     | fecal pellet                              | Mc     | ventral wall of the mantle cavity     |
| Cl    | columellar muscle                         | Pi     | pigment                               |
| D     | digestive gland                           | Pr     | prostate                              |
| Es    | esophagus                                 | Sh     | shell fragment                        |
| $G_1$ | large, white "granular" units             | St     | stomach                               |
| Go    | gonad                                     | $Vd_1$ | posterior portion of the vas deferens |
| Gu    | a single glandular unit from the prostate | $Vd_2$ | anterior portion of the vas deferens  |
| In    | intestine                                 | Ver    | verge covered by mantle wall          |
| $K_1$ | kidney                                    | Vg     | visceral ganglion                     |
| M     | edge of the mantle                        |        |                                       |

ganglion (Vg); (2) the mid-body region extending from the end of the mantle cavity to the posterior portion of the stomach and in which are observed the stomach (St; Pst, Ast), kidney ( $K_1$ ), part of the intestine (In), a segment of the esophagus (Es), and a portion of the oviduct or vas deferens (Ov or  $Vd_1$ , respectively); (3) the digestive gland (D) which continues posteriorly from the stomach and contains the gonad (Go) in its left ventral surface beneath the epithelium just posterior to the stomach.

In the female (Pl. 5), the pronounced pallial oviduct (Po) traverses the anterior portion of the mid-body and the length of the mantle cavity. In the male (Pl. 6), the prostate (Pr) lies over the 2 areas but does not extend anteriorly the whole length of the mantle. The bursa copulatrix (B) is prominent in the mid-body of the female. The columellar muscle (Cl) emerges from the "neck" area of the head (Pl. 4) on the ventral surface and is pressed against the ventral exterior mantle cavity wall which is exposed in Plates 5 and 6. Connective tissue usually binds the columellar muscle in place but it has been torn and the muscle pulled away from the mantle cavity wall to expose that area and the associated structures, i.e., the visceral ganglion (Vg), anterior section of the vas deferens in the male ( $Vd_2$ ) and the spermathecal duct in the female (Sd).

The various organs which were pointed out in the plates discussed above are readily observed, although no connective tissue has been removed, because of their position just beneath the connective tissues, their size and bulk, or color and texture. The gonads in the living animals stand out bright yellow, as does the bursa copulatrix (B, Pl. 5). The kidney ( $K_1$ ) is visible because the ventral tissues of this organ are rather transparent. The organ is sac-like and filled with fluid. Numerous white granules are in constant agitation and can be observed through the membranes. The total effect is that the kidney appears quite white compared

with the surrounding tissues of other organs.

The mantle cavity and pallial oviduct are flecked with pigment. Imbedded within the connective tissue all over the ventral surface are what appear to be white granules (some appear under the compound microscope to be glandular as shown in Pl. 11, Fig. 6). These are particularly concentrated in several areas (Pl. 5): the triangular area from the mantle edge to the point where the anterior portion of the pallial oviduct disappears into the mantle cavity, the connective tissue sheet between the bursa copulatrix and the posterior edge of the mantle cavity. The space over the style sac between the left edge of the anteroventral arm of the kidney and the esophagus is frequently crowded with granules as is the V-shaped area between the anterior (Ast) and posterior (Pst) chambers of the stomach. Granules are scattered over the ventral tissue of the digestive gland.

In the male the reduced size of the prostate, as compared with the female pallial oviduct, permits a clearer external view of the intestine (In), which crosses the mantle cavity and is often filled with fecal pellets (F) in a characteristic manner (Pl. 6). Likewise, characteristic for the male, from an external view, is the dorsal swelling of the mantle cavity corresponding to the large verge coiled within.

### 3. The Mantle Cavity.

The mantle cavity was opened by cutting posteriorly along the right lateral margin of the mantle wall where it fuses with the "neck" (Pl. 4) or posteriorly just to the right of the mid-dorsal mantle wall (Pl. 11, Fig. 1). In Plate 4 the columellar muscle is shown pulled away from the mantle cavity wall and downward; the mantle edge (M) is pulled forward to stretch the left wall, enabling a clear view of the ctenidium (Ct) and osphradium (Opi, osphradial pit and Og, osphradial ganglion within).

Organs and structures associated with

the mantle cavity are the ctenidium, osphradium, anterior wall of the pericardium (Pe), opening of the kidney (Or, Pl. 8, Fig. 1), openings of the anus (A), pallial oviduct (Opo) and spermathecal duct (Osd, Pl. 9, Figs. 4, 7). In males, the verge is housed within the cavity. The reproductive structures and their association with the mantle cavity will be discussed in sections dealing with the reproductive systems.

Ctenidium. The ctenidium is composed of triangular gill filaments characteristically of a hydrobiid nature (Pl. 11, Fig. 1; Pl. 13, Fig. 3). Dundee (1957) stated that there were 15-20 filaments while Abbott (1948a) stated that there were 27-29 lamellae. In a study of over 50 mature specimens without regard to sex the number found varied between 20-28 with an average of 24. Males characteristically had fewer lamellae than females, an average of  $22 \pm 2$  for the former and  $25 \pm 3$  for the latter. This difference is correlated with sexual dimorphism, the males being smaller than the females. A blood channel (V, Pl. 4) is noted in the mantle collar connected with a blood sinus (Si) running along the anterior intestine. Near the anus the tissues of the sinus make a collar around the intestine and send a tubular passage to the blood channel in the mantle edge. The gill lamellae connect with the sinus (Si, subintestinal sinus). The base of each lamella connects with a vessel (V) which runs to the auricle (Au) (Pl. 11, Fig. 1; Pl. 8, Fig. 1).

Osphradium. The osphradium is an elliptical groove or pit (Opi) located at the base of the gills near the anterior end of the mantle cavity as shown in Plate 4 and Plate 11, Fig. 1. The edges of the groove are swollen and lip-like, packed with small white granules (possibly glands). Swelling up within the groove is the osphradial ganglion covered with an epithelium which itself appears swollen and filled with fluid (Og, Pl. 4; Pl. 11, Fig. 1). The osphradial nerve (On, Pl. 11) enters the ganglion just anterior to

the mid-ventral line. The osphradium is  $0.59 \pm 0.12$  mm long and  $0.33 \pm 0.02$  mm wide.

Visceral ganglion. The visceral ganglion (Vg) is observed imbedded within the tissues of the floor of the mantle cavity at the base of the "neck" (Pl. 11, Fig. 1). As mentioned previously, the ganglion is just as readily observed from the external surface of the ventral mantle wall where it is imbedded in the connective tissues (Pls. 5, 6). The sub-visceral and supravisceral connectives (Sbv, Suv, Pl. 11, Fig. 1) are seen running anteriorly on either side of the "neck" to disappear into the epithelium covering the anterolateral portions of the "neck."

Mucoid glands. There is no distinct hypobranchial gland, such as is figured by Fretter & Graham (1962) for *Littorina littorea*, which serves in producing a copious supply of mucus. There are, however, posterior to the gills at the posterior mantle cavity, under the area hidden by the spermathecal duct (Sd, Pl. 4), numerous, individual, large, spheroidal, glandular units within which one can observe, under the compound microscope, numerous tiny granules all in high agitation. Mucus is liberated upon disrupting these units. In many cases these glandular units are so thick that they appear coalesced into a large glandular sheet covering the epithelium of the intestine and right wall of the mantle within the posterior recess of the cavity.

Posterior Mantle Cavity. The mantle cavity narrows posteriorly and its terminal epithelium is appressed against 2 organs, the pericardium and the kidney. In Plate 11, Fig. 1, the pericardium (Pe) appears at the posterior end of the "neck." The opening of the kidney (not shown) is, in this figure, above the pericardium. In Plate 8, Fig. 1, one can see that these organs are appressed against the dorsal surface of the body tube and that the pericardium lies on the left dorsolateral curvature while the anterior kidney wall arises

from the right dorsolateral curvature. The wall of the kidney abuts on the pericardium. The opening of the kidney (Or) is a slit-like aperture bounded by a pair of "whitish tumid lips" (Dundee, 1957). The lips are provided with a sphincter muscle.

#### 4. Female Reproductive System.

Dundee (1957) provides a useful table of terms used by various authors for the different organs of the female reproductive system as found in prosobranch snails. Further comparative material is found in Fretter & Graham (1962) where the schematic diagrams of reproductive systems from various prosobranch genera are presented. In the overall scheme, oocytes pass from the gonad and travel along the oviduct past the entrance of the seminal receptacle and sperm duct to enter the posterior end of the pallial oviduct. In theory the eggs travel down the pallial oviduct to emerge over the omniphoric groove. Actually, no one has recorded the passage of an egg through the pallial oviduct. Sperm enter the spermathecal duct which opens into the mantle cavity near the anterior end of the pallial oviduct. They travel to the bursa copulatrix or pass into the sperm duct at the entrance of the bursa and move into the oviduct and then into the seminal receptacle. The spermathecal duct and the pallial oviduct are not fused, but separate structures.

Gonad (Pls. 5, 10). The female gonad is a plastic tubular structure about 1.2-1.5 mm long and 0.57-0.59 mm wide. It is characterized by few branches, the style of branching being plastic and variable. The outer epithelium was removed from one of the ovaries (Pl. 10) to demonstrate how the gonad can be gorged with oocytes. The epithelium of the gonad is often extremely stretched by gonadal products. The anterior edge of the ovary is generally not further than 0.2-0.3 mm from the posterior edge of the stomach. The oviduct runs anteriorly from the gonad as shown (Pl. 5)

and crosses the edge of the stomach at a point beneath which the digestive gland opens into the stomach. Passing over the posterior portion of the esophagus (Es) the oviduct runs below the left ventrolateral edge of the kidney and seems to disappear at the posterior end of the mantle cavity. Up to this point, all along the oviduct, oocytes can be frequently observed characteristically squeezed and flattened into elongate spheres (Pl. 5, Fig. 2; Pl. 7, Fig. 2). Along this length (1.4-1.8 mm) the oviduct is about 0.06-0.10 mm wide. The mid-region of the body, the anterior portion of which seemingly engulfs the oviduct, is complex in its interrelationships of organs. The juxtaposition of kidney, pericardium, nerves, reproductive tubes and connective tissue layers is of such a complex nature that some space is devoted to describing this region.

Mid-Region of the Body (Pls. 5, 7, 8). The posterior end of the mantle cavity (x, Pl. 5) is readily defined by a marked crease where the mantle cavity wall folds dorsally along with the distinctive kidney tissue of the ventral anterior arm of that organ. At the edge of the pallial oviduct between the bursa copulatrix (B) and the mantle cavity (Mc) one observes a thick layer of connective tissue which occludes the posterior portion of the spermathecal duct (Sd). This tissue is generally full of large white granules; it runs as a sheet to the left ventral curvature of the body tube and folds under the area traversed by the esophagus (Es). It is into this connective tissue that the oviduct turns dorsally just at the edge of the mantle cavity.

The ventral surface of the kidney on the right side stretches between the edge of the anterior portion of the stomach and the posterior end of the pallial oviduct. It surrounds the posterior end of the bursa copulatrix (B) and sends an arm anteriorly between the left edge of the bursa and the oviduct. This ventral anterior arm, like the oviduct, turns dorsally at the posterior edge of the mantle.

In Plate 7, Fig. 1, the connective tissue sheet between the bursa and the mantle was peeled away. Staining the living organism with neutral red aided in revealing more clearly the underlying structures. The spermathecal duct (Sd) runs dorsal to the point where the oviduct (Ov<sub>2</sub>) enters the pallial oviduct (Po). At the junction of the spermathecal duct (Sd) and the bursa copulatrix (B), there arises a tube from the left side, which first turns left, then turns to the right over the spermathecal duct, then left again to enter the oviduct. This is the sperm duct (Sdu).

As previously described, the kidney (K) is a thin walled, fluid filled sac. It is molded around and between organs from the posterior edge of the mantle to the anterior chamber of the stomach (Ast, Pl. 5). As the kidney fills the residual space within the bounds described, it is like a second body cavity, the ventral wall of which is shown in Plates 4-7. Opening the ventral wall and peeling it away (Pl. 7, Fig. 2) exposes the cavity of the kidney. The bursa copulatrix (B) is shown pulled slightly outward and rotated about 45° to the right. The posteroventral and all of the dorsal surfaces of the bursa are covered with kidney wall. Pulling the bursa outwards exposes the oviduct lying coiled dorsal to the bursa, likewise wrapped in kidney tissue. The dorsal wall of the kidney (K<sub>2</sub>, Pl. 7, Fig. 2) covers the style sac (Sts), which is better shown in Plate 8, Fig. 1. The style sac arises from the anterior chamber of the stomach, runs anteriorly for about 1.44 mm, not quite reaching the end of the mantle cavity. This structure is about 0.96 mm wide at its posterior end. From its left ventrolateral surface at a point (Oi) about 0.45 mm from the edge of the anterior chamber of the stomach, there arises the intestine with a width of 0.36 mm. The intestine runs anteriorly (In<sub>1</sub>), swings over the rounded ventral and anterior tip of the style sac and turns dorso-posteriorly, still appressed against the style sac. The intestine then makes a

sharp turn swinging anteroventrally again (In<sub>2</sub>). The fecal pellet compressor is located in this sharp turn. The dorsal wall of the kidney is appressed and molded around these structures. A deep crevice is formed to the right of the point where the intestine turns dorso-posteriorly over the style sac. The crevice runs down towards the dorsal surface (Cr, Pl. 7, Fig. 2). This deepened portion of the kidney extends anteriorly up to the anterior wall of the kidney abutting on the rear of the mantle cavity.

In Plate 8, Fig. 1 the kidney tissue was cleared from the oviduct (Ov<sub>1</sub>, Ov<sub>2</sub>), the pallial oviduct (Po) was cut anterior to the point where the oviduct (Ov<sub>2</sub>) entered it and the posterior portion of the pallial oviduct with bursa copulatrix (B) and tubes was lifted up and outward, thereby exposing structures otherwise hidden by that complex. In that same figure the ventral wall of the mantle cavity was removed. The anterior wall of the kidney is shown abutting on the epithelium of the posterior mantle cavity (W). The left edge of the anterior wall abuts on the pericardium (Pe), the right edge abuts on the intestine (In<sub>3</sub>). The opening of the kidney (Or) is shown on the right side of the pericardium.

The pericardium lies anterior to the style sac (Sts) and pushes out into the mantle cavity (Dmc). The auricle (Au) is shown and the vessel (V) which brings blood from the ctenidium to the auricle. Just posterolateral to the point where the auricle joins the ventricle a thin tube, the gonopericardial duct (Gp), connects the pericardium with the oviduct (Ov<sub>1</sub>). The gonopericardial duct has not been previously mentioned for *Pomatiopsis*. It arises from the dorsal surface of the oviduct where the latter turns into the body tube under the anteroventral arm of the kidney (Pls. 7, 8).

This area is generally occluded by pigmented connective tissue and the gonadal nerve (Gn, Pl. 7, Figs. 1, 2) which runs posteriorly over the oviduct at this point and is bound to the latter

PLATE 7. Mid-body region of *Pomatiopsis lapidaria*.

FIG. 1. The ventral surface of the kidney (K) is shown. Connective tissue was removed to reveal the point where the sperm duct and spermathecal duct connect and their relationship to the bursa copulatrix (B).

FIG. 2. The ventral wall of the kidney was slit open to reveal the cavity of the kidney. The bursa copulatrix (B) was pulled out of the cavity to show how it and the coiled oviduct (Ov) were wrapped in kidney tissue.

- B bursa copulatrix
- Cl columellar muscle
- Cr the deep crevice between the right edge of the style sac and the anteroventrally running intestine. This is the deepest portion of the kidney.
- E<sub>3</sub> external mantle cavity nerve 3
- Es esophagus
- F fecal pellet
- Gn gonadal nerve
- Gr white granules found in epithelium
- In intestine
- K kidney
- K<sub>1</sub> ventral surface of the kidney
- K<sub>2</sub> portion of kidney wall adjacent to the style sac
- Ke cut edge of kidney wall
- Oo oocyte
- Ov coiled portion of the oviduct
- Ov<sub>1</sub> oviduct posterior to the gonopericardial duct
- Ov<sub>2</sub> portion of the oviduct entering the pallial oviduct
- Po pallial oviduct
- Sbv subvisceral connective
- Sd spermathecal duct
- Sdu sperm duct
- St stomach
- Suv supravisceral connective
- Vg visceral ganglion

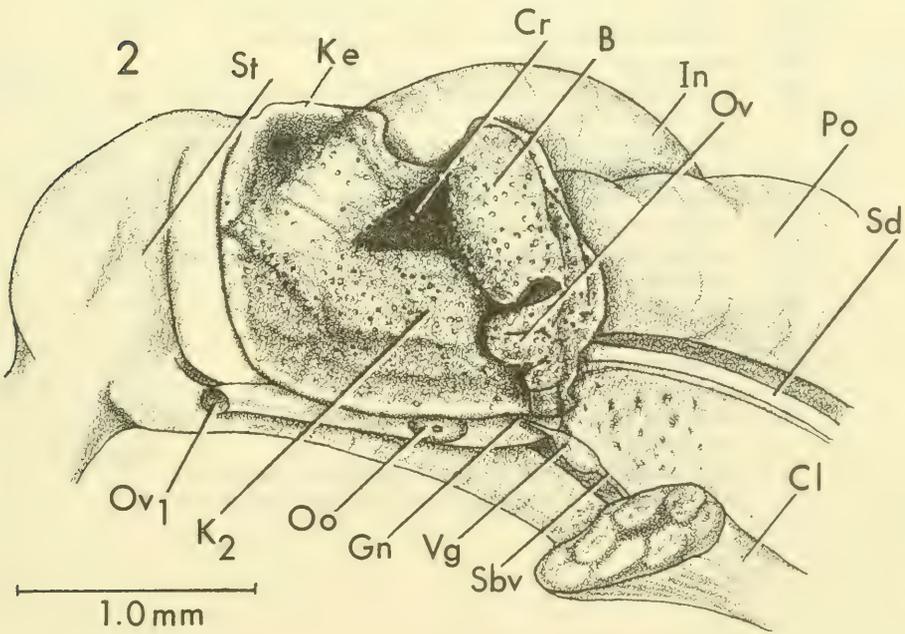
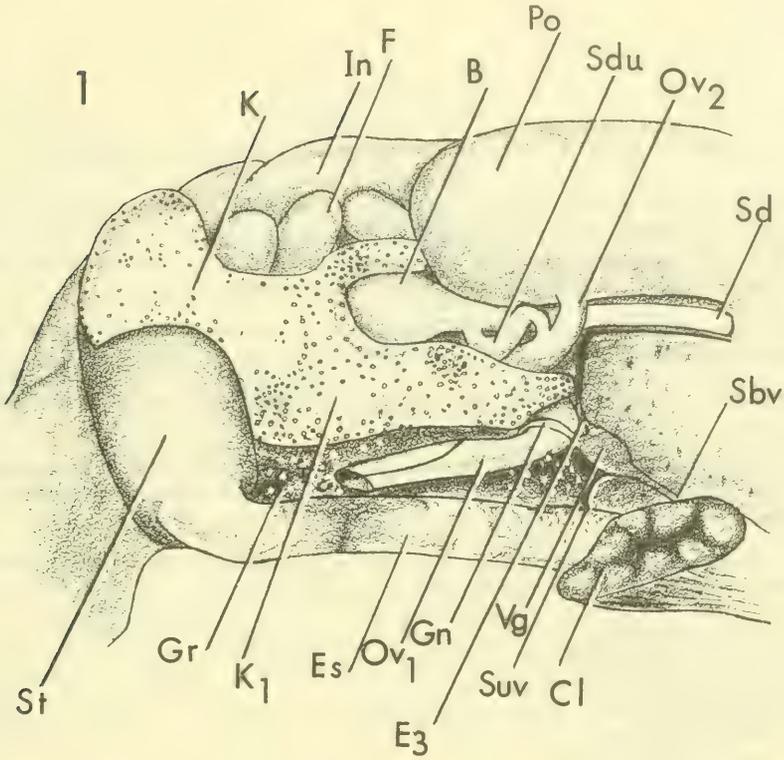


PLATE 8. Female reproductive system of *Pomatiopsis lapidaria*.

- FIG. 1. The ventral wall of the kidney was removed as well as the ventral wall of the mantle cavity. All kidney tissue was removed from the reproductive structures. The pallial oviduct and spermathecal duct were cut and the posterior portion of the reproductive system was lifted from the body tube to show underlying structures.
- FIG. 2. The bursa copulatrix with the left lateral "crest" showing, and the oviduct between the bursa and gonopericardial duct pulled out like a spring to show the nature of coiling.
- FIG. 3. The ventral surface of the bursa copulatrix exposed with the oviduct pulled out as in Fig. 2.
- FIG. 4. The relationship of the seminal receptacle (Sr) to the bursa copulatrix is shown as well as the coiled portion of the oviduct. Note the spatial relationship between the gonopericardial duct (Gp) and the seminal receptacle (Sr).
- FIG. 5. The relationship of the sperm duct and bursa copulatrix showing the position of the opening of the seminal receptacle (Osr) into the oviduct.

Au	auricle
B	bursa copulatrix
Cl	columellar muscle
Dmc	dorsal wall of the mantle cavity
Es	esophagus
Gp	gonopericardial duct
In <sub>1</sub>	portion of the intestine circling over the tip of the style sac
In <sub>2</sub>	intestine anteroventral to the pellet compressor
In <sub>3</sub>	intestine running along side the pallial oviduct
Ke	cut edge of the kidney wall
Oi	the point where the intestine arises from the posterior portion of the style sac
Or	opening of the kidney into the posterior mantle cavity
Osr	opening of the seminal receptacle into the oviduct
Ov	coiled portion of oviduct
Ov <sub>1</sub>	oviduct posterior to the gonopericardial duct
Ov <sub>2</sub>	portion of the oviduct entering the pallial oviduct
Pe	pericardium
Po	pallial oviduct
Sd	spermathecal duct
Sdu	sperm duct
Sr	seminal receptacle
Sts	style sac (here partly covered by a remnant of the dorsal wall of the kidney)
V	vein draining the ctenidium and leading to the auricle
W	posterior wall of the mantle cavity abutting on the kidney

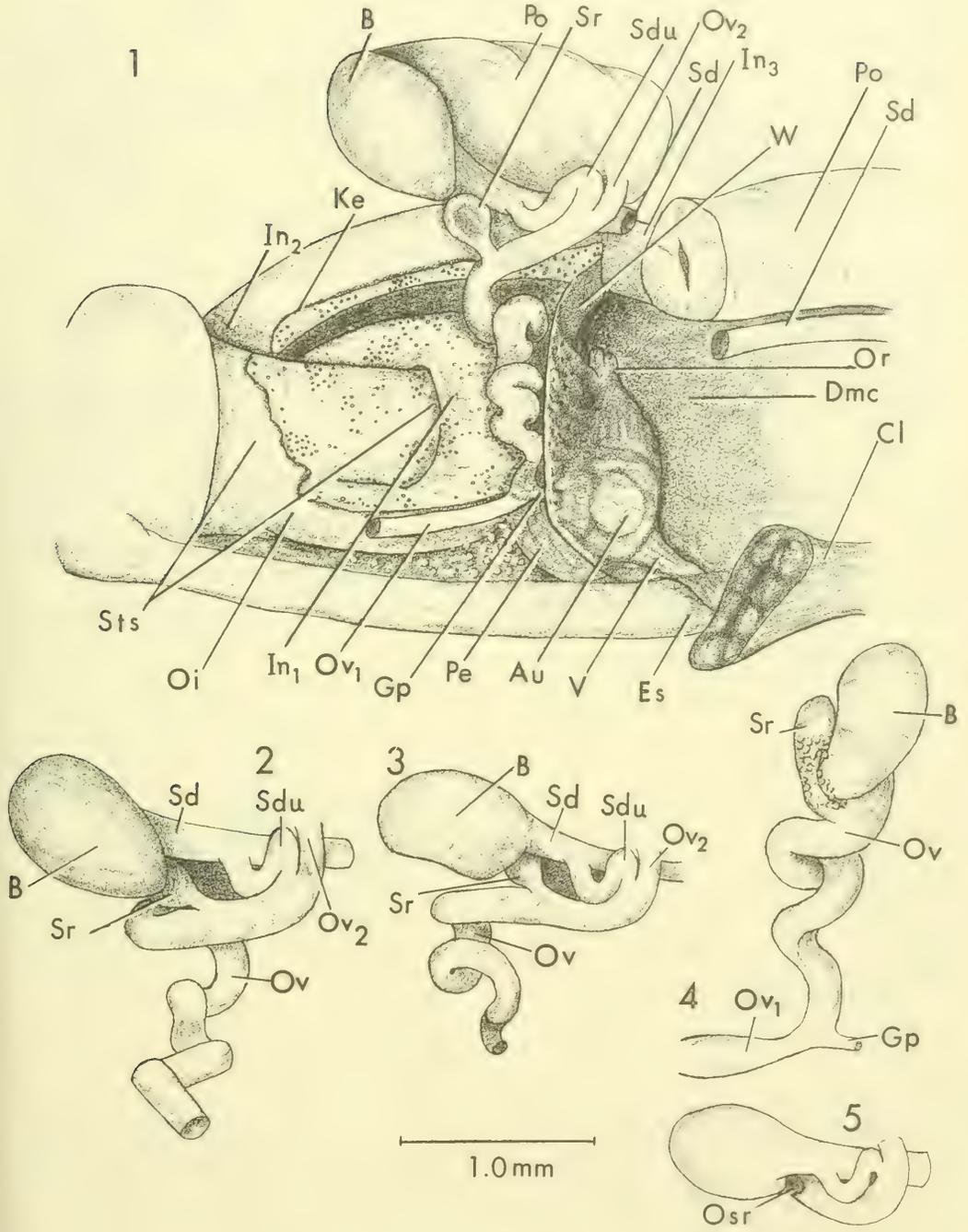
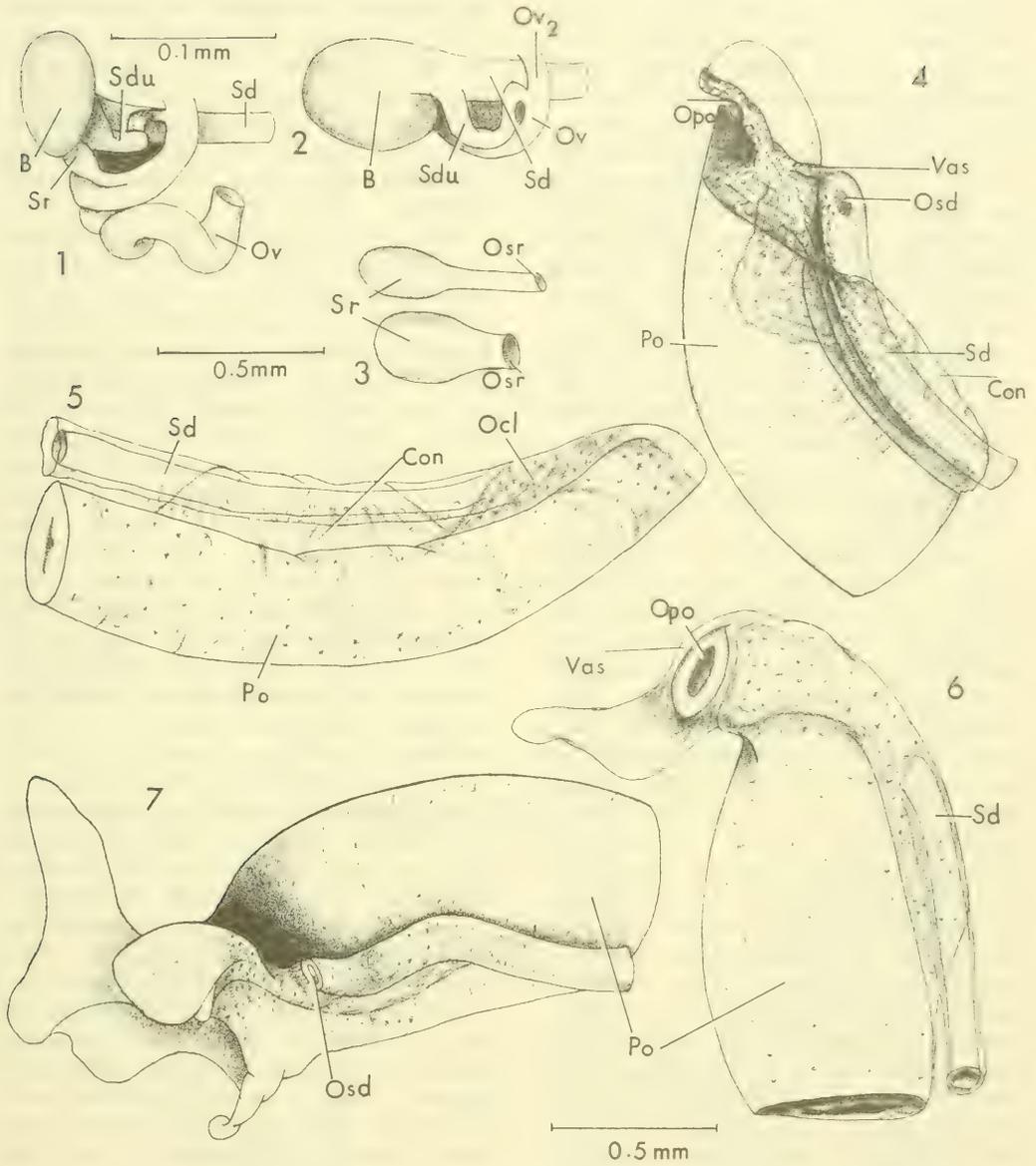


PLATE 9. Female reproductive system of *Pomatiopsis lapidaria*.

- FIGS. 1, 2. Different views and variations of the bursa copulatrix and associated ducts.
- FIG. 3. Variation in the seminal receptacle.
- FIG. 4. The pallial oviduct and the spermathecal duct oriented to show the opening of the spermathecal duct and the dense connective tissue sheets binding the spermathecal duct to the pallial oviduct.
- FIG. 5. Terminal portion of pallial oviduct and the spermathecal duct viewed as in Plate 4, but with connective tissues not removed and in greater detail.
- FIG. 6. The pallial oviduct oriented to show the female orifice as well as the connective tissue "tubes" encircling the lips and running into the mantle tissue.
- FIG. 7. The pallial oviduct and spermathecal duct oriented to show the opening of the spermathecal duct.



B bursa copulatrix  
 Con connective tissue sheets  
 Ocl anterior end of the spermathecal duct  
 occluded by heavy strands of the connective tissue  
 Opo opening of the pallial oviduct  
 Osd opening of the spermathecal duct  
 Osr opening of the seminal receptacle  
 Ov oviduct

Ov<sub>2</sub> portion of the oviduct passing ventral to the spermathecal duct to enter the pallial oviduct  
 Po pallial oviduct  
 Sd spermathecal duct  
 Sdu sperm duct  
 Sr seminal receptacle  
 Vas vascular channels in the connective tissue sheets

by tenacious connective tissue.

Lifting the bursa and pallial oviduct and displacing them (Pl. 8, Fig. 1) reveals the coiled nature of the oviduct between the gonopericardial duct and the bursa copulatrix. In this plate the relationship of the seminal receptacle (Sr) to the bursa copulatrix is shown and the point where the sperm duct (Sdu) enters the oviduct. The coiled portion of the oviduct readily fits into the space between the end of the style sac and the posterior slope of the pericardium. As can be seen in Plate 5, Fig. 2, all of the coils of the oviduct are packed dorso-laterally to the point where the spermathecal duct (Sd) enters the bursa copulatrix (B).

Gonopericardial Duct to Pallial Oviduct  
(Pls. 5, 7-9).

*Gonopericardial Region and Coiled Oviduct.* The oviduct narrows just posterior to the gonopericardial duct (Pl. 8, Fig. 4). Oocytes have not been seen past this point although they may be found lined up, one behind the other, posteriorly, right back to the gonad. The small section of the oviduct from which the gonopericardial duct arises is distinct in that it is characteristically swollen (Pl. 5, Fig. 2); it is about 0.24 mm long and 0.19 mm wide. The duct penetrates connective tissue layers to enter the pericardium and is open at both ends. It is about 0.096 mm long and 0.048 mm wide. The connective tissues occluding the reproductive tract between the bursa copulatrix (B) and the oviduct (Ov) shown in Plate 5, Fig. 1, were removed. The exposed structures (Pl. 5, Fig. 2) are presented as observed, with one exception. The gonopericardial duct arises more dorsally than is shown and would, therefore, be barely visible.

The coiled portion of the oviduct forms a very compact cylinder some 0.31 mm in diameter and 0.48-0.36 mm in length depending upon whether there are 4 or 3 coils in the tube. The tube in the coil is up to 0.17 mm wide. Uncoiled, the length of the oviduct between the gono-

pericardial duct and the point of entry of the seminal receptacle into the oviduct is about 2.0 mm. Coiling may be regular or with irregular twists. In Plate 8, Figs. 1-4, the oviduct has been slightly stretched out as one would stretch a spring to demonstrate the nature of the coils and twists commonly found in that section.

*Seminal Receptacle.* The seminal receptacle (Sr) is not observable from the ventral surface (Pl. 5, Fig. 2; Pl. 8, Fig. 5; Pl. 9, Fig. 2) although the point where it enters the oviduct may be seen (Pl. 8, Fig. 5). This small, spherical, sac-like organ is bound to the anterior dorsal surface of the bursa copulatrix by a connective tissue sheath in which numerous white granules are often densely imbedded (Pl. 8, Fig. 4). The seminal receptacle does not communicate directly with the bursa copulatrix. By slowly rotating the bursa from its normal position (Pl. 5, Figs. 1, 2), like turning the page in a book, one gradually exposes the seminal receptacle (sequence in Pl. 8: Figs. 5, 3, 2, 4; but with different specimens).

The shape of the seminal receptacle varies greatly depending upon the extent to which it is gorged with sperm and fluid. It may be elliptical or circular with gradations between (Sr, Pl. 8, Figs. 4, 1, respectively). It often appears to have a dense hard core (Fig. 1). The duct leading to the oviduct varies in length (Pl. 8, Figs. 1-4; Pl. 9, Fig. 3) from 0.12 to 0.25 mm, while the width varies from 0.06 to 0.15 mm. The longer slender duct is most commonly encountered. The spherical portion of the organ varies in length from 0.20 to 0.24 mm and the width from 0.17 to 0.24 mm. The duct enters the oviduct about 0.48 mm from the opening of the sperm duct (Pl. 8, Figs. 1-3, 5; Pl. 9, Fig. 1).

*Sperm Duct.* The sperm duct (Sdu, Pl. 8, Figs. 1-3, 5) connects the spermathecal duct and the oviduct. It may arise abruptly from the spermathecal duct (Sd) at its juncture with the bursa copulatrix (B, Pl. 9, Figs. 1, 2) or out along the

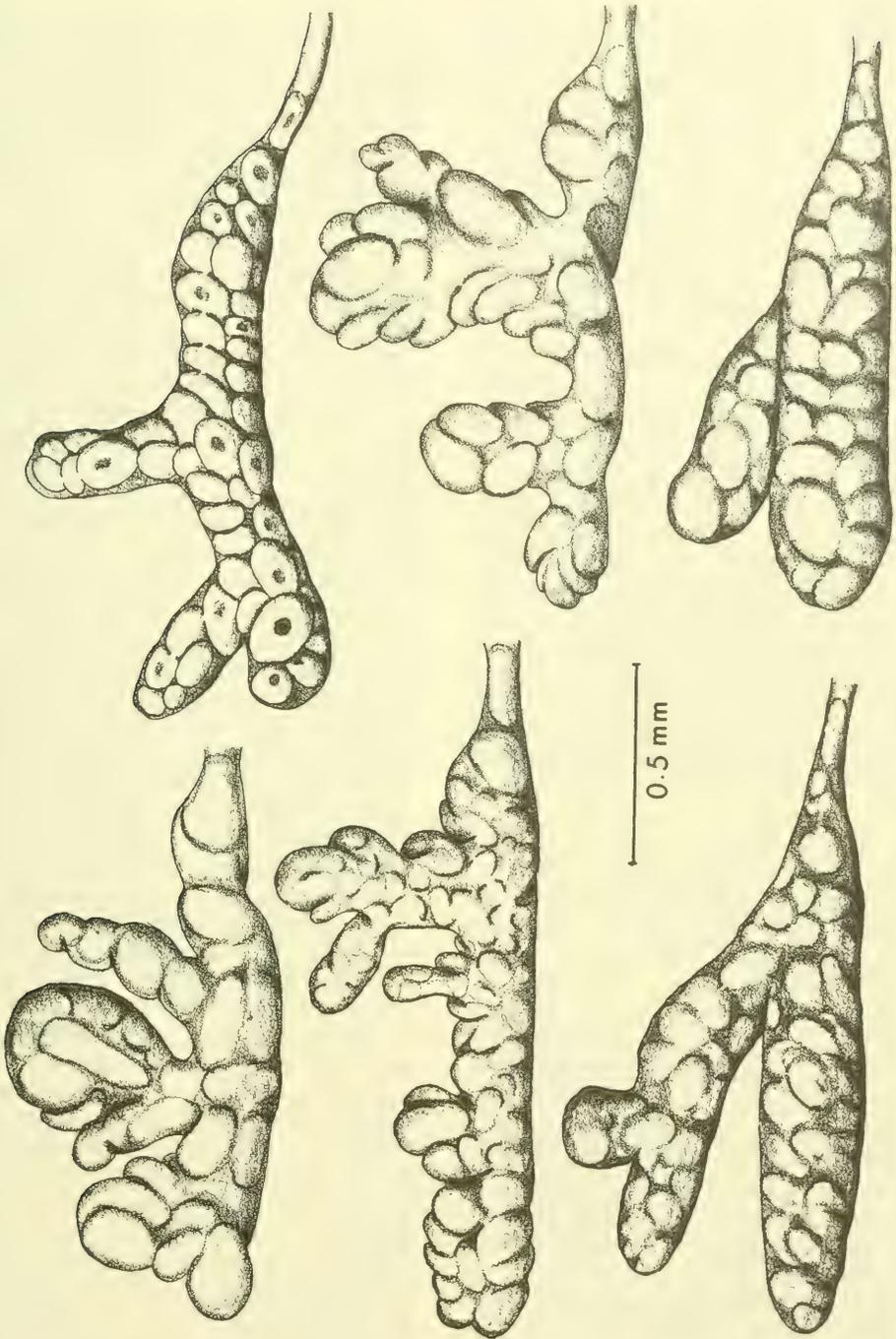


PLATE 10. Variations in the gonad of female *Pomatiopsis lapidaria*.

The ovarian follicles are gorged with oocytes. The membrane has been pulled away in the upper right-hand drawing to show the nature of the oocytes.

spermathecal duct as far as 0.5 mm from the anterior portion of the bursa (Pl. 8, Fig. 2). It varies in length from 1.2 to 0.4 mm, but is, on the average and most commonly, 0.70 mm long. The width varies between 0.14 and 0.09 mm. The degree of convolution of the sperm duct (Sdu) varies between the sinuous condition shown in Plate 8, Fig. 1, and the almost straight (Pl. 5, Fig. 2), the latter condition being rather rare.

The oviduct, beyond the entry of the sperm duct (Ov<sub>2</sub>, Pl. 8, Fig. 1), is short, some 0.17 mm long and 0.12 mm wide. It passes into the pallial oviduct (Po) 1.0-1.7 mm from the posterior end of the latter, not in the mid-length of that organ as stated by Dundee (1957). This point is  $0.65 \pm 0.16$  mm from the posterior end of the bursa copulatrix (B) and is covered by the sheet of connective tissue discussed above (Pl. 5, Fig. 1). The oviduct (Ov<sub>2</sub>) passes ventral to the spermathecal duct (Sd) and does not communicate with it, although both are closely bound together by connective tissue.

*Bursa Copulatrix.* The bursa copulatrix (B), as viewed from the ventral side, lies over the anterior tip of the style sac (Sts) with part of the curvature often within the kidney space on the right of the style sac (Pl. 5, Fig. 1; Pl. 7, Figs. 1, 2; Pl. 8, Fig. 1). As shown in Plate 5, Fig. 2, the ventral surface is evenly rounded, the medio-lateral surface is not rounded but narrows to a crest (Pl. 8, Figs. 1, 2; Pl. 9, Fig. 1). This organ is characteristically appressed against the posterior end of the pallial oviduct as shown in Plate 8, Fig. 1. At times the end of the bursa projects beyond the edge of the pallial oviduct (Pl. 7, Fig. 1) but this is rare. In another variation the tip of the pallial oviduct swings slightly away from the bursa (Pl. 5, Fig. 1). The bursa has never been found posterior to the tip of the pallial oviduct as shown by Dundee (1957, Pl. 11). The general positions of the bursa and the pallial oviduct in

relationship to the organs in the mid-body region shown in Plates 5, 7 and 8 were found to be invariable. The length of the bursa along its ventral surface averaged  $0.72 \pm 0.14$  mm and the width averaged  $0.48 \pm 0.03$  mm.

#### Pallial Oviduct and Spermathecal Duct (Pls. 5, 7, 8, 9).

*Spermathecal duct.* The spermathecal duct (Sd) arises from the anterior tip of the bursa copulatrix (B) as a tube 0.096-0.168 mm wide. It runs anteriorly, closely appressed to the ventromedial edge of the pallial oviduct (Po) and passes dorsal to the oviduct (Ov<sub>2</sub>, Pl. 8, Fig. 1) where the latter enters the pallial oviduct. Passing over the end of the mantle cavity, the spermathecal duct narrows to 0.07-0.10 mm and runs separately at a distance of 0.02-0.07 mm from the pallial oviduct. As shown in Plate 5, the spermathecal duct (Sd) is readily observed following the curve of the pallial oviduct (Po) imbedded within the ventral superficial tissues of the mantle cavity wall. Anteriorly, with the sharp curve of the mantle cavity and pallial oviduct, the spermathecal duct turns towards the pallial oviduct, becomes more slender, and disappears under the ventromedial edge of the pallial oviduct. The area where the spermathecal duct disappears from view is about 0.48 mm from the point where the pallial oviduct is observed to disappear within the mantle cavity (Pl. 5, Fig. 1).

The gross aspects of the relationship between the termination of the spermathecal duct and pallial oviduct within the mantle cavity are shown in Plate 4. It would seem, from this gross aspect, that the spermathecal duct enters the tip of the pallial oviduct. About 0.7 mm posterior to the opening of the pallial oviduct (Opo) the spermathecal duct becomes closely bound to the pallial oviduct by sheets of connective tissue; however, the opening of the spermathecal duct is not evident. A number of whole mount slides were prepared of the anterior 1.0 mm portion of the spermathecal duct, pallial oviduct and intestine,

using CMC-10 (Michelson, 1960). Water mounts were also made. Under the compound microscope it was found that the spermathecal duct did not open into the pallial oviduct as stated by Dundee (1957), but that the stout connective tissue layers continuing to the tip of the pallial oviduct obscured the point where the spermathecal duct does open into the mantle cavity. In Plate 9, Fig. 5, the structures under discussion are shown as they are oriented in Plate 4. In Plate 4 the opening of the pallial oviduct (Opo) has been indicated only to demonstrate the point where it opens. In reality what would be seen is the lip-like tip (outer lips) of the pallial oviduct (Pl. 9, Fig. 5) appressed to the inner mantle wall by muscular contraction, thereby sealing off the opening of the pallial oviduct. Both lips around the opening (Pl. 9, Fig. 6) are muscular, and thickened by connective tissue strands which encircle the edges of the lips. The connective tissue anterior to the opening of the spermathecal duct is heavily pigmented, full of whitish granules, and forms the tubular channel (Vas) for the flow of blood to the tip of the pallial oviduct and around the lips (Pl. 9, Fig. 4). This vascular tube is thick and so dense that one can perceive only with the greatest difficulty where the spermathecal duct ends and the connective tissue tube begins (Pl. 9, Fig. 5). The vascular channels running down each side of the lips of the pallial oviduct end in sinuses within the mantle edge.

By rotating the connected spermathecal duct and pallial oviduct shown in Plate 4, and Plate 9, Fig. 5, as turning a page in a book from left to right, one can observe with the aid of a bright light, that the spermathecal duct terminates about 0.58 mm from the tip of the pallial oviduct. The opening of the spermathecal duct (Osd) is permanent (Pl. 9, Figs. 4,7) but, due to the rather thin edge of the tube at this point, it is not distinct. Note that the opening of the spermathecal duct is not oriented in the same direction as that of the pallial ovi-

duct but rotated  $90^{\circ}$ . In the intact animal, where the mantle is in its normal position, the opening of the pallial oviduct is appressed against the right ventrolateral mantle wall, while the spermathecal duct opens towards the "neck."

The opening of the spermathecal duct is about  $71\mu$  long and  $25\mu$  wide. In some specimens it appears slit-like measuring about  $60 \times 12\mu$ ; in others it was round with a diameter of  $35\mu$ . In Plate 9, Fig. 6, the tips of the organs are rotated still further, exposing the thickened lips of the opening of the pallial oviduct (Opo) but causing the opening of the spermathecal duct to disappear from sight.

*Pallial Oviduct.* The pallial oviduct is the largest organ of the body with a length of 4.3-5.0 mm and a width of 0.72 mm towards the posterior regions. In the living animal the organ appears divisible into 2 parts of equal length; the posterior half appears greyish, thick and quite glandular, and the anterior portion is whitish, narrower and more smooth. These observations correspond to Dundee's (1957) histological findings.

## 5. Male Reproductive System

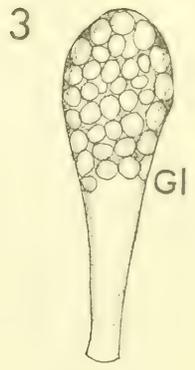
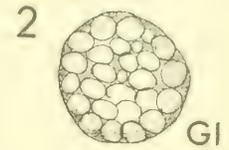
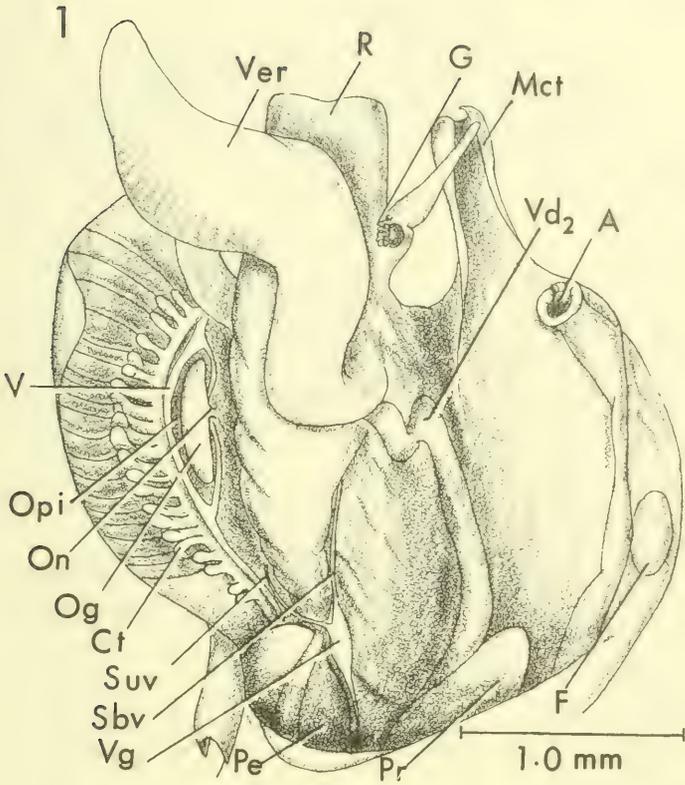
A male snail is shown uncoiled in Plate 6, as was the female previously described. Sperm from the gonad (Go) travel via the vas deferens (Vd<sub>1</sub>) to the prostate (Pr), enter the prostate, and leave through the anterior portion of the vas deferens (Vd<sub>2</sub>) which leads into the base of the verge, along the length of the verge (Ver) to exit at its tip (Pl. 11, Fig. 1).

*Gonad* (Go, Pls. 6, 12). The testis, observed through the ventral epithelium of the digestive gland (Pl. 6, Fig. 1), does not appear as distinct as did the gonad of the female. The reason for this vagueness is that the lobes of the gonad are small and that it is finely branched. The main collecting duct, the vas deferens, is dorsal to the gonad and therefore hidden from sight until it turns ventrally near the edge of the stomach. Peeling off the ventral epithelium reveals the structure of the testis (Pl. 12, Fig. 1).

PLATE 11. Male reproductive system of *Pomatiopsis lapidaria*.

- FIG. 1. Head, verge and mantle cavity. The verge is shown uncoiled and partially extended.
- FIG. 2. Head of the gland shown in Fig. 3.
- FIG. 3. Longitudinal view of the glands which pack the area between the vas deferens and the concave curvature of the verge (Gl of Fig. 5).
- FIG. 4. Gland type commonly found near the tip of the verge, appearing dense or black under direct illumination. They are oriented with the circular muscle fibers (Gl<sub>1</sub> of Fig. 5).
- FIG. 5. Verge showing the vas deferens and glandular areas.
- FIG. 6. Gland type (Gl<sub>2</sub>) clustered about the vas deferens.

A	anus
Ct	ctenidium
F	fecal pellet
G	glandular units around the dorsomedial surface of the eye
Gl	glandular types shown in Figs. 2, 3
Gl <sub>1</sub>	gland type shown in Figs. 4, 5
Gl <sub>2</sub>	gland type shown in Figs. 5, 6
Mct	cut edge of the mantle
Mvd	thick layers of circular muscles encircling the vas deferens at the base of the verge
On	osphradial nerve
Og	osphradial ganglion
Opi	osphradial pit
Pe	pericardium
Pr	prostate
R	rostrum
Sbv	subvisceral connective
Suv	supravisceral connective
V	blood vessel at the base of the ctenidium
Vd <sub>2</sub>	anterior portion of the vas deferens
Ver	verge
Vg	visceral ganglion



0.01 mm

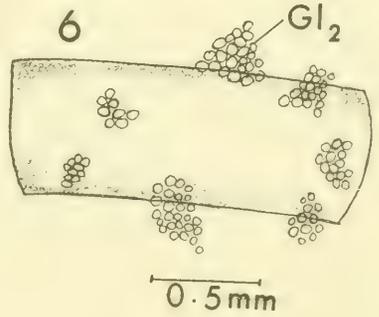
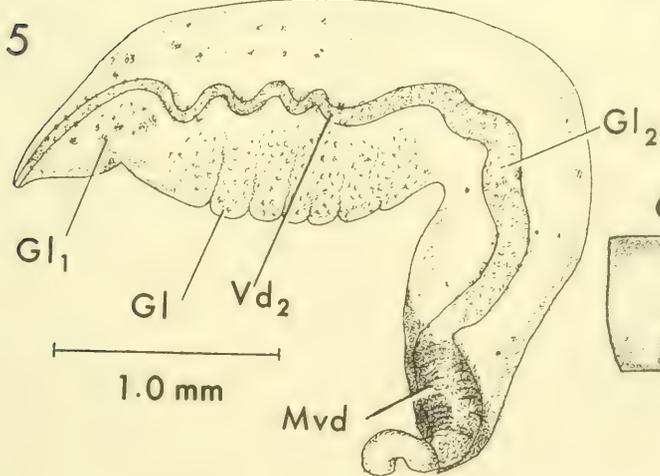
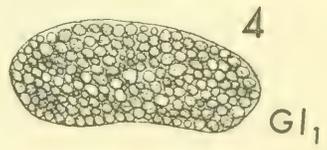
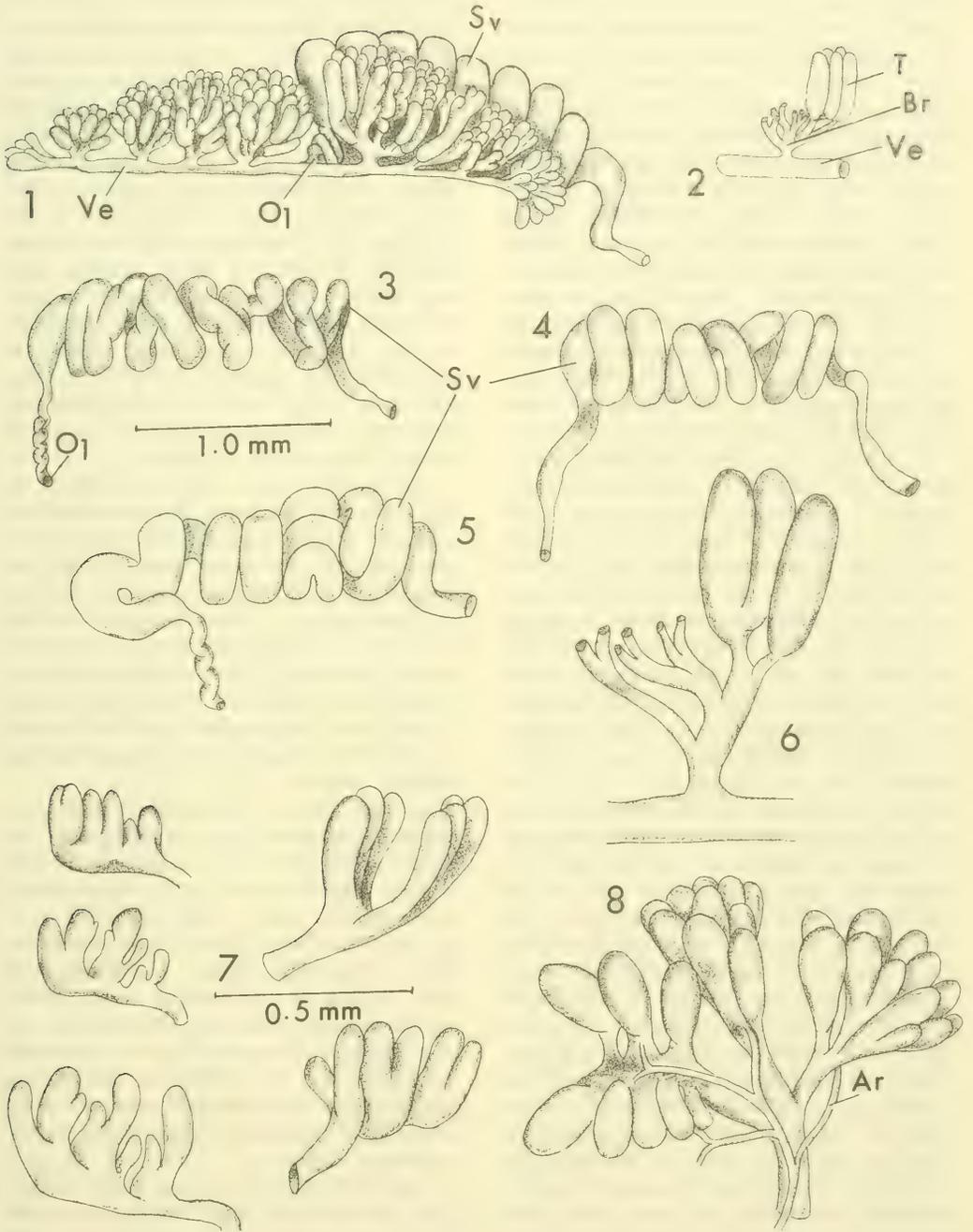


PLATE 12. Male reproductive system of *Pomatiopsis lapidaria*.

- FIG. 1. Gonad showing 9 multibranched units and the coiled "seminal vesicle" behind.
- FIG. 2. A single multibranched unit arising from the vas efferens.
- FIGS. 3, 4, 5. Variation in the coiling of the "seminal vesicle."
- FIG. 6. An enlarged view of a single multibranched unit supporting testicular lobes.
- FIG. 7. Variation in the structure of the testicular lobes seen laterally.
- FIG. 8. A multibranched unit showing testicular lobes and how the lobes are vascularized.

Ar artery to the testicular lobes  
Br branch arising from the vas efferens  
O<sub>1</sub> point where the vas deferens arises from the vas efferens  
and leads to the "seminal vesicle"  
Sv seminal vesicle  
T testicle lobe  
Ve vas efferens



With a length of about 2.4-2.8 mm, it is longer than the female gonad. The width of the gonad at its widest part, which is usually in its anterior third, is about 0.7 mm. From a narrow collecting duct (0.05-0.09 mm wide), the vas efferens (Ve), there arise 8-9 multi-branched units, whose nature is shown in Plate 12, Figs. 2, 6 and 8. At the end of each branch are lobes of varying size. The lengths and widths of the lobes depend upon the degree to which they are filled with gonadal products. A single tubular branch may support several interconnecting lobes (Fig. 8). In that figure vascularization of 3 groups of testicular lobes is shown. The whole gonad appears very bright yellow when all of the testicular lobes are productive.

Posterior Vas Deferens (Pls. 6, 12).

A narrow tube (0.048 mm diameter), which is extremely convoluted in most cases, arises just anterior to the mid-length of the vas efferens (Ve). This is the beginning of the section of the posterior vas deferens called the "seminal vesicle" by Dundee (1957). As shown in Plate 12, Fig. 1, the tube arises at a place ( $O_1$ ) which would otherwise be filled by a multibranched unit. The "seminal vesicle" is coiled in a characteristic manner (Sv, Pl. 12, Figs. 3, 4, 5). The initial slender convoluted portion thickens within 0.6 mm of its origin, forming a series of coils which are regular like those of a spring when the tube is not overloaded with gonadal products (Pl. 12, Figs. 3, 4), but bulge out of position when fully loaded, so as to disrupt the neatly coiled pattern (Pl. 12, Fig. 5). The length of the coil is 1.4-1.9 mm; its width about the same as that of the gonad. Fully uncoiled, the tubing making up the "seminal vesicle" measures up to 6.0 mm in length. The tube may have a diameter of 0.24 mm. At the anterior end of the testis the "seminal vesicle" narrows to about 0.07 mm and turns ventrally to emerge from the dorsal side of the gonad, as shown in Plate 6. The vas deferens ( $Vd_1$ ), as the oviduct, takes an anterior course toward the rear

of the mantle cavity. At the place where the oviduct turned dorsally, the vas deferens continues along the ventral surface across the body tube, posterior to the mantle cavity, without either coiling, connecting with the pericardium, or great involvement with kidney tissue. The vas deferens moves to the prostate (Pr) to enter that organ just posterior to the place where the anterior portion of the vas deferens ( $Vd_2$ ) is seen leaving (Pl. 6, Fig. 1). The tube is difficult to see because it becomes more slender as it approaches the prostate and is surrounded in connective tissue. In Plate 6, Fig. 2, the prostate was turned over to reveal the point of entry of the vas deferens ( $Vd_1$ ) into its dorsal surface. At this point the vas deferens measures about 0.024 mm in diameter.

Prostate (Pls. 6, 11). As seen from the ventral surface, the prostate is a kidney shaped organ, white in color, situated in the same position as the female pallial oviduct but not as long as that organ. The prostate is 1.68-1.75 mm long and the greatest width is about 0.70 mm. The posterior end projects about 0.48 mm beyond the mantle cavity and is encircled by kidney tissue, as was the female bursa copulatrix and pallial oviduct.

In contrast to the pallial oviduct, the prostate surface is quite irregular, due to discrete glandular units which were readily separated (Pl. 6, Fig. 3). Viewing the opposite side of the prostate, it is evident that the glandular units drain into a narrow tubular collecting tube (Pl. 6, Fig. 2). The posterior portion of the vas deferens ( $Vd_1$ ) enters the collecting tube of the prostate 0.60 mm from its posterior end, while the larger anterior portion of the vas deferens ( $Vd_2$ ) leaves the prostate 0.17 mm anterior to the entry of  $Vd_1$ .

Anterior Vas Deferens (Pls. 6, 11). The anterior vas deferens ( $Vd_2$ ) emerges from the dorsal side of the prostate (Pl. 6) as a tube 0.96-0.12 mm wide. It usually follows along the edge of the prostate until the anterior end of that

organ before running obliquely over the mantle cavity towards the columellar muscle, but occasionally it diverges immediately upon leaving the prostate as shown in Plate 6, Fig. 1. The vas deferens enters the mantle cavity behind the columellar muscle, 1.4 mm from the edge of the mantle. Plate 11, Fig. 1, shows the anterior tip of the prostate (Pr) and the vas deferens ( $Vd_2$ ) running anteriorly on the floor of the mantle cavity along the right side of the "neck" to a point slightly anterior to the base of the verge (Ver); it then turns back and enters the "neck" under the base of the verge.

**Verge** (Pl. 11). The verge of *Pomatiopsis lapidaria* is very characteristic for the species. The verge would be called simple in much of the malacological literature, as it has no appendages and since the vas deferens terminates at the tip, which has no papilla. The verge has, however, a number of significant details of importance to a systematic discussion. As Dundee (1957) stated, the verge is very flattened; it is relatively thin bladed. It arises from the "neck" to the right of the mid-line and is carried, coiled counter-clock-wise over the "neck." It measures up to 3.4 mm in length in the extended condition, but it can possibly be expanded further. The animal shown in Plate 11, Fig. 1 is a relatively small snail; the verge shown in Fig. 5 is from a larger male.

Abbott (1948a) described the verge as varying considerably, "ranging from a simple, flattened, tapering cylinder to a prong with a meat-chopper blade on one side." I found the "simple" verge only in immature males. In adults the verge is characteristically of the shape shown in Figs. 1 and 5. In very rare cases (less than 1%) the inner curvature near the anterior end has a fleshy lobe (Pel, Pl. 13, Fig. 2) reminiscent of a penial appendage common in some other hydrobiid snails. The inner margin of the mid-verge is swollen out into a convex curve which appears scal-

loped. The scalloped effect (Pl. 11, Fig. 5) is due to the rounded ends of blunt, finger-like, lobes with parallel sides. The verge was cut from the body deep at its base so as to include the vas deferens where it entered. This organ was studied in a drop of saline, gently placing a coverslip on it, and viewing it through the compound microscope. The vas deferens ( $Vd_2$ ) runs along the base of the verge before turning up into the mid-portion of that organ. The basal portion of the vas deferens is greatly thickened because of a pronounced layer of dense circular muscles (Mvd, Fig. 5). Beyond the base, the vas deferens takes an anterior course either along the mid-line of the verge or slightly displaced towards the outer curvature. The tube, loosely undulating, begins to narrow noticeably about mid-verge. At the tip the vas is only about 0.024 mm wide.

The area of the verge between the crenulated edge and the vas deferens is highly glandular with ducts and fissures running beneath the epithelium, along the edges of the "lobes," towards the edge of the verge. At 150X distinct glandular units were noted pushing against the epithelium so that the whole area seemed pustulate. The glands (Gl, Pl. 11, Figs. 2, 3, 5) were club-shaped, the diameter of the heads (Fig. 2) measuring 10-30 $\mu$ . The head of such a gland appears full of vesicles (8-20 per head) measuring 2-10 $\mu$  in diameter. The length of the glands was about 160 $\mu$ ; the basal portions were without vesicles (Fig. 3). The entire area was full of these glands, the whole length of which could often be seen lying under the epithelium.

Two other glandular types can be found in the verge. Along the vas deferens are numerous crowded groups of vesicles (Gl<sub>2</sub>, Pl. 11, Figs. 5, 6). Also near the anterior end of the verge one sometimes finds dense spheres of minute vesicles which look like black spots under the dissecting microscope (Gl<sub>1</sub>, Pl. 11, Figs. 4, 5). The longitudinal axes of these dense spheres are oriented lengthwise

PLATE 13. Structures of the muscular, respiratory, reproductive, digestive and nervous systems in *Oncomelania* and *Pomatiopsis*.

FIG. 1. Muscles arising in the pedal haemocoel at a level below the pedal ganglion. The drawing was made from *O. hupensis formosana* and shows a cross section through the pedal haemocoel. The pedal ganglion on the right is transected. The right propodial and metapodial ganglia are shown. The main structures to be pointed out are the mid-ventral protractors arising from the anterior wall of the pedal haemocoel.

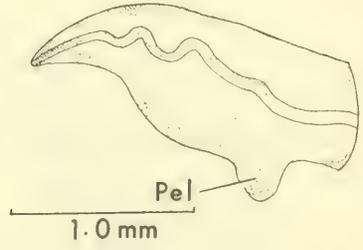
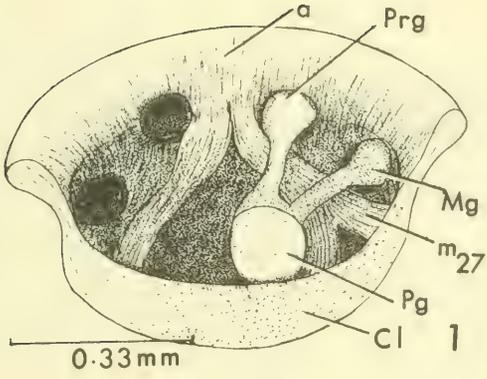
FIG. 2. Verge of *P. lapidaria* showing a rarely found penial lobe (Pel).

FIG. 3. The structure of 2 gill filaments from *P. lapidaria*.

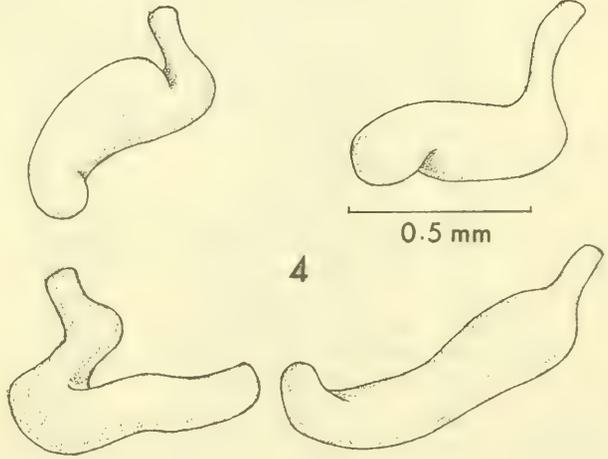
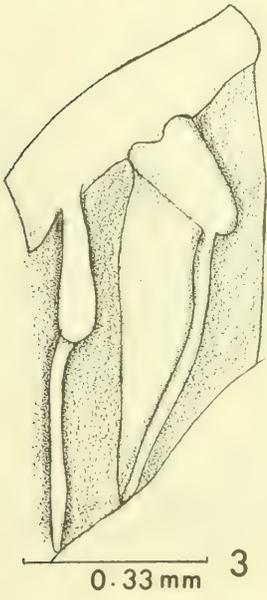
FIG. 4. Variation in the salivary glands of *P. lapidaria*.

FIG. 5. Variation in the nerves associated with the buccal ganglion.

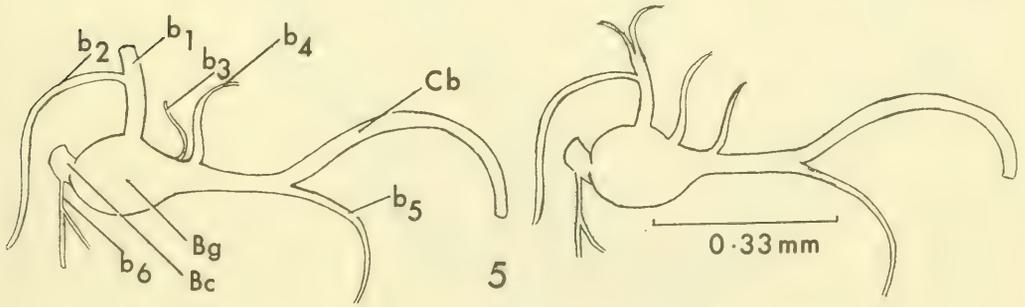
- a anterior wall of pedal haemocoel
- b<sub>1</sub> dorsal buccal nerve
- b<sub>2</sub> esophageal nerve
- b<sub>3</sub> central buccal nerve
- b<sub>4</sub> anterior buccal nerve
- b<sub>5</sub> odontophoral nerve
- b<sub>6</sub> posterior buccal nerve
- Bc buccal commissure
- Bg buccal ganglion
- Cb cerebro-buccal connective
- Cl columellar muscle
- Mg metapodial ganglion
- m<sub>27</sub> mid-ventral protractor
- Pel rare penial lobe
- Pg pedal ganglion
- Prg propodial ganglion



2



4



5

PLATE 14. Dorsal buccal mass and associated organs in *Pomatiopsis lapidaria*.

Bg	buccal ganglion
Bm	buccal mass
Cc	cerebral commissure
Cg	cerebral ganglion
Cl	columellar muscle
Es	esophagus
m <sub>5</sub>	buccal protractor muscle
m <sub>16</sub>	preventral dilator muscles
m <sub>17</sub>	suspensors of the buccal mass
m <sub>22</sub>	lateral cephalic retractor muscle
ML <sub>1</sub>	median labial nerve 1
ML <sub>2</sub>	median labial nerve 2
Mn <sub>2</sub> *	mantle nerve 2
On*	osphradial nerve
Opt	optic nerve
pp <sub>3</sub>	lateral nerve 3
Psc	pleuro-supraesophageal connective
Sa	salivary gland
Sl	supralabial nerve
Sug	supraesophageal ganglion
Suv	supravisceral connective
Tn	tentacular nerve

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\* As a rule a single large trunk, the joint osphradio-mantle nerve, emerges from the supraesophageal ganglion, which then bifurcates to form the osphradial nerve (On) and mantle nerve 2 (Mn<sub>2</sub>) (see p 79).

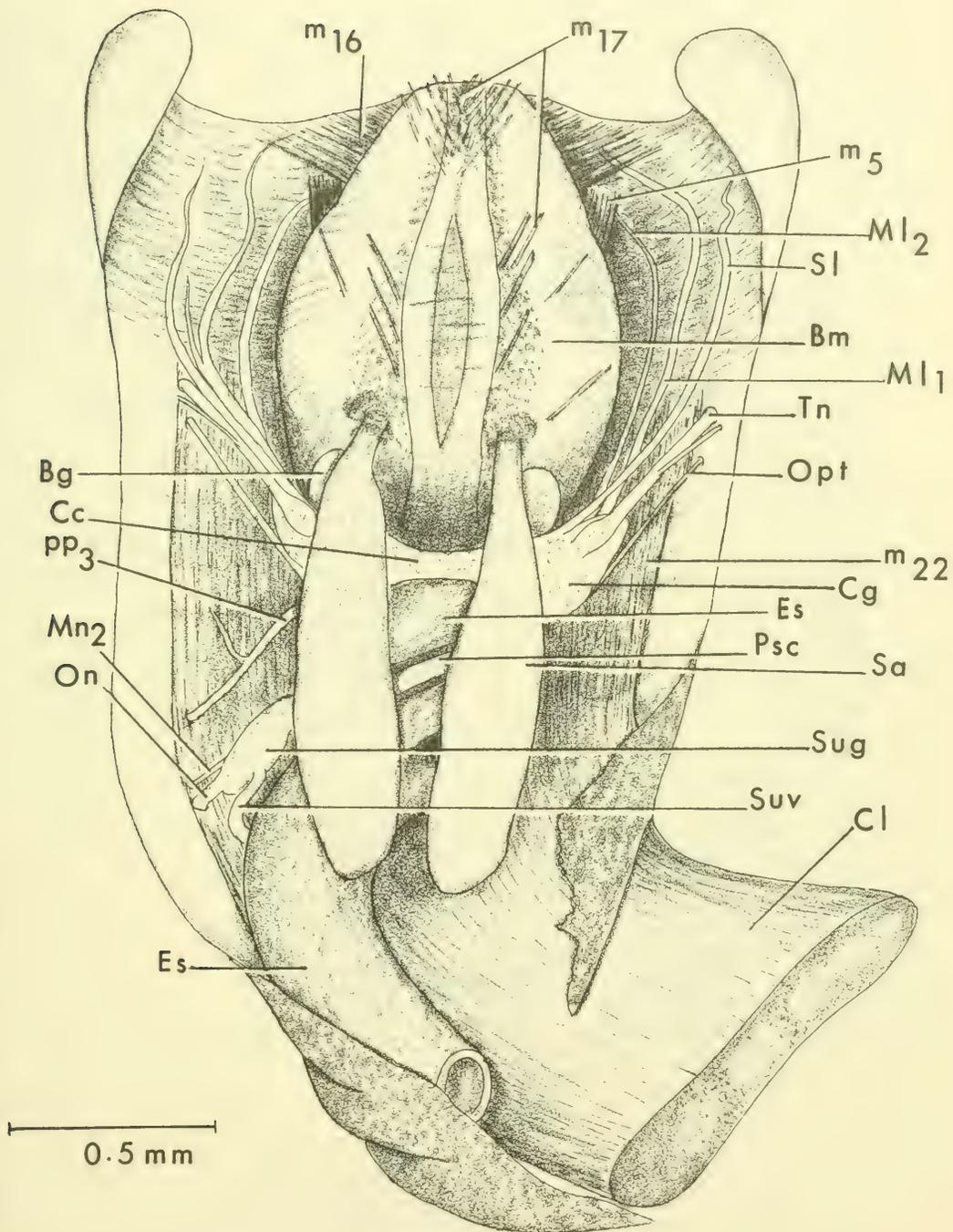


PLATE 15. Muscular, nervous and digestive systems in the cephalic region of *Pomatiopsis lapidaria*.

FIG. 1. The lateral aspect of the buccal mass with associated neural structures.

FIG. 2. The posterodorsal portion of the pedal haemocoel showing the relationship of the columellar muscle (Cl), mid-columellar supportive (m<sub>23</sub>), and pedal ganglia in anterior view (compare with Pl. 20, Fig. 2).

a	nerve characteristically arising from P <sub>6</sub>	m <sub>25</sub>	dorsal pedal tensor
b	nerve characteristically arising from P <sub>6</sub> ventral to "a"	m <sub>26</sub>	dorsal propodial retractor
b <sub>1</sub>	dorsal buccal nerve	Mg	metapodial ganglion
b <sub>2</sub>	esophageal nerve	Ml <sub>1</sub>	medial labial nerve 1
b <sub>3</sub>	central buccal nerve	Ml <sub>2</sub>	median labial nerve 2
b <sub>4</sub>	anterior buccal nerve	Opt	optic nerve
Bg	buccal ganglion	P <sub>1</sub>	lateral retractor nerve
Cb	cerebro-buccal connective	P <sub>2</sub>	pedal nerve to the anteroventral wall of pedal haemocoel
Cg	cerebral ganglion	P <sub>3</sub>	major lateral nerve
Cl	columellar muscle	P <sub>4</sub>	propodial connective
Cp	cerebro-pedal connective	P <sub>6</sub>	metapodial connective
C <sub>8</sub>	cerebro-tensor nerve	P <sub>7</sub>	dorsolateral pedal nerve
Eo	external odontophore membrane	Pg	pedal ganglion
Es	esophagus	Pp	pleuropedal connective
m <sub>5</sub>	buccal protractor muscle	pp <sub>1</sub>	lateral nerve 1
m <sub>6</sub>	preventral protractors	pp <sub>2</sub>	penial nerve
m <sub>8</sub>	anterior jugalis	pp <sub>3</sub>	lateral nerve 3
m <sub>9</sub>	buccal constrictor	pp <sub>4</sub>	lateral nerve 4
m <sub>11</sub>	dorsolateral buccal protractor	Prg	propodial ganglion
m <sub>12</sub>	buccal retractor	Rp	right pleural ganglion
m <sub>13</sub>	membranous jugalis	Sa	salivary gland
m <sub>16</sub>	preventral dilators	Sl	supralabial nerve
m <sub>17</sub>	suspensors of the buccal mass	Sta	statolith
m <sub>20</sub>	rostral retractors	Stc	statocyst
m <sub>22</sub>	lateral cephalic retractors	Sul	sublabial nerve
m <sub>23</sub>	mid-columellar supportive	Tn	tentacular nerve



PLATE 16. Musculature and pharyngeal structures in *Pomatiopsis lapidaria*.

- FIG. 1. Posterior portion of the pharyngeal tube showing the dorsal aspect of the odontophore with the radula removed. The odontophore divaricator muscle ( $m_3$ ) is not shown as it is vaguely defined when the dorsal surface of the odontophore is viewed, due to its ventro-lateral position (fig. 4).
- FIG. 2. Left buccal cartilage in dorsal view wrapped in intrinsic muscles. Muscle  $m_1$  is not shown.
- FIG. 3. Lateral aspect of the left buccal cartilage showing muscles arising or inserting on the cartilage. The position of the cartilage is as in Fig. 4.
- FIG. 4. Schematic representation of the odontophore within the posterior portion of the pharyngeal tube. The left side of the odontophore is shown.
- FIG. 5. The fused cup-like muscles making up the mediolateral cartilage tensor ( $m_2$ ) and the subradular membrane which continues from this muscle. The medial radular retractor ( $m_4$ ) arises from the postero-ventral curvature of this muscle.

a	a thickened portion of the subradular membrane	$m_{17}$	suspensor of buccal mass
Bca	buccal cavity	$m_{20}$	rostral retractor
Bf	floor of pharyngeal tube	$m_{21}$	oral sphinctor
Bpl	bending plane of the radula (=tip of odontophore)	Mo	mouth
Ca	cartilage	Ol	outer lip
Es	esophagus	Ot	oral tube
Ev	esophageal valve	Ra	radula
Fg	food groove	Ras	radular shield
$m_1$	lateral cartilage tensor	Rb	pharyngeal roof, or roof of the buccal mass
$m_2$	mediolateral cartilage tensor	Rf	roof of the rostrum
$m_3$	odontophore divaricator	Rfl	floor of the rostrum
$m_4$	medial radular retractor	Rpc	rostral portion of cephalic haemocoel
$m_5$	buccal protractor	Rs	radular sac
$m_6$	preventral protractor	Sp	sublingual space
$m_{15}$	suspensor of radular sac	Sur	subradular membrane
		Vf	ventral fold

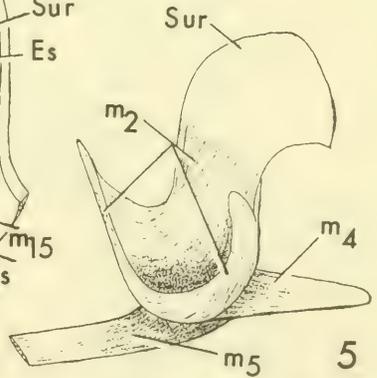
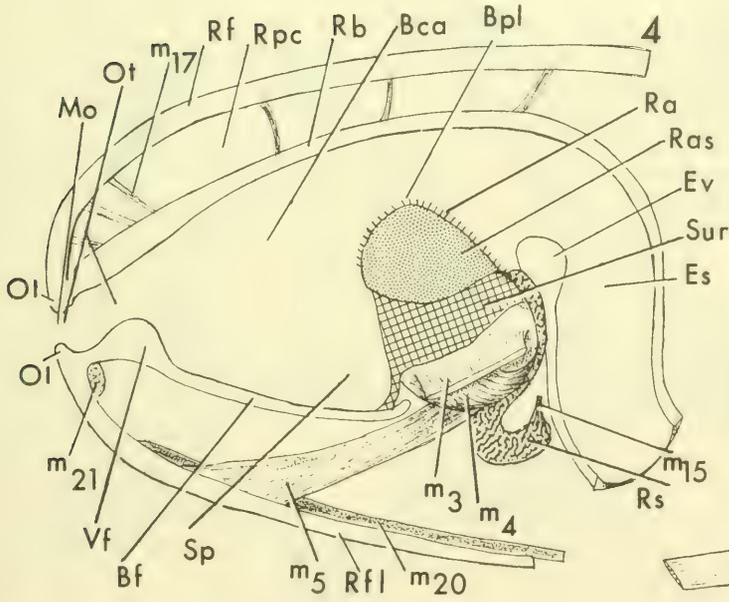
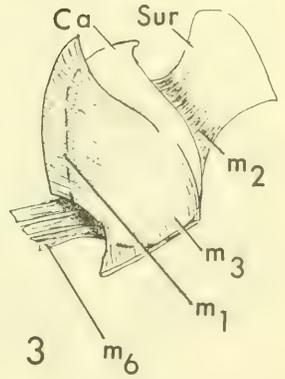
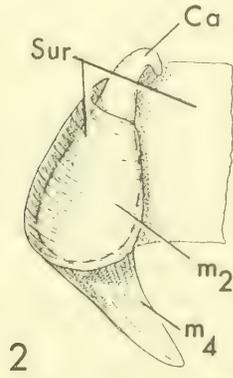
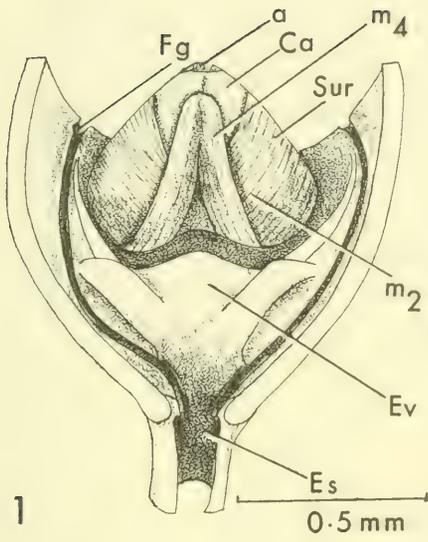
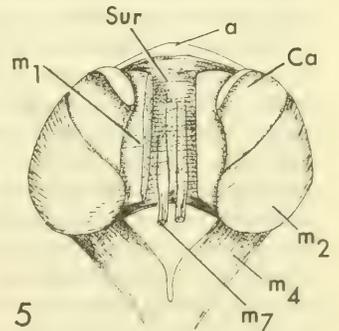
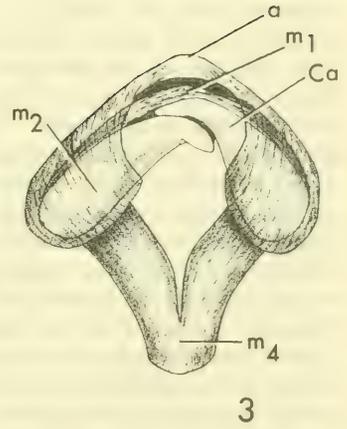
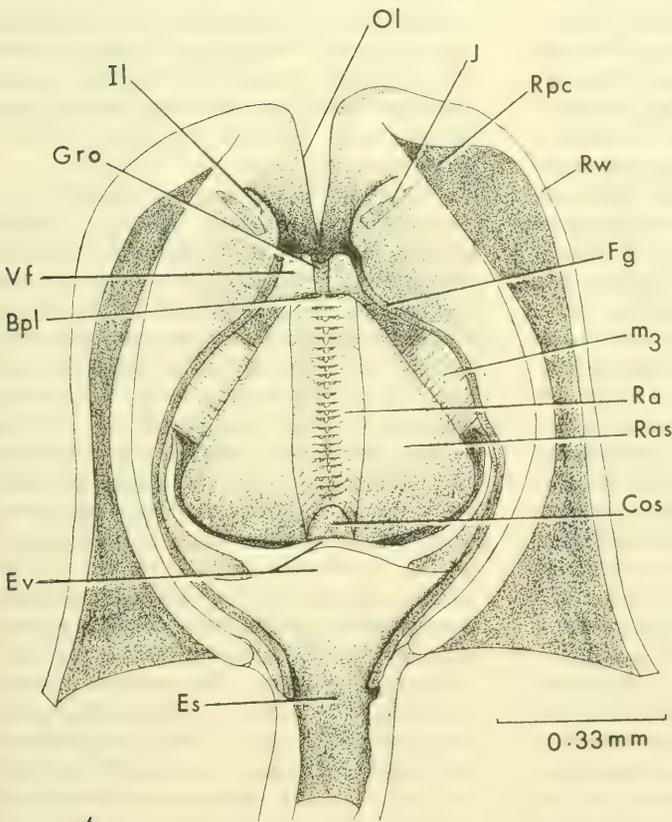
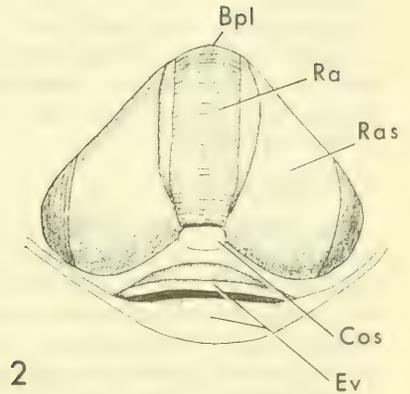
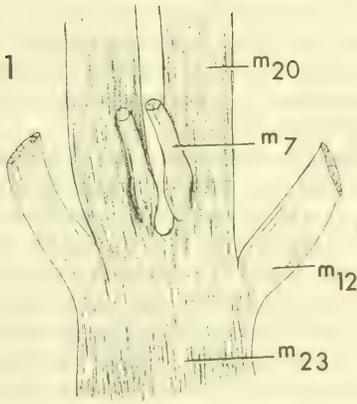


PLATE 17. Buccal and rostral musculature of *Pomatiopsis lapidaria*.

- FIG. 1. Muscles arising from the mid-columellar supportive muscle ( $m_{23}$ ).
- FIG. 2. Dorsal view of the odontophore before removing the radula.
- FIG. 3. Dorsal view of the odontophore with the radula removed and the medial radular retractor pulled backwards from the odontophore.
- FIG. 4. Dorsal aspect of the opened pharyngeal tube showing the odontophore.
- FIG. 5. The cartilages are shown separated and folded out to show the medial surface of the cartilages wrapped in the medio-lateral cartilage tensor muscle.

a	thickened anterior portion of the subradular membrane
Bpl	bending plane of the radula (=tip of the odontophore)
Ca	cartilage
Cos	collostyle tip
Es	esophagus
Ev	esophageal valve
Fg	food groove
Gro	central groove in the ventral fold
Il	inner lip
J	jaw
$m_1$	lateral cartilage tensor
$m_2$	mediolateral cartilage tensor
$m_3$	odontophore divaricator
$m_4$	medial radular retractor
$m_7$	radular protractor
$m_{12}$	buccal retractor
$m_{20}$	rostral retractor
$m_{23}$	mid-columellar supportive
Ol	outer lip
Ra	radula
Ras	radular sac
Rpc	rostral portion of the cephalic haemocoel
Rw	rostral wall
Sur	subradular membrane
Vf	ventral fold



4

2

3

5

with the circular muscle fibers encasing the verge.

Anteriorly, the verge has a characteristic indentation on the inner curvature (Pl. 11, Figs. 1, 5). The edge of the verge appears to have no cilia when viewed at 600X.

## 6. Buccal Mass

In Plate 14 the rostrum is shown opened along the mid-dorsal line. The epithelium of the "neck" was slit open revealing the wide, powerful columellar muscle (Cl). Structures are shown as they appeared upon opening this area with 2 exceptions. First the salivary glands (Sa) are usually folded and tucked ventrolaterally around and about the cerebral commissure (Cc), esophagus (Es) and cerebral ganglia (Cg) in a variable manner (Pl. 15, Fig. 1). They were pulled upward and straight back to permit at least a limited view of the relationships of the buccal mass (Bm) with the dorsal area above the cerebral ganglia (Cg), esophagus (Es) and underlying musculature. Second, the area dorsal to the cerebral ganglia (Cg), esophagus (Es) and pleuro-supraesophageal connective (Psc) is hidden by transverse strands of connective tissue just beneath the roof of the cephalic area. These strands support the cephalic aorta which travels along the esophagus dorsally, crossing from left to right, to descend, near the right cerebral ganglion, into the pedal haemocoel. These structures were removed to permit observation of the underlying organs.

Dorsally viewed the buccal mass (Bm) is somewhat pyriform. In the contracted state it varies in length from 0.85-1.08 mm; in width from 0.79-0.84 mm. It fills the rostral portion of the cephalic haemocoel. The posterior end turns sharply ventrally at the beginning of the esophagus, the mid-posterior curve of which is pressed against the posteroventral part of the buccal mass (Pl. 15) by the cerebral commissure (Cc). The position of the cerebral commissure corresponds to the mid-dorsoventral axis

of the posterior buccal mass. The esophagus again turns dorsally, under the pleuro-supraesophageal connective (Psc) and swings left to follow the left edge of the columellar muscle (Cl) posteriorly, lying on that muscle. The esophagus is a large tube 0.31-0.24 mm in diameter.

Muscles arising from the dorsal and dorsolateral surface of the buccal mass ( $m_{17}$ ) are irregularly placed and serve to suspend the buccal mass from the rostral roof. The dorsal posterior portion of the buccal mass appears fleshy and laced with only a few muscle fibers. The mid-dorsal portion appears as if 2 fleshy folds were pressed together and bound in position by superficial layers of connective tissue. Each lip-like edge is the dorsal termination of a hemisphere of tissue arising from the lateral and ventrolateral faces of the buccal mass. Studying the lateral aspect of the buccal mass (Pl. 15, Fig. 1) one observes that the anterior, lateral and dorsolateral regions are highly muscular ( $m_g, m_g, m_g$ ).

The buccal mass may be considered a composite of 2 main parts; (1) the pharyngeal tube and attendant muscles which swell between the mouth and the esophagus; (2) the odontophore apparatus which projects into the ventral posterior portion of the pharyngeal tube (Pl. 16, Fig. 4). The odontophore is a neatly delineated structure made up of the buccal cartilages, and the musculatures binding together the cartilages as well as the odontophore to the pharyngeal tube, radula, and radular sac. Connected to the posterior dorsal region of the buccal mass are the paired salivary glands which drain into the pharyngeal tube above the odontophore apparatus.

Salivary Glands. The salivary glands are characteristically simple, flattened structures (Pl. 14). They are delicate and easily damaged. As shown in Plate 15, Fig. 1, they arise from the dorsolateral surface of the buccal mass 0.24-0.36 mm from the mid-dorsal line. The duct leading from the main expanded

glandular blade varies in length and is closely appressed to the tissue of the buccal mass. The anterior 0.36 mm are generally covered by a sheet of connective tissue. The total length of gland and duct may reach 1.37 mm but is generally less, about 1.0 mm. The width of the gland, which varies considerably, averages about 0.20 mm. Variation in the shape of these glands is shown in Plate 13, Fig. 4; Plate 14; Plate 15, Fig. 1.

Interior Buccal Mass. Slitting the epithelium between the dorsal lips of the buccal mass and pulling aside the hemispheres of the pharyngeal tube exposes the inner regions of the tube and the odontophore apparatus (Pl. 17, Fig. 4). The large triangularly shaped odontophore, upon full contraction of the buccal mass, causes the dorsal lips of the buccal mass to stretch apart (Pl. 14). The odontophore imparts shape to the buccal mass and gives support to the pharyngeal tube and the initial portions of the esophagus.

A longitudinal section of the pharyngeal tube is shown in Plate 16, Fig. 4. This figure differs from the other illustrations in that the mouth lies to the left. The unit at the posterior end of the buccal cavity (Bca) is the odontophore apparatus which comprises the cartilages (not visible) covered by the medial radular retractor ( $m_4$ ), subradular membrane (Sur), radular shield (Ras) and radula (Ra). The long axis of the cartilages runs obliquely from posteroventral to anterodorsal. The radula runs along the mid-dorsal surface of the odontophore (Pl. 17, Fig. 4) and bends over the anterior odontophore tip called the bending plane of the radula (Bpl, Pl. 16, Fig. 4). The radula teeth are formed on a chitinous membrane, the radular shield (Ras) which tightly caps the anterior, anterolateral, and dorsolateral portion of the odontophore (Pl. 16, Fig. 4; Pl. 17, Fig. 4).

Posteriorly, the radula emerges from the radular sac (Rs), which bends ventrally between the cartilages (Pl. 16,

Fig. 4) and turns dorsally again at its tip. The ventral aspects of the radular sac and odontophore are shown in Plate 18. There is a fleshy papilla, the collostyle tip (Carriker, 1946a) at the point where the radula leaves the sac (Cos, Pl. 17, Figs. 2, 4). The collostyle is an elongate plug of dense white tissue between the radular teeth and the posterodorsal curvature of the radular sac epithelium. Only the tip protrudes beyond the opening of the radular sac.

A sheet of membranes from both posterolateral sides of the inner buccal mass meet, coalesce, and form the radular sac. The posterolateral continuation of this sheet thickens noticeably and forms the esophageal valve (Ev, Pl. 16, Figs. 1, 4; Pl. 17, Figs. 2, 4). This structure, quite variable in form between individuals, is extensively discussed by Fretter & Graham (1962). The anterodorsal edge of the valve is pressed against the radular sac. Together with the collostyle tip it serves to keep material from dropping into the radular sac or other ventral spaces behind the odontophore. The lateral portions of the valve send membrano-tendonous folds of tissue anteroventrally along either side of the odontophore on the sides of the pharynx (Pl. 16, Fig. 1; Pl. 17, Fig. 4). These cross over the odontophore divaricator muscles ( $m_3$ ) to run into the loosened membranes arising from the pharynx floor in front of the odontophore under the sublingual space (Sp, Pl. 16, Fig. 4). Part of this same membrane system sweeps over the divaricator muscles to join the radular sac.

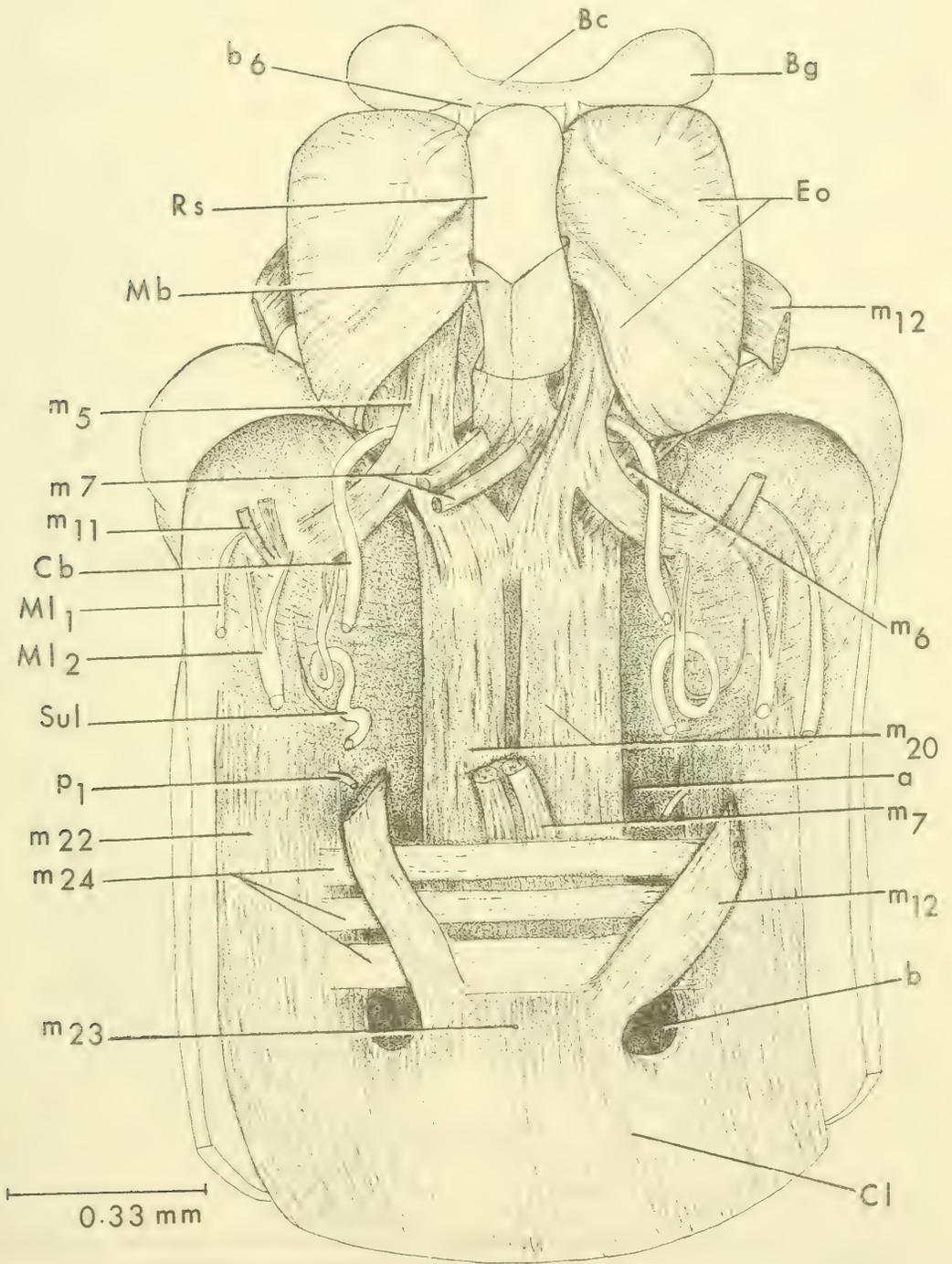
Dorsally, the esophageal valve may be folded with an internal anterior lip (anterior portion of Ev., Pl. 17, Fig. 2). The dorsal valvular structure may be folded in various ways (Pl. 16, Fig. 1; Pl. 17, Figs. 2, 4). The esophageal valve also serves as the floor for the initial portion of the esophagus.

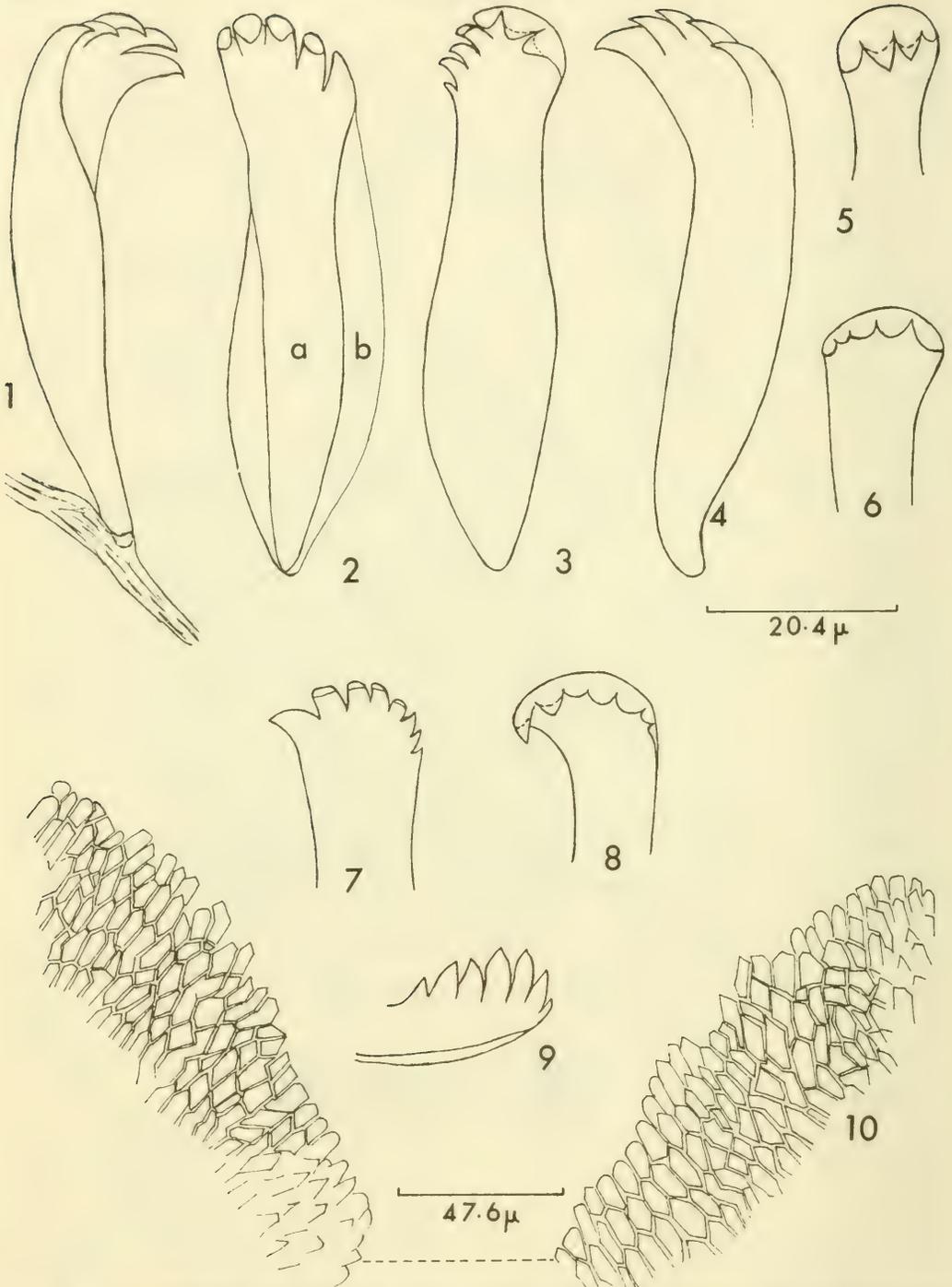
The mouth is bounded by the outer lips (Ol, Pl. 16, Fig. 4; Pl. 17, Fig. 4). It opens into a short oral tube (Ot)

PLATE 18. Musculature ventral to the buccal mass of *Pomatiopsis lapidaria*.

The buccal mass was pulled out of the rostrum (opened along the mid-dorsal line) to expose the muscles and nerve endings underneath.

- a area where the floor of the rostrum slopes downward as the anterior wall of the pedal haemocoel
- b posterodorsal gap of the pedal haemocoel
- b<sub>6</sub> posterior buccal nerve
- Bc buccal commissure
- Bg buccal ganglion
- Cb cerebrobuccal connective
- Cl columellar muscle
- Eo external odontophore membrane
- m<sub>5</sub> buccal protractor
- m<sub>6</sub> preventral protractor
- m<sub>7</sub> radular protractor
- m<sub>11</sub> dorsolateral buccal protractor
- m<sub>12</sub> buccal retractor
- m<sub>20</sub> rostral retractor
- m<sub>22</sub> lateral cephalic retractor
- m<sub>23</sub> mid-columellar supportive
- m<sub>24</sub> tensor magnus
- Mb membrane around the radular sac
- MI<sub>1</sub> median labial nerve 1
- MI<sub>2</sub> median labial nerve 2
- p<sub>1</sub> lateral retractor nerve from the pedal ganglion
- Rs radular sac
- Sul sublabial nerve





TEXT FIG. 1. Marginal teeth and jaw of *Pomatiopsis lapidaria*. Variation in the outer marginals is illustrated in 1-6, that in the inner marginals in 7-9. The jaws are shown in 10.

- a thickened central core of the peduncle  
 b thin, wing-like expanded portion of the peduncle

TABLE 6. Radular statistics

Feature	<i>Pomatiopsis lapidaria</i> (fr. 9 radulae)			<i>Oncomelania hupensis</i> <i>formosana</i> (fr. 16 radulae)		
	$\bar{X}$	S	Se	$\bar{X}$	S	Se
Radular length (mm)	1.133	0.085	0.016	0.976	0.098	0.024
Radular width (mm)	0.125	0.005	0.002	0.120	0.008	0.002
Total no. of rows of teeth	94	8.6	2.5	84	7.5	1.9
No. of rows in the formative stage	19	5.6	1.9	19	5.3	1.4

$\bar{X}$  = Mean

S = Standard deviation

Se = Standard error of the mean

which terminates at the 2 lateral outswellings of the pharynx wall, the inner lips (Il, Pl. 17, Fig. 4), and a pronounced transverse ventral fold (Vf). The inner lips bear the paired jaws (J). The ventral fold is centrally grooved (Gr) and is the threshold to the buccal cavity, i.e., the entire space of the pharyngeal tube. The portion of the buccal cavity beneath the anterior tip of the odontophore is the sublingual space (Sp, Pl. 16, Fig. 4).

The ventral fold is traversed on either side by ventrolateral ciliated grooves, the food grooves (Fg, Pl. 16, Fig. 1; Pl. 17, Fig. 4). They traverse the ventrolateral edge of the pharyngeal tube, pass up and over the large odontophore divaricator muscles, and over the lateral edges of the esophageal valve into the dorsal portion of the anterior section of the esophagus.

Jaw. F. C. Baker (1928) showed a small section of the jaw patterned like bricks in a wall. Dundee (1957) stated that the jaw consisted of 25-30 cuticular plates. She derived this number from a longitudinal, histological section of the jaw area which included the inner lips. A camera lucida drawing of each jaw is shown in Text Figure 1 (10). The edge of each jaw alone is composed of at least 27 cuticular plates and the whole jaw of many more, arranged in a narrow sheet. The length of this sheet is 0.13-

0.16 mm and its width varies around 0.06 mm.

Radula. The radula is of the typical taenioglossate type, i.e., with numerous rows of teeth, each row consisting of 7 teeth: a central, flanked on either side by a lateral, an inner and an outer marginal.

Nine radula ribbons were straightened out on slides and studied. The statistics on radular length, width, total rows of teeth and rows of teeth in the formative state are presented in Table 6. Rows of teeth within the radular sac are in various stages of formation, those closer to the end of the sac being the least formed. In this posterior area the central may not yet be formed, although the peduncles of the laterals could be counted. The total number of rows was determined by counting all the centrals until they became too indistinct to count and then all the remaining rows of peduncles. Rows of teeth were considered to be in the formative stages unless all the cusps could be discerned on each of the 7 teeth.

Each of the 7 teeth showed considerable variation in the number of cusps not only between specimens from a single population but also along the same radular ribbon: this variability is not one due to wear and tear, which is readily observed for what it is. The formulas for the cusp arrangements have

TABLE 7. A general formula for the most common cusp arrangement in *Pomatiopsis lapidaria*

Tooth	Cusp Formula	Snails* in which arrangement occurred in at least 90% of individual teeth %
Central (anter. & basal cusps)	$\frac{1-1-1}{3-3}$	62
Lateral	2-1-2(3)	75
Inner Marginal	6 ± 1	84
Outer Marginal	5 - 6	90

\* 50 snails from 3 populations.

been considered important throughout systematic molluscan literature. Reviewing the literature it becomes evident that, on the one hand, this variability is not generally appreciated, and, on the other hand, many who have observed variability often discredit the use of cusp arrangement as a major characteristic of either genera or species.

Knowledge of variability is essential and the presence of variation is not a problem if all the classes of variation can be adequately accounted for. It is, therefore, not sufficient to describe 1, 2, or even a dozen radulae, until the full range of variation is documented. In the study of this species it was necessary to study 50 radulae from 3 distinct populations in order to adequately encompass variation in cusp number (Tables 7 and 8).

In addition to cusp number, basic tooth morphology also may vary between taenioglossate radulae of various genera, subfamilies and families. Some of the variation in the morphology of the central tooth has already been discussed above in the section on systematics.

In Plate 19, the top row of teeth de-

TABLE 8. The various types of cusp arrangement for the different teeth among 50 radulae of *Pomatiopsis lapidaria* and the percentage of radulae showing that arrangement at least once

Central		Lateral	
Arrangement of cusps:		Arrangement of cusps:	
<u>anterior</u> basal	%		%
$\frac{1-1-1}{3-3}$	62	2-2-2	40
$\frac{1-1-1}{2-2}$	40	2-1-3	39
$\frac{1-1-0}{2-2}$	2	1-1-2	18
$\frac{1-1-2}{3-3}$	2	2-1-4	6
$\frac{2-1-2}{3-3}$	2	2-1-2	6
$\frac{1-1-2}{2-2}$	2	2-1-2 one side	12
0	2	2-1-3 other side	
Inner Marginal		Outer Marginal	
Number of cusps	%	Number of cusps	%
6	60	5	90
7	60	6	56
8	16	4	6
5	13	7	6

picts the relationship between teeth as seen on the lingual ribbon, i.e., shows tooth folded over tooth. All other teeth are shown disarticulated from the membrane and were drawn from various planes and views. The teeth were chosen to represent variations observed among the centrals and laterals. A series of inner and outer marginals is shown in Text Fig. 1. For an accurate count of

the cusps on the laterals and marginals it is advisable to fold the teeth back far enough to expose all the cusps (Pl. 19, lateral 5; Text Fig. 1, teeth 2-4, 7-9).

Each cusp is composed of a thickened, rounded supporting piece (Sup) and a thin, blade-like cutting edge (Cu). In radular preparations the cutting edges are often torn away leaving only the supports (Text Fig. 1, tooth 6). Where the cutting edge is drawn, the edge of the underlying support is represented by a dashed line (Pl. 19; Text Fig. 1). The body of the central tooth supports both anterior and basal (posterior) cusps. The supports for the basal cusps of the central have been discussed in the section of systematics (p 13). In Plate 19, central 6, note the characteristic shape of the posterior edge of the support (Slb). It can be discerned by focusing down through the large medial basal cusps. In central 6, the large cusps are represented only as dashed lines to make the shape of the supports clear. The plane of focus on centrals 3-5 is on the upper surface of the basal cusps so that the underlying supports are not evident. The supports for the large medial basal cusps give the face (Fa) of the tooth a square appearance.

There is no thickened tongue-like projection from the face of the central tooth, as figured for some hydrobiids (e.g., by Berry, 1943, for "*Amnicola integra*", Pl. 3, Fig. 4). As shown in central 6, the posterior contour of the tooth is slightly concave towards the lateral angle (La); it is bowed out posteriorly at the center as a tongue-shaped structure (Bp) which is neither thickened nor obvious. This structure is the membranous attachment of the basal edge of the central tooth to the lingual membrane. Viewing a row of central teeth on the radula, the attachment is often not noted, although it is there, e.g., centrals 1-5.

The laterals and marginals are attached to the membrane by the base of the slender peduncle (Pd, Pl. 19; Text

Fig. 1, tooth 1). The lateral tooth has a distinctive thickening (Th, Pl. 19, lateral 3), which arises from the comparatively massive, swollen support for the innermost cusps, i.e., in a lateral tooth with the cusps formula 2-1-3 (See Table 8) the "2-1" cusps. The thickened ridge runs posteriorly over the medial face of the lateral tooth and turns sharply to run along the peduncle of the tooth. Posterior to this ridge the peduncle is very thin and membranous.

In Table 7, the arrangement of cusps most commonly found on the various teeth is shown along with the percentage of radulae on which that arrangement was found to occur in at least 90% of the teeth. Only 61% of the snails had the representative formula as a whole, i.e., that shown in column 2 of Table 7; but inevitably there were some teeth in these radulae which were not representative types. In Table 8, every cusp arrangement found for each of the teeth is tabulated, with the percentage of radulae on which it was found, regardless of the frequency with which it occurred. The only significant difference between populations was found in the central tooth. Centrals from the radulae of snails from the Parker Mill population had a formula of 1-1-1/2-2 in 61% of the populations, while in the Hog Back and Barton populations 80% of the population of snails had a central with a formula of 1-1-1/3-3. A rare case was found which had no centrals at all. An unusually high percentage (12%) had a lateral tooth formula of 2-1-3 on 1 side of the central and 2-1-2 on the other side. This arrangement would continue the length of the ribbon.

Variation within the same lingual ribbon was greatest among the cusps of the marginal teeth. In 60% of radulae, variation within a ribbon was limited to the marginals. Whether a marginal had 7 or 8 cusps would often depend on the presence or absence of a tiny lateral spur as shown in Text Fig. 1, tooth 7. In only 20% of the radulae was variation but minor, i.e., did a few outer

PLATE 19. Variation in the radular teeth of *Pomatiopsis lapidaria*.

The horizontal top row of teeth shows 1 row of teeth on the radula in natural position.\* In some of the teeth the cutting edges of the cusps (Cu) have been torn off and only their thickened supports (Sup) are visible; when the cutting edges are shown, the supports are indicated by broken lines.

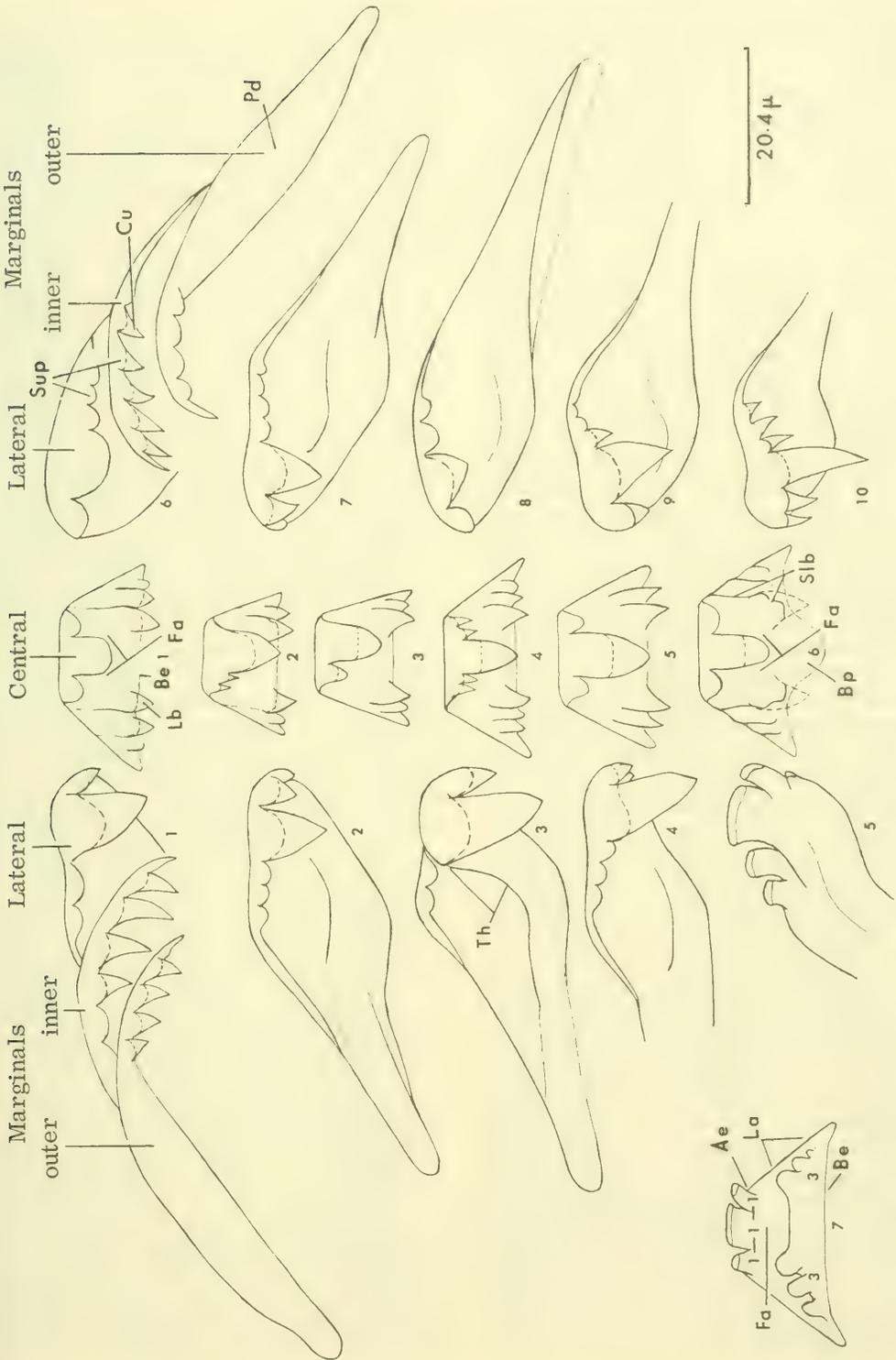
The central column shows variation in 6 central teeth. Focus on centrals 1-5 is on the upper surfaces of the large, medial basal cusps (Lb). On this plane of focus the supports (Sup) for the basal cusps are not clearly discerned (centrals 1, 2) or not seen (centrals 3-5). The posterior, basal, edge of the tooth (Be) appears straight. The plane of focus is lowered on central 6 so that the characteristics of the supports for the basal cusps (Slb) can be observed. The large, medial basal cutting edges are shown as dashed lines to make more clear the shape of the basal supports. At this level the basal process (Bp), a tongue-shaped attachment of the posterior tooth to the lingual membrane, can be seen. Central 7 (to the bottom, left) is shown with its posterior edge lifted up, showing how the basal supports are thickened and knob-like. To help interpret the cusp formulae quoted in the text, the numbers are marked on central 7 near the cusps involved.

The 2 columns of lateral teeth numbered 1-5 (on the left) and 6-10 (right) show variations in cusp number and shape of the cutting edges. Lateral tooth 5 is shown with the anterior edge depressed so that each cusp is clearly observed for counts.

Ae	anterior edge of the tooth
Be	basal (posterior) edge of the tooth
Bp	basal process attaching central tooth to the lingual membrane
Cu	cutting edge of the cusp
Fa	face of the tooth
La	lateral angle
Lb	large medial basal cusp of central tooth
Pd	peduncle
Slb	supportive for large medial basal cusp
Sup	supportive for cutting edge (dashed when shown under cusp)
Th	molded thickening which gives the anterior medial part of the tooth a distinctive curvature

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\*When the plate is turned sideways so that the labels are horizontal.



marginals vary between 5 or 6 cusps. In the 40% of the snails where the central or lateral tooth varied along the ribbon, a small stretch of about 10 centrals might have one formula while the next stretch of 15 or so would have another. In other cases only 5 or 6 centrals along the whole ribbon would vary.

In Table 3 (p 12) are listed the authors who have previously figured or discussed the radula of *Pomatiopsis lapidaria*. Also shown are the formulas they have figured or presented. The variations discussed in this paper encompass the formulas presented by Stimpson (1865), Thiele (1928), Abbott (1948a), and Dundee (1957). Baker (1902) studying the work of Stimpson (1865), discussed the 2 incurved basal denticles on each side of the central tooth. In 1926, however, in his family diagnosis for the Pomatiopsidae, he mentions only 2 large basal denticles for the central and it is this arrangement he figures in 1928, i.e., 1 large basal cusp on either side of the central. In the light of the weight of evidence of the other workers, it is evident that Baker (1926, 1928) either did not observe the other cusps which were present or that he examined an extremely rare variant. Further, he probably reversed the inner and outer marginals; it is more likely that the inner marginal had 9 and the outer marginal 6 cusps in the specimen that he studied.

Annandale (1924) reports the highly improbable number of 10 cusps for the inner and outer marginals. These can only be considered as misinterpretations. The central tooth formula for the anterior cusps, i.e., 2-1-2, could only represent a rare variant. Thiele (1931) gives a formula intended for the genus, hence including species other than *P. lapidaria*, with a lower limit of the number of cusps in the marginals. Inner and outer marginals with 4 and 3 cusps, respectively, are characteristic of *P. cincinnatiensis*.

## 7. Musculature

### Muscles of the Odontophore and Buccal Mass

It is beyond the scope of this paper to attempt the detailed study on buccal mass musculature and function such as presented by Carriker (1946a) or by Nisbet (1953), as presented by Fretter & Graham (1962), on the functional relationships in *Monodonta lineata*. For a full review of the literature pertaining to the buccal mass musculature one should consult Herrick (1906), Carriker (1946a), and Fretter & Graham (1962). There is not much literature on the buccal mass musculature of taenioglossate snails. Johansson (1939) presented a noteworthy paper on the musculature of *Littorina littorea*; Krause (1949) discussed the anatomy of *Lithoglyphus naticoides*. Fretter & Graham (1962) show several figures of the buccal mass musculature of *Viviparus viviparus*. The last authors review other literature which pertains to this group. As much of the musculature shown by these authors apparently does not pertain to the muscles described in this study, muscle nomenclature becomes a problem. Johansson (1939) avoided this problem by numbering the muscles. I have named all the muscles discussed on the basis of position or inferred function.

### Intrinsic Muscles of the Odontophore

The intrinsic muscles of the odontophore are those intimately associated with the cartilages. Removing the radula, radular shield and radular sac from the odontophore (Pl. 16, Fig. 1) and viewing its dorsal aspect, one observes 2 muscles ( $m_4$ ) each running from a posterior, ventrolateral position towards the mid-anterodorsal line of the odontophore. They fuse and form a trough which supports the radular sac. This muscle, the medial radular retractor, is readily lifted from the odontophore and pulled backward exposing the tips of the buccal

carilages (Ca, Pl. 17, Fig. 3), which overlap with either the right over the left tip, or the reverse. Each cartilage is about 0.6 mm long, with a height of about 0.35 mm, and swells posteriorly, reaching a width of 0.26 mm. The shape of the medial surface of the cartilages is shown in Plate 17, Fig. 5. The dorsal outline of the (left) cartilage, seen through the enveloping musculature, is shown in Plate 16, Fig. 2. The posterior ends of the cartilages are widely separated (Pl. 17, Fig. 3) leaving adequate room for the radular sac to nestle between.

1) *Lateral Cartilage Tensor* ( $m_1$ , Pl. 16, Fig. 3; Pl. 17, Figs. 3, 5). The lateral cartilage tensor passes as an arc-like band about 0.36 mm wide about the ventroanterior end of the 2 cartilages, binding the cartilages together. The muscle fastens along the exterior lateral edges of the cartilages (Pl. 16, Fig. 3).

2) *Mediolateral Cartilage Tensor* ( $m_2$ , Pl. 16, Figs. 1, 2, 3, 5; Pl. 17, Figs. 3, 5). The mediolateral cartilage tensor is a pronounced muscle seen arising from the ventral edge of each cartilage, as well as the ventral and posterolateral edge, thus forming a cup-like structure encasing the posterior end of each cartilage (Pl. 16, Fig. 5; Pl. 17, Fig. 5). The muscle runs dorsally and anteriorly over the medial surface of each cartilage and appears thickened at the dorsal edge of the cartilages. From this thickening appears to arise a membranous continuation (Sur), called the subradular membrane, which continues as a wide, thin band down laterally, and around the anterior edge of the cartilages, covering the lateral cartilage tensor (Pl. 16; Pl. 17, Figs. 3, 5). The mid-ventral crest of the membrane is thickened (a, Pl. 17, Figs. 3, 5) and serves as the place for the insertion of the radular protractor muscles ( $m_7$ , Pl. 17, Fig. 5). The radular shield (Ras) is tightly appressed to the subradular membrane (Pl. 16, Fig. 4). Ventrally the subradular membrane is con-

tinuous with the floor of the pharynx (Bf) to which it becomes tightly and inseparably appressed (Pl. 16, Fig. 4).

On either side of the ventral crest of the subradular membrane a thin muscle ( $m_{18}$ , not figured) inserts: the lateral membrane protractor. Its origin is on the posteroventral edge of the cartilages.

3) *Odontophore Divaricator* ( $m_3$ , Pl. 16, Figs. 3, 4; Pl. 17, Fig. 4). The odontophore divaricator arises from the posterolateral edge of each cartilage. It is a band which runs laterally to the pharynx wall and serves, in part, as the major source of support and attachment of the pharynx wall to the odontophore.

4) *Medial Radular Retractor* ( $m_4$ , Pl. 16, Figs. 1, 2, 4, 5; Pl. 17, Figs. 3, 5). The origin of this muscle is from the posterior, ventrolateral portion of the mediolateral cartilage tensor ( $m_2$ ). As mentioned above, this muscle runs dorsally between the cartilages, over the medial surface of the mediolateral cartilage tensor, fuses with its counterpart from the other cartilage, and rests with its anterodorsal tip on the crossed anterior ends of the buccal cartilages. The paired muscles insert on the anterior, ventrolateral portion of the radular sac at a point  $1/5$  the way posteroventrally from the tip of the muscle. The muscle is trough-like and serves to support the radular sac as well as to retract it.

Not figured is a pair of thin muscles ( $m_{19}$ ) which run from the midventrolateral edge of the style sac, between the cartilages, under the anteroventral edge of the lateral cartilage tensor ( $m_1$ ), where they insert on the subradular membrane to either side of the radular protractor ( $m_7$ ). This tiny muscle is the ventral membrane protractor.

#### Extrinsic Muscles of the Odontophore

5) *Buccal Protractors* ( $m_5$ , Pl. 14; Pl. 15, Fig. 1; Pl. 16, Figs. 4, 5; Pl. 18). The origin of these paired muscles is shown in Plate 18. There are characteristically 2 slips for each of the protractors; one from the rostral re-

tractor ( $m_{20}$ ) and the other from the anterior, ventrolateral rostral wall. The slips arising from the rostral retractors are usually fused as shown. Occasionally all the slips are fused in a single arc of muscle, but this occurs rarely. Each muscle inserts partially on the posteroventral surface of the medial radular retractor ( $m_4$ ) and partially on the mediolateral cartilage tensor ( $m_2$ , Pl. 16, Figs. 4, 5). These broad, heavy muscles serve to pull the odontophore anteriorly as well as to depress the posterior end of the odontophore.

6) *Preventral Protractors* ( $m_6$ , Pl. 15, Fig. 1; Pl. 16, Fig. 3; Pl. 18). These muscles originate at the tip of the rostrum immediately adjacent to the oral aperture (Pl. 15, Fig. 1). They insert on the posterior ventrolateral edge of each cartilage (Pl. 16, Fig. 3). These bands of muscle are most readily observed from the lateral, external view of the buccal mass (Pl. 15). They serve to protract the odontophore as well as the pharyngeal walls. The number of muscle bands is variable.

7) *Radular Protractor* ( $m_7$ , Pl. 17, Figs. 1, 5; Pl. 18). The paired radular protractors arise from the base of the rostral retractors ( $m_{20}$ ) as shown in Plate 17, Fig. 1. They run anteriorly side by side, bound in a connective tissue sheath with the main vascular supply to the buccal mass. They pass over the point where the fused medial bands of the buccal protractors ( $m_5$ ) separate (Pl. 18), pass into the connective tissue sheet (Mb) which surrounds the radular sac, and run between the cartilages to insert on the central crest of the subradular membrane (Sur, Pl. 17, Fig. 5). These muscles serve to depress the tip of the odontophore while protracting the radula slightly over the bending plane of the odontophore.

#### Extrinsic Muscles of the Buccal Mass

8) *Anterior Jugalis* ( $m_8$ , Pl. 15, Fig. 1). Arising from the anterior dorsal crest of the buccal mass, the muscle band, about 0.40 mm wide, runs obliquely

posteroventrally covering the posterior portion of the buccal constrictor ( $m_9$ ). It inserts on the odontophore divaricator ( $m_3$ ) and the ventrolateral edge of the cartilage. The cerebrobuccal connective (Cb) passes between this muscle and the buccal constrictor ( $m_9$ ).

9) *Buccal Constrictor* ( $m_9$ , Pl. 15, Fig. 1). The buccal constrictor surrounds the anterior end of the buccal mass, encasing the fleshy walls of the oral tube in a sheath of muscles. This muscular sheath extends from the anterior oral tube posteriorly to the rear of the pharyngeal tube to a point corresponding to the place where the subradular membrane (Sur, Pl. 16, Fig. 4) fuses with the floor of the pharyngeal tube. The posterior portion of this muscle is covered by the anterior jugalis ( $m_8$ ).

10) *Odontophore Levator* (not figured). The odontophore levator runs obliquely from the odontophore to the dorsal portion of the buccal constrictor ( $m_9$ ) between the buccal constrictor and the anterior jugalis ( $m_8$ ). The insertion of this slender muscle is with the anterior jugalis.

11) *Dorsolateral Buccal Protractor* ( $m_{11}$ , Pl. 15, Fig. 1; Pl. 18). The muscle arises from each side of the anterolateral rostral wall (as shown in Pl. 18) as a single or as 2 thin parallel strands, which run to an insertion on the odontophore divaricator ( $m_3$ ) or the buccal retractor ( $m_{12}$ ). The insertion of this muscle is hidden by the buccal retractor ( $m_{12}$ , Pl. 15, Fig. 1). Commonly the protractor bifurcates, the anterior slip fusing with the anterior slip of the retractor, both inserting on the divaricator muscle. The posterior protractor slip runs posteroventrally over the exterior odontophore membrane to fuse with fibers from the tensor of the odontophore membrane mentioned below (under 14).

12) *Buccal Retractor* ( $m_{12}$ , Pl. 15, Figs. 1, 2; Pl. 17, Fig. 1; Pl. 18). These pronounced paired muscles arise from the basal part of the mid-columnar supportive ( $m_{23}$ , Pl. 17, Fig. 1;

Pl. 18). They run anteriorly passing the medial surfaces of the cerebral ganglia (Cg), the pleuro-pedal (Pp) and the cerebro-pedal (Cp) connectives to insert on the odontophore divaricator muscle ( $m_3$ ) or the lateral cartilage surface. Before inserting, this broad muscle bifurcates, sending the anterior slip to an insertion on the odontophore; the posterior slip sends fibers into the membranous jugalis ( $m_{13}$ ).

13) *Membranous Jugalis* ( $m_{13}$ , Pl. 15, Fig. 1; Pl. 14). Posterior to the anterior jugalis ( $m_8$ ) and dorsal to the external odontophore membrane (Eo), the buccal mass is quite fleshy; its surface is laced with thin and irregularly oriented muscle strands suggesting a thin membranous network rather than a distinct, stout muscular layer. The salivary glands enter this tissue; the esophagus arises from its posterior continuations.

14) *Tensor of the Odontophore Membrane* (not figured). The lateral musculature of the odontophore bulges outward making the contour of the odontophore evident to one observing the lateral buccal mass (Pl. 15, Fig. 1). This musculature is hidden from view as it is wrapped in a membrane, the external odontophore membrane (Eo, Pl. 18), which is continuous between the 2 halves of the odontophore. Posteroventrally this membrane passes between the ventral, protruding, recurved end of the radular sac and the odontophore musculature.

A slender muscle, the tensor of the odontophore membrane, runs from side to side across the external odontophore membrane through the angle formed by the emerging esophagus and the membrane. This muscle sends branches over the ventrolateral surface of the membrane.

15) *Suspensor of the Radular Sac* ( $m_{15}$ , Pl. 16, Fig. 4). The origin of this muscle is the thickened tensor of the odontophore membrane at the point where the latter passes over the recurved ventral tip of the radular sac. It inserts on the membranes of the tip of the radular

sac. The muscle may be forked, i.e., its origins on the tensor of the odontophore membrane are slightly separated while it has a common point of insertion on the tip of the radular sac.

16) *Preventral Dilator Muscles* ( $m_{16}$ , Pl. 14; Pl. 15, Fig. 1). Numerous thin muscle strands run from the buccal constrictor muscle ( $m_9$ ) and the preventral protractors ( $m_6$ ) to the antero-lateral rostral wall.

17) *Suspensors of the Buccal Mass* ( $m_{17}$ , Pl. 14; Pl. 15, Fig. 1; Pl. 16, Fig. 4). Irregularly placed muscle strands run from the dorsal and dorso-lateral surface of the buccal mass to the rostral roof. Anteriorly, from the dorsal crest of the buccal constrictor ( $m_9$ ) and the anterior jugalis ( $m_8$ ), these muscles are more dense and some of them undoubtedly serve to protract the buccal mass.

18) *Lateral Membrane Protractor* ( $m_{18}$ , discussed under 2).

19) *Ventral Membrane Protractor* ( $m_{19}$ , discussed under 4).

#### Body Musculature

The principal muscle of the body is the columellar muscle (Cl). In Plates 5 and 6 this muscle is observed emerging from the ventral mantle tissue behind the collar (M). Only a portion of this wide muscle is shown. As previously mentioned, the muscle is normally pressed and bound to the ventral wall of the mantle cavity. It is fused with the columella of the shell at a level corresponding to the posterior end of the mantle cavity.

In Plate 4, the muscle is shown emerging from the epithelium covering the "neck." In Plate 14, the rostral and "neck" epithelium are slit and folded back to reveal this broad muscle, which is the basis of support for the head-foot region. To show the underlying features (Pl. 18), the esophagus was cut at the level of the pleuro-supraesophageal connective (Psc), the cerebral commissure (Cc) was cut, and also the posterior region of the buccal retractors ( $m_{12}$ ). The posterior end of

the buccal mass was then pulled upward out of the rostral portion of the cephalic haemocoel and forward, using the origins of the buccal protractors ( $m_5$ ) as a hinge. The radular protractors ( $m_7$ ) were then cut and the buccal mass pulled forward completely. Finally, removing the dorsal nervous system, the musculature (Pl. 18) underlying the organs shown in Plate 14 can be observed.

The columellar muscle, beneath the point where the pleuro-supraesophageal connective (Psc) crosses the esophagus (Pl. 14) sends 3 pronounced bands anteriorly, while it sweeps ventrally in an arc as shown in Plate 15, Fig. 2. The 3 bands are the 2 lateral cephalic retractors ( $m_{22}$ ) and the centrally positioned mid-columellar supportive ( $m_{23}$ , Pl. 15, Fig. 2; Pl. 18).

The mid-columellar supportive muscle ( $m_{23}$ ) serves as the origin for a number of important muscles. On either side of the origin of this muscle is noted a cavity (b, Pl. 18), leading into the posterior portion of the pedal haemocoel, whose posterior wall and roof are formed by the ventrally curved columellar muscle and the mid-columellar supportive, respectively. Laterally there is a space between the mid-columellar supportive and the lateral cephalic retractors ( $m_{22}$ ) which marks the dorsolateral edges of the pedal haemocoel. The mid-columellar supportive bifurcates anteriorly into 2 band-like muscles, the rostral retractors ( $m_{20}$ ), which continue across the dorsal pedal haemocoel and run anteriorly over the floor of the rostrum (Pl. 17, Fig. 1; Pl. 18).

Johansson (1939) has an excellent photograph of a gross dissection of the rostral area of *Littorina littorea* showing these paired muscles running toward the oral aperture as they do in *Pomatiopsis lapidaria*. Anteriorly the retractors pass beneath the point where the medial slips of the buccal protractors ( $m_5$ ) take their origin; they send numerous inserting slips around the oral aperture on the rostral floor.

The origin of the radular protractors ( $m_7$ ) is at the dorsomedial base of the rostral retractors ( $m_{20}$ , Pl. 17, Fig. 1). They are characteristically slightly swollen at their base. The buccal retractors ( $m_{12}$ ) arise at the dorsal, posterolateral base of the mid-columellar supportive muscle ( $m_{23}$ , Pl. 17, Fig. 1; Pl. 18).

Around the oral aperture, at the rostral tip, is a thin circular band of muscles best observed by clearing the rostral floor of the buccal protractors ( $m_5$ ). This band is the labial sphincter ( $m_{21}$ , Pl. 16, Fig. 4). Anterior to the origin of the buccal retractors is a sheet of muscles running from side to side between the lateral cephalic retractors ( $m_{22}$ ). Characteristically, this sheet, the tensor magnus ( $m_{24}$ ), is split up into 3-5 discrete bands (Pl. 18). The sheet is 0.36 mm wide and about 0.96 mm long. It rests upon the mid-columellar supportive muscles ( $m_{23}$ ) as well as the posterior portion of the rostral retractors ( $m_{20}$ ) and supports the posterior portion of the contracted buccal mass, providing a supportive framework for the cerebral ganglia as well. The tensor magnus and the mid-columellar supportive form a sort of roof over the pedal haemocoel separating it in a loose manner from the cephalic haemocoel. The paired pleuropedal connectives (Pp), one on either side of the mid-columellar supportive, pass from the cerebral area between the posterior and mid-slips of the tensor magnus down into the pedal haemocoel to connect with the pedal ganglia. The cerebropedal connectives (Cp) do likewise, passing between the mid and anterior slips of that muscle (Pl. 18). The origin of the buccal retractor ( $m_{12}$ ) is sometimes split so that an anterior slip appears to arise from the posterior slip of the tensor magnus (Pl. 18).

The lateral cephalic retractors are powerful bands ( $m_{22}$ ) giving support to the posteroventral wall of the rostrum. They terminate at about the point where the rostral floor turns ventrally forming

the anterior wall of the pedal haemocoel (Pl. 18). The relationship of the ventrally curving columellar muscle (C1), mid-columellar supportive ( $m_{23}$ ) and lateral cephalic retractor ( $m_{22}$ ) is shown with regard to the posterior pedal haemocoel and the pedal ganglia (Pl. 15, Fig. 2). The columellar muscle sweeps ventroposteriorly beneath the operculum. Where the rostral floor slopes down to form the anterior wall of the pedal haemocoel some transverse muscle fibers are noted passing between the lateral cephalic retractors ( $m_{22}$ ). These muscles, the dorsal pedal tensors ( $m_{25}$ ), lie against the anterior wall of the pedal haemocoel and pass across the mid-anterior surface of the pedal ganglia or a little dorsal to the mid-length of these ganglia.

A single muscle arises from the ventral origin of the rostral retractors ( $m_{20}$ ), the dorsal propodial retractor ( $m_{26}$ , Pl. 15, Fig. 2). This muscle passes anteroventrally between the pedal ganglia over the pedal commissure, forks, and sends a slip ventrolaterally to the anterior haemocoel wall beneath the dorsal pedal tensor ( $m_{25}$ ).

At the level of the propodial ganglia (Prg), from the mid-anterior haemocoel wall, arises a muscle about 0.15 mm wide which bifurcates and sends a slip laterally, right and left respectively, under the point where the propodial ganglia enter the anterior musculature, back under the metapodial connective, to the posterolateral wall of the descending columellar muscle. This is the mid-ventral protractor ( $m_{27}$ ) shown for *Oncomelania hupensis formosana*, which has the same arrangement (Pl. 13, Fig. 1).

## 8. Nervous System

In the study of neural anatomy for comparative, systematic use, considerable attention was directed towards the position of ganglia and their dimensions, the number of nerves and their respective points of origin on a given ganglion, the lengths of the major com-

missures and connectives, and especially the amount of variation encountered in all of the above. Secondary and especially tertiary branches of nerves were found to be highly variable and were not generally considered for this comparative study.

There are major discrepancies between the previous work on the neural anatomy of *Pomatiopsis lapidaria* (Dundee, 1957) and my findings. The present work is derived entirely from my own observations made on over 100 snails dissected especially for neural structure.

A classical, extensive study on comparative prosobranch neural anatomy is that of Bouvier (1887). General prosobranch neural anatomy is reviewed by Fretter & Graham (1962). Johansson (1939) presented some unique photographs of the gross dissections of the central nervous system of *Littorina littorea*. Krull (1935) gave data on prosobranch nervous systems and their relevance to prosobranch phylogeny. Krause (1949) published excellent drawings on the nervous system of *Lithoglyphus naticoides*. Further references are reviewed by these authors.

There are 6 major ganglionic complexes, the cerebral, buccal, pedal, pleural, parietal, and visceral.

### Cerebral Complex

a. *Dorsal Aspect*. Opening the rostral cavity from the dorsal side one exposes the buccal mass and associated structures. Particularly noticeable is the heavy pigment dusted over the ganglia and nerves seen from the dorsal surface. The paired cerebral ganglia (Cg, Pl. 14; Pl. 20, Fig. 1) connected by the cerebral commissure (Cc) are pressed against the mid-posterior curvature of the esophagus where the latter bends ventrally (Pl. 15, Fig. 1). The anterior tips of the ganglia press against the mid- or ventral, posterolateral wall of the buccal mass, in particular against the anterior end of the buccal retractor muscle ( $m_{12}$ , Pl. 15, Fig. 1).

The cerebral commissure (Cc) is 0.14

PLATE 20. Nervous system of *Pomatiopsis lapidaria*

FIG. 1. Dorsal aspect of the "brain" or central portion of the nervous system lifted out of the rostrum.

FIG. 2. Anterior aspect of the pedal ganglia.

FIG. 3. A rare variant in that a distinct pleuro-subesophageal connective is found between the left pleural ganglion and the subesophageal ganglion.

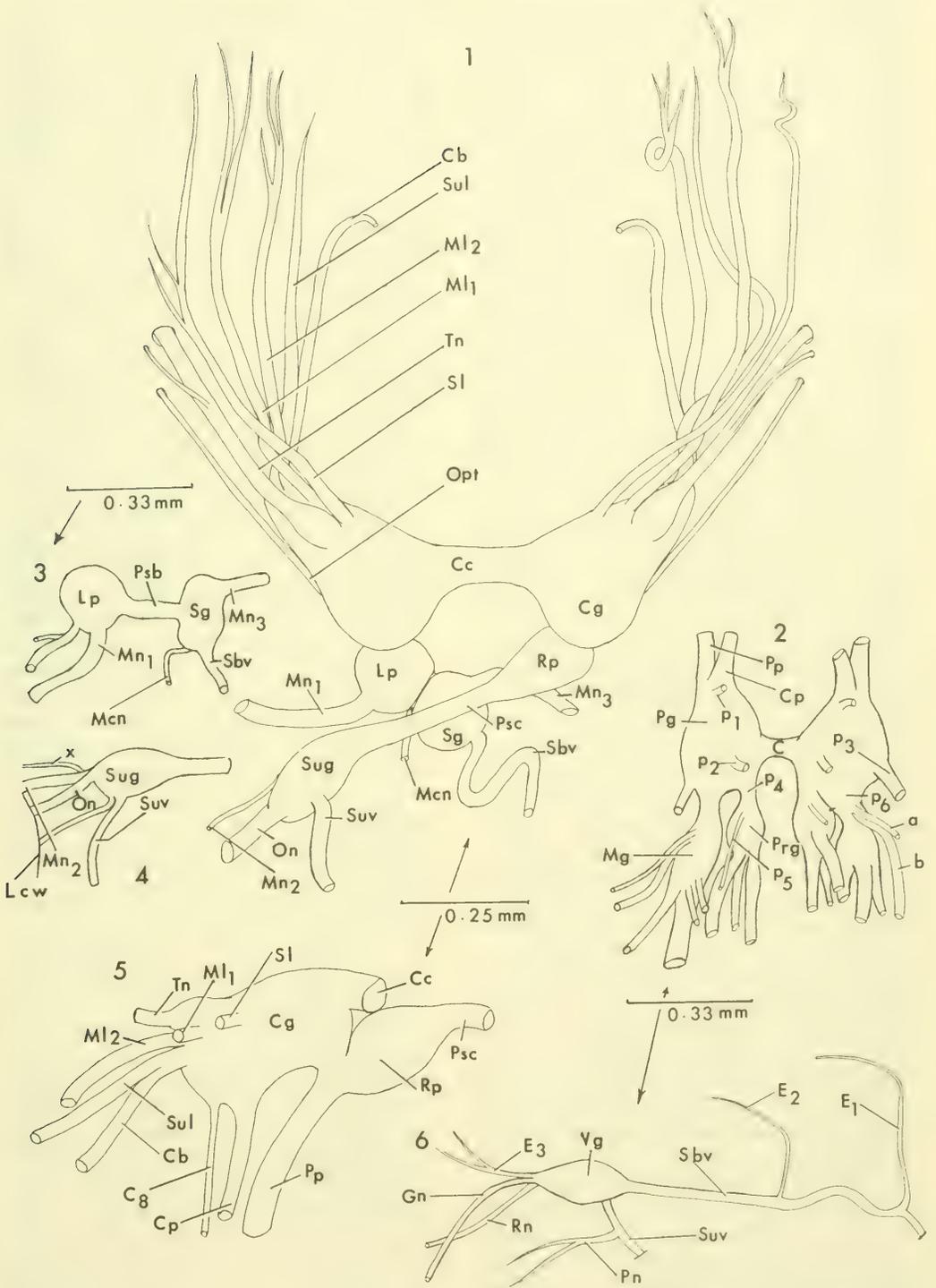
FIG. 4. A variant in the arrangement of nerves leaving the supraesophageal ganglion (same scale as Fig. 3).

FIG. 5. Medial surface of the right cerebral ganglion, showing the exact positions where the nerves from that ganglion arise.

FIG. 6. Visceral ganglion viewed from the ventral side.

a	nerve from p <sub>6</sub>	Opt	optic nerve
b	nerve from p <sub>6</sub>	p <sub>1</sub>	lateral retractor nerve from the pedal ganglion
C	pedal commissure	p <sub>2</sub>	nerve to the anteroventral wall of the pedal haemocoel
Cb	cerebro-buccal connective	p <sub>3</sub>	major lateral nerve of pedal ganglion
Cc	cerebral commissure	p <sub>4</sub>	propodial connective
Cg	cerebral ganglion	p <sub>5</sub>	mid-propodial nerve
Cp	cerebro-pedal connective	p <sub>6</sub>	metapodial connective
C <sub>8</sub>	cerebro-tensor nerve	Pg	pedal ganglion
E <sub>1</sub>	external mantle cavity nerve 1	Pn	pericardial nerve
E <sub>2</sub>	external mantle cavity nerve 2	Pp	pleuro-pedal connective
E <sub>3</sub>	external mantle cavity nerve 3	Prg	propodial ganglion
Gn	gonadal nerve	Psc	pleuro-supraesophageal connective
Lcw	left lateral cephalic wall	Psb	pleuro-subesophageal connective
Lp	left pleural ganglion	Rn	renal nerve
Mcn	mid-columellar nerve	Rp	right pleural ganglion
Mg	metapodial ganglion	Sl	supralabial nerve
Ml <sub>1</sub>	median labial nerve 1	Sg	subesophageal ganglion
Ml <sub>2</sub>	median labial nerve 2	Sbv	subvisceral connective
Mn <sub>1</sub>	mantle nerve 1, from the left pleural ganglion	Sug	supraesophageal ganglion
Mn <sub>2</sub> *	mantle nerve 2, from the supraesophageal ganglion	Sul	sublabial nerve
Mn <sub>3</sub>	mantle nerve 3, from the subesophageal ganglion	Suv	supravisceral connective
On*	osphradial nerve	Tn	tentacular nerve
		Vg	visceral ganglion
		x	variant branch of Mn <sub>2</sub>

\* Usually a single large trunk emerges from the supraesophageal ganglion the common osphradio-mantle nerve, which then bifurcates to form the osphradial nerve (On) and mantle nerve 2 (Mn<sub>2</sub>) (see p 79).



mm long, but in some specimens, a length of 0.19 mm was found. The width is 0.05-0.06 mm. In dorsal view the cerebral ganglia are 0.36-0.29 mm long. Eight nerves arise from each cerebral ganglion. Seven of these nerves are pronounced and run anteriorly, while 1 is quite thin and has a ventral course. Upon opening the dorsal mid-line, 5 of the most conspicuous nerves appear, jumbled, intermixed, or appressed to the buccal mass (Pl. 14) while the 2 remaining ones are hidden from view beneath it. In all cases the nerves are easily untangled and separated from the buccal mass. In a rare case the right salivary gland was found tucked down between the cerebral ganglion and buccal mass, intermixed in the nerves arising from the ganglion. With the buccal mass removed, one can observe all 7 of the pronounced nerves (Pl. 20, Fig. 1) although, for most of them, their origin on the ganglion cannot be observed.

1) Tentacular Nerve. The tentacular nerve (Tn) is the most prominent nerve rising from the cerebral ganglion (Pl. 14; Pl. 15, Fig. 1; Pl. 20, Figs. 1, 5). It arises from a marked swelling, the tentacular bulb, on the anterodorsal end of each cerebral ganglion and runs anterolaterally into the lateral rostral wall, through the wall and into the tentacle. Arising from the tentacular nerve about half way out towards the rostral wall is a slender nerve which runs into the rostral wall apart from the entry of the tentacular nerve. Occasionally 1 or 2 other nerves are seen emerging from the tentacular nerve at a point 0.24 mm beyond the tentacular bulb. These last mentioned nerves are rarely found. When they occur they enter the rostral wall posterior to the tentacular nerve.

2) Optic Nerve. This nerve (Opt) arises from the mid-, ventrolateral surface of each ganglion and runs anteroventrally to enter the rostral wall about 0.12 mm posterior to the tentacular nerve. The nerve is quite

slender. It innervates the eyes (Pl. 14; Pl. 15, Fig. 1; Pl. 20, Fig. 1).

3) Supralabial Nerve. The origins of the remaining nerves that arise from the cerebral ganglia are displayed in Plate 20, Fig. 5, which shows the medial surface of the right cerebral ganglion. The supralabial nerve (Sl) sweeps dorsally from the anteromedial surface of the cerebral ganglion crosses over the other nerves from the ganglion, and runs dorsolaterally to the rostral wall. It becomes bound to the dorsolateral rostral wall about 0.12 mm anterior to the point where the tentacular nerve (Tn) enters the wall. Travelling anteriorly, dorsal to the other labial nerves, it becomes more slender and, in the dissection shown, undulates near its anterior end due to the contracted state of the rostrum (Pl. 14, Sl). The nerve terminates more posteriorly than the other labial nerves. The supralabial often sends off a small branch near the point where it fuses with the rostral wall (Pl. 20, Fig. 1).

4) Median Labial Nerve 1. Arising slightly anteroventrally of the previous nerve (Pl. 20, Fig. 5), the median labial (Ml<sub>1</sub>) runs anteriorly unfused with the rostrum but usually lying against the rostral wall. Anteriorly it passes above the origin of the dorsolateral buccal protractor muscle (m<sub>11</sub>) and runs to the tip of the rostrum beneath the pre-ventral dilators (m<sub>16</sub>, Pl. 15, Fig. 1; Pl. 18; Pl. 20, Figs. 1, 5).

5) Median Labial Nerve 2. Median labial 2 (Ml<sub>2</sub>) arises from the anterior edge of the cerebral ganglion beneath the tentacular bulb (Pl. 20, Fig. 5). It runs anteroventrally to median labial 1, likewise unfused with either the rostral wall or buccal mass. It characteristically forks anteriorly sending a root to either side of the origin of the dorsolateral buccal protractor (m<sub>11</sub>); one root innervates this muscle.

6) Sublabial Nerve. This nerve (Sul) arises closely appressed to the cerebro-buccal connective (Cb) and sometimes these 2 nerves arise as a

fused, inseparable trunk before branching. The sublabial travels freely along the floor of the rostrum. In the contracted rostrum, the nerve coils. From the coiled part a branch arises to innervate the anterior rostral floor (Pl. 18). Anteriorly the nerve forks; one branch passes to the origin of the buccal protractor ( $m_5$ ) while the other passes beneath that muscle to travel anteriorly to the rostral tip (Pl. 15, Fig. 1; Pl. 18; Pl. 20, Figs. 1, 5).

7) Cerebro-Buccal Connective. This nerve (Cb) runs anteriorly over the lateral edge of the rostral retractor muscle ( $m_{20}$ , Pl. 18), up under and behind the buccal protractors ( $m_5$ ) before the latter disappear beneath the external odontophore membrane. As shown in Plate 15, Fig. 1, the cerebro-buccal connective travels between the anterior jugalis ( $m_8$ ) and the buccal constrictor ( $m_9$ ) over the anterolateral edge of the odontophore, emerges over the surface of the membranous jugalis ( $m_{13}$ ) to run posteriorly into the anterior tip of the buccal ganglion (Bg). Before reaching the buccal ganglion it passes beneath the inserting fibers of the posterior slip of the buccal retractor ( $m_{12}$ ).

8) Cerebro-Tensor Nerve. This nerve ( $C_8$ ) arises from the anteroventral edge of the cerebral ganglion (Pl. 20, Fig. 5). It is a slender nerve which runs directly ventrally to innervate the anterior slip of the tensor magnus muscle ( $m_{24}$ ).

b. *Right Lateral Aspect of the Cerebral Complex.* Two connectives leave the ventral surface of the cerebral ganglia (Pl. 15, Fig. 1; Pl. 20, Fig. 5), drop ventrally and converge on the dorsal surface of the pedal ganglia (Pg). The posterior connective is the pleuro-pedal (Pp), the anterior one is the cerebro-pedal (Cp). The former is about 0.19-0.22 mm long and 0.048-0.050 mm wide. The latter is about 0.19 mm long with a width of about 0.03 mm. No nerves arise from along the length of the cerebro-pedal connective. From the

juncture of the pleuro-pedal connective and the cerebral ganglion arises the pleural ganglion (Rp, Pl. 15, Fig. 1; Pl. 20, Figs. 1, 5). A few nerves, usually 4, labeled  $pp_{1-4}$ , arise from the pleuro-pedal connective.

1) Lateral Nerve 1. At the ventral juncture of the pleural ganglion with the connective a nerve arises ( $pp_1$ , Pl. 15, Fig. 1) which runs ventrolaterally under the penial nerve ( $pp_2$ ) to enter the musculature of the rostral wall just above the lateral insertion of the posterior slip of the tensor magnus muscle ( $m_{24}$ ) in the lateral cephalic retractor ( $m_{22}$ ). This nerve may be absent; when present its thickness is found to vary considerably. It is 0.024 mm in diameter or thinner.

2) Penial Nerve. In males this nerve ( $pp_2$ , Pl. 15, Fig. 1) is greatly thickened (0.036 mm wide). It arises from the mid-length of the connective (in some cases slightly lower, e.g., Pl. 15, Fig. 1). Passing posteriorly as well as dorsolaterally to the cephalic wall above  $pp_1$ , it becomes thinner and proceeds to the mid-cephalic roof and then into the basal musculature of the verge. In females the nerve is slender, about 0.024 mm wide, and passes dorsolaterally to the cephalic wall.

3) Lateral Nerve 3. Slightly ventral and lateral to the penial nerve a nerve arises from the pleuro-pedal connective ( $pp_3$ , Pl. 15, Fig. 1) which initially travels anteriorly and ventrolaterally, then dorsolaterally. Near its end it forks and sends both branches into the lateral rostral wall just posterior to the point where the tentacular nerve enters the wall. The optic nerve occasionally passes between the bifurcation. The points where the branches of this nerve enter the wall are variable and may be ventral or posterior to the positions described. The nerve itself is markedly variable. Instead of a single nerve arising from the connective, 2 closely associated nerves often arise and run directly to the lateral rostral wall. In rare instances 2 minute

nerves just ventral to  $pp_3$  arise from the connective and run laterally to the rostral wall.

4) Lateral Nerve 4. When dissecting under Bouin's solution with the aid of a very bright light, I often found arising from the ventral surface of the cerebral ganglion between the pleuro-pedal connective and the cerebro-pedal connective a thin strand ( $pp_4$ , Pl. 15, Fig. 1) appressed to or fused with the pleuro-pedal connective, or distinctly separate so as to traverse the space between the connectives. It is suspected that this nerve, when not observed, is incorporated in the pleuro-pedal connective. The nerve passed into the area where the  $pp_2$  and  $pp_3$  nerves arose, but its final destination was not ascertained.

c. *Left Lateral Aspect of the Cerebral Complex.* The counterpart of  $pp_1$ , found on the right side, was rarely present on the left. When encountered, it was a thin strand running ventrolaterally into the lateral cephalic retractor muscle ( $m_{22}$ ) near the posterior edge of the attachment of the tensor magnus ( $m_{24}$ ). The counterpart of the penial nerve is regularly present. It runs dorsolaterally to enter the cephalic wall just behind the entrance of the optic nerve where the latter enters the wall. The counterpart to  $pp_3$  runs laterally and only slightly dorsally, bifurcates, each branch entering the ventrolateral cephalic wall opposite the pleuro-pedal connective or posterior to that point ( $pp_3$ , Pl. 14). Again, variation is commonly encountered in the arrangement of these lateral nerves from the pleuro-pedal connective. In unusual instances one finds, instead of  $pp_1$ , a nerve arising from the posterior surface of the pleuro-pedal connective below the point where the pleural ganglion arises. The nerve runs ventrally and innervates the posterior slip of the tensor magnus. In contrast to the right side,  $pp_2$  and  $pp_3$  arise from the ventral portion of the connective.

### Pedal Complex

The pedal complex consists of 3 pairs of ganglia. Each of the large dorsal pedal ganglia connects ventrally with an antero-medial propodial ganglion and a postero-lateral metapodial ganglion. The statocyst is part of the pedal complex. It is appressed to the dorso-posterior surface of each pedal ganglion at the base of the pleuro-pedal connective.

The *pedal ganglia* are quite large and fill the pedal haemocoel beneath the tensor magnus ( $m_{24}$ ). Each ganglion seen from the anterior face (Pg, Pl. 15, Fig. 2; Pl. 20, Fig. 2) is about 0.31 mm long and 0.24 mm wide. They are connected, as shown, by a commissure (C) which varies in length from 0.08-0.04 mm. The right and left ganglia are similar with regard to the number of nerves which arise, their position, and variability. The description below pertains to either ganglion. Seven nerves, labeled  $p_{1-7}$  (including the propodial and metapodial connectives) arise from the pedal ganglia.

1) Lateral Retractor Nerve. The lateral retractor nerve ( $p_1$ ) arises anteroventrally with respect to the cerebro-pedal connective (Cp) as a slender nerve (Pl. 15, Fig. 2; Pl. 20, Fig. 2). It runs anteriorly (Pl. 15, Fig. 1) to enter the lateral cephalic retractor ( $m_{22}$ ) at its ventral edge near the area where the rostral retractors emerge from beneath the tensor magnus ( $m_{24}$ , Pl. 18).

2) Nerve to the Anteroventral Wall. A slender nerve ( $p_2$ ) leaves the anterior face of the pedal ganglion ventral to the above nerve, and just dorsal to the propodial connective ( $p_4$ ). It runs anteriorly to the anterior wall of the pedal haemocoel beneath the dorsal pedal tensor ( $m_{25}$ ).

3) Major Lateral Nerve. This stout nerve ( $p_3$ ), often 0.048 mm wide, arises from the ventrolateral edge of the pedal ganglion and runs posterolaterally back through the musculature to an area be-

neath the operculum. The minor lateral nerve (not figured) was found only a few times. It branches off between  $p_3$  and the metapodial connective ( $p_6$ ) on the lateral edge of the ganglion and follows  $p_3$  into the lateral musculature of the foot.

4) Propodial Connective. This short, stout connective ( $p_4$ ) joining the pedal ganglion (Pg) to the propodial ganglion (Prg) arises from the ventromedial edge of the former. It is devoid of emergent nerves. It usually swings dorsally from the long axis of the pedal ganglion and enters the anteroventral foot musculature (Pl. 13, Fig. 1).

The *Propodial Ganglion* (Prg). Within about 0.07 mm from the pedal ganglion the propodial connective swells into the propodial ganglion which is about 0.12 mm in diameter or slightly less. The foot musculature forms a spheroidal cavity encasing the propodial ganglion. From the distal tip of that ganglion there arise 3 nerves of about equal size. These nerves are, however, variable and at times only 1 of them is particularly pronounced.

5) Mid-Propodial Nerve. A thin nerve ( $p_5$ , Pl. 20, Fig. 2) emerges from the anterior, mid-surface of the propodial ganglion. It runs anteroventrally into the foot musculature.

6) Metapodial Connective. This stout connective ( $p_6$ ) between the pedal ganglion (Pg) and the metapodial ganglion (Mg) takes off lateral to the propodial connective and runs ventrolaterally to it. Between the pedal and metapodial ganglia 2 slender nerves arise from the connective (a, b, Pl. 20, Fig. 2). Commonly they do so next to each other at the dorsal end of the metapodial connective, although the exact point of emergence is quite variable. The dorsal strand (a) may actually, at times, originate at the point where the pedal ganglion gives rise to the connective and at the posterior side. The ventral strand (b) may arise from the mid-anterior connective near the pedal ganglion. These 2 nerves are tightly

appressed to each other and against the connective, the metapodial ganglion, and nerves leaving the distal end of the metapodial ganglion.

The *metapodial ganglion* (Mg) is not spherical as the propodial ganglion, but more like an elongate swelling of the connective. It is about 0.19 mm long and 0.10 mm wide. Nerves leaving the distal tip vary as to number and thickness. Generally, the ganglion narrows to a central wide and thin band flanked on either side by a thinner nerve. Often the medial of the thinner nerves is replaced by 2 nerve strands. The metapodial ganglion (Mg) and distal nerves enter a well-defined, roomy channel in the ventrolateral wall of the pedal haemocoel below the level of the more medially placed propodial ganglion (Pl. 13, Fig. 1).

7) Dorsolateral Pedal Nerve. Viewing the pedal ganglion laterally (Pl. 15, Fig. 1) a nerve ( $p_7$ ) is seen to arise just ventral to the point where the pleuro-pedal connective joins the pedal ganglion. This point of origin is just anterior to the mid-point of the statocyst (Stc) where the latter is pressed against the dorsoposterior wall of the pedal ganglion. The nerve soon bifurcates and each branch runs laterally to the haemocoel wall. The nerve is variable and 2 nerves might arise from the ganglion instead of 1. In one case  $p_7$  arose from the pleuro-pedal connective just dorsal to the pedal ganglion as a thick, single nerve. It ran posteroventrally for 0.07 mm and then branched into 3 distinct nerves each of which traveled laterally to the haemocoel wall.

The *statocyst* (Stc, Pl. 15, Fig. 1) is 0.12 mm in diameter and contains a single statolith (Sta) 0.07 mm in diameter. Upon full contraction of the rostrum these spheres slide up within the openings (b) shown in Plate 18, lateral to the base of the mid-columnar supportive ( $m_{23}$ ).

#### Buccal Complex

From the dorsal aspect, the *buccal ganglia* (Bg) are just visible in each

angle of the anterodorsal cerebral ganglion and the outer edge of the salivary glands (Pl. 14). It is evident from the lateral view (Pl. 15, Fig. 1) that these ganglia lie in the depression where the esophagus presses against the posterior buccal mass just after its origin. The ganglia are slightly elongate, the long axis generally projecting slightly anterodorsally when the buccal mass is horizontal. Each ganglion is about 0.19 mm long, with a width of 0.14 mm. The 2 buccal ganglia are connected by the buccal commissure which is about 0.24 mm long and 0.048 mm wide. The commissure passes between the esophagus where it leaves the buccal mass and the posterior buccal mass. The commissure is shown ventrally in Plate 18 (Bc). The cerebro-buccal connective (Cb) has been discussed (p 75). A number of nerves, labeled  $b_{1-6}$  arise from these ganglia and from the cerebro-buccal connective.

1) Dorsal Buccal Nerve. This nerve ( $b_1$ ) arises from the mid-dorsal surface of the buccal ganglion as a stout strand which runs dorsally and bifurcates beneath the emerging salivary glands. Each branch runs to the dorsal crest of the buccal mass over the area of the esophageal valve (Pl. 15, Fig. 1; Pl. 13, Fig. 5).

2) Esophageal Nerve. The esophageal nerve ( $b_2$ ) arises from the dorsal buccal nerve before the latter passes beneath the salivary gland. It runs posteriorly along the mid-esophageal surface (Pl. 13, Fig. 5; Pl. 15, Fig. 1).

3) Central Buccal Nerve. This nerve ( $b_3$ , Pl. 13, Fig. 5) is variable in position. It may arise from the anterodorsal surface of the ganglion, from the posterior base of  $b_4$ , or as a branch of  $b_4$ . The nerve runs dorsally to enter the buccal mass just posterior to the root of the salivary gland (Pl. 15, Fig. 1).

4) Anterior Buccal Nerve. This nerve ( $b_4$ , Pl. 13, Fig. 5; Pl. 15, Fig. 1) arises from the dorsal surface of the emerging cerebro-buccal connective. It runs dorsally to disappear beneath the

root of the salivary gland.

5) Odontophore Nerve. About 0.19 mm anterior to the buccal ganglion a nerve ( $b_5$ ) emerges from the ventral surface of the cerebro-buccal connective. This nerve curves over the external lateral odontophore musculature beneath the external odontophore membrane (Pl. 13, Fig. 5).

6) Posterior Buccal Nerve. At the point where each buccal ganglion (Bg) gives rise to the buccal commissure (Bc) a nerve ( $b_6$ ) emerges from the commissure to run ventrally over the external odontophore membrane (Pl. 13, Fig. 5; Pl. 18).

#### Pleural Complex

The pleural ganglia (Rp, Lp, Pl. 20, Figs. 1, 2) arise from the pleuro-pedal connective (Pp) immediately at the juncture of the cerebral ganglia and the connective (Pl. 15, Fig. 1) and are, therefore, partially pressed beneath the posteroventral curvature of the cerebral ganglia and intimately associated with them (Pl. 20). As a result of the characteristic prosobranch streptoneurous condition the posterior tip of the *right pleural ganglion* (Rp) is drawn up over the edge of the esophagus (Pl. 15, Fig. 1) where it gives rise to the pleuro-supraesophageal connective (Psc, Pl. 20, Fig. 1). The connective crosses the esophagus to the left side of the body. The shape of the ganglion, corresponding to this stretching, is not round like its counterpart, but drawn out, with a length of 0.24 mm and width of 0.12 mm. The pleuro-supraesophageal connective (Psc) often lies in a crease in the esophagus, crossing at a distance of 0.24 mm or less behind the cerebral commissure (Pl. 14). The connective has a length of  $0.34 \pm 0.048$  mm. It enlarges at the left lateral extremity into the supraesophageal ganglion (Sug, Pl. 14; Pl. 20, Figs. 1, 4).

The *left pleural ganglion* (Lp), resting on the mid-columellar supportive muscle is round with a diameter of 0.17 mm. Posteriorly it gives rise to a pronounced nerve, mantle nerve 1 (Mn<sub>1</sub>,

Pl. 20, Figs. 1, 3). This nerve runs laterally to the left ventrolateral cephalic wall and enters it at a point just anterior to the point where mantle nerve 2 ( $Mn_2$ ) from the supraesophageal ganglion (Sug) enters the wall. The left pleural ganglion is tightly appressed to and connected with the subesophageal ganglion (Sg). The latter is characteristically not separated from the former by more than 0.04 mm. In only one instance was a short, pronounced pleuro-subesophageal connective noted (Psb, Pl. 20, Fig. 3). In that same instance an unusual second nerve arose from the left pleural ganglion just anterolateral to mantle nerve 1 and ran to the left ventrolateral cephalic wall. No corresponding mantle nerves arise from the right pleural ganglion.

Parietal Complex

The parietal ganglionic complex is composed of 3 ganglia, the supra- and sub-esophageal ganglia and the osphradial ganglion. The latter has already been discussed in the section on the mantle cavity (p 27).

The *supraesophageal ganglion* (Sug, Pl. 14; Pl. 20, Figs. 1, 4) is about 0.24 mm long and 0.12 mm wide. The tip of the ganglion is very close to the ventrolateral cephalic wall (Lcw, Pl. 20, Fig. 4 and also Pl. 14), generally only 0.12 mm or less. From the area of the ganglion tip a few nerves arise in a variable fashion. The *supravisceral connective* (Suv) leaves the posterolateral surface of the ganglion. It consistently leaves the ganglion at this point, travels posteriorly along the left edge of the columellar muscle against the base of the "neck" wall, emerges from the left side of the "neck," and runs posteriorly to join the visceral ganglion (Vg, Pl. 11), thereby completing one side of the "visceral loop."

The *osphradiomantle nerve* arises from the tip of the supraesophageal ganglion (Sug) and bifurcates into the osphradial nerve (On) and mantle nerve ( $Mn_2$ ). The point of bifurcation, however, is quite variable. In Plate 20, Fig. 1, the mantle nerve 2 and osphradial nerve

are shown to arise separately, the former emerging from the ganglion as a very thin fiber. This condition is rare. Commonly the 2 nerves emerge, equally thick, as separate but closely associated trunks. Equally common, a single large trunk emerges from the ganglion and bifurcates immediately before entering the cephalic wall. Occasionally bifurcation occurs within the wall.

The *osphradial nerve* (On, Pl. 14; Pl. 20, Figs. 1, 4) runs laterally to the osphradium to enter the mid-ventral surface of the osphradial ganglion (Og, Pl. 11). The *mantle nerve* ( $Mn_2$ ) runs anteriorly towards the mantle edge after it enters the lateral cephalic wall. This nerve sends a branch to connect in a dialyneury with mantle nerve 1 from the left pleural ganglion. An extreme variant as regards the manner in which nerves arise from the supraesophageal ganglion is shown in Pl. 20, Fig. 4. A branch (x) arose from mantle nerve 2 at the point where the latter emerged from the supraesophageal ganglion. In this same rare variant specimen, a nerve also arose from the base of the supra-visceral connective (Suv) and ran laterally to the cephalic wall.

The *subesophageal ganglion* (Sg, Pl. 20 Figs. 1, 3) is of the same dimension as the left pleural ganglion (Lp). It lies beneath the esophagus closely appressed against the left pleural ganglion as previously mentioned. Three nerves characteristically emerge from this ganglion.

1) The *subvisceral connective* (Sbv) arises from the posterolateral curvature. It forms a loop when the rostral area is contracted and then runs posteriorly along the mid-line of the columellar muscle (Cl, Pl. 7, Fig. 2) or slightly to the right of the mid-line. It enters that muscle in the posterior "neck" region and appears emerging from the "neck" on the right side (Pl. 11, Fig. 1). The connective runs to the visceral ganglion (Vg) thereby completing the other arm of the "visceral loop."

2) *Mantle nerve 3* ( $Mn_3$ ) arises from the anterolateral curvature of the subesophageal ganglion as a strong nerve running laterally across the columellar muscle just as the columellar muscle turns ventrally to form the posterior wall of the pedal haemocoel. This nerve tends to slope downward at the right edge of the columellar muscle and then turns to enter the right wall.

3) The *mid-columellar nerve* ( $Mcn$ ) arises from the left, ventral curvature of the subesophageal ganglion as a slender fiber which slips around the left edge of the mid-columellar supportive ( $m_{23}$ ), turns ventrally and enters the columellar muscle.

#### Visceral Complex

The *visceral ganglion* ( $Vg$ , Pl. 5; Pl. 6, Fig. 1; Pl. 7, Figs. 1, 2; Pl. 11, Fig. 1; Pl. 20, Fig. 6) is a single structure about 0.24 mm long and 0.096 mm wide. It is exposed by pulling back the columellar muscle of the uncoiled snail, as shown in Plates 5, 6 and 7. Also exposed is the subvisceral connective ( $Sbv$ ) where it emerges dorsal to that muscle and runs into the ganglion. Two nerves are seen arising from the subvisceral connective (Pl. 20, Fig. 6) at points usually covered by the columellar muscle. The anterior nerve, *external mantle cavity nerve 1* ( $E_1$ ), is stouter. It runs directly over the mantle cavity, often crossing the spermathecal duct, towards its right edge, to the pallial oviduct in the females or to the intestine in the males. The posterior nerve, *external mantle cavity nerve 2* ( $E_2$ ), is variable in size and at times is absent. It takes off about 0.32 mm posterior to  $E_1$ , runs to the mid-ventral external wall of the mantle cavity, and turns posteriorly, becoming slender and finely branched. Between these 2 nerves the subvisceral connective is characteristically kinked or undulating.

The *supravisceral connective* ( $Suv$ ) arises from the dorsal surface of the visceral ganglion near its anterior end (Pl. 20, Fig. 6). About 0.07 mm from

the ganglion, the *pericardial nerve* ( $Pn$ ) leaves the supravisceral connective, runs posteriorly over the pericardium, forks and sends 2 branches over the pericardial surface. In Plate 20, Fig. 6, the visceral ganglion is shown as observed in Plates 5-7. From the posterior tip of the ganglion, arise 2 major nerves and one minor nerve. Most readily observed is the ventrally located *gonadal nerve* ( $Gn$ ) which travels along the vas deferens or oviduct towards the gonadal area (Pl. 7, Fig. 1).

Arising alongside of the gonadal nerve or as an early branch of the gonadal nerve is a minor nerve, the *external mantle cavity nerve 3* ( $E_3$ ). It runs over the area covering the end of the mantle cavity towards the right.

The *renal nerve* ( $Rn$ ) can only be observed from the ventral surface by cutting the gonadal nerve and lifting the posterior tip of the visceral ganglion up. From the dorsal surface of the ganglion near the posterior tip this stout nerve arises and runs into the body tube between the posterior mantle cavity epithelium and the kidney epithelium pressed against the posterior wall of the mantle cavity. The nerve passes to the dorsal surface of the body tube and bifurcates, sending roots to the kidney.

Occasionally very fine fibers were observed emerging from the right lateral surface of the ganglion which ran onto the external mantle cavity epithelium.

#### D. *Oncomelania hupensis formosana*

##### 1. Shell

Pilsbry & Hirase (1905) described this species as *Blanfordia formosana*. The shell was described as: "perforate, light brown, rather solid, turritid-conic, the outline of the spire straight, apex rather acute. Whorls 6 3/4, quite convex and parted by well-impressed sutures, smooth except for faint growth lines. The last whorl has a rounded and rather strong crest or varix behind the outer lip. The aperture is ovate,

TABLE 9. Conchological measurements of *Oncomelania hupensis formosana*

Structures measured	Number of snails	Length in mm			Number of snails	Width in mm		
		$\bar{X}$	S	Se		$\bar{X}$	S	Se
Shell 7.0 whorls	6	5.76	0.58	0.23	6	2.82	0.34	0.14
Shell 7.5 whorls	25	6.30	0.25	0.05	25	3.00	0.22	0.04
Aperture	25	2.40	0.19	0.04				
Callus	25	1.08	0.14	0.03				
Apical whorl					25	0.34	0.03	0.005
Tip of apical whorl					25	0.12	-	-

$\bar{X}$  = Mean

S = Standard deviation

Se = Standar error of the mean.

brown within; peristome brown-edged, the columella concave and somewhat thickened, whitish. Length 7, diam. 3.25 mm, length of aperture with peristome 2.8 mm."

Others who described the shell of this species were primarily Bartsch (1936), Annandale (1924) and Abbott (1948b). Annandale included *Blanfordia formosana*, *Katayama nosophora* and *Oncomelania hupensis* within the single genus *Oncomelania*. Abbott also included the genus *Schistosomophora* (*S. quadrasi*) as discussed by Bartsch (1936). The descriptions presented by these authors need to be expanded and in some cases modified.

Adult shells, i.e., those with a varix, have 7.0-7.5 whorls (Pl. 2B). The nuclear whorls are 2.5, white, glossy, set off from the yellow-horn of the remaining whorls. The first nuclear whorl is emergent.

The sutures are moderately impressed and the whorls slightly convex. Pilsbry & Hirase (1905) correctly stated that the outline of the spire was straight; the comparatively flattened whorls aid in giving this impression. The aperture is ovate, elongate, narrowed apically. The inner lip is slightly reflected over the narrow umbilicus and is connected with

the outer lip by a long parietal callus (Pl. 2; Pl. 3, Figs. 7, 8). The outer lip is thin and strong. Observing the outer lip with the shell rotated 90° to the left of the apertural view, one observes that it is sinuate as is the thickened varix behind the lip (Pl. 3, Fig. 4).

The base of the shell, as shown in Plate 3, Figs. 7, 8, is not rounded but appears truncate. The surface of the shell has fine growth lines in contrast to the roughened microsculpture of *Pomatiopsis lapidaria*. Only occasionally is a line here and there more pronounced. Cleaning the shell with "Clorox" removes the periostracum and the "brown-edged" peristome.

Conchological measurements for 31 adults are presented in Table 9. Shells with 7.5 whorls had an average length of 6.3 mm and a width of 3.0 mm; those of 7.0 whorls measured 5.76 mm and 2.82 mm, respectively. The average length of the aperture for 7.5 mm shells was 2.4 mm and the average callus length was 1.08 mm. The width of the first nuclear whorl was 0.34 mm and the tip of the first nuclear whorls measured 0.12 mm in diameter. The width of the tip of the first nuclear whorls was almost constant.

Several features of the shell of

*Oncomelania hupensis formosana* separate this species from *Pomatiopsis lapidaria*:

(1) The apical whorls of *P. lapidaria* are considerably larger than those of *O. hupensis formosana* (Pl. 3, Figs. 6, 9).

(2) The straight lip of the former contrasts with the sinuate lip of the latter.

(3) The lack of varix formation in the former differs considerably from the pronounced varix in the latter.

(4) The wide umbilicus and short parietal callus in *P. lapidaria* contrasts with the narrow umbilicus and long parietal callus in *O. hupensis formosana*.

(5) The roughened microsculpture, deeply impressed sutures, and pronounced convex whorls of the former are in contrast to the comparatively smooth shell, moderately impressed sutures and moderately convex whorls of the latter.

(6) When both species are fully mature and have the same shell length, *O. h. formosana* has 1 more whorl than *P. lapidaria* (compare Tables 4 and 9 for the number of whorls at a shell length of 6.2-6.3 mm). Few *P. lapidaria* reach 7 whorls while many *O. h. formosana* have 7.5 whorls.

## 2. External Morphology and Topography

The folds and grooves of the head as well as the mode of progression are as described for *Pomatiopsis lapidaria*.

**Pigmentation.** The pattern of pigmentation is somewhat different. *Oncomelania hupensis formosana* shows sexual dimorphism in that the integument of the apical whorls of the male has an intense black pigment which is lacking in the females. This was initially evident, through the shell; the apical whorls of the male appeared black while those of the female appeared light brown and peppered with the usual white granular bodies.

In the males the pronounced band of pigment starts at the beginning of the digestive gland (Pl. 24, Fig. 2) with a width of 0.48 mm. The apical edge of the band is smooth and regular in contrast to the flammulate pattern found

in *Pomatiopsis lapidaria*. The lower edge of the band is irregular but not deeply lobed or flammulate.

Anteriorly the dorsal surface of the stomach is lightly dusted with pigment. No pigmented strip underlines the intestine crossing the body whorl in either sex. Viewing the animals of both sexes through the shell in apertural view one notes a very dark pigmented area to the left. This area corresponds to the dorsal surface of the mantle which is densely pigmented all the way back to the rear of the ctenidium.

The dorsal rostrum and head are gray-black, the intensity of the pigment varying between individuals. The pigment is evenly dusted over the epithelial surfaces. Below the suprapedal fold on the sides of the foot there is very light pigmentation in contrast to the darker pigment found in *P. lapidaria*. Lack of dark pigment in this area is one reason for the decreased prominence of the pedal crease.

The sole of the foot differs from *P. lapidaria*: the entire surface appears white due to the densely packed large, white, glandular units, which were found mainly at the posterior part of the foot in *P. lapidaria*. The lateral indentation is slightly less pronounced.

**Tentacles and Eyes.** The tentacles of *Oncomelania hupensis formosana* are more elongate than those of *Pomatiopsis lapidaria*. A series of adult specimens was placed under water with adult *P. lapidaria*, observed as they moved about, and the tentacular length beyond the eye measured. The tentacles of both species, although capable of great expansion and contraction, are carried in a characteristic fashion, usually just short of full extension. In *O. h. formosana* the length measured between 0.96 and 1.20 mm; in *P. lapidaria* the length varied between 0.60 and 0.90 mm. The width of the tentacle at the base was about 0.12 mm in both species.

The "glandular units" partly covering the dorsomedial surface of the eyes formed units about the same length and

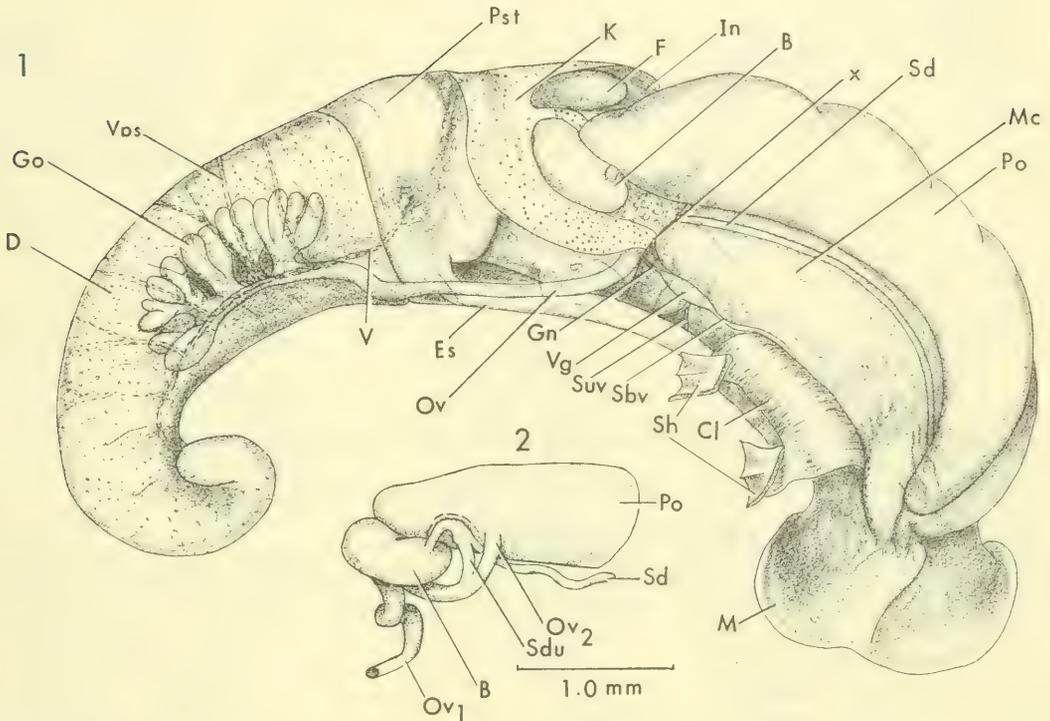


PLATE 21. Uncoiled female *Oncomelania hupensis formosana*

FIG. 1. The uncoiled female. Numerous organs are evident through the epithelium.

FIG. 2. A portion of the female reproductive system exposed by removing connective tissue and kidney tissue visible in Fig. 1 anterior to and to the left of the bursa copulatrix (B). A portion of the pallial oviduct is cut away (dashed line) to show the tubes of the spermathecal and sperm ducts which are overgrown by tissue of the pallial oviduct.

- |                 |   |         |   |
|-----------------|---|---------|---|
| B               | bursa copulatrix                        | oviduct |   |
| Cl              | columellar muscle                       | Po      | pallial oviduct   |
| D               | digestive gland                         | Pst     | posterior chamber of stomach                                |
| Es              | esophagus                               | Sbv     | subvisceral connective                                      |
| F               | fecal pellet                            | Sd      | spermathecal duct   |
| Go              | gonad                                   | Sdu     | sperm duct  |
| Gn              | gonadal nerve                           | Sh      | shell fragments   |
| In              | intestine                               | Suv     | supravisceral connective                                    |
| K               | kidney                                  | V       | visceral artery   |
| M               | edge of the mantle                      | Vas     | vascular element running laterally over the digestive gland |
| Mc              | ventral wall of mantle cavity           | Vg      | visceral ganglion   |
| Ov              | oviduct                                 | x       | end of the mantle cavity                                    |
| Ov <sub>1</sub> | coiled portion of oviduct               |         |   |
| Ov <sub>2</sub> | portion of oviduct passing into pallial |         |   |

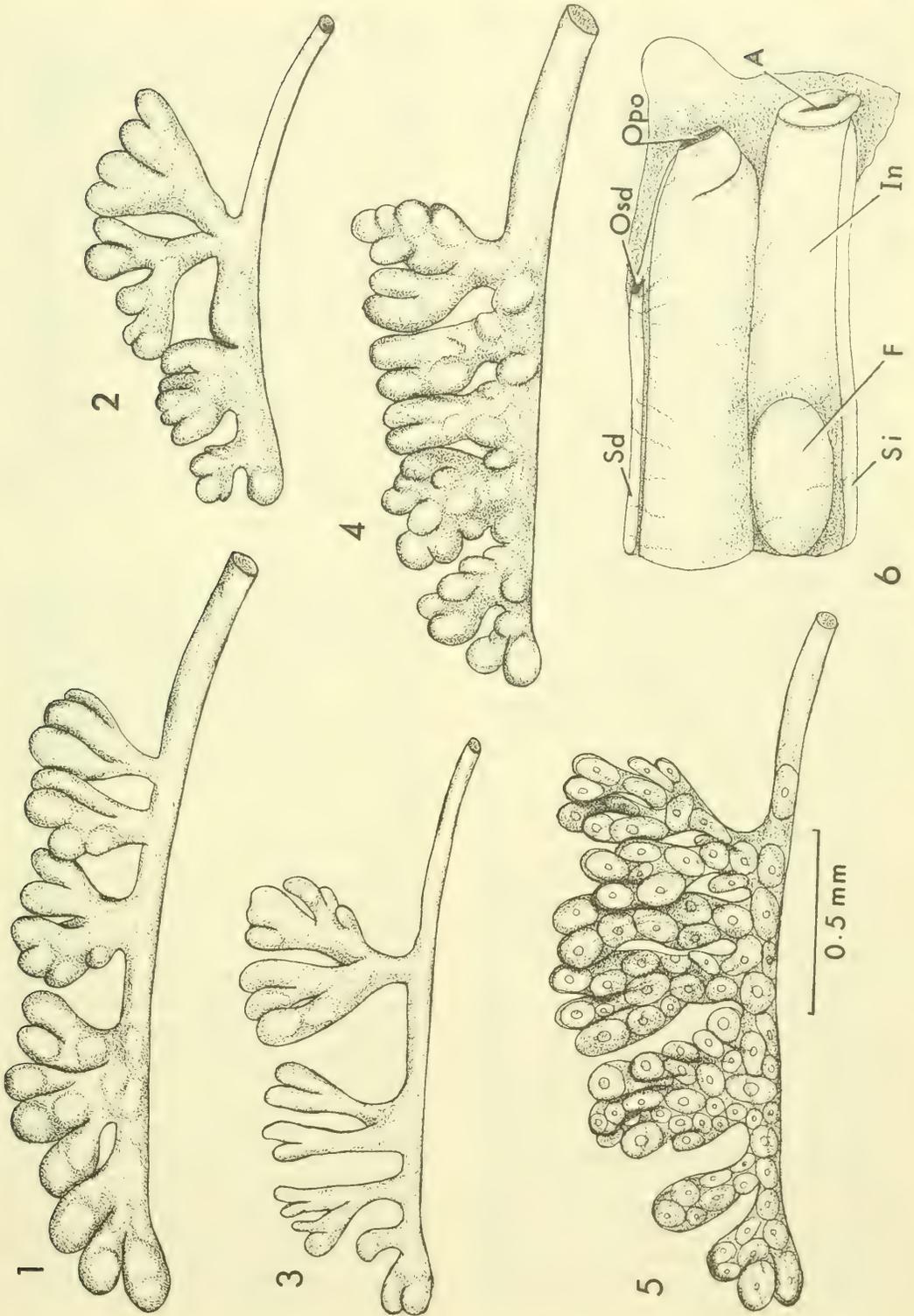
PLATE 22. Female reproductive system in *Oncomelania hupensis formosana*

FIGS. 1, 4, 5. Mature gonads. Membrane was removed from Fig. 5 to show oocytes.

FIGS. 2, 3. Underdeveloped gonads from adult sized snails.

FIG. 6. The relationship of the spermathecal duct, pallial oviduct and intestine in the area where the ducts open into the mantle cavity.

A     anus  
F     fecal pellet  
In    intestine  
Opo   opening of the pallial duct  
Osd   opening of the spermathecal duct  
Sd    spermathecal duct  
Si    subintestinal sinus



width in both species. The glandular patches vary in size. They may continue anteriorly by as much as 0.09 mm beyond the eye. Abbott (1948b) states these granules to be bright yellow in this species, as in *Oncomelania hupensis quadrasi*. In reality, the color varies from light, pale yellow to a white-yellow. Very few individuals have been found which have the bright yellow colored glandular units found in *O. h. quadrasi*. While the yellow tinge is not common in *Pomatiopsis lapidaria*, pure white granules are very uncommon in *O. h. formosana*.

General Topography. The position and arrangement of organs in both species are the same. The detailed descriptions of the relationships of organs in *Pomatiopsis lapidaria* likewise pertain to this species. Differences between the 2 snails are mainly ones of structural or dimensional modifications of homologous organs. The descriptions which follow deal mainly with those features which are different as compared with *P. lapidaria*. The organ systems of *Oncomelania hupensis formosana* are presented in Plates 21-31 in an analogous manner, so that a general comparison with *P. lapidaria* can be readily made.

One feature of the digestive gland of this species deserves attention: the way in which the vascular elements stand out beneath the external epithelium (vas, V, Pls. 21, 24). The blood vessels are so evident because they are outlined by pigment as well as by the white "granules" imbedded in the ventral surface of the digestive gland. The main artery supplying this portion of the vascular system runs under the epithelium above the left ventral edge of the digestive gland. It runs over the edge of the gonad and can be traced to an area on the stomach where the anterior and posterior chambers of the stomach join (Pls. 21, 24). At this point there is a slight depression covered by dense epithelium studded with numerous "granules." From here the artery can be traced anteriorly to the right where it parallels

the esophagus and gonoduct until the mid-region of the right anterior arm of the kidney beneath which it disappears. It passes over the style sac under the kidney tissue, beneath the intestine where the intestine loops over the tip of the style sac and connects with the aorta soon after the aorta sends a main branch anteriorly towards the head. This artery is present in *Pomatiopsis lapidaria* but never stood out prominently, as it did in this species.

The vessels from the artery running along the gonad pass from left to right, perhaps branching once before passing over the right edge of the digestive gland. These same vessels are not pronounced in *P. lapidaria* and are discerned with comparative difficulty, since the pronounced pigmented outlines are lacking.

### 3. Mantle Cavity

The gill filaments are numerous:  $46 \pm 4$ . Abbott (1948b) stated that 40-60 ctenidial filaments are characteristic for the genus *Oncomelania*. The osphradium is  $0.5 \pm 0.09$  mm long and its width is about  $0.14 \pm 0.024$  mm. The osphradium (Pl. 24, Fig. 1) of this species is definitely more narrow than that of *Pomatiopsis lapidaria*.

### 4. Female Reproductive System

The uncoiled female is shown in Plate 21, Fig. 1. The general arrangement of organs and tubes of the reproductive system is similar to that described for *Pomatiopsis lapidaria*. There are, however, distinct differences to be found in the structure of the gonad, the coiling of the oviduct posterior to the bursa copulatrix, and the arrangement of the ducts leaving the bursa.

Gonad. The ovary is multibranching (Pl. 21, Fig. 1). The average length of this organ is about  $1.39 \pm 0.19$  mm and the width is  $0.53 \pm 0.096$  mm at the widest portion. The lobed nature of the gonad of *Oncomelania hupensis quadrasi* was shown schematically by Abbott (1948b). Roth & Wagner (1957) published

a schematic drawing of the gonad from *O. h. nosophora*. Roth (1960) presented a schematic diagram for the female reproductive tract of *O. h. formosana* depicting a gonad composed of lobe-like structures.

Variation in the size and shape of the gonad of *O. h. formosana* is shown in Plate 22. Gonads in Figures 2 and 3 show rudimentary development although they were taken from otherwise adult specimens. The gonads of both of the species investigated showed no variation corresponding to the time of the year, i.e., no swelling or decrease in productivity throughout the year.

The gonad is rather delicate compared with that of *Pomatiopsis lapidaria* (Pl. 10). The branches, in the latter species, arise from a large swollen tube while those in the former arise from a slender collecting duct. The fully matured ovaries of *Oncomelania hupensis formosana* have 5 or 6 branched units, each supporting several terminal lobes. The posterior end of the gonad ends in one of the multibranched units.

The oocytes of *Pomatiopsis lapidaria* are distinctly larger than those of this species, the larger oocytes measuring about 0.17 mm in the former against about 0.11 mm in the latter.

Coiled section of the Oviduct. The length of the oviduct between the gonopericardial duct and the opening of the seminal receptacle into the oviduct measures about 1.7-2.1 mm. The tube is narrower than that of *Pomatiopsis lapidaria*; the diameter measures up to 0.12 mm. The convoluted oviduct in this region does not form the relatively compact cylinder found in *P. lapidaria*. It forms 1 or 2 irregular loops ( $Ov_1$ ) as shown in Plate 23, Fig. 3. In Figures 3 and 4 the oviduct is shown just beyond the gonopericardial duct. After making 2 irregular coils the oviduct circles under the bursa (B) and encircles the seminal receptacle (Sr) which is appressed against the bursa. The oviduct does not encircle the seminal receptacle in *P. lapidaria* (Pl. 8, Figs. 2, 3).

Roth & Wagner (1957) show the coil encircling the seminal receptacle in a similar manner in *Oncomelania hupensis nosophora*.

Seminal Receptacle. The seminal receptacle (Sr, Pl. 23, Figs. 3, 4) varies in shape and dimension as it does in *Pomatiopsis lapidaria*. The spherical or elliptical portion of the organ varies in length from 0.17-0.31 mm and the width from 0.12-0.24 mm. A dense central core is often observed within the central portion of the organ. The duct leading to the oviduct varies in length from 0.14-0.19 mm. It is slender, with a width of 0.048 mm. The duct enters the oviduct about 0.14 mm from the opening of the sperm duct. In one case (Pl. 23, Fig. 3) the duct entered the oviduct right at the base of the entrance of the sperm duct (Sdu).

Bursa Copulatrix. The bursa, viewed from the ventral surface (Pl. 21, Figs. 1, 2; Pl. 23, Fig. 2) measures  $0.84 \pm 0.096$  mm in length and  $0.38 \pm 0.048$  mm in width. The spermathecal duct (Sd) does not arise from the anterior tip of the bursa as it does in *Pomatiopsis lapidaria*. It arises anteriorly, together with the sperm duct (Sdu) 0.12 mm from the end of the bursa, or the right side (Pl. 21, Figs. 1, 2; z, Pl. 23, Fig. 2). Study of this area as a whole mount in CMC-10 revealed that the spermathecal duct (Sd) and the sperm duct (Sdu) emerge from the bursa as 2 distinct tubes bound together in a common sheath of connective tissue (z, Pl. 23, Fig. 5), which at magnifications of 60X appeared as 1 tube (z, Pl. 23, Fig. 2). The 2 tubes loop to the right and anteriorly. They emerge from the connective tissue sheath just posterior to the point where the oviduct ( $Ov_2$ ) passes ventral to the spermathecal duct (Sd; Pl. 21, Fig. 2; Pl. 23, Figs. 2, 3, 5). In Plate 23, Fig. 5, the tubes within the connective tissue sheath are shown as dashed lines. In one specimen the sheath was lacking altogether. Where the tubes loop anteriorly, they are overgrown by tissue of the pallial oviduct (Po, Pl. 21, Fig. 2). The sperm duct

PLATE 23. Female reproductive system of *Oncomelania hupensis formosana*

- FIG. 1. The terminal portions of the anus, pallial oviduct and the spermathecal duct.
- FIG. 2. Mid-body region cleared of kidney tissue showing the relationship of the tip of the style sac (Sts) to the pericardium (Pe). Note the point where the gonopericardial duct (Gp) enters the pericardium. Note how the commonly bound spermathecal and sperm ducts leaving the bursa (z) seem to enter the pallial oviduct.
- FIG. 3. The bursa has been removed to show the relationship of tubes and structures associated with the bursa. Note the coil about the seminal receptacle (Sr).
- FIG. 4. The bursa has been rotated to show how the oviduct coils about the seminal receptacle.
- FIG. 5. The point where the sperm duct and the spermathecal duct leave the bursa in the common connective tissue sheath is shown. The tubes are separate within the sheath (dashed lines).

A	anus	Ov <sub>2</sub>	portion of the oviduct entering the pallial oviduct
Ast	anterior chamber of the stomach	Pe	pericardium
B	bursa copulatrix	Pn	pericardial nerve
Cl	columellar muscle	Po	pallial oviduct
Ct	ctenidium	Pst	posterior chamber of the stomach
D	digestive gland	Rn	renal nerve
Es	esophagus	Sbv	subvisceral connective
Gn	gonadal nerve	Sd	spermathecal duct
Gp	gonopericardial duct	Sdu	sperm duct
In	intestine	Si	subintestinal sinus
In <sub>1</sub>	intestine starting to swing over the tip of the style sac	Sr	seminal receptacle
In <sub>2</sub>	intestine at the hair-pin turn, the point of the pellet compressor	Sts	style sac
M	edge of the mantle	Suv	supraviscceral connective
Mc	ventral wall of the mantle cavity	Vg	visceral ganglion
Osd	opening of the spermathecal duct	y	passage from the stomach to the digestive gland
Ov <sub>1</sub>	coiled section of the oviduct beyond the gonopericardial duct	z	sheathed tubes leaving the bursa copulatrix

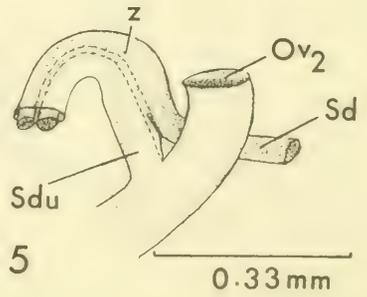
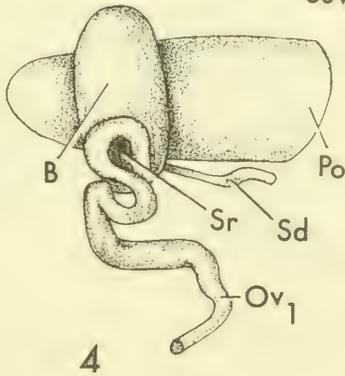
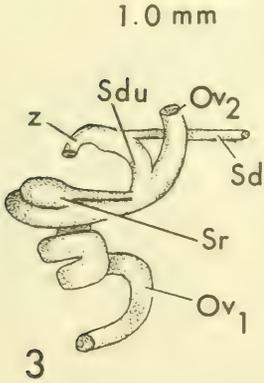
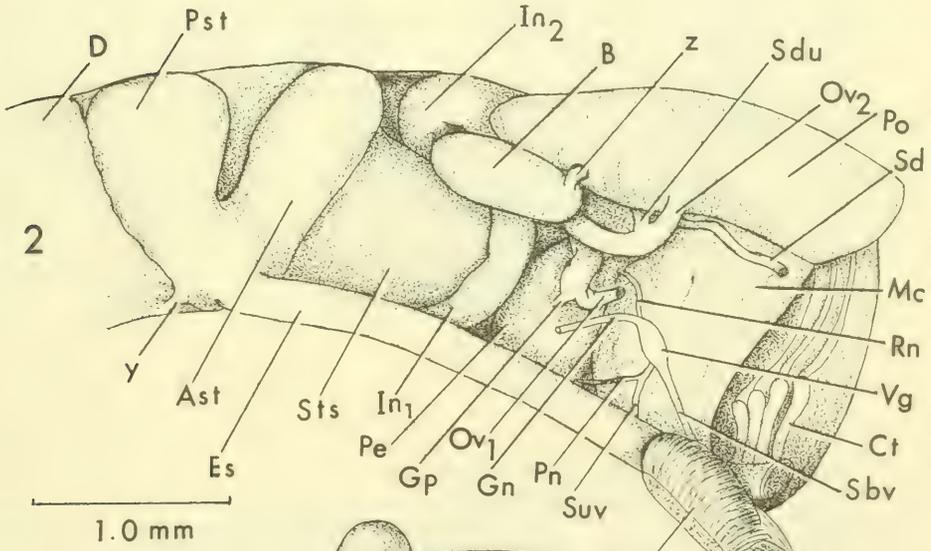
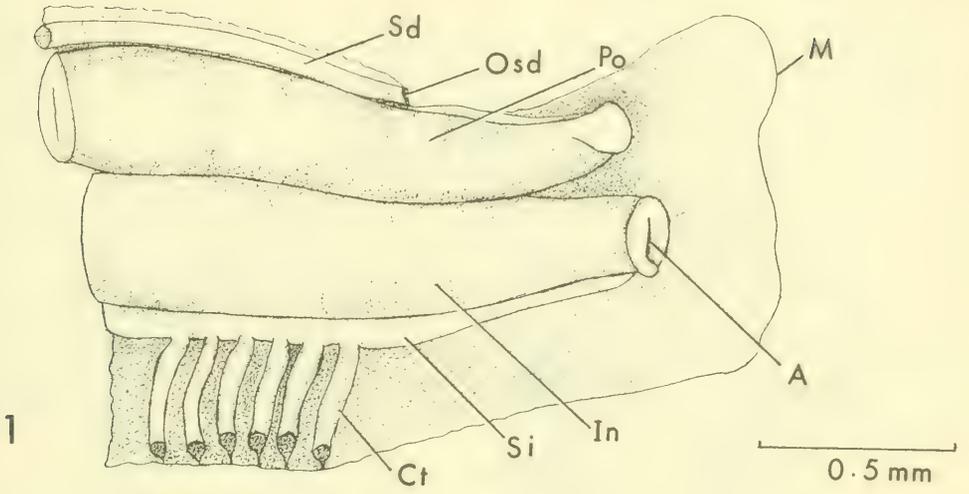


PLATE 24. Male reproductive system of *Oncomelania hupensis formosana*

FIG. 1. Head, verge and mantle cavity.

FIG. 2. Stomach and digestive gland as observed without removal of external epithelia. Note the "seminal vesicle" (Sv) irregularly coiled and protruding from behind the gonad (Go).

A	anus
Ast	anterior chamber of stomach
Ct	ctenidium
D	digestive gland
Es	esophagus
F	fecal pellet
G	glandular units
Go	gonad
M	edge of the mantle
N	the "neck"
Og	osphradial ganglion in osphradial pit
Pe	pericardium
Pi	pigment
Pr	prostate
Pst	posterior chamber of stomach
R	rostrum
Sbv	subvisceral connective
Suv	supravisceral connective
Sv	seminal vesicle
V	visceral artery
Vd <sub>1</sub>	posterior portion of the vas deferens
Vd <sub>2</sub>	anterior portion of vas deferens
Ver	verge
Vg	visceral ganglion

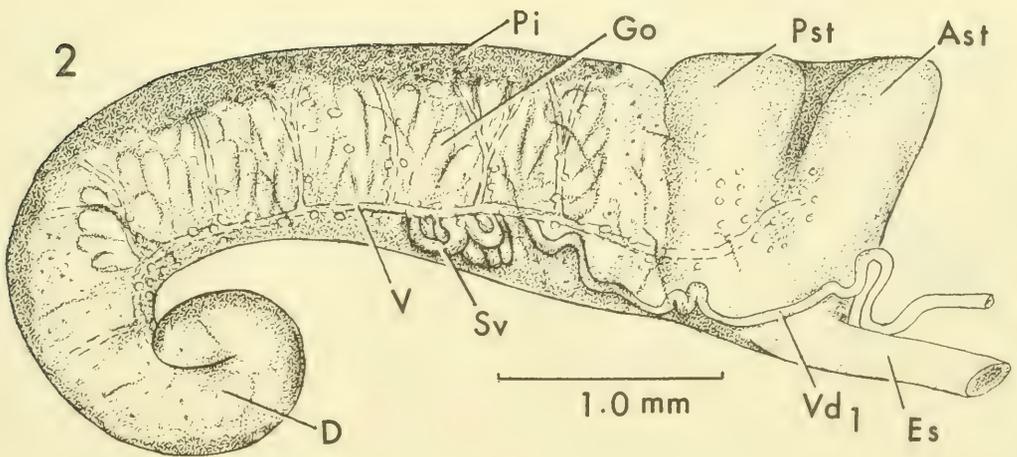
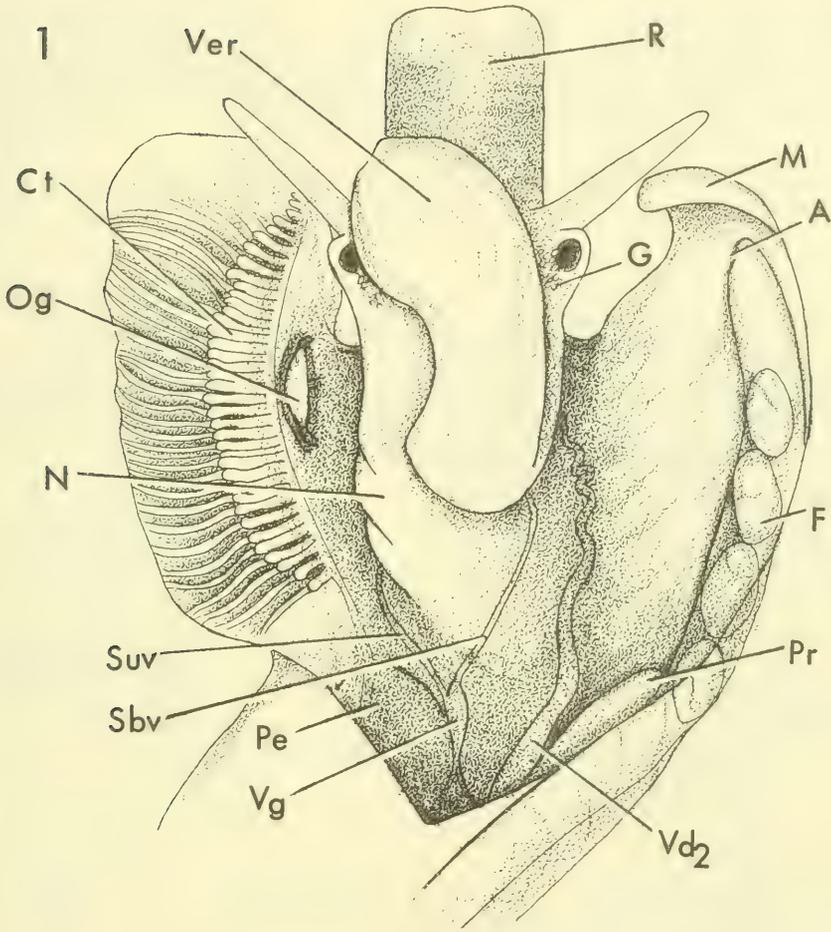


PLATE 25. Male reproductive system of *Oncomelania lupensis formosana*

- FIG. 1. The gonad with several of the multibranched units removed to reveal the structure of the seminal vesicle.
- FIG. 2. The gonad as viewed from the ventral surface.
- FIG. 3. The single glandular type found in the verge.
- FIG. 4. The prostate as seen from the ventral surface A and turned over, B, to show the points where the vas deferens enters and leaves the organ. The scale is the same as Fig. 2.
- FIG. 5. The structure of the testicular lobes.
- FIG. 6. Verge.
- FIG. 7. The tip of the verge magnified several times to show the strips of cilia on either side of the papilla.

Mvd thick layer of circular muscles encircling the vas deferens  
at the base of the verge.

O<sub>1</sub> initial portion of the vas deferens

Pa papilla at the end of the verge

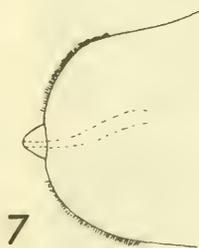
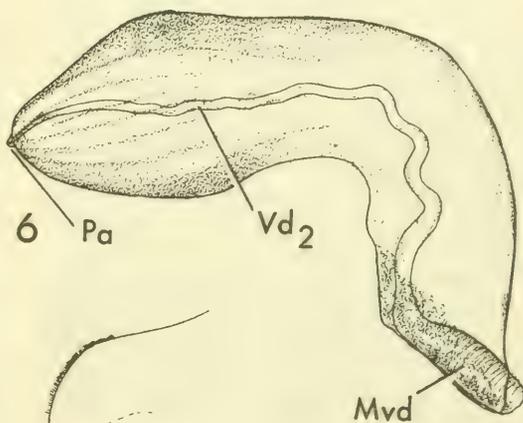
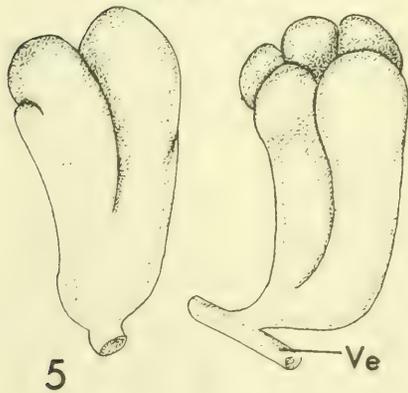
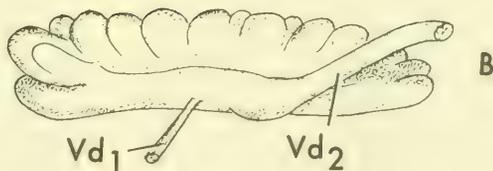
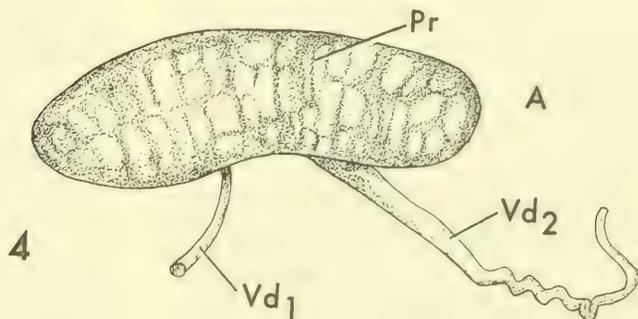
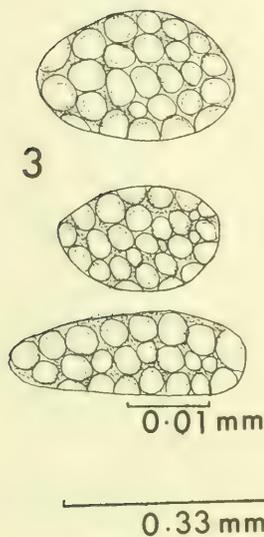
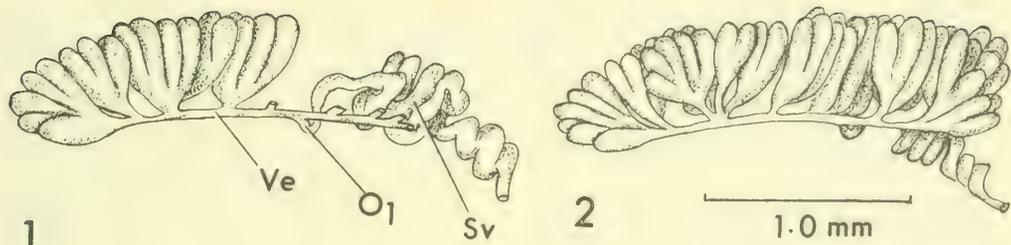
Pr prostate

Sv seminal vesicle

Vd<sub>1</sub> posterior portion of the vas deferens

Vd<sub>2</sub> anterior portion of the vas deferens

Ve vas efferens



(Sdu), as it enters the oviduct, measures about 0.12 mm in diameter. Upon entering the common sheath with the spermathecal duct it narrows noticeably to 0.048 mm. Both tubes, emerging from the bursa, have a collective diameter of about 0.036 mm.

Roth & Wagner (1957) describe the spermathecal duct as the "vagina" and the sperm duct as the "duct of the bursa." They figure the ducts arising from the mid-portion of the bursa in *Oncomelania hupensis nosophora*. A more thorough investigation of that snail has verified that the arrangement of tubes leaving the bursa is as described for *O. h. formosana*.

**Pallial Oviduct and Spermathecal Duct.** The spermathecal duct (Sd) is a slender tube in this species, with a diameter of about 0.048 mm. Its opening (Osd, Pl. 22, Fig. 6; Pl. 23, Fig. 1) is quite evident at 60X. The complications found in *Pomatiopsis lapidaria* with the thick, heavily pigmented connective tissue sheets are not encountered here. The opening of the duct is 0.48-0.57 mm posterior to the opening of the pallial oviduct (Opo). The opening of the spermathecal duct is  $25\mu$  wide and is surrounded by lips  $12.5\mu$  thick.

The pallial oviduct (Po) is about 3.36-4.00 mm long and up to 0.60 mm wide. This organ is distinctly smaller than that of *P. lapidaria*. The width of the pallial oviduct at the anterior end of this species is 0.14 mm as against 0.27 mm for *P. lapidaria*. For a distance of 0.12 mm posterior to the opening, the pallial oviduct is very slender and non-glandular.

In both species snails of adult size may possess an underdeveloped pallial oviduct, only 0.28 mm wide for the whole of its length, but this occurs more frequently in *Oncomelania hupensis formosana*.

There has been some confusion concerning the area near the bursa where the oviduct (Ov<sub>2</sub>) passes ventral to the spermathecal duct (Sd). Itagaki (1955) shows the ducts intercommunicating in

*O. h. nosophora*. Current investigations, as well as those of Roth & Wagner (1957), clearly show that these ducts do not intercommunicate. Itagaki (1955) does not mention the sperm duct. He states that the spermathecal duct enters the pallial oviduct near the latter's terminus, as did Dundee (1957) for *Pomatiopsis lapidaria*. As Roth & Wagner (1957) showed, this is not the case in *O. h. nosophora*; the spermathecal duct and the pallial oviduct have separate openings into the mantle cavity.

## 5. Male Reproductive System

**Gonad.** The testis appears as indistinct as that of *Pomatiopsis lapidaria* when viewed through the ventral epithelium of the uncoiled digestive gland (Pl. 24, Fig. 2). The distinctly outlined vascular pathways further tend to obscure the gonad. The structure of this organ is revealed by removing the ventral epithelium from the digestive gland (Pl. 25, Fig. 2). Comparison of the gonad of *P. lapidaria* (Pl. 12) with that of this species shows several differences. Although there are about the same number of multibranched units (7-9) arising from the vas efferens (Ve), the units of *Oncomelania hupensis formosana* lack the many finely branched tubes supporting testicular lobes at their tips. The testicular lobes in this species are thick and elongate, tending to rise from wider, more basal branches very close to the vas efferens (Pl. 25, Fig. 5).

The length of the gonad is  $1.92 \pm 0.24$  mm and the width is  $0.55 \pm 0.10$  mm. The vas deferens arises from the vas efferens at a position (O<sub>1</sub>) similar to that described for *Pomatiopsis lapidaria*. The initial tube is not tightly coiled but runs directly into the "seminal vesicle." The "seminal vesicle" (Sv) is very characteristic for this species. It never forms the neatly delineated coil described for *P. lapidaria* (Pl. 12, Figs. 3, 4) but forms a knot, a spherical mass of intertwined tubes bound together by connective tissue strands and laced with vascular elements (Pl. 25, Fig. 1). The

left edge of this confused mass of tubing projecting out from the dorsal surface of the gonad is generally visible in ventral view (Pls. 24, 25, Fig. 2). The tubes of the "seminal vesicle" are quite narrow, not more than about 0.096 mm wide, but often more slender. The "knot" is about 0.60 mm long and 0.40 mm wide. Uncoiled, the vas deferens, up to the prostate, is about 2.4 mm long.

**Prostate Gland.** The prostate measures 2.20-2.25 mm in length and 0.62-0.72 mm in width. The relationship of the anterior and posterior portions of the vas deferens to the prostate is the same as that found in *Pomatiopsis lapidaria* (compare Pr, Pl. 25, Fig. 4A, B, with Pl. 6, Figs. 2, 3).

**Verge.** Although the verge is characterized as "simple" in the literature, it has a number of distinctive features which are of value for comparative purposes. The length of the extended verge is  $3.36 \pm 0.12$  mm and the greatest width (near the tip) is about 0.80 mm. The verge is thicker and more muscular than that of *Pomatiopsis lapidaria*. The anterior end is so muscularly thickened that it has a pronounced convex curvature (Pl. 24, Fig. 1; Pl. 25, Fig. 6). The anterior portion of the verge has a distinct pinkish, salmon color which was also observed by Itagaki (1955) for *Oncomelania hupensis nosophora*. In this area longitudinal muscle strands are readily observed. The tip is blunt and flattened, or in the contracted state, a little concave (Ver, Pl. 24, Fig. 1).

When the verge is expanded a distinctly fleshy papilla is pushed beyond the tip (Pa, Pl. 25, Figs. 6, 7). At a magnification of 600X, it is evident that the tip of the verge is ciliated on either side of the papilla (Pl. 25, Fig. 7). The ciliary bands extend, invariably, only about  $50\mu$  on each side of the papilla; they project beyond the epithelium by  $3.8\mu$  and actively beat posteriorly.

The inner curvature of the verge does not have the expanded glandular area so pronounced in *Pomatiopsis lapidaria*. Only 1 glandular type was found scattered

throughout the verge (Pl. 25, Fig. 3). The verge differs from that of *P. lapidaria* in the following:

- (1) The tip is ciliated; (2) it possesses a distinct protrudable papilla; (3) there is 1 glandular type; (4) there is a pronounced musculature which is "pink" in color; (5) it lacks the glandular edge; and (6) the anterior end is not crimped and thereby set off from the rest of the verge (compare with *P. lapidaria*, Pl. 11, Fig. 5).

Roth & Wagner (1957) noted the papilla at the tip of the verge as well as the "several small white lines running parallel to the axis of the penis ... in the tapering portions" for *Oncomelania hupensis nosophora*. Those "white lines" represent fibers of longitudinal muscle.

## 6. Buccal Mass

The buccal mass and associated structures are similar in the 2 taxa under consideration. The most noticeable difference is one of size. *Oncomelania hupensis formosana* is uniformly smaller when one compares dimensions of the buccal mass, ganglia, muscles, etc. The length of the buccal mass is  $0.79 \pm 0.07$  mm and the width is  $0.53 \pm 0.05$  mm. The dorsal view of the buccal mass is shown in Plate 26; the opened pharyngeal tube in Plate 28, Fig. 2.

**Jaw.** The jaws are similar to those of *Pomatiopsis lapidaria*, except that they are slightly longer. The plates of the jaw are comparatively larger [compare Pl. 30, Fig. 7, with Text Fig. 1 (10)].

**Radula.** The structure of the radula is the same in both species. A series of 16 radulae was straightened out on slides and studied (Table 6). It can be seen that the radula of this species is shorter, less wide and has fewer rows of teeth than *P. lapidaria*. There was no significant difference in the number of rows of teeth in the formative stages.

The cusp formula most commonly encountered for each tooth in 50 snails investigated is presented in Table 10, along with the percentage of radulae on

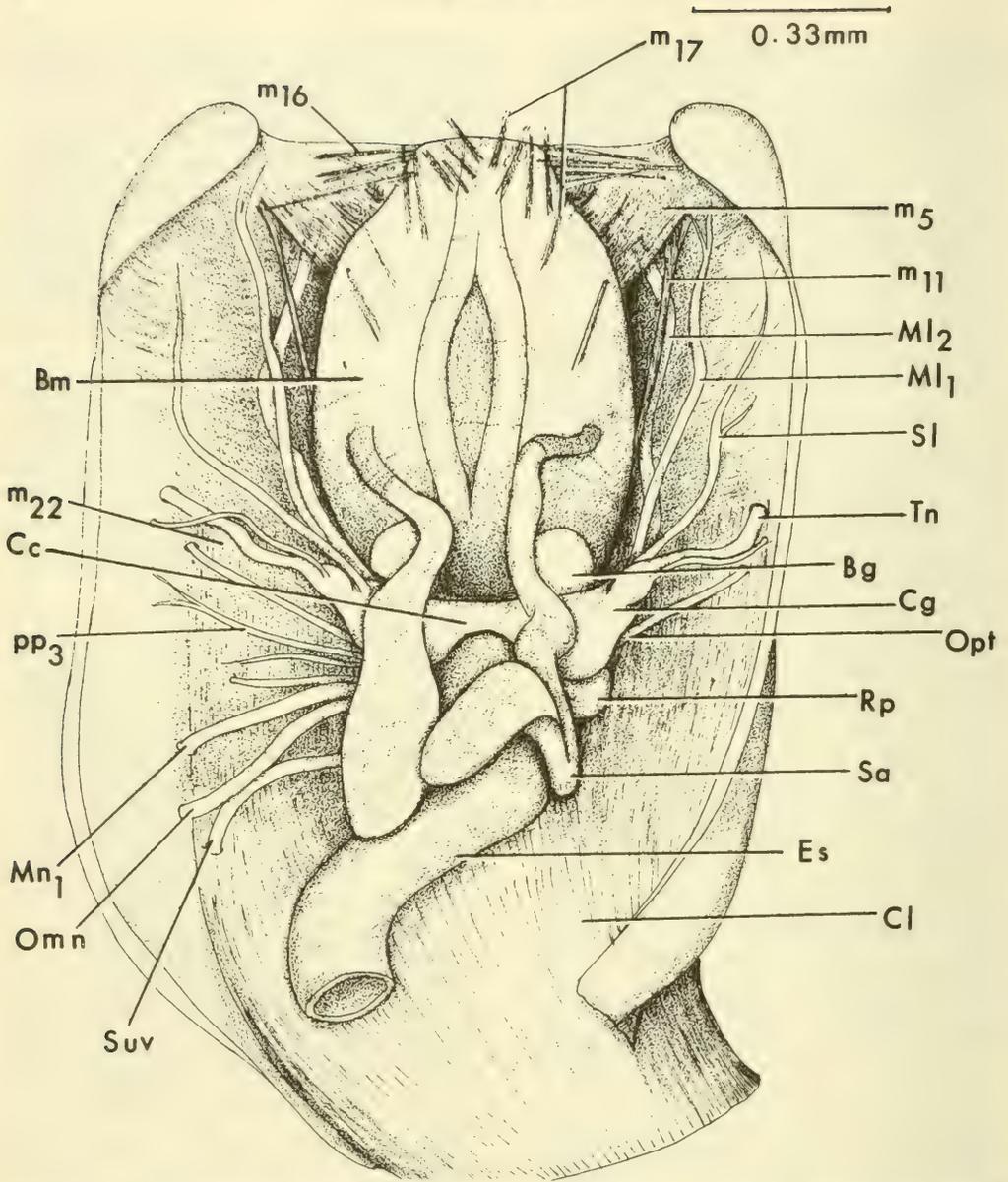


PLATE 26. Dorsal aspect of the buccal mass and associated structures in *Oncomelania hupensis formosana*

Bg buccal ganglion  
 Bm buccal mass  
 Cc cerebral commissure  
 Cg cerebral ganglion  
 Cl columellar muscle

Es esophagus  
 m<sub>5</sub> buccal protractor  
 m<sub>11</sub> dorsolateral buccal protractor  
 m<sub>16</sub> preventoral dilators  
 m<sub>17</sub> suspensors of the buccal mass  
 m<sub>22</sub> lateral cephalic retractor

Ml<sub>1</sub> median labial nerve 1  
 Ml<sub>2</sub> median labial nerve 2  
 Mn<sub>1</sub> mantle nerve 1  
 Omn osphradiomantle nerve  
 Opt optic nerve  
 pp<sub>3</sub> lateral nerve 3

Rp right pleural ganglion  
 Sa salivary gland  
 Sl supralabial nerve  
 Suv supravisceral connective  
 Tn tentacular nerve

TABLE 10. A general formula for the most common cusp arrangement found in *Oncomelania hupensis formosana* (from 50 radulae).

Tooth	Cusp Formula	Snails in which arrangement occurred in at least 90% of individual teeth %
Central (anter. & basal cusps)	$\frac{1-1-1}{2-2}$	62
Lateral	2-1-2(3)	74
Inner Marginal	8-9	100
Outer Marginal	6-5	91

which the arrangement was found for at least 90% of the teeth of each category. Only 53% of these 50 radulae had the representative formula shown in column 2 of Table 10. Table 11 shows every cusp arrangement found for each tooth type, with the percentage of radulae on which it occurred at least once.

Studies on the radulae of the subspecies of *Oncomelania* by Abbott (1948b), Mao & Li (1948) and Kuo & Mao (1957) reveal the fact that variation in radular formulas for a single subspecies of *Oncomelania hupensis* includes the cusp arrangements of the other subspecies as well as of *Pomatiopsis lapidaria*. However, those studies did not generally consider the frequency of occurrence of cusp arrangement. In the radulae (snails) investigated in this study, 70% of *O. h. formosana* had a central tooth formula of 1-1-1/2-2 (at least once; Table 11) and 62% of the snails had this formula represented in at least 90% of their central teeth. In *Pomatiopsis lapidaria* only 40% of the radulae had central teeth with this formula (Table 8) and the formula was not as common as that of 1-1-1/3-3 (Table 7). Considering the inner marginals, 5-7 cusps were common in *P. lapidaria* (84%, Table 7)

TABLE 11. The various types of cusp arrangement for the different teeth in 50 radulae of *Oncomelania hupensis formosana* and the percentage of radulae showing that arrangement at least once.

Central		Lateral	
Arrangmt. of cusps anterior basal	%	Arrangmt. of cusps	%
$\frac{1-1-1}{2-2}$	70	2-1-3	50
$\frac{1-1-1}{3-3}$	30	2-1-2	40
$\frac{2-1-2}{2-2}$	20	2-1-4	10
$\frac{2-1-2}{3-3}$	10	2-1-0	10
$\frac{1-1-2}{2-2}$	30	3-1-3	10
$\frac{1-1-2}{3-3}$	10	3-1-4	10
$\frac{3-1-2}{3-3}$	10	one side, 2-1-2; other side, 2-1-3	20
		one side, 2-1-3; other side, 2-1-4	10
Inner marginal		Outer marginal	
No. of cusps	%	No. of cusps	%
9	100	6	90
10	20	5	60
11	40	4	10
8	4		

while 8-9 were the rule in *O. h. formosana* (100%, Table 10). No radulae of *P. lapidaria* were observed where any inner marginal had 9 cusps (Table 8). The question arises, whether other populations of *Oncomelania* conform to the pattern found in the laboratory population studied here. For the most part that

question remains unanswered, except for data on 1 population of *O. h. hupensis* in China presented by Mao & Li (1948). The central had a formula 1-1-1/2-2 for 75% of the population. Centrals with 3 basal denticles occurred in only 26% of the snails. There were 8-9 cusps on the inner marginals in 87% of the populations, the remainder having 7.

In spite of the overlapping variations between the subspecies of *Oncomelania* and *Pomatiopsis lapidaria*, it appears that *P. lapidaria* is separable from *Oncomelania* on the basis of cusp formula in that there is a pronounced tendency in the former to have centrals with 3 basal cusps on each side as compared with 2, and 5-8 cusps on the inner marginal, as compared with 8-11.

Aside from the differences in length, width, and number of rows of teeth, there are 2 other small differences. The large mediobasal cusps of *Oncomelania hupensis formosana* are more widely separated than those of *Pomatiopsis lapidaria* (compare Pl. 19 with Pl. 29). Also, there is a pronounced tendency in the outer marginal to have an enlarged and swollen outer cusp (Pls. 29, 30), a condition rarely observed in *P. lapidaria*.

The manner in which the teeth in both species are attached to the membrane is shown in Plate 30, Fig. 6.

## 7. Musculature

The musculature is shown in Plate 13, Fig. 1, and Plates 26-28. The patterns and arrangements of muscles are the same in both species with few exceptions (compare with Pls. 14-18). There are grades of variation in muscular structures which reach extremes in *Oncomelania hupensis formosana*. The preventral protractors ( $m_6$ , Pl. 28, Fig. 1) tend to be more strongly developed than in *Pomatiopsis lapidaria*, although this is variable. The suspensors of the buccal mass ( $m_{17}$ , Pl. 26; Pl. 28, Fig. 1) tend to be more stout and fewer in number than those found in *P. lapidaria*, although there is a gradation to the exact condition found in the latter species. In

several cases stout muscle strands from the anterior jugalis ( $m_9$ , Pl. 28) were observed running anterodorsally to the anterior rostral roof, a condition never observed in *P. lapidaria*.

There are 3 distinct differences between the musculature of these 2 species:

(1) The rostral retractors ( $m_{20}$ , Pl. 27) terminate shortly after passing over the pedal haemocoel. They do not pass under the buccal protractors ( $m_5$ ) to insert about the oral aperture at the floor of the tip of the rostrum as they do in *Pomatiopsis lapidaria*.

(2) The buccal protractors commonly fuse in a single sheet which takes its origin from the rostral floor ( $m_5$ , Pl. 27), in contrast to the condition normally found in *P. lapidaria* ( $m_5$ , Pl. 18). There also are mediolateral slips, not found in *P. lapidaria*, which have their origin on each side of the oral aperture and run posteriorly, and then dorsally, to unite with the buccal protractor near the rostral floor before the muscle bifurcates; but as they are anterior to the main sheet of  $m_5$ , they cannot be seen in Plate 27. The 2  $m_5$  labels in Plate 28, Fig. 1, refer to the mediolateral slip and the posterolateral portion of the sheet, respectively.

(3) The mediolateral slips are crossed by a circular band of muscles ( $m$ , Pl. 28) which arise from the floor of the rostrum at either side of the oral aperture and sweep around in an arc over the mediolateral slips of the buccal protractors. This muscle band does not correspond to the oral sphincter muscle. Usually this arc of muscle is posterior to the oral sphincter.

## 8. Nervous System

Several differences are found between the species with regard to the nervous system.

(1) Upon opening the rostral cavity it is seen that the ganglia and nerves are not dusted with the heavy pigment, as was the case in *Pomatiopsis lapidaria*.

(2) The cerebral commissure (Cc, Pl. 31) is short in *Oncomelania hupensis*

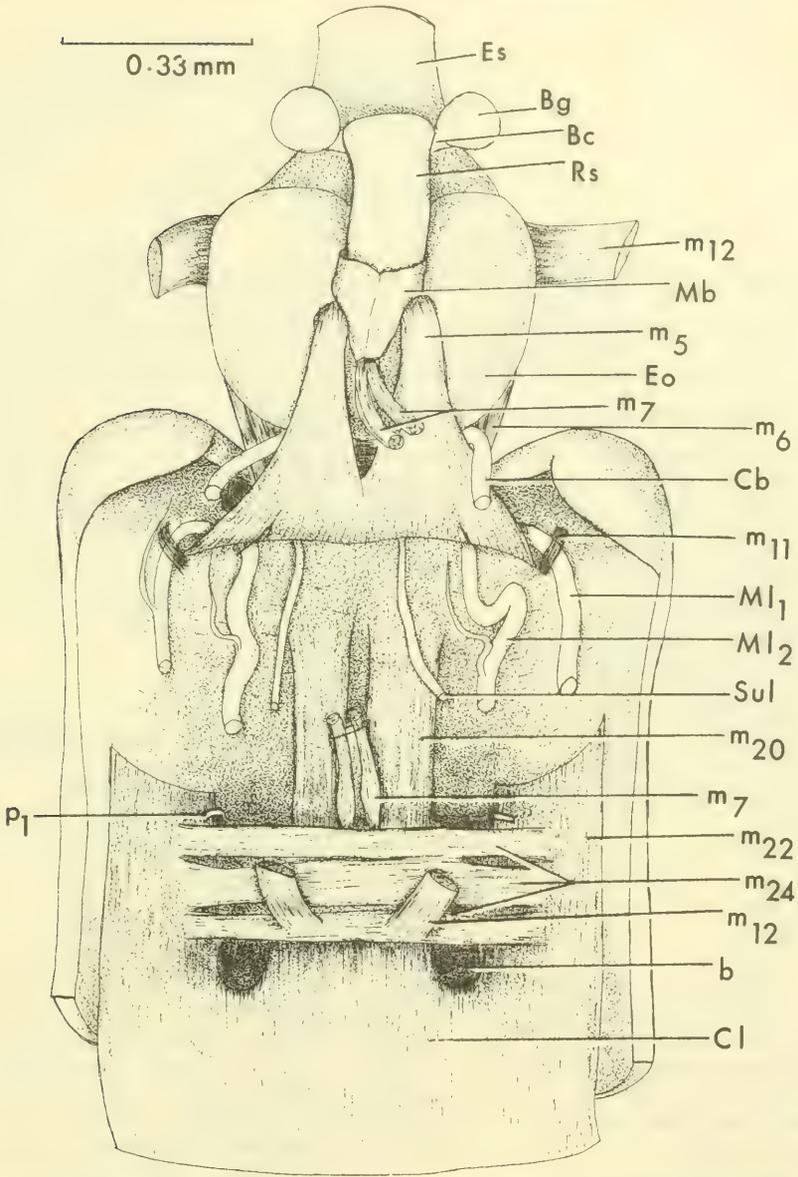


PLATE 27. Musculature of the cephalic region of *Oncomelania hupensis formosana*

- |                 |  |                 |   |
|-----------------|--|-----------------|---|
| b               | posterodorsal gap of the pedal haemocoel | m <sub>12</sub> | buccal retractor                                |
| Bc              | buccal commissure                        | m <sub>20</sub> | rostral retractor                               |
| Bg              | buccal ganglion                          | m <sub>22</sub> | lateral cephalic retractor                      |
| Cb              | cerebrobuccal connective                 | m <sub>24</sub> | tensor magnus                                   |
| Cl              | columellar muscle                        | Mb              | membrane around the radular sac                 |
| Eo              | external odontophore membrane            | Ml <sub>1</sub> | median labial nerve 1                           |
| Es              | esophagus                                | Ml <sub>2</sub> | median labial nerve 2                           |
| m <sub>5</sub>  | buccal protractor                        | p <sub>1</sub>  | lateral retractor nerve from the pedal ganglion |
| m <sub>6</sub>  | preventral protractor                    | Rs              | radular sac                                     |
| m <sub>7</sub>  | radular protractor                       | Sul             | sublabial nerve                                 |
| m <sub>11</sub> | dorsolateral buccal protractor           |                 |   |

PLATE 28. Musculature and nerves of the cephalic region of *Oncomelania hupensis formosana*

FIG. 1. The lateral view of the buccal mass with the central portion of the nervous system.

FIG. 2. Dorsal aspect of the opened pharyngeal tube, showing the dorsal odontophore.

FIG. 3. The right buccal cartilage and associated intrinsic muscles.

FIG. 4. The overlapping tips of the cartilages are pulled apart to expose their medial surfaces, which are wrapped in the intrinsic muscles of the odontophore.

b <sub>1</sub>	dorsal buccal nerve	m <sub>13</sub>	membranous jugalis
b <sub>4</sub>	anterior buccal nerve	m <sub>16</sub>	preventral dilators
b <sub>5</sub>	odontophoral nerve	m <sub>17</sub>	suspensors of the buccal mass
Bg	buccal ganglion	MI <sub>1</sub>	median labial nerve 1
C <sub>8</sub>	cerebro-tensor nerve	MI <sub>2</sub>	median labial nerve 2
Ca	cartilage	OI	outer lip
Cb	cerebro-buccal connective	Opt	optic nerve
Cg	cerebral ganglion	p <sub>1</sub>	lateral retractor nerve
Cos	collostyle tip	p <sub>2</sub>	pedal nerve to the anteroventral wall
Cp	cerebro-pedal connective	p <sub>3</sub>	major lateral nerve
Eo	external odontophore membrane	p <sub>7</sub>	dorsolateral pedal nerve
Es	esophagus	Pg	pedal ganglion
Ev	esophageal valve	Pp	pleuro-pedal connective
Fg	food groove	pp <sub>1</sub>	lateral nerve 1
Gro	central groove in the ventral fold	pp <sub>2</sub>	penial nerve
Il	inner lip	pp <sub>3</sub>	lateral nerve 3
J	jaw	pp <sub>4</sub>	lateral nerve 4
m	circular muscle running over the medial slips of the buccal protractors	Ra	radula
m <sub>1</sub>	lateral cartilage tensor	Ras	radular shield
m <sub>2</sub>	mediolateral cartilage tensor	Rp	right pleural ganglion
m <sub>3</sub>	odontophore divaricator	Rpc	rostral portion of the cephalic haemocoel
m <sub>4</sub>	medial radular retractor	Rw	rostral wall
m <sub>5</sub>	buccal protractor	Sa	salivary gland
m <sub>6</sub>	preventral protractor	Sur	subradular membrane
m <sub>7</sub>	radular protractor	Sta	statolith
m <sub>8</sub>	anterior jugalis	Stc	statocyst
m <sub>9</sub>	buccal constrictor	Sul	sublabial nerve
m <sub>11</sub>	dorsolateral buccal protractor	Tn	tentacular nerve
m <sub>12</sub>	buccal retractor	Vf	ventral fold

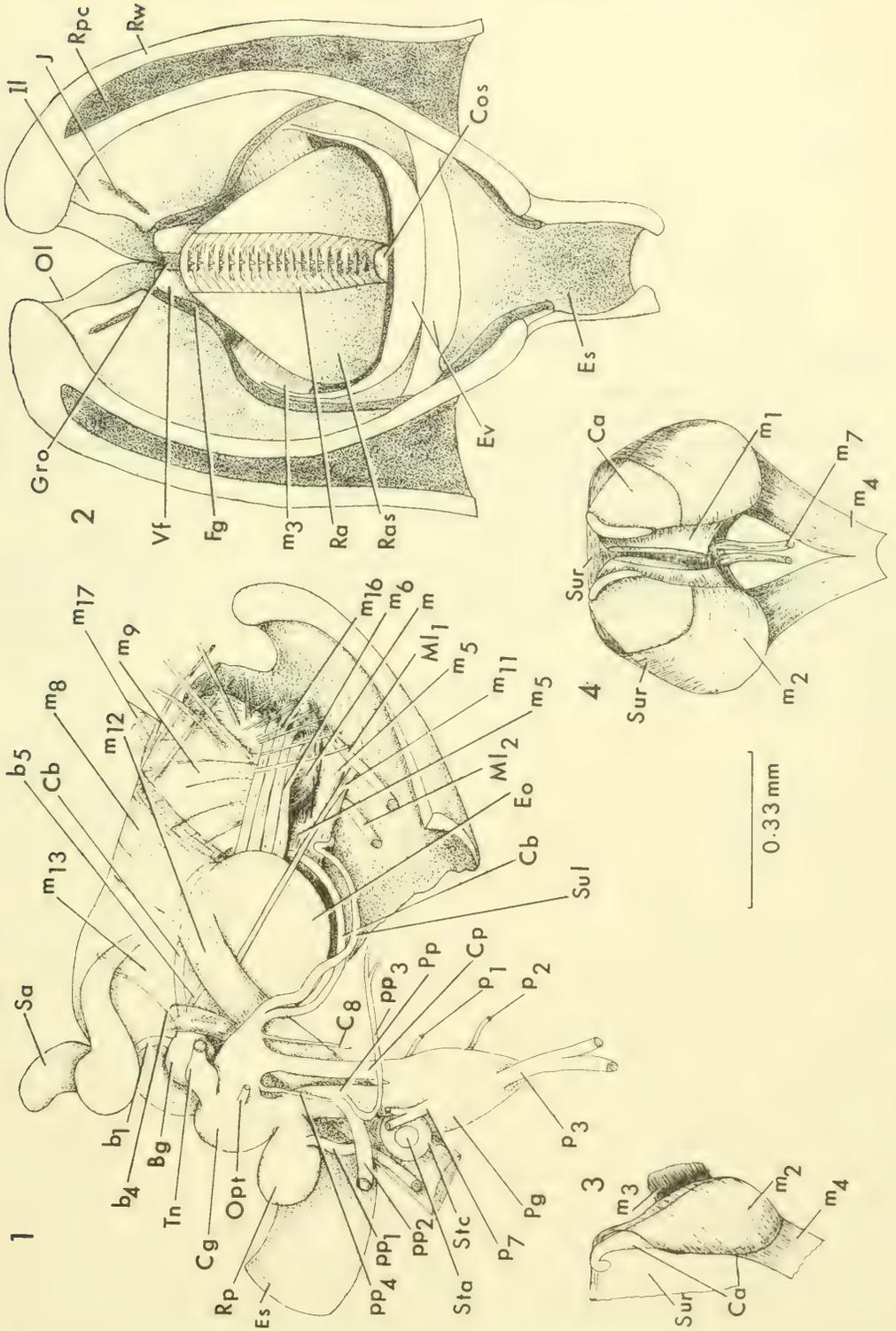


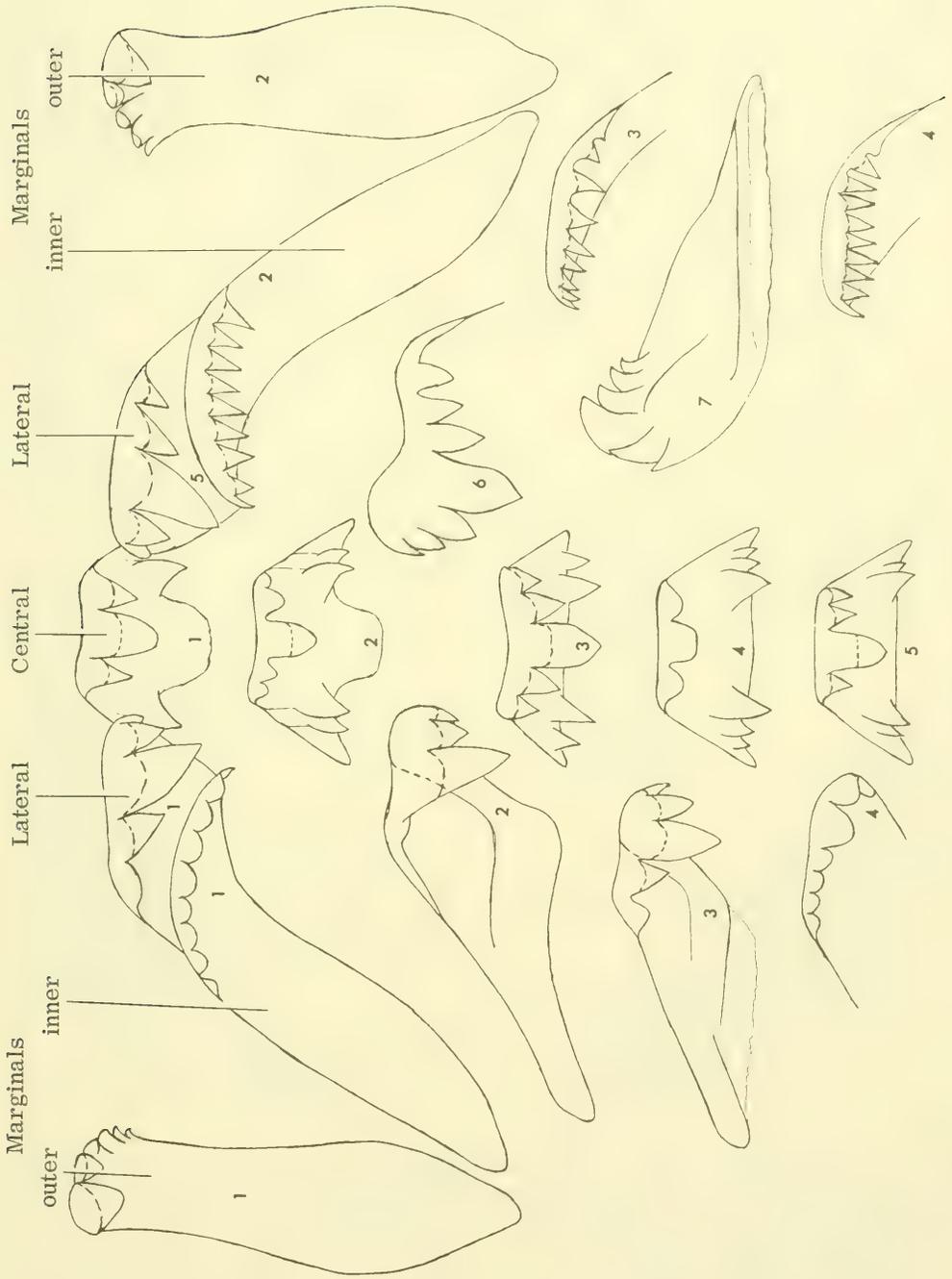
PLATE 29. Variation in the radular teeth of *Oncomelania hupensis formosana*

The horizontal top row of teeth displays 1 radular row in natural position,\* except for the outer marginals 1 (left) and 2 (right) which are erected to expose the exact number of cusps. Variation is shown for the other teeth. The description for *Pomatiopsis lapidaria* given for Plate 19 generally applies and should be consulted. The plane of focus on central teeth 1-5 is on the upper surfaces of the medial basal cusps. The supports for those cusps are not shown but are similar to those of *P. lapidaria*. The tongue-shaped attachment (basal process) described for central 6 of Plate 19 is shown diagrammatically for centrals 1-2. The anterior end of central 3 is raised thereby demonstrating the dagger-like cusps as seen from this orientation.

Lateral teeth 1-4 (left) and 5-7 (right) show variation in cusp number and shape. Lateral 6 shows only the cusps of the tooth; the anterior edge of the tooth is lifted upwards. Lateral 7 is shown with peduncle (Pd, Pl. 19) turned to the right 90° from the normal position to show the hook-like nature of the cusps. Inner marginals 1 (left) and 2-4 (right) are all shown in normal position.

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\*When the plate is turned sideways so that the labels are horizontal.



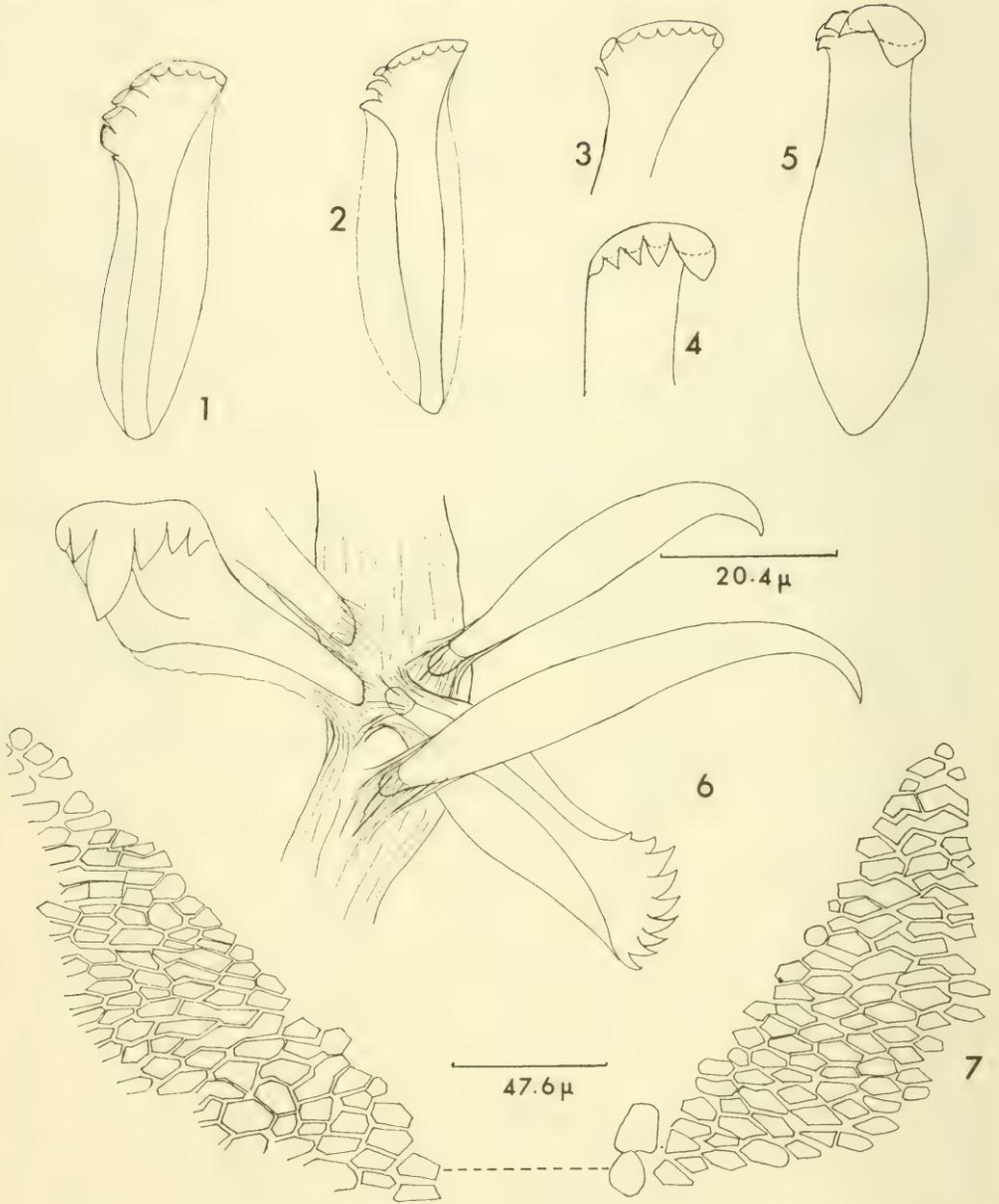


PLATE 30. Radula of *Oncomelania hupensis formosana*

FIGS. 1, 2, 3. Inner marginals.

FIGS. 4, 5. Outer marginals.

FIG. 6. The manner in which the teeth are attached to the radular membrane.

FIG. 7. Jaw.

TABLE 12. Anatomical differences between *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* considered to be of major importance

Character	<i>P. lapidaria</i>	<i>O. hupensis formosana</i>
Shell		
1. umbilicus	wide	narrow
2. varix	-	+
3. sutures	deep	moderately deep
4. apex	wide	narrow
5. lip	straight	sinuate
6. whorls	6.5-7.0	7.0-7.5
Gill filaments	30 or less	35 or more
Tentacles	short	long
Female Reproductive System		
1. gonad branches	few	several
2. gonad collecting duct	wide	slender
3. oviduct coils encircle the seminal receptacle	-	+
4. spermathecal duct leaves bursa copulatrix	at anterior end	laterally (in common sheath w. sperm duct)
5. sperm duct arises from	spermathecal duct	bursa copulatrix
Male Reproductive System		
1. verge with papilla	-	+
2. verge ciliated	-	+
3. verge with glandular edge pronounced	+	-
4. verge with pronounced musculature	-	+
5. seminal vesicle	thick; neatly coiled	slender, knotted tube
Nervous System		
1. cerebral commissure	long	short
2. pleuro-supraesophageal connective	long	short
3. Supravisceral connective arises on supraesophageal ganglion from	posterolateral surface	tip
4. osphradial and mantle nerves: distance from origin to lateral cephalic wall	short; bifurcating soon after origin to run separately	long and running jointly

+ = yes

- = no

*formosana*. It is  $0.07 \pm 0.024$  mm long and about 0.06 mm wide. In *P. lapidaria* it is at least 0.12 mm long and 0.07 mm wide. This difference in length is not necessarily due to the generally smaller structures in *O. h. formosana*.

(3) The pleuro-supraesophageal connective (Psc, Pl. 31, Fig. 1) is short, measuring  $0.168 \pm 0.048$  mm as against at least 0.288 mm in *Pomatiopsis lapi-*

*daria*. As a result of the shortened connective the supraesophageal ganglion (Sug) rests on the dorsolateral surface of the esophagus. The osphradio-mantle nerve (Omn) and the supravisceral connective (Suv) arise from the tip of the ganglion and travel about 0.39 mm to the left lateral body wall (Pl. 26). In *P. lapidaria*, due to the lengthened pleuro-supraesophageal connective, the tip of the

PLATE 31. Nervous system of *Oncomelania hupensis formosana*

FIG. 1. Dorsal aspect of the central nervous system or "brain."

FIG. 2. Anterior aspect of the pedal ganglia.

FIG. 3. Medial aspect of the right cerebral ganglion showing the position where each nerve arises.

FIG. 4. Ventral aspect of the visceral ganglion and associated nerves.

a	nerve from p <sub>6</sub>	p <sub>1</sub>	lateral retractor nerve, from the pedal ganglion
b	nerve from p <sub>6</sub>	p <sub>2</sub>	nerve to the anterioventral wall of the pedal haemocoel
C	pedal commissure	p <sub>3</sub>	major lateral nerve of the pedal ganglion
Cc	cerebral commissure	p <sub>4</sub>	propodial connective
Cb	cerebro-buccal connective	p <sub>6</sub>	metapodial connective
Cg	cerebral ganglion	p <sub>8</sub>	minor lateral nerve of the pedal ganglion
Cp	cerebro-pedal connective	Pg	pedal ganglion
C <sub>8</sub>	cerebro-tensor nerve	Pn	pericardial nerve
E <sub>1</sub>	external mantle cavity nerve 1	Pp	pleuro-pedal connective
E <sub>2</sub>	external mantle cavity nerve 2	Prg	propodial ganglion
E <sub>3</sub>	external mantle cavity nerve 3	Psc	pleuro-supraesophageal connective
E <sub>4</sub>	external mantle cavity nerve 4	Rn	renal nerve
Gn	gonadal nerve	Rp	right pleural ganglion
Lp	left pleural ganglion	Sl	supralabial nerve
Mc <sub>n</sub>	mid-columellar nerve	Sg	subesophageal ganglion
Mg	metapodial ganglion	Sbv	subvisceral connective
Ml <sub>1</sub>	median labial nerve 1	Sug	supraesophageal ganglion
Ml <sub>2</sub>	median labial nerve 2	Sul	sublabial nerve
Mn <sub>1</sub>	mantle nerve 1, from the left pleural ganglion	Suv	supravisceral connective
Mn <sub>3</sub>	mantle nerve 3, from the subesophageal ganglion	Tn	tentacular nerve
Omn	osphradio-mantle nerve	Vg	visceral ganglion
Opt	optic nerve		

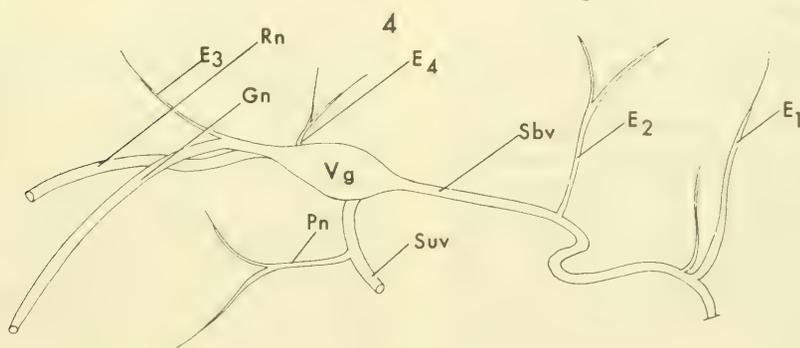
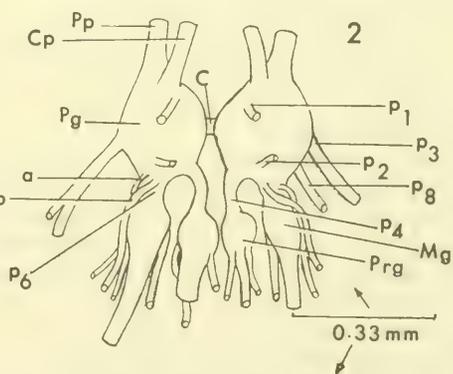
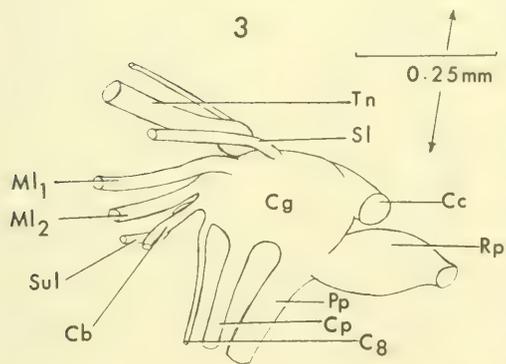
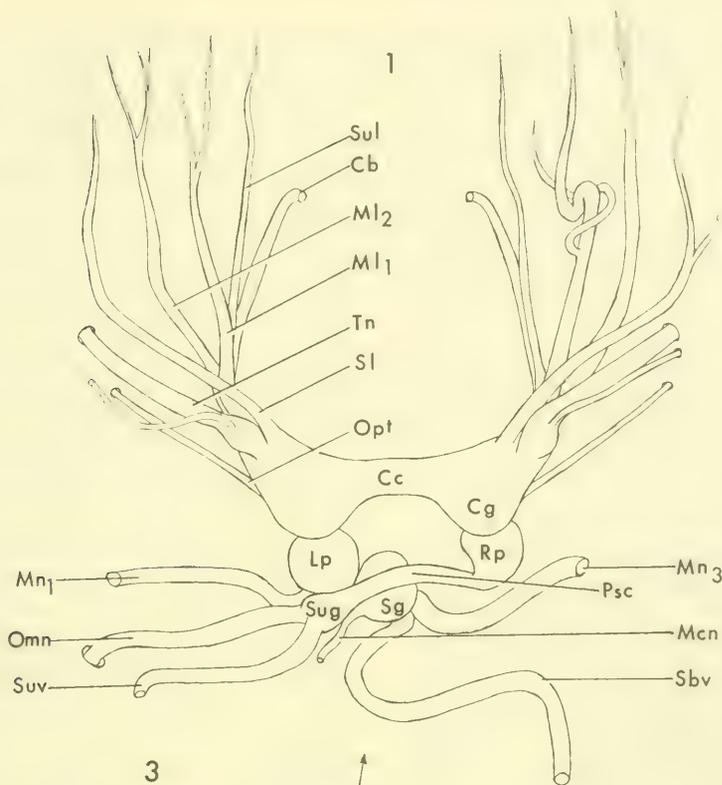


TABLE 13. Anatomical differences between *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* indicative of specific rank

Characteristic	<i>P. lapidaria</i>	<i>O. hupensis formosana</i>
Pigmentation	no dimorphism	sexual dimorphism
Glands at edge of eyes	white-yellow, white	yellow, white-yellow
Digestive gland with prominent vascularization	-	+
Radula		
1. length	1.13 mm	0.98 mm
2. width	0.13 mm	0.12 mm
3. total rows of teeth	94	84
Size of organs	larger	smaller
Osphradium	wider	narrower
Muscles		
1. short rostral retractor	-	+
2. mediolateral slip from buccal protractor	-	+
3. circular muscle over buccal protractor	-	+
Male Reproductive System		
1. gland types in verge	3	1
2. testicular lobes	short and slender	elongate and thickened
Female Reproductive System		
1. opening of the spermathecal duct obscure	+	-
2. ova	larger	smaller
Nervous System		
1. buccal nerve 3	+	-
2. external mantle cavity nerve 4	-	+
3. branch of tentacular nerve arises at	mid nerve	base

+ = yes

- = no

supraesophageal ganglion almost touches the lateral body wall (Pl. 14); the supra-visceral connective arises from the posterior side of the ganglion near its tip, but not from the tip (Pls. 14, 20). The osphradio-mantle nerve bifurcates to form the osphradial and mantle nerves just after leaving the ganglion and before entering the lateral wall (see p 79).

(4) The main branch of the tentacular nerve (Tn, Pl. 31) arises from the basal swelling of the tentacular nerve, not from the mid-length of the nerve as in *Po-*

*matiopsis lapidaria*.

(5) Buccal nerve 3 (b<sub>3</sub>, Pl. 15, Fig. 1) was not found in *Oncomelania hupensis formosana*.

(6) The other differences are mainly ones of size (compare Pls. 31 and 20). A few minor differences might be mentioned. The minor lateral nerve (p<sub>8</sub>, Pl. 31, Fig. 2) from the pedal ganglion was frequently encountered in *O. h. formosana* while it was infrequently found in *P. lapidaria*. The external mantle cavity nerve 4 (E<sub>4</sub>, Pl. 31, Fig. 4), not

found in *P. lapidaria*, was observed although it varied in strength and was sometimes absent. It ran anterolaterally over the mantle wall epithelium.

### E. Summary and Discussion

Abbott (1948a) stated that the reproductive and nervous systems of *Pomatiopsis* and *Oncomelania* showed few differences. Actually, the major differences found between the species were in these systems, especially in the reproductive systems. In Table 12 are listed those differences which I feel are important in defining the generic separation of *Oncomelania* and *Pomatiopsis*. Differences of a specific nature are listed in Table 13. Because many of the characters listed are unknown for the other species of *Pomatiopsis* as well as for the subspecies of *Oncomelania*, only future investigations of these other forms can show whether these characters are specific or representative of the genus as a whole.

Differences in the shell clearly separate *Oncomelania* from the species of *Pomatiopsis*. *P. binneyi*, however, has a shell quite unlike those of either *Oncomelania* or the other species of *Pomatiopsis*. It is tiny, about 3.0 mm high; imperforate, and the inner and outer lips are continuous, i.e., there is no parietal callus and the lips are slightly separated from the body whorl. *P. binneyi* will be discussed in the final summary statements, as this form is also aberrant in other ways from the other species of *Pomatiopsis*.

The higher number of gill filaments also clearly separates *Oncomelania* from species of *Pomatiopsis*. All the species of *Pomatiopsis* have shorter tentacles, relative to the length of the rostrum, than *Oncomelania*.

None of the 4 species of *Pomatiopsis* investigated have the terminal papilla found in the verge of *Oncomelania*, *Blanfordia* and *Tomichia*. Two species of *Pomatiopsis*, *P. cincinnatiensis* and *P. californica*, have penial filaments,

unknown in the above genera.

*Oncomelania* and *Blanfordia* have the characteristic ciliation of the tip of the verge described for *O. hupensis formosana*. *Tomichia ventricosa* has the same active cilia, but in this species the bands of cilia extend half way back along the verge. In these 3 genera the cilia are about 4 $\mu$  in length and beat actively.

The verges of *Pomatiopsis lapidaria* and *P. cincinnatiensis* lack cilia. In initial studies on *P. binneyi* and *P. californica*, I found that the tips of the verges have bristle-like cilia. Unlike those of *Oncomelania*, they are not active, a cilium here or there beating slowly once in a while. They were bushy, irregularly oriented, and 6-8 $\mu$  in length. In *P. californica* they extended part way out on the penial filament.

There are clear-cut differences in the origin and the relation of the spermathecal and sperm ducts. The spermathecal duct arises at the anterior end of the bursa copulatrix in *P. lapidaria* and from its right ventro-lateral surface in *Oncomelania* and in 2 other members of the Pomatiopsinae I have studied, *Blanfordia japonica* and *Tomichia ventricosa*. The spermathecal and sperm ducts originate together from the bursa, in one common sheath, in *Oncomelania* and *Blanfordia*, while in *Pomatiopsis* and *Tomichia* the sperm duct arises from the spermathecal duct near its junction with the bursa. Thus, *Tomichia* shows an intermediate position, in that the sperm duct arises from the spermathecal duct, as in *Pomatiopsis*, but the spermathecal duct arises laterally from the bursa, as in *Oncomelania*, and not at the anterior end. The bursa in *Tomichia* is about twice as long as in *Pomatiopsis lapidaria* and in the subspecies of *Oncomelania hupensis*. The arrangement of bursa and ducts in *Pomatiopsis* might possibly be derived from the condition found in *Tomichia* by a reduction in length of the anterior end of the bursa (the reverse derivation being likewise open to consideration). How the tubes arise from the bursa is unknown for *P. californica* and

*P. binneyi*.

The slender collecting duct of the ovary and the numerous branches separate *Oncomelania* from *Pomatiopsis lapidaria* and *P. cincinnatiensis*. The condition is unknown for *P. californica* and *P. binneyi*.

The oviduct coils around the seminal receptacle in a characteristic manner in the subspecies *Oncomelania hupensis quadrasi*, *O. h. formosana* and *O. h. nosophora*. The condition is unknown in *O. h. hupensis*. The arrangement does not occur in *Pomatiopsis lapidaria* and *P. cincinnatiensis*. The condition is unknown for *P. californica* and *P. binneyi*.

The pleuro-supraesophageal connective in all the subspecies of *Oncomelania* is relatively short, as described, a condition also found in *Blanfordia japonica*. In these snails the common osphradio-mantle nerve leaving the tip of the supraesophageal ganglion is correspondingly long and usually does not bifurcate before entering the wall of the "neck." The supravisceral connective arises also from the tip of that ganglion. In the species of *Pomatiopsis* studied, on the other hand, the pleuro-supraesophageal connective is elongate: van der Schalie & Dundee (1956) show a long connective for *P. cincinnatiensis*, and I also found it so in *P. binneyi* and *P. lapidaria*. In these 3 species of *Pomatiopsis* the tip of the ganglion is close to the lateral wall, and the mantle and osphradial nerves, which frequently bifurcate soon after leaving the tip of the ganglion, have a very short length before entering the lateral wall. In *P. binneyi*, as in *P. lapidaria*, the supravisceral connective arose from the posterolateral surface of the supraesophageal ganglion near, but not from, the tip. *P. californica* has not been studied.

## HYBRIDIZATION STUDIES

Success in hybridizing the subspecies of *Oncomelania* was reviewed by Davis et al. (1965). Van der Schalie, Getz & Dazo (1962) reported success in hybridization experiments when male

*Pomatiopsis lapidaria* were placed in culture with virgin female *O. hupensis quadrasi* and *O. hupensis formosana*. They did not report success with crosses involving female *P. lapidaria* and male *Oncomelania*.

Cross cultures were again set up between male *Pomatiopsis lapidaria* and virgin females of the various subspecies of *Oncomelania* in order to obtain hybrids for anatomical studies. Three different sets of experiments were established over a total of 26 months in which adult, male *P. lapidaria* were maintained under various culture conditions with virgin female *Oncomelania*. In all the experiments males which died were replaced. Females that died were removed from the culture but not replaced. Cultures which deteriorated due to soil erosion, mold, or algal accumulation were replaced by fresh cultures. The deteriorated cultures were maintained for at least 1 month in order to observe if any young had hatched from eggs laid just prior to changing the culture. All cultures were maintained at  $24^{\circ} \pm 2^{\circ} \text{C}$ .

## Experiment 1.

(A) Seven plastic tray cultures (see p 118) were established with 10-20 females in each, along with 13-40 males, no culture having more than 50 snails. The cultures were maintained in normal room level light during the day and were in the dark at night.

One culture was set up with female *Oncomelania hupensis nosophora*, 2 with *O. h. quadrasi* and 4 with *O. h. formosana*.

(B) Eight cultures were set up using 3 inch diameter clay flower pots, 1 inch deep, partially filled with loam and maintained exactly as those described by van der Schalie et al. (1962). Five specimens of each sex were maintained in the cultures. The only females used were *O. h. formosana*.

In both (A) and (B), the cultures were routinely serviced each day along with the 80 other stock cultures on hand. Within 3-7 months 5 of the plastic tray

cultures contained young. Upon re-determining the sex of all the adults in the cultures (methods of Wong & Wagner, 1954) it was demonstrated that in every case where young were produced, 1 or 2 males of *O. h. formosana* or *O. h. quadrasi* were present. Such contamination had occurred in 2 cultures in the 7th month, after the sexes of all the snails had been rechecked in the 5th.

In the 7th month all the cultures were placed in isolation and serviced with tools set aside for the cross cultures only. Those cultures, in which contamination had occurred, were discontinued. When these precautions were taken, no further young were produced, although the cultures were maintained and constantly observed until the 20th to the 26th month.

#### Experiment 2

Thirteen cultures were established in medium size clay pots, a type of vivarium found to provide optimal conditions for the production of young *Oncomelania* (van der Schalie & Davis, 1968, in press. See p 119, e).

Five specimens of each sex were placed in each culture. Two of the cultures contained female *O. h. quadrasi*; 11 of the cultures contained female *O. h. formosana*. Five of the cultures were maintained under constant light provided by cool, white fluorescent bulbs. The intensity of light was 75 ft. candles. All cultures were carefully maintained in isolation. The experiment was discontinued after 14 months. No young were produced. All control cultures with *Oncomelania* males produced young within 2 to 3 months of being established. Male *Pomatiopsis lapidaria* were observed in copulation with the *Oncomelania* females many times and spot examination of the male gonads showed them to be fully mature and productive.

#### Experiment 3

Another 16 medium clay pot cultures were established and separated into blocks of 4, each block being provided with male *Pomatiopsis lapidaria* from a

different population. These were collected and placed in culture in the spring, a time of year when the species exhibits pronounced sexual activity. Two cultures of each block were established with virgin *O. h. quadrasi* and 2 with virgin *O. h. formosana*. Two females were uniformly used in all the cultures with alternately 2 or 4 males. The cultures were maintained in isolation. Copulation was noted frequently but no young were produced over a period of 5 months. At the end of 5 months the cultures were discontinued.

Females removed from the terminated cultures were fixed in Bouin's solution, sectioned, stained with standard Hematoxylin and Eosin and studied to determine if the ovaries were productive and if the seminal receptacles were storing sperm. In no case was sperm noted in the seminal receptacle or bursa copulatrix. Oocytes posterior to the gonopericardial duct often showed signs of atrophy and deterioration.

#### Summary and Discussion

As a result of these experiments it was concluded that *Pomatiopsis lapidaria* will not produce an hybrid F<sub>1</sub> generation when interbred with *Oncomelania*. It is felt that the results of previous experiments reporting success in such crosses were possibly due to contamination of the cultures with male *Oncomelania* during routine maintenance.

Burch (1960b) reported that the spermatogonial cells of *P. lapidaria* have 33 chromosomes, 16 pairs and "a heterochromatic chromosome, presumably a sex chromosome." The oögonial cells of *Oncomelania hupensis quadrasi* and *O. h. nosophora* have 34 chromosomes (17 pairs). The spermatogonial cells of *Pomatiopsis cincinnatiensis* have 30 chromosomes plus a heteromorphic pair. According to Patterson (1963) the sex determining mechanism in *P. lapidaria* is of an XO type while in *P. cincinnatiensis* and *O. h. formosana* the sex determining mechanism appears to be an XY type. Although a cross between individuals with  $2n=32$  and  $2n=34$  is possible, a

successful cross between *P. cincinnatensis* ( $2n = 32$ ), having a heteromorphic pair of sex chromosomes, and *Oncomelania* ( $2n = 34$ ), which lacks a corresponding heteromorphic pair, is highly improbable. Crosses between *P. lapidaria* and subspecies of *O. hupensis* are improbable because of the apparent difference in the sex determining mechanism. Also, other karyotypic differences in the chromosomes of the 2 species of *Pomatiopsis* and subspecies of *Oncomelania hupensis* indicate that there would probably not be a sufficient number of similar homologues between them to permit successful hybridization (Burch, personal communication).

## ELECTROPHORETIC STUDIES

### A. Introduction

Little is known about electrophoresis as applied to molluscan systematics. Cheng (1964) and Davis & Lindsay (1967) review previous work pertaining to mollusks. Cheng, using membrane electrophoresis, investigated several species of marine and freshwater gastropods as well as a few marine pelecypods (also 1 sphaeriid). The gastropods were widely separated taxonomically. Using serum, he obtained up to 5 fractions, although there were generally only 1-3 fractions. From his results he concluded that each "species" could be identified by its serum electrophoretic pattern. He states that "undoubtedly much more extensive surveys of the serum proteins of mollusks must be made before useful taxonomic information will be obtained."

Wright & Ross (1959) found that paper electrophoretic analysis of proteins in snail blood was not satisfactory and began using cellulose-acetate electrophoresis (1959, 1963). Their studies turned to gastropod egg proteins (1963, 1965) to provide characters of use in the taxonomy of planorbid snails.

In 1963 they published data showing that blood proteins and haemoglobin varied considerably both quantitatively and qualitatively with progressive growth

and development of sexual maturity in *Biomphalaria glabrata* (= *Australorbis glabratus*). As a result they stated that "the results of this work confirm earlier doubts concerning the taxonomic value of molluscan blood proteins...."

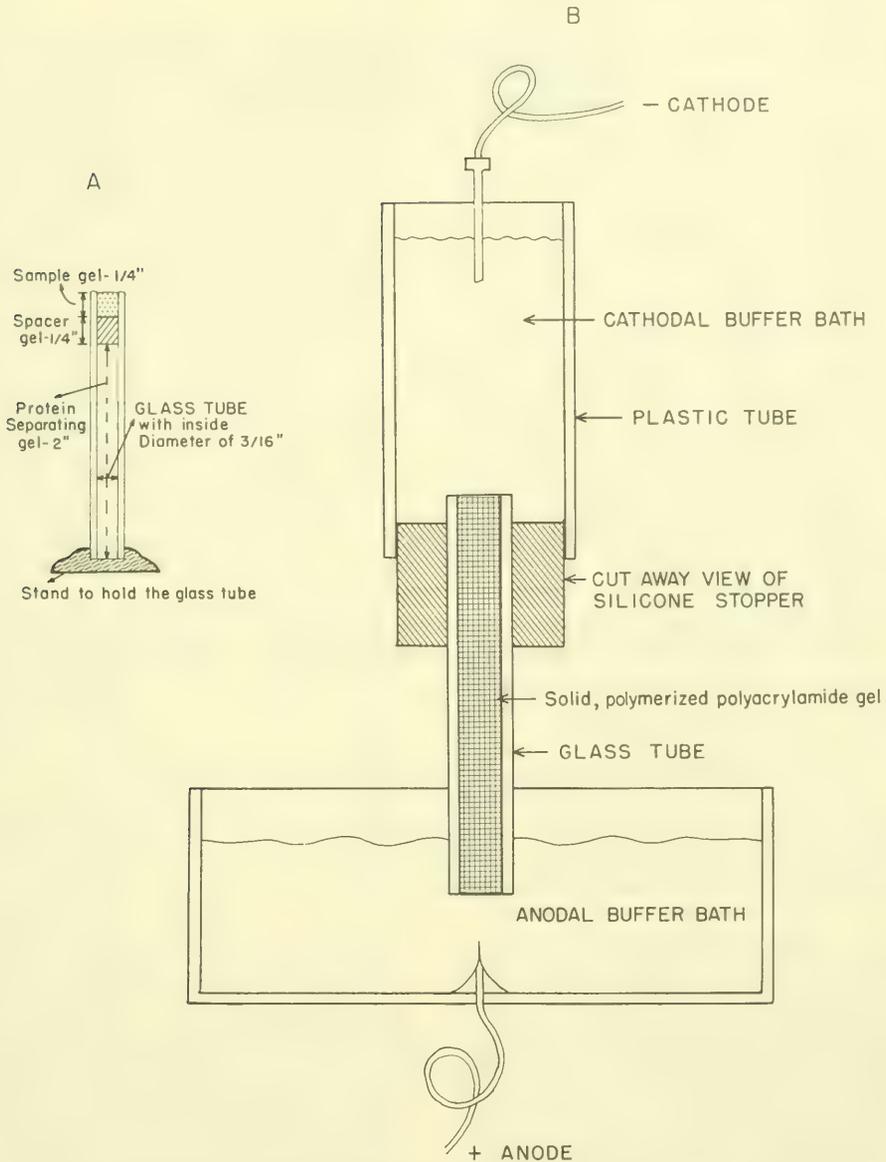
Davis & Lindsay (1964, 1967) studied proteins from foot muscle and blood of *Helix pomatia* using polyacrylamide electrophoresis. Proteins from the blood yielded 12, from foot muscle extract 20, components, which showed no qualitative changes with snails of different size (age). However, when size of snail was correlated with protein density, there was a significant inverse quantitative change with haemolymph, but not with foot muscle extract. They (1967) also showed that different populations of *Pomatiopsis lapidaria* had population-specific protein patterns. Despite significant variation between populations, the species was characterized by a densitometric pattern clearly recognized in each population.

Michelson (1966) studied the haemolymph of *Biomphalaria glabrata* using polyacrylamide electrophoresis. He reviews literature pertaining especially to mollusks involved in host-parasite relationships. Michelson reported that size did not affect qualitative results but that density in blood proteins in *B. glabrata* increased with increased size. His results regarding increased density of blood proteins with larger snails are contrary to those of Davis & Lindsay (1964, 1967) with haemolymph of *Helix pomatia*.

The purpose of the present investigation was to study the electrophoretic patterns of *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* in order to determine whether distinct taxon characterizing patterns could be obtained. This investigation was undertaken as a preliminary step for comparative studies involving the other subspecies of *Oncomelania hupensis* and species of *Pomatiopsis*.

### B. Materials and Methods

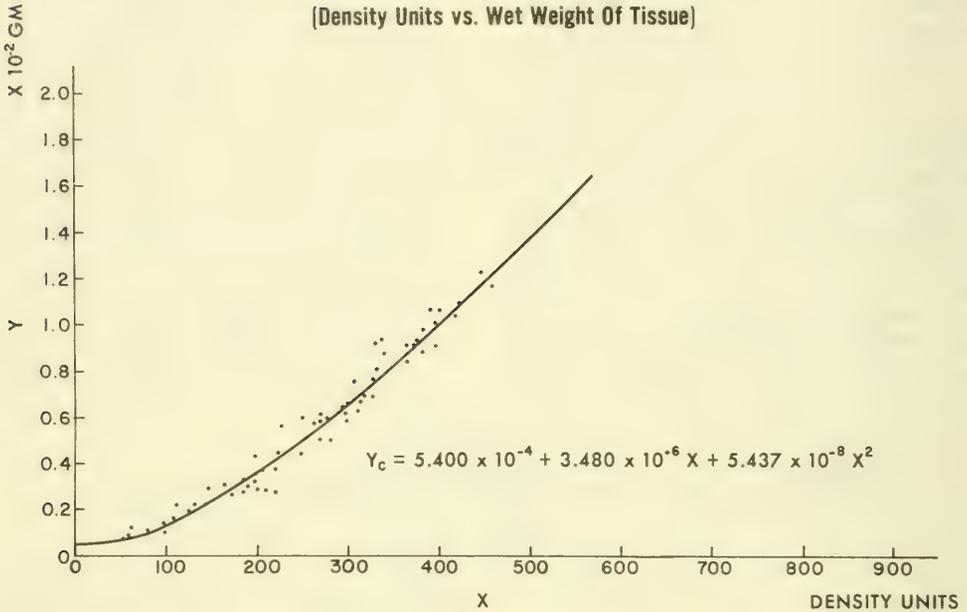
Disc electrophoresis. The technique



TEXT FIG. 2. Diagrammatic set-up for disc electrophoresis

- A. A glass tube is set upright in a supporting base stand. Three gel solutions are poured into the tube, one at a time. Each solution is allowed to polymerize before the next is added. The protein separating gel is the standard 7.5% acrylamide gel. When the uppermost solution is polymerized, the glass tube with the internal gel column is removed from the base stand.
- B. The glass tube containing the gel column is held in place in the upper cylindrical cathodal buffer bath by means of a perforated silicone stopper while the lower end hangs freely in the anodal buffer bath. Usually, as many as 12 cathodal units were used in a common anodal bath.

FOLIN-CIOCALTEU PROTEIN ESTIMATION  
(Density Units vs. Wet Weight Of Tissue)



TEXT FIG. 3. Estimation of protein in foot muscle tissue

The density units are direct readings from the scale of the Klett colorimeter. Results were not linear with smallest weights of tissue: with X taken at zero the Y intercept ( $Y_c$ ) was  $5.400 \times 10^{-4}$  grams of wet (but blotted) weight of tissue.

of disc electrophoresis was described in detail by Ornstein (1962, 1964) and B. J. Davis (1964). The reader is also referred to bibliographies on work pertaining to disc electrophoresis available through the Canalco Corporation, Rockville, Maryland, U. S. A.

The methods used in this study were discussed fully by Davis & Lindsay (1967). The standard 7.5% acrylamide gel was used. In Text Fig. 2 is shown a schematic drawing of the arrangement of gels polymerized in the glass supporting tube (A) and a drawing of how one such tube is positioned so as to bridge the 2 buffer baths (B).

The buffer was a tris\*-glycine mix-

ture with a pH of 8.2-8.4. A constant current of 5 milliamperes was passed through each tube and was maintained by hand regulation of a Heathkit power supply. Bromphenol blue dye was added to the cathodal bath prior to starting the current through the gels and served as a tracking dye to indicate the position of the front or leading band moving through the separating gel towards the anode. When the front band had moved 33 mm into the protein separating gel, the current was turned off; the gels were removed from the glass tubes and placed in Amido-Schwartz stain. After 2 hours of staining, the gels were destained in acetic acid.

Preparation of sample. Proteins from foot muscle tissue were extracted in Carriker's (1946b) physiological saline.

\*tris = 2-amino-2-hydroxymethyl-1,3-propanediol

The reasons for using foot muscle are fully discussed by Davis & Lindsay (1964, 1967). The tissue of 20-50 snails was pooled and 0.15 gm (wet weight, blotted tissue) were homogenized in 1.0 ml saline using a Servall microhomogenizer (50,000 rpm). The tissue was homogenized for 30 seconds and then examined to see whether all the tissue was "taken." If some pieces remained the muscle was homogenized for another 30 seconds. All operations were carried out at 2<sup>0</sup>-3<sup>0</sup> C.

The homogenate was centrifuged at 1200 rpm (250X G) for 5 minutes. Supernatant was mixed with the sample gel in a ratio of 1:2. Only 100 lambda of the mixture were polymerized above the spacer gel in each glass tube (A, Text Fig. 2).

An estimate was made of the relative amount of protein present in the supernatant fluid after centrifugation as well as how much actually was separated in the electrophoretic runs. The Folin-Ciocalteu reagent test, based on colorimetric procedures, was applied to determine the relative weight of protein in the supernatant and in the electrophoretic runs. Shreds of foot tissue were weighed on a Mettler Microbalance and subsequently submitted to the Folin test. Wet weight (blotted shreds) of tissue was plotted against Klett colorimetric readings (Text Fig. 3). From the resulting curve it was possible to determine the relative amount of protein in the supernatant fluid in relation to the initial wet weight of tissue. The tissue, of course, was not entirely protein, i.e., some weight was contributed by carbohydrates and fat, but the relative approximation of weights gives a useful indication of the fate of homogenized tissue. It was determined that 27% of the initial wet weight of tissue are found in the total volume of supernatant after homogenization and centrifugation. When supernatant was mixed with upper gel and electrophoresis was completed, it was found that 45% of the proteins in the sample gel were too crude to pass into

the spacer gel, 21% remained in the spacer gel, and 33% migrated into the separating gel.

At least 20 experiments were performed for each species. Each experiment consisted of 4-5 tubes loaded with aliquots of a single muscle preparation.

Analysis of results. Densitometric tracings of the distribution of the protein fractions in the gels were made using the unmodified Canalco Model E microdensitometer. Later studies (Davis & Lindsay, 1967; published before the present account) were made with the densitometer modified to give an expanded tracing with a more clearly defined densitometric pattern.

Rf values for the fractions were determined from the densitometric tracings when peaks were pronounced; they were calculated from direct measurement of the gels when a band was diffuse or faint. An Rf value is the ratio of the distance from the origin to a given fraction and the distance from the origin to the front band. It serves to minimize differences in band migration due to small differences in the length of the "run" which did not always measure exactly 33 mm. Measurements were made using a ruler accurately calibrated in 0.5 mm units.

Results were analyzed by studying the differences between Rf values and densitometric patterns of the taxa. More detailed information on the use of Rf values in comparing taxa can be found in Davis & Lindsay (1967). When Rf values of fractions in different taxa varied by 0.014 or less, they were considered analogous because this value represented the greatest error for value determination when different people measured the same component from the gel.

### C. Results

The results of the study are illustrated in Plate 32, Figs. 1, 2 for *Pomatiopsis lapidaria* (Parker Mill population) and *Oncomelania hupensis formosana*, re-

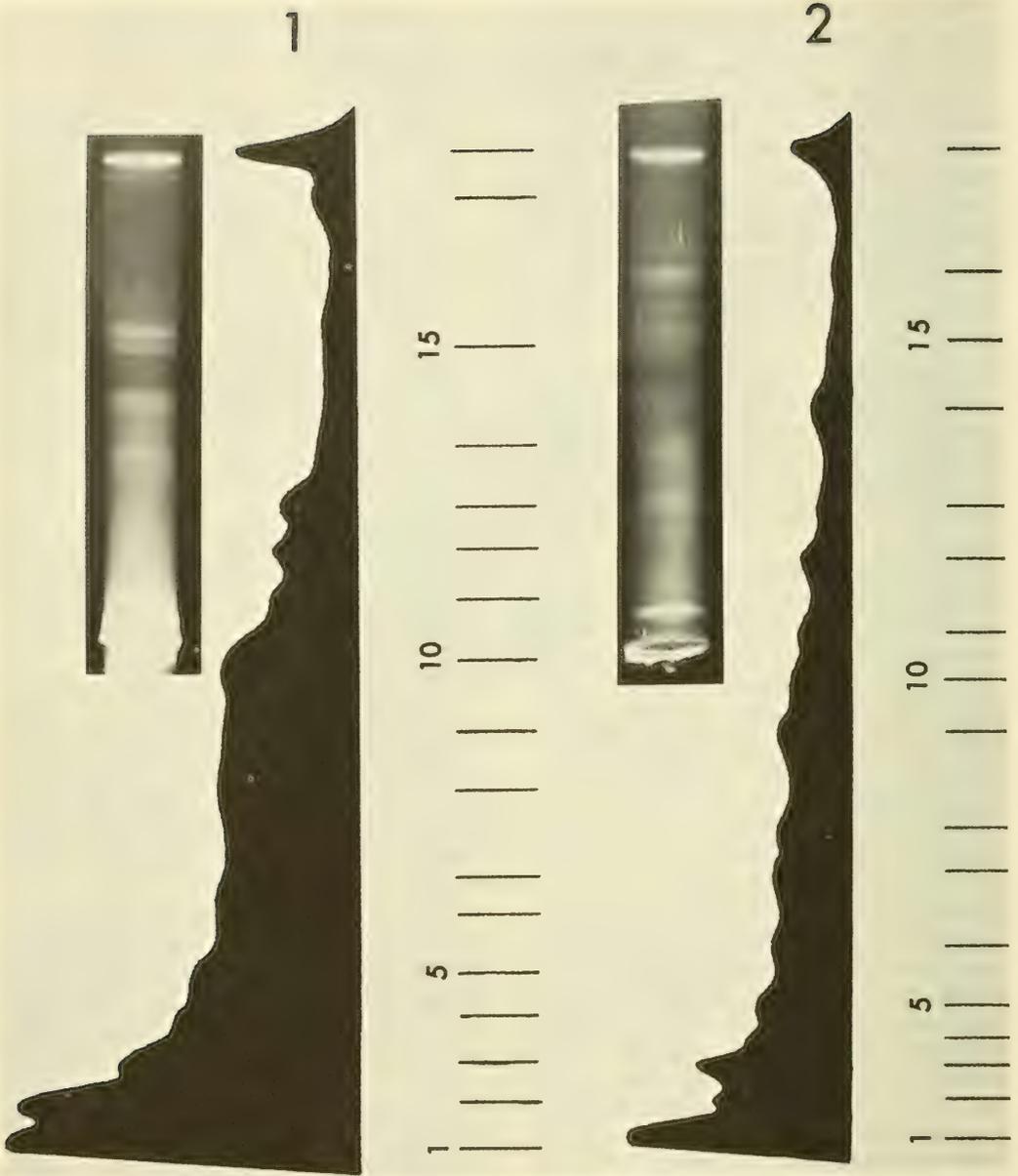


PLATE 32. Electrophoretic comparison of *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana*

1. *P. lapidaria*
2. *O. h. formosana*

The vertical lines beneath the densitometric tracings (solid black) represent the separated protein components from the foot muscle. The photographs above each tracing were made by using the stained gels as negatives and placing them under the enlarger. Prints were made directly from the gels.

TABLE 14. Representative Rf values\* for the separated protein components illustrated in Plate 32

Band	<i>Pomatiopsis lapidaria</i>	<i>Oncomelania hupensis formosana</i>
1	0.014	0.014 (1)**
2	0.056	0.042 (2)
3	0.099	0.070
4	0.141	0.098 (3)
5	0.196	0.133 (4)
6	0.244	0.198 (5)
7	0.296	0.266
8	0.350, 0.390	0.314
9	0.440	0.410
10	0.497	0.464
11	0.552	0.515
12	0.601	0.649 (13)
13	0.640	0.691
14	0.718	0.765
15	0.818	0.821 (15)
16	0.965	0.896
17	1.000	1.000 (17)

\* Averages of data from numerous tubes.

\*\* The number to the right of the Rf value refers to the band of *P. lapidaria* with which the Rf is analogous, i. e., differs by 0.014 or less.

spectively. The lines beneath the peaks of the 2 typical densitometric graphs represent the linear spacing of 17 protein fractions indicating the components distinguished. The tubes and tracings are representative and reliably portray the species differences despite the small variations that do occur between different experiments on homologous preparations.

Average Rf values for the separated fractions are listed in Table 14. Comparison shows that only about 44% of the components were analogous in the 2 species. The reader is reminded that analogy does not mean homology, and that homology must be proved by biochemical and/or immunological means. As stated by Davis & Lindsay (1967), *P. lapidaria* is particularly characterized by (1) fractions 11-13 with the

twin dense peaks at 12 and 13; (2) by the fact that the area from band 13 to the front is devoid of high density components.

In *P. lapidaria*, band 8 was frequently split into 2 bands (Table 14).

*O. h. formosana* is characterized by bands 12-15, bands 14 and 15 being close to each other and 15 being the less dense. Band 13 is always very faint while 12 is dense. Bands 9-11 are wide and diffuse. Generally bands 1 and 3 are very dense and wide so as to hide band 2. Results in Plate 32, Fig. 2, where band 1 is not dense, are the exception.

#### D. Discussion

Initial studies with the other subspecies of *Oncomelania hupensis* revealed that in contrast to *Pomatiopsis lapidaria* all had 1 or 2 dense fast-moving protein fractions in the region beyond Rf 0.75 (after band 13). The group of subspecies includes the so-called *Tricula chiuvi* which was referred to the genus *Oncomelania* by Davis & Chiu (1964). This snail is currently considered to be *O. hupensis chiuvi* (see footnote 5, p 133).

The subspecies of *Oncomelania hupensis* are further separated from *P. lapidaria* electrophoretically by the fact that they have distinctive densitometric patterns in the gel region between Rf 0.601 and 0.850 (includes bands 12-15 in Table 14). The characteristic fractions for *P. lapidaria* are found in the gel region between Rf 0.338 and 0.656 (includes bands 8-13 in Table 14) while the gel region beyond Rf 0.656 is devoid of dense components. The limits here given for the gel regions include the variations found for various populations of *P. lapidaria* (Davis & Lindsay, 1967) and subspecies of *O. hupensis* (Davis, unpublished).

#### LABORATORY ECOLOGY

##### A. Introduction

Modifications of Vogel's (1948)

aquaterrarium have been used with varying degrees of success for rearing the subspecies of *Oncomelania hupensis*. References to such vivaria are found in Stunkard (1946), Ward et al. (1947), DeWitt (1952), Wagner & Wong (1956) and Moose et al. (1962). These and others have noted a marked contrast between the relative ease in rearing *Oncomelania* and the difficulties in maintaining and rearing species of *Pomatiopsis*.

Stunkard (1946) noted that *Pomatiopsis cincinnatiensis* did not survive well in the laboratory and that, although *P. lapidaria* remained alive many weeks, it did not reproduce. Ward et al. (1947) failed to maintain *P. lapidaria* on moist mud in shallow pans. They employed large aquaterraria with a sloping mud bank covered with dry maple leaves, but found that stocks died after several months. Berry & Rue (1948) stated in an abstract that laboratory breeding of *P. lapidaria* was successful. They noted egg laying, followed by hatching after 3 weeks. No other data were given. DeWitt (1952) stated that he was successful in maintaining *P. lapidaria* from 1944 to 1952, using an "aquaterrarium." He gave no data on reproduction, growth of young, or if an  $F_1$  generation was reared to maturity in the laboratory.

Dundee (1957) described a large flower pot container (12 inches in diameter) which she used "successfully" as a vivarium. She reported reproduction and egg hatching, but did not mention whether the young were reared to maturity. Van der Schalie & Dundee (1955), van der Schalie et al. (1962) and van der Schalie & Getz (1962, 1963) discuss various aspects relating to the difficulties in maintaining species of *Pomatiopsis* in the laboratory.

Few quantitative data have been presented comparing *Oncomelania* and *Pomatiopsis* with regard to survival in the laboratory, natality, growth rates of the young produced, or survival of the young in the laboratory. Van der Schalie & Getz (1963) provided comparative data

on temperature and moisture responses between "species" of the 2 genera.

In this study the survivorship of field collected *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* was investigated when these snails were placed in different standard vivaria. The production of young was noted and the growth rates of the young recorded. Records were maintained on the survivorship of the young in culture.

## B. Materials and Methods

### 1. Vivaria Utilized in the Investigations

a. Plastic Tray Container. This vivarium is a modification of the aquaterrarium used by DeWitt (1952) and was discussed in detail by van der Schalie & Davis (1965). It is briefly described as follows: the measurements of the plastic tray were 28 x 19 x 6.5 cm. At one end was a soil bank and the other a water reservoir with about 250 cc capacity. The water in the reservoir was constantly aerated. The tray was covered by a sheet of plexiglass bored with many small holes to permit gaseous exchange. Filter paper was added as food, as prescribed by van der Schalie et al. (1962).

b. Tall Clay Pot. The set up in a tall, unglazed, clay flower pot (5 1/2 inches deep and 7 inches in diameter at the top) was described by van der Schalie & Getz (1962). It was designed to decrease or regulate soil moisture, since other vivaria with saturated soils were particularly detrimental to *Pomatiopsis cincinnatiensis*. This unit was modified slightly for this study: the filter paper wick was replaced by a thick roll of cheesecloth which projected up into the pot about 2 inches. The pot was filled with sand up to within 3 inches of the top. The sand was covered with 1 1/2 inches of loam. The packed loam was dusted with finely ground dried loam to provide a surface of particularly fine particles.

c. Battery Jar. Cylindrical glass containers 10 inches deep and 8 inches in

diameter were utilized. The bottom of each jar was covered with a double thickness of No. 500 filter paper. A glass plate 3 inches by 4 inches was placed in the center of the jar and was used to support a small mound of mud. The filter paper was continually soaked with water so that a residue of 15-20 cc was present. The jar was covered with a plate of glass.

d. Petri Dish Culture. Nine centimeter Petri Dishes were used as cultures as described by van der Schalie & Davis (1968, in press). A mound of soil was placed in the center of the dish so that a space of 1.5 cm remained between the edge of the soil and the walls of the dish. About 40 ml of pond water were added to the culture. This type of vivarium was used only for rearing the young, newly-hatched snails to maturity. The vivarium was maintained under a 40 watt, white, "cool," fluorescent tube suspended 10 inches above the cultures. The light, providing 100-150 ft. candles, was cycled 10-12 hrs. per day.

e. Medium Clay Pot. Wagner & Wong (1956) used unglazed flower pot 5 inches in diameter in which a slope of "soil" was packed "high on one edge and terminated before reaching the other edge." The "soil" was a mixture containing soil, gravel and sand mixed in the ratio of 2:1:1. Filter paper and dried leaf were added as food.

A modification of the Wagner-Wong vivarium was found to provide optimal conditions for the production of young *Oncomelania* (van der Schalie & Davis, 1968, in press). In this modification the containers were unglazed, shallow Clay Pots with a diameter of 13 cm and a depth of 4 cm. A central mound of mud was placed on a large disc of filter paper. The bottom of the culture was covered with water, so that 5-6 cc were always present. Five males and 5 females were maintained in each unit of this culture type.

## 2. Conditions of Temperature and Light

The cultures, with the exception of the Petri Dish cultures, were maintained

at  $24^{\circ} \pm 2^{\circ}$  C. As the Petri Dish vivaria were placed closer to the source of illumination, the temperature was generally  $25^{\circ} \pm 2^{\circ}$  C.

The cultures were maintained under different lighting conditions. Those maintained in "room level light" (normal daylight + usual overhead lights) were exposed to  $60 \pm 10$  ft. candles over an 8-hour period. They were in the dark at night. Cultures under "alternating light" were exposed to  $100 \pm 10$  ft. candles for periods of 10-12 hours except for the Petri dishes, which were exposed to fluorescent light as described above. Cultures under constant light were exposed to  $140 \pm 20$  ft. candles for 24 hours.

## 3. Routine Maintenance and Collection of Data

All cultures were routinely checked each day to secure proper water levels, knock down the snails from the sides of the vivaria (these snails show a pronounced negative geotropism), and observe general culture conditions. Every 2 weeks each culture (except the Petri Dish cultures) was thoroughly examined. Dead snails were removed and recorded; young were removed and the number at each whorl stage was recorded. At this time water reservoirs clogged with soil particles were cleaned. The young were placed in Petri Dish cultures where they remained for at least 8 weeks. In some cases 2-5 young were placed in Medium Clay Pots and observed.

In the growth experiments young of *Oncomelania hupensis formosana* were measured every 3 days using a dissecting microscope and a Nippon Kogaku sliding ocular micrometer. The young of *Pomatiopsis lapidaria* were initially measured every 3 days but it soon became evident that monthly measurements were sufficient.

## C. Experiments

### 1. Survivorship

a. *Oncomelania hupensis formosana*. From a shipment of about 2,000 speci-

TABLE 15. The arrangement of vivaria and the number of snails used per culture

Species	Culture condition*	Light condition	No. of cultures	Total no. of snails	No. of snails per culture
<i>Oncomelania lupensis formosana</i>	PT	Alternating	5	600	120
	BJ	Room level	7	1188	170
<i>Pomatiopsis lapidaria</i>	PT	Alternating	6	156	26
		Constant	11	286	26
	BJ	Room level	5	500	100
		MCP	Alternating	5	50
	MCP	Constant	9	90	10
		Room level	21	210	10
		TCP	Alternating	14	140
<i>Pomatiopsis cincin-natiensis</i>	PT	Alternating	6	156	26
		Constant	5	130	26
	MCP	Alternating	5	50	10
		Constant	6	60	10
		Room level	5	50	10
	TCP	Alternating	15	150	10
		Constant	7	70	10

\*BJ = Battery Jar

MCP = Medium Clay Pot

PT = Plastic Tray

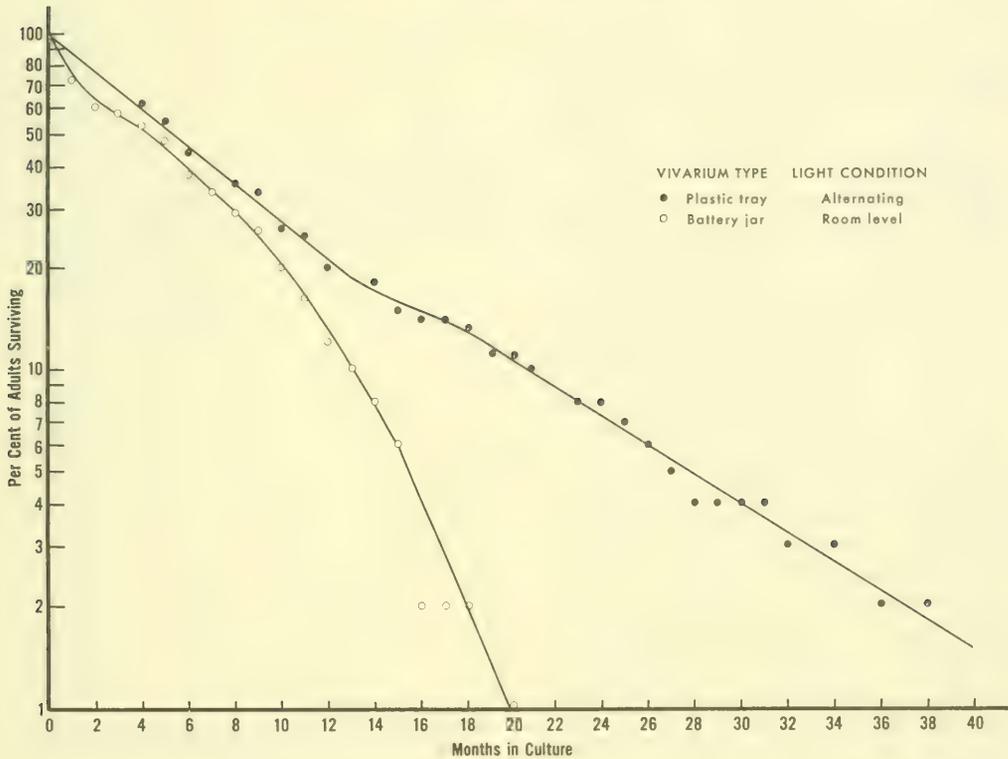
TCP = Tall Clay Pot

mens received from Taiwan, 1,788 adults were sorted out, i. e., animals with shells possessing varices. Shells showing extreme erosion were not used. From the adult size, the condition of shell and from information in the literature the age of the snails was estimated at about 1/2-1 year. Although Sugiura (1933) has demonstrated that *O. h. nosophora* was capable of living about 5 years in the field and McMullen et al. (1951) have stated that this subspecies could live more than 2 years, other evidence indicates that the average life expectancy for subspecies of *Oncomelania* reaching maturity is less than 2 years. While Li (1953) could not indicate how long field *O. h. formosana* lived, he concluded from his data that a large number of adults of the previous year died during or soon after the most active breeding season, and calculated the life span to be about 1 year for the vast majority of snails.

Pesigan et al. (1958) reported that the average rate of daily mortality for field females of *O. h. quadrasi* was 0.76%, and that the average female lived 65.8 days after reaching maturity. From their data on the growth rate of this subspecies, the average female would succumb in about the 7th-8th month in life.

As for the adults of *O. h. formosana* of the present collection, it was calculated from Li's (1953) growth rate in the field that none of the adults received were less than 5 months old, while from the condition of the shells, it was thought that the minimal age was most likely 7-8 months.

The snails were placed in Battery Jar and Plastic Tray vivaria; the former were maintained at "room level light," the latter under alternating light. The total number of *Oncomelania* snails placed in each type of culture along with the total number of cultures are tabulated

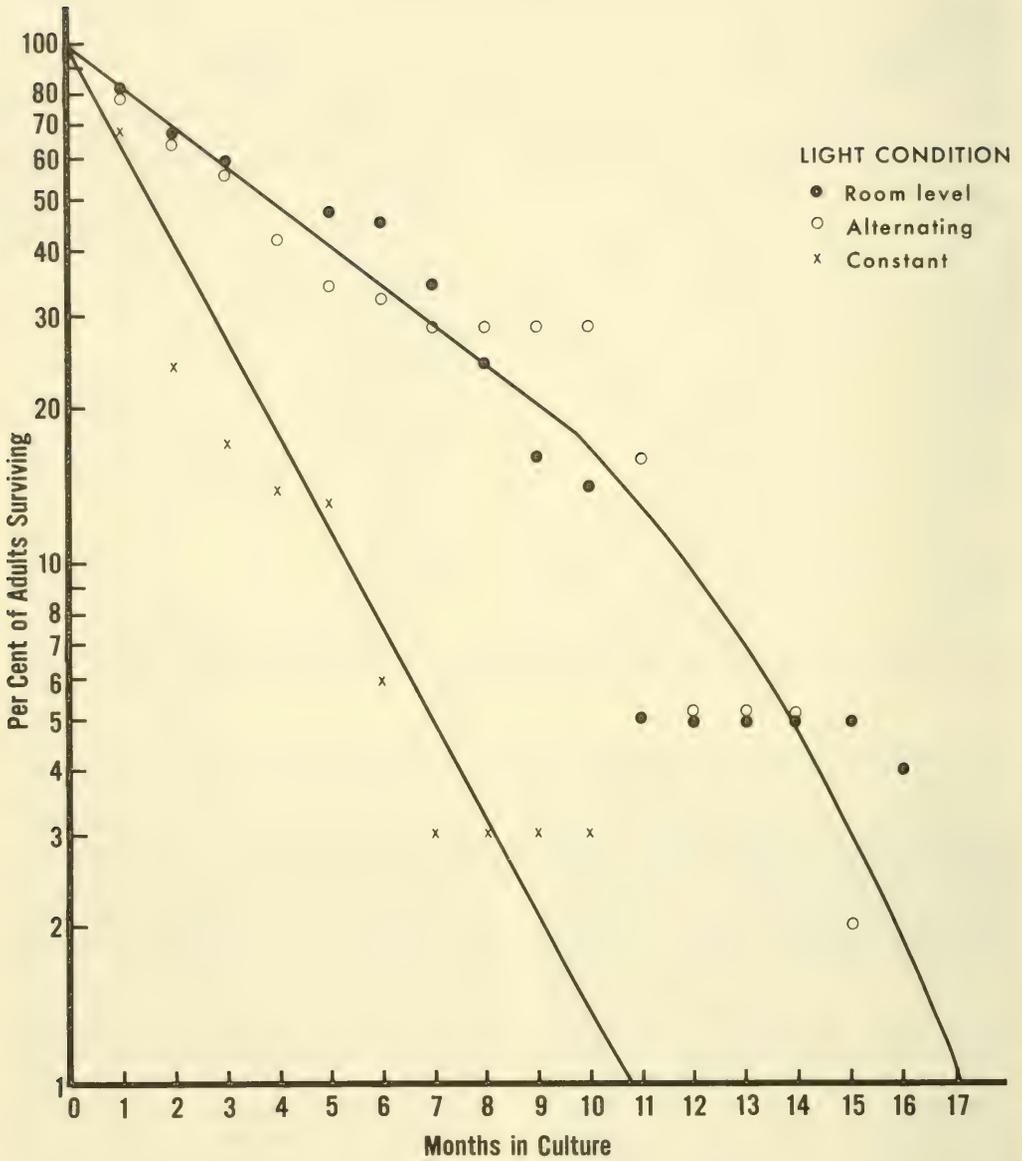


TEXT FIG. 4. Survival of *Oncomelania hupensis formosana* in 2 types of vivaria

in Table 15. The initial proportion of females in each culture varied between 47-55%. The percentage of adults surviving each month is shown in Text Fig. 4. It is evident that a constant fraction of the snails in the Plastic Tray vivarium died each month (exponential rate of 0.127), while the snails maintained in the Battery Jars showed an increasing rate of mortality, which indicates an unfavorable environment. The snails were apparently less able to subsist in that environment with advancing age, although the cultures were cleaned every 2 weeks and the filter paper was regularly replaced. Extreme erosion of the shells in snails maintained in the Battery Jars for 8-9 months also gave evidence that this environment was not optimal. As a result of the poor environment, 50% of the adults died within 4 months and only 1% survived until the 32nd month. In

the Plastic Tray vivaria, 50% survived until the 6th month and 2% were surviving at the end of 38 months.

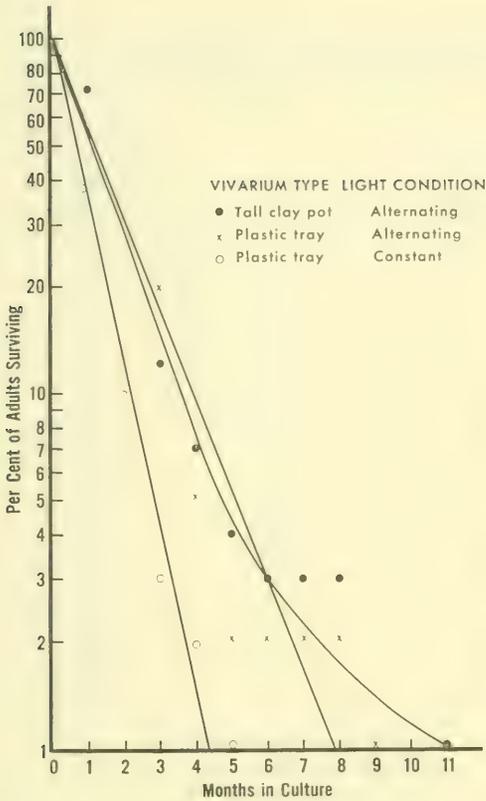
b. *Pomatiopsis lapidaria*. Adults of this species were collected from the stations previously mentioned (p 15). The growth rate of this species in nature has been given as 0.20 mm per week by Dundee (1957). At that rate of growth, adults used in this study were at least 7 1/2 months old. As the snails over-winter and are inactive for at least 4 months, the snails were most likely a minimal 11 1/2 months of age. Dundee also indicated that the life span of this species in the field is about 2 years. As the adults were collected primarily in July and August, they had over-wintered and had presumably grown from young hatched in June or July of the previous year. The snails were thus assumed to be 11-12 months of age.



TEXT FIG. 5. Survival of *Pomatiopsis lapidaria* in Medium Clay Pots under varying conditions of light

Experiments with *Pomatiopsis* were conducted using a larger array of culture chambers than used for *O. h. formosana*. The reasons for this are several. (1)

The survivorship of *P. lapidaria* was not tested in the laboratory until 1 year after the establishment of *Oncomelania*. In that period of time van der Schalie



TEXT FIG. 6. Survival of *Pomatiopsis lapidaria* in different vivaria

& Davis (1968, in press) had found that the various subspecies of *Oncomelania* survived better and produced more young per female when cultured in Medium Clay Pot vivaria. (2) The Tall Clay Pot had been found more suitable for rearing *P. cincinnatiensis*. (3) Additional field specimens of *O. h. formosana* were not immediately available for testing in the new types of culture chambers.

The number of cultures used for *P. lapidaria* under the varying conditions of light as well as the number of snails in each culture are listed in Table 15. The sex ratio was 1 in all these cultures. The survivorship curves for this species in 6 different environments are presented in Text Figs. 5 and 6. The lowest mortality rate was found in the snails

maintained in Medium Clay Pots, at either room level or alternating light. In these environments the exponential rate of mortality was 0.177 over the first 10 months, after which time it progressively increased.

In Table 16 are listed the exponential rates and finite rates of mortality for *P. lapidaria* in the 6 environments tested. The rates were calculated for the first 4-7 months in culture. It is evident that optimal survival is correlated with the Medium Clay Pot vivarium type. It also appears that constant light is correlated with increased mortality.

Survivorship in the Medium Clay Pot in room level or alternating light was compatible with the 2 year life expectancy in the field. The increasing rate of mortality after the snails were about 2 years old perhaps reflects the natural consequence of old age rather than deteriorating culture conditions. All the other environments are clearly unsuitable for maintaining *P. lapidaria*. Mortality rates were rapid, the exponential rate exceeding 0.40 per month, i.e., a finite rate of over 33% of the snails per month.

*c. Pomatiopsis cincinnatiensis*. Data presented by van der Schalie & Dundee (1955) indicated that the number of adults decreases in the field at the end of August and that the vast majority of young hatch early in August. These authors have shown that the life span is 16-18 months. In this study adults were collected in July and August; a few cultures were established with snails collected in May. These snails had presumably hatched in August or October of the previous year and had over-wintered. The snails were considered to be about 10-12 months old. The number of snails per culture type is listed in Table 15. The sex ratio was 1. Survivorship curves for *P. cincinnatiensis* in 7 different environments are presented in Text Figs. 7, 8 and 9.

Optimal survival was obtained in the Medium Clay Pot cultures under constant light (Text Fig. 8) where the exponential rate of mortality was 0.17

TABLE 16. Exponential and finite rates of mortality for *Pomatiopsis lapidaria*, *P. cincinnatiensis*, and *Oncomelania hupensis formosana* under varying environmental conditions

Species	Exponential* death rate per month (first 4-7 months)	Finite <sup>+</sup> death rate per month (%)	Vivarium type	Light condition
<i>O. hupensis formosana</i>	0.127	12	PT	Altern.
<i>P. lapidaria</i>	0.177	16	MCP	Room
	0.177	16	MCP	Altern.
	0.42	34	MCP	Const.
	0.60	45	PT	Altern.
	0.66	49	TCP	Altern.
	1.08	66	PT	Const.
<i>P. cincinnatiensis</i>	0.17	16	MCP	Const.
	0.40	33	PT	Const.
	0.42	34	MCP	Altern.
	0.60	45	TCP	Const.
	0.60	45	PT	Altern.
	0.60	45	TCP	Altern.
	0.92	60	MCP	Room

\*  $I_x = e^{-ax}$ +  $1 - e^{-a}$ 

MCP = Medium Clay Pot

PT = Plastic Tray

TCP = Tall Clay Pot

Altern. = Alternating

Const. = Constant

Room = Room Level

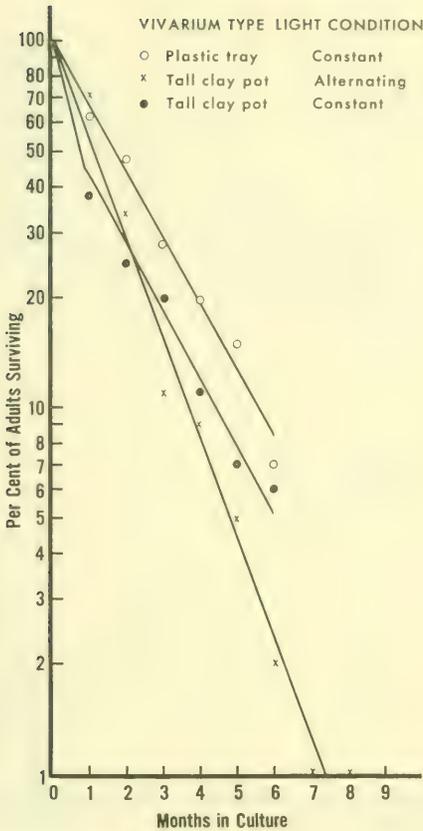
for the first 3 months and 0.82 thereafter. Only 10% of the snails were alive at the end of 6 months. In Table 16 cultures and lighting conditions are listed in order of increasing exponential rates of mortality. It is evident that survival is correlated with lighting conditions and not culture type. Optimal survival occurred under constant light. The least favorable environment was that of room level light in a vivarium which provided optimal survival in constant light, i.e., the Medium Clay Pot. In all but the 1 optimal condition, the finite rate of mortality was 33% or greater per month. After 6 months, cultures were down to 1-4 snails with the exception of those maintained under constant light where there were 6-10 snails per culture.

The patterns of survival of *P. cincinnatiensis* in the laboratory indicate that this species is an "annual" as observed by van der Schalie & Dundee (1955) in

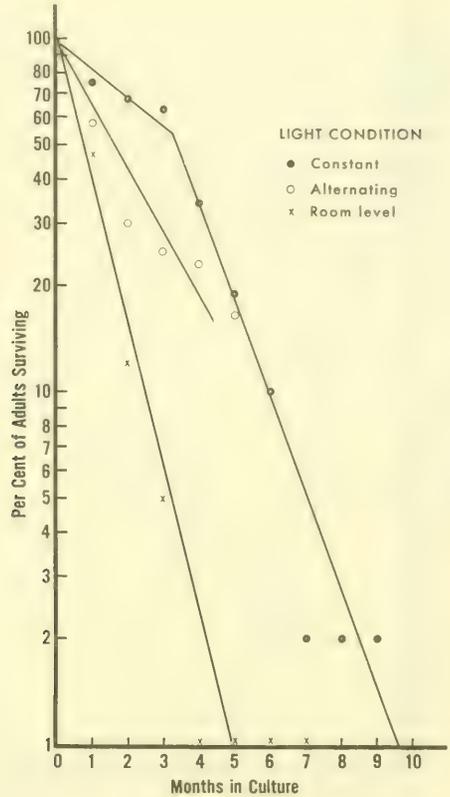
the field. Constant high rates of mortality might be expected for adults collected at the end of summer.

d. *Pomatiopsis californica* and *P. binneyi*. Nothing is known of the life history of these snails, which are native to California. Although exact data were not kept for these species, attempts at maintaining them in Plastic Tray or Medium Clay Pot vivaria have not been successful so far. No young were produced nor did survival generally exceed 5 months. Over 200 specimens of each species were involved in attempts to establish these species in the laboratory.

e. *Interspecific comparisons*. It is of value to compare the survivorship curves of the different species in the same environment. In only 1 case was a comparison possible between the 2 species of *Pomatiopsis* and *Oncomelania hupensis formosana*: in the Plastic Tray vivarium under alternating light, a con-



TEXT FIG. 7. Survival of *Pomatiopsis cincinnatiensis* in different vivaria



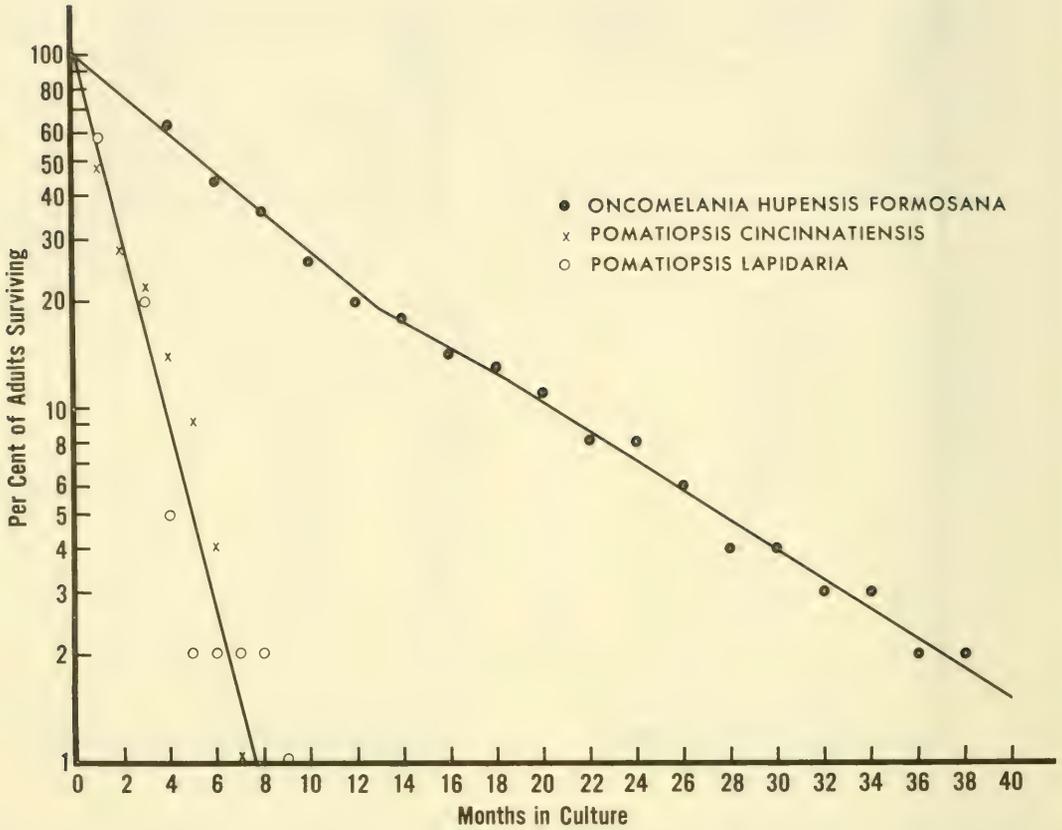
TEXT FIG. 8. Survival of *Pomatiopsis cincinnatiensis* in Medium Clay Pots under varying conditions of light

dition providing only an intermediate type of survival for the species of *Pomatiopsis* (Text Figs. 6, 9). The finite rate of mortality for *O. h. formosana* was 12% per month while the rate was 45% per month for both species of *Pomatiopsis*.

Survivorship curves for *P. cincinnatiensis* and *P. lapidaria* are compared for 5 different environments in Text Figs. 10-13. *P. lapidaria* is distinctly separated from *P. cincinnatiensis*, on the basis of superior survivorship, when maintained in the Medium Clay Pot vivarium under alternating or room level light. *P. cincinnatiensis* survives in that vivarium under constant light (Text Fig. 11) as well as does *P. lapidaria* at room level light (Text Fig. 10) for 3

months; however, its rate of mortality increases markedly in the 4th month. This indicates that the 2 species have a comparable rate of mortality in their respective optimal environments but that the specific differences in longevity account for the difference in rates of mortality after the 4th month. In the first few months both species have a finite monthly rate of mortality of 16% comparable to that of 12% for *O. h. formosana* in the Plastic Tray vivarium.

*Pomatiopsis lapidaria* has greater rates of mortality than *P. cincinnatiensis*, when both are maintained in the same vivarium types, under constant light (Text Figs. 11, 12). Constant light



TEXT FIG. 9. Comparative survivorship in the Plastic Tray vivaria under alternating light

was as detrimental for *P. lapidaria* in the Plastic Tray culture (Text Fig. 12), as room level light was for *P. cincinnatiensis* in the culture condition proving to be optimal for the former, i.e., the Medium Clay Pot (Text Fig. 10).

Plastic Tray and Tall Clay Pot vivaria under alternating light provided an environment in which an intermediate level of survival occurred for both species, i.e., an exponential rate of mortality of about 0.60, corresponding to a finite rate of mortality of about 45% per month (Text Figs. 9, 13).

The survivorship of *P. lapidaria* under "optimal" laboratory conditions very closely approximate that of *O. h. formosana* in the Battery Jar environment, considered detrimental to the latter

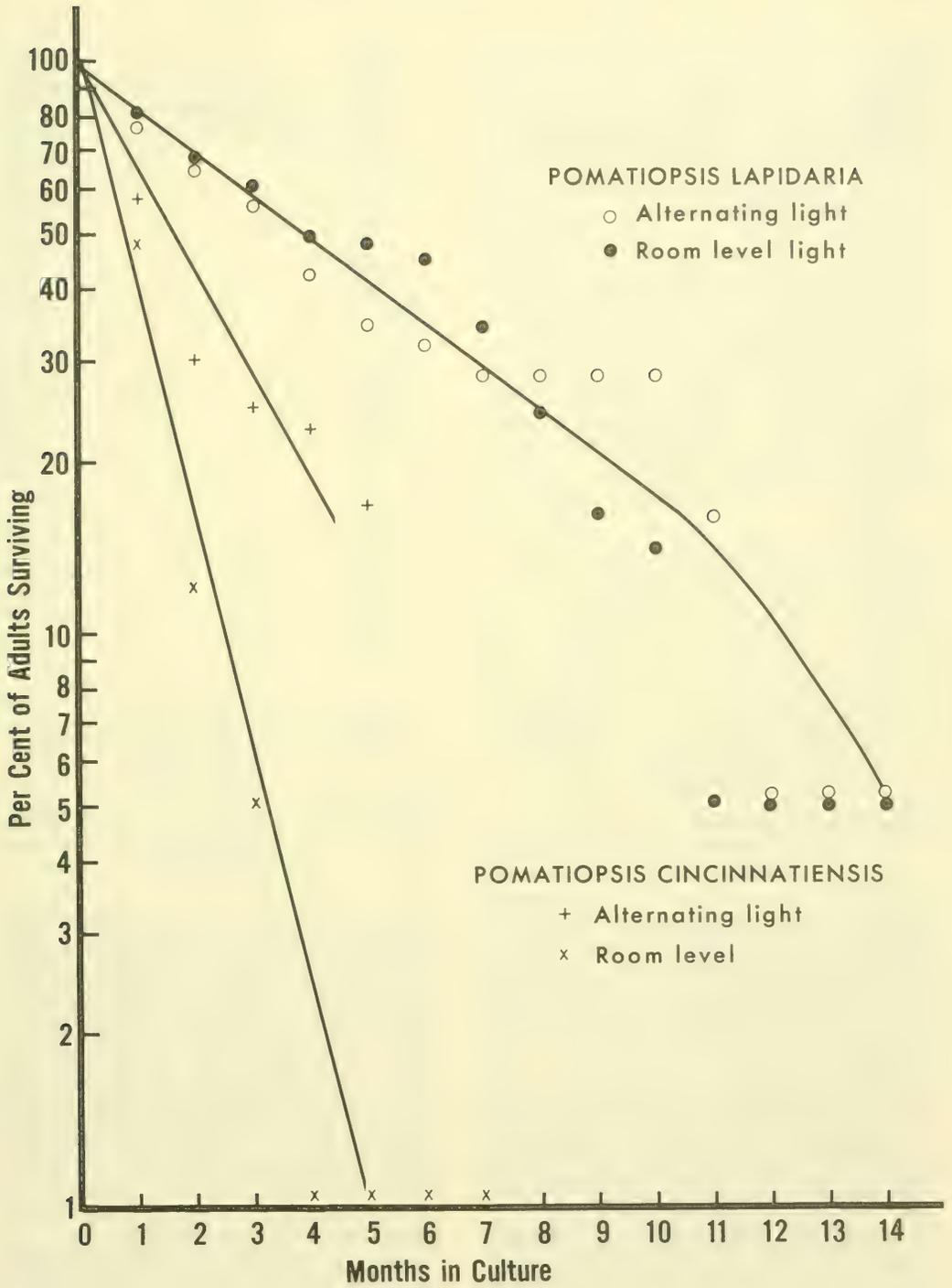
species. In the Battery Jar vivaria, *P. lapidaria* suffered a 50% mortality within 1 month and none survived past 4 months.

## 2. Productivity

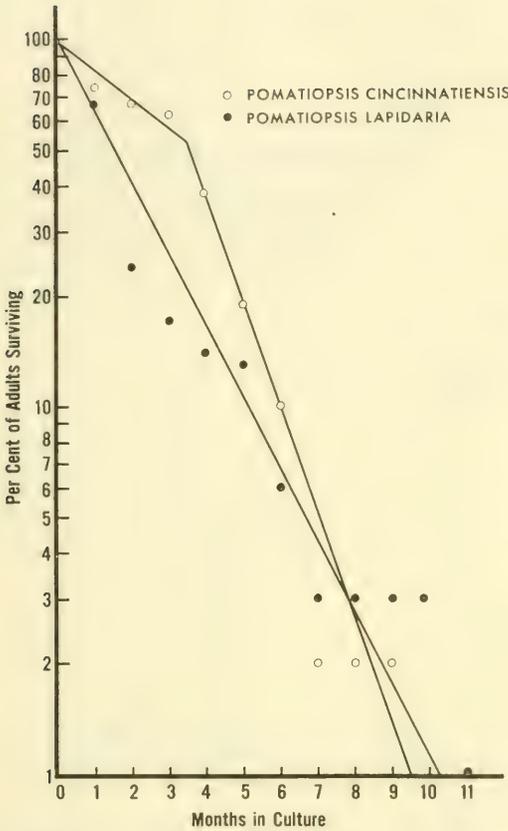
The production of young is a function of survivorship of the female, of the capacity of the female to lay a number of eggs per unit time over her lifespan and of a suitable environment which encourages egg laying and hatching.

Table 17 gives for each species, expressed in percentages, the proportion of producing vivaria for each type of environment provided, the distribution of young in those types and the average number of young per producing aquarium.

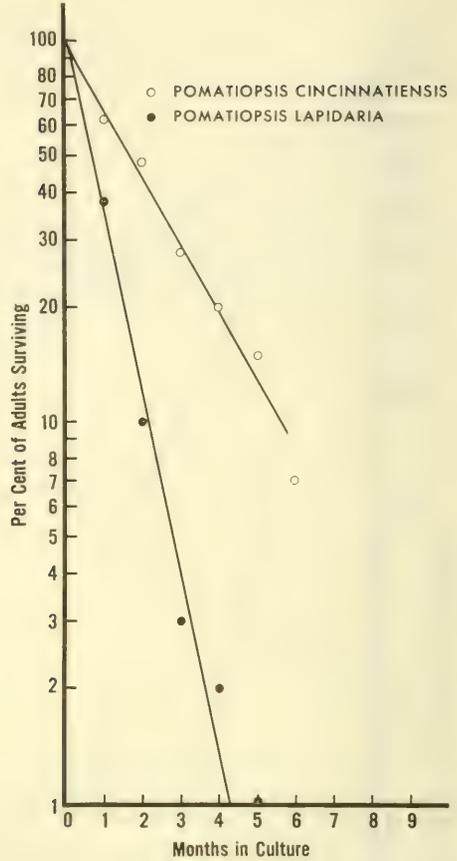
Hatchlings of *Oncomelania hupensis*



TEXT FIG. 10. Comparative survivorship in Medium Clay Pot vivaria under different lighting conditions



TEXT FIG. 11. Comparative survivorship in Medium Clay Pot vivaria under constant light



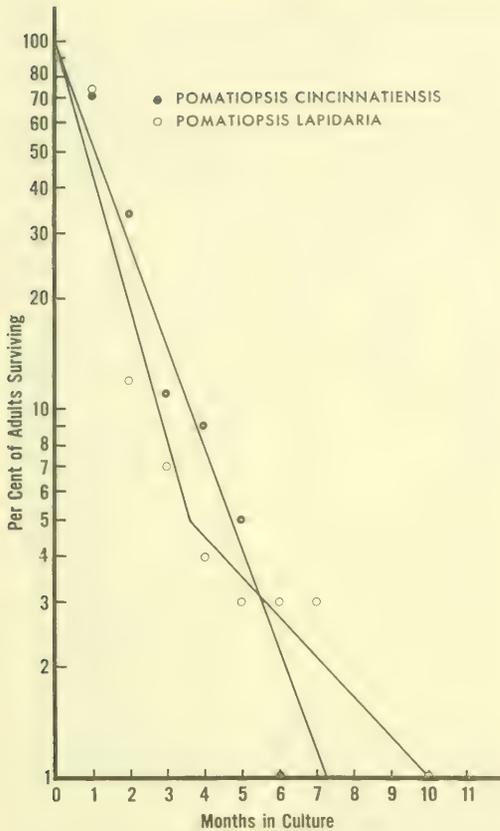
TEXT FIG. 12. Comparative survivorship in Plastic Tray vivaria under constant light

*formosana* were obtained in each of the 2 vivarium types used: 54% of them in Plastic Tray cultures and 46% in Battery Jar cultures. The average number per culture brought forth was 243 in the former and 148 in the latter, in spite of the fact that initially there were more females in the latter type of culture.

As regards *Pomatiopsis lapidaria*, only 50% of the Medium Clay Pots in room level light yielded offspring, and 40% of those in alternating light. Only a relatively small percentage of young were bred in the other culture types. Young from producing Medium Clay Pots in room level light accounted for 68% of all the hatchings, with an average of 9.4 per culture. The second highest

percentage was 19, the result of 1 Plastic Tray culture in alternating light, which yielded 27 juveniles. Although survival of *P. lapidaria* in Medium Clay Pots was about equal in alternating light and in room level light, only 1% of the young were produced under the former condition, but 68% under the latter. That the difference in the initial number of females did not account for the higher rate of reproduction is shown by the percentage of young per initial female, which was 0.04% against 0.65%.

Among the vivaria housing *Pomatiopsis cincinnatiensis*, 60% of the Plastic Tray vivaria in constant light, and 67% of all the Tall Clay Pots in alternating light were producers. However, only



TEXT FIG. 13. Comparative survivorship in Tall Clay Pot vivaria under alternating light

16% of the progeny originated in the former, while 80% came from the latter, with an average of 8.3 young in each of the producing cultures of the former and an average of 14.2 in the latter. The initial number of females was about equal in each type of culture, although there were about twice as many females per culture in the Plastic Tray vivaria. The greatest multiplication occurred in a vivarium where there was an intermediate rate of mortality. No young were generated in the culture providing the best rate of survival, i.e., Medium Clay Pots in constant light.

These comparisons become more meaningful when the number of young per female per unit time is considered.

Young per female per month were calculated for those cultures yielding 46% or more of the young (Table 18). The calculations are based on the survival of the females. Exact survivorship was not recorded for the females in particular, but spot checks on the cultures indicated a general trend of equal rates of mortality for both sexes. The data presented in Table 18, are therefore, only a rough estimate, but nonetheless serve to show real differences between the 3 species listed. *Oncomelania hupensis formosana* shows sustained production of offspring at much higher numbers of young/female/month than found for the other species. In the most productive month this number was 5 for *Pomatiopsis lapidaria* and 10 for *O. h. formosana*.

Accurate data are available for each of the 4 subspecies of *Oncomelania* maintained in Medium Clay Pots in room level light. With snails at 1 year of age, the greatest number of young/female/month in the respective optimum month was, for *O. h. hupensis*, 23; for *O. h. nosophora*, 22; for *O. h. quadrasi*, 51; and for *O. h. formosana*, 44.

It appears that the peak multiplication in the field at a given season does not carry over in the laboratory (Table 19). In that table, the percentage of the young produced each month is listed for *Oncomelania hupensis formosana*, *Pomatiopsis lapidaria* and *P. cincinnatiensis*. It was found that hatchlings of *P. lapidaria* appeared in culture 4-5 months after the culture was initiated. As most of the cultures were established in July or August, the greatest percentage of young were found in December and January. Offspring of *P. cincinnatiensis* were present in cultures 3-4 months after the cultures were established in August or September. Six cultures were set up in May and young appeared 1-2 months later. Due to rapid rates of mortality in the adults, no young were produced during a number of months.

TABLE 17. Vivaria in which young were found

Species	Vivarium type*	Light condition**	% of vivarium type producing young	% of young in producing vivarium	Average No. young per producing vivarium	
<i>Oncomelania hupensis formosana</i>	PT	Altern.	100	54	243	
	BJ	Room	100	46	148	
<i>Pomatiopsis lapidaria</i>	PT	Altern.	17	19	27	
		Const.	0	0	0	
	BJ	Room	0	0	0	
		MCP	Altern.	40	1	1
			Const.	22	7	4.5
	Room	50	68	9.4		
	TCP	Altern.	21	5	2.5	
<i>Pomatiopsis cincinnatiensis</i>	PT	Altern.	17	2	4.0	
		Const.	60	16	8.3	
	MCP	Altern.	0	0	0	
		Const.	0	0	0	
		Room	25	2	2	
	TCP	Altern.	67	80	14	
		Const.	0	0	0	

\* BJ = Battery Jar  
MCP = Medium Clay Pot  
PT = Plastic Tray  
TCP = Tall Clay Pot

\*\* Altern. = Alternating  
Const. = Constant  
Room = Room level

TABLE 18. The production of young per female per month in cultures which yielded 46% or more of the young

Species	Culture* condition	Light** condition	y/f/m	No. of months in production	Highest y/f/m in any month
<i>Oncomelania hupensis formosana</i>	PT	Altern.	0.88	26	2.19
	BJ	Room	2.12	21	10.0
<i>Pomatiopsis lapidaria</i>	MCP	Altern.	0.22	2	0.22
		Room	0.22	9	0.64
	PT	Altern.	. . .	1	5.0
<i>Pomatiopsis cincinnatiensis</i>	PT	Const.	0.39	3	0.48
	TCP	Altern.	0.50	1.5	1.29

\* BJ = Battery Jar  
MCP = Medium Clay Pot  
PT = Plastic Tray  
TCP = Tall Clay Pot

\*\* Altern. = Alternating  
Const. = Constant  
Room = Room Level

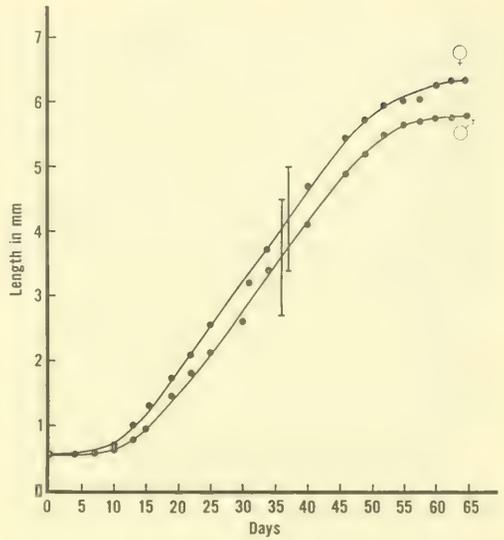
TABLE 19. The percentage of the young produced in the laboratory in each month of the year

Month	<i>Oncomelania</i>	<i>Pomatiopsis</i>	
	<i>hupensis</i> <i>formosana</i>	<i>lapidaria</i>	<i>cincinnatiensis</i>
Jan.	5	16	31
Feb.	8	10	1
Mar.	8	11	1
April	5	1	0
May	7	11	0
June	11	17	26
July	18	10	9
Aug.	10	7	0
Sept.	7	3	0
Oct.	3	3	0
Nov.	9	3	9
Dec.	9	31	24

### 3. Growth Rates and Survivorship of Young

Van der Schalie & Davis (1964, 1965) found that newly hatched young of *Oncomelania hupensis formosana*, maintained 1 or 2 per Petri Dish culture, grew more rapidly than snails reared in any other fashion. Petri Dish vivaria provided an environment where the snails grew, on the average, 0.65 mm per week for the first 8 weeks with a mortality below 10%. In Text Fig. 14, the growth curves for male and female *O. h. formosana* are presented. The measurements were made using 25 snails of each sex. The snails were maintained 1 per dish under constant light. It was later found that the same optimal growth occurred under alternate light.

Under the same conditions young *Pomatiopsis lapidaria* grew at a slow rate with a high mortality. Within 3 months 50% were dead and little growth had taken place (Text Fig. 15). Only 15% were alive at the end of 6 months. At the end of 1 year 5% remained of the 40 snails which started. Over a period of 6 months the average weekly increment in length was 0.13 mm. This rate is about the same as that given by Dundee (1957) for the



TEXT FIG. 14. Growth curves for male and female *Oncomelania hupensis formosana*

growth of this species in the laboratory.

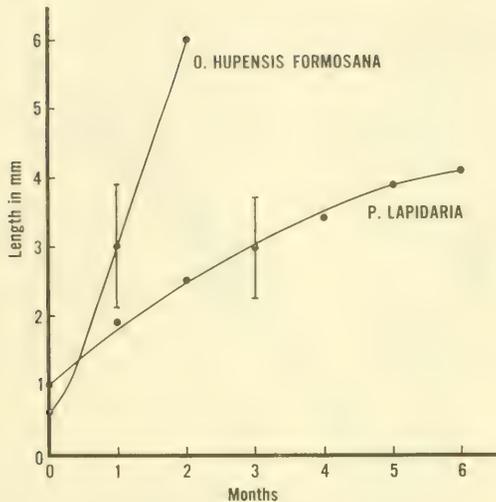
Comparing the growth of the 2 species between the ages of 1 and 1.5 months, *O. h. formosana* was found to grow at a rate of 0.95 mm per week as against 0.20 mm for *P. lapidaria*.

It was discovered that survival of the young could be increased by placing 5 young *P. lapidaria* in a Medium Clay Pot in room level light, or by leaving the young in the parental cultures. Of 15 snails observed under these conditions, 50% survived until the 9th month and at the end of 13 months 40% were still surviving. The growth rate, however, was somewhat lower, i.e., about 0.10 mm per week for the first 6 months (as against 0.13 mm).

*P. cincinnatiensis* barely lasted 5 months in Petri Dish vivaria. Of the 20 young snails studied, 50% were dead in 3 1/2 months and none survived past 5 months. In that period the average increment in length was 0.12 mm per week.

### D. Discussion

As has been reported by a number of



TEXT FIG. 15. Growth curves for *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana*

authors, the subspecies of *Oncomelania hupensis* can survive and reproduce under a variety of conditions which are not optimal environments. *O. h. formosana* survived well in the vivaria provided and produced young at an adequate rate to assure survival of the species in the laboratory. Van der Schalie & Davis (1968, in press) found that survival and productivity increased when the 4 subspecies were maintained in Medium Clay Pot vivaria.

There was a uniform, large gap between *Oncomelania* and *Pomatiopsis* with regard to longevity, production of young, growth rates of young and survival of the young in the laboratory. The 4 species of *Pomatiopsis* did not survive well in any of the vivaria provided, with 2 exceptions. *P. lapidaria* and *P. cincinnatiensis* showed survival in "optimal" environments, i.e., in Medium Clay Pots, at room level light for the former and at constant light for the latter, corresponding to what might have been predicted from knowledge of their life span in the field.

*P. lapidaria* produced more young per female in the environment which pro-

vided optimal conditions for survival. Although *P. cincinnatiensis* survived better under constant light, this species produced more young per female in the Tall Clay Pot vivarium type under alternating light, an environment in which the adults showed an intermediate rate of survival.

Data on the production of young per female per month as well as the greatest production per female for any given month indicate that 1 or several critical factors in the laboratory environments were either absent or detrimental. For instance, in the field *P. cincinnatiensis* reproduces throughout the summer with pronounced hatches of young in August and October. In August, I found 20 females and 155 young in a quadrat of 2 x 3 feet. The following month I found in the same quadrat 24 females and 525 young. This condition could be duplicated up and down the river banks for 30 feet in either direction and concerned a population known to be in a steady state for over 10 years. It is obvious that production of young per female per month in the field was many times that found for this species in the laboratory.

The 4 subspecies of *Oncomelania hupensis* appear more closely allied in their performance in the laboratory than the 2 species of *Pomatiopsis* here discussed in detail. Data from the laboratory studies show that *P. lapidaria* survives best under room level light, produces the greatest number of young in this optimal environment, and had a finite rate of mortality of 16% over 8-9 months. *P. cincinnatiensis* survives best under constant light, but produces more young in a Tall Clay Pot under alternating light, a condition where the finite mortality rate was 49% per month for 7 months.

#### CONCLUDING DISCUSSION

A large number of similarities between *Oncomelania* and *Pomatiopsis* have been discussed by many authors. These similarities have mainly pertained to

subfamily characteristics such as the general arrangement of organs in the body, an amphibious mode of existence, eggs laid singly out of water and coated with a mud capsule, the distinctive step-like manner of progression, and the structure of the central tooth of the radula where the basal cusps arise from the face of the tooth instead of from the lateral angle.

Enough data are now available to state that *Oncomelania* and *Pomatiopsis* are distinct genera within the Pomatiopsinae, a hydrobiid subfamily which also includes *Tomichia* from South Africa and *Blanfordia* from Japan.

The genera *Pomatiopsis* and *Oncomelania* will not hybridize. Reference was made to the cytological differences, such as sex determining mechanisms, between *Oncomelania* and *P. lapidaria* and *P. cincinnatiensis*.

Major anatomical differences between the 2 genera are listed in Table 12. Of particular importance are the facts that: 1) In *Oncomelania* the sperm and spermathecal ducts arise together from the right ventrolateral surface of the bursa copulatrix, bound together as slender tubes in a connective tissue sheath while, in *Pomatiopsis*, the sperm duct arises from the spermathecal duct near the point where the latter emerges as a broad tube from the anterior end of the bursa copulatrix. 2) Unlike *Pomatiopsis*, the verge of *Oncomelania* has a protrudable papilla and the tip of the verge is quite muscular. Some species of *Pomatiopsis* have a penial filament.

In the laboratory *Oncomelania* is characterized by the comparative ease with which it adapts to laboratory culture and by the rapid growth of young. *Pomatiopsis* (all species) does not adapt to the laboratory environment in which *Oncomelania* thrives; the growth rate of the young is extremely slow compared with that of young *Oncomelania*.

Van der Schalie & Getz (1963) pointed out that *Pomatiopsis lapidaria* and *P. cincinnatiensis* were more tolerant of

low temperatures ( $-5^{\circ}$  to  $-7^{\circ}$  C) and less tolerant of high temperatures ( $41^{\circ}$  to  $44^{\circ}$  C) than *Oncomelania*; these 2 species of *Pomatiopsis* were less resistant to drowning than *Oncomelania*.

Several of the "specific" differences are most probably indicative of characters applicable to the generic level. For instance the electrophoretic differences between *O. h. formosana* and *Pomatiopsis lapidaria* are of no more than specific order; however, preliminary data for the other subspecies of *Oncomelania hupensis* indicate similarities among these latter in the dense, fast moving proteins, which do not occur in *P. lapidaria*.

Davis (1964) indicated a distinct difference between *P. lapidaria* and *O. h. formosana* in the potential for shell regeneration. *O. h. formosana* rapidly regenerated a shell in the apical whorls with low mortality, while *P. lapidaria* had a high mortality and showed no signs of shell regeneration.

*Oncomelania* is a genus with but 1 species; it has 4 well established<sup>5</sup> subspecies. The subspecies of *Oncomelania* do not differ greatly in their internal anatomy. External differences are mainly those of size, ribs on the shells of some populations of *O. hupensis hupensis*, and variance in the intensity of external pigmentation and the yellow coloration of the granules surrounding the medial surface of the eye.

In *Oncomelania* the shell is usually smooth (except for the above mentioned ribs), with moderately deep sutures and with moderately convex whorls. The outer lip of the shell has a tendency to form a varix which is usually quite pronounced and is sinuate. The umbilicus is narrow and the apical whorl narrow.

<sup>5</sup>Davis (1968, in press) discussed the systematic position of the so-called *Tricula chiui*; this taxon is now assigned to *Oncomelania*, as a 5th subspecies of *O. hupensis*, *O. hupensis chiui*, on the basis of anatomical, electrophoretic and serological data.

The parietal callus is elongate. There are at least 35 gill filaments, usually 45 or more. The verge is muscular, the tip has short strips of actively beating cilia and a distinct protrudable papilla. The pleuro-supraesophageal connective is relatively short. The osphradio-mantle nerve and supravisceral connective both arise from the tip of the supraesophageal ganglion. The former nerve is elongate and most commonly bifurcates only within the lateral wall of the "neck" into the osphradial nerve and mantle nerve. The sperm and spermathecal ducts arise together from the ventral, right anterolateral surface of the bursa copulatrix. The female gonad is multibranched and the collecting duct of the gonad relatively slender. The oviduct encircles the seminal receptacle in a characteristic manner. The seminal vesicle is composed of a slender tube which is characteristically knotted. The verge has a single glandular type. The cerebral commissure is short. The tentacles are elongate relative to the length of the rostrum.

*Pomatiopsis* is a genus composed of 4 distinct species of which only *P. lapidaria* and *P. cincinnatiensis* are known in terms of anatomy and life history. *P. californica* and *P. binneyi* are virtually unknown except for general habitat and shell. A 5th form, *P. chacei*, is most like *P. californica*; the type description given by Pilsbry (1936) is not sufficient to separate this form as a distinct species. Another species, *P. robusta* Walker, was listed by Abbott (1948a) with *Pomatiopsis*. However, Pilsbry (1933) had removed this species from *Pomatiopsis* with justification, as the radula is clearly not of the type found in the Pomatiopsinae. He assigned it to *Amnicola*. Gregg & Taylor (1965) have placed this species in a new genus and subgenus, *Fontelicella (Natricola) robusta*.

*Pomatiopsis binneyi* is different from the other species in the genus on the basis of shell and habitat, although there are similarities on the basis of some

subfamily characteristics. The shell is only about 3 mm high, without an umbilicus; the inner and outer lips meet without forming a parietal callus and are generally slightly separated from the parietal surface of the body whorl. The species lives high on Mt. Tamalpais in Marin County, California. The snails live in dense shade on the surfaces of rocks and leaves, either in the path of trickling water or sprayed by water streaming down steep canyon slopes. This habitat is markedly different from those inhabited by the other species of *Pomatiopsis*. *P. californica* lives on shallow mud banks and marshy seepages leading into shallow streams. The area I observed was a lowland habitat on the edge of Bolinas Bay, Marin County, California. *P. cincinnatiensis* lives on a narrow margin of river bank while *P. lapidaria* survives in marshy *Typha* swamps, grassy seepages leading into rivers, or wooded lowlands seasonally swampy due to stream overflow. Much of the swampy areas inhabited by *P. lapidaria* closely resemble the habitat of *Oncomelania hupensis quadrasi* observed in the Philippines.

Because *Pomatiopsis binneyi* appears so different on the basis of shell and habitat it should be studied thoroughly to determine whether it is, indeed, a species of *Pomatiopsis*.

*Pomatiopsis* is currently defined as a genus in which the shell has a roughened microsculpture, the lip is sharp, straight, and there is no tendency to form a varix. The apical whorls are wide. The umbilicus is wide and pronounced. The sutures are deeply impressed and the whorls very convex. There are less than 30 gill filaments. The verge does not have the pronounced musculature or papilla found in *Oncomelania*. Penial cilia are lacking in 2 species (*P. lapidaria* and *P. cincinnatiensis*); in the 2 other species they are bushy and generally not active. Two species have penial filaments (*P. cincinnatiensis* and *P. californica*), a feature not found in *Oncomelania*, *Blanfordia*,

or *Tomichia*. The verge has 3 glandular types (known for *P. lapidaria*). The pleuro-supraesophageal connective is elongate. The distance from the tip of the supraesophageal ganglion to the lateral cephalic wall is very short. The osphradiomantle nerve is quite short and frequently bifurcates, forming the osphradial and mantle nerves, before entering the lateral wall. The supravisceral connective leaves the posterior edge of the ganglion, not the tip (known only for *P. lapidaria* and 1 specimen of *P. binneyi*). The spermathecal duct arises from the anterior end of the bursa copulatrix. The female gonad is little branched and the collecting duct is very wide. The oviduct does not encircle the seminal receptacle. The seminal vesicle is a thick, regularly coiled tube, not a "knot" of tubes. The tentacles are relatively short, compared to the length of the rostrum.

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## RESUMEN

RELACIONES SISTEMATICAS DE *POMATIOPSIS LAPIDARIA* Y  
*ONCOMELANIA HUPENSIS FORMOSANA* (PROSOBRANCHIA)

G. M. Davis

*Pomatiopsis lapidaria* (Say) de Norte América y la *Oncomelania hupensis formosana* (Pilsbry & Hirase) oriental, se eligieron como representantes de dos géneros relacionados de hydróbridos. Se estudió la anatomía comparada, hibridación potencial, propiedades electroforéticas y ecología de laboratorio, para determinar en que alcance pueden encontrarse valores sistematicos.

En base a sus anatomías *Pomatiopsis* y *Oncomelania*, se juzgan como géneros distintos dentro de la misma subfamilia Pomatiopsinae.

En el género *Oncomelania* (considerado como formado por una especie con 4 subespecies), la concha es lisa (excepto en la forma costulada *O. hupensis hupensis*) con suturas de profundidad moderada y anfractos convexos. El labio externo tiene tendencia a formar una varice muy pronunciada. El ombligo es estrecho, así como el anfracto apical. El callo parietal es alargado. Tiene por los menos 35 filamentos branquiales y generalmente 45 o más. La verga es muscular, la punta con bandas de activas ciliias y una papila sobresaliente. Los conectivos pleuro-supraesofágicos comparativamente cortos: en consecuencia el nervio manto-osfrádico elevándose de la punta del ganglio supraesofágico es relativamente largo: generalmente no se bifurca hasta estar adentro de la pared cefálica. El conectivo supravisceral también surge de la punta del ganglio. El espermoducto y ducto de la espermateca surgen de la derecha en una sola vaina, en la superficie antero lateral de la bursa copulatrix. El oviducto rodea el receptáculo seminal en una manera característica. La verga tiene un tipo glandular único (estudiada en *O. h. formosana* y *H. quadrasi*). La comisura cerebral es corta. Los tentáculos son alargados comparados con la longitud del rostro.

Comparada con *Oncomelania*, la concha de *Pomatiopsis* tiene una microescultura rugosa, el labio es agudo y notiene tendencia a formar varice. En las cuatro especies las vueltas apicales son anchas. El ombligo es dilatado y pronunciado, suturas profundamente impresas y los anfractos muy convexos (excepto in *P. binneyi*). In *P. lapidaria* y *P. cincinnatiensis* hay menos de 30 filamentos branquiales. La verga no tiene una musculatura pronunciada o papila, en las cuatro especies; ciliias peniales faltan en 2 especies (*P. lapidaria* y *cincinnatiensis*); cuando aparecen ciliias estan aglomeradas y generalmente inactivas (*P. californica* y *binneyi*). Dos especies (*cincinnatiensis* y *californica*) tienen filamentos peniales, una condición que no se encuentra en *Oncomelania*. La verga (tal como es conocida en *P. lapidaria*) tiene tres tipos glandulares. El conectivo pleuro-esofágico es alargado, el ganglio supraesofágico descansa cerca de la pared cefálica lateral y los nervios del manto y osfrádico, el cual generalmente se bifurca a partir de la punta del ganglio, son correspondientemente mas cortos. El conectivo supravisceral, en *P. lapidaria*, surge del lateral, borde posterior del ganglio supraesofágico, no de la punta. El oviducto no encierra la vesícula seminal. El ducto de la espermateca surge del extremo anterior de la bursa copulatrix (*P. lapidaria* y *P. cincinnatiensis*), y el ducto espermático del ducto de la espermateca. La gonada femenina es un poco ramificada, y el ducto colector bastante ancho. La vesícula seminal forma un tubo grueso regularmente arrollado. Los tentáculos son cortos, en relación a la longitud del rostro.

No se conocen híbridos entre *Pomatiopsis lapidaria* y *Oncomelania*.

Estudios disco-electroforéticos de proteína fresca del músculo pedal de los dos taxa representativos mostró que cada taxón tiene un patrón específico. Todas las subespecies de *Oncomelania* tienen 1 o más componentes protéicos densos con valores Rf (proporción de la distancia desde el origen a el centro de cada banda y del origen al frente) mayor que 0.75. *Pomatiopsis lapidaria* no tiene proteínas densas de movimientos rápidos más allá de un Rf de 0.75.

Las cuatro subespecies de *Oncomelania* se caracterizan por adaptabilidad a las condiciones de cultivo en laboratorio. En 12 meses, bajo condiciones menos que óptimas, la mortalidad (caracoles de natural habitat cerca de 1 año de edad) fué de 12% por mes. Los jóvenes crecieron 0.65 mm por semana con mortalidad baja. Las hembras produjeron crías en proporción de 2.12 mensualmente por más de 2 años.

Las 4 especies de *Pomatiopsis* investigadas no se adaptaron bien a condiciones de laboratorio. *P. californica* y *P. binneyi* murieron rapidamente sin reproducirse. *P. lapidaria* y *P. cincinnatiensis* (de habitat natural, 1 año de edad) tuvieron 16% de mortalidad por mes en condiciones "óptimas" sobre un periodo de 10 meses para el primero y 3 meses para el segundo, después de lo cual la mortalidad aumentó rapidamente, en parte por razón del más corto término de vida de esos caracoles. Jóvenes crecieron menos de 0.14 mm por semana con mortalidad excediendo el 30% en 2 meses. Jóvenes fueron producidos en proporción de menos de 0.51 por hembra mensualmente por periodos cortos.

#### АБСТРАКТ

СИСТЕМАТИЧЕСКИЕ ВЗАИМОТНОШЕНИЯ МЕЖДУ *POMATIOPSIS LAPIDARIA*  
И *ONCOMELANIA HUPENSIS FORMOSANA* (PROSOBRANCHIA; HYDROBIIAE)

Г. М. ДЕВИС

Для исследования были выбраны представители двух родственных родов гидробиид: северо-американский вид *Pomatiopsis lapidaria* (Say) и восточный *Oncomelania hupensis formosana* (Pilsbry & Hirase). Изучалась сравнительная анатомия этих форм, потенциальные возможности для гибридизации, электрофоретические свойства и лабораторная экология; все это должно было определить, насколько имеющиеся различия имеют ценность для систематики.

С точки зрения анатомии *Pomatiopsis* и *Oncomelania* считаются вполне самостоятельными родами одного и того же подсемейства *Pomatiopsinae*.

У представителей рода *Oncomelania* (имеющего 1 вид с 4 подвидами), раковина гладкая (исключая ребристую форму *O. hupensis hupensis*), с умеренно-глубокими швами и умеренно-выпуклыми оборотами. Наружная губа раковины имеет тенденцию образовывать поперечный гребень (*varix*), обычно довольно выдающийся. Пупок (*umbilicus*) узкий, как и апикальный оборот. Париетальный каллус удлинённый. Имеется по крайней мере 35 жаберных филаментов, обычно 45 или больше. Край мантии мускулистый, на конце имеются короткие ряды активно-работающих ресничек и ясно-выдающаяся папилла. Плевронадглоточная коннектива сравнительно короткая; соответственно, осфрадийно-мантийный нерв, отходящий от верхушки надглоточного ганглия, относительно длинный; он обычно не раздваивается вплоть до внутренней части цефалической стенки. Суправисцеральная коннектива также отходит от верхушки

ганглия. Семепровод и семеприемник находятся в общей оболочке и отходят от передне-боковой поверхности совокупительной сумки. Яйцевод характерным образом опоясывает семеприемник. Железа края мантии единого типа (изучена на *O. h. formosana* и *O. h. quadrasi*). Церебральная комиссура короткая. Тентакулы, по сравнению с длиной рострума, удлиненные.

По сравнению с *Oncomelania*, раковина *Pomatiopsis* имеет более грубую микроскульптуру; губа раковины острая и не имеет тенденции образовывать гребень (*varix*). Пупок широкий и хорошо развитый; швы глубоко вдавленные, обороты очень выпуклые (кроме *P. binneyi*). У *P. lapidaria* и *P. cincinnatiensis* имеется менее 30 жаберных филаментов. У 4 видов край мантии не имеет хорошо выраженной мускулатуры и папилл; у 2 видов (*P. lapidaria* и *P. cincinnatiensis*) пениальные реснички отсутствуют; когда реснички имеются, они обычно густые и мало активные (*P. californica* и *P. binneyi*). У двух видов (*P. cincinnatiensis* и *P. californica*) имеются пениальные филаменты, отсутствующие у *Oncomelania*. Край мантии имеет железы трех типов (*P. lapidaria*). Плевро-надглоточная коннектива удлиненная, надглоточный ганглий расположен близ боковой части цефалической стенки, а осфрадийный и мантийный нервы, которые обычно раздваиваются сразу после отхода их от верхушки ганглия, соответственно довольно короткие. Суправисцеральная коннектива у *P. lapidaria* отходит от бокового заднего края надглоточного ганглия, а не от его верхушки. Яйцевод не опоясывает семенной пузырек; протока сперматеки отходит от переднего конца совокупительной сумки (*P. lapidaria* и *P. cincinnatiensis*), а семепровод - от протока сперматеки. Женская половая железа слабо-разветвленная; собирающий проток довольно широкий. Семенной пузырек в виде толстой правильно-извитой трубки. Тентакулы относительно длины рострума короткие.

Гибридизация между *Pomatiopsis lapidaria* и *Oncomelania* не наблюдается.

Исследование белков из свежего ножного мускула у представителей двух групп моллюсков методом дискового электрофореза показало, что каждый таксон имеет в этом отношении свои специфические компоненты. Все подвиды *Oncomelania* имеют 1 или более характерных плотных протеиновых компонентов с величинами *Rf* (отношение расстояния от источника до центра в каждой полосе и от источника до переднего края) более 0.75. *Pomatiopsis lapidaria* не имеет плотных быстро "двигающихся" белков, со значениями *Rf* более 0.75.

Все 4 подвиды *Oncomelania* характеризуются способностью адаптироваться к искусственным лабораторным условиям. За 12 месяцев при условиях, далеко не оптимальных, конечная скорость отмирания улиток (взятых из природных условий в возрасте 1 года), составляла 12% в месяц. Темп роста молоди был около 0.65 мм в неделю, при малой смертности. Непрерывно в течение более 2 лет продукция молоди составляла 2.12 на 1

самку в месяц. Четыре исследованных вида *Pomatiopsis* не адаптировались достаточно хорошо к лабораторным условиям. *Pomatiopsis californica* и *P. binneyi* быстро отмирали, не давая потомства. *P. lapidaria* и *P. cincinnatiensis* (собранные в возрасте около 1 года) имели конечную скорость отмирания 16% в месяц, при "оптимальных" условиях за период более 10 месяцев для первого вида и за 3 месяца для второго; после этого периода темп отмирания быстро возрастал, отчасти благодаря более короткому жизненному циклу этих видов. Прирост молоди составлял по крайней мере 0.14 мм в неделю, а смертность - более 30% за 2 месяца. Продукция молоди была 0.51 на 1 самку в месяц за довольно короткий промежуток времени.



SUSCEPTIBILITY OF *ONCOMELANIA HUPENSIS CHIUI* TO  
INFECTION WITH *SCHISTOSOMA JAPONICUM* <sup>1</sup>

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ABSTRACT

A small amphibious hydrobiid snail, *Oncomelania hupensis chiui* (originally described as *Tricola chiui*) from the northern tip of Taiwan, known to act as the snail host of *Paragonimus iloktsuenensis*, was found to be able to serve as a good intermediate host for both the zoophilic (Formosan-Changhua) and human (Japanese) strains of *Schistosoma japonicum*. Since the snail host (*O. h. formosana*) of *S. japonicum* in Taiwan from different locations is either refractory or only slightly susceptible to human strains of *S. japonicum* from Japan and the Philippines, this discovery of an efficient potential host brings the establishment of human schistosomiasis within the range of possibility in Taiwan.

The experiments indicated that this snail showed a varying degree of susceptibility to the 2 geographic strains of *S. japonicum* and is, on the whole, a more suitable host for the Changhua strain than for the Japanese strain. As high an infection rate as 100% was obtained for the Changhua strain of *S. japonicum*, when cercariae were shed from the snails 95 days after exposure to 6 miracidia individually. Interesting results were obtained for the Japanese strain. Snails exposed in pairs to 20 miracidia were 100% infected but did not produce infective cercariae. Only the snails exposed to 5-7 miracidia individually, and infected at the rate of 22.2%, shed cercariae 105 days after infection.

INTRODUCTION

In the course of a study on the trematode genus *Paragonimus* in Taiwan, a small amphibious hydrobiid snail was incriminated as the first intermediate host of *P. iloktsuenensis* (Chiu, 1961, 1965b). This snail was subsequently named *Tricola chiui* by Habe & Miyazaki in 1962. Habe & Miyazaki also stated that the species was allied to *Oncomelania formosana*, the snail host of *Schistosoma japonicum* in Taiwan. This raised the

question of whether each of these snails would prove susceptible to the trematode parasite of the other. As a result, it was found that the snail host of the lung fluke could indeed also carry the blood fluke (Chiu, 1965a). While investigations were in process, a systematic malacological study was made on this snail by Dr. G. M. Davis. He came to the conclusion that the so-called *Tricola* was in fact *Oncomelania* (Davis, 1968). Since he considers all so-called "species" of *Oncomelania* as subspecies of *O.*

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*hupensis*, this new species is now called *O. hupensis chiuvi*.

The present paper gives further detail on the experimental infection of this snail with the non-human Formosan-Changhua strain of *S. japonicum* and, in addition, reports susceptibility to the human Japanese strain of schistosome.

#### MATERIALS AND METHODS

*Oncomelania hupensis chiuvi* (Habe et Miyazaki) were collected from the type locality, Alilao village, Taipei County (northern tip of Taiwan) where schistosomes have not been found. The eggs of the zoophilic Changhua strain (central part of the western coastal plain of Taiwan) of *Schistosoma japonicum*, used for infecting the snails, were obtained from the liver of a rabbit and a mouse that had been experimentally infected and kept in the laboratory for 40-50 days after infection. Eggs of the human Japanese strain of *S. japonicum* were secured from the liver of a mouse with a 39 day old infection. The infected *Oncomelania nosophora* used for infecting the mouse were obtained from the 406th Medical Laboratory in Japan. These snails were experimentally infected with a strain of *S. japonicum* originally from Yamanashi, Japan.

Mature *Oncomelania hupensis chiuvi* snails collected from the field were exposed to miracidia either individually or in pairs in small glasses, allowing 3-5 hours for penetration at  $26^{\circ} \pm 2^{\circ}\text{C}$ . After exposure, the snails were kept in clay flower-pots in the laboratory as previously described (Chiu, 1965b) at room temperatures varying between  $15^{\circ}$  and  $32^{\circ}\text{C}$ . Snails were crushed at different times after exposure to miracidia, to determine infection. Cercarial emergence was checked by isolating snails in a small glass of water for 5 hours in the morning.

#### EXPERIMENTS

##### Experiment 1.

A preliminary experiment designed to investigate the susceptibility of *Oncomelania hupensis chiuvi* to infection with the Changhua strain of *Schistosoma japonicum* was made as follows: 25 snails were exposed individually to 2-3 miracidia for 5 hours on April 9, 1964. The miracidia were hatched from eggs obtained from the liver of a rabbit which had been infected in the laboratory with cercariae of *S. japonicum* shed from naturally infected snails. The results obtained from this experiment have been partly reported before (Chiu, 1965a).

Upon 1st examination, 79 days after infection (Table 1), schistosome sporocysts were found in both snails dissected. These sporocysts contained embryos in various stages. Immature cercariae were seen within some daughter sporocysts. Mature cercariae were found emerging from the snails 95 days after exposure to miracidia, and 2 snails crushed contained sporocysts and cercariae. Similar positive findings were made on the 121st day after infection, in 1 of 3 snails dissected and on the 127th as well as on the 153rd days in 2 of 3 snails crushed. Cercarial emergence was still observed among the 5 surviving snails on the 243rd day after infection. However, on the 282nd day, the 3 surviving snails failed to shed the cercaria and these snails were found dead a month later.

In summary, 9 of 13 snails crushed (69.2%) were found infected with the schistosomes. This experiment showed that *O. h. chiuvi* is readily infected with the Changhua strain of *Schistosoma japonicum*.

The infectivity of the cercariae shed from *Oncomelania hupensis chiuvi* was confirmed by means of animal infection.

TABLE 1. Examination of *Oncomelania hupensis chiui* snails exposed to 2 - 3 miracidia each of the Changhua strain of *Schistosoma japonicum*

Date examined 1964-1965	Days after infection	No. snails examined*	No. snails infected	Larval stages found
June 27	79	2	2	Sporocysts
July 13	95	(21)	(Shedding)	Cercariae
		2	2	Sporocysts & Cercariae
Aug. 8	121	3	1	Sporocysts & Cercariae
Aug. 14	127	3	2	Sporocysts & Cercariae
Sept. 9	153	3	2	Sporocysts & Cercariae
Oct. 30	204	(9)	(Shedding only)	Cercariae
Dec. 8	243	(5)	(Shedding only)	Cercariae
Jan. 16	282	(3)	(Shedding only)	(-)
Total dissected		13	9 (69.2%)	

\*Numbers in parentheses designate snails that were not dissected.

TABLE 2. Examination of *Oncomelania hupensis chiui* snails exposed to 6 miracidia each of the Changhua strain of *Schistosoma japonicum*

Date examined 1964	Days after infection	No. snails examined	No. snails infected	Larval stages found
Sept. 20	27	1	1	Sporocysts
Oct. 4	41	1	1	Sporocysts
Nov. 27	95	(6)	(Shedding only)	Cercariae
Dec. 7	105	6	6	Sporocysts & Cercariae
Total dissected		8	8 (100%)	

Three mice were exposed to an undetermined number of cercariae from 2 positive snails for 2 hours, and at autopsy, 40 days later, adult *Schistosoma japonicum* were collected.

#### Experiment 2.

Another experiment, with heavier exposure to miracidia, was made to gain further insight concerning the infectivity of *Oncomelania hupensis chiui* as regards the Changhua strain of *Schistosoma japonicum* in the laboratory. Fifteen snails were exposed individually to 6 miracidia for 4 hours on August 24,

1964. The miracidia were hatched from eggs obtained from the liver of a mouse infected in Experiment 1.

Upon dissection of 1 snail each on the 27th day and the 41st day after infection, sporocysts were encountered in both (Table 2). On the 95th day, cercarial shedding was demonstrated in the 6 surviving snails. Ten days later, these were dissected, and all 6 were found positive for sporocysts and cercariae.

The infection rate among those snails that had survived in this experiment was 100%. This high rate indicates that *Oncomelania hupensis chiui* pos-

TABLE 3. Examination of 2 groups of *Oncomelania hupensis chiui* snails exposed to different number of miracidia of the Japanese strain of *Schistosoma japonicum*

Date examined 1965	Days after infection	A exposed to 5-7 miracidia each			B exposed to 10 miracidia each on the average		
		No. exam.	No. infect.	Larval stages found	No. exam.	No. infect.	Larval stages found
March 20	4	-	-		1*	?	(-)
March 22	6	-	-		1*	?	(-)
March 25	9	-	-		1	1	Sporocysts
April 8	23	3	1	Sporocysts	2	2	Sporocysts
April 15	30	1	1	Sporocysts	1	1	Sporocysts
April 22	37	-	-		1	1	Sporocysts
May 4	49	2	1	Sporocysts	-	-	
May 11	56	7	1	Sporocysts	1	1	Sporocysts
May 18	63	2	1	Sporocysts	-	-	
May 27	72	-	-		1	1	Sporocysts
June 19	95	(30)	(Shedding)	(-)	(10)	(Shedding)	(-)
June 29	105	(20)	(Shedding)	Cercariae (Sporocysts & Cercariae Sporocysts & Cercariae	(7)	(Shedding)	(-)
July 5	111	5	2		1	1	Sporocysts
July 12	118	20	3		1	1	Sporocysts
Sept. 6	174	5	0	(-)	2	2	(Sporocysts & de- generating cercariae
Totals dissected		45	10 (22.2%)		11*	11 (100%)	

\*The two snails examined as early as the 4th and 6th days after infection are excluded from the total.

sesses a high degree of susceptibility to infection with the Changhua strain of *Schistosoma japonicum*. The snail should be an excellent host for maintaining the life cycle of the Changhua strain of *S. japonicum* in the laboratory unless the schistosome should adapt specifically to *O. h. chiui*.

### Experiment 3.

The susceptibility of *Oncomelania hupensis chiui* to the Japanese strain of *Schistosoma japonicum* was tested

on 2 groups of snails, A and B, using 2 different doses of miracidial exposure: 75 snails in Group A were exposed individually to 5-7 miracidia, and 42 snails in Group B were exposed in pairs to 20 miracidia, for 3 hours, on March 16, 1965.

Sporocysts were first detected in the internal organs of a snail from Group B 9 days after infection (Table 3), none having been encountered in the 2 snails examined earlier. However, these 2 snails were very possibly infected, since

TABLE 4. Infection percentages of *Oncomelania*\* from different geographic locations with 4 strains of *Schistosoma japonicum* reported by various workers

Species of <i>Oncomelania</i>	<i>Schistosoma japonicum</i> strain			
	Chinese	Japanese	Philippine	Formosan (Changhua)
	%	%	%	%
<i>O. h. hupensis</i> (China)	34 (D)	13 (D)	20 (HH)	0 (D)
<i>O. h. nosophora</i> (Japan)	0 (D)	21 (D) 44.4 (HRO)	9.6 (HH)	21 (D)
<i>O. h. quadrasi</i> (Philippines)	0 (D)	35.7-43.8 (MW) 0 (D)	44-75 (P) 28.7-45.0 (MW)	6.4 (D)
<i>O. h. formasana</i> (Taiwan)				
- Changhua	0 (D)	0 (D) 0.8 (HRO)	0 (HH)	35 (D) 18.0-36.2 (MW)
- Ilan	-	5.6 (MW)	5 (MW)	1 (MW)
- Kaohsiung	-	0 (MW)	0 (MW)	0-1.8 (MW)
<i>O. h. chiui</i> (Taiwan)				
- Alilao	-	22.2-100 (C)	-	69.2-100 (C)

\*The "species" of *Oncomelania* are here all considered to be subspecies of *O. hupensis*.

Abbreviations:

(D) = DeWitt, 1954;

(HRO) = Hunter, Ritchie & Otori, 1952;

(MW) = Moose & Williams, 1963, 1964;

(P) = Pesigan et al., 1958;

(C) = Chiu, this paper;

(HH) = Hsü & Hsü, 1960.

a 100% infection rate was later shown to prevail in this heavily exposed group. The negative finding suggests that schistosome larvae may stay in the head-foot muscle for a while after penetration. Lower infection rates (22.2% on the average) were observed in Group A, exposed to fewer cercariae. Sporocysts were discovered in 5 out of 15 snails dissected 23-63 days after infection. On the 95th day, shedding failed to occur in both groups. On the 105th day, cercariae were shed by snails of Group A, but not of Group B. Six days later, 2 of 5 snails crushed in Group A harbored sporocysts and cercariae, while 1 snail examined in Group B still harbored nothing but sporocysts. On the 118th day, 3 of 20 snails dissected in Group A were found infected, but only 1 snail harbored cercariae, the other 2 merely sporocysts. In a snail from Group B, again only sporocysts were detected on the 111th and 118th days. The 7 surviving

snails were dissected on the 174th day. None of 5 snails crushed in Group A were infected with schistosomes. In contrast, the 2 surviving snails from Group B were parasitized with sporocysts and a few cercariae, but these were degenerated and apparently non-infective.

In summary, 10 of 45 snails dissected (22.2%) were infected with the parasite in Group A. On the other hand, although presumably a 100% infection rate obtained in Group B, no snail was capable of producing infective cercaria. It was also noted that the snail death rate was significantly higher in Group B (69%) than in Group A (40%). These observations, as compared with those for the Changhua strain of *Schistosoma japonicum*, suggest that *Oncomelania hupensis chiui* is a less suitable host for the Japanese than for the Changhua strain of *S. japonicum*. The infectivity of schistosome cercariae of the Japanese

strain shed from *Oncomelania hupensis chiui* was also confirmed by animal infections. Two mice were exposed to an undetermined number of cercariae for 2 hours, and at autopsy, 42-49 days after infection, adults of *Schistosoma japonicum* were recovered.

#### DISCUSSION

It is well known that oncomelanid snails from various geographic locations possess a varying degree of susceptibility to infection with different strains of *Schistosoma japonicum* (Hunter et al., 1952; DeWitt, 1954; Pesigan et al., 1958; Hsü & Hsü, 1960; Moose & Williams, 1963, 1964). The knowledge available is summarized in Table 4. It is seen that *Oncomelania hupensis hupensis* from China was susceptible to the Chinese, Japanese and Philippine strains of *S. japonicum*, but refractory to the Formosan-Changhua strain; *O. h. nosophora* from Japan was susceptible to the Japanese, Philippine and Changhua schistosome strains, but not to the Chinese strain; *O. h. quadrasii* from the Philippines to the Philippine and Changhua strains, but not to the other 2 strains; *O. h. formosana* from Changhua, Taiwan, was susceptible to the Changhua strain, was faintly infected by, but an unsuitable host for, the Japanese schistosome strain, and refractory to the other 2 strains. Recent findings by Moose & Williams (1963, 1964) indicate that *O. h. formosana* from Ilan, in north eastern Taiwan, another endemic area for *Schistosoma japonicum*, recently discovered by Kuntz (1965), was relatively susceptible to the Japanese and Philippine strains of schistosome, but exceedingly resistant to the Changhua strain; whereas snails from Kaohsiung, in southern Taiwan, were altogether unsuitable as hosts and resistant to the Changhua, Japanese and Philippine strains.

The author (1965a) has already reported the fact of susceptibility of "*Tricola (=Oncomelania) chiui*" to in-

fection with the Changhua strain of *Schistosoma japonicum*. In the present study, *Oncomelania hupensis chiui* was not only confirmed as a good potential intermediate host for the Changhua strain but also found capable of transmitting the Japanese strain of *S. japonicum*. In other words, the snail can serve as an efficient host for both the non-human and human strains of *S. japonicum*. For the Changhua strain of *S. japonicum* an infection rate of 100% could be obtained. The results for the Japanese strain were interesting, in that moderately heavy exposure (5-7 miracidia per snail) resulted in functional infection at the rate of 22.2%, whereas, with heavier exposure (an average of 10 miracidia per snail), even though the infection rate amounted to 100%, not a single mature infective cercaria could be found throughout the 5 month duration of the experiment. This interrelationship between the cercaria producing capacity of *O. h. chiui* and the number of miracidia to which it was exposed remains to be understood. The results of this study have further demonstrated that *O. h. chiui* also showed a varying degree of susceptibility to 2 geographic strains of *S. japonicum*, as do the other oncomelanid snails, i.e., at approximately equal exposure, infection rates were about 100% for the Changhua strain of *S. japonicum* and 22% for the Japanese schistosome (Tables 2 and 3A). The variety in response now realized to exist in local strains of *O. h. formosana* - from refractive to the indigenous non-human schistosome to slightly susceptible to the foreign human schistosomes - and the discovery of the new subspecies *O. h. chiui*, susceptible to both these schistosomes, already entails a revision of our former views, in particular of the view that Taiwan was safe from the threat of human schistosomiasis, because there was no potential snail host for the human strain of *S. japonicum*. No doubt further investigation will provide further evidence of variation in the snail-parasite relation-

ship.

Among other related hydrobiid snails, DeWitt (1954) reported that the North American *Pomatiopsis lapidaria* was capable of infection with the Chinese (1%) and the Changhua (3%) strains of *Schistosoma japonicum*.

It is also of interest to note that Hunter & Abbott (1949) reported on an infection experiment with *Tricula minima* from Japan and the Japanese strain of *Schistosoma japonicum*. They found that the miracidia could not develop to the cercarial stage in that snail; only a few degenerating mother sporocysts were discovered 7-71 days after infection.

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#### ADDENDUM

Further experiments have indicated

that *Oncomelania hupensis chiui* is also susceptible to infection with the Formosan-Ilan, Japanese-Kurume, Philippine and Chinese strains of *Schistosoma japonicum*, at the rates of 56.5-100%, 87.5-100%, 43.8-100% and 66.7-87.0% respectively. The exposure per snail ranged from 1 to 15 miracidia.

#### RESUMEN

#### SUSCEPTIBILIDAD DE ONCOMELANIA HUPENSIS CHIUI A LA INFECCION POR SCHISTOSOMA JAPONICUM

Jui-Kuang Chiu

Se ha descubierto que el pequeño caracol hydróbido, anfibio, *Oncomelania hupensis chiui* (descrito originalmente como *Tricula chiui*) del extremo norte de Taiwan (Formosa), conocido como huésped de *Paragonimus iloktsuenensis*, es también capaz de servir de intermediario tanto para la raza zoofílica (Formosa-Changhua), como para la raza que ataca al hombre (japonesa) de *Schistosoma japonicum*. Desde que el caracol huésped (*O. h. formosana*) de *S. japonicum* en Taiwan de diferentes localidades, es refractario o muy ligeramente susceptible a las razas de infección humana de *S. japonicum*, este descubrimiento de un huésped potencialmente eficiente, trae la esquistosomiasis humana dentro del área de posible establecimiento en Taiwan.

Los experimentos indicaron para este caracol un grado variable de susceptibilidad a las dos razas geográficas de *S. japonicum*, y es, en general, un huésped más adaptable a la raza Changhua que a la japonesa. El linaje Changhua mostró una proporción infecciosa del 100%, y los caracoles libraron cercarias 95 días después de ser expuestos a 6 miracidios por individuo. Resultados interesantes se obtuvieron del linaje japonés. Caracoles expuestos en parejas a 20 miracidios, fueron 100% infectados pero no produjeron cercarias infecciosas. Sólo aquellos expuestos individualmente a 5-7 miracidios, infectados en una proporción del 22.2% libraron cercarias 105 días después de la infección.

#### АБСТРАКТ

#### ВОСПРИИМЧИВОСТЬ ONCOMELANIA HUPENSIS CHIUI К ЗАРАЖЕНИЮ SCHISTOSOMA JAPONICUM

ДЖУ-КУАНГ-ШИУ

Мелкая амфибийная гидробия *Oncomelania hupensis chiui* (первоначально описанная как *Tricula chiui*) с северной оконечности Тайваня известна как промежуточный хозяин *Paragonimus iloktsuenensis*; было найдено также, что она может служить хорошим промежуточным хозяином как для зоофильного штамма (из формозы-Чангуа), так и для человеческого (японского) *Schistosoma japonicum*. Поскольку улитка-хозяин (*O. h. formosana*) паразита *S. japonicum* из различных мест Тайваня является устойчивой или лишь слабо-восприимчивой к человеческой форме *S. japonicum* из Японии и Филиппин, открытие весьма эффективного потенциального хозяина *Schistosoma* позволяет ожидать развития человеческого шистозомиазиса на Тайване.

Эксперименты показывают, что этот моллюск имеет различную степень восприимчивости в двум географическим штаммам *S. japonicum* и, в целом, является более подходящим хозяином для штамма из Чангуа, чем для японского штамма. 100% заражение было получено для штамма *S. japonicum* из Чангуа, когда церкарии выходили из моллюсков через 95 дней после индивидуального заражения их 6 мирацидиями каждый. Интересные результаты были получены для японского штамма. Улитки заражались попарно 20 мирацидиями и давали 100% заражение, но не давали заражающих церкарий. Улитки, зараженные 5-7 мирацидиями индивидуально и давшие только 22.2% заражения, давали церкарии через 105 дней после заражения.



THE ANATOMY AND RELATIONSHIPS OF A  
SOUTH AFRICAN *FERRISSIA* (BASOMMATOPHORA: ANCYLIDAE)

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ABSTRACT

*Ferrissia*, introduced by Walker (1903) to accommodate *Ancylus rivularis* Say of North America, has subsequently been regarded as a worldwide genus of freshwater limpets with fine radial sculpture on the apex of the shell. On anatomical evidence, the New and Old World species were classified in the subgenera *Ferrissia* s.s. and *F. (Pettancyclus)* respectively by Hubendick (1964). The genus *Gundlachia* Pfeiffer, originally characterised by the septate shell, was restricted by Hubendick to comprise ancylics of Central and South America; African species previously assigned to that genus are, apparently, septate forms of *Ferrissia*.

The range of *Ferrissia* in Africa extends from the Mediterranean to the South African Cape. Published information about the anatomy of African species is restricted to the radula, jaw and external characters. The present account deals with certain features of the internal and external anatomy of non-septate specimens of *F. burnupi* (Walker, 1912) collected in Natal province, Republic of South Africa. One object of the study was to obtain information for comparison with other Old World *Ferrissia* species, in particular *F. tenuis* (Bourguignat) which has been reported to transmit human schistosomiasis in India.

Features of *Ferrissia burnupi* indicating a close relationship with the *Pettancyclus* group of species are: the poor pigmentation, the lack of fusion between the plates composing the jaw, the large uterine gland complex, and, above all, the structure of the male copulatory organ with its small penis and vestigial penis sheath into which opens a long flagellum. Although the differences between *F. burnupi* and *F. tenuis* in the radular teeth, seminal vesicle and flagellum may be of specific rank, *F. burnupi* may be worthy of consideration as a possible intermediate host of *Schistosoma haematobium* in Natal.

Anatomically *Ferrissia burnupi* resembles the non-septate Italian form of *Watsonula wautieri* Mirolli, according to information given by Mirolli (1960) and Hubendick (1964), but the copulatory organ differs from that described by Wautier et al. (1966) for French specimens identified as *Gundlachia wautieri*.

The left pleural ganglion of *Ferrissia burnupi* is distinguishable from, though intimately connected with, the left parietal ganglion. Fusion between the corresponding ganglia of the right side has been carried even further. The lateral ganglia in the visceral loop are named left and right pleuro-parietal ganglia and the asymmetrically placed ganglion lying between them the abdominal ganglion, a terminology which expresses the composition and function of these 3 ganglia in *Ferrissia* more accurately than various previous terminologies, and may be applicable to some other ancylics. There are separate connectives between the pedal ganglion and the cerebral and pleuro-parietal ganglia on each side.

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## INTRODUCTION

*Ferrissia* was introduced by Walker (1903) as a section of *Ancylus* for the North American species *A. rivularis* Say. It has subsequently been regarded as a genus comprising freshwater limpets with fine radial sculpture on the apex of the shell from many parts of the world. Hubendick (1964) defined 2 subgenera with different male copulatory organs and gave the distribution of *Ferrissia* s.s. as North and Middle America and the West Indies; the species of Africa, South and East Asia, Australia and Oceania were placed, together with *Watsonula wautieri* Mirolli of Italy in *F. (Pettancyclus)*, with *Ancylus tasmanicus* Tenison-Woods as type species. At least some species of each subgenus are capable of forming septate shells, in which an upper and a lower portion are partially separated.

The presence of a shell septum closing the posterior part of the aperture has been regarded by many authors as a characteristic of *Gundlachia* Pfeiffer, 1849, and for this reason several African ancylids have been classified in that genus. In the case of the South African species *equeefensis* (Walker) and *G. burnupi* Walker, Walker (1923, 1926) believed that their generic position was confirmed by the structure of the radular teeth. However, the central radular tooth of *G. equeefensis*, redescribed by H. Watson (in Connolly, 1939), and that of *G. burnupi*, illustrated by H. B. Baker (in Walker, 1926), have, like the *Ferrissia* species of South Africa, symmetrical cusps instead of the asymmetrical cusps found in *Gundlachia* species of South and Central America. North American septate ancylids placed in *Gundlachia* by some authors were transferred to *Ferrissia* by Basch (1959a, 1963), who concluded that South American species belonging to the former genus are distinguished by the asymmetrical cusps on the central radular teeth. Likewise, Hubendick (1964) restricted *Gundlachia* to comprise Neotropical species with asymmetrical cusps on the central

tooth; these species are further differentiated by the structure of the male copulatory organ. Although no description of the copulatory organ of a septate African ancylid has been published, it appears unlikely that *Gundlachia* occurs in the southern part of the continent, and the present author follows Connolly (1939) by including in *Ferrissia* the South African forms that were formerly placed in *Gundlachia*.

The range of *Ferrissia* in Africa extends from Egypt to the South African Cape, although, in spite of considerable collecting activity, no species have been recorded from the Congo or Angola (Pilsbry & Bequaert, 1927; Wright, 1963). Twelve species have been described from South Africa, Rhodesia, and Mozambique (Walker, 1912, 1923, 1926; Connolly, 1925), and the genus appears to be particularly diverse and abundant in southern Africa.

Of the other 2 ancylid genera occurring in Africa, *Ancylus* reaches the southern limits of its range in Ethiopia. The species of *Burnupia* Walker, 1912, known from Africa only, have punctate shell apices.

Little information has been published about the anatomy of African *Ferrissia* species. The radulae of 2 South African forms have been described (Walker, 1923; Watson, in Connolly, 1939), and the radula and jaw of *F. junodi* Connolly, from Mozambique (Connolly, 1925). H. B. Baker (in Walker, 1926) gave an account of some external features and also the radula of *Gundlachia burnupi* (transferred to *Ferrissia* and renamed *F. cliffdeni* by Connolly, 1939). Brown (1965) described the radulae of aphyllid Ethiopian specimens belonging to 2 species. The present account deals with certain features of the external and internal anatomy of non-septate specimens of *Ferrissia burnupi* (Walker, 1912) that were collected from a single population in Natal province, Republic of South Africa. Future revision may show that some or all of the other 5 species of *Ferrissia* recorded from Natal (Connolly, 1939)

are synonymous with *F. burnupi*, which is the senior species described from that region. One reason for undertaking the study was to obtain information for comparison with other Old World *Ferrissia* species, in particular *F. tenuis* (Bourguignat) of India.

*Ferrissia tenuis* has been reported to serve as an intermediate host of *Schistosoma haematobium* in a focus 250 km south of Bombay (Gadgil & Shah, 1955; Gadgil, 1963); it is consequently desirable to compare *F. tenuis* with African species of *Ferrissia* since these ancyliids are widely distributed in the regions of Africa where human urinary schistosomiasis is endemic. In South Africa, *Ferrissia* occurs abundantly in a variety of habitats including permanent lakes, temporary pools, and small stony streams. Porter (1938) examined 45 "wild" specimens for trematode cercariae and reported a "furcocercous monostome" and a "holostome" from *F. burnupi*. Cercariae of African schistosomes have not been reported from African *Ferrissia*, although, as far as the author is aware, the susceptibility of the snails has not been tested experimentally.

#### MATERIAL AND METHODS

The specimens of *Ferrissia burnupi* used in this study were collected by the author from Amahlongwa River, 5 miles northeast of Umzinto, Natal province, Republic of South Africa; at bridge on road between Umzinto and Umkomaas, within Umahlangwa Mission Reserve (South Africa 1: 250,000 topo-cadastral map, sheet 3030 Port Shepstone). Coordinates: south 30° 15', east 30° 43'. 17 July 1964. Collector's number 359. National Snail Collection (Institute for Zoological Research, Potchefstroom University, South Africa) catalogue No. 68.48.66.

The limpets were collected from dead leaves in residual pools on sandbanks when the river was at a low level. The majority of shells were thickly encrusted

with a black deposit, which was removable with oxalic acid solution.

External features were observed in snails that were active or had been narcotised in a 5% solution of Nembutal. Dissections were made of 30 specimens fixed in 5% formol saline at 60° C, and preserved in 80% ethanol. Two specimens were fixed in Bouin's Fluid, serially sectioned and stained with Mallory's Triple stain for histological study.

#### OBSERVATIONS

##### Shell (Figs. 1-4)

The largest shells were 4.0 mm long. No septate specimens were found. The lateral margins are straight or slightly convex. There are numerous fine radial ribs on the apex, and coarser ribs on the anterior slope, which extend to the shell margin in some small specimens. Irregularly placed circular ridges are conspicuous on some parts of the shells.

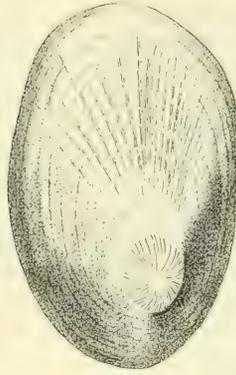
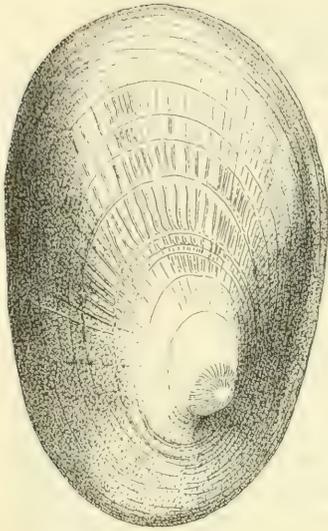
In their height, the present shells resemble *Ancylus* (*Ferrissia*) *burnupi* Walker, 1912, and *A. (F.) equeefensis* Walker, 1912, both described from Equeefa River, which lies approximately 10 miles southwest of the Amahlongwa River at Nkwifa. The apex, in its shape and position relative to the posterior margin of the shell, is most similar to that of *F. equeefensis*. Walker (1923) stressed the importance of differences between the radulae of these species, but he appears to have illustrated worn teeth of *F. equeefensis*. In view of the variation in shell form that may be observed in series of *Ferrissia* from a single locality, and of the fact that *F. burnupi* and *F. equeefensis* have a common type locality, the former name, which has page priority, is employed for the present material.

##### Animal (Figs. 5, 8, 9)

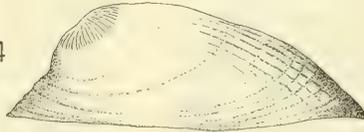
In an active animal both the anterior and posterior ends of the foot are bluntly rounded. No grooves or macroscopic glandular openings were observed on the

1

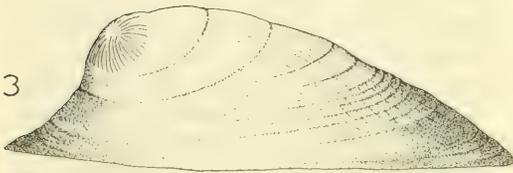
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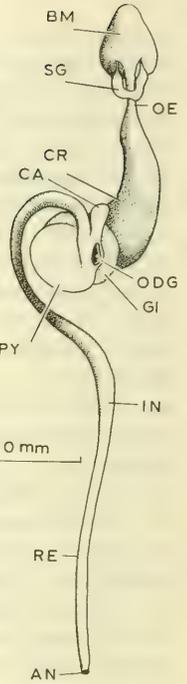
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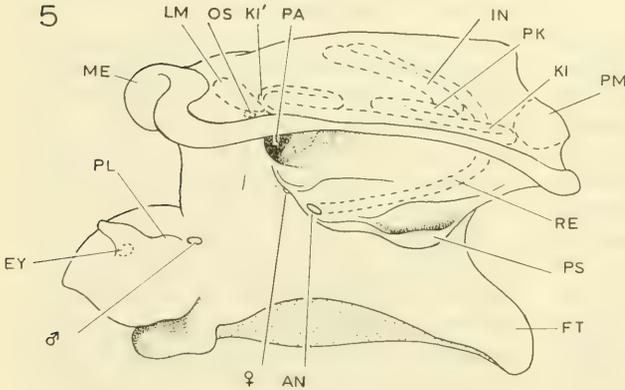


1 mm

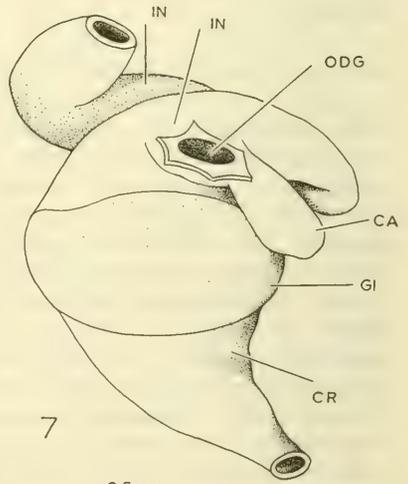


6

5



1.0 mm



7

0.5 mm

FIGS. 1-7. Figs. 1-4. *Ferrissia burmupi*. Dorsal and lateral views of shells. Fig. 5. External features of left side of animal removed from shell. Fig. 6. Alimentary canal. Fig. 7. Stomach viewed from right side. ♀: female genital aperture; ♂: male genital aperture.

## List of abbreviations (except nervous system)

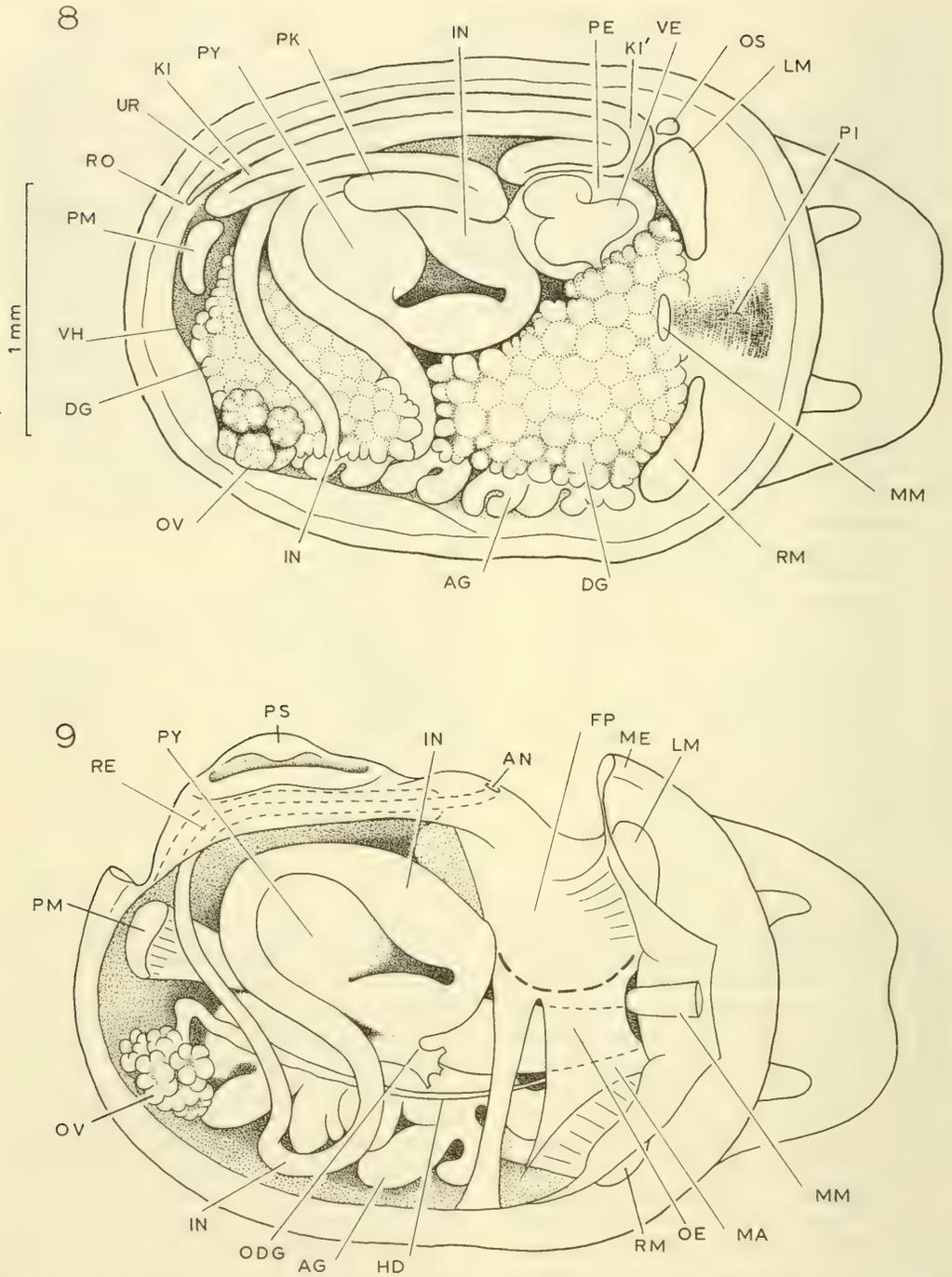
AG	albumen gland	OS	osphradium
AN	anus	OV	ovotestis
BM	buccal mass	P	penis
BW	cut edge of body wall	PA	pulmonary aperture
C	carrefour	PC	pulmonary cavity
CA	caecum	PE	pericardium
CI	cilia	PI	pigmented band
CR	crop	PK	proximal sac of kidney
DG	digestive gland	PL	post-tentacular lappet
EG	egg	PM	posterior shell muscle
EY	eye	PR	preputium
FL	flagellum	PS	pseudobranch
FP	floor of pulmonary cavity	PSH	penis sheath
FT	foot	PY	pyloric stomach
GI	gizzard	QM	quaternary membrane
GU	glandular uterus	RCG	right cerebral ganglion
HD	hermaphrodite duct	RE	rectum
IN	intestine	RO	position of renal opening on ventral surface of mantle
KI	posterior loop of kidney tubule	SA	sarcobelum
KI'	anterior loop of kidney tubule	SG	salivary glands
LM	left anterior shell muscle	SP	spermatheca
MA	membrane of André	TS	thin strip in dorsal wall of uterus
ME	mantle edge	TT	terminal tail of egg capsule
MM	median muscle	UR	ureter
MU	muscular part of uterus	UT	uterus
NG	nidamental gland	VA	vagina
NR	non-glandular ridge of uterus	VD	vas deferens
OD	oviduct	VE	ventricle
ODG	opening of digestive gland	VH	external surface of visceral hump
OE	oesophagus		
OP	operculate suture of egg capsule		

foot. A small post-tentacular lappet (PL, Fig. 5) is attached to the postero-lateral base of each tentacle. Each eye (EY) is situated within the median anterior part of the tentacle base. The female genital aperture lies beneath the anterior end of the pseudobranch. When the male copulatory organ is present there is a male aperture behind the left tentacle close to the post-tentacular lappet.

The term pseudobranch may be applied to the whole of the flap that projects from the body between the left anterior shell muscle (LM) and the posterior shell muscle (PM). The posterior part of the pseudobranch (PS, Figs. 5, 9), consisting of a single lobe with 2 longitudinal folds ("auriform lobe" of Mirolli, 1960),

probably performs a respiratory function. The rectum (RE) passes forwards through the anterior part of the pseudobranch, which may be called the anal lobe, to the anus (AN) on the dorso-lateral surface of this lobe.

In living animals a capacious hypopleural cavity (a cavity protected by the mantle but lying outside the pulmonary aperture; Harry, 1964) is formed by the extension of the peripheral region of the mantle to the edge of the shell. The pulmonary aperture (PA, Figs. 5, 10) is situated behind the left anterior shell muscle (LM), and leads to a pulmonary cavity that is bounded dorsally and posteriorly by the pericardium (PE) and the anterior loop of the kidney tubule (KI'),



FIGS. 8 & 9. Dorsal view of *Ferrissia burnupi* removed from shell. Fig. 8. The organs seen *in situ* as if the mantle were transparent. Fig. 9. Organs *in situ* after the mantle, kidney, pericardium, and digestive gland have been removed. Median wall of pulmonary cavity indicated by heavy broken line.

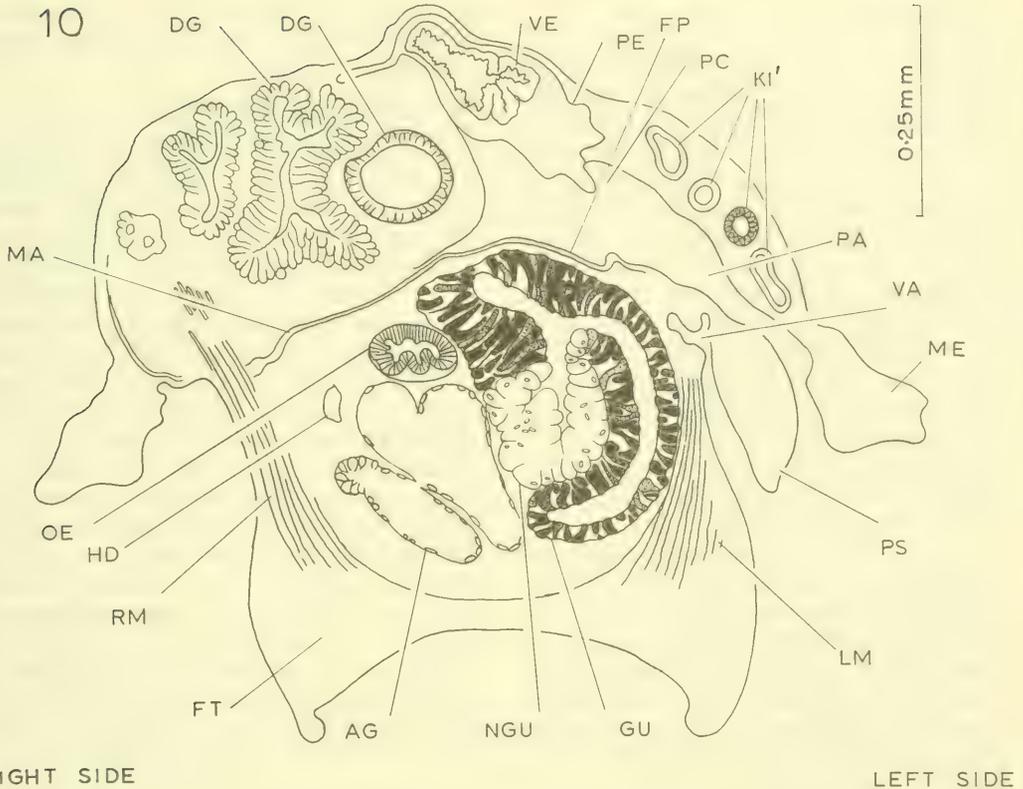


FIG. 10. *Ferrissia burnupi*. Transverse section at vaginal opening (viewed towards the posterior end of the animal).

and medianly by the anterior part of the digestive gland (DG, Figs. 8, 10).

The osphradium (OS, Figs. 5, 8) may be observed through the dorsal surface of the mantle, slightly anterior to the lateral edge of the left anterior shell muscle; it is an invagination from the ventral surface of the mantle that is lined with ciliated epithelium.

There is little dark pigment in the foot, the tentacles, or the superficial tissues of the cephalic region, and pigment is most densely concentrated in the labial palps. Internally there are numerous flecks of dark pigment in the tissue sheathing the buccal mass and in the lining of the body cavity. In the majority of specimens pigmentation of the mantle is confined to a short median band on the dorsal surface between the anterior shell

muscles (PI, Fig. 8), but in one animal there was a wide band anterior to these muscles.

Many shining bodies resembling oil droplets were observed in the tissue of the mantle edge in living animals, and also present in some individuals were small opaque white granules. These granules do not appear to be comparable to relatively large white glands in the mantle edge of *Burnupia caffra* (Krauss), which discharge a milky secretion when the animal is prised from the substratum (unpublished observations).

There are many cilia on the external surfaces of the body, particularly on the pseudobranch. Fine particles were observed to be drawn under the anterior edge of the shell of stationary animals and expelled from beneath the posterior

shell margin.

Organs of the visceral hump (Figs. 5, 8, 9)

The organs lying within the visceral hump may be seen through the overlying tissue without dissection (Fig. 8). Most of the space on the right side of the visceral hump is occupied by the digestive gland (DG), beneath which lies the albumen gland (AG). The oesophagus is deflected to the left side, so that the stomach (PY) lies in the left side of the body. The apex of the visceral hump is occupied by the ovotestis (OV), which is surrounded by the posterior part of the digestive gland.

The dorsal attachment of the posterior shell muscle (PM, Fig. 8) lies to the left of the median line. The 2 anterior shell muscles (LM, RM) are widely separated and between them the connection between the mantle and the body wall of the cephalic region is relatively thick. A small median muscle (MM), which is inserted in the cephalic body wall at the base of the connection with the mantle, passes between the anterior shell muscles into the cavity of the visceral hump; its dorsal end is attached to epithelium at the posterior end of the median band of pigment on the mantle (PI).

The kidney tubule (KI, KI', Figs. 5, 8) extends posteriorly from the anterior end of the proximal sac (PK), forms a posterior loop (KI) near the posterior shell muscle and then runs forward to near the left anterior shell muscle. Here, it makes a double anterior loop (KI'), and continues as ureter (UR, Fig. 8) posteriorly once more to the renal opening (RO), which lies on the ventral side of the mantle lateral to the posterior shell muscle. A large part of the kidney tubule is contained within the eave-like projection of the mantle that overlies the pulmonary aperture and the pseudobranch (Fig. 10). The posterior part of the pericardium (PE, Fig. 8) extends towards the proximal sac of the kidney (PK), to which it is probably connected by a reno-pericardial duct as observed by Hubendick (1958, 1960b, 1964) in other species of *Pettancylus*;

in transverse sections of *F. burnupi* long cilia were observed at the presumable position of this duct.

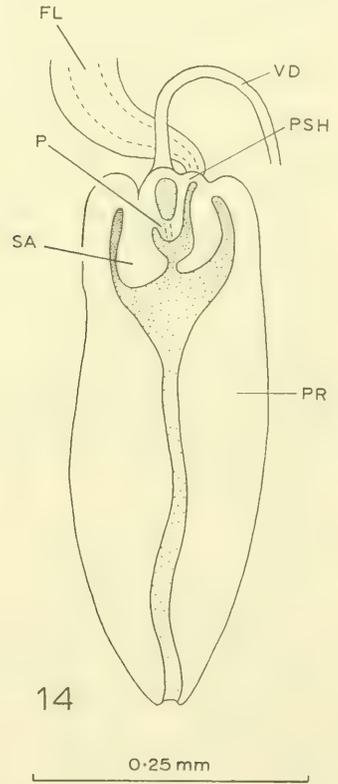
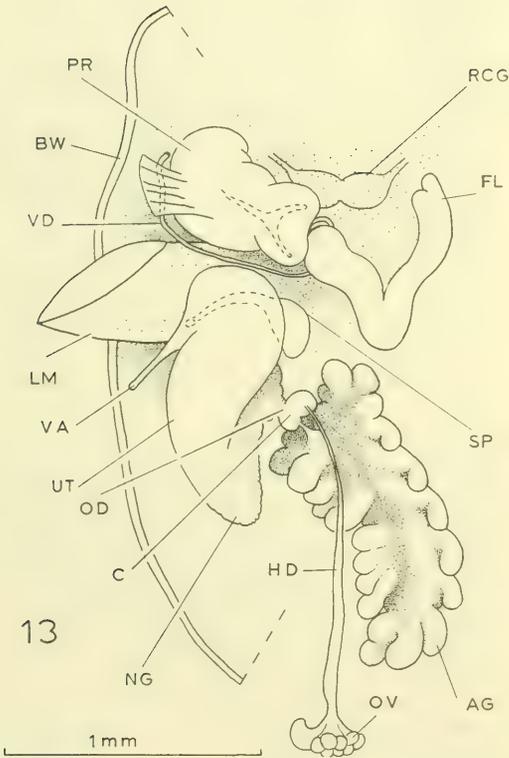
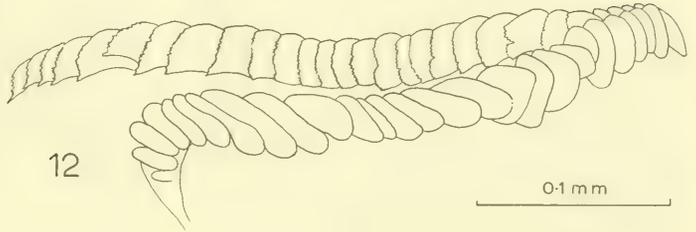
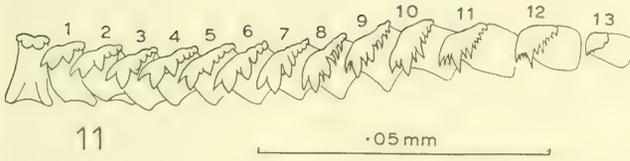
The cephalo-pedal cavity

The cavities of the visceral hump and the cephalo-pedal region are partially separated by horizontal membranes divided into anterior and posterior parts (MA, Fig. 9), which extends between the floor of the pulmonary cavity and the right side of the body, where it is attached to the inner surface of the right shell muscle and to the body wall behind that muscle. A similar membrane in *Ferrissia tarda* was named the membrane of André by Hoff (1940). The median muscle (MM) passes through a gap between this membrane and the junction between the mantle and the body wall of the cephalic region. Beneath the membrane of André lie the distal genital organs, oesophagus, and the anterior lobes of the albumen gland. The right side of the pedal cavity is largely occupied by the albumen gland (AG), and the left side by the uterus (GU, Fig. 10) and nidamental gland (NG).

The alimentary canal (Figs. 6-9, 11, 12)

The jaw (Fig. 12) consists of a single row of numerous overlapping, but unfused, plates with fine teeth on their median edges; in an animal of 3.2 mm shell length there are approximately 25 plates in each side of the jaw.

The radula sac lies beneath the visceral loop of the central nervous system and extends no further posteriorly than the pedal ganglia. In 5 radulae examined the number of teeth in a transverse row is 27 or 29 (Fig. 11). The cusps of the central tooth are extremely small, but it appears that 2 symmetrical median cusps are present and perhaps also a pair of lateral cusps. There are 3 main cusps on the inner lateral teeth. The crown of each tooth is progressively elongated towards the lateral margin of the radula, additional small cusps appear on the lateral part of the crown, and an interstitial cusp arises between the ectocone and the mesocone. This interstitial cusp is best



FIGS. 11-14. *Ferrissia burnupi*. Fig. 11. Radula (half of a complete transverse row of teeth). Fig. 12. Jaw (oblique lateral view). Fig. 13. Reproductive organs *in situ*. Fig. 14. Diagrammatic longitudinal section of male copulatory organ reconstructed from serial sections (details of the internal folds of the preputium omitted).

developed in teeth 9-12. Marginal teeth numbers 11 and 12 bear about 10 cusps of which the largest correspond to the 3 major cusps of the inner lateral teeth.

The salivary glands (SG, Fig. 6) are attached to the dorsal surface of the buccal mass close to the origin of the oesophagus and are joined above the oesophagus. They do not pass through the central nerve ring. The oesophagus lies dorsal to the buccal commissure, and after passing between the cerebral and the visceral commissures, turns dorsally and gradually widens. The crop (CR) leads to the muscular gizzard (GI), which is succeeded by the relatively thin-walled pyloric region (PY) of the stomach (Figs. 6, 7). Circular and longitudinal muscle fibres are visible externally on the wall of the gizzard, which contains pieces of grit measuring up to 0.25 x 0.20 mm in an animal of 3.5 mm shell length. A caecum (CA) opens into the pyloric stomach at the attachment of the intestine. The main anterior and posterior ducts from the digestive gland unite just before their entry (ODG) near the junction of the caecum. The intestine (IN, Figs. 6, 9) forms a loop on the left side of the visceral hump followed by a loop on the extreme right; the rectum (RE) follows the median side of the auriform lobe of the pseudobranch, and passes through the anal lobe to the anus situated on its dorso-lateral surface (Figs. 5, 9).

#### Reproductive system (Figs. 9, 10, 13-15)

The ovotestis consists of 3-5 lobes connected to a common atrium, each lobe being formed of a group of 5-7 acini (OV, Figs. 9, 13). The proximal half of the hermaphrodite duct (HD) is translucent and relatively wide, but possesses no definite seminal vesicle; the duct becomes very narrow before entering a swelling of the carrefour (C, Fig. 13).

The albumen gland (AG, Fig. 13) is translucent, bluntly lobed, and has a hard texture in animals preserved in alcohol. Most of the volume of the albumen glands that were serially sectioned is

made up of secretion, cell nuclei being confined to a superficial layer (Fig. 10). A narrow albumen duct enters a swelling of the carrefour that is separate from the one at the entrance of the hermaphrodite duct.

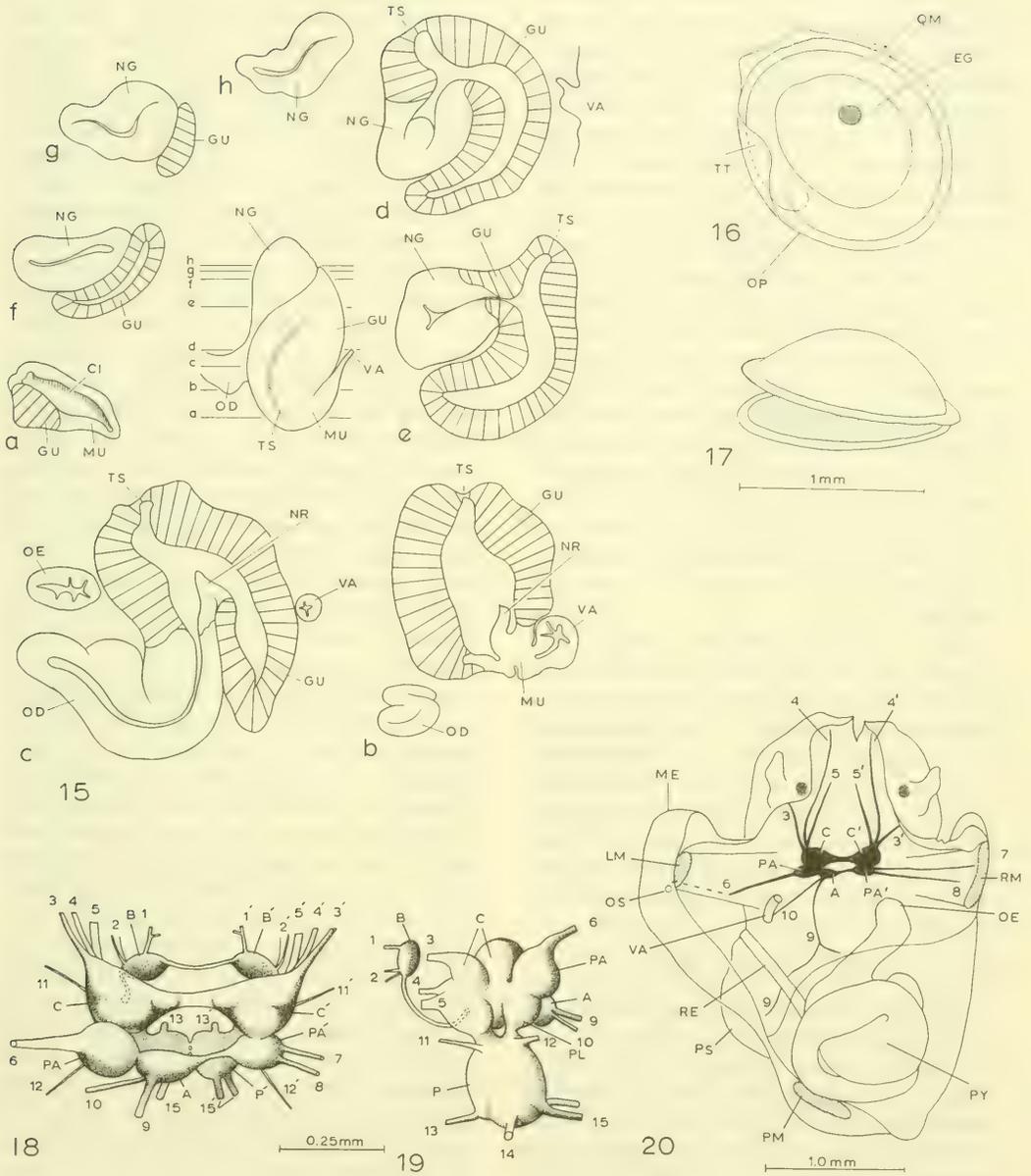
A very short oviduct (OD, Fig. 13) leads from the carrefour to the uterus (UT), which is sharply bent at its distal end, so that the slender vagina (VA) passes in a postero-lateral direction to the body wall. A narrow strip (TS, Fig. 15) along the dorsal surface of the uterus is thin-walled and broken open in many preserved specimens.

Two distinct glandular regions are present in the uterine complex: (1) except for the dorsal strip and near the vagina, most of the uterus wall is thickened with glandular tissue (GU, Figs. 10, 15), and (2) the posterior part of the uterine complex is formed by the nidamental gland (NG), cells of which also occur in the wall of the distal part of the oviduct. These regions correspond to the "glandular region of the uterus" of Mirolli (1960) and the "nidamental gland" of Hoff (1940) and of Mirolli. There is a wide connection between the lumina of the uterus and the nidamental gland (Fig. 15, sections d, e) and they are separate only in the posterior part of the uterine complex (Fig. 15, sections f-h).

Muscular tissue is developed at the distal end of the uterus (MU, Fig. 15) near its junction with the vagina; there is a ridge of non-glandular tissue (NR, sections b, c) on the floor of the anterior part of the uterus. Cilia (CI, Fig. 15, section a) were present on the internal antero-lateral surface of the uterus and also in the lumen of the oviduct.

The spermathecal duct attaches to the proximal end of the vagina, near to the uterus (Fig. 13). The duct is slightly longer than the club-shaped spermatheca (SP).

A male copulatory organ was present in 3 specimens (including one that was serially sectioned) out of 30 that were examined. The penis sheath (PSH, Fig.



FIGS. 15-20. *Ferrissia burnupi*. Fig. 15. Dorsal view of uterus and transverse sections at positions a-h. Fig. 16. Dorsal view of egg capsule. Fig. 17. Lateral view of hatched egg capsule. FIGS. 18-20. Nervous system of *Ferrissia burnupi*. Fig. 18. Dorsal view of central ganglia. Fig. 19. Left lateral view of central ganglia. Fig. 20. The peripheral nerves of the cerebral, pleuro-parietal, and abdominal ganglia seen in dissection. A, abdominal ganglion; B, B', buccal ganglia; C, C', cerebral ganglia; P, P', pedal ganglia; PA, PA', parietal regions of pleuro-parietal ganglia; PL, pleural region of left pleuro-parietal ganglion; 1, 1', n. gastricus; 2, 2', n. pharyngeales; 3, 3', n. tentacularis; 4, 4', n. frontolabialis superior; 5, 5', n. labialis medius; 6, n. pallialis sinister; 7, n. pallialis dexter anterior; 8, n. pallialis dexter posterior; 9, n. analis; 10, n. genitalis; 11, 11', n. cervicalis superior; 12, 12', n. cervicalis inferior; 13, 13', n. pedalis superior; 14, 14', n. pedalis medius; 15, 15', n. pedalis inferior.

14) is reduced to a vestige; it cannot be distinguished from the preputium externally (PR), and appears to be permanently invaginated within the preputium. A large flagellum (FL, Figs. 13, 14) is attached to the penis sheath close to the vas deferens (VD); the lumen of the flagellum opens into the lumen of the penis sheath at the base of the penis (P). The penis is small and encircled by a ridge, the sarcobelum (SA), which projects internally from the junction between the penis sheath and the preputium.

The vas deferens (VD, Fig. 13) emerges from the body wall laterally to the attachment of the preputium, and follows the length of the preputium before joining the proximal end of the copulatory organ. Neither the proximal part of the vas deferens nor the prostate gland were found in the 3 euphallic specimens examined, nor in any aphyallic snails.

Egg capsules collected from dead leaves, or laid on glass surfaces in the laboratory, were approximately circular in outline and contained a single egg each (EG, Figs. 16, 17). The capsule opens by a suture (OP) at its edge ("operculate suture" of Bondeson, 1950) and appears to possess a terminal tail (TT) and quaternary membrane (QM) as described by that author for *Ancylus fluviatilis* Müller.

#### Nervous system (Figs. 18-20)

Paired cerebral, buccal and pedal ganglia are present (CC', BB' and PP', Fig. 18). A small pleural ganglion (PL) may be distinguished beneath the left parietal ganglion (PA, Fig. 19). As the dorsal part of this pleural ganglion is intimately connected with the parietal ganglion, and fusion between the pleural and parietal ganglia has been carried even further on the right side, it is convenient to refer to a pleuro-parietal ganglion on each side of the animal. A single abdominal ganglion (A, Fig. 18) is situated asymmetrically on the left side of the visceral loop between the pleuro-parietal ganglia.

The ganglia and their peripheral nerves will be described successively. The terminology of the nerves is that used by Lever et al. (1965) in their description of the nervous system of *Australorbis* (= *Biomphalaria*) *glabratus*.

The median surfaces of the buccal ganglia (B, B') are connected by a long, slender commissure which passes under the oesophagus. In *Biomphalaria glabrata* a *n. receptacularis radulae* originates from each point of attachment of the commissure to a buccal ganglion. The presence of these nerves in *Ferrissia burnupi* was not definitely established, but is suggested by strands of pigmented tissue. From the dorso-anterior surface of each ganglion originates a *n. gastricus* (1, 1') giving one branch to the oesophagus and another to the buccal mass. At least 2 *nervi pharyngeales* (2, 2') originate from the ventro-lateral surface of each buccal ganglion, near to the attachment of the cerebro-buccal connective.

The left cerebral ganglion (C) is slightly larger than the right (C'). Mediodorsal bodies are present near to the attachment of the cerebral commissure. There are swellings at the bases of the peripheral nerves, but large lateral lobes of the kind described by Lever (1957) and Wautier et al. (1961) were not observed. Three nerves pass anteriorly from each cerebral ganglion: a *n. tentacularis* (3, 3') originates from the dorso-lateral surface of each ganglion and innervates the post-tentacular lappet; a *n. fronto-labialis superior* (4, 4') has a more ventral origin and runs to the anterior edge of the dorsal buccal lip; the most ventral in origin and stoutest of all, the *n. labialis medius* (5, 5') goes to the ventral part of the lateral buccal lip. Despite careful dissection in the vicinity of the eye, a *n. opticus* could not be found. It is thought that the eye may be innervated by a fine branch from the *n. tentacularis* (3) or from the *n. fronto-labialis superior* (4), which passes close to the eye. The cerebro-buccal connectives are attached to the median

surfaces of the cerebral ganglia (Figs. 18, 19). A cerebro-pedal connective originates from the antero-ventral parts of each cerebral ganglion, and a connective joins the posterior part of each cerebral ganglion to a pleuro-parietal ganglion.

The position of the *left pleural ganglion* (PL, Fig. 19) is indicated by a swelling, better developed in some individuals than in others, beneath the *left parietal ganglion* (PA). In whole mounts at a magnification of x 160, the cerebro-pedal and pleuro-parietal to pedal connectives were readily distinguishable, and also fibres passing from the pleural region of the left pleural-parietal ganglion to the cerebral ganglion. It was not possible to determine whether the latter fibres originated within the pleural region, in which case they would constitute a pleuro-cerebral connective, or passed directly from the parietal to the cerebral ganglion. A relatively small swelling beneath the right parietal ganglion appears to represent the pleural region of the right pleuro-parietal ganglion. No nerves originate from the pleural regions of either pleuro-parietal ganglion.

The dorsal (parietal) part of the left pleuro-parietal ganglion is conspicuously bigger than the right one (PA, Fig. 18). It bears a single, stout *n. pallialis sinister* (6) which passes dorso-laterally through a cleft in the left anterior shell muscle to the lower surface of the mantle where one of its branches innervates the osphradium (OS, Fig. 20). From the right pleuro-parietal ganglion originate 2 nerves, a *n. pallialis dexter anterior* (7) and a *n. pallialis dexter posterior* (8), which both run towards the body wall and mantle of the right side. The former was traced as far as the dorsal edge of the median surface of the right shell muscle, and the latter was observed to pass through this muscle in a dorso-lateral direction.

The *abdominal ganglion* (A) is attached to the pleuro-parietal ganglia by a short connective on the left side, and a long one

on the right side. It bears 2 nerves on its posterior surface, of which the thickest appears to correspond to the *n. analis* (9) of *Biomphalaria glabrata*, proceeding posteriorly in the body cavity beneath the viscera to the inner surface of the ventral wall of the pseudobranch, and giving at least one branch to the viscera (Fig. 20). The other nerve extends to the vagina and may correspond to the *n. genitalis* (10) of *B. glabrata*. Neither the *n. intestinalis* nor the *n. cutaneus pallialis* of *B. glabrata* were observed in *Ferrissia burnupi*.

The pedal ganglia (P, P') lie close together and are connected by 2 fine commissures (Fig. 18). Two nerves originate from the dorso-lateral surface of each pedal ganglion, a *n. cervicalis superior* (11, Fig. 19) passing antero-laterally to the body wall, and a *n. cervicalis inferior* (12) extending laterally to the body wall (Fig. 19). No nerve corresponding to the *n. columellaris* of *Biomphalaria glabrata* was observed. The ventral region of each pedal ganglion bears 4 nerves which innervate the musculature of the foot: an anterior *n. pedalis superior* (13), a lateral *n. pedalis medius* (14), and 2 posterior *nervi pedales inferiori* (15).

A statocyst is situated on the lateral median surface of each pedal ganglion. The aorta lies beneath the left pleuro-parietal ganglion and runs across the median surface of the left cerebral ganglion to a haemocoel lying anterior to the pedal ganglia ("preganglionic sinus" of Boer & Lever, 1959). In *Ferrissia burnupi*, the wall of this haemocoel is darkly pigmented and overlies the anterior nerves originating from the pedal ganglia.

## DISCUSSION

Various terminologies have been used by different authors to describe the ganglia of the visceral loop in the central nervous systems of *Ferrissia* and other Ancyliidae. According to the basic theory of the gastropod nervous system

TABLE 1. Some anatomical features of *Ferrissia (Ferrissia) rivularis* and 3 forms belonging to *F. (Pettancyclus)*.

Feature	<i>Ferrissia rivularis</i> Hoff (1940), Hubendick (1964) North America	<i>Ferrissia australica</i> Hubendick (1960 b, 1964) Australia	<i>Ferrissia burnupi</i> South Africa	<i>Ferrissia tenuis</i> Hubendick (1958, 1964) India
Radula	21-1-21 (maximum), central bicuspid (Hoff); 19-1-19, central with 2 major and 2 minor cusps (Hubendick)	13-1-13, central with 2 major and 2 minor cusps	14-1-14 (maximum), central probably with 4 minute cusps	17-1-17, central with 2 major and 2 minor cusps
Jaw	Dorsal scales partially fused (Hubendick)	Not fused	Not fused	Not fused
Salivary glands	Separate (Hoff); joined (Hubendick)	Joined	Joined	Joined
Pseudobranch	Unfolded or slightly folded (Hoff); folded (Hubendick)	Slightly folded	Folded	Folded (1964)
Digestive gland	Single opening into stomach (Hoff) and (Hubendick)	Single opening into stomach	Single opening into stomach	Single opening into stomach (1964)
Ovotestis	5-7 lobes (Hoff) 5 - many lobes (Hubendick)	2 lobes	3-5 lobes	4-5 lobes (1964)
Seminal vesicle	Diverticulum from hermaphrodite duct (Hoff)	Thin walled, bladder-like diverticulum	Slight dilatation of hermaphrodite duct	Similar to <i>F. australica</i> (1964)
Nidamental gland	Attached to uterus by narrow duct (Hoff)	No information	Intimately associated with uterus	No information
Uterus	Small, with little glandular development (Hoff)	Large uterine gland complex	Large, with glandular walls	No information
Spermathecal duct	Slightly longer than spermatheca (Hoff)	Slightly longer than spermatheca	Slightly longer than spermatheca	No information

TABLE 1. (continued)

Feature	<i>Ferrissia rivularis</i> Hoff (1940), Hubendick (1964) North America	<i>Ferrissia australica</i> Hubendick (1960 b, 1964) Australia	<i>Ferrissia burnupi</i>  South Africa	<i>Ferrissia tenuis</i> Hubendick (1958, 1964) India
Copulatory organ	Flagellum attached to proximal end of preputium (Hoff); short pear-shaped flagellum opening into long invaginated penis sheath, moderately long penis (Hubendick)	Long cylindrical flagellum opening into vestigial penis sheath, at base of short penis	Long cylindrical flagellum opening into vestigial penis sheath, at base of short penis	Moderately long cylindrical flagellum opening into vestigial penis sheath, at base of short penis
Aphallic individuals	Reported for <i>F. (F.) fragilis</i> by Basch (1963)	Many	Many	Many

(Pelseneer, 1906), the parietal ganglion is peculiar to the Euthyneura and may be regarded as having arisen, to provide an origin for pallial nerves, as a result of fusion between the infra-intestinal and abdominal ganglia. The visceral loop of a dextral basommatophoran contains 3 ganglia, which are from left to right, named with regard to homology with other Gastropoda: parietal, abdominal,<sup>2</sup> and supra-intestinal. This terminology has been simplified by later authors (e.g., Fretter & Graham, 1962; Morton, 1964; Lever et al., 1965) who refer to the lateral ganglia as left and right parietals.

In the Ancyliidae and other higher Euthyneura the cerebral, pleural, and parietal ganglia on each side become closely associated and fusions may take place. In the unfused condition, the cerebral and pleural ganglia of one side are connected to each other, and each is connected separately to the pedal ganglion of the same side; the parietal

ganglion, being situated in the visceral loop, has no direct connection with the pedal ganglion. The pleural ganglion may be fused, partly or completely, with either the cerebral or the parietal ganglia, and the connectives to the pedal ganglion provide evidence of which process has occurred. The presence of 2 separate connectives to the pedal ganglion indicates that the pleural has fused with the parietal ganglion, since if the pleural had fused with the cerebral ganglion it is likely that their combined connectives would be indistinguishable. To designate the composite pleuro-parietal ganglia as pleural ganglia (Hubendick, 1964, in the case of the American *Ferrissia tarda*) is unsatisfactory, because the pleural ganglia are regarded as bearing peripheral nerves only in the more primitive forms of Euthyneura.

The condition of the post-cerebral ganglia in *Ferrissia burnupi* is similar to that described by Pelseneer (1901) for *Gundlachia* sp. from New Zealand, which, according to Hubendick (1964) belongs to *F. (Pettancyllus)*. Pelseneer recognised 3 ganglia in the visceral loop,

<sup>2</sup>The name abdominal is preferable to visceral because this is a composite ganglion innervating the body wall as well as the viscera.

namely, a left pleural + parietal, an abdominal, and a supra-intestinal + right pleural. The lateral ganglia thus appear to be homologous with those termed pleuro-parietal in *F. burnupi*.

Hoff (1940) used the terms left anterior visceral, left posterior visceral, and right visceral for the 3 post-cerebral ganglia of *Ferrissia tarda*. His description and figure showing 2 connectives with the pedal ganglion suggest that his left anterior visceral and right visceral ganglia represent fused pleural and parietal ganglia. Two parietal ganglia and one abdominal ganglion were illustrated by Lever (1957) in *Ferrissia* sp. of North American origin (identified as *F. shimekii* (Pilsbry) by Lever, unpublished), but no information was given about the pedal connectives. That form shows marked differences to *F. burnupi* in the position of attachment of the cerebro-pedal connectives to the cerebral ganglia, in the peripheral nerves arising from the cerebral ganglia, and in the presence on these ganglia of large lateral lobes.

Three post-cerebral ganglia similar in size and arrangement to those of *Ferrissia burnupi* were illustrated by Mirolli (1960) for *Watsonula wautieri* from Italy. These ganglia were named left and right parietal and visceral by Wautier et al. (1961) in French material, later identified (Wautier, 1964) as *Gundlachia wautieri*; no information about pedal connectives was given.

Although the nature of the lateral ganglia in the visceral loop can be interpreted with confidence in no more than a few species of *Ferrissia*, it appears likely, from the general similarity of these ganglia in all the forms that have been studied, that partial fusion of the pleural and parietal ganglia is common in this genus. Pleuro-parietal ganglia lying on either side of an abdominal ganglion have been observed in *Ancyclus tapirulus* by Hubendick (1960a), in *Burnupia caffra* by Brown (unpublished observations), and in the Acroloxidae by Hubendick (1962). *An-*

*cyclus tapirulus* resembles *Ferrissia burnupi* in the presence of a small left pleural ganglion, which is distinct from the pedal ganglion but is closely associated with the cerebral and parietal ganglia. While the formation of pleuro-parietal ganglia appears to occur frequently in freshwater limpets, the single connective to the right pedal ganglion of *Laevapex fuscus* illustrated by Basch (1959b) suggests that fusion of the cerebral and pleural ganglia can occur.

The anatomical characteristics of representatives of the 2 subgenera of *Ferrissia* may be compared in Table 1. Information for *F. rivularis*, the type species of the genus, is derived from Hubendick (1964) and from the account by Hoff (1940) for *F. tarda*, which is synonymous with *F. rivularis* according to Basch (1963). Anatomical differences described between the various North American species of *Ferrissia* s.s. are only slight and lie mainly in the size of the flagellum and the pseudobranch (Basch, 1963). Included in Table 1 are *F. burnupi*, *F. tenuis*, and *F. australica* (Tate), which, according to Hubendick (1964), is closely related to the type species of *Pettancylus*.

*Ferrissia burnupi* resembles *F. australica*, but differs from *F. rivularis*, in respect of its radular formula (small number of teeth), jaw (separate scales), large uterine gland complex, and copulatory organ (long flagellum attached to vestigial penis sheath containing short penis). These resemblances particularly in the copulatory organ, are reasons for classifying *F. burnupi* in the subgenus *Pettancylus* as defined by Hubendick (1964). In addition, *F. burnupi* is poorly pigmented, which is the normal condition in that group according to Hubendick.

The relations between *Ferrissia burnupi* and *F. tenuis* are not easily determined in the absence of any analysis of variation in taxonomic characters at the specific level within the subgenus *Pettancylus*. The cusps on the radular teeth of *F. burnupi* are less pointed, and there are fewer teeth in a transverse

row than in *F. tenuis*. No distinct seminal vesicle was observed in *F. burnupi*; the flagellum attached to the copulatory organ is somewhat larger in that species. At present, these differences may be regarded as of specific rank, although it is possible that differences in organs associated with the reproductive system are related to different phases of reproductive activity. In view of the close relationship between the South African *F. burnupi* and the Indian *F. tenuis*, and the presence in Natal of a human Indian population that might be susceptible to the Indian strain of *Schistosoma haematobium* if it were introduced, *F. burnupi* is worthy of consideration as a potential intermediate host.

*Ferrissia burnupi* should be compared with *Watsonula wautieri* Mirolli of Italy, since Hubendick (1964) regarded the latter as a member of *Pettancyclus* and suggested that it had been introduced into Europe in recent time. Africa is an obvious source of such an introduction. According to the information given by Mirolli (1960) and Hubendick (1964) for aphyllid, non-septate specimens of *W. wautieri*, it appears that *F. burnupi* has fewer ovotestis lobes and a shorter spermathecal duct, while in other respects the anatomies of the 2 species are similar. However, from the description of the copulatory organ by Wautier et al. (1966) for 3 ephyllid specimens from France, identified as *Gundlachia wautieri*, that species resembles *Ferrissia* s.s. rather than *F. (Pettancyclus)* in respect of the well developed penis sheath and moderately long penis (0.2 - 0.3 mm). It can only be concluded that the relations between the Italian and French limpets, and their affinities with other ancyliids deserve further study.

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## RESUMEN

ANATOMIA Y RELACIONES DE UNA *FERRISSIA* SUDAFRICANA  
(BASOMMATOPHORA: ANCYLIDAE)

D. S. Brown

El género *Ferrissia*, introducido por Walker (1903) para acomodar *Ancyclus rivularis* Say de Norte América, ha sido subsecuentemente reconocido como un género de distribución mundial, caracterizado por la fina escultura radial del ápice. Por evidencia anatómica, las especies del Nuevo y Viejo Mundo fueron clasificadas respectivamente en los subgéneros *Ferrissia* s.s. y *F. (Pettancyclus)* por Hubendick (1964). El género *Gundlachia* Pfeiffer, originalmente caracterizado por el septum de la conchilla, fue restringido por Hubendick para ancylicos de Central y Sud América: especies africanas previamente asignadas a este género son, aparentemente, formas septadas de *Ferrissia*.

La distribución de *Ferrissia* en Africa se extiende desde el Mediterráneo al Cabo de Buena Esperanza. La información publicada sobre la anatomía de las especies africanas, tratan sólo la rádula, mandíbula y caracteres externos. El presente trabajo trata ciertos aspectos de la anatomía interna y externa de los individuos no septados de *F. burnupi* (Walker, 1912), colectados en la Provincia de Natal, República de Sud Africa. Un objeto del estudio era obtener información para comparar esta con otras especies de *Ferrissia* del Viejo Mundo, en particular *F. tenuis* (Bourguignat), que ha sido indicada como trasmisora de esquistosomiasis humana en India.

Aspectos de *Ferrissia burnupi* que indican estrecha relación con el grupo *Pettancyclus*, son: la escasa pigmentación, la falta de fusión entre las placas que componen la mandíbula, el largo complejo glandular uterino y, sobre todo, la estructura del órgano copulador masculino con su pene pequeño y vestigios de una vaina penial en la cual se abre un largo flagelo. Aunque las diferencias entre *F. burnupi* y *F. tenuis* en la rádula, vesícula seminal y flagelo pueden ser de rango específico, *F. burnupi* merece consideración como un posible huesped intermedio de *Schistosoma haematobium* en Natal.

Anatómicamente *Ferrissia burnupi* se asemeja a las formas italianas no septadas de *Watsonula wautieri* Mirolli, de acuerdo a Mirolli (1960) y Hubendick (1964), pero el órgano copulatorio difiere de aquel descrito por Wautier y otros (1966) para ejemplares de Francia identificados como *Gundlachia wautieri*.

El ganglio pleural izquierdo de *F. burnupi* no se puede distinguir del parietal izquierdo, aunque está íntimamente conectado con éste. La fusión entre los ganglios correspondientes del lado derecho es más avanzada. Los ganglios laterales en la torsión visceral son llamados pleuro-parietales izquierdo y derecho y el asimétricamente colocado entre ellos, ganglio abdominal, una terminología que expresa la composición y función de esos tres ganglios en *Ferrissia* más seguramente que en previa terminología, y puede aplicarse a otros ancylicos. Hay conectivos separados entre los ganglios pedal, cerebral y pleuro-parietal en cada lado.

## АБСТРАКТ

АНАТОМИЯ И РОДСТВЕННЫЕ ВЗАИМООТНОШЕНИЯ ЮЖНО-АФРИКАНСКИХ  
*FERRISSIA* (BASOMMATOPHORA: ANCYLIDAE)

Д. С. БРОУН

Род *Ferrissia*, установленный Уолкером (Walker, 1903) для *Ancyclus rivularis* Say из Северной Америки, рассматривался как всесветно-распространенный род пресноводных улиток, махушка раковины у которых обладает тонкой радиальной скульптурой. По своему анатомическому строению виды *Ferrissia* из Старого и Нового

Света были отнесены Хубендиком (Hubendick, 1964) к подродам *Ferrissia* s.s. и *F. (Pettancylus)*, соответственно. Род *Gundlachia* Pfeiffer, первоначально характеризовавшийся раковиной с септами, был сужен Хубендиком до объема анциллид Центральной и Южной Америки; африканские виды, ранее относимые к этому роду, являются, видимо видами рода *Ferrissia*, обладающими септами.

В Африке *Ferrissia* распространены от Средиземного моря до южной ее оконечности. Имеющиеся опубликованные данные по анатомии африканских видов сводятся к строению радулы, челюстей и к наружным признакам. Настоящая работа касается внутренней и внешней анатомии без-септовых форм *Ferrissia burnupi* (Walker, 1912), собранных в провинции Наталь, Южно-Африканская Республика. Целью настоящего исследования было получение данных для сравнения этих форм с другими видами *Ferrissia* Старого Света, особенно с *F. tenuis* (Bourguinat), который стал известен, как промежуточный хозяин возбудителя человеческого шистозомиазиса в Индии. Признаками, указывающими на близкое родство *Ferrissia burnupi* с видами группы *Pettancylus*, являются следующие: слабая пигментация, отсутствие слияния между пластинками челюстей, крупный маточный железистый комплекс и, кроме того, структура мужского копулятивного органа с его маленьким penisом и рудиментарной его оболочкой, куда входит длинный флагеллум. Хотя различия между *F. burnupi* и *F. tenuis* в строении зубцов радулы семенного пузырька и флагеллума могут иметь видовое значение, *F. burnupi* заслуживает внимания, как возможный промежуточный хозяин *Schistosoma haematobium* в Натале.

Анатомически *Ferrissia burnupi* похожа на бессептовую итальянскую форму *Watsonula wautieri* Mirolli как это следует из работ Миролли (Mirolli, 1960) и Хубендика (Hubendick, 1964), но её копулятивный орган отличается от описанного Вотье и др. (Wautier et al, 1966) для французских экземпляров, определенных как *Gundlachia wautieri*.

Левый плевральный ганглий у *Ferrissia burnupi* отличим от левого париетального ганглия, хотя и тесно с ним связан. Слияние между соответствующими ганглиями правой стороны происходит даже еще дальше. Боковые ганглии в висцеральной петле названы левым и правым плевро-париетальными ганглиями, а ассимметрически-расположенный ганглий, лежащий между ними - абдоминальным ганглием; эта терминология более точно выражает состав и функцию этих трех ганглиев у *Ferrissia*, чем предыдущие названия и может быть применена и к некоторым другим анциллидам. Имеются отдельные коннективы между ножным ганглием, церебральным и плевро-париетальными ганглиями с каждой стороны.

CHROMOSOME NUMBERS IN RELATION TO OTHER MORPHOLOGICAL  
CHARACTERS OF SOME SOUTHERN AFRICAN *BULINUS*  
(BASOMMATOPHORA: PLANORBIDAE)<sup>1</sup>

D. S. Brown<sup>2</sup>, C. H. J. Schutte<sup>3</sup>, J. B. Burch<sup>4</sup> and R. Natarajan<sup>5</sup>

ABSTRACT

Chromosomes were counted in preparations of ovotestis tissue from *Bulinus* (*Bulinus*) collected at 87 localities in southern Africa. A basic haploid set of 18 chromosomes was present in all samples. In 3 samples of the group of *B. natalensis*, 1-3 extra chromosomes were observed, with different numbers of chromosomes occurring in different meiotic cells of the same individuals in one sample. Two samples of laboratory specimens of *B. truncatus* from Egypt and Iran were, as expected, tetraploid:  $n=36$ .

Previous work has suggested that a haploid chromosome number of  $n=18$  is characteristic of the *tropicus* species group and  $n=36$  (or higher multiples) is characteristic of the *truncatus* species group. Although all the southern African specimens studied possessed a basic chromosome number of 18, some populations had smoothly curved mesocones of the 1st lateral teeth of the radula associated with the *tropicus* group, while others had the angular-sided mesocones of the *truncatus* group and in some cases included aphyllid specimens, also associated with that group. Thus, chromosome numbers do not always show a correlation with these other characters in respect of the 2 established species groups. Therefore the *natalensis* species group is introduced to contain forms which are morphologically similar to the *truncatus* group but possess only 18 chromosomes. To this end the samples studied were divided according to the shapes of the mesocones (which showed some correlation with shell shape) into the *tropicus* or *natalensis* groups, or were placed in an 'intermediate' category.

The importance of the separation of species groups of *Bulinus* lies in the fact that some of the intermediate hosts of *Schistosoma* spp. with terminal-spined ova are included in the *truncatus* group, and may possibly exist in the *natalensis* group, while members of the *tropicus* group apparently do not transmit human bilharziasis.

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<sup>4</sup>Museum and Department of Zoology, University of Michigan, Ann Arbor, U. S. A. Supported by a research grant (AI 07279) and Research Career Program Award (5-K3-AI-19, 451) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

<sup>5</sup>Museum of Zoology, University of Michigan, Ann Arbor, U. S. A. Supported by a research grant (GB-787) from the National Science Foundation, Washington D. C., U. S. A.

The *tropicus* group was found to occur over the greater part of South Africa including western Cape Province, whereas the *natalensis* group is apparently confined to the warmer moderate and lower altitudes of the northern and eastern parts of the area.

## INTRODUCTION

This paper deals with southern African material referable to the *tropicus* and *truncatus* species groups (Mandahl-Barth, 1957) of the planorbid genus *Bulinus*, which constitute the subgenus *Bulinus* s.s. according to the usage of Walter (1962), Burch (1964) and Natarajan, Burch & Gismann (1965). Few of the taxa that have been established within these species groups can be unequivocally defined, and the limits of the groups themselves are not entirely clear. Since some members of the *truncatus* group serve as intermediate hosts of schistosomes with terminal-spined ova and members of the *tropicus* group apparently do not transmit human bilharziasis, an improvement in our knowledge and classification of the bulinine snails may contribute to a better understanding of their parasites.

Species of the *tropicus* group occur in Africa south of the Sahara and are unknown in countries bordering the Mediterranean Sea. The northwestern limit of the range of this group appears to lie in West Cameroon (Wright, 1965), and it should be noted that a considerable part of the range formerly attributed to it in northwest Africa (Mandahl-Barth, 1957) was based upon records of *Bulinus guernei* (Dautzenberg), which is now regarded as belonging to the *truncatus* group by Wright (1965) and Mandahl-Barth (1965). In eastern Africa the *tropicus* group extends from Kenya to the coast of Cape Province (Mandahl-Barth, 1957; Azevedo et al., 1961; van Eeden, Brown & Oberholzer, 1965). The *truncatus* group, as formerly understood, occurs primarily in many Mediterranean and Middle Eastern countries, and also in central and southern Africa where the southernmost recorded localities are in

South West Africa (*B. natalensis*; Mandahl-Barth, 1965), Transvaal (*B. depressus*; van Eeden, 1964; Schutte, 1966), and Natal (*B. depressus*; van Eeden et al., 1965).

Burch (1964) and Natarajan et al. (1965) have reported on 35 samples of *Bulinus* from central Africa, several countries of the Mediterranean area, from Asia minor, and Western Aden Protectorate, and found that the species of the *tropicus* and the *truncatus* groups investigated possessed haploid sets of 18 and 36 chromosomes respectively. Burch (1964) commented on the combination in the *truncatus* group of a haploid set of 36 or higher multiples of 18, with the capacity to transmit *Schistosoma haematobium*, and suggested that chromosome numbers might help in identifying potential intermediate hosts.

The present paper describes the results of an investigation of chromosome numbers in relation to several taxonomic characters previously employed, i.e., the shell, radula (shape of the mesocone of the first lateral tooth) and copulatory organ (commonly absent in the *truncatus* group). The purpose of our work was to investigate the distribution in South Africa of *Bulinus* populations apparently belonging to the *truncatus* group, and to determine whether or not a correlation existed between chromosome numbers and the morphological characters mentioned above. Identification of our material is based upon the radula, which has been given importance in definitions of the *truncatus* and *tropicus* groups (Mandahl-Barth, 1957).

## MATERIAL AND METHODS

Specimens intended for cytological examination, collected between June 25,

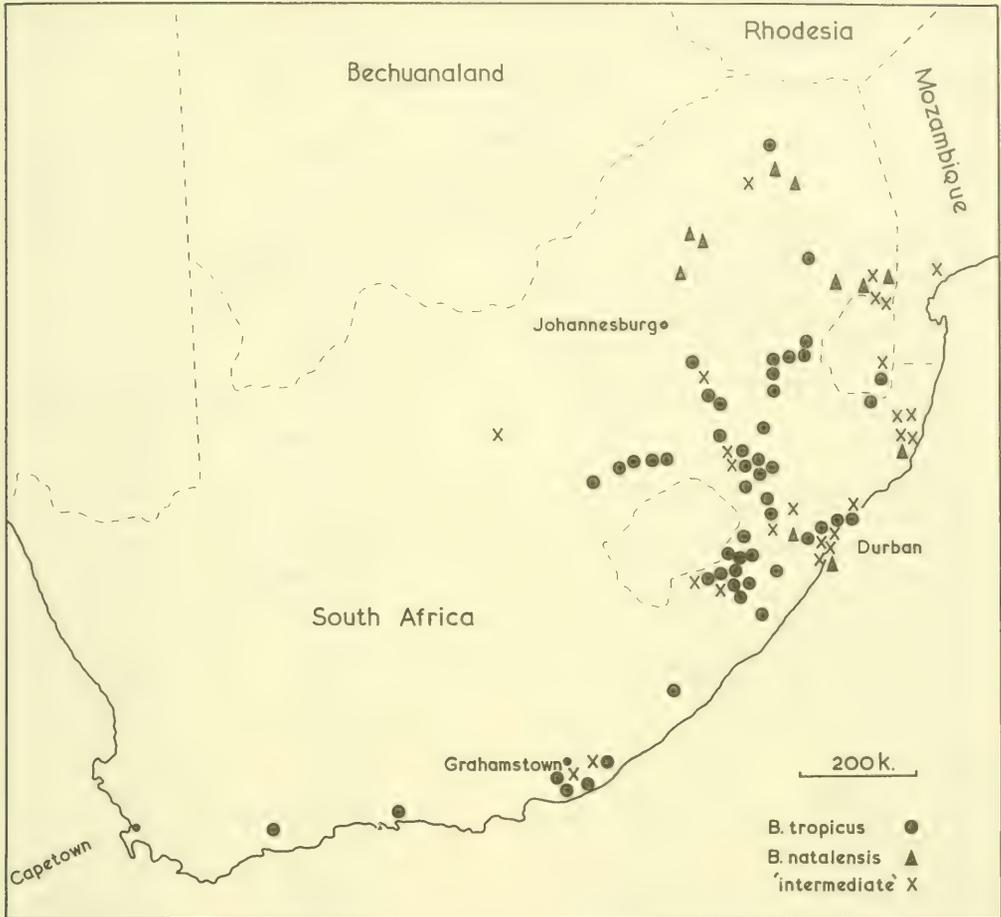


FIG. 1. Localities in southern Africa from which samples of *Bulinus* were examined and assigned to the species groups of *B. tropicus*, *B. natalensis* or to an 'intermediate' category, on radular characteristics.

1964 and March 28, 1965, were killed and preserved in Newcomer's (1953) fluid after the apices of the shells had been cracked to allow rapid penetration of the fixative. Chromosomes were observed in ovotestis tissue prepared by the acetic-orcein squash technique (La Cour, 1941). Observations were made with Nikon microscopes using 100X (n.a. 1.25) oil immersion objectives and 10, 20 and 30X oculars.

The damaged shell and the animal of each specimen were retained and labelled so that they could be correlated with the appropriate ovotestis preparation.

Each animal was dissected to determine whether the copulatory organ was present or absent, and an unstained preparation was made of each radula in the manner described by Schutte (1965); further observations on shells, radulae and copulatory organs were made from specimens preserved in alcohol.

Specimens were obtained from a total of 87 localities situated in the Republic of South Africa, Swaziland or Mozambique (Fig. 1 and Table 1); records containing full details of these localities have been deposited in the Experimental Taxonomy Section of the Zoology De-

TABLE 1. Chromosome numbers and other morphological features of various populations of South African *Bulinus*

Locality*	Chromosome number		Radula type	Copulatory organ	
	n	2n		Nos. studied	Nos. aphallic
1. Mhlangana R., Durban (N)	18-21	-	intermediate	155	0
2. Mhlatuzane R., Durban (N)	18	-	intermediate	75	0
3. Umsunduzi R., Valley of Thousand Hills (N)	18	-	<i>tropicus</i>	10	0
4. Pietermaritzburg (N)	18	36	<i>natalensis</i>	51	7
5. Mooi River at town (N)	18	-	<i>tropicus</i>	10	0
6. Newcastle (N)	18	-	<i>tropicus</i>	25	0
7. Bisana (CP)	18	-	<i>tropicus</i>	60	0
8. Tongaat (N)	18	-	intermediate	100	0
9. Wewe R., Tongaat (N)	18	36	<i>tropicus</i>	100	0
10. Shakaskraal (N)	18	36	<i>tropicus</i>	4	0
11. Ncanaweni R., Stanger (N)	18	-	<i>tropicus</i>	8	0
12. Stanger (N)	18	-	intermediate	100	0
13. Big Bend, Swaziland	18	-	intermediate	5	0
14. Big Bend, Swaziland	18	36	<i>tropicus</i>	3	0
15. Pongola Settlement (T)	18	-	<i>tropicus</i>	8	0
16. Sterkspruit, Lydenberg (T)	18	-	<i>tropicus</i>	50	0
17. Kokstad (CP)	18	-	<i>tropicus</i>	14	0
18. Ixopo R., at town (N)	18	-	<i>tropicus</i>	25	0
19. Kokstad (CP)	18	-	<i>tropicus</i>	7	0
20. Cedarville (CP)	18	-	<i>tropicus</i>	23	0
21. Cedarville (CP)	18	-	intermediate	18	0
22. Matatiele (CP)	18	-	<i>tropicus</i>	53	0
23. Matatiele (CP)	18	-	<i>tropicus</i>	4	0
24. Swartberg (CP)	18	-	<i>tropicus</i>	25	0
25. Swartberg (CP)	18	-	<i>tropicus</i>	14	0
26. Swartberg (CP)	18	-	<i>tropicus</i>	13	0
27. 23 km N. Swartberg (CP)	18	-	<i>tropicus</i>	28	0
28. 40 km N. Swartberg (N)	18	-	<i>tropicus</i>	24	0
29. Mtubatuba (N)	18	-	<i>natalensis</i>	50	0
30. Ncemane R., Hluhluwe (N)	18	36	intermediate	100	0
31. Msunduzi R., Hluhluwe (N)	18	-	intermediate	20	0
32. Msunduzi R., Hluhluwe (N)	18	-	intermediate	5	0
33. Mzinene R., Hluhluwe (N)	19	-	intermediate	15	0
34, 35. Ladysmith (N)	18	-	<i>tropicus</i>	120	0
36. Bethlehem (OFS)	18	-	<i>tropicus</i>	20	0
37. 46 km W. of Bethlehem (OFS)	18	-	<i>tropicus</i>	20	0
38. 70 km W. of Bethlehem (OFS)	18	36	<i>tropicus</i>	50	0
39. 96 km W. of Bethlehem (OFS)	18	36	<i>tropicus</i>	11	0
40. Winburg (OFS)	18	-	<i>tropicus</i>	15	0
41. Christiana (T)	18	-	intermediate	18	0
42. Pietersburg (T)	18	36	intermediate	50	0
43. Bandoleirkop (T)	18	-	<i>tropicus</i>	20	0
44. Thabina R., Tzaneen (T)	18	-	<i>natalensis</i>	10	8
45. Nylstroom (T)	18	-	<i>natalensis</i>	10	6
46. Palmeira, Mozambique	18	-	intermediate	100	0

\*Except for localities 60 and 61, which are from the Middle East, provinces of South Africa are abbreviated as follows: Natal (N), Cape Province (CP), Orange Free State (OFS), Transvaal (T).

Table 1 (continued)

Locality*	Chromosome number		Radula type	Copulatory organ	
	n	2n		Nos. studied	Nos. aphallic
47. Winterton (N)	18	-	<i>tropicus</i>	4	0
48. Estcourt (N)	18	36	<i>tropicus</i>	1	0
49. Nottingham Road (N)	18	36	intermediate	6	0
50. Roodepoort, Warmbaths (T)	18	-	<i>natalensis</i>	6	0 <sup>+</sup>
51. Leeupoort, Nylstroom (T)	18	-	<i>natalensis</i>	4	0 <sup>+</sup>
52. Nylstroom (T)	18	-	intermediate	5	0
53. Nelspruit aquaria (T)	18	-	<i>natalensis</i>	100	0
54. Buffelspruit, Malelane (T)	18	-	<i>natalensis</i>	13	0
55. Thankerton Creek, Hector-spruit (T)	18	-	intermediate	10	0
56. Ngwetspruit, Komatipoort (T)	18	-	<i>natalensis</i>	10	0
57. Border Gate, Swaziland	18	-	intermediate	10	0
58. Border Gate, Swaziland	18	-	intermediate	5	0
59. Simondsdal, Lake Chrissie (T)	18	36	<i>tropicus</i>	5	0
60. Abis, Egypt	36	-	<i>truncatus</i>	5	0
61. Iran	36	-	<i>truncatus</i>	5	4
62. Merebank, Durban (N)	18-20	-	<i>natalensis</i>	9	2
63. Durban (N)	18	-	intermediate	55	0
64. Rietvlei (N)	18	-	intermediate	50	0
65. Ladysmith (N)	18	-	<i>tropicus</i>	50	0
66. Van Reenen (OFS)	18	-	intermediate	50	0
67. Van Reenen (OFS)	18	-	<i>tropicus</i>	50	0
68. Harrismith (OFS)	18	-	intermediate	30	0
69. Harrismith (OFS)	18	-	<i>tropicus</i>	50	0
70. 113 km NW of Harrismith (OFS)	18	-	<i>tropicus</i>	27	0
71. Villiers (T)	18	-	<i>tropicus</i>	15	0
72. Villiers (T)	18	-	intermediate	50	0
73. Villiers (T)	18	36	<i>tropicus</i>	50	0
74. Tzaneen (T)	18	-	<i>natalensis</i>	11	5
75. Idutywa (CP)	18	-	<i>tropicus</i>	25	0
76. Heidelberg (CP)	18	-	<i>tropicus</i>	20	0
77. Knysna (CP)	18	-	<i>tropicus</i>	20	0
78. Grahamstown (CP)	18	-	<i>tropicus</i>	30	0
79. Port Alfred (CP)	18	-	<i>tropicus</i>	14	0
80. Alexandria (CP)	18	-	<i>tropicus</i>	25	0
81. Grahamstown (CP)	18	-	intermediate	14	0
82. Breakfastvlei (CP)	18	-	intermediate	29	0
83. Peddie (CP)	18	-	<i>tropicus</i>	3	0
84. 46 km NW Piet Retief (T)	18	-	<i>tropicus</i>	18	0
85. Lothair (T)	18	-	<i>tropicus</i>	20	0
86. Ermelo (T)	18	-	<i>tropicus</i>	25	0
87. Ermelo (T)	18	-	<i>tropicus</i>	25	0
88. Amersfoort (T)	18	-	<i>tropicus</i>	24	0
89. Sani Pass (N)	18	-	<i>tropicus</i>	31	0

+Aphallic specimens were present in the original samples from which these specimens were descended.

partment, British Museum (Natural History), and in the Institute for Zoological Research, University of Potchefstroom, South Africa.

Most of the samples were fixed in the field, but laboratory-bred descendants of snails collected at localities 50, 51, 52, 53 and 59 were used. Observations were also made on laboratory stocks of *Bulinus truncatus truncatus* originally obtained in Egypt and Iran (60 and 61).

## OBSERVATIONS

### Chromosomes (Table 1)

A constant number of 18 chromosomes was present in meiotic (haploid) cells of all but 3 of the 87 samples from southern Africa. Between 1 and 16 individuals were examined from each sample. Eighteen pairs of chromosomes were observed whenever it was possible to make counts in diploid cells.

In the 3 exceptional samples, although the basic number was also 18, between 1 and 3 extra chromosomes were observed in meiotic cells. All 3 specimens of sample 33 had 19 chromosomes. Sixteen specimens of sample 1 were examined having 18, 19, 20 and 21 chromosomes in individual specimens in the proportions 5:5:5:1. Different numbers of chromosomes were present in different meiotic cells from each of the 2 specimens examined from locality 62; 18, 19 and 20 in one individual, and 18 and 19 in the other. Such supernumerary chromosomes have already been reported for *B. natalensis* from Southern Rhodesia (Burch, 1964).

Two samples of *Bulinus truncatus truncatus* from Egypt and Iran (localities 60 and 61) possessed 36 chromosomes in meiotic cells. This polyploid number has been reported before for *B. t. truncatus* from these 2 countries by Burch (1964).

### Radula (Figs. 2 and 3)

Each radula was classified according to the most frequent shape of the mesocones (middle cusps) of the first lateral

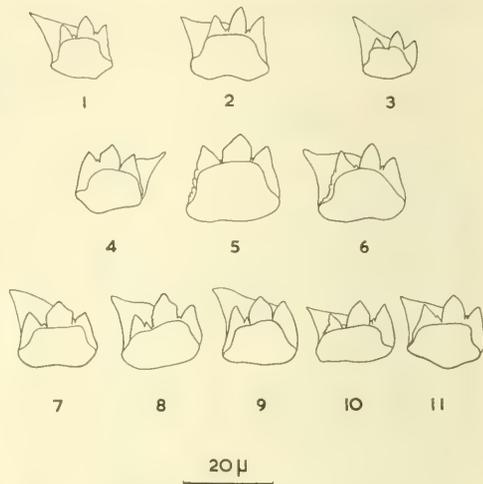


FIG. 2. First lateral teeth of radulae of:

*Bulinus* of the *tropicus* group. 1, locality 88; 2, locality 67; 3, locality 25 (the mesocones are typical of the *tropicus* type).

*Bulinus* of the 'intermediate' group. 4-6, locality 68; 7-11, locality 1 (the mesocones of 4, 8 and 10 are of the intermediate type; 5 and 7 are of the *natalensis* type; 6, 9 and 11 are of the *tropicus* type).

teeth into 1 of the 3 following categories:

1. *tropicus* group: Sides of mesocone smoothly curved (Fig. 2: 1-3, 6; Fig. 3: 4, 19), or slightly angular (Fig. 2: 9, 11). These shapes most closely resemble the "triangular" mesocone described by Mandahl-Barth (1957) for the *tropicus* group.
2. *natalensis* group: Both sides of mesocone angular, with the sides usually converging towards the base (Fig. 2: 5, 7; Fig. 3: 1-3, 5-14, 16-18). This shape resembles the "arrow-head" mesocone described by Mandahl-Barth (1957) for the *truncatus* group.
3. Intermediate group: One side of the mesocone angular (Fig. 2: 4, 10; Fig. 3: 15). Other mesocones which, because of the fluting of their edges, could not be classified in either of the 2 preceding groups (Fig. 2: 8;

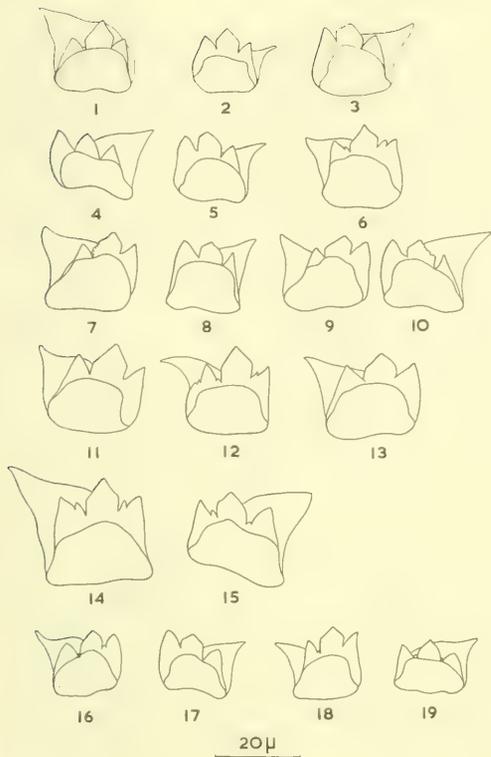


FIG. 3. First lateral teeth of radulae of:

*Bulinus* of the *natalensis* group. 1, locality 29; 2, locality 4; 3, locality 44; 4, 5, locality 45; 6, locality 53; 7, 8, locality 50; 9, 10, locality 51; 11, locality 54; 12, locality 56; 16, 17, locality 75; 18, 19, locality 62 (most mesocones are typical; 4 and 19 are of the *tropicus* type).

*Bulinus truncatus truncatus*. 13, locality 60; 14, 15, locality 61 (mesocone 15 is of the intermediate type).

see also Schutte, 1965), are placed here.

Whenever possible 5 radulae were prepared from each sample of snails. The mesocones on the first lateral teeth in the unworn rows were examined. Individual radulae usually had mesocones of all the 3 types described above and were classified in the group of whichever type of mesocone was prevalent. Each sample was then classified according to its most frequent type of radula.

Data on radulae of other specimens from localities 16, 50, 51, 53, 55, 59 and 60 of the present series were given by Schutte (1965), who presented a detailed analysis of the frequencies of various shapes of mesocone. No detailed analysis was carried out in the present investigation, but in material from the same localities the most frequent shapes of mesocone were found, with one exception, to correspond well with those reported by Schutte. Material from the exceptional locality (55: Thankerton Creek, Hectorspruit) was classified by Schutte in the *tropicus* group but is included in our 'intermediate' category. This difference may be due to the fact that whereas Schutte determined the prevalent type of mesocone in each sample of radulae considered as a whole, our identifications are based on separately classified radulae.

More than 50% of the samples classified in the *natalensis* group and approximately 25% of those placed in the *tropicus* group included radulae of the intermediate category, and about 80% of the samples placed in the intermediate group included radulae of either or both the *tropicus* and *natalensis* types. However, no *natalensis* sample possessed any *tropicus* radulae although mesocones of this type were present in some radulae. Similarly, no *tropicus* sample contained any *natalensis* radulae although mesocones of this type were present in some radulae.

Shell (Figs. 4 and 5)

The *tropicus* and *natalensis* groups of samples possess in general distinct forms of shell, but an attempt to express accurately the degree of correlation between mesocone shape and shell form cannot be made until the variation of these characters has been studied further. Characteristics commonly present in *tropicus* group shells are: the long spire which is conical and sharply pointed, the evenly rounded whorls, and the acute upper angle of the aperture. These features are illustrated in shells

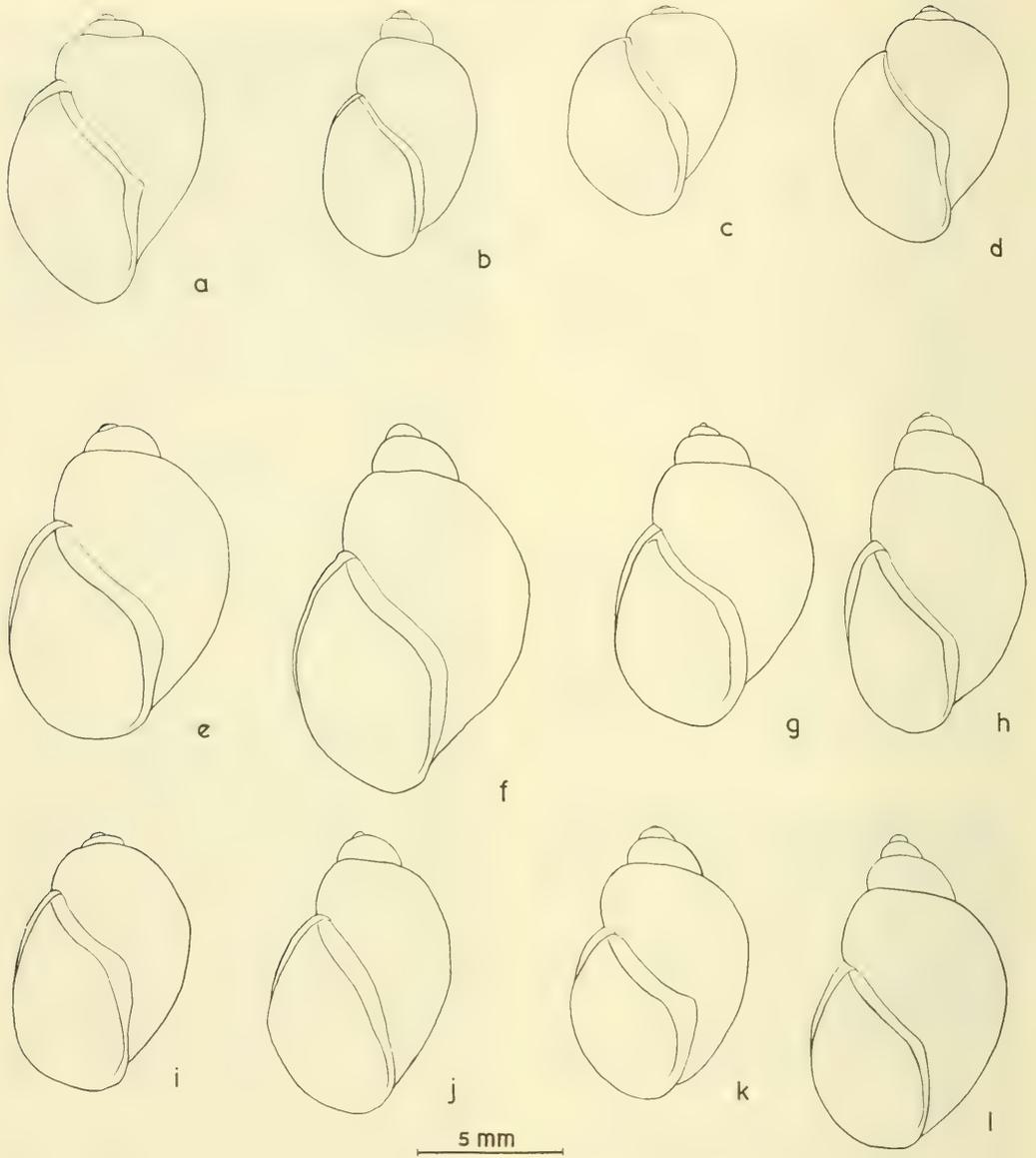


FIG. 4. Shells of *Bulinus* classified according to the prevalent shape of mesocone on the first lateral teeth of the radula.

*tropicus* group: e, f, locality 20; g, h, locality 22; i, j, locality 34; k, l, locality 90 (these shells are of the form commonly found in the *tropicus* group of samples, and also represent the range of variation in their particular samples).

'intermediate' group: a-d, locality 1 (these shells illustrate the wide variation present in some samples possessing radulae of the 'intermediate' type).

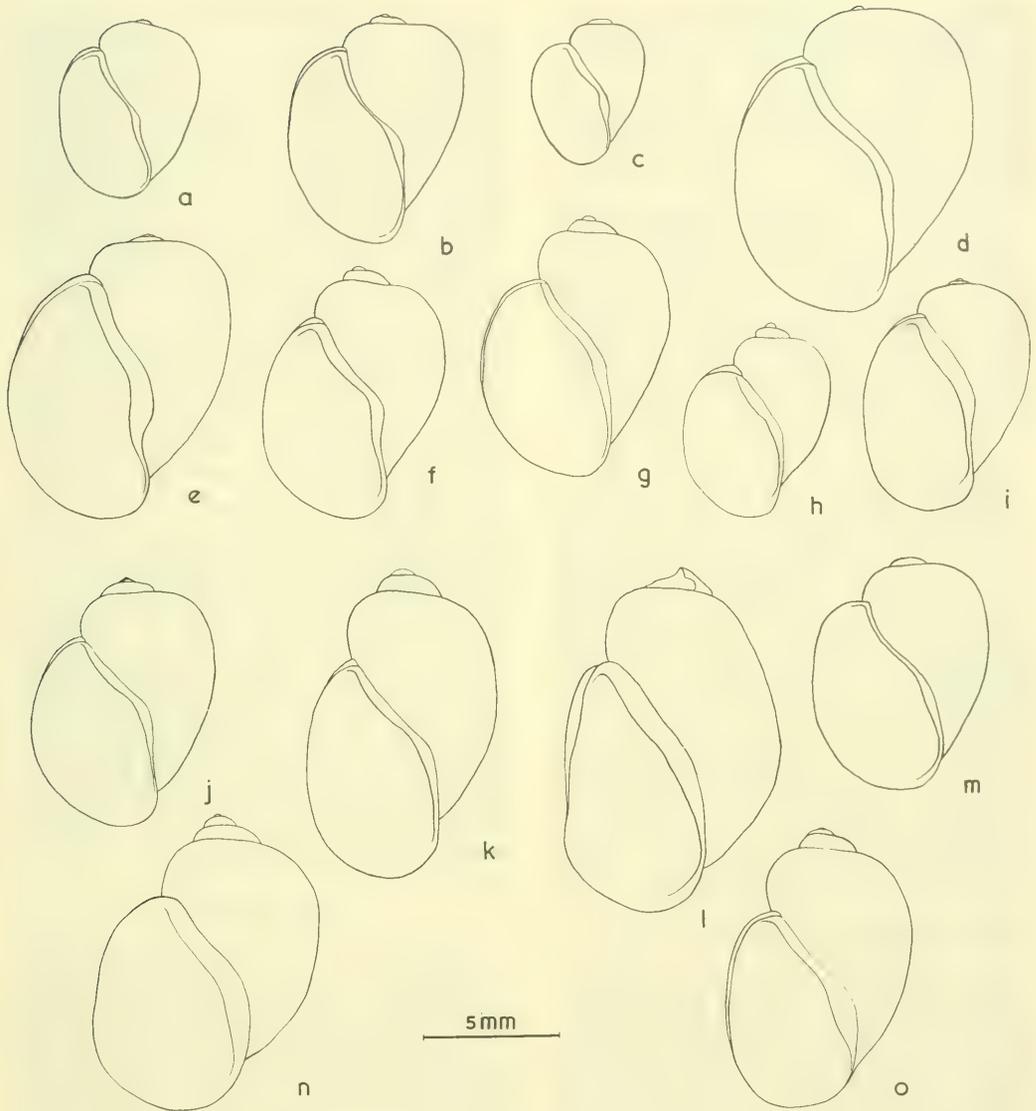


FIG. 5. Shells of *Bulinus* classified according to the prevalent shape of mesocone on the first lateral teeth of the radula.

*natalensis* group: a, m, locality 75; b, c, locality 62; d, e, f, locality 29; g, h, locality 44; i, j, locality 45; k, locality 50; l, locality 51; m, n, o, locality 53.

representing the extremes of variation in 4 samples of the *tropicus* group (Fig. 4, e-l). A microsculpture of fine spiral lines is present on the post-embryonic

whorls of some shells.

The majority of the shells in the *natalensis* group samples have comparatively short spires, and in further

contrast to the *tropicus* group have shouldered whorls and an obtuse upper angle of the aperture (Fig. 5). A microsculpture of fine spiral lines, better developed than in the *tropicus* group, is frequently present on the post-embryonic whorls.

Some of the samples classified as intermediate on the basis of their radulae possessed shells of the *tropicus* type, while others had shells of the *natalensis* type. Still other samples contained a wide variety of shells, including forms resembling both the *tropicus* and *natalensis* types (Fig. 4, a-d).

#### Copulatory organ (Table 1)

Aphallic individuals were present in 5 out of the 12 samples of the *natalensis* group (localities 4, 44, 45, 62 and 74) and in 2 further cases had been present in the original stock from which the euphallic specimens here examined were descended (localities 50 and 51). Aphallic specimens were also present in the samples of *B. truncatus* from Iran (locality 61). Some specimens of the *tropicus* group that were heavily infected with larval trematodes possessed a copulatory organ much reduced in size, but no aphallic specimens were found.

#### IDENTIFICATION OF MATERIAL

Many shells in samples classified in the group in which non-angular mesocones were prevalent have a pointed, relatively high spire and a smoothly curved columella, in these respects resembling *Physa tropica* Krauss 1848. It is possible that distinct but related forms are present in South Africa (see Mandahl-Barth, 1957, for synonymy of *B. tropicus*) and were represented in our samples and therefore our material is referred to the *tropicus* group without specific identification.

Some of the samples classified in our group in which angular-sided mesocones were prevalent have shells with a short spire and a straight or twisted columella, thus resembling *Physa natalensis* Küster

1841; several of these samples were collected near the type locality of that species in the valley of the Umgeni River in Natal. Other samples in this group have shells with considerably shorter spires, resembling in this respect *Bulinus hemprichii depressus* Haas 1936, which was included in the synonymy of *Bulinus natalensis* as a member of the *truncatus* group by Mandahl-Barth (1965). In the prevalence of angular-sided mesocones and the presence in some populations of aphallic individuals our samples resemble the forms included by Mandahl-Barth (1957) in the *truncatus* group; cytologically, however, the South African material, with 18 pairs of chromosomes, is diploid, in contrast to the polyploid chromosome numbers possessed by all the northern African or Middle Eastern material of the *truncatus* group that has been investigated (Burch, 1964; Natarajan et al., 1965; Burch, 1967). Schutte (1966) suggested that the South African *Bulinus* apparently belonging to the *truncatus* group examined by him should be placed in a separate group and it seems appropriate to refer our samples to the species group of *Bulinus natalensis*.

#### DISCUSSION

Snails from the northern Transvaal identified as *Bulinus depressus* Haas by van Eeden (1964) have characters of the '*truncatus*' group according to van Eeden (unpublished observations) and Schutte (1966). *B. depressus* is recorded from the eastern Transvaal and northern Natal by van Eeden et al. (1965). Since shells with short spires in some of our *natalensis* group samples are similar to the form identified as *B. depressus* from eastern and northern Transvaal (G. Oberholzer, personal communication; Fig. 5, a-j and m), it seems probable that bulinines with some morphological characters of the '*truncatus*' group, but having 18 pairs of chromosomes, are widely distributed in north eastern South Africa at moderate and low altitudes.

A form of '*B. tropicus*' collected at Grahamstown, Eastern Cape having angular mesocones on the lateral radular teeth (Stiglingh, van Eeden & Ryke, 1962), appears to be the southernmost population known to have that characteristic of the '*truncatus*' group. The distribution pattern of the *B. natalensis* group in South Africa suggests that these snails are adapted to warm climatic conditions, in contrast to the *tropicus* group which is more widely distributed, occurring in the subtropical region and also at relatively high altitudes in the temperate climatic region.

The existence of populations which we have classified as 'intermediate' could be regarded as evidence that genetic exchange between *Bulinus tropicus* and *B. natalensis* may take place. However, the nature of these populations is obscure and it is possible that a more detailed analysis of morphological characters would in some cases reveal 2 or more distinct forms. Some of these samples could be classified in either one or the other of the *tropicus* or *natalensis* groups depending on which taxonomic character was given most importance, i.e. shell and/or shape of the mesocone of the first lateral tooth vs. egg mass proteins and/or chromosome number. For example, specimens from Mhlangana River (locality 2) near Durban have been identified according to their shells and radulae as *B. natalensis* belonging to the *truncatus* group (Mandahl-Barth, personal communication), but according to biochemical evidence this population is most closely related to the *B. tropicus* group (Wright & Ross, 1965). Further, it has 18 pairs of chromosomes, which, according to Burch (1964) places it in the *B. tropicus* group. The apparent lack of correlation between these taxonomic characters suggests that further study of the "subgenus *Bulinus* s.s." in southern Africa will provide a significant contribution to our understanding of the relationship existing between the characters that are at present applied in the distinction of species groups.

A form of *Bulinus* with  $n=18$  occurring in Ethiopia (Lake Awasa) was listed by Natarajan et al. (1965) as *Bulinus* "? *sericinus*" following information supplied with the specimens by C. A. Wright, but considered to belong to the *tropicus* species group according to the chromosome numbers. The same form was classified as *Bulinus* sp. belonging to the *truncatus* group by Brown (1965) according to the shapes of the radular mesocones and the existence of aplanic individuals. This Ethiopian bulinine snail conforms to our definition of the *B. natalensis* group, which therefore appears to be widely distributed in Africa.

Little information has been published on the ability of *Bulinus* of the *natalensis* group to transmit species of *Schistosoma*. Laboratory-bred descendants of snails ( $n=18$ ) collected in Lake Awasa, Ethiopia, could not be infected with *S. haematobium* from Western Aden Protectorate (Brown, 1964). Attempts to infect South African '*B. depressus*' (Table 1, 50 and 51) with strains of *S. haematobium* and *S. mattheei* from the eastern Transvaal were also unsuccessful (Schutte, 1966). Positive results have been reported by Pitchford (1965: 109, 114) and Schutte (loc. cit.) in the case of snails obtained from outdoor aquaria at the Nelspruit laboratory, that were found to be capable of transmitting South African strains of *S. haematobium* and *S. mattheei*, and also Iranian strains of *S. haematobium* and *S. bovis*. Pitchford and Schutte referred to these snails as *Bulinus* sp. belonging to the *truncatus* group and believed them to be descended from specimens collected locally in South Africa. The several specimens of this stock from these outdoor aquaria that were examined cytologically possessed 18 pairs of chromosomes (Table 1, 53) and it therefore may be possible that naturally occurring populations of the *Bulinus natalensis* group are capable of transmitting schistosomes.

One or more extra chromosomes have been reported in meiotic cells of South

African and Southern Rhodesian specimens of the *B. natalensis* group; these represent approximately 4% of all the populations of African *Bulinus* s.s. that have been examined (Burch, 1964; Natarajan et al., 1965; present paper). The nature and origin of these extra chromosomes are, at present, unknown.

Although extra chromosomes occur in a few populations, all "*Bulinus* s.s." in southern Africa have been found to be basically diploid, i.e., to have 18 pairs of chromosomes. Polyploidy has not been found to occur in any specimens south of Tanzania. This homogeneity contrasts with the remarkable variety of haploid chromosome numbers, i.e., 18, 36, 54 and 72, known in Ethiopian populations (Burch, 1967).

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## RESUMEN

EL NUMERO CROMOSOMATICO EN RELACION A OTROS CARACTERES MORFOLOGICOS DE ALGUNOS *BULINUS* DEL AFRICA MERIDIONAL (BASOMMATOPHORA: PLANORBIIDAE)

D. S. Brown, C. H. J. Schutte, J. B. Burch and R. Natarajan

Se contaron los cromosomas en preparaciones de tejidos del ovotestis de *Bulinus* s.s., colectados en 87 localidades del Africa meridional. Un juego básico de 18 cromosomas estaba presente en todas las muestras. En 3 muestras del grupo de *B. natalensis* se observaron de 1 a 3 cromosomas extras, con números diferentes en distintas células meióticas de la mismos individuos de una muestra. En dos muestras de ejemplares de laboratorio de *B. truncatus* del Egipto e Irán, el número, como se esperaba, fué tetraploide: n=36.

Trabajos previos han sugerido que el número de cromosomas haploideos n=18, es característico del grupo de especies de *B. tropicus* y n=36 (o mayores múltiples) del grupo *truncatus*. Aunque todos los ejemplares estudiados del sur de Africa, poseian un número básico de 18, algunas poblaciones tenian los mesoconos del primer diente lateral de la rádula, suavemente curvos, asociados con el grupo *tropicus*, mientras que en otros los mesoconos tenian costados angulosos como en el grupo de *truncatus*, y en algunos casos incluían ejemplares afálicos también asociados a ese grupo. De tal manera, el número cromosomático no siempre muestra una correlación con esos u otros caracteres de los dos grupos establecidos. Por consiguiente, introducimos aquí, un grupo de especies de *B. natalensis*, para incluir las formas que son morfológicamente similares al grupo de *truncatus*, pero que sólo poseen 18 cromosomas. Con esta finalidad las muestras estudiadas fueron divididas de acuerdo a la forma de los mesoconos (que mostraron correlación con la forma de la concha) dentro de los grupos *tropicus* o *natalensis*, o fueron colocados como una categoría intermedia.

La importancia de la separación de *Bulinus* en grupos de especies, se destaca por el hecho de que algunos de los huéspedes de *Schistosoma* spp., con ova terminando en espinas, estan incluidos en el grupo de *truncatus*, y posiblemente existe en el grupo de *natalensis*, mientras que los miembros del grupo de *tropicus* aparentemente no transmiten bilharziasis humana.

El grupo de *tropicus* se encontró en la mayor parte de Sud Africa, incluyendo la Provincia del Cabo, mientras que el grupo de *natalensis* parece estar confinado a las áreas de menor o moderada, altitud, más calidas, y más al norte y al oeste de aquella región.

## АБСТРАКТ

ОТНОШЕНИЕ ЧИСЛА ХРОСОМ К ДРУГИМ МОРФОЛОГИЧЕСКИМ  
 ПРИЗНАКАМ НЕКОТОРЫХ ЮЖНО-АФРИКАНСКИХ  
*BULINUS* (BASOMMATOPHORA: PLANORVIDAE)

Д. С. БРОУН, К. Х. ШЮТТЕ, ДЖ. Б. БЁРЧ И Р. НАТАРАДЖАН

При исследовании тканей гермафродитной железы у *Bulinus* (*Bulinus*), собранных в 87 местах Южной Африки, у них было также подсчитано число хромосом. Основной гаплоидный набор из 18 хромосом имелся у моллюсков из всех проб. В 3 пробах в группе *B. natalensis* были обнаружены 1-3 дополнительных хромосом, при различном числе хромосом, встречаемых в различных мейотических клетках одних и тех же экземпляров животных из одной и той же пробы. Две пробы лабораторных экземпляров *B. truncatus* из Египта и Ирана были, как и ожидалось тетраплоидными:  $n = 36$ .

Из предыдущих работ следовало, что число гаплоидных хромосом равно 18, характерно для видов из группы *tropicus*, а  $n = 36$  (или больше множественных хромосом), свойственно видам из группы *truncatus*. Хотя у всех изученных южно-африканских видов основное число хромосом было 18, некоторые популяции имели гладко-изогнутые мезоконы первый боковых зубцов радулы, что присуще группе видов *tropicus*, в то время, как другие имели угловатые сбоку мезоконы, характерные для группы *truncatus*, и включали в некоторых случаях афалические экземпляры, также связанные с этой группой. Таким образом, число хромосом не всегда коррелирует с другими признаками, как это было установлено для 2, указанных выше групп видов. Поэтому группа видов *natalensis* представляется, как имеющая формы, морфологически сходные с видами группы *truncatus*, но обладающая 18 хромосомами. Исходя из этого, изученные пробы моллюсков были разделены (в соответствии с формой мезоконов, которые имеют некоторую корреляцию с формой раковины) на группы *tropicus* или *natalensis* или были отнесены к "промежуточной" категории.

Важность деления видовых групп *Bulinus* заключается в том, что некоторые моллюски - промежуточные хозяева *Schistosoma* spp. (с заостренными на конце яйцами) включались в группу *truncatus* и, возможно могут оказаться среди форм из группы *natalensis*, в то время, как моллюски из группы *tropicus*, видимо не являются переносчиками человеческого билхарциоза.

Было найдено, что виды группы *tropicus* встречаются на большей части Южной Африки, включая западную часть Калской провинции, в то время как группа *natalensis*, видимо связана с более теплыми, умеренными и низкими широтами северной и восточной частей ареала.

DISTRIBUTION OF CYTOLOGICALLY DIFFERENT  
POPULATIONS OF THE GENUS *BULINUS*  
(BASOMMATOPHORA: PLANORBIDAE) IN ETHIOPIA

D. S. Brown<sup>1</sup> and J. B. Burch<sup>2</sup>

ABSTRACT

The genus *Bulinus* has, like the rest of the Planorbidae, a basic chromosomal complement of 18 pairs of chromosomes. However, in the *B. truncatus* species group there are tetraploid species with 36 pairs and some populations, apparently belonging to that group, have 54 or 72 pairs of chromosomes. The species groups of *B. tropicus* and *B. natalensis* have 18 pairs of chromosomes and are referred to in the present paper, together with the *B. truncatus* group, as the subgenus "*Bulinus* s.s." The diploid ( $n=18$ ) members of the complex occur predominantly in southern Africa, and are known as far north as Lake Tana in Ethiopia and as far west as Senegal. The tetraploid ( $n=36$ ) species occur predominantly in northern Africa and the Mediterranean and Middle Eastern regions, and extend southwards to Mauritania, Senegal, Ghana and Tanzania. The hexaploid ( $n=54$ ) has been recorded only from Ethiopia and the octoploid ( $n=72$ ) from Ethiopia and Western Aden Protectorate. Apparently only the tetraploid, and perhaps the octoploid, serve as intermediate hosts to *Schistosoma haematobium* under natural conditions.

The present paper combines the results of recent cytological observations on 22 samples of "*Bulinus* s.s." collected by the authors in Ethiopia with earlier data. From a total of 28 localities, cytologically different forms have been obtained with the following frequencies:  $n=18$  (10),  $n=36$  (8),  $n=54$  (4) and  $n=72$  (6). Diploid and tetraploid populations are widely distributed, while the hexaploid and octoploid forms have been found only on the highland plateau northwest of the Rift Valley.

Ethiopian "*Bulinus* s.s." have been classified previously in the *B. truncatus* species group. In different populations formerly identified as *B. truncatus sericinus* we have observed diploid, tetraploid and octoploid numbers. It therefore seems likely that study of morphology in relation to chromosome number will lead to a revised classification of these snails.

Both diploid and tetraploid "*Bulinus* s.s." occur in lakes Awasa and Zwai; the 2 forms have distinctive shells and each is apparently restricted to a characteristic habitat. Cytologically different "*Bulinus* s.s." have otherwise been recorded from the same waterbodies only in Tanzania. The fact that different forms have not been found living together in any Ethiopian locality may be due to competition between the forms, based on adaptation to particular ecological conditions.

The tetraploid form of "*Bulinus* s.s." appears to be most relevant in relation to the transmission of *Schistosoma haematobium* in Ethiopia, and its apparent rarity may serve, in conjunction with other epidemiological factors, as a barrier to the establishment of foci of urinary bilharziasis.

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It is possible that future study will lead to the detection of some cytological form, resistant to *S. haematobium*, and successful enough in competition with susceptible snails to serve as a means for their biological control.

## INTRODUCTION

The planorbid genus *Bulinus* inhabits freshwaters in the African continent and the Mediterranean region, extending eastwards in southwestern Asia to Iran. These snails are the subject of intensive study because some forms serve as intermediate hosts to *Schistosoma* species causing human vesical and bovine bilharziasis. The genus *Bulinus* is unusual among Basommatophora in that it includes forms having widely different chromosome numbers, which appear to constitute a polyploid series.

The basic haploid chromosome number in the genus *Bulinus* is 18, as in other Planorbidae (Burch, 1965). However, in the *B. truncatus* (Audouin) group there are tetraploid species with 36 pairs and some populations, apparently belonging to that group, have 54 and 72 pairs (Burch, 1964, 1967). The *B. truncatus* group was defined by Mandahl-Barth (1958) with particular reference to the arrowhead shaped mesocones on the lateral radular teeth. The polyploid *B. truncatus* group appears to belong to a complex of closely related forms that includes 2 diploid ( $n=18$ ,  $2n=36$ ) species groups, i.e., the *B. tropicus* (Krauss) group that has mesocones of triangular shape (Mandahl-Barth, 1958), and the *B. natalensis* (Külster) group, which has arrowhead-shaped radular mesocones resembling those of *B. truncatus* (Brown, Schutte, Burch & Natarajan, 1967). The morphological distinctions between these 3 species groups are not entirely clear and it seems advisable to consider them together, from the cytological point of view. All snails of this complex from populations in Africa known or suspected to serve as intermediate hosts of *Schistosoma haematobium* under natural conditions, that have been examined, have been found to be tetraploid ( $n=36$ ;

$2n=72$ ).

The species groups mentioned above may be referred to briefly as the subgenus "*Bulinus* s.s.". Although nomenclatural objections may be raised to this usage, it provides a means of distinguishing the complex at present under consideration from the other members of the genus *Bulinus*, contained in the species groups of *B. (=Physopsis) africanus* (Krauss) and *B. forskalii* (Ehrenberg) (Mandahl-Barth, 1958). Those 2 groups are, so far as is known, diploid, but do contain species that serve as intermediate hosts to *Schistosoma haematobium*.

Observations on the chromosome numbers of "*Bulinus* s.s." have been published by Burch (1964, 1967), Natarajan, Burch & Gismann (1965) and Brown et al. (1967). A total of approximately 130 samples have been examined from Africa, the Mediterranean region and southwestern Asia, including 8 from 7 Ethiopian localities (Burch, 1964, 1967). From these observations, the diploid and various polyploid forms appear to have different distribution patterns (Fig. 1), although our knowledge of these is far from complete. However, differences between the 2 most intensively sampled countries, South Africa and Ethiopia, are striking: from South Africa only the diploid number ( $n=18$ ) with occasionally 1-3 extra chromosomes has been reported, but in Ethiopia the diploid and all of the 3 known polyploid numbers are present.

The present paper gives the results of cytological observations on 22 samples of *Bulinus* collected in Ethiopia by the authors, separately or together, in 1965. Observations on the ecology of snails in the Ethiopian Rift Valley lakes were made by one of us (Brown) in that year, and also in 1962, with the help of Dr. M. V. Prosser (Haile Sellassie I University).

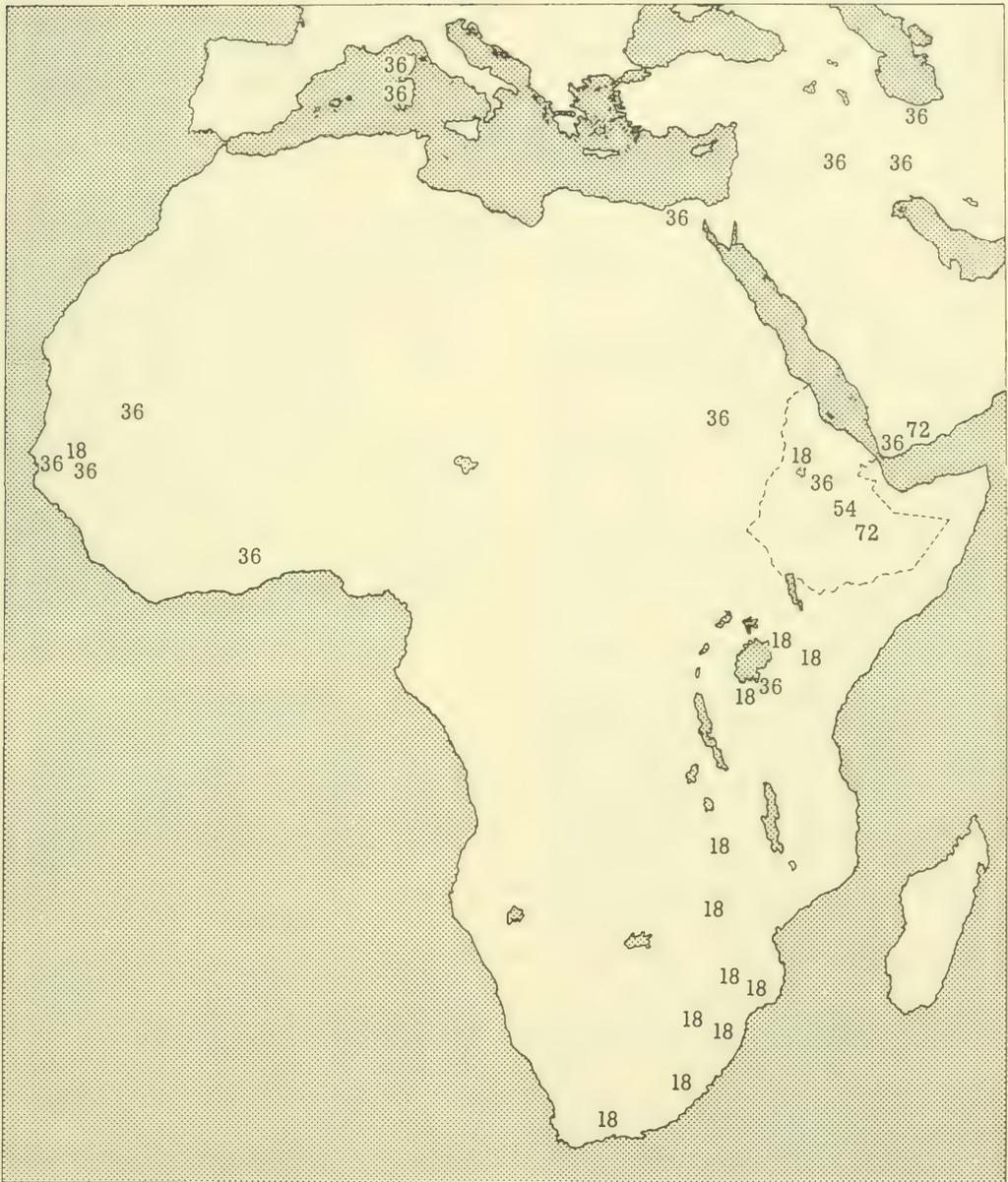


FIG. 1. Haploid chromosome numbers of "*Bulinus s.s.*", comprising the species groups of *truncatus* Audouin, *tropicus* Krauss and *natalensis* Küster. Data are from about 130 samples and cover Corsica, Sardinia, Iran, Iraq, Egypt, Sudan, Mauritania, Senegal, Gambia, Ghana, Ethiopia, Western Aden Protectorate, Kenya, Tanzania, Zambia, Rhodesia, Mozambique and South Africa, from Burch (1964, 1967, and unpublished in the case of Corsica, Mauritania and Gambia); Natarajan, Burch & Gismann (1965); and Brown, Schutte, Burch & Natarajan (1967). Distribution in Ethiopia (indicated by broken line) is shown in Fig. 2.

TABLE 1. Chromosome numbers of "*Bulinus s.s.*" from 22 Ethiopian localities

Collection number	Locality	Date 1965	Haploid chromosome number
65/8	15 km west of Webbe Shibeli bridge on road between Shashamanna and Dodolo, Arussi province	25 July	18
65/14*	Lake at Langhei village, Harar province	29 July	18
65/28	Western shore of Lake Zwai, Arussi province	11 Aug.	18
65/44	43 km northeast of Dangila on Bahar Dar road, Gojjam province	21 Aug.	18
65/61*	Northeastern shore of Lake Ashangi, Tigre province	27 Aug.	18
65/62*	Southern shore of Lake Haik, Wallo province	27 Aug.	18
65/65*	Lake Bishoftu (Biete Mengest), Shoa province	7 Sept.	18
65/77	30 km towards Shashamanna from Soddu/Aba Minch road junction, Sidamo province	9 Sept.	18
65/29	Northeastern shore of Lake Awasa at entrance of Black River, Sidamo province	12 Aug.	36
65/46	Bahar Dar town, Gojjam province	21 Aug.	36
65/50	50 km northeast of Bahar Dar town on Gondar road, Begemedir province	23 Aug.	36
65/53	37 km south of Gondar on Gorgora road, Begemedir province	23 Aug.	36
65/58	1 km west of Aduwa road junction on Aksum road, Tigre province	25 Aug.	36
65/66*	Northwestern shore of Lake Zwai, Arussi province	7 Sept.	36
65/22	15 km west of Debra Birhan on Jihur road, Shoa province	4 Aug.	54
65/40	21 km southeast of Engiabaia (Injibara) on Debra Markos road, Gojjam province	20 Aug.	54
65/85	21 km north of Shano, Shoa province	15 Sept.	54
65/54*	38 km north of Gondar on Asmara road, Begemedir province	24 Aug.	72
65/84	14 km north of Shano, Shoa province	15 Sept.	72
65/86	1 km north of Debra Birhan, Shoa province	16 Sept.	72
65/87	3 km north of Debra Birhan, Shoa province	16 Sept.	72
65/89	4 km south of Shano, Shoa province	16 Sept.	72

\* The asterisks designate populations previously classified in *B. truncatus sericinus* by Brown (1965).

The new observations are discussed in relation to information previously published for Ethiopia and other parts of Africa. We are indebted to Mrs. A. Gismann for her valuable criticism of this paper.

#### METHODS

Material for cytological examination was fixed in Newcomer's (1953) fluid immediately after collection. Cytological techniques employed were those used in our previous cytological studies, i.e., acetic-orcein squash preparations (La Cour, 1941) observed with Nikon microscopes using oil immersion objectives (n.a. 1.25) and 10X and 30X oculars. Chromosomes were counted in up to 5 individuals from each locality. Material for morphological study is preserved in alcohol and has been deposited in the Experimental Taxonomy Section of the Zoology Department, British Museum (Natural History).

#### OBSERVATIONS

Details for samples of *Bulinus*, collected between the end of July and the middle of September, 1965, are listed in Table 1. Their distribution is shown in Fig. 2.

#### DISCUSSION

Because of the presence of arrowhead-shaped mesocones on the lateral radular teeth and the frequent absence of the male copulatory organ, Ethiopian bulinines with various shell forms have been classified in the *Bulinus truncatus* species group, as *B. truncatus sericinus* (Jickeli) and *Bulinus* sp., by Brown (1965) and Mandahl-Barth (1965). Burch (1967) found topotypical material of *B. t. schackoi* (Jickeli) (= *sericinus*, according to Brown, 1965; Mandahl-Barth, 1965) to be tetraploid ( $n=36$ ), which is the usual condition in the *B. truncatus* group. Our cytological studies have shown some of the populations previously classified in

*B. t. sericinus* to be diploid, while others are polyploid (Table 1), and it thus seems likely that a study of morphology in relation to chromosome number will lead to a revised classification of these snails. The Ethiopian diploid populations that have been examined have arrowhead shaped mesocones on the lateral radular teeth, and are therefore comparable to the *B. natalensis* group of forms occurring in southern Africa (Brown et al., 1967).

In the total of 28 Ethiopian localities from which "*Bulinus* s.s." have been examined cytologically, different forms have been obtained with the following frequencies:  $n=18(10)$ ,  $n=36(8)$ ,  $n=54(4)$  and  $n=72(6)$ . The total of localities is made up of 1 reported by Burch (1964, Lake Awasa), 6 given by Burch (1967, excluding a further sample from the same part of Lake Awasa), and 21 reported in the present paper (excluding Lake Bishoftu, from which material was examined by Burch, 1967). The duplicate samples had, for each lake, the same numbers of chromosomes. Both diploid and tetraploid snails are widely distributed (Fig. 2), although diploid populations have not been found north of Lake Ashangi or Lake Tana. The hexaploid ( $n=54$ ) and the octoploid ( $n=72$ ) are comparatively restricted in occurrence, but have extensive ranges on the highland plateau northwest of the Rift Valley (Fig. 2). *B. "sericinus"* with 72 pairs of chromosomes has also been reported from Tarbak in the highland of Western Aden Protectorate (Natarajan et al., 1965).

In each of the lakes Awasa and Zwai, situated in the southern Ethiopian Rift Valley (Fig. 2), occur 2 cytologically different forms of "*Bulinus* s.s.": the diploid *Bulinus* sp. previously reported from Lake Awasa by Brown (1965), and a tetraploid form. These 2 bulinines have distinctive shells and each is apparently restricted to a characteristic habitat. Collections were made on the eastern shore of Lake Awasa and the western shore of Lake Zwai: on each shore 2

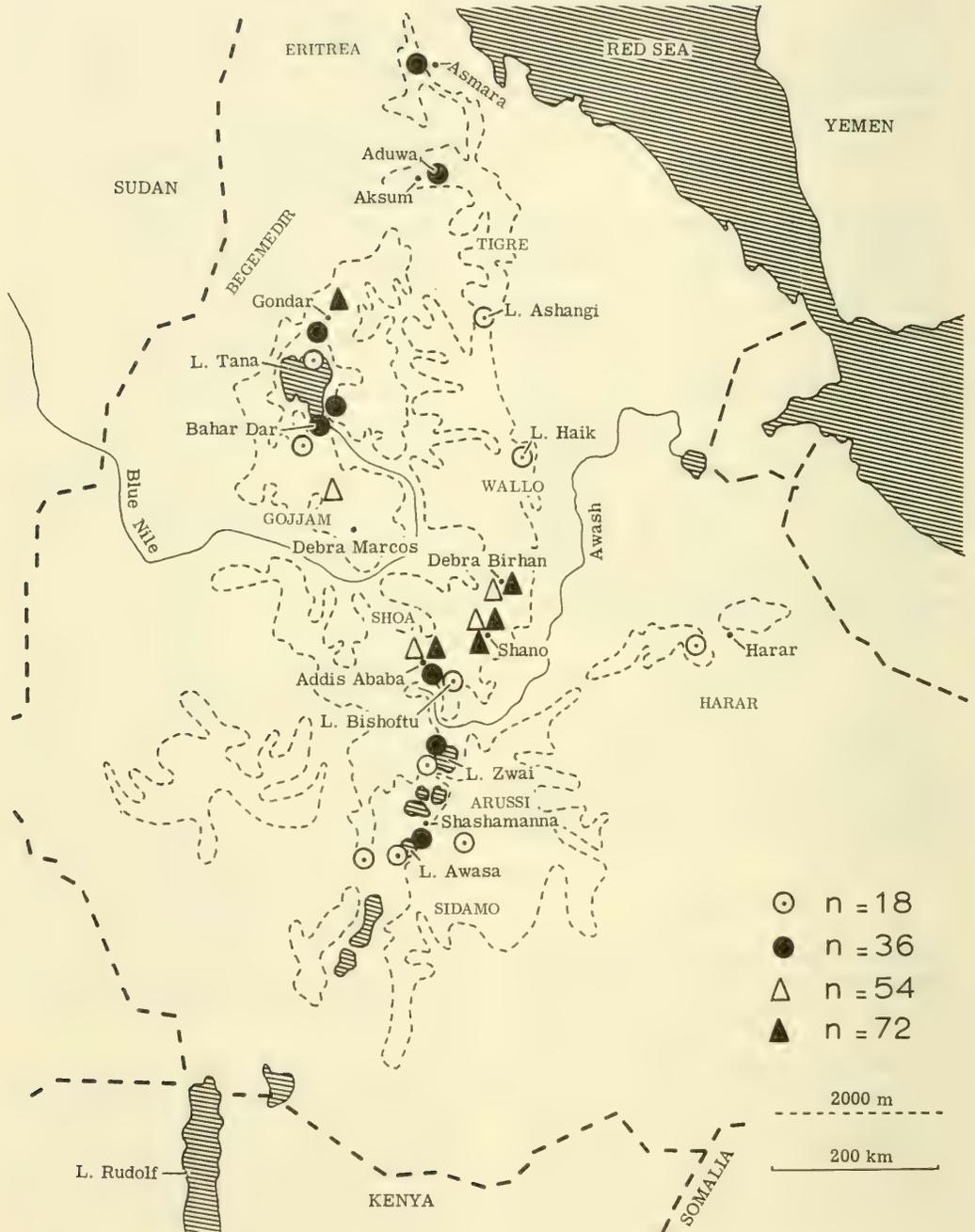


FIG. 2. Distribution in Ethiopia of diploid ( $n=18$ ) and various polyploid populations of "*Bulinus s.s.*" (from Burch, 1967 and present paper). Each symbol represents 1 locality, except for the octoploid near Debra Birhan where 2 localities are represented by 1 symbol.

types of littoral habitat were investigated.

1) On gently shelving bottoms exposed to wave action, beds of aquatic grass extend from the edge of the water into a depth of about 2 m, i.e., to as much as 100 m offshore in Lake Zwai. The substratum is composed largely of sand and fine gravel. The molluscan fauna comprises few species: *Melanoides tuberculatus* (Müller), *Lymnaea natalensis* Krauss (found in Lake Zwai only), *Gyraulus bicarinatus* Mandahl-Barth (Brown, 1967), and *Bulinus* sp. with 18 pairs of chromosomes. *Bulinus* sp. was very abundant in both lakes.

2) In situations protected from wave action there are swamps with luxuriant vegetation, including *Papyrus* and *Nymphaea* spp., which in some places forms a floating bog. At the times of the observations the water was brown in colour and clear, in contrast to the cloudy water of the open lakes. The substratum is composed largely of dark mud with a high organic content. The molluscan fauna is comparatively rich, comprising: *Biomphalaria sudanica* (Martens), *Segmentorbis* sp., *Lentorbis* sp., *Anisus coretus* (de Blainville) (Brown, 1967), *A. natalensis* (Krauss) (found in Lake Zwai only), *Lymnaea natalensis*, *Sphaerium* sp. (found in Lake Awasa only), and *Bulinus* sp. with 36 pairs of chromosomes. *Bulinus* sp. was rare in both lakes.

In view of the demonstrated differences between the habitats of the cytologically different bulinines in lakes Awasa and Zwai, and the fact that no more than 1 cytological form has been obtained from any locality in Ethiopia, it is believed that different forms might well be adapted to particular ecological conditions. All the known localities for the hexaploid and octoploid forms in Ethiopia are situated at an altitude of more than 2000 m, and those reported in the present paper are small, cold-water streams like those described by Burch (1967). However, the numbers of individuals in our samples are not large, and it is possible that cytological examination of more specimens might reveal that different

forms occur together in Ethiopia, as found by Burch (1967) in several small bodies of water in Tanzania. Even so, the homogeneity we have found in each sample suggests that other cytological forms, if present in these localities, are rare. A form might have a low density in some localities because it had recently originated or arrived there, but, with regard to the diploid and tetraploid forms, which are widely distributed with overlapping ranges, it seems that the apparent dominance of one or other form in any habitat may depend on whichever is better adapted to the particular ecological conditions, and perhaps be the outcome of competition between the forms.

Because of the apparently close relationship between *Bulinus "truncatus sericinus"* and *B. truncatus truncatus* of Egypt, these snails have been regarded as an actual or potential intermediate host of *Schistosoma haematobium* in Ethiopia (Ayad, 1956; Brown, 1964). There are, however, few records of human infection with *S. haematobium* from areas where *B. "truncatus sericinus"* has been recorded, i.e., the highland plateaux and the southern part of the Rift Valley. The *B. ("Physopsis") africanus* species group, which is of great epidemiological importance in some parts of Africa, is comparatively rare in Ethiopia. Endemic foci of urinary bilharziasis have been reported reliably only from the lower Awash River valley (Russell, 1958; Akililu Lemma, 1965), where the intermediate host is probably *B. ("Physopsis") abyssinicus* (Martens) (Brown, 1967), as is also the case in the lower Webbe Shibeli river valley of Somalia (Ayad, 1956). With regard to the apparent rarity of *S. haematobium* in Ethiopia, Ayad suggested, among other epidemiological considerations, that *B. "truncatus sericinus"* in Eritrea might not be susceptible to infection; Brown (1964) pointed out that the great variability in shell form of that taxon in Ethiopia, considered as a whole, indicated the existence of local races that might also differ in their physiology and capacity to act as intermediate hosts.

Support for those early speculations is provided by our finding that *B. "truncatus sericinus"* comprises several cytologically different forms. Of these, the tetraploid (n=36) may be capable of serving as an intermediate host to *S. haematobium*, in view of the fact that the members of the *B. truncatus* group known to do so in northern Africa and southwestern Asia are tetraploid (Burch, 1964). Ethiopian octoploid (n=72) populations may also be susceptible to infection, as evidence obtained by Wright (personal communication, in Burch, 1964) implicates an octoploid form of "*Bulinus* s.s." in the transmission of *S. haematobium* in Western Aden Protectorate. There is no evidence that a naturally occurring diploid (n=18) population of the species groups here discussed serves as an intermediate host in Ethiopia or elsewhere. The tetraploid appears to deserve most consideration in relation to the epidemiology of urinary bilharziasis in Ethiopia, since the known distribution of the higher polyploids is confined to a high altitude zone where the climate is, it may reasonably be assumed, generally too cool to permit full development of larval *S. haematobium*. At lower altitudes, where the climate may be suitable for that parasite, a greater number of diploid than tetraploid populations has been found. It therefore appears that the low frequency of tetraploid populations may serve, in conjunction with other factors such as a low density of human populations, as a barrier to the establishment of foci of urinary bilharziasis.

A detailed study of the susceptibility to schistosome infection of cytologically different "*Bulinus* s.s.", from Ethiopia and elsewhere, and of their ecology, seems worthwhile, as the detection of forms resistant to infection and successful in competition with susceptible snails might be of potential practical value. The introduction of a non-susceptible form into natural habitats or newly created water bodies might serve as a means for the biological control of sus-

ceptible snails. This possibility seems most likely of realization using diploid forms, which are apparently resistant to infection and live in a variety of habitats and climatic zones.

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#### АБСТРАКТ

#### РАСПРОСТРАНЕНИЕ ЦИТОЛОГИЧЕСКИ-РАЗЛИЧНЫХ ПОПУЛЯЦИЙ РОДА *BULINUS* (BASOMMATOPHORA: PLANORBIDAE) В ЭФИОПИИ

Д. С. БРОУН И ДЖ. Б. БЁРЧ

Основное число гаплоидных хромосом у видов рода *Bulinus*, как и у других Planorbidae равно 18, но в группе видов *Bulinus truncatus* встречаются формы с полиплоидным числом ( $n = 36$ ); возможно, что среди этих форм встречаются и такие, которые имеют  $n = 54$  и  $n = 72$ . Эта группа, а также группы видов *B. tropicus* и *B. natalensis* являющиеся диплоидными ( $n = 18$ ) могут рассматриваться, как подрод "*Bulinus s.s.*". Диплоидные и различные полиплоидные формы внутри этого комплекса имеют и несколько различные районы распространения. Диплоидные виды встречаются преимущественно в Южной Африке и на север вплоть до Эритреи, а на запад - до Мавритании. Тетраплоидные виды ( $n = 36$ ) встречаются, главным образом в Северной Африке, в Средиземноморском и Средне-Восточном районах, а также в Мавритании, Сенегале, Гане, и Танзанье. Гексаплоидные ( $n = 54$ ) были найдены только в Эфиопии, а октоплоидные ( $n = 72$ ) в районах Эфиопии и Западного Аденского протектората.

В природе, повидимому лишь тетраплоидные и, возможно, октоплоидные формы служат промежуточными хозяевами *Schistosoma haematobium*. В настоящей работе сведены результаты как современных цитологических наблюдений, сделанных на 22 пробах "*Bulinus s.s.*", собранных авторами в Эфиопии, так и имеющиеся более ранние данные. В целом, цитологически-различные формы из 28 мест были получены со следующей частотой встречаемости:  $n = 18$  (10),  $n = 36$  (8),  $n = 54$  (4) и  $n = 72$  (6). Диплоидные и тетраплоидные популяции распространены более широко, в то время, как гексаплоидные и октоплоидные формы были найдены лишь на высокогорном плато, к северо-западу от Рифтовой долины.

Эфиопские формы "*Bulinus s.s.*" ранее относили к группе видов *B. truncatus*. В различных популяциях, ранее определенных как *B. truncatus sericinus*, мы нашли диплоидные, тетраплоидные и октоплоидные формы по числу хромосом. Нам кажется поэтому,

что изучение морфологии моллюсков в связи с количеством хромосом приведет к пересмотру их классификации.

Как диплоидные, так и тетраплоидные "*Bulinus s.s.*" встречаются на озерах Эйвеза и Цвейи; из них 2 формы имели различные друг от друга раковины и каждая была, видимо приурочена к определенному местообитанию. Цитологически-различные "*Bulinus s.s.*" были найдены в одном и том же водоеме только в Танзанье. Тот факт, что различные формы этой группы не были найдены живущими вместе ни в одном месте в Эфиопии может быть происходит из за конкуренции между отдельными формами и основано на адаптации их к определенным экологическим условиям.

Тетраплоидная форма "*Bulinus s.s.*" кажется наиболее подходящей, как передатчик *Schistosoma haematobium* в Эфиопии, однако ее очевидная редкость распространения может здесь служить (вместе с другими эпидемиологическими факторами) барьером для образования очагов уринарного билхарциозиса.

SOME OPISTHOBRANCHS FROM SAPELO ISLAND,  
GEORGIA, U. S. A.<sup>1</sup>Eveline Marcus and Ernst Marcus<sup>2</sup>

## ABSTRACT

The paper deals with 2 pelagic and 10 intertidal and subtidal opisthobranchs from the southeastern coast of the U.S.A. Four species are described: *Okenia sapelona*, *Doridella burchi*, *Tritonia (Candiella) bayeri misa* and *Armina wattla*. This last species differs from the solely American species of *Armina* by its strong caruncle, and from *A. undulata* and the other species with strong caruncle by the radula. *Okenia sapelona* resembles *O. mediterranea*, hence belongs to the opisthobranchs whose range can be traced from the Tertiary Tethys Sea. The remaining 2 new forms are related to species from the warm water region of the western Atlantic. The known littoral species of the present collection are inhabitants of that region too, with the exception of *Doris verrucosa*, which occurs also in the eastern Atlantic.

The subspecies *Pleurobranchaea hedgpethi hamva* is suppressed, because in the present material the direction of the flap over the genital apertures was often found to be oblique, i. e. neither dorsal (*P. hedgpethi hamva*) nor anterior (*P. h. hedgpethi*).

In 1962, Dr. J. B. Burch of the University of Michigan initiated a study of the mollusks of the southeastern U.S.A. for the Sapelo Island Research Foundation and the Institute of Malacology. This paper is based on the specimens collected by Dr. Burch and his students, and by Mr. Milton S. Gray, professional collector for the Marine Institute, University of Georgia. The zoological materials described here are part of the collections housed by the Marine Institute, Sapelo Island, Georgia, U.S.A.

## SYSTEMATICS AND DISTRIBUTION

## List of species

Order Anaspeida, Superfamily Aplysiacea

Aplysiidae, Aplysiinae

1. *Aplysia (Varria) morio* Ver-rill, 1910

Order Notaspeida, Superfamily Pleurobranchacea

Pleurobranchidae, Pleurobranchinae

2. *Pleurobranchaea hedgpethi* Abbott, 1952

Order Doridoidea,

Suborder Eudoridoidea

Infraorder Cryptobranchiata

Dorididae, Doridinae

3. *Doris verrucosa* Cuvier, 1804, Fig. 1

Infraorder Phanerobranchiata,

Superfamily Suctoria

Goniodorididae

4. *Okenia sapelona*, new species, Figs. 2-6

<sup>1</sup>Contribution No. 143 from the Marine Institute, University of Georgia, Sapelo Island, Georgia, U. S. A., and No. 4 from the Southeast American Mollusks Program, Institute of Malacology. The collection of material on which this publication is based was supported by funds from the Sapelo Island Research Foundation.

<sup>2</sup>Caixa Postal 6994, São Paulo, Brazil.

## Corambidae

5. *Doridella burchi*, new species, Figs. 7-12

## Suborder Porostomata

## Dendrodorididae

6. *Dendrodoris krebsii* (Mörch, 1863)  
7. *Doriopsilla pharpha* Marcus, 1961

## Order Dendronotoidea

## Tritoniidae

8. *Tritonia (Candiella) bayermisa*, new subspecies, Figs. 13-14

## Scyllaeidae

9. *Scyllaea pelagica* Linné, 1758, Fig. 15

## Order Arminoidea

## Suborder Euarminoidea

## Arminidae

10. *Armina wattla*, new species, Figs. 16-20

## Order Aeolidioidea

## Suborder Acleioprocta

## Fionidae

11. *Fiona pinnata* (Eschscholtz, 1831)

## Suborder Cleioprocta

## Favorinidae, Facalaninae

12. *Dondice occidentalis* (Engel, 1925)

1. *Aplysia (Varria) morio* Verrill, 1901

*Aplysia morio* Verrill, 1901: 25, pl. 3, figs. 5, 5a.

? *Aplysia modesta* Thiele, 1910: 124, pl. 9, figs. 17, 17a.

*Aplysia (Varria) morio* Eales, 1960: 328-332, figs. 28-29.

Occurrence: Sapelo Island, 31° 02 min. 00 sec. N, 80° 02 min. 25 sec. W, 42.4 m depth, 1 young specimen, collected on July 9, 1963.

Further distribution: Bermuda (original locality), and from Rhode Island to Florida and Texas.

Since a single young, colorless specimen, 12 mm in length, 8 mm in width, and 6 mm in height cannot be classified

with certainty, the following characters were considered as decisive for the classification: soft skin; large and thin parapodia; short, elliptical tail; closed, invisible mantle foramen; rhachidian tooth with excavated head, long cusp and large basal denticles; details of the denticulation of the lateral teeth; and the caecum lying flat on the surface of the digestive gland.

The juvenile specimen of *Aplysia modesta* was included in the list of synonyms on the authority of Eales. In Thiele's short description nothing contrasts with the characters of *A. morio*. The locality, Lovango Cay, between St. John and St. Thomas, Virgin Islands, was not included in the range of *A. morio* by Dr. Eales.

2. *Pleurobranchaea hedgpethi* Abbott, 1952

*Pleurobranchaea hedgpethi* Abbott, 1952: 1-2, pl. 1, figs. 1-8.

*Pleurobranchaea hamva* Marcus, 1957a: 21-27, figs. 40-52.

*Pleurobranchaea hedgpethi* Marcus, 1960b: 253-254, fig. 6.

*Pleurobranchaea hedgpethi hamva* Marcus, 1961b: 141; 1967a (in press), fig. 56.

*Pleurobranchaea hedgpethi* Nijssen-Meyer, 1965: 143-145, figs. 1-2.

Occurrences: Sapelo Island, Georgia, between 31° 33 min. 30 sec. to 30° 55 min. N and 79° 37 min. 30 sec. to 80° 24 min. W., 30-88 m depth; a total of 24 specimens collected in February and October, 1961, May, 1962, and June, July and August, 1963.

Further distribution: Point of Cape Hatteras, North Carolina off Savannah Beach, Georgia, 70-95 m depth; Dry Tortugas, Florida, 51 m depth; Gulf of Mexico from Port Aransas (original locality) to the Bay of Campeche; coast of Surinam, 28 m depth; coast of Rio de Janeiro and São Paulo to 25° S., Brazil.

The preserved specimens are 5-43 mm

in length, 3.5-22 mm in width and 3-13 mm in height. Where the pigment is preserved, the caudal spur is black. In some specimens there are residues of a brown dorsal network. The flap above the genital apertures either has a dorsal direction or a direction intermediate between dorsal (as in *Pleurobranchaea hamva*) and anterior (as in *P. hedgpethi*). This oblique position, also observed in Nijssen-Meyer's slug from Surinam, leads us to abandon a special designation for specimens with a dorsally directed flap.

### 3. *Doris verrucosa* Cuvier, 1804 (Fig. 1)

*Doris verrucosa* Cuvier, 1804: 451, 467, pl. 73, figs. 4-7;

Eliot, 1910: 96-98 (incl. var. *mollis*), not the reference (: 147) to Alder and Hancock, 1856, Family 1, Pl. 11;

v. Ihering, 1915: 142;

Pruvot-Fol, 1954: 232-233, figs. 86a-e (*verrucosa*), figs. 87a-h (*januarii*);

Marcus, 1955: 127-131, figs. 102-108; 1957b: 420, fig. 90 (São Paulo).

*Staurodoris verrucosa* Bergh, 1878: 579-583, pl. 63, figs. 20-23, pl. 64, figs. 2-7;

v. Ihering, 1886: 230-232 (Sta. Catarina);

Bergh, 1894: 161-162, pl. 5, figs. 16-18 (western Florida); 1904: 38-39 (South Carolina);

Eliot, 1906: 337-339 (incl. var. *mollis*);

Bergh, 1907: 46-47, pl. 11, figs. 26-27;

Nobre, 1938-1940: 52, pl. 10, fig. 3.

*Staurodoris januarii* Bergh, 1878: 583-585, pl. 63, fig. 24, pl. 64, figs. 8-12 (Rio de Janeiro); 1880: 37-40, pl. C, figs. 13-25, pl. D, fig. 22 (Rio de Janeiro);

v. Ihering, 1886: 230 (synonymized with *verrucosa*).

*Doridigitata de relicta* O'Donoghue, 1929: 763;

Johnson, 1934: 158;

Lange de Morretes, 1949: 116.

Occurrences: 1) Sapelo Sound, Georgia, November 30, 1961, 3 specimens; 2) Sapelo Sound, 12 m depth, January 26, 1962, 2 specimens.

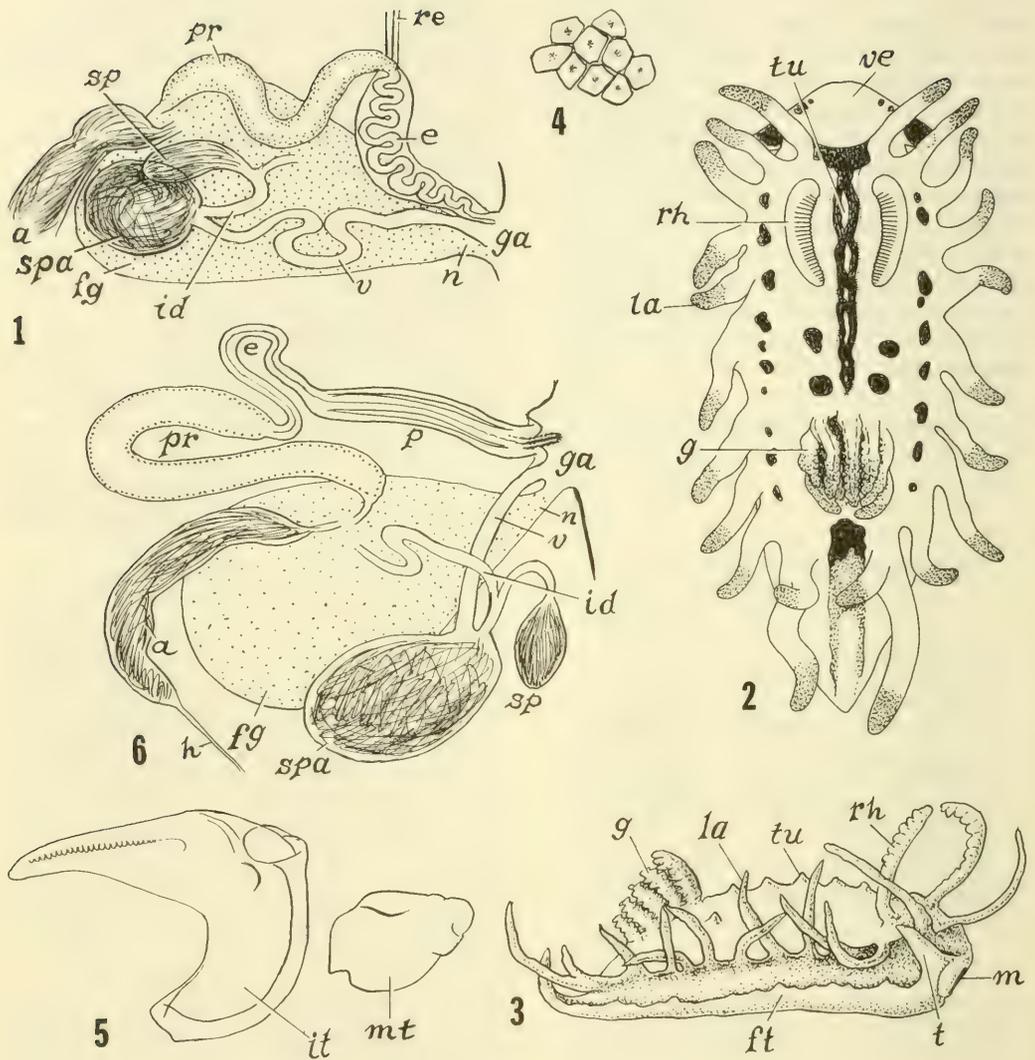
Further distribution: West Atlantic: South Carolina; Manatee Bay, western Florida; Rio de Janeiro, São Paulo, Santa Catarina, Brazil. East Atlantic: British Isles, France, Portugal; Mediterranean; South Africa. Original locality unknown (Bergh, 1878, p 579-580), possibly Mediterranean.

The largest of the present preserved specimens is 35 mm long, 20 mm broad, and 13 mm high. Its sole is 28x14 mm. The big vesicular warts, up to 1.8 mm in diameter, are separated from one another and do not coalesce. The anterior pedal border is bilabiate and slightly notched.

The color of the live animals was brownish-yellow (locality 1) and yellowish-green (locality 2); preserved specimens are whitish. In one slug the spicules are preserved and form ridges between the warts. The tentacles are barrel-shaped and grooved. The rhinophores have 16 perfoliations. The rhinophoral pits bear one large papilla on either side and smaller ones in front and behind. There are up to 14 gills. The rim of the branchial pit has a variable number of papillae, up to 9 big ones and 9 small ones.

The labial cuticle, the radula, and the gut are as previously described.

A major distinction between *Doris* and *Archidoris* Bergh, 1878, lies in the prostatic ental portion (Fig. 1, pr) of the male duct, which is not thickened in *Archidoris*. The latter genus has a pleurembolic penis, while the penis is the acrembolic type in *Doris*. The ectal part of the efferent duct (e) of *Doris* is coiled within a muscular sheath, at the inner end of which inserts a retractor muscle (re). In the present material, and also in a re-examined specimen from São Paulo, we found the disposition of the seminal receptacles slightly different from our earlier diagram (Marcus, 1955, fig. 106). The insemination duct (id) begins at the



FIGS. 1-6. Fig. 1. *Doris verrucosa*. Diagram of reproductive organs. FIGS. 2-6. *Okenia sapelona*, n. sp. Fig. 2. Dorsal view of living slug, drawn by Dr. J. B. Burch; stippled parts: pale yellow; black parts: bright yellow. Fig. 3. Side view of preserved slug. Fig. 4. Polyagonal jaw elements, conical in profile. Fig. 5. Half-row of radula. Fig. 6. Diagram of reproductive organs.

entrance of the vagina (v) into the spermatheca (spa), similar to Bergh's figure 23 (1880, pl. C) of material from Rio de Janeiro.

#### Discussion of *Doris verrucosa*

Pruvot-Fol (1954: 234) maintains *Doris ocelligera* Bergh, 1881a (p 95-98,

## KEY TO LETTERING IN FIGURES

a	ampulla	mu	mucus gland
ag	albumen gland	mt	marginal tooth
an	anus	n	nidamental duct (outer oviduct)
b	brain	no	notum
c	caruncle (wattle)	nr	notal ridges
e	efferent duct	o	ovotestis
fg	female gland mass (i. e. albumen and mucus glands)	p	penis
ft	foot	pr	prostate
ftg	foot gland	r	rhachidian tooth
g	gill	ra	radula
ga	genital aperture(s)	re	retractor muscle
h	hermaphrodite duct	rh	rhizophore
hn	hyponotum	rha	rhizophoral appendage
id	insemination duct	rp	renal pore
io	inner oviduct	sp	spermatocyst
it	intermediate tooth (or inner lateral tooth)	spa	spermatheca
j	jaw	spo	spermoviduct
l	lateral lamellae	t	tentacle
la	lateral appendages	tu	tubercle
lt	lateral tooth (teeth)	v	vagina
m	mouth	va	velar appendage
ma	male atrium	ve	veil

pl. 4, figs. 11-21) as a separate species. The original material came from Trieste; v. Ihering had a specimen 4 mm long from Naples (1886: 232-233), and Pruvot-Fol had several slugs 10-12 mm long from Banyuls, France. The length of the living animals in the original diagnosis, 0.5 cm, is a misprint for 5 cm. The descriptions of Bergh, v. Ihering, and Pruvot-Fol are not quite uniform, but hardly justify a specific separation of *D. ocelligera*. The reproductive organs were recorded only by Bergh.

*Staurodoris pseudoverrucosa* v. Ihering (1886: 233) from Naples with conical tubercles and 5 bipinnate gills cannot be united with *Doris verrucosa*. Its reproductive organs were not described, so that its generic position remains unknown.

#### 4. *Okenia (Okenia) sapelona*, new species (Figs. 2-6)

Occurrence: Sapelo Island, Georgia, November, 1963; 2 specimens. Holotype, UMMZ 230616; Paratype UMMZ 230617.

The general color is an iridescent pale blue; the rhizophores and some spots on the gills are maroon. The following areas or spots are bright yellow (Fig. 2): dots on the lateral margins and the corners of the veil (ve), a broad fleck between the first appendages, a center stripe excluding the bases of 5 dorso-median pale blue tubercles (tu), 2 pairs of larger roundish spots in front of the gills (g), smaller irregular spots forming a row on each side of the back, and a median blotch behind the gills. The tips of the lateral appendages (la) and of the gills are pale

yellow, and the postbranchial bright yellow area passes into a light yellow one which extends backwards with 2 lines.

The measurements of the 2 live specimens (Holotype and Paratype respectively) were: body length 7.6 and 5.3 mm; width of body 2.2 and 1.9 mm; length of rhinophores 1.7 and 1.4 mm; diameter of rhinophores 0.3 and 0.2 mm; length of the longest gill in both specimens 1.1 mm; length of central pigmented stripe 3.4 and 2.0 mm; Holotype, UMMZ 230616; Paratype, UMMZ 230617.

The shape of the veil is different in the 2 specimens. In the larger specimen its anterior border is convex (Fig. 2); in the smaller one it is straight and slightly concave in the middle. The tentacles are triangular flaps. The foot corners are rounded, not projecting. The tails of the preserved specimens end with a longer point than they showed in life; the foot is narrower than the back. The pallial ridge bears 11 soft appendages on each side in the larger slug, 9 in the smaller slug. These appendages have blunt tips when alive; when preserved they are pointed. The hindmost appendages are double. In the smaller slug the 6th right papilla is also double. Two pairs of the appendages stand in front of the rhinophores and 2 behind the branchiae. The gills are unipinnate, the larger animal has 9, the smaller animal has 7. Five low, pointed tubercles stand on the center stripe (Fig. 3). In front of the gills there is a pair of low bosses, whose position corresponds to the posterior pair of the large, roundish spots mentioned above.

The labial cuticle bears a ring-shaped thickening around the mouth composed of smooth, polygonal elements (Fig. 4), which are conical in side view. Sometimes their tips are slightly curved. The radula (Fig. 5) of the larger slug consists of 12 rows of teeth (radular formula: 1.1.0.1.1). The inner (intermediate or lateral) tooth is  $94\mu$  long, the outer (marginal) tooth  $28\mu$  long. The

former bears 20-24 denticles on its inner side, of which those near the tip are stronger than those near the base. There is a boss near the root of the cusp similar to Vayssière's figure 15 (1919: pl. 4) of *Okenia dautzenbergi*. The scale-shaped marginal tooth has a single short point.

The reproductive organs (Fig. 6) are similar to those in other species of *Okenia*. The hermaphrodite duct (h) is thin, the ampulla (a) cylindrical in the dissected slug. A narrow spermoviduct merges into the female gland mass (fg). From there the male duct goes out and soon becomes prostatic (pr). This glandular portion of the duct bends back on itself and is sharply set off from the narrow efferent duct (e), which is sheathed by an outer muscle layer. It functions as an acrembolic penis (p), and its terminal section bears cuticular spines.

The nidamental duct (n) and the vagina (v) open together. The vagina leads to a spacious spermatheca (spa). The long and curved insemination duct (id) leaves the spermatheca near the entrance of the vagina. These 2 spermathecal ducts correspond to what is called the serial type of the seminal receptacles. The canal of the spermatocyst (sp) arises from the insemination duct.

#### Discussion of *Okenia* (*Okenia*) *sapelona*

For the principal literature and the valid species of *Okenia* Menke, 1830, we refer to our list (Marcus, 1957b: 438). Since then 3 new species, all from Japan, have been added to the genus (Baba, 1960: 80-81; Hamatani, 1961: 363-365). One of these Japanese species, *O. plana*, has recently also been found in San Francisco Bay (Steinberg, 1963: 65, 71). These new Pacific species belong to the subgenus *Okenia*, characterized by appendages on the central area of the back between rhinophores and gills. When these appendages are poorly developed, they can only be seen in side view as low tubercles (Fig. 3). The 2 species of *Okenia* described from western

Atlantic warm waters belong to the subgenus *Okenia* s.s. (Marcus, 1957b: 434-442), while those reported from the New England area (Johnson, 1934: 156) belong to the subgenus *Idaliella* Bergh, 1881. This latter subgenus does not have appendages in the center of the dorsum. The species from western Atlantic warm waters are *Okenia evelinae* and *O. impexa*. *O. evelinae* has been recorded from the southern middle Brazilian coast and Miami (Marcus, 1960a: 162). *O. impexa* has been recorded from Brazil and Beaufort, North Carolina (Marcus, 1961b: 144).

The labial cuticle of *Okenia impexa* is thin and smooth, and its marginal tooth bears a sharp principal cusp and 2 further basal secondary cusps. The thick labial cuticle of *O. evelinae* projects with a kind of bilabiate beak on each side of the mouth; this beak is simple, not composed of separate elements. The lateral appendages do not extend backwards beyond the level of the gills. In front of the latter there are 2 median unpaired papillae and 1 lateral pair. The male copulatory organ is pleurembolic, without spines, and ends with a penial papilla.

Several of the East Atlantic and Mediterranean species of *Okenia* in Pruvot-Fol's classification (1954: 308-311), *O. elegans*, *O. dautzenbergi*, and *O. mediterranea*, must be compared with *O. sapelona*. In Vayssi re's last publication (1930) *O. dautzenbergi* is annexed to *O. elegans*. The marginal tooth of *O. elegans elegans* and *O. elegans dautzenbergi*, although 7-8 times smaller than the lateral tooth, is rather similar to it in shape. *O. e. elegans* has 17-22 gills, *O. e. dautzenbergi* 14 gills. The broad foot appears to project beyond the pallial ridge in dorsal view. *O. mediterranea*, the species nearest to *O. sapelona*, has no papillae down the midline, but 2 pairs of tubercles in front of the gills, and the surface of the labial elements bears numerous points and tubercles. Pruvot-Fol (1954: 311) said that *O. mediterranea* and *O. amoenula*,

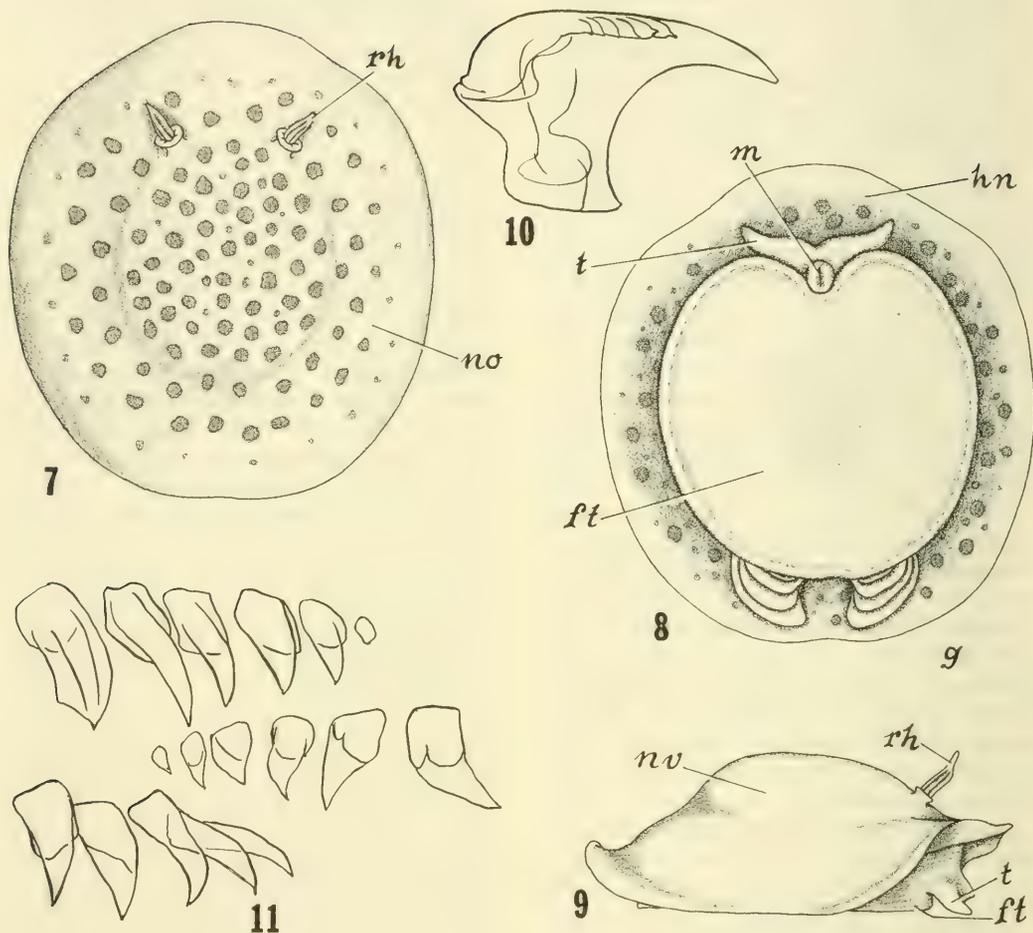
a South African species, are similar, but according to Macnae (1958: 368-369), the latter species belongs to the subgenus *Idaliella*.

#### 5. *Doridella burchi*, new species (Figs. 7-12)

Occurrences: Georgia, 1) off Cabretta Island, 4.3 m depth, November 28, 1962; 2a) Blackbeard Creek, between Blackbeard and Sapelo Islands, about 1/4 mi. from Raccoon Bluff, tide flat, December 1962; 2b) same locality, May 14, 1964; 3) Sapelo Sound, 16-26 m depth, April 10, 1963; 4) off Nanny Goat Beach, Sapelo Island, May 17, 1963 (type locality). A total of 182 specimens, all on *Alcyonidium* (Bryozoa, Ctenostomata). Holotype, UMMZ 230618; Paratypes, UMMZ 230619.

Living slugs are up to 8 mm long, but the average size of these specimens was somewhat less than 7 mm. The largest preserved specimen was 7 mm in length, 5 mm in width and 3 mm in height. The collection also contained many smaller animals, some as little as 1.5 mm in length. The notum of this species is transparent, slightly opaque, the foot rather opaque. Through the notum the following organs and structures were seen: the pumping heart in the hind part of the visceral mass, the dark brown digestive gland and white lobes around it, which are the follicles of the ovotestis, the yellowish-tan outline of the visceral mass, and the outline of the foot. Round brown spots are numerous in the center (Fig. 7), decreasing in number towards the periphery. Many of these spots are about 150 $\mu$  in diameter, but much smaller spots also occur. The ramified pigment cells forming these spots lie in the deepest layer of the notum, and therefore they appear near the surface of the hyponotum in ventral view (Fig. 8). Yellow marks are scattered between the brown spots. In preserved animals the foot, the rhinophores, and the gills are whitish.

The notum is almost circular when



FIGS. 7-11. *Doridella burchi*, n. sp. Fig. 7. Dorsal view of living slug. Fig. 8. Ventral view of same. Fig. 9. Side view of same. Figs. 7-9 drawn by Dr. J. B. Burch. Fig. 10. Intermediate tooth of radula. Fig. 11. Three rows of marginal (or outer lateral) teeth viewed from different angles.

the animal is at rest, but pointed behind in locomotion (Fig. 9). The notal border is not notched behind, but a bundle of muscles that originates between the gills and inserts on the border of the hypnotum, may produce a temporary emargination of the notum. As in other corambids the integument consists of a deciduous cuticle which is known to be periodically shed and renewed (MacFarland & O'Donoghue, 1929: 9), an epidermis which secretes the cuticle and

its pegs, and a thick layer of connective tissue without spicules. The pegs in the present species are broader than those in *Corambe pacifica* MacFarland & O'Donoghue, 1929, (pl. 1, Figs. 3-4). The cells of the connective tissue are scarce. Numerous large glands are sunk into the connective tissue.

The foot (ft) is cordate and bilabiate in front (Fig. 8), rounded behind, though sometimes narrower, sometimes broader, according to contraction and re-

laxation. At rest the foot and the head and its veil (ve) are covered by the notum. In locomotion (Fig. 9) the head is protruded. In the gliding animal the tentacles touch the substrate. As in most other corambids, the rhinophores bear 2 lamellae on either side. They are similar to those of *Corambella baratariae* (Harry, 1953: fig. 6), which according to Franz (1967, p 75) is a synonym of *Doridella obscura* Verrill, 1870. In the present species, however, the border of the rhinophoral pit is not scalloped in our specimens of *C. burchi*. In these the border is a broad collar whose outer edge is smooth, while the inner edge has some slight radial folds (Fig. 7). In the preserved specimens the rhinophores are completely withdrawn. The eyes lie deep under the skin, a little in front of the cerebral ganglia, on either side of the crop. On account of the coalescence of the cerebral and pleural ganglia, the central nervous system agrees better with that of *Corambe testudinaria* (Fischer, 1889 (Hoffmann, 1936: figs. 554 A, B) than with that of *C. pacifica* (MacFarland & O'Donoghue, 1929: pl. 2, figs. 8, 9).

In the groove between the hind end of the notum and the foot, which represents an extremely reduced mantle cavity (Hoffmann, 1934: 330), a pair of gills (g) lies on either side of the midline (Fig. 8). Each pair consists of a smaller anterior, more ventral, and a larger, posterior, dorsal plate, the former with about 4 dorsal and 4 ventral leaves, the latter with about 9 leaves on either face. The anus opens between the gills, not on a papilla; the renal pore lies beside the anus. The muscle fibres connecting the anal region with the hind margin of the hyponotum have already been mentioned. There are 3 branchial glands at the base of each pair of branchiae. The genital apertures lie on a lobed papilla immediately behind the right tentacle. A high epithelium covers the genital papilla.

The cavity of the mouth is lined with

a thick cuticle. The radula consists of about 40 rows. Each half-row contains 1 inner lateral or intermediate tooth and 5-6 (in the older portion of the radula 4) outer lateral or marginal teeth. The intermediate tooth (Fig. 10) is  $88\mu$  long,  $47\mu$  high, and bears 3-7 denticles on the inner surface of the hook. The first marginal tooth is  $34\mu$  long. The different aspects of the marginal teeth viewed from different angles are shown by the 3 half-rows of Fig. 11.

The male and female follicles of the ovotestis are separate as in *Doridella obscura* and *C. carambola*, but in contrast to *Corambe evelinae*. The hermaphrodite duct (Fig. 12, h) and the ampulla (a) have the usual characters. The spermoviduct (spo) divides outside the female gland mass (fg). The male branch is glandular (pr) in its entire length, from the bifurcation of the spermoviduct to the root of the conical penis (p). The base of the latter is thick, cushion-like, its tip pointed, its epithelium ciliated. The ciliated vagina (v) runs straight inwards from the female genital aperture and has several constrictions. It begins with its own outer opening immediately next to the penis and ends in a spacious spermatheca (spa) containing unorientated sperm. The insemination duct (id) leaves the spermatheca laterally, well away from the entrance of the vagina. Its coiled course before it enters the oviduct is simplified in the diagram (Fig. 12). The spermato-cyst (sp) which lodges orientated sperms is annexed to the ental portion of the inner oviduct (io). The latter passes into the glandular oviduct whose convolutions constitute the so-called female gland mass (fg). The outer oviduct or nidamental duct (n) opens separately from the vagina. As mentioned above, the papilla on which the 3 genital apertures lie, has a high, folded epithelium.

The egg string described by Dr. J. B. Burch (personal communication) corresponds to a single planed coil of clear jelly, similar to that observed in other

corambids, e.g., *Doridella obscura* (Verrill, 1873: 30) and *Corambe pacifica* (MacFarland & O'Donoghue, 1929: 20). The 3 turns observed in the present species sometimes overlap slightly. They contain 2-3 layers of eggs, each egg in its own capsule. The eggs are spaced from one another by distances equal to their own diameter. MacFarland & O'Donoghue calculated 1500 eggs in the egg string of *C. pacifica*.

The corambids feed upon Bryozoa. The bryozoan on which *Doridella burchi* was found was identified as *Alcyonidium* cf. *verrilli* by Mr. Milton S. Gray of the Marine Institute, University of Georgia. As this erect, branching bryozoan species is recognizable by a much firmer consistency than that of *A. gelatinosum* and *A. hirsutum*, both of which grow in a similar form (Osburn, 1932: 444), we presume that this identification is correct, although we are not aware of other records of *A. verrilli* south of Chesapeake Bay (Osburn, 1944: 14).

The species is named for Dr. J. B. Burch, who first recognized it as an undescribed species and who provided drawings of the living animals and many observations which were included in our description.

#### Discussion of *Doridella burchi*

The family Corambidae comprises 11 species known from the European west coast (Netherlands, France) and the Atlantic and Pacific coasts of North and South America. The bibliographic records up to 1929 can be found in MacFarland & O'Donoghue (1929: 2-3). Further species were described by Harry (1953), Marcus (1955, 1958a, 1959), and Lance (1962a). The 2 genera currently distinguished are *Corambe* Bergh (1869: 359, footnote), and *Doridella* Verrill (1870, p 405). In *Corambe* there is a median notch in the posterior border of the notum, in *Doridella* the border is not notched.

The posterior notch of *Corambe* has been observed in living *C. testudinaria*, *C. pacifica* and *C. evelinae* as a con-

stant structure. In preserved *C. evelinae* and in *C. lucea*, which is not known alive, the notch may be contracted and reduced to a mere suture. In living *Doridella* the contraction of the notal border may produce a temporary emargination, probably for the better flow of water around the gills, but preserved specimens of *Doridella* showing a notch have not been described.

The first corambid described having an entire notal border is "*Doridella obscura* Verrill, 1870, a species which occurs from Vineyard Sound, Massachusetts to New Jersey. (Verrill published a more detailed description in 1873: 307, 400-401, 664, pl. 25, fig. 173a, b). The type species of *Corambella*, *C. depressa* Balch, 1899, was first collected at Long Island, New York, hence within the range of *D. obscura*. The gills of *C. depressa* are plate-like, as probably those of the type species of *Corambe*, *C. sargassicola* Bergh (1871: 1295, pl. 11, fig. 24, pl. 12, fig. 1). The same type of gills occurs in *Doridella obscura* (Franz, 1967, fig. 1A). All other species with posterior notal notches have plume-like gills. If the gills were used for the separation of the genera, *Doridella* would become a synonym of *Corambe*. Then the species with plume-like gills would have to be united under a new name. One species with an entire notal border, *Doridella steinbergae* Lance, 1962a (35), would come under this new genus.

Thiele (1931: 430) mentioned the twisted inner lateral tooth of *Corambella depressa* Balch (1899: pl. 1, fig. 15) in the diagnosis of the genus, but we do not accept it as a specific feature. Evidently this tooth had been deformed by too strong a pressure of the cover glass. The reticulate pattern of the notum, the single pair of lamellae surrounding the central cone of the rhinophore, and the anal opening on a papilla are perhaps specific characters of *C. depressa*; the genital aperture on the left side is an anomaly or a misinterpretation.

*Doridella steinbergae* differs widely from all other species of *Doridella* by the plume-like branchiae and the smooth rhinophores.

*Doridella obscura* Verrill, 1870, *D. baratariae* (Harry, 1953), *D. carambola* Marcus, 1955, and *D. burchi* are related to one another; Franz (1967: 74) even united *D. obscura* and *D. baratariae*. The minute penial papilla of *D. carambola* and its 2 pairs of branchial glands separate this species from the others. The shape of the marginal teeth can hardly be used for specific separation, because their aspect varies when viewed from different angles. Both *D. carambola* and *D. baratariae* are smaller species than *D. burchi*. A preserved specimen of *D. baratariae* from Biscayne Bay, Florida, is 3 mm long and 2.25-2.4 mm wide. Its penis is 0.3 mm in length and does not have the dilated base (Marcus, 1960b: fig. 13) of *D. burchi* (Fig. 12). The vagina of *D. carambola* is narrow and the margins of the rhinophoral pits are scalloped. The discrepancy in the length of the lateral and first marginal tooth is less in *D. baratariae* (Harry, 1953: fig. 5) than in *D. burchi*.

The entire notal margin of *Doridella batava* (Kerbert, 1886; van Benthem Jutting, 1922: 400-401, figs. 5-6a, b) led Engel (1936: 106-107) to the correct generic allocation, but this species is still placed in the genus *Corambe* by some authors (e.g., Swennen, 1961: 205). *Doridella obscura* and *D. batava* are probably different species, because the former is pointed behind, while the latter is broadly rounded. But the data available are hardly sufficient for separating the remaining species of *Doridella* from *D. batava*. However, the very weak denticulation of the intermediate tooth and the broad irregularly shaped marginal teeth without cusps seem to be peculiar characters of *D. batava*.

6. *Dendrodoris krebsii* (Mörch, 1863)

*Rhacodoris Krebsii* Mörch, 1863: 34.

*Doriopsis Krebsii* Bergh, 1875a: 87-

91, pl. 11, figs. 8-23.

*Doriopsis Krebsii* var. *pallida* Bergh,

1879: 44-49.

*Doriopsis atropos* Bergh, 1879: 49-64.

*Dendrodoris atropos* Marcus, 1957b: 443-447, figs. 146-154; 1962, fig. 19.

*Dendrodoris krebsii* Marcus, 1963: 35 (*D. atropos* synonymized).

*Dendrodoris atropos* Collier & Farmer, 1964: 389-391, figs. 2 G-H and pl. 5.

*Dendrodoris krebsii* Marcus, 1967a; 1967b: figs. 62, 63 (in press).

Occurrence: Dredged of Sapelo Island, Georgia, September 12, 1963; 1 specimen.

Further distribution: Atlantic Ocean: Florida; Bahamas; Virgin Islands (original localities); Antilles; Curaçao; coast of southern middle Brazil. Pacific coast of Lower California; Gulf of California; mainland of Mexico.

At present, Sapelo Island and Puerto Peñasco, Sonora, Mexico, are the northernmost localities for *D. krebsii*, whose range testifies to the central American marine continuity admitted even for the Lower Pliocene (Ekman, 1953: 37).

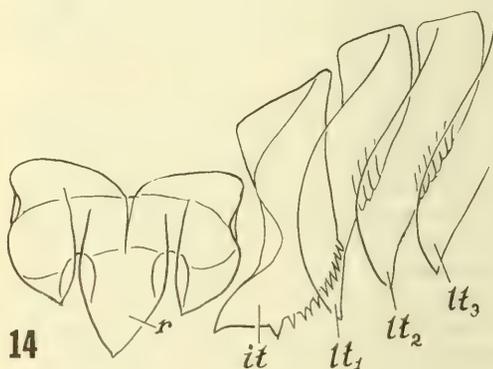
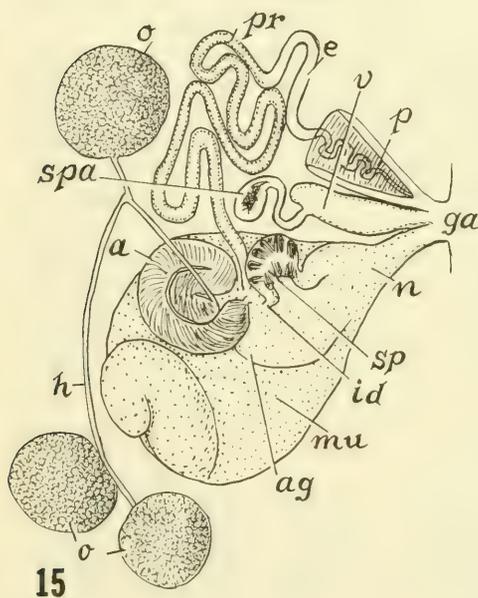
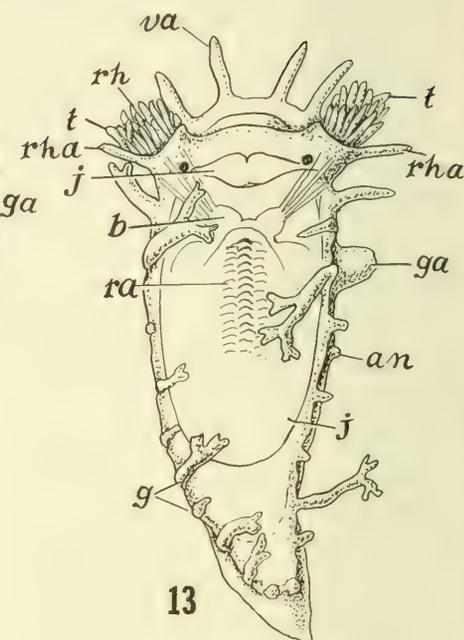
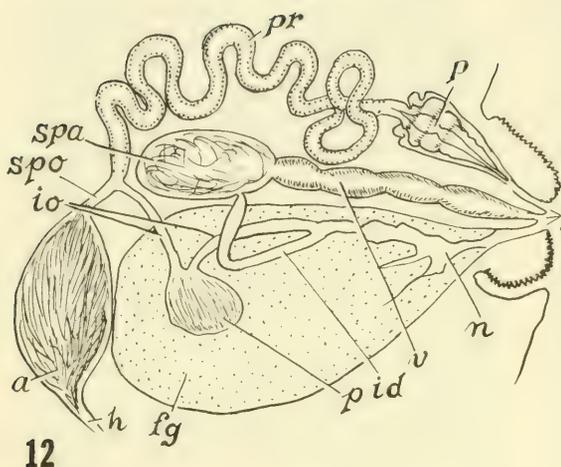
The preserved slug is 30 mm long, 15 mm broad and 14 mm high. Its notum and foot have frilled borders. The rhinophore has about 20 leaves; there are 6 tripinnate gills. The surface of the notum and the borders of the rhinophoral and branchial pockets are smooth.

Collier & Farmer found the base of the penis thickened in their east Pacific material. We also found this part to be thicker in specimens from the Gulf of California, collected by Dr. Peter E. Pickens, than in our Atlantic specimens from the Lesser Antilles and Brazil. This character could not be examined in the present specimen, because part of the hook-bearing section of the penial papilla is protruded, and only completely retracted penes are comparable.

7. *Doriopsilla pharpha* Marcus, 1961

*Doriopsilla pharpha* Marcus, 1961b: 146, figs. 19-21.

Occurrences: Georgia, 1) Sapelo



FIGS. 12-15. Fig. 12. *Dovidella burchi*, n. sp.. Diagram of reproductive organs. FIGS. 13-14. *Tritonia bayeri misa*, n. ssp. Fig. 13. Preserved slug, dorsal view. Fig. 14. Inner teeth of radula. Fig. 15. *Scyllaea pelagica*. Diagram of reproductive organs.

Sound, November 30, 1961, 1 specimen;  
2) Wallburg Creek, 18-23 m depth,  
February 20, 1962, 4 specimens; 3)  
Sapelo Island, 1-2 miles east of sea  
buoy, dredged from 18-23 m depth, 2  
specimens.

Further distribution: Beaufort, North

Carolina.

The biggest of the preserved speci-  
mens is 18 mm long, 10 mm wide and  
4 mm high. The foot is 16 mm long,  
7 mm broad. The living slugs were  
yellow, but in the preserved specimens  
this ground color had disappeared. How-

ever, in preserved specimens the dark brown chromatophores of the connective tissue, mentioned in the first description, were visible. The present material agrees with that from Beaufort, N. C., except that the spermatheca is larger and the prostatic section of the efferent duct is considerably wider. These characters are functional and not of systematic value.

The dark specks and the 12 rhinophoral perfoliations distinguish *D. pharpha* from the 2 other species of *Doriopsilla* found in American Atlantic warm waters, *D. leia* Marcus (1961b: 144) and *D. areolata* Bergh, 1880. The latter 2 species lack the specks, and have 8 (*D. leia*) and 20-25 (*D. areolata*) perfoliations. Moreover, *D. leia* is soft and smooth, *D. pharpha* firm and slightly bossed on the notum. The pedal commissure of *D. leia* is distinct, and in *D. pharpha* the pedal ganglia are contiguous. The notal bosses of *D. areolata* are more distinctly set off than those of *D. pharpha*, and the hindmost muscular section of the oesophagus (Marcus, 1962: fig. 18, zi) is only half as long. When the white epidermal net of *D. areolata* is present, this species is easily identified. But sometimes this net is absent, making identification more difficult, as was the case in the single specimen of *D. areolata* reported from the West Atlantic Ocean (Marcus, 1962: 472).

In *Dendrodoris* and *Doriopsilla*, which suck their food, the anterior gut is so much modified that the term "oral tube" and "buccal bulb" are inadequate and should be replaced by "oral vestibule" and "pharynx" respectively. In both genera the oral vestibule is more or less dilatable, and the anterior portion of the tubular pharynx often protrudes into the posterior part of the vestibule. In *Dendrodoris*, a bilobed posterior oral gland (ptyaline gland, Bergh), or a pair of glands, opens into the vestibule. In *Doriopsilla* such glands are absent. Where ptyaline glands have been erroneously described for species of *Doriopsilla*, they are really the ductless

lymphatic blood glands. The buccal ganglia lie far from the nerve ring in *Dendrodoris*, whose cerebro-buccal connectives are long, while they are apposed to the pedal ganglia in *Doriopsilla*.

Other morphological characters frequently mentioned in the literature are subject to variations and therefore cannot be used as diagnostic taxonomic characters. They are: soft consistency of the body in *Dendrodoris* and a stiff, leathery one in *Doriopsilla*; a nearly smooth (*Dendrodoris*) or strongly warty notum (*Doriopsilla*); spicules scarce (*Dendrodoris*) and abundant (*Doriopsilla*). The pharyngeal or salivary glands cannot be used taxonomically in view of Pruvot-Fol's (1952: 414) and our (Marcus, 1962: 474) negative search for them in east and west Atlantic material of the type species of *Doriopsilla*.

8. *Tritonia* (*Candiella*) *bayeri* *n. sp.*,  
new subspecies  
(Figs. 13-14)

Occurrences: Off Sapelo Island, Georgia, 1) 31° 33 min. 30 sec. N, 79° 37 min. 30 sec. W, 77 m depth, August 4, 1962, 1 specimen; 2) 31° 26 min. 32 sec. N, 79° 42 min. 13 sec. W, 89-77 m depth, August 4, 1963, 2 specimens (type locality). Holotype, UMMZ 230620; Paratype, UMMZ 230621.

The preserved animals are 2.4, 3.0, and 3.5 mm in length, the last one is 2 mm broad without the short gills. The back is smooth. The foot is nearly as broad as the body, round and bilabiate in front, tapering behind.

The cephalic veil is entire, not bilobed. There are 4 digitiform velar appendages (Fig. 13, va) between the grooved tentacles (t). The smooth rim of the rhinophore sheath bears a single process (rha). The 9 branchial tufts (g) on either side alternate in length, the longer gills dichotomize. The genital aperture (ga) lies under the 3rd right tuft, the anus (an) between the 4th and 5th, behind the middle of the body.

The length of the jaws (j) is more than half that of the body. The masticatory

border is set with several rows of conical teeth. The radula (Fig. 14) comprises 26 rows with 10 teeth per half-row (radular formula: 9.1.1.1.9). The median cusp of the tricuspidate rhachidian tooth (r) is a little larger than the lateral cusps. The intermediate tooth (it) has an inner concavity and a row of outer denticles. The outermost of these is stronger than the others. The following teeth (lt) are hook-shaped, but the 1st of them (lt 1), sometimes also the 2nd, (lt 2), bears a small denticle between cusp and base.

In spite of the small size of the slugs, their reproductive organs contained mature sperms. The genital system agrees with that of *T. (C.) bayeri bayeri* Marcus, 1967a, found in the area of Miami. The longish form of the ampulla (curved, sausage-shaped) differs from the globular one in *T. (C.) b. bayeri*.

The name of this subspecies is the latinized form of the French *Mise*, an abbreviation of *Marquise*, wife of a *Marquis*.

#### Discussion of *Tritonia (Candiella) bayeri mise*

The new form has 4 velar appendages against 2 in the larger *T. (C.) b. bayeri*, which is preserved 7-11 mm in length. Since, in the tritoniids, the number of these appendages is known to increase with growth, the larger number in the smaller form is a distinctive character. Minor peculiarities of *T. (C.) b. mise* are the strong 1st denticle of the intermediate tooth (it) and the occasional occurrence of denticles on the 2nd lateral tooth (lt 2). The (longish, not globular) shape of the ampulla is a functional character with no systematic importance.

#### 9. *Scyllaea pelagica* Linné, 1758 (Fig. 15)

Alder & Hancock, 1848: family 2, pl. 5 (anatomy); 1855: pl. 46, suppl. fig. 27 (radula);

Bergh, 1875b: 319-342 (including the

varieties *marginata*, *ghomfodensis*, *sinesis*, *orientalis*), pls. 40, 42-43, 44, figs. 1-18, pl. 45, figs. 16-18;

Odhner, 1936: 1097 (color after Verrill, 1878), 1098 (synopsis of species of *Scyllaea*), figs. 7, 30, 31a;

Baba, 1949: 89, 168-169, figs. 112-113, pl. 36, fig. 130 (colored);

Pruvot-Fol, 1954: 367-368, figs. 143a-j;

Marcus, 1963: 36-37, figs. 65-66;

Abe, 1964: 87, pl. 29, fig. 101 (colored).

Occurrence: Off Georgia coast in Gulf-weed drift, 31° 04 min. N, 80° 28 min. W, 4 specimens.

Further distribution: Pelagic in warm and warm-temperate waters, clinging to floating seaweed and feeding on hydroids. Occasionally farther north (Marcus, 1961b: 148).

Living slugs reach 60 mm in length (Barnard, 1927: 210) when extended; the largest preserved specimen at hand is 30 mm long, 16 mm high including the lobes, and 8 mm broad.

As 2 figures (Odhner, 1936; Baba, 1949) of the reproductive organs of *S. pelagica* are published in papers not easily accessible, and the reproduction of the 3rd figure (Pruvot-Fol, 1954) is mediocre, we give a new diagrammatic drawing (Fig. 15) of this peculiar system. The hermaphrodite glands (o) are globular. The specimen we dissected had 3 of these glands, but up to 6 have been recorded (Baba, 1949). The hermaphrodite ducts (h) unite, so that a single duct enters the tubular, coiled ampulla (a). The short spermoviduct (spo) goes into the female gland mass (albumen gland, ag), in which the male and female ducts separate.

The male duct is glandular, prostatic (pr) in its inner, and muscular in its outer course (e). The outermost part, the ejaculatory duct, winds through a muscular, unarmed, conical penis (p) lodged in a narrow male atrium.

The wide vagina (v) leads to the spermatheca (spa) which is small, though bigger than in Odhner's figure (1936:

fig. 30). It contains debris, probably remains of sperm and male secretion, so that it is not functionless (loc. cit.: 1068). The chambered spermatocyst (sp) is a small organ, apposed to the albumen gland (ag). It is connected with the gland mass by a short insemination duct (id) near the entrance of the sperm-oviduct (spo). Some folds of the glandular oviduct project as a spiral over the surface of the mucus gland (mu). The genital apertures lie between the right rhinophore and the first dorsal lobe on the side of the body.

10. *Armina wattla*, new species  
(Figs. 16-20)

Occurrences: Sapelo Island, Georgia, 11-19.5 miles from sea buoy, 16.5-19 m depth, January 31, February 13 and March 13, 1961; a total of 6 specimens. Holotype, UMMZ 230622; Paratype, UMMZ 230623.

The largest of the animals was 24 mm long, 15 mm broad and 8 mm high. The sole measured 20 mm in length, 9 mm in width. The smallest slug measured 15 mm. The preserved slugs were whitish with black pigment in the furrows between the notal ridges, on the base of the rhinophores, in the folds of the caruncle, in the middle of the veil, on the sides of the foot, and on the sole. Preserved, the crests of the notal ridges are white, but they may have been yellow alive, as some vestiges indicate.

There are about 36 notal ridges (nr) in the biggest animal, which run parallel to the mid-line. Broad and narrow ridges generally alternate on the sides, while in the middle the narrow ridge is often absent. On the anterior border of the notum there begin 20-24 broad and narrow ridges, the rest originate farther behind.

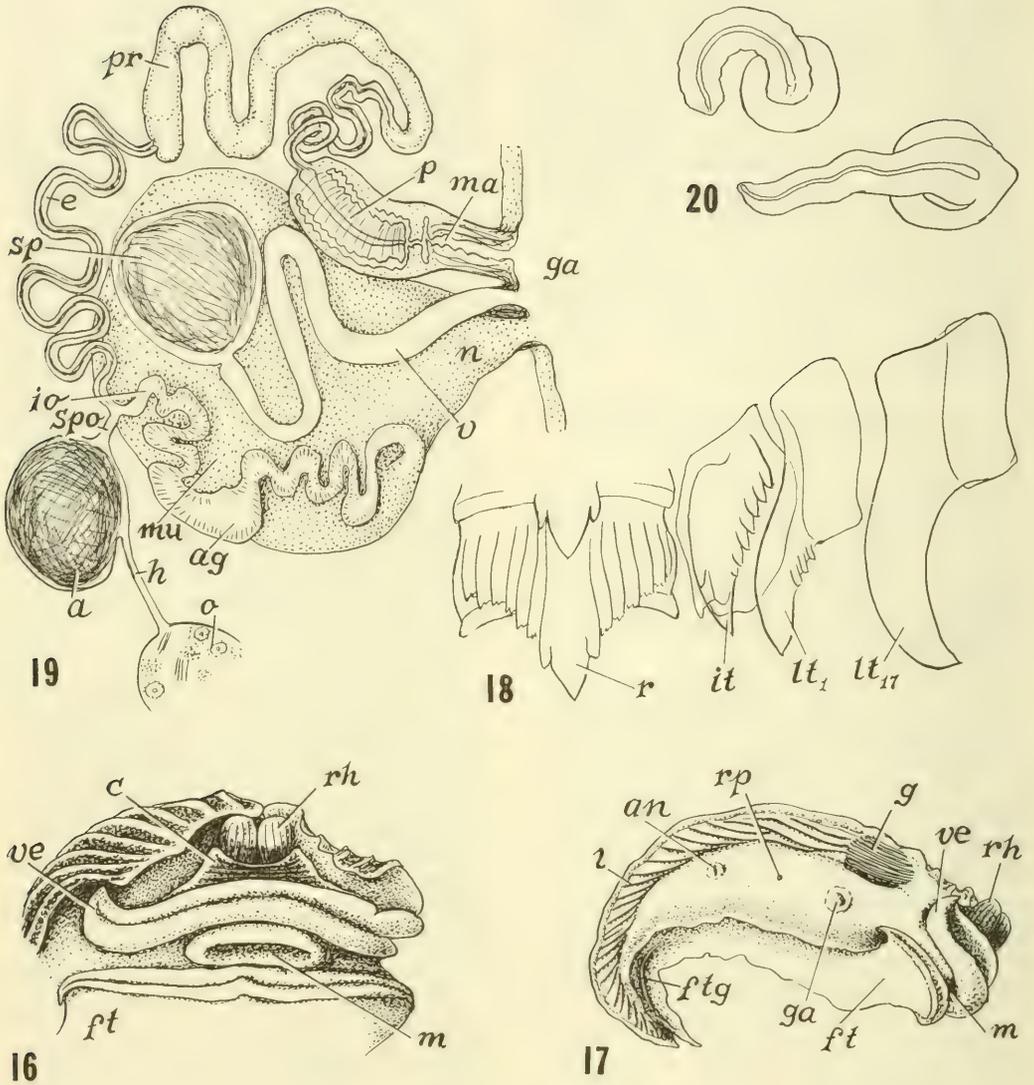
In front (Fig. 16) the notum is frilled by the ridges and notched in the middle; it is pointed behind. The pores of the marginal glands, Bergh's cnidopores, are numerous, but in most specimens they are difficult to see.

There are about 28 branchial leaves (Fig. 17, g), the innermost of which lie in an open pocket over the viscera; 3-4 lateral lamellae (l) arise from the branchiae. Farther behind there are 18-22 or, when all primordia are counted, up to 29 lamellae. They all run obliquely outwards, the posterior ones in a more pronounced way.

The semilunar veil (Fig. 16, ve) is as broad as the foot, its corners are bent upwards. Its upper border is weakly undulate. The dark middle of the veil contrasts with the colorless margins. Nuchal papillae are not developed, but a strong caruncle (c) arises from the upper or posterior border of the veil. The caruncle is folded transversely, is concave behind, where it borders the rhinophoral pits, and ends with a point on either side of the rhinophores (rh). These have 12 longitudinal leaves which are confluent on the tip and further divided downwards.

The anterior border of the foot (ft) is bilabiate and notched; its corners are slightly prominent and rolled upwards. The pedal gland (ftg, Fig. 17) is marked by a furrow 5 mm long. The shape of the buccal lip (m) varies, being either trapezoid or elliptic. The genital aperture (ga) lies under the gills, the anus (an) behind the middle or in the 2nd third of the body. The renal pore (rp) is between the anal and genital openings, about equally distant from both.

The yellow jaws are small, about 3x 1.5 mm; the masticatory process is undulate after treatment with KOH. The cutting edge has 3-4 rows of denticles in front. These denticles look like corn (maize) on the cob. The rows are more numerous in the rear; on the free process there are about 10 rows of pointed denticles measuring up to 40 $\mu$  in length. The radula (Fig. 18) comprises 40 rows with about 46 lateral teeth (lt) per half-row. The rhachidian tooth is 140 $\mu$  broad, 90 $\mu$  high. The median cusp is flanked by 5-7 denticles, 1-2 of which sit on the central cusp. The intermediate tooth (it)



FIGS. 16-20. *Armina wattla*, n. sp. Fig. 16. Anterior end of animal, frontal view. Fig. 17. View from the right side. Fig. 18. Inner teeth of radula. Fig. 19. Diagram of reproductive organs. Fig. 20. Two everted penes.

has a broad base and about 7 outer denticles. Furthermore there are 1-2 big inner points which lie farther behind than the outer denticles. They are difficult to see, because they are overlapped by the cusp. The 3 first lateral teeth may bear up to 4, exceptionally 5,

denticles. There are, however, many half-rows without any denticles. The lateral teeth increase in size towards the middle of the half-row and then decrease outwards.

The hermaphrodite duct (Fig. 19, h) is rather short, the ampulla (a) globular.

The short spermoviduct (spo) bifurcates into the inner oviduct (io) and the sperm duct. The latter begins as a long, winding efferent duct (e) followed by a prostatic portion (pr). The ectal, 3rd part is convoluted and thin; it reaches the muscular penis (p). The penis is lodged in the male atrium (ma); in 2 specimens it was protruded. The shape of the everted male organs (Fig. 20), conical in one animal, cylindrical in the other, shows that it cannot be used as a specific character.

The inner oviduct (io) passes into the inner portion of the glandular oviduct, the albumen gland. This organ is simplified in the diagram (Fig. 19, ag); it is tubular as in the species examined previously (Marcus, 1960a, fig. 67; 1961a, figs. 148, 154). The mucus gland (mu) is wide and richly folded. The long vagina (v) leads from the external aperture to an ample, spherical seminal receptacle, the spermatocyst (sp). From there the sperm descend again, enter the nidamental duct (n) and pass inward to the inner oviduct, where the eggs are fertilized (Marcus, 1960a, fig. 67, f).

The broad bicuspid caruncle (wattle) suggested the name of this species.

#### Discussion of *Armina wattla*

The only previously known *Armina* of the Atlantic coasts of the Americas is *A. mülleri* (v. Ihering, 1886: 223-230, pl. 9, fig. 1) from Santa Catarina, São Paulo, and north of Rio de Janeiro (Marcus, 1960a: 170; 1967a). Evidently Nijssen-Meyer's specimen from Surinam also belongs to that species; differences she mentioned (1965: 149) can be considered as intraspecific variations. For the intermediate tooth Nijssen-Meyer (: 148) indicates: "...at least 6 denticles on the inner side of the cusp". As her figure 4 shows, this is a lapsus for "outer side". *A. mülleri* has 2 small but recognizable caruncles and a median boss between them. Therefore we can not unite it with *A. semperi* (Bergh, 1866: 37-42, pl. 3), whose caruncle is so minute (:39) that it is almost invisible

on the cited figure 1. Pruvot-Fol (1933), the only one of the later authors who dealt with *A. semperi* and mentioned the caruncle, also called it "presque nulle". The original locality of *A. semperi* lies on the southwestern coast of Mindanao; it has been further reported from Japan, the Arabian Sea (Gulf of Oman), the Gulf of Aden, and the northern Red Sea.

*Armina mülleri* differs from *A. wattla* by the shape of the caruncle, which in the former, consists of 2 swellings without folds that are separated by a median boss. The rhachidian tooth of *A. mülleri* has a width ranging from 0.2 mm in a preserved slug 31 mm long, to 0.25 mm in preserved animals 39 and 16 mm long, against 0.14 mm in a 24 mm specimen of *A. wattla*. In the latter species, the lateral denticles of the rhachidian tooth are a little more numerous. In Nijssen-Meyer's and in our material of *A. mülleri* the reduction of the denticulation of the lateral teeth is less pronounced than in *A. wattla* (see Fig. 18). However, v. Ihering's description of *A. mülleri* does not show this difference.

A species of the Pacific South American coast, *Armina cuvieri* (d'Orbigny, 1837: 198, pl. 17, figs. 1-3) from Valparaiso is practically unknown; its pyriform male copulatory organ cannot be evaluated, because the above description of *A. wattla* as well as the literature (v. Ihering, 1886: 225; Marcus, 1960a: 173; 1961a: 44) show that the shape of the penis is variable, at least in preserved animals, probably due to contraction.

In an earlier exposition (Marcus, 1961a: 44) we discussed the 4 following species of *Armina* from the west coast of North America and indicated their bibliography: *A. californica* (Cooper, 1862), *A. vancouverensis* (Bergh, 1876), *A. columbiana* O'Donoghue, 1924, and *A. digueti* Pruvot-Fol, 1955. Lance (1962b: 51-54) has since described *Armina convolvula* from the northern part of the Gulf of California, and has recognized the isolated position of that species. In

fact, *A. convolvula* belongs to *Histiomena* Mörch, 1859, known from the Pacific coast of Nicaragua (Marcus, 1966: 189). While Bergh (1881b: 172), v. Ihering (1886: 226), Eliot (1905: 238), and Pruvot-Fol (1933: 114) did not admit a broad intra-specific variability of the radula in *Armina*, Steinberg (1963: 65) does. She unites the 4 species of *Armina* of the North American Pacific coast from Panama to Vancouver Island under the oldest name. Her opinion will probably be accepted, though a comparison of the reproductive organs of several specimens is still desirable. For our purposes, i.e. the distinction of *A. wattla* from the warm temperate western Atlantic, which has so many faunal relationships with the eastern Pacific, it will be sufficient to note the weak caruncles of the 3 first Pacific species. As for *A. digueti* Pruvot-Fol, 1955, whose caruncle is not described, it differs from *A. wattla* by its coarse white ridges, among the broad interspaces of which there course thin black ridges.

Comparing the further species of *Armina* with *A. wattla*, we found a strongly developed caruncle with transverse folds in *A. tigrina* Rafinesque, Bergh's *Pleurophyllidia undulata* Meckel, 1823 (1866: 18-19, pl. 1). This species is recorded from the western, central (Sargasso Sea) and eastern warm and warm temperate Atlantic Ocean (for range see Marcus, 1966: 191). The radula of *A. tigrina* differs widely from that of *A. wattla*, especially by the high and narrow rhachidian tooth with 15-30 lateral denticles on either side of the median cusp. Another species that should be compared with *A. wattla* is *A. natalensis* (Bergh, 1866: 34; Barnard, 1927: 213) from the coast of Natal. It has a similar strong caruncle, but its rhachidian tooth (Bergh, 1866: pl. 6 B, fig. 7) is very broad, and the number of lateral lamellae is much higher than in *A. wattla*. Lateral teeth nearly without denticles, as in *A. natalensis*, occur also in several other species (Bergh, 1907: 102-103), but all of these have small caruncles, or (Baba,

1949: 162, *A. major*) longitudinal ridges on the veil.

#### 11. *Fiona pinnata* (Eschscholtz, 1831)

Marcus, 1961a: 50-51, figs. 173-179, references, distribution, description;

Bayer, 1963: 460-465, figs. 5-7, behavior, feeding, growth and reproduction.

Occurrences: Off the Georgia Coast in Gulf Stream Drift, 31° 01 min. N, 79° 52 min. W, May 1, 1962; numerous specimens together with a pre-adult male of the pycnogonid, *Anoplodactylus brasiliensis* Hedgpeth, 1948 (: 222, 224).

Further distribution: Pelagic and gregarious in warm and temperate seas, original locality: Sitka, Alaska, on a piece of wood washed ashore. Dr. Wolfram Noodt and Rudolf Röttger collected this species and its egg masses on floating feathers with barnacles about 250 km off Peru in October, 1965 while on board the research ship "Anton Bruun".

The largest of the preserved specimens at hand is 17 mm long, which corresponds to the maximum length known of living animals, 25 mm.

#### 12. *Dondice occidentalis* (Engel, 1925)

*Caloria occidentalis* Engel, 1925: 73-76, figs. 7-15;

*Dondice occidentalis* Marcus, 1958b: 62-65, figs. 97 (:54), 98-104 (: 63); 1960a: 186-187, figs. 87-90; 1963: 48; Edmunds, 1964: 27-28.

Occurrences: Georgia, 1) Sapelo Sound, 16-26 m depth, April 16, 1963, 2 specimens; 2) 17 1/2-15 1/2 mi. 102° from Sea buoy, 15 m depth, 11 specimens.

Further distribution: Beaufort, North Carolina; Miami, Florida; Jamaica; St. Martin, Bonaire, Lesser Antilles; Guanta, Venezuela; São Paulo, Brazil.

The preserved specimens reach 20 mm in length. The jaws are covered with a

black epithelium. The radula has 17 teeth (radular formula: 0.1.0), whose median cusp is flanked by 4-7 denticles. As in Edmunds' material, the gonopores lie immediately behind the arch of cerata from the anterior liver, at the front of the interhepatic space.

#### ZOOGEOGRAPHIC REMARKS

Two species of the present collection, *Scyllaea pelagica* and *Fiona pinnata*, are widely distributed pelagic species and occur in all seas of middle and low latitudes. Seven are littoral species peculiar to the warm western Atlantic region, which extends from Cape Hatteras to southern Brazil, probably to northern Santa Catarina. The new form *Tritonia bayeri misa* belongs to this West Indian group, because it is related to *I. b. bayeri* from the Miami area, Florida. *Doridella burchi* is near the widely distributed *D. obscura*, whose range extends, according to Franz (1967: 75), from Massachusetts to Texas. Only 1 species of the present West Indian element, *Dendrodois krebsii*, is also known from the tropical west coast of North America. Relicts of the Tethys Sea, which existed up to Middle Tertiary times, are *Doris verrucosa* and *Okenia sapelona*. The former is known from South Carolina to Santa Catarina, and from the British Isles to South Africa. The latter is related to a species, *O. mediterranea*, known from Naples and the French Mediterranean coast.

One species of the present collection cannot be allotted to any of these groups: *Armina wattla*. It differs by its caruncle from the solely American species of the genus, and is separated from the remaining species of *Armina* by its radula.

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## ADDENDUM

The disposition of the holotype and paratype specimens of the opisthobranchiate mollusks described in Marcus & Burch (1965, Marine euthyneuran Gastropoda from Eniwetok Atoll, western Pacific, MALACOLOGIA, 3(2): 235-262) is as follows [catalogue numbers are those of the Museum of Zoology, University of Michigan, Ann Arbor, Michigan, U. S. A.]

- Haminoea musetta* Marcus & Burch, Holotype, UMMZ 230624
- Haminoea musetta* Marcus & Burch, Paratypes, UMMZ 230625
- Haminoea linda* Marcus & Burch, Holotype, UMMZ 230626
- Haminoea linda* Marcus & Burch, Paratypes, UMMZ 230627
- Chromodoris briqua* Marcus & Burch, Holotype, UMMZ 230629
- Herviella mietta* Marcus & Burch, Holotype, UMMZ 230630
- Herviella mietta* Marcus & Burch, Paratype, UMMZ 230631
- Onchidella evelinae* Marcus & Burch, Holotype, UMMZ 230632
- Onchidella evelinae* Marcus & Burch, Paratype, UMMZ 230633

## RESUMEN

ALGUNOS OPISTOBRANQUIOS DE LA ISLA SAPELO,  
GEORGIA, ESTADOS UNIDOS

E. Marcus and E. Marcus

Este trabajo trata sobre 2 opistobranquios pelágicos, y otros 10 de las zonas entre mareas y sub-mareas, de la costa sudeste de Estados Unidos. Se describen cuatro especies: *Okenia sapelona*, *Doridella burchi*, *Tritonia (Candiella) bayeri misa*, y *Armina wattla*. La última especie difiere de la única conocida *Armina americana* por su fuerte carúncula, y de *undulata* y otras especies por su rádula. *Okenia sapelona* se asemeja a *O. mediterranea*, y por lo tanto pertenece a los opistobranquios cuya existencia puede trazarse desde el Mar Tethys del Terciario. Las dos restantes son de la región de aguas cálidas del Atlántico occidental. Las especies litorales de la presente colección habitan también aquellas aguas, con excepción de *Doris verrucosa* que aparece también en el Atlántico oriental.

La subespecie *Pleurobranchaea hedgpethi hamva* se elimina, porque en el presente material, la dirección del plegado sobre los orificios genitales es con frecuencia oblicua, no dorsal (*P. h. hamva*) ni tampoco anterior (*P. h. hedgpethi*).

## ZUSAMMENFASSUNG

ÜBER EINIGE OPISTHOBRANCHIER VON DER SAPELO INSEL,  
GEORGIA

E. and E. Marcus

Die Arbeit behandelt 2 pelagische Opisthobranchier und 10 aus der Gezeitenzone und dem Sublitoral von der Sapelo Insel, Georgia. Neu sind: *Okenia sapelona*, *Doridella burchi*, *Tritonia (Candiella) bayeri misa* und *Armina wattla*. Die letzte unterscheidet sich durch die starke Karunkel von den rein amerikanischen *Armina*-Arten sowie durch die Radula von *A. undulata* und den anderen Arten mit starker Karunkel. *O. sapelona* ähnelt der *O. mediterranea*, gehört also zu den Opisthobranchiern, deren Verbreitung auf das tertiäre Tethysmeer zurückgeführt werden kann. Die 2 übrigen neuen Formen sind mit Arten der westatlantischen Warmwasserregion verwandt. Gleichfalls Bewohner dieser Region sind die bekannten litoralen Arten der vorliegenden Sammlung, mit Ausnahme von *Doris verrucosa*, die auch im Ostatlantik vorkommt.

Die Unterart *Pleurobranchaea hedgpethi hamva* wird aufgegeben, weil im vorliegenden Material der Fortsatz über den Geschlechtsöffnungen oft schräg gerichtet ist, d. h. weder nach oben (*P. h. hamva*), noch nach vorn (*P. h. hedgpethi*).

## RESUMO

SOBRE ALGUNS OPISTOBRANQUIOS DE ILHA DE SAPELO,  
GEORGIA

E. e E. Marcus

O trabalho trata de dois opistobrânquios pelágicos e dez litorais, da zona das marés e abaixo desta, da ilha de Sapelo, Georgia. Formas novas são: *Okenia sapelona*, *Doridella burchi*, *Tritonia (Candiella) bayeri misa*, e *Armina wattla*. A última difere das

espécies puramente americanas de *Armina* pela carúncula forte e pela rádula de *A. undulata* e das outras espécies com carúncula forte. *O. sapelona* assemelha-se a *O. mediterranea*, por isso pertence aos opistobrânquios cuja distribuição pode ser reconduzida ao mar Terciário da Tethys. As duas novas formas restantes são aparentadas com espécies da região quente do Atlântico ocidental. Também as espécies já conhecidas da presente coleção são habitantes desta região, com exceção de *Doris verrucosa* que ocorre no Atlântico ocidental e oriental.

A subespécie *Pleurobranchaea hedgpethi hamva* foi suprimida, porque a direção do lóbulo em cima das aberturas genitais é frequentemente oblíqua, nem para cima (*P. h. hamva*), nem para diante (*P. h. hedgpethi*).

#### АБСТРАКТ

#### О НЕКОТОРЫХ ОПИСТХОБРАНЧИА ИЗ РАЙОНА О. САПЕЛО (ДЖОРДЖИЯ, С. Ш. А.)

Э. МАРКУС И Э. МАРКУС

В работе рассматриваются 2 пелагических и 10 литоральных и сублиторальных видов *Opisthobranchia* из вод, омывающих восточное побережье С. Ш. А. Описываются 4 вида: *Okenia sapelona*, *Doridella burchi*, *Tritonia (Candiella) bayeri missa* и *Armina wattila*. Последний вид отличается от американского вида рода *Armina* сильно развитым карункулом, а от *A. undulata* и других видов, имеющих большой карункул - радулой. *Okenia sapeloma* похожа на *O. mediterranea*, следовательно относится к *Opisthobranchia*, распространение которых прослеживается, начиная с третичного моря Тетис. Остальные 2 новых формы родственны видам из тепловодного района западной Атлантики. Все известные литоральные виды настоящей коллекции - также обитатели этого района, за исключением *Doris verrucosa*, который встречается также и в восточной Атлантике. Подвид *Pleurobranchaea hedgpethi hamva* - закрывается, поскольку в настоящем материале кожный вырост над генитальными отверстиями часто был направлен косо т.е. ни дорзально (как у *P. hedgpethi hamva*), ни кпереди (как у *P. h. hedgpethi*).

REVISION OF THE GENUS *HERVIELLA*  
(OPISTHOBRANCHIA: EOLIDACEA)

Robert Burn<sup>1</sup>

ABSTRACT

*Hervietta* Baba (1949) (Opisthobranchia: Eolidacea) is especially characterized by a single row of cerata in the right liver, a penial stylet and a 'serial' spermatheca. *Muessia* Marcus (1965), with the same characteristics, is a synonym. Eight species are known from the western Pacific Ocean including the new species *H. burchi* described in this paper. Two subgenera are distinguished; *Hervietta* s. s., with the central radular cusp longer than the lateral denticles, contains the species *H. yatsui* (Baba) type species, *H. affinis* Baba, *H. burchi* sp. nov., *H. evelinae* (Marcus), *H. claror* Burn and *H. exigua* (Risbec); *Marciella* subgen. nov., with the lateral denticles nearly or as long as the central cusp, contains the species *H. mietta* Marcus (type species) and *H. albida* Baba.

*Noumeaella* Risbec (1937) with similar genital characters and an arch of cerata in the right liver appears to be closely related to *Hervietta*. The 2 genera form a distinct group within the family Favorinidae, and with some reservations are placed together in a new subfamily, *Herviellinae*.

Cleioproct eolids with a single row of cerata in the anterior or right liver are few in number and their known distribution is restricted to the western Pacific Ocean. Eight species appear to be united by this taxonomically important anatomical characteristic. The current revision of both the generic and specific units involved is derived from the literature, examination of preserved specimens of *H. burchi* and *H. mietta*, together with field notes on living specimens of these 2 species, and a study of living specimens of *H. claror*.

The writer is indebted to Dr. J. B. Burch, Museum of Zoology, University of Michigan, Ann Arbor, Michigan, U. S. A., for the opportunity to examine some of the *Hervietta* material described by Dr. Ernst Marcus and himself (1965), as well as to study his field notes made at the time of collection. This research was undertaken while the writer was a recipient of a grant from the Science and Industry Endowment Fund,

Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia.

THE GENUS *HERVIELLA*

The primary generic unit involved in this revision is *Hervietta* Baba (1949: 107, 180), the type of which, *H. yatsui* (Baba, 1930), is now known anatomically (Baba, 1966b). Based upon the type species, it appears that the following characteristics are diagnostic for the genus: a single row of cerata in the right liver; anterior of foot expanded and rounded; rhinophores simple; jaws high anteriorly and narrow posteriorly, masticatory edge with a single row of denticles; penial stylet present; and female ducts with the spermatheca 'serial' (i.e., it is formed by a swelling of the oviduct or vagina).

The recently constituted genus *Muessia* Marcus (1965: 282), type *M. evelinae* Marcus (1965: 283) described from a single very small preserved specimen,

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is similar to *Herviella*, except that the rhinophores and tentacles are annulate in the preserved Holotype, and the jaws are stated to be oblong in shape. Annulate rhinophores and tentacles occur in a preserved specimen of *H. mietta* examined for this revision, but field notes and published descriptions indicate that these appendages are smooth in life. Therefore, it is presumed that living *Muessa* have smooth rhinophores and tentacles. The oblong shape of the jaws depends upon how they are orientated for observation. In *Muessa* (Marcus, 1965: fig. 38) the jaws are shown with the masticatory border in the horizontal position. If the figure is turned so that the upper anterior margin of the jaw is in the vertical position, then when compared with the figure of the jaw of *H. yatsui* (Baba, 1966: pl. 1, fig. 4), it can be seen that the differences are not objective. Consequently, I regard *Muessa* to be identical with and a junior synonym of *Herviella*.

As a result of this synonymy, there are 7 species that definitely can be assigned to the genus *Herviella*. An eighth species, *Aeolidia exigua* Risbec (1928: 245), in which the position of the anus is not known, is tentatively ascribed to *Herviella* (Burn, 1963: 18; Marcus & Burch, 1965: 251). These 8 species are sharply divided in the shape of the radular teeth. In *H. yatsui*, *H. affinis*, *H. burchi*, *H. evelinae*, *H. claror* and *H. exigua* the median cusp is longer than the 3 to 5 denticles on each side. *Herviella mietta* and *H. albida* have the outermost of the 4 to 9 denticles on each side nearly or as long as the median cusp and with the intermediary denticles shorter. These 2 species can be separated by this characteristic into a subgenus *Marciella* subgen. nov., with *H. mietta* Marcus (1965) designated as the type species.

The jaw of *Herviella burchi* is inter-

mediate in shape between those of *H. yatsui*, *H. evelinae* and *H. claror*, and therefore it does not seem justified to distinguish the latter 2 species even subgenerically on jaw shapes.

Marcus & Burch's *Herviella claror* (1965: 251) differs from *H. claror* Burn (1963: 18) in colour pattern and the shape of the jaw and radular teeth. Here it is described as *H. burchi* sp. nov.

The following characterizations of the species of *Herviella* are drawn from the literature unless otherwise stated. By means of line drawings, Fig. 1 shows specific differences as they occur in the colour pattern of the anterior portion of the body and the cerata (columns 1 & 2), the jaws and masticatory border (column 3), and the radular teeth (column 4).

Subgenus *Herviella* s.s. Type species: *H. yatsui* (Baba, 1930).

The lateral denticles of the radular teeth are shorter than the central cusp and they generally decrease in height toward the lateral margins.

*H. yatsui* (Baba, 1930: 121; 1937: 328; 1949: 107, 180; 1966b: 1; Abe, 1964: 70). Japan; Pacific and Japan Sea coasts, common. Body yellowish white; black U-shaped pigmentation pattern on the head continuing on to the tentacles; black specks occur on the median part of the back; rhinophores with a black band at their mid-length; cerata with an upper and lower cluster or ring of black spots and an opaque white band at the tip. Jaws high anteriorly, tapering sharply behind, concave dorsally, with 15-20 denticles. Radula with 18-25 teeth; central cusp short; 4-5 denticles on each side. Penial stylet with 3-6 spines along the concave side; spermatheca spherical.

Like *Herviella mietta* and *H. albida*, the denticles of the masticatory borders are conical. The spines on the penial stylet of *H. yatsui* are unique

FIG. 1. Specific differences of the species of *Herviella*. Colours are indicated as follows: fine stippling, opaque white; heavy stippling, black; oblique hatching, red, orange or yellow.

	Color pattern of anterior body	Color pattern and shape of cerrata	Jaw shape (a) and masticatory border (b)	Radular teeth
<b>H. yatsui</b> after Baba 1966b				
<b>H. affinis</b> after Baba 1966b				
<b>H. burchi</b> after Marcus and Burch 1965 (ceras drawn from field notes)				
<b>H. evelinae</b> after Marcus 1965 (Anterior body reconstructed)		?		
<b>H. claror</b> after Burn 1963				
<b>H. exigua</b> after Risbec 1928			?	
<b>H. mietta</b> after Marcus and Burch 1965 (Jaw drawn from own observations)				
<b>H. albida</b> after Baba 1966a				

among the Eolidacea.

*H. affinis* Baba (1960: 303; 1966b: 4; Abe, 1964: 71). Japan; Pacific and Japan Sea coasts, not common. Body yellowish-white, without U-shaped pigmentation pattern on the head, but instead with black specks covering the head, back, sides and lower half of the cerata; rhinophores with a black band at their mid-length; cerata with an orange ring below their tips. Jaws high anteriorly, tapering behind, dorsally concave; with 10-12 large oblique denticles. Radula with 13-14 teeth, central cusp long and wide, 3-4 denticles on each side. Penial stylet long and curved.

Oblique or raking denticles occur also in *Herviella burchi*, *H. evelinae* and *H. claror*. All 4 species have black speckling on the body and 3-4 denticles on each side of the wide central cusp of the radula. Shape of the jaw and colour patterning, particularly on the cerata, separate these 4 species.

*H. burchi* sp. nov. (*H. claror* Marcus & Burch, 1965: 251, fig. 28-30; non *H. claror* Burn, 1963: 18). Marshall Islands; Eniwetok Island, three specimens. Body white, without U-shaped pigment pattern on the head, with black specks and white spots on the back and side of the body (clear transverse areas occur between the cerata groups on opposite sides of the body); the lower part of the rhinophores and tentacles speckled with black pigment; cerata with an orange ring at the distal one-third and an opaque white band above and below this. Jaws high anteriorly, tapering slightly behind, slightly convex dorsally; with 6 large inclined denticles. Radula with 11 teeth, central cusp broad and blunt, 3-4 lateral denticles on each side. Penial organ not known.

The Holotype is a preserved specimen 4.5 mm long in the collections of the Museum of Zoology, University of Michigan, (cat. no. 230634). A total of 3 specimens were collected by Dr.

William H. Heard, April 2-12, 1960. Only the Holotype was available for study, the other 2 specimens having been preserved for cytological studies.

The new species differs from *Herviella claror* Burn (and other species of the genus) in colour pattern, jaw shape and the blunt central radular tooth. The ovoid shape of the jaws is especially distinctive. The species is named for Dr. J. B. Burch, who allowed me to examine the type specimen and his field notes, sketches and photographs made at Eniwetok Atoll.

*H. evelinae* (Marcus, 1965: 283). Caroline Islands; Ifaluk Island, one specimen. Body yellowish (?) in life, with black specks on the back, head, tentacles and rhinophores. The jaws narrowed behind, with 8 large oblique denticles having rough edges. Radula with 14 teeth, central cusp broad with curved sides and 3-4 lateral denticles on each side. Penis with a long stylet; the spermatheca exists only as a small dilation.

The broad radular teeth, rough-edged masticatory denticles and the posteriorly narrowed jaws (referred to as 'oblong' by Marcus, 1965: 282) are the diagnostic characteristics of *H. evelinae*.

*H. claror* Burn (1963: 18). Australia; northern New South Wales (Woody Head), 2 specimens. Body white with black speckles on the back, head, tentacles and rhinophores; cerata with an orange band below the tip and black speckling on the anterior side. Jaws slightly narrowed behind; with 6 large oblique denticles having smooth edges. Radula with 13 teeth, central cusp long with straight sides and having 3 denticles on each side. Penis with a curved stylet.

*Herviella claror* and *H. evelinae* have similarly shaped jaws, but those of *H. claror* are broader posteriorly and the masticatory denticles have smooth edges. The long taper of the central radular tooth is unlike that of

any other *HervIELla*. An orange band on the cerata occurs also in *H. affinis*, and *H. burchi*, and a red one occurs on the cerata of *H. exigua*.

*H. exigua* (Risbec, 1928: 245; 1953: 134). New Caledonia; Kouaoua Bay, 3 specimens. Body yellowish with minute black speckles grouped together to form greyish areas, tentacles and rhinophores with a black band in their middle portion, cerata with a red band below their tips. Jaws with a single row of strong denticles. Radula with about 12 teeth, the central cusp long and tapering and having 3 denticles of equal length on each side. The penial stylet is long and curved.

The position of the anus is not known for *HervIELla exigua*, therefore its placement in the genus *HervIELla* is somewhat doubtful. The red band on the cerata and even height of the lateral denticles are the distinctive characteristics for the species.

Subgenus *Marciella* subgen. nov. Type species: *H. mietta* Marcus & Burch (1965).

The marginal lateral denticles of the radular teeth are nearly or as long as the central cusp; intermediary lateral denticles are shorter than the marginal denticles.

The subgenus is named for Dr. Ernst Marcus of Brazil who has added immensely to the knowledge of the Eolidacea and other Opisthobranchia.

*H. mietta* Marcus & Burch (1965: 251). Marshall Islands; Eniwetok Island, not uncommon. Body white below, black above, the pigmentation extending on to the cerata (except at the tips), head, tentacles (dorso-median line) and rhinophores (middle third black, with a short pigmentation line below). Jaws high anteriorly, narrow behind and deeply concave dorsally; with at least 30 sharp denticles, the largest below (personal observation). Radula with 18 teeth, the central cusp short and pointed, with 8-9 thin denticles on each side, the outermost denticle as long as the central cusp. Penial

stylet curved.

The jaws figured for *H. mietta* in Fig. 1 are from the Holotype (University of Michigan, Museum of Zoology, cat. no. 230630); they measured 0.9 mm in both length and height. The Holotype is a specimen with heavy black pigment which, in the original description, is called the second colour type. The first colour type (University of Michigan, Museum of Zoology, cat. no. 230631) has morphologically defective jaws by being deeply incised (Marcus & Burch, 1965: fig. 34; confirmed by personal observation). Radular and other characteristics are in accord despite the fact that this first colour type has a light and transparent body, white granules and sometimes black pigment on the back, the head with a black pattern, the rhinophores with a black band and the cerata clear with yellow digestive glands.

This is the most distinctive species of the genus. The important diagnostic characters are carrot-shaped cerata that are predominantly black in colour, black pigment on the body, and 8-9 denticles on each side of the small central cusp. The jaws are unlike those of any other *HervIELla* (see Fig. 1, column 3); the anterior margin is more erect and evenly convex, the masticatory border is deeper and bears more (about 30) denticles, the dorsal margin is deeply concave, and the posterior end is narrow and squarely truncate. Both *H. mietta* and *H. albida* have a somewhat similar pattern of black pigment on the head, otherwise they are quite different.

It is doubtful that the next species, *HervIELla albida*, should be classified in the subgenus *Marciella* with *H. mietta*. Except for the long marginal denticles of the radular teeth, *H. albida* belongs to the subgenus *HervIELla s.s.*, as indicated by the fusiform cerata, the configuration of the anterior edge of the jaws and the long 'legs' of the radular tooth. Raising *Marciella* to a

full genus may be justified when more knowledge is available, particularly concerning the reproductive organs. For the present, however, it is maintained as a subgenus, solely to include the 2 species with long marginal denticles.

*H. albida* Baba (1966a: 361). Japan; Inland Sea (Seto, Kii), one specimen. Body yellowish-white with scattered white spots on the head and back, black pigment present in a U-shape pattern on the head, a pigment line on the tentacles, a pigment band on the rhinophores and in lines laterally between the groups of cerata; cerata with 2 bands of opaque white in their upper halves. Jaws high anteriorly, narrow behind, deeply concave dorsally; with 16-18 pointed denticles. Radula with 20 teeth, the central cusp with a long taper, and with 3-4 denticles on each side and nearly as long as the central cusp. The penial stylet is short and curved.

The other 2 Japanese species, *Herviella yatsui* and *H. affinis*, closely resemble *H. albida* in body shape, general body colouration and shape of the jaws. *Herviella albida* is separated from these 2 species by the long marginal lateral denticles of the radular teeth. Fewer lateral denticles (3-4) and much less black pigment distinguish *H. albida* from *H. mietta*.

#### DISCUSSION

*Herviella* shows an unusual character in the structure of the female reproductive ducts. The spermatheca is a dilation of the vagina with 2 separate openings, one to the vagina proper and the other to the oviduct and gland mass. Thus it may be termed 'serial' following the terminology of similar parts in the doridacean opisthobranchs. In the species of the Eolidacea, the spermatheca is a blind sac with a narrower stalk attached at the inner end of the vagina (Cleioprocta) or nearer the outer end (Acleioprocta), or it

may have a separate external opening near that of the vagina (some, but not all, Pleuroprocta).

From the literature, there appears to be only 2 cleioproct species comparable to *Herviella*: *Palisa papillata* Edmunds (1964: 12, fig. 10A; = *Moridilla kris-tenseni* Marcus & Marcus, 1963: 44) and *Noumeaella rehderi* Marcus (1965: 282, fig. 35). In *P. papillata* the spermatheca is a dilated section of the vagina; in *N. rehderi* it is lobated. A similar 'serial' spermatheca is reported in a number of species of the dendronotacean genus *Doto* (Marcus, 1957; 1961: 36-41, figs. 129, 134, 138, 140, 146; Marcus, E. & E., 1960: 166, fig. 57), which, like *Herviella*, grow to little more than 10 mm in length and have very slender bodies. Therefore, it would seem that smallness of size may have led to this parallel development within related groups.

*Palisa* has 5 or 6 rows of cerata in the right liver and, therefore, in the present eolid classification, belongs to the family Facelinidae. Like *Noumeaella*, *Palisa* has rhinophores that are thickly papillate on their rear edges, but unlike *Noumeaella*, the penis is unarmed.

The present classification places both *Herviella* and *Noumeaella* among the genera of the family Favorinidae (characterized by the right liver in an arch or single row), subfamily Favorininae (characterized by cerata in a single series). In *Noumeaella* the right liver forms an arch, the rhinophores are papillate as mentioned above and the foot corners are tentaculiform. The similarity of the genital organs, both with penial stylet and serial spermatheca, suggest that these 2 favorinids should be grouped together, perhaps even so far as to warrant a subfamily of their own, *Herviellinae*.

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## RESUMEN

REVISION DEL GENERO *HERVIELLA* (OPISTOBRANCHIA: EOLIDACEA)

R. Burn

*Herviella* Baba 1949 (Opisthobranchia: Eolidacea) se caracteriza especialmente por una hilera de "cerata" en el hígado derecho, un estilote penial y una espermateca "serial". *Muessa* Marcus 1965, con las mismas características es un sinónimo. Ocho especies son conocidas del Pacífico occidental, incluyendo la nueva *H. burchi* aquí descripta. Se distinguen dos subgéneros: *Herviella* s.s. con la cúspide del diente raquídeo más larga que los dentículos laterales, contiene las especies *H. yatsui* (Baba) tipo, *H. affinis* Baba, *H. burchi* sp. n., *H. evelinae* (Marcus), *H. claror* Burn y *H. exigua* (Risbec); *Marciella* subgénero nuevo, con los dentículos laterales casi tan largos como la cúspide central, contiene la especie *H. mietta* Marcus (tipo), y *H. albida* Baba.

*Noumaeaella* Risbec 1937 con características genitales similares y un arco de "cerata" en el hígado derecho, parece estar muy relacionada a *Herviella*. Los dos géneros forman un grupo distinto dentro de la familia Favorinidae, y con algunas reservas se juntan en una nueva subfamilia, *Herviellinae*.

## АБСТРАКТ

РЕВИЗИЯ РОДА *HERVIELLA*  
(OPISTHOBRANCHIA: EOLIDACEA)

Р. БЁРН

Род *Hervietta* Baba (1949) (Opisthobranchia: Eolidacea) характеризуется следующими особенностями: один ряд папилл (*cerata*) в правой печени, пениальный стилет и "сериальная" сперматека (семеприемник). Род *Muessia* (Marcus, 1965), имеющий те же признаки, является синонимом.

Из западной части Тихого океана известно 8 видов рода *Hervietta*, включая новый вид *H. burchi*, описанный в настоящей работе. Различаются 2 подрода: *Hervietta* s.s., у которого центральный зубец радулярной пластинки длиннее латеральных зубчиков. Сюда относятся - *H. yatsui* (Baba) тип; *H. affinis* Baba, *H. burchi* sp. nov., *H. evelinae* (Marcus), *H. claror* Burn, *H. exigua* (Risbec); *Marciella* subgen. nov., с латеральными зубчиками почти или такой же длины, как и центральный зубец; он включает 2 вида: *H. mietta* Marcus (типовой вид) и *H. albida* Baba.

*Noumeaella* Risbec (1937) со сходным строением гениталий и с дугообразным расположением папилл в правой печени, видимо представляет собою род, близко-родственный *Hervietta*. Эти 2 рода образуют хорошо-очерченную группу внутри семейства *Favorinidae* и, с некоторыми оговорками, выделены в новое подсемейство *Herviellinae*.

*ERVILIA CONCENTRICA* AND *MESODESMA CONCENTRICA*:  
CLARIFICATION OF SYNONYMY<sup>1</sup>

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ABSTRACT

A small pelecypod, *Mesodesma concentrica* Holmes (1860), was described from fossil material at Simmons Bluff, Yorges Island, South Carolina, U. S. A. A similar mollusk, *Ervilia concentrica* Gould (1862), was described from dredgings on the North Carolina coast. Comparison of hinge structure, pallial markings and external characteristics of lectotypes shows that these 2 forms are identical and synonymous. Furthermore, comparison of features possessed by Holmes' type material and specimens of *M. arctatum* Conrad shows no basis for including the synonymized species in the genus *Mesodesma*. Thus, a newly designated lectotype is described for *E. concentrica* along with a corrected taxonomic citation.

Nomenclatural Synonymy

*Ervilia* (Turton) 1822, *Conchylia Dithyra Insularum Britannicarum: The Bi-valve Shells of the British Islands*. p 55-56, pl. 19, fig. 4.

*Ervilia concentrica* (Holmes) Plate II, Figs. 3-6

*Mesodesma concentrica* Holmes 1860, *Post-Pliocene Fossils of South Carolina*, p 44, pl. 6, fig. 10. Simmons Bluff, Yorges Island, South Carolina.

*Ervilia concentrica* Gould 1862, *Proc. Boston Soc. nat. History*, 8: 281-282. [No figure.] Coast of North Carolina.

*Ervilia concentrica* Gould 1862, *Otia Conchologia*, p 239. [No figure.] Coast of North Carolina.

GENERIC CONSIDERATIONS

The genus *Ervilia* was established by Turton (1822) to accommodate a lentil-shaped shell previously described as *Mya nitens* by Montagu (1808). Thus the type for the genus is *M. nitens* (by monotypy). The genus has a world-wide distribution in tropical and temperate waters; fossil forms are known beginning with the Tertiary.

All species of *Ervilia*, fossil and recent, have certain characteristics in common. The valves are small, rarely

exceeding 10 mm in length, and they are somewhat compressed and have concentric striations (Fig. 1). Radiating striae are present on most species, but these usually are reduced mid-laterally. Occasionally, the radiating striae are restricted to the posterior portion of the shell. Variability of these striae are probably determined in part by erosion. The valves are fragile and often translucent.

The umbo is usually slightly closer to the anterior end, although still close enough to the mid-point to give the shell

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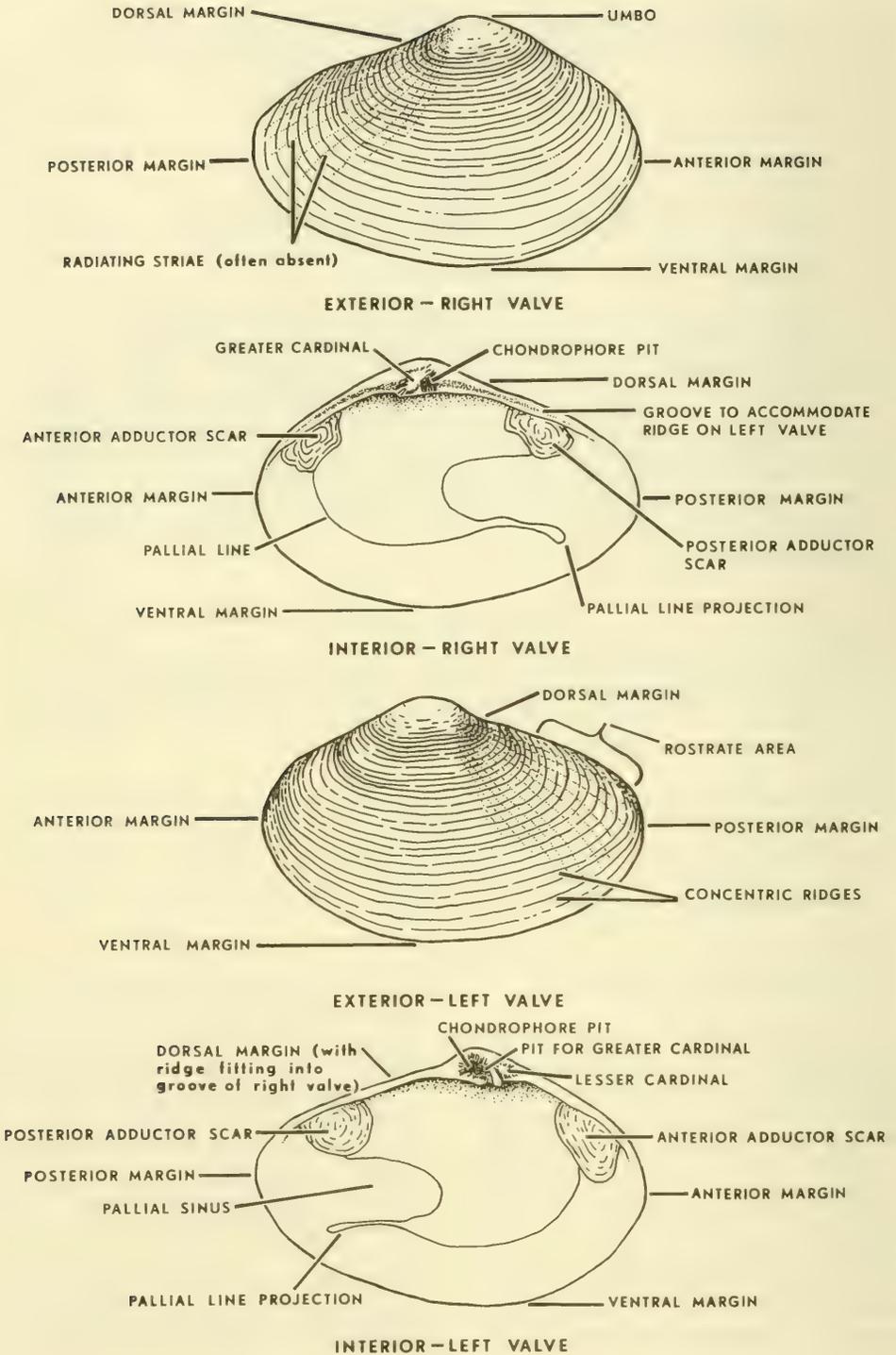


FIG. 1. General shell morphology of *Ervilia concentrica* (Holmes).

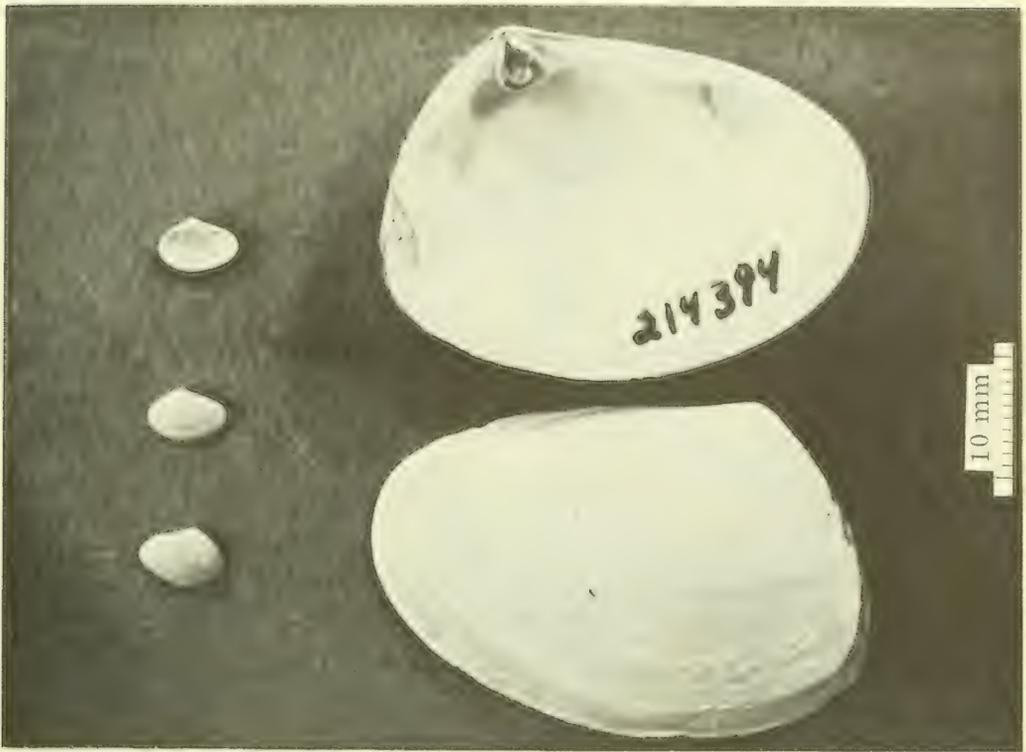


FIG. 2. Comparison of *Ervilia concentrica* and *Mesodesma arctatum*. Left column: *E. concentrica*; the upper 2 valves are the new lectotype selected from the Holmes' material (AMNH 11291); the bottom valve is the paratype selected by Whitfield & Hovey (1901) as a representative specimen of the same collection. Right column; *M. arctatum* (MCZ 214394) from Nauset Beach, Orleans, Cape Cod, Massachusetts. Scale in millimeters.

an oval shape. Some species are rostrate posteriorly, which tends to accentuate the displacement of the umbo toward the anterior end.

The hinge region is relatively simple. The right valve has a prominent cardinal tooth just anterior to a large chondrophore pit. Posterior to this pit is a smaller depression for the lesser cardinal tooth of the left valve. The left valve has a pit anteriorly for the greater cardinal tooth of the right valve. Adjacent and posterior to this pit is the chondrophore pit followed by the lesser cardinal tooth. There are essentially no lateral teeth, and the ligament is much reduced.

The inner surface of the valves displays a distinctive pattern. The pallial sinus is deep, extending nearly to beneath the umbo. Posteriorly, where the

lower edge of the pallial sinus merges with the pallial line, the fused lines bend downward and outward toward the margin of the valve.

In summary, *Ervilia* is characterized by concentric ridges on small, compressed, oval valves; radiating striae that are often restricted to the posterior region; a very large cardinal tooth in the right valve; the lack of lateral teeth; a deep pallial sinus; and by a downward-projecting combination of the pallial line and the ventral sinus margin. This last feature is perhaps the most distinctive diagnostic feature for generic identification of both fossil and recent specimens.

Characteristics of the genus *Mesodesma* in the western North Atlantic have been reviewed elsewhere (Davis,

1964, 1965), but because that genus is also involved in this paper a few morphological characters of both fossil and recent forms will be mentioned. Members of the genus *Mesodesma* are much larger than those of *Ervilia* (see Fig. 2). The 2 species of *Mesodesma* found in the western North Atlantic, *M. deauratum* (Turton) 1822, and *M. arctatum* (Conrad) 1831, are commonly about 35 to 40 mm long and occasionally reach 50 mm in length. The shells are acutely truncate posteriorly (the most distinctive diagnostic feature) and quite thick and only moderately compressed. Serrated lateral teeth are present and the cardinal teeth are somewhat reduced, but a large chondrophore is present. Features of the hinge area are shown in Pl. 1, Figs. 1 & 2.

#### DISCUSSION

Holmes (1860) described a new fossil pelecypod taken from material at Simmons Bluff, Yonges Island, South Carolina, and named it *Mesodesma concentrica*. The original Holmes description follows:

"Small shell, very inequilateral, concentrically and finely ribbed. Anal margin compressed. Posterior extremity of shell prolonged, narrowed, wedge-shaped.

"This shell closely resembles *Mesodesma arctata* Gould; the anterior extremity is not truncated, but regularly rounded. The concentric striae are quite characteristic. Found in sand of sea beaches."

Two years later, Gould (1862) described a small bivalve which he named *Ervilia concentrica*. His description follows:

"*Ervilia concentrica*. T. minuta, oblongo-ovata, pellucida, nitida, (senioribus, incrassatis, margaritaccis) confertim sed profecto concentricè arata; umbonibus paullo postmedianis; extremitate antico acutiori quam extremitate postico. Long. 6+; alt. 4; lat. 3 milim.

"Dredged off the coast of North Carolina. Coast Survey.

"This little shell, which seems to be abundant along the whole southern coast, is quite different from anything before described."

Dall & Simpson (1902) later gave the following, more informative description under the same name:

"Shell small, scarcely inflated. Posterior end narrower. Surface finely concentrically ridged. Having delicate radial ribs most conspicuous on the anterior end.

"Hinge — right valve with single triangular tooth in front of the small triangular resilium and a feeble one behind it. Left valve with a double cardinal. Pallial sinus faint, deep. Color whitish or pink. Length 5, height 3.5, diam. 2 mm."

Later workers have frequently questioned the validity of these 2 species, and it is the purpose of this paper to examine this taxonomic problem and show that *Mesodesma concentrica* and *Ervilia concentrica* are, indeed, both synonyms and homonyms and that the species involved should be excluded from the genus *Mesodesma*.

As noted above, when Holmes described the species *Mesodesma concentrica* in 1860, he concluded his remarks by saying, "Found in sand of sea beaches". Yet his description is included as a part of his survey of the Post-Pliocene Mollusca of South Carolina. A search for the shells used in the original description led to a lot of specimens in the paleontological collections of the American Museum of Natural History, New York City. The information accompanying the material reads as follows:

"No. 11291 Type Am. Mus. nat. Hist. Holmes

*Mesodesma concentrica* Hol.

P. P. Foss. Post-Pliocene Fossils of S. C., p. 44, Pl. 6, Fig. 10.

Up. Miocene, Simmons' S. C."

Whitfield & Hovey (1901) indicated that they considered this lot to be the "type" of *Mesodesma concentrica*. When I first studied this material a single

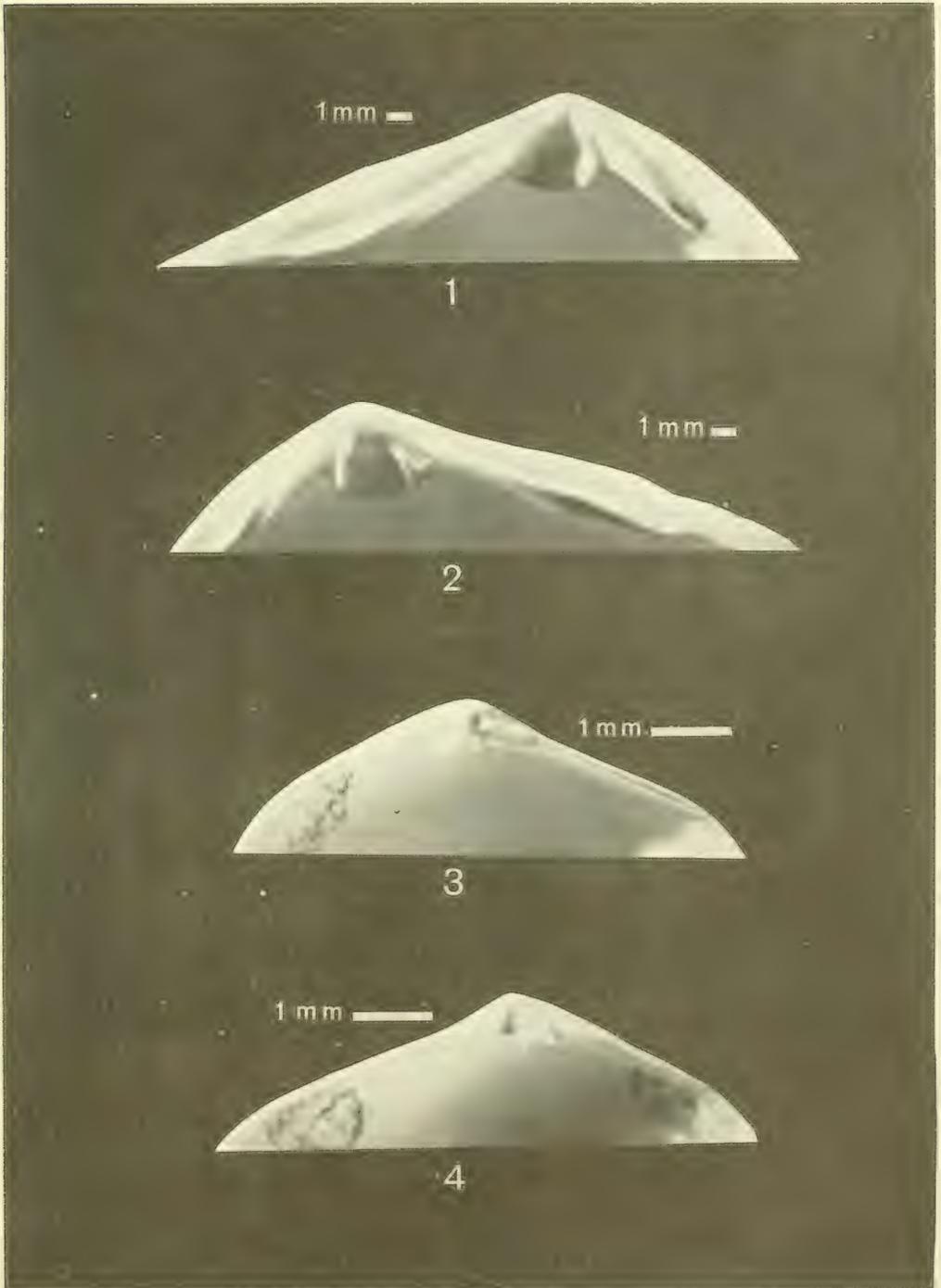


PLATE 1. Comparison of hinge structure in *Mesodesma arctatum* and *Ervilia concentrica*. Fig. 1. Right valve of *M. arctatum*. Fig. 2. Left valve of *M. arctatum*. Fig. 3. Right valve of *E. concentrica*. Fig. 4. Left valve of *E. concentrica*. Scale as indicated.

right valve was glued to a small diamond-shaped piece of green cardboard. Dr. Norman Newell, Curator of Fossil Invertebrates at the American Museum of Natural History, informed me that the cardboard was probably fastened to the valve by Whitfield and Hovey. Newell further informed me that Whitfield and Hovey placed a question mark after the listing of this material in the American Museum catalogue. It is Newell's opinion that this notation may indicate Whitfield and Hovey were not sure that this was the type material. It is my opinion, however, that they were probably questioning the validity of the genus and not the type. It is known that much, if not all, of the Holmes' material did eventually come to reside in the collections of the American Museum of Natural History. The presence of the word "type" and Holmes' name in the upper right corner of the label strongly suggests that either this is the original type material or it at least came from the describer's collection and represents a series of paratypes. In either case, it represents a starting point for discussion of the species, *M. concentrica*.

After careful study of the material I disagree with Holmes' contention that the species "... closely resembles *Mesodesma arctata* Gould". In fact, there is little to suggest any relationship between the 2 forms. Most obvious is the difference in size, readily apparent in Fig. 2. The valves in the Holmes lot of *M. concentrica* do not exceed 10 mm

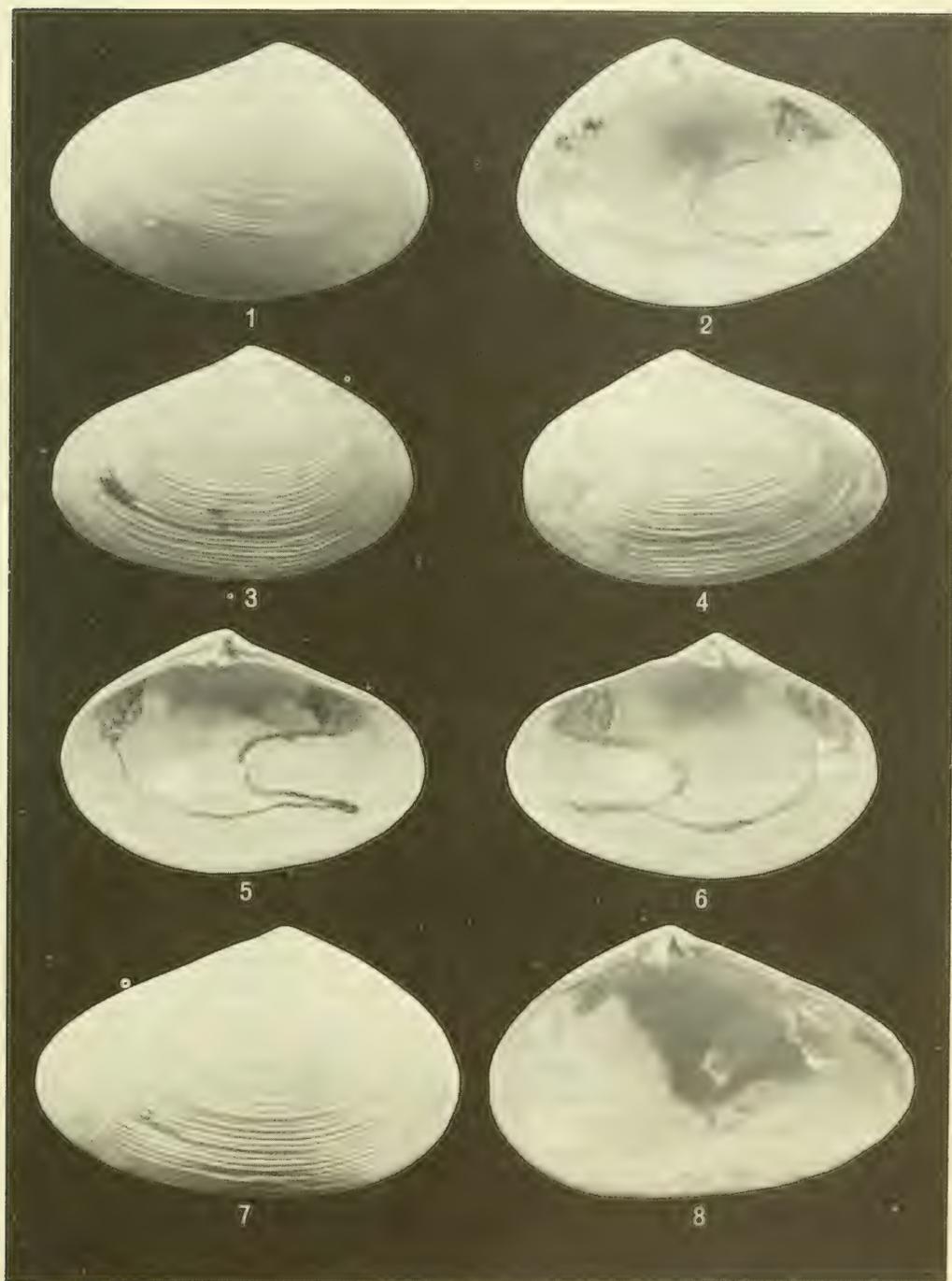
in length. On the other hand, the original type of *M. arctatum*, designated by Conrad — not Gould, has apparently been lost, but the lectotype designated by Davis (1964) has dimensions of length 25.2 mm, height 18.0 mm.

Comparison of other features confirms further this lack of similarity. Study of the hinge areas, as shown in Pl. 1, reveal little similarity between the 2 forms. The order and arrangement of teeth and pits are entirely dissimilar. In addition, shells of *Mesodesma arctatum* are much thicker and different in form. For example, *M. arctatum*, as indicated previously, has a truncate posterior margin. By contrast, it is the posterior margin of valves in the Holmes lot that is drawn out — the exact opposite of the situation in *M. arctatum*. Comparison of pallial lines and sinuses complete the picture; there appear to be relatively few similarities between these 2 bivalves. Therefore, the species so named *M. concentrica* by Holmes must be reconsidered as it cannot be accepted as a representative of the genus *Mesodesma*.

As indicated previously, Gould described *Ervilia concentrica* 2 years later. Although his description was not overly informative, the name persisted and the Dall & Simpson description (1902) strengthened the identity of the bivalve involved. Gould's type material was dredged up off the coast of North Carolina in the Coast Survey. According to Johnson (1964), after Gould's death the original Gould material was sold to the

PLATE 2. *Ervilia concentrica* (Holmes) 1860.

- FIGS. 1-2. Paratype selected as representative by Whitfield & Hovey (1901) from AMNH 11291 (AMNH 11291/1:3); length 7.2 mm, height 4.9 mm. Fig. 1, exterior — right valve. Fig. 2, interior — right valve.
- FIGS. 3-6. Lectotype designated from AMNH 11291 (AMNH 11291/1:1, AMNH 11291/1:2); length 6.6 mm, height 4.3 mm. Fig. 3, exterior — right valve. Fig. 4, exterior — left valve. Fig. 5, interior — right valve. Fig. 6, interior — left valve.
- FIGS. 7-8. Lectotype designated by Johnson (1964) from Gould Type Collection, MCZ 169092; length 6.4 mm, height 4.1 mm. Fig. 7, exterior — right valve. Fig. 8, interior — right valve.



New York State Museum. In 1959, that portion of the collection described as "The Gould Type Collection" was placed on permanent loan to the Museum of Comparative Zoology, Cambridge, Massachusetts. From this material Johnson designated the lectotype of *E. concentrica* as the lot numbered MCZ 169092. He also designated 3 paratypes, Museum of Comparative Zoology (MCZ) cat. no. 169093, and 1 paratype, U. S. National Museum (USNM) cat. no. 611263, all from the original lot.

Examination of the lectotype designated by Johnson reveals that *Ervilia concentrica* possesses all of the generic features discussed previously (the radiate striae are reduced but still visible). The most significant aspect is encountered when the Gould lectotype of *E. concentrica* is compared with the Holmes material from the American Museum of Natural History. I have done this with great care, and it was readily apparent that all specimens are identical. All features are the same — shape, dimensional proportions, hinge structure, pallial lines and pallial sinuses. These features can be compared in Plate 2.

Thus, without question, *Mesodesma concentrica* Holmes and *Ervilia concentrica* Gould are synonyms (and homonyms also — an unusual combination). Holmes discovered the species first but assigned it to the wrong genus. Gould encountered the shell 2 years later during marine dredging and assigned it to the correct genus, *Ervilia*, and at the same time gave it the same species name which Holmes had given to his so-called species of *Mesodesma*. Therefore, Holmes named the species but Gould put it in the correct genus.

On the basis of Holmes' earlier description and the assumption that the material in lot AMNH (American Museum of Natural History) cat. no. 11291 has direct lineage, at least, from the Holmes Collection, I have designated 2 matching right and left valves from this lot as the lectotype for *Ervilia concentrica*. The

additional valves (including the single right valve to which Whitfield & Hovey (1901) attached the green cardboard marker) have been designated paratypes. The valves of the lectotype have been cataloged under AMNH 11291/1: 1 (right valve) and AMNH 11291/1:2 (left valve). The single right valve designated by Whitfield and Hovey is identified as a paratype AMNH 11291/1:3. The remaining paratypes are cataloged under the original number. The lectotype was found to be the only pair of matching left and right valves in the lot and was selected to provide representation of the entire shell.

The paired matching valves are 6.6 mm long and 4.3 mm high at the umbo. The valves are opaque white with many concentric ridges of equal height. Radiating striae are absent. The valves are moderately compressed and unequilateral (therefore being unevenly oval in shape). The posterior end is fairly rostrate, placing the umbo anteriorly to the midpoint of the shell. The beaks are turned inward and somewhat posteriorly.

Internally, the hinge area of the right valve possesses a large cardinal tooth slanting obliquely downward and forward anteriorly to a fairly large chondrophore. There are no lateral teeth. The pallial sinus is deep, extending nearly beneath the umbo. Posteriorly, the pallial line bends up to join the ventral margin of the sinus. These 2 fused lines then curve downward and project out toward the ventral margin (see Pl. 2, Figs. 5 and 6).

The hinge area of the left valve is somewhat different. The dorsal valve margin produces a projection anterior to the pit accommodating the large cardinal tooth of the opposite valve. Posterior to this pit is the chondrophore with no intervening ridge. Further posterior another extension of the margin protrudes like a very small cardinal tooth. There are no lateral teeth, but the dorsal margin of the left valve has a laterally-projecting ridge

which is accommodated in a corresponding groove in the right valve.

A brief description of the single valve previously singled out by Whitfield & Hovey (1901) is provided for purposes of identification and comparison. It is a right valve and is 7.2 mm long and 4.9 mm high at the umbo. It is opaque buff-white with many concentric ridges all of about equal height. Radiating striae are not visible. Like the lectotype, the valve is moderately compressed and unequilateral with an uneven oval shape. Other features, external and internal, are essentially identical to the features described for the right valve of the lectotype. Some crystallized glue is attached to part of the exterior where the green marker was fastened by Whitfield and Hovey.

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## RESUMEN

*ERVILIA CONCENTRICA* Y *MESODESMA CONCENTRICA*  
CLARIFICACION DE SINONIMIA

J. D. Davis

Un pequeño pelecípodo, *Mesodesma concentrica* Holmes (1860), fué descrito sobre materiales fósiles de Simmons Bluff, Isla Yongues, Carolina del Sur, Estados Unidos. Otro molusco similar, *Ervilia concentrica* Gould, se describió en 1862, proveniente de rastreos en la costa de Carolina del Norte. La comparación de la estructura de las charnelas, marcas paleales, y características externas de los lectotipos, demuestra que esas formas son idénticas y sinónimas. También se demuestra que, comparando los caracteres que poseen los materiales tipo de Holmes, con ejemplares de *M. arctatum*, no hay base para incluir la especie sinonimizada en el genero *Mesodesma*. Así, un nuevo lectotipo designado se describe para *E. concentrica*, junto con la corrección de la cita taxonómica.

Sinonimia Nomenclatural

*Ervilia* (Turton) 1822, *Conchylia Dithyra Insularum Britannicarum: The Bivalve Shells of the British Islands.* p 55-56, pl. 19, fig. 4.

*Ervilia concentrica* (Holmes) Plate II, figs. 3-6.

*Mesodesma concentrica* Holmes, 1860, *Post-Pliocene Fossils of South Carolina.* p. 44, pl. 6, fig. 10. Simmons Bluff, Yonges Island, South Carolina.

*Ervilia concentrica* Gould, 1862, *Proc. Boston Soc. nat. Hist.*, 8: 281-282. [No figure.] Coast of North Carolina.

*Ervilia concentrica* Gould, 1862, *Otia Conchologica*, p. 239. [No figure.] Coast of North Carolina.

АБСТРАКТ

*ERVILIA CONCENTRICA* И *MESODESMA CONCENTRICA*  
(ПО ПОВОДУ ИХ СИНОНИМИИ)

ДЖ. Д. ДЭВИС

Мелкий двустворчатый моллюск *Mesodesma concentrica* Holmes (1860) был описан из отложений Симмонс Влафф, о. Йонгс, Южная Каролина, США. Сходная форма *Ervilia concentrica* Gould (1862) была описана из драгажных сборов у берегов Северной Каролины. Сравнение строения замка и синуса, а также наружных признаков лектотипа показывает, что эти 2 формы идентичны и являются синонимами. Кроме того, сравнение признаков экземпляров из типовой коллекции Холмса и экземпляров *M. arctatum* Conrad не дает оснований включать эти виды-синонимы в род *Mesodesma*. Таким образом, вновь установленный лектотип описан для *E. concentrica* и приводятся исправленные ссылки на систематические указания.

## Номенклатурная синонимия

*Ervilia* (Turton) 1822, Conchylia Dithyra Insularum Britannicarum: The bivalve Shells of the British Islands. p 55-66, pl. 19, fig. 4.

*Ervilia concentrica* (Holmes) Plate II, figs. 3-6.

*Mesodesma concentrica* Holmes 1860, Post-Pliocene Fossils of South Carolina. p 44, pl. 6, fig. 10. Simmons Bluff, Yorges Island, South Carolina.

*Ervilia concentrica* Gould 1862, Proc. Boston Soc. nat. Hist., 8: 281-282. [No figure.] Coast of North Carolina.

*Ervilia concentrica* Gould 1862, Otia Conchologica, p 239. [No figure.] Coast of North Carolina.



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АБСТРАКТ

Изучение неогеновых наземных моллюсков Предкавказья показало их большое разнообразие и широкое распространение в осадках среднего и верхнего миоцена и верхнего плиоцена. По своей зоогеографической структуре эти моллюски принадлежат к 4 разным группам: 1) группе тропических психро- и термофилов, вымерших на Кавказе, в Европе и северной Азии, 2) группе восточного Средиземноморья, 3) европейской группе и 4) группе видов, тождественных с современными северокавказскими. На протяжении неогена роль тропической группы угасала и увеличивался удельный вес средиземноморских и европейских видов, а общий облик фауны приближался к современному. Анализ распространения разных групп моллюсков позволяет в общих чертах восстановить историю изменения климатических условий Кавказа за неогеновое время.

Отечественная палеонтология располагает весьма скудными, можно сказать ничтожными, сведениями о фауне наземных моллюсков неогенового времени. В континентальных отложениях неогена, широко распространенных на территории Сибири и Средней Азии, остатки раковин этой группы моллюсков по-видимому встречаются сравнительно редко. Иначе обстоит дело в Крымско-Кавказской провинции, где ископаемые остатки наземных моллюсков встречаются в изобилии и имеют хорошую сохранность. Указания на их присутствие в Предкавказье и Закавказье, а также в Крыму, на Украине и в Молдавии можно найти в работах еще прошлого столетия. До сих пор, однако, эта интересная группа моллюсков не подвергалась у нас специальному изучению, а те редкие отрывочные описания, которые появились в нашей научной литературе за последние 70-80 лет (Эйхвальд, 1850; Синцов, 1875, 1877, 1897; Андрусов, 1902; Ализаде, 1936, 1954; Волкова, 1939, 1953; Коробков и Смирнов, 1959), включая и наиболее полную (с описанием 16 видов) работу В. В. Богачева по Куринской низменности (Богачев, 1935), не дают об этой фауне сколько-нибудь ясного представления. Естественно поэтому, что исследователям, трактующим вопросы истории малакофауны территории Советского Союза, приходится опираться лишь на данные палеонтологов Западной Европы, где ископаемые наземные моллюски собираются и изучаются уже более 100 лет.

Попыткой в какой-то степени восполнить этот пробел является проводимая мной в последние годы работа по исследованию остатков наземных моллюсков из континентальных неогеновых отложений Предкавказья (Стеклов, 1959, 1961, 1962а, б, 1964). Изучение ископаемых наземных брюхоногих

Предкавказья показало большое разнообразие их по систематическому составу. В ископаемом состоянии в неогене найдены представители почти всех семейств, составляющих ныне богатейшую малакофауну Кавказской провинции. Из известных на Кавказе 20 семейств в неогене не найдены лишь представители *Oleacinidae*, *Endodontidae*, *Bradybaenidae*, *Vitrinidae* и *Trigonochlamiidae*, то есть 5 семейств. Что касается двух последних, то можно предполагать их присутствие в собранной коллекции. Кроме того, в ней присутствуют виды *Strobilopsidae* и *Subulinidae* - семейств, вымерших ныне не только на Кавказе, но и во всей Европе.

Наибольшим распространением в неогене пользовались *Pomatiasidae*, *Ellobiidae*, *Valloniidae*, *Limacidae*, *Pupillidae*, *Enidae*, *Clausiliidae* и *Helicidae*. Первые 3 семейства представлены каждое 1 родом, как и в современной фауне. Остатки слизней, по понятным причинам не сохраняющиеся в ископаемом состоянии во всем разнообразии видов, пока систематически не обработаны. Зато *Pupillidae*, *Enidae*, *Clausiliidae* и *Helicidae* характеризуются значительным разнообразием, охватывающим в общей сложности до 30 родов и более 70 видов, что составляет примерно 60% всего видового состава фауны. Остатки представителей *Aciculidae*, *Succineidae*, *Cochlicopidae*, *Ferussaciidae*, *Zonitidae* и *Parmacellidae* встречаются редко.

Брюхоно среднего миоцена собраны в единственном местонахождении у станции Костромской в Майкопо-Пабинском районе. Встреченный здесь комплекс отличается большим своеобразием и насчитывает 21 вид, 12 родов, 7 семейств: *Cochlicopa* sp., *Gastrocopta (Albinula) cf. acuminata* Klein, *G. (Sinalbinula) fissidens* Sandberger, *G. (Sinalbinula) nouletiana* Dupuy, *G. (Sinalbinula) farcimen* Sandberger, *Vertigo (Vertigo) cf. ovatula* Sandberger, *V. (Vertilla) angulifera* O. Boettger, *Truncatellina* sp., *Pupilla triplicatoidea* Steklov, *P. signataeformis* Steklov, *Microstele wenzi* Fischer, *M. caucasica* Steklov, *M. buryaki* Steklov, *Pupilorcula karaganica* Steklov, *Vallonia sandbergeri* Deshayes, *V. subcyclophorella* Gottschick, *Chondrula (Mastus) forcarti* Steklov, *Caecilioides* sp., *Opeas minutum* Klein, *Zootecus insularis causicus* Steklov, *Caucasotachea kubanica* Steklov.

Остатки верхнемиоценовых улиток собраны во многих местонахождениях Дагестана, Чечено-Ингушетии, Северной Осетии, Кабардино-Балкарии, а также к востоку от Ставрополя, в Майкопо-Лабинском районе и у станции Верхне-Баканской в районе между Анапой и Крымском. Кроме того, интересные находки были сделаны в неотических отложениях на Керченском полуострове и в нижнем сармате южной Украины (в последнем местонахождении материал был собран доктором Л. С. Белокрысом). Среди очень разнообразных верхнемиоценовых улиток чаще встречаются и особенно характерны *Caspicyclo-terus praesieversi* Steklov, *Pomatias rivulare* Eichwald, *Carychium plicatum* Steklov, *Gastrocopta (Vertigopsis) magna* Steklov, *G. (Albinula) acuminata* Klein, *G. (Albinula) ukrainica* Steklov, *G. (Sinalbinula) nouletiana* Dupuy, *G. (Sinalbinula) fissidens* Sandberger, *Vertigo (Vertigo) ovatula* Sandberger, *V. (Vertigo) anti-vertigo callosa* Reuss, *Pupilla mutabilis* Steklov, *Microstele wenzi* Fisher, *Vallonia ex gr. lepida* Reuss, *V. subcyclophorella* Gottschick, *Strobilops (Strobilops) ukrainica* Steklov, *S. (Strobilops) costata* Clessin, *S. (Eostrobilops) caucasica* Steklov, *Zebrina gumsiana* Steklov, *Chondrula (Mastus) caucasica strigata* Steklov, *Retouskia matyokini* Steklov, *Euxinophaedusa volkovae* Likharev, *Serrulina nazranica* Likharev, *Quadruplicata farsica* Likharev et Steklov, *Hawaiiia antiqua* Riedel, *Nesovitrea petronella* L. Pfeiffer, *Monacha (?) externa* Steklov, *Caucasotachea andrussovi* Steklov, *C. fortangense* Steklov. Кроме названных, реже встречаются еще другие виды *Acicula*, *Carychium*, *Vertigo*, *Negulus*, *Truncatellina*, *Pupilla*, *Microstele*, *Chondrus*, *Jaminia*, *Chondrula (Mastus)*,

*Euxinophaedusa*, *Laeviphaedusa*, *Pontophaedusa*, *Oxychilus*, *Daudebardia*, "*Limax*," *Helicella*, *Tropidomphalus*, *Helicodonta*, *Caracollina*, *Helix*.

Плиоценовые (акчагылские и апшеронские) брюхоногие собраны также из многих местонахождений, главным образом Дагестана и районов Сунженского хребта, хотя в меньшем количестве они встречаются и западнее (к северу от Минеральных Вод, на Кубани ниже Армавира и в других местах). В плиоценовых отложениях особенно часто встречаются *Chondrula* (*Chondrula*) *microtraga psedachica* Steklov, *C.* (*Chondrula*) *microtraga sunzhica* Steklov, *C.* (*Chondrula*) *tchetchenica* Steklov, *Euxina* aff. *somchetica* L. Pfeiffer, *Helicella* *sunzhica* Steklov, *H. libidinosa* Steklov, *H. crenimargo* L. Pfeiffer, *Monacha* (?) *praeorientalis* Steklov, *Tropidomphalus psedachica* Steklov, а также, более редко - *Pomatias rivulare* Eichwald, *Gastrocopta* (*Albinula*) *zamankulense* Steklov, *G.* (*Sinalbinula*) *calumniosa* Steklov, *Vertigo* (*Vertigo*) *antivertigo antivertigo* Droparnaud, *V.* (*Vertilla*) *angustior* Jeffreys, *Truncatellina cylindrica* Ferussac, *T. dentata* Steklov, *Vallonia* aff. *pulchella* Muller, *Jaminia* (*Bollingeria*) *pupoides* Krynicki, *Retowskia schlaeflii pliocenica* Steklov, *Quadruplicata intermedia* Likharev, *Caucasotachea* (?) *maslovae* Steklov, *Helix* cf. *buchi* L. Pfeiffer и др.

Пытаясь обобщить имеющийся материал, можно наметить в составе неогеновой малакофауны Предкавказья несколько разнородных групп, различающихся в зависимости от зоогеографических связей и древности входящих в них видов.

1. Группа древних видов, вымерших в настоящее время не только на Кавказе, но и в Европе и северной Азии, ближайшие которым - преимущественно психро- и термофилы, обитатели тропических и субтропических лесов, - сохранились в юго-восточной, южной и изредка центральной частях Азии, в Америке, Африке, Австралии (*Gastrocopta*, *Microstele*, *Pupilla mutabilis*, *P. belokrysi*, *Negulus*, *Strobilops*, *Hawaiiia*, *Zootecus*, *Opeas*). К этой же группе приходится причислить и такие, не имеющие прямых аналогов в современной фауне формы, как *Euxinophaedusa*.

2. Группа видов, вымерших в настоящее время на Северном Кавказе, ближайшие которым распространены преимущественно в области восточного Средиземноморья (Турция, Иран, Закавказье, Греция, реже - Балканы и север Африки). Эта группа охватывает преимущественно обитателей засушливых и жарких областей (аридных субтропиков) - *Chondrula* (кроме *C. tchetchenica* и *C. caucasica strigata*), *Jaminia ledereri*, *Imparietula*, *Monacha*, - и в меньшей степени субтропических мезо- и психрофилов, таких как *Caspicyclotus*, *Retowskia*, *Pontophaedusa*, *Laeviphaedusa*, *Quadruplicata* и др.

3. Группа видов, ближайшие которым ныне распространены преимущественно в Европе, а также в большинстве (а некоторые почти исключительно) и на Кавказе (*Acicula*, *Zebrina*, *Parmacella*, *Helicella*, *Caucasotachea* и др., а также такие виды, как *Vertigo angulifera*, *Pupilla triplicatoidea*, *Chondrula tchetchenica*, *C. caucasica strigata*). С экологической точки зрения эта группа охватывает как виды умеренно-теплых степей (*Chondrula*, *Helicella*), так и обитателей смешанных лесов (*Vertigo*, *Caucasotachea*) и горных областей (*Pupilla triplicatoidea*, *Daudebardia*).

4. Группа видов, тождественных с обитающими и ныне на Северном Кавказе (*Pomatias rivulare*, *Vertigo antivertigo antivertigo*, *V. angustior*, *Truncatellina cylindrica*, *Jaminia pupoides*, *Euxina somchetica*, *Nesovitrea petronella*, *Helicella crenimargo* и др.) и таких, ближайшие которым пользуются широким распространением в Палеарктике, или Голарктике (*Carychium suevicum*, *Pupilla submuscorum*, *Vallonia subcyclophorella*, *V. aff. pulchella* и др.).

Хотя намеченное подразделение является условным и даже в какой-то степени искусственным, так как оно, не отражая в полной мере зоогеографической природы ассоциаций моллюсков разных моментов геологического времени, существенным своим критерием имеет степень близости форм - обитающим ныне на Северном Кавказе, - оно тем не менее дает возможность отчетливо продемонстрировать процесс перестройки кавказской малакофауны на протяжении неогена.

Группа широко распространенных и тождественных северокавказским видов не имеет представителей в среднемиоценовой малакофауне. Начиная же с верхнего миоцена и особенно в плиоцене она представлена уже целым рядом видов. В верхнем плиоцене вполне отчетлива близость ископаемых форм рецентным вплоть до полной идентификации некоторых видов (*Vertigo antivertigo*, *V. pusilla*, *Truncatellina cylindrica*, *Jamnia pupoides*, *Zebrina hohenackeri*, *Euxina tschetschenica*, *Helicella crenimargo* и др.).

Группа видов восточного Средиземноморья представлена в среднем миоцене двумя видами - *Pupilla signataeformis* и *Chondrula forcarti*, а в верхнем миоцене - семью: *Caspicyclotus praesieversi*, *Jamnia ledereri*, *Retowskia matyokini*, *Pontophaedusa praefiniculum*, *Laeviphaedusa miocaenica*, *Serrulina sieversi*, *Pagodulina*. Из верхнеплиоценовых видов к этой группе принадлежат *Retowskia schlaesfli pliocenica*, *Chondrula likharevi*, *C. ex gr. microtraga*, *Imparietula*, *Zebrina hohenackeri*.

Группа древних тропических видов составляет ядро среднемиоценовой малакофауны Предкавказья (*Microstele wenzii*, *M. buryaki*, *M. caucasica*, *Gastrocopta fissidens*, *G. nouletiana*, *G. farcimen*, *Opeas minutum*, *Zootecus insularis causicus*) и широко представлена в верхнем миоцене (*Gastrocopta magna*, *G. acuminata*, *G. ukrainica*, *Pupilla mutabilis*, *P. belokrysi*, *Microstele*, *Strobilops caucasica*, *S. costata*, *S. ukrainica*, *Euxinophaedusa volkovae*, *E. steklovi*, *Negulus*, *Hawaiiia antiqua* и др.) В плиоцене роль этой группы сходит на нет. К ней можно отнести всего лишь два вида *Gastrocopta*.

Группу древних тропических видов составляют в основном формы, относящиеся по систематическому положению к родам, полностью вымершим в Европе и северной Азии, или имеющим в фауне этих областей единичных представителей обычно с разорванным ареалом распространения, подчеркивающим их реликтовый характер. В меньшей степени сюда входят вымершие виды родов, пользующихся более широким распространением. Так, род *Microstele*, в настоящее время известен на Цейлоне, в Индии и восточной Африке, *Negulus*, - только в Африке, *Opeas* - в тропической зоне Азии, Африки и Америки, *Zootecus* - в Индии, Северной Африке и на островах Зеленого Мыса. Семейство *Strobilopsidae*, богато представленное в позднем палеогене и неогене Европы, а также в миоцене Предкавказья и Южной Украины, в настоящее время распространено в юго-восточной Азии и обеих Америках. Гастрокопты из подродов *Albinula* и *Vertigopsis* сохранились только в Америке.

Сравнивая относительный объем и структуру каждой из выделенных групп в разные моменты неогенового времени, мы отчетливо видим, как на протяжении неогена с одной стороны угасала роль экзотических тропических элементов в кавказской малакофауне и возрастала роль группы форм, связанных со Средиземноморьем, и как, с другой стороны, общий облик фауны приближался к современному. Если в среднемиоценовой фауне тропические виды составляли более 60% всего ее состава, а в верхнемиоценовой - половину, то в верхнем плиоцене удельный вес этой группы не превышает

5-6%. Наоборот, удельный вес средиземноморских видов (в целом) возрастает с 33% в среднем миоцене до 60% в верхнем плиоцене.

Все средиземноморские виды среднего миоцена входят в группу, связанную с восточным Средиземноморьем. В верхнем миоцене влияние последней группы уменьшается, и соответствующие виды составляют около половины всех средиземноморских, в верхнем же плиоцене - еще меньше.

Факт распространения в миоцене на большой площади Европы и Кавказа полностью вымерших ныне на этой территории (или сохранившихся в виде единичных и редких реликтов) родов и даже семейств, представленных близкими и тождественными видами, позволяет сделать вывод об общности неогеновой малакофауны всей этой области. Можно допускать, что экзотическая группа в миоценовой фауне Кавказа является дериватом некогда единой тропического типа фауны, распространенной в пределах палеогеновой, или еще более древней суши на месте Евразии. Последние ее потомки выжили и сохранились главным образом в юго-восточной области Азиатского материка, где, очевидно, удержались наиболее благоприятные для этого ландшафтные, в первую очередь климатические условия. Эти соображения согласуются с представлением о мало дифференцированном тропическом климате Евразии в начале миоцена. Субтропическими чертами, вероятно, отличался климат Европы и Кавказа и в течение всего миоцена. В это время уже происходил процесс вытеснения тропических элементов фауны группой нового, средиземноморского типа. С первыми средиземноморскими элементами мы сталкиваемся в среднем миоцене. При этом обращает на себя внимание, что среднемиоценовые представители группы ближе всего стоят к рецентным видам, обитающим в области жаркого и засушливого климата в основном в странах Ближнего Востока и Закавказья. Так, *Pupilla signataeformis* близка *P. signata* Mouss., обитающей в Закавказье, Средней Азии и Иране (а также - на севере Китая). Современные родичи *Chondrula (Mastus) forcarti* обитают в Греции и Турции. Тем самым, находит подтверждение идея некоторых исследователей о возникновении очагов ксеротермизации в центре Евразии еще в глубокой древности (Давиташвили, 1956).

В верхнем миоцене на Северном Кавказе еще обитает ряд форм, близких видам, сохранившимся ныне только в Закавказье: *Caspicyclotus praesieversi*, *Retowskia matyokini*, *Jaminia ledereri*, *Pontophaedusa praefuniculum*, *Serrulina sieversi*. Верхнемиоценовый климат Северного Кавказа был по-видимому, влажным, субтропическим. Об этом свидетельствует не только разнообразие и относительное богатство азиатской группы в составе фауны верхнего миоцена, не только присутствие форм, близких закавказским видам, но и расцвет мезо- и психрофильных групп средиземноморского типа - обилие *Pomatias*, крупных уплощенных гелицид, *Clausiliidae*, и др. Попутно можно отметить интересный факт бедности кавказской верхнесарматской и меотической фауны настоящими *Helix*, тогда как в Крыму и на южной Украине *Helix* является преобладающим элементом фауны этого времени. В этом факте можно видеть свидетельство достаточно отчетливой климатической дифференциации, возникшей к концу миоцена. Территория северного побережья Сарматского моря имела по-видимому климат гораздо более умеренный, чем кавказский остров, а в дальнейшем полуостров. Последнее обстоятельство обычно не учитывается палеонтологами, изучающими морскую сарматскую фауну, а его следовало бы принимать в расчет. Кроме того намечается дифференциация ландшафтной обстановки в области самого Предкавказья, достаточно отчетливо

проявляющаяся с конца сарматского времени и особенно в меотическом веке. Видимо, уже в конце миоцена заложены те черты различия климатических условий двух противоположных оконечностей Кавказского полуострова, которые наиболее ярко проявили себя в конце понтической эпохи (время образования керченских железных руд и первой половины балаханской серии Азербайджана). Верхнесарматские и меотические ассоциации западной части Предкавказья содержат в преобладающем количестве виды психрофильных групп. Среди верхнесарматских моллюсков района р. Аргудан (между Нальчиком и Орджоникидзе) уже появляется значительная примесь таких мезофильных лесных элементов, как *Pomatias* и *Caucasotachea*, и вместе отмечается относительное сокращение роли видов субтропической группировки (*Gastrocopta*, *Strobilops*, и др.). Меотический же комплекс р. Фортанги (к востоку от Орджоникидзе) характеризуется резким преобладанием мезофилов и даже существенной примесью ксерофильных элементов (*Mastus*, *Zebrina*, *Jaminia*, *Monacha*, *Helicella*), играющих заметную роль и в других местонахождениях восточного Предкавказья.

Таким образом, прогрессирующая климатическая дифференциация и возникновение очагов ксеротермизации привели в миоцене к распаду единой тропической фауны палеогена, к вымиранию ее в Европе и на Кавказе и выработке новой фауны средиземноморского типа, окончательно сформировавшейся на рубеже миоцена и плиоцена.

Верхнеплиоценовая фауна особенно резко отличается по своей структуре от более древних, и приближается по типу к современной кавказской, сохраняя при этом и отчетливые черты своеобразия - присутствие реликтов древней тропической группы и форм, очень близких рецентным видам Закавказья. Ядро верхнеплиоценовой фауны составляют виды средиземноморской группы, часть которых конхиологически тождественны рецентным. Большинство этих видов, однако, встречается довольно редко. Чаще же и в изобилии встречаются остатки немногих видов *Chondrula s. str.* и *Helicella*. Это обстоятельство само по себе свидетельствует о широком развитии в верхнем плиоцене в Предкавказье степных ландшафтов. Анализ морфологических адаптаций раковины акчагыльских и апшеронских *Chondrula* приводит к выводу о высокой ксеротермности предкавказского климата акчагыла, в значительной степени затем сглаживающейся с началом второй половины верхнеплиоценового времени.

В заключение этого краткого обзора можно сказать, что наши знания о неогеновых наземных моллюсках юга СССР еще очень ограничены. Изложенное показывает, между тем, что изучение этой группы фауны может дать интересный материал и для биостратиграфии континентальных отложений, и для палеоклиматологии, и для истории развития фауны.

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## ABSTRACT

DEVELOPMENT STAGES OF NEOGENE TERRESTRIAL  
MOLLUSKS OF CISCAUCASIA

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A study of the Neogene terrestrial Mollusca of Ciscaucasia has revealed the variability and wide distribution of these mollusks in sediments of Middle and Upper Miocene and Upper Pliocene age. These mollusks belong to 4 different groups in regard to their zoogeographical structure: 1) a group of tropical psychro- and thermophylls now extinct in the Caucasus, Europe and North Asia, 2) a group of the eastern Mediterranean, 3) a European group, and 4) a group of species identical to the recent North-Caucasian ones. Throughout the Neogene period the role of the tropical group decreased and the relative importance of the Mediterranean and European species increased, approaching the composition of the recent fauna. An analysis of the distribution of various mollusk groups enables us to reproduce in general the history of the climatic changes of the Caucasus during the Neogene time.

## RESUMEN

PERIODOS DE DESARROLLO DE LOS MOLUSCOS NEOGENICOS  
DE CISCAUCASIA

A. A. Steklov

El estudio de los moluscos del epígrafe reveló su amplia distribución y variabilidad en sedimentos del Mioceno Medio-Superior y Plioceno Superior. Estos moluscos pertenecen a 4 grupos diferenciados en su estructura zoogeográfica: 1) psicro- y termo-filo tropical al presente extintos en el Cáucaso, Europa y norte de Asia. 2) grupo del Mediterráneo oriental. 3) grupo europeo, y 4) un grupo de especies idénticas a las del Reciente Nor-Caucásico. A través del período Neógeno, el rol del grupo tropical decreció, aumentando la importancia relativa de los mediterráneos y europeos, acercándose así a la composición actual. Un análisis de la distribución de los diferentes grupos nos capacita para reconstruir, en general, la historia de los cambios climáticos del Cáucaso durante el Neógeno.



PLANORBULA CAMPESTRIS (GASTROPODA: PLANORBIDAE)  
FROM THE CUDAHY FAUNA (KANSAN) OF MEADE COUNTY, KANSAS,  
WITH NOTES ON THE STATUS OF  
THE SUBGENERIC CATEGORIES OF PLANORBULA

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ABSTRACT

Over 200 fossil shells of *Planorbula campestris* (Dawson) have been collected from 2 localities of the Cudahy fauna (Kansan), Meade County, Kansas. Study of shell sculpture and internal lamellae in these materials and in all available Recent lots of *P. campestris* and *P. armigera* in collections of the U. S. National Museum and Museum of Zoology, University of Michigan, revealed several characteristics which have not been previously reported.

These include: (1) the presence of multiple sets of denticles in both *Planorbula campestris* (as many as 4) and *P. armigera* (as many as 2); (2) a maximum number of denticles per set, which consistently never exceeded 5 in *P. campestris* and 6 in *P. armigera*; and (3) 2 distinctive types of sculpture, consisting in *P. campestris* of strongly developed, continuous, incised spiral striae which interrupt the slightly raised, evenly spaced, incremental growth lines. In *P. armigera* surface ornamentation consists of less regularly spaced growth lines which are crossed by unevenly spaced, weakly developed, and discontinuous spiral striae and fine lirae.

The shell characteristics here recognized to separate these 2 species, placed by Baker (1945) in the subgenus *Planorbula* s. s., are at least as important as the shape of the lower palatal lamella used by him to distinguish the 2 subgeneric categories of *Planorbula*, *Planorbula* s. s. and *Haldemanina*. It is suggested that Baker's subgeneric categories of *Planorbula* are probably not valid.

INTRODUCTION

During the summer of 1958, field parties from the University of Michigan Museum of Paleontology made collections from 2 localities of the Cudahy fauna in Meade County, Kansas. A total of 41 species of mollusks (Table 1) were recovered from the matrix collected from the Cudahy Ash Mine, SE 1/4 sec. 2, T. 31 S., R 28 W<sup>2</sup> (Meade Co., K. U. Loc. 10), and Sunbrite Ash

Mine, NE 1/4 sec. 26, T. 32 S., R. 28 W<sup>2</sup> (Meade Co., K. U. Loc. 17), Meade County, Kansas. Included among these mollusks were over 200 shells of *Planorbula campestris*.

The present report is based on a study of this fossil material, and of Recent lots of *Planorbula campestris* and *P. armigera* examined in the collections of the United States National Museum (USNM) and the University of Michigan Museum of Zoology (UMMZ). Its pur-

<sup>1</sup>Contribution Number 5, Department of Geology, Kent State University.

<sup>2</sup>Interpretation of the U. S. Geological Survey Topographical maps here quoted is explained in *Malacologia*, 1966, 4(1): 27-28; the position of Meade County is shown. *Ibid*: 193.

TABLE 1. Mollusks collected during the summer of 1958 from the Sunbrite and Cudahy localities of the Cudahy fauna, Meade County, Kansas

Species	Sunbrite		Cudahy	
	UMMZ Cat. No.	No. of specimens*	UMMZ Cat. No.	No. of specimens*
<i>Pisidium casertanum</i>	216747	(7/2)	-	
<i>P. obtusale</i>	218322	(8/2)	-	
<i>Carychium exiguum</i>	216753	(121)	216705	(220)
<i>Stagnicola caperata</i>	216751	(14)	216727	(7)
<i>S. exilis</i>	218320	(55)	-	
<i>Fossaria dalli</i>	216752	(16)	216722	(38)
<i>Omalodiscus pattersoni</i>	216731	(79)	216725	(9)
<i>Cyraulus circumstriatus</i>	216755	(34)	216702	(21)
<i>G. deflectus</i>	216754	(13)	216700	(175)
<i>G. parvus</i>	216756	(54)	216703	(64)
<i>Helisoma trivolvis</i>	216732	(105)	-	
<i>Planorbula campestris</i>	216730	(200)	216723	(3)
<i>P. cf. P. armigera</i>	-		216724	(3)
<i>Promenetus exacuus kansasensis</i>	218321	(170)	-	
<i>P. umbilicatellus</i>	216745	(5)	216701	(16)
<i>Ferrissia parallela</i>	218324	(25)	-	
<i>Physa skimmeri</i>	216737	(250)	-	
<i>Physa</i> sp. (immature)	216760	(9)	-	
<i>Aplexa hypnorum</i>	216757	(12)	216721	(36)
<i>Cionella lubrica</i>	-		216718	(400)
<i>Strobilops labyrinthica</i>	216735	(28)	216729	(350)
<i>Gastrocopta armifera</i>	216738	(25)	-	
<i>G. holzingeri</i>	216759	(1)	216711	(9)
<i>G. tappaniana</i>	216739	(23)	216726	(70)
<i>Pupoides albilabris</i>	216742	(4)	-	
<i>Pupilla muscorum</i>	216741	(39)	216720	(75)
<i>Vertigo elatior</i>	218325	(7)	216713	(60)
<i>V. milium</i>	216750	(53)	216712	(100)
<i>V. ovata</i>	216749	(45)	216716	(16)
<i>Vallonia cyclophorella</i>	216746	(29)	-	
<i>V. gracilicosta</i>	216743	(28)	216709	(175)
<i>V. pulchella</i>	216744	(6)	216710	(30)
<i>Oxyloma</i> sp.	218323	(14)	216728	(9)
<i>Discus cronkhitei</i>	216734	(24)	216715	(30)
<i>Deroceras aenigma</i>	216740	(525)	216719	(350)
<i>Euconulus fulvus</i>	216736	(20)	216714	(85)
<i>Punctum minutissimum</i>	-		216706	(45)
<i>Nesovitrea electrina</i>	216733	(25)	216708	(45)
<i>Hawaiiia minuscula</i>	216758	(20)	216704	(33)
<i>Zonitoides arboreus</i>	216748	(9)	216707	(23)
<i>Stenotrema leai</i>	-		216717	(150)

\* Numbers in excess of 100 have been estimated volumetrically.

pose is to: (1) record the first unequivocal fossil occurrences of *P. campestris* and to list the mollusks with which they were found associated; (2) present the results of observations made on the shell characters of *P. campestris*; and (3) bring together information on the ecology and geographic distribution of *P. campestris*.

#### METHODS AND MATERIALS

All of the Recent lots of *P. campestris* examined in the U. S. National Museum collections (20 lots, 68 specimens) were studied through transmitted light to determine the number of lamellar sets present in each shell. A technique suggested by Walter (1962) was followed to make the shells more translucent. The shells were soaked several minutes in a full-strength solution of sodium hypochlorite which usually removed sufficient amounts of dirt, organic material and periostracum to permit viewing internal shell structures through the outer wall. Seventeen lots (73 specimens) of *Planorbula armigera* from the U. S. National Museum collections were similarly examined. Two individuals, one in lot USNM 8970 and USNM 511386, had 2 lamellar sets.

Twenty-nine fossil shells of *Planorbula campestris* from the Sunbrite Ash Mine locality (UMMZ 216730) were selected for study of their internal lamellae. These shells were permitted to stand over-night in a commercially prepared oil (refractive index 1.60) used in the determination of the index of refraction in minerals and sold under the trade name "Shillaber's Certified Index of Refraction Liquids". All but 2 shells were made sufficiently translucent by this treatment to permit viewing of the internal lamellae without destroying the shell.

During examination each shell was viewed alternately from the right and left sides<sup>3</sup>, with the light passing

through the shell at right angles to the plane of coiling. In these 2 positions the location of the large transverse basal and smaller upper palatal lamellae could be readily established when these lamellae occurred several whorls back from the aperture. Shells in which more than one set of lamellae were visible within the last whorl contained a parietal lamella, as well as a basal, lower and upper palatal lamella within each set. In the earlier whorls it was not possible to see all the lamellae in a set.

In these instances it was assumed that when an upper and basal palatal lamellae were observed in the same region of the shell, a complete set of lamellae was probably present at this position. The results of these observations are summarized in Tables 2 and 3.

#### SYSTEMATIC DISCUSSION

Class Gastropoda  
Subclass Euthyneura  
Order Basommatophora  
Superfamily Ancyloidea  
Family Planorbidae

Genus *Planorbula* Haldeman, 1840  
*Planorbula campestris* (Dawson) 1875  
Plate I, Figs. 1-4, 6-8

*Segmentina armigera* var. *campestris*  
Dawson, 1875, p 349-350.

*Segmentina (Planorbula) christyi* Dall,  
1905, p 99, Pl. II, Figs. 10, 11.

*Planorbula (Planorbula) campestris*  
(Dawson), Baker, 1945, p 176, pl. 118,  
Fig. 8; Pl. 119, Figs. 13-15.

#### Original Description

"*Segmentina armigera* var. *cam-*

<sup>3</sup>Right and left side refer to orientation of the shell with respect to the living animal, wherein the outer lip of the aperture is positioned anteriorly.

TABLE 2. Measurements of 68 Recent *Planorbula campestris* in the United States National Museum collections. The development of lamellae in the set situated nearest the aperture is indicated by: A=absent; P=present; W=weakly developed. The number of sets of lamellae in excess of 1 are indicated by the number in parenthesis following the catalog number.

USNM catalog number	Apertural lamellae					No. of whorls	Height mm	Diameter mm
	Parietal tubercle	Parietal	Palatal					
			Basal	Lower	Upper			
63393 <sup>1</sup>	A	A	A	A	A	6-3/8	3.2	8.9
" 2	A	A	A	A	A	6-1/4	3.2	8.6
"	P	P	P	P	P	6-1/2	3.1	8.5
" (2)	P	P	P	P	P	6-1/3	2.9	8.0
"	P	P	P	P	P	5-1/2 <sup>3</sup>	2.8	6.7
"	P	P	P	P	P	6 <sup>3</sup>	3.0	8.5
474763	A	A	A	A	A	7 <sup>3</sup>	4.0	10.9
"	A	A	A	A	A	6-3/4	3.9	11.7
"	A	A	A	A	A	5-3/4	3.3	8.1
"	A	A	A	A	A	6-1/2 <sup>3</sup>	3.4	9.0
"	A	A	A	A	A	6-5/8	3.7	9.7
"	A	A	A	A	A	6-1/2	3.7	10.2
"	A	A	A	A	A	5-3/4 <sup>3</sup>	2.3	5.5
"	A	A	A	A	A	5-7/8	3.0	8.6
"	A	A	A	A	A	6-1/2	3.4	8.9
"	A	A	A	A	A	5-1/2	3.0	8.3
"	A	A	A	A	A	6-5/8	3.1	8.5
180300	A	W	A	A	A	6	3.2	8.3
382128	A	A	A	A	A	5-3/4 <sup>3</sup>	3.5	8.7
"	A	A	A	A	A	5-3/4	3.2	7.7
"	A	A	A	A	A	6-1/4	3.3	8.3
"	A	A	A	A	A	6-1/4	3.8	9.2
"	A	A	A	A	A	6-1/4	3.3	8.4
"	A	W	A	A	A	6	3.0	7.9
382129	A	A	A	A	A	5-7/8	2.9	7.9
"	A	A	A	A	A	6	3.0	7.7
"	A	A	A	A	A	6	3.1	8.2
"	A	A	A	A	A	4-3/4 <sup>3</sup>	2.3	4.6
"	A	A	A	A	A	6 <sup>3</sup>	3.2	7.3
252447	A	A	A	A	A	6-1/8 <sup>3</sup>	3.0	8.2
"	A	A	A	A	A	6-3/8	3.3	9.6
519925	A	P	A	A	A	5-3/4 <sup>3</sup>	2.8	7.7
471342	A	A	A	A	A	6-1/2	3.1	7.8
470842	A	A	A	W	W	6-3/8	3.1	9.1
"	A	A	A	A	A	6-1/4	3.4	9.0
471327	A	A	A	A	A	7-1/2	4.2	12.1
471253	A	A	A	A	A	6-3/8	3.4	9.6
"	A	A	A	A	A	6-1/4	3.0	9.0
" (4)	P	P	P	P	P	5-1/4	2.0	5.6
" (3)	P	P	P	P	P	5-7/8	2.4	6.1
471251	A	A	A	A	A	6-1/2	3.5	9.4
471317	A	A	A	A	A	5-3/4 <sup>3</sup>	2.9	7.0

Table 2 (continued)

USNM catalog number	Apertural lamellae					No. of whorls	Height mm	Diameter mm
	Parietal tubercle	Parietal	Palatal					
			Basal	Lower	Upper			
470483	A	A	A	A	A	6-1/2	3.6	9.8
601439	A	A	A	A	A	6-1/4	3.0	8.8
"	A	A	A	A	A	6-1/4	3.5	9.3
" (2)	A	P	P	P	P	6-3/8	3.6	9.6
601437	A	A	A	A	A	6	2.9	8.2
"	A	A	A	A	A	4-1/2 <sup>3</sup>	1.8	3.2
471252	A	A	A	A	A	7-3/8	5.2 <sup>7</sup>	12.0
"	A	A	A	A	A	5-1/2 <sup>3</sup>	2.5	6.4
"	A	P	P	P	W	6	3.3	8.2
"	A	A	A	A	A	5-7/8	3.7	9.8
" 4	A	P	P	P	P	6-1/4	3.1	9.7
" 5	A	P	P	P	P	6-1/8	3.2	9.4
"	A	W	A	A	A	5-1/2	3.2	7.1
"	A	P	W	P	P	5-5/8 <sup>3</sup>	2.5	6.7
"	A	W	A	W	W	5-3/4	3.0	7.2
"	A	A	A	A	A	5-5/8	2.5	5.2
" (2) <sup>6</sup>	A	A	P	P	P	5-3/4	2.9	6.7
"	A	A	A	A	A	5-3/4	2.6	6.5
601438	A	A	A	A	A	6-1/4	3.7	10.0
"	A	P	P	P	P	6-7/8	2.5	6.1
" (2)	A	P	P	P	P	5-1/2	2.3	6.7
"	A	P	P	P	P	5-3/4	2.2	6.2
"	A	P	P	P	P	6-1/8 <sup>3</sup>	2.4	7.2
"	A	P	P	P	P	6-7/8	2.4	6.3
" (2)	A	P	P	P	P	5-1/4	2.2	5.4
"	A	P	P	P	P	5-3/4	2.4	6.4

1 Holotype of *Planorbula christyi*

2 Paratypes of *P. christyi*

3 Shell broken

4 Lamellae located 7/8 whorl behind aperture

5 Lamellae located 1-1/8 whorls behind aperture

6 Second set of lamellae weakly developed

7 Aperture deflected downward

*pestris*, Pointe du Chêne. Dufferin. Traders' Road. 500 mile Lake. This is a large fine variety characteristic of the prairie region, which I have distinguished by the above varietal name. The normal form, with the usual number of whorls (4) is abundant in the Lake of the Woods, and surrounding wooded region. Specimens seldom at all exceed 6.5 mm. The variety *campestris* occurs abundantly in some pools and coulees of the Red River Valley, and prairie region westward. They are

much larger, with more whorls, and only in young specimens show the teeth. Colour generally wax yellow or pale brown. Diameter of largest specimens from 10.5 mm to 12.5 mm, whorls often six, specimens to 7.5 mm often, but not invariably show teeth; above this size no teeth were recognized" (Dawson, 1875: 349-350).

Emended Description

Shell medium, ultradextral. Whorls rounded, about 6 1/2, slowly increasing

## PLATE I

- FIGS. 1-3. *Planorbula campestris* (Dawson) right (=umbilical), left (=spire-pit) and apertural views. Cudahy fauna (Kansan), Meade County, UMMZ 216730a. X 10.
- FIG. 4. *Planorbula campestris* (Dawson), view showing apertural lamellae. Same locality. UMMZ 216730b. X 10.
- FIG. 5. *Planorbula armigera* (Say), view of portion of right side of shell showing details of sculpture. Recent, Hudson, Summit County, Ohio. X 40.
- FIG. 6. *Planorbula campestris* (Dawson), enlarged view of right side of shell showing details of sculpture. Cudahy fauna (Kansan), Meade County, Kansas. UMMZ 216730c. X 40.
- FIGS. 7-8. *Planorbula campestris* (Dawson), peripheral and left (spire-pit) view of shell showing distribution of lamellae. Two transverse basal palatal lamellae can be seen as white bars in left view. Recent, Belleville, Ontario. UMMZ 185968a. X 8.



TABLE 3. Measurements of 29 fossil shells of *Planorbula campestris* (UMMZ 216730) from the Sunbrite Ash Mine locality

No. of whorls	Height mm	Diameter mm	No. of lamellar sets
4-1/8	1.7	3.5	3*
4	1.5	4.5	1 <sup>1</sup>
5-1/8	2.5	6.7	1*
5	7.25	5.5	1*
4-1/2	2.0	4.7	none*
4-1/4	2.5	5.5	2*
5-1/2	2.7	6.75	none*
4-3/4	2.0	5.0	1* <sup>1</sup>
4	2.5	5.5	2*
5-1/2	2.5	6.5	none*
3-1/2	1.75	3.25	none*
5-1/8	2.3	5.8	none*
5-1/2	2.25	5.8	none*
3-1/2	1.25	2.75	none*
4	1.6	4.0	?* <sup>2</sup>
5	1.75	4.1	2
4-1/4	2.1	4.6	none*
4-7/8	2.75	7.0	none*
5-5/8	2.8	7.5	none*
5	2.5	6.5	none*
4-1/4	1.1	3.25	2*
4-1/4	1.5	3.75	1*
4-1/4	1.0	3.5	none*
4-3/4	1.0	4.0	4 <sup>3</sup>
4-1/4	1.75	3.75	none*
5-1/8	1.5	4.5	2* <sup>4</sup>
4-1/2	1.6	4.0	1 <sup>1</sup>
4-1/2	1.25	3.0	2 <sup>5</sup>
6	3.0	8.5	? <sup>2</sup>

\* Shell broken

<sup>1</sup> One set in last whorl

<sup>2</sup> Shell too opaque to permit examination by transmitted light.

<sup>3</sup> Three sets in last whorl

<sup>4</sup> First set 1/4 whorl behind aperture; 2nd set 1-1/4 whorls behind aperture

<sup>5</sup> Two set in last whorl.

in diameter, coiled in same plane, overlapping, the last whorl not deflected to left near aperture; right umbilical

side flat to slightly depressed below the general plane; periphery rounded; left side rounded to subcarinate around edge of spire-pit. Spire-pit rounded, shallow, about 1/2 of total shell diameter, exhibiting all of the earlier whorls. Sutures weakly impressed. Apertural lip thickened within. Sculpture consisting of fine, close, evenly spaced, incremental growth lines which are incised by spiral striae. Apertural denticles, when all are present, are 5 in number and usually 1/5 of a whorl back from aperture. There are 2 parietal denticles: a large sigmoidal parietal lamella, ascending at about an angle of 70° to axis of shell; below the parietal there may be a small tubercle. There are never more than 3 palatal lamellae: a large slightly transverse basal palatal; a large lower palatal on the periphery of the outer wall, which descends at an angle of about 25° from the plane of coiling; and a short, thick upper palatal lamella which descends from the plane of coiling at an angle of about 45°. As many as 4 sets of lamellae may be present.

#### Measurements

See Tables 2 and 3.

#### Geologic Range

Taylor (1966) has referred some poorly preserved specimens of *Planorbula*, collected from a number of Pliocene and Pleistocene age deposits in Wyoming and Idaho, to *P. campestris*. The oldest of these occurrences is from the Middle Pliocene Teewinot Formation. The oldest unequivocal fossil occurrences of this species are from the Cudahy and Sunbrite Ash Mine localities of the Cudahy fauna (Middle Pleistocene, Crooked Creek Formation) (Taylor, 1965; Miller, this report).

#### Distribution

Southeastern Ontario, west to British Columbia, north to the Great Slave Lake, south in the Great Plains to east central South Dakota.



The accompanying map (Fig. 1) is based on published records and materials examined in the United States National Museum (USNM) and University of Michigan Museum of Zoology (UMMZ) collections. The following is a list of peripheral localities:

- Ontario: Belleville (UMMZ 185968).  
 South Dakota: Duell County, slough, Coteau Hills (UMMZ 90359).  
 North Dakota: Ramsey County, road pond west of Garske (UMMZ 30094).  
 Wyoming: Grand Teton National Park, Moran, small pond south of dam (UMMZ 184678).  
 Montana: Flathead County, Kalispell (USNM 519925).  
 British Columbia: Nulki Lake, small marsh near Vanderhoof (USNM 601437; 601438).  
 Mackenzie District: Fort Smith, MacKenzie River (USNM 180300); MacKenzie River, 30 miles above Fort Providence (Whittaker, 1924).

### Ecology

*Planorbula campestris* is typically a species of temporary ponds that occur in relatively open grassland areas. It is often associated in these situations with *Stagnicola caperata*, *S. palustris*, *Aplexa hypnorum*, *Promenetus exacuus*, *P. umbilicatellus* and *Planorbula armigera* (Mozley, 1938).

It has been collected in Grand Teton National Park from small ephemeral ponds that are subject to considerable fluctuations in water level (Beetle, 1965). The ponds at these localities are situated in open meadowland at an elevation of about 6700 feet and apparently represent habitats that are quite similar to those from which *P. campestris* has been collected in the northern Plains (Mozley, 1938).

### Remarks

*Planorbula campestris* is distinct from the 3 other species of *Planorbula* s.s.,

*P. armigera*, "*P. crassilabris*" and "*P. jenkinsii*", considered valid by Baker (1945: 176) in the: (1) nature of its shell sculpture; (2) possession of never more than 3 palatal lamellae; and (3) absence of a flexure in the last whorl near the aperture. In *P. campestris*, spiral striae incise and interrupt the slightly raised incremental growth lines, producing an evenly distributed reticulated pattern that covers the entire shell exterior (Pl. I, Fig. 6). Sculpture in the other species of *Planorbula* s.s. is usually weakly developed and unevenly distributed, and consists of discontinuous spiral striae and fine lirae (Pl. I, Fig. 5).

The lots of *Planorbula campestris* and *P. armigera* examined exhibited a great deal of variation in the number of sets of lamellae present in individual shells, the location of lamellar sets and in the degree of development of the individual denticles in each set. The number of lamellar sets in *P. armigera* was found to range from 0-2, and, in *P. campestris*, from 0-4. To the writer's knowledge, this is the first report of multiple sets of lamellae in *Planorbula*. In some individuals of *P. campestris* only one set of lamellae occurs and may be located as much as 1 - 1/8 whorls behind the aperture. Although the minimum number of denticles per set in both *P. armigera* and *P. campestris* is not constant, the maximum number of denticles per set never exceeded 6 (2 parietal and 4 palatal) in *P. armigera* and 5 (2 parietal and 3 palatal) in *P. campestris* (Fig. 2).

### DISCUSSION AND CONCLUSIONS

The shells of *Planorbula campestris* that have been recovered from 2 fossil localities of the Cudahy fauna of Meade County, Kansas represent the first reported fossil record of this species in the southern Great Plains and extends its known range to the Kansan.

The present study of the internal lamellae and shell ornamentation from a representative series of this fossil material and of all the available lots

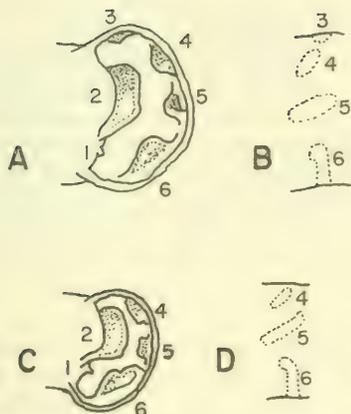


FIG. 2. A, B, *Planorbula armigera* (Say). A. Apertural view of lamellae. B. Lamellae as they appear from outside of shell.

C, D, *P. campestris* (Dawson). C. Apertural view of lamellae. D. Lamellae as they appear on outside of shell.

The lamellae are indicated by numbers: (1) Parietal tubercle, (2) Parietal, (3) Suprapalatal (absent in *P. campestris*), (4) Upper Palatal, (5) Lower Palatal, (6) Basal Palatal (modified from Winslow, 1921).

of Recent *Planorbula campestris* and *P. armigera* in the collections of the U. S. National Museum and University of Michigan revealed shell characteristics not previously reported, and indicates that the description of the shell characteristics attributed to the subgenus *Planorbula* s.s. by F. C. Baker (1945) in his monograph on the Planorbidae, is in need of emendation and consequently his subgeneric classification in need of revision.

Baker proposed 2 subgeneric categories, *Planorbula* s.s. and *Haldemanina*, a separation based on the difference in the lower palatal lamella. The latter subgenus, containing the 1 species *P. wheatleyi*, was differentiated from all the other species of the genus by the complexity of this lamella which "... is about twice as long as in *armigera* ... the upper part is bent upward almost at right angles to the transverse lower part, so that this end is on a line with

the upper palatal lamella, the whole lamella being bent like an Australian boomerang" (Baker, 1945: 177). As regards *Planorbula* s.s., which contains *P. campestris*, *P. armigera*, "*P. crassilabris*", "*P. jenkinsii*", as well as several doubtful fossil species, Baker states in his description of that group (1945: 173-174) that there are 6 lamellae "... a large parietal lamella ... a small tubercular lamella ... and 4 palatal lamellae .... Only one set of lamellae occurs in each shell ... the old set appearing to be absorbed before the new one is formed as the shell increases through growth."

From my observations it appears that, while the number of denticles was maximally 6 in *P. armigera*, it consistently never exceeded 5 in *P. campestris*. Moreover, the old sets of lamellae are not regularly all absorbed, but multiple sets occur in some individuals of both species, with a maximum of 4 sets observed in *P. campestris* and 2 in *P. armigera*. These 2 species are further distinguished by 2 types of shell sculpture, consisting, in *P. campestris*, of continuous incised spiral striae that interrupt the slightly raised, evenly spaced incremental growth lines. In contrast, the incremental growth lines in *P. armigera* are less regular, and are crossed by irregularly spaced, weakly developed, discontinuous spiral striae and fine lirae. The shell sculpture in "*P. crassilabris*", "*P. jenkinsii*" and *P. wheatleyi* is similar to that of *P. armigera*.

In my opinion the difference between the *Haldemanina* and *Planorbula* groups is no greater than the differences which distinguish *P. campestris* from the other species within the *Planorbula* s.s. group. To treat the size and shape of the lower palatal armature in *P. wheatleyi* as a subgeneric character would justify the creation of a 3rd subgenus to accommodate the shell characters found in *P. campestris*. Such a proliferation of subgeneric categories in a genus that probably contains no more than 3 valid living species, *P. campestris*, *P. armigera* and *P. wheatleyi*, seems an un-

necessary complication. In these 3 species the shell characters in question probably represent no more than good specific differences.

#### ACKNOWLEDGEMENTS

Appreciation is expressed to Dr. Harald A. Rehder, U. S. National Museum and Dr. Henry van der Schalie, Museum of Zoology, University of Michigan, for the use of their facilities. Dr. Claude W. Hibbard, Museum of Paleontology, University of Michigan, provided the fossil material from the Cudahy fauna.

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#### RESUMEN

*PLANORBULA CAMPESTRIS* (GASTROPODA: PLANORBIIDAE)  
DE LA FAUNA CUDAHY (EADADE KANSANA), DISTRITO MEADE, KANSAS,  
CON NOTAS SOBRE EL STATUS DE LAS CATEGORIAS  
SUBGENERICAS DE *PLANORBULA*

B. B. Miller

Se colectaron mas de 200 ejemplares fósiles de *Planorbula campestris* (Dawson), en el condado de Meade, Kansas, en dos localidades de la Fauna Cudahy de edad Kansana. El estudio de la escultura de las conchillas y los denticulos internos, en estos asi como en todos los lotes Recientes disponibles de *P. campestris* y *P. armigera*, en las colecciones del U. S. Nat. Museum y del Mus. de Zool. de la Universidad de Michigan, revelaron las siguientes caracteriaticas que no se habian informado previamente:

(1) Presencia de denticulos en series múltiples en *P. campestris* (hasta 4) y *P. armigera* (hasta 2); (2) Número máximo de denticulos por serie que nunca excede

de 5 en *P. campestris* o 6 en *P. armigera*; (3) dos tipos distintos de escultura consistentes en *P. campestris* de continuas estrias espirales de fuerte desarrollo, que se interrumpen en las líneas un poco elevadas, de crecimiento y regularmente espaciadas. En *P. armigera* las líneas están espaciadas con menos regularidad, pero cruzadas por estrias y fina liración discontinua débiles e irregularmente espaciadas.

Tales características, aquí reconocidas, para separar las especies ubicadas por Baker (1945) en el subgen. *Planorbula* s.s., son tan importantes por lo menos como la forma de las lamelas palatales inferiores que dicho autor usó para distinguir las dos categorías subgenéricas, *Planorbula* s.s. y *Haldemanina*. Se sugiere que las categorías subgenéricas de Baker para *Planorbula*, probablemente no son válidas.

#### АБСТРАКТ

*PLANORBULA CAMPESTRIS* (GASTROPODA: PLANORBIDAE) ФАУНЫ  
КЬЮДЕХИ (КАНЗАН) ИЗ РАЙОНА МИД КАУНТИ, КАНЗАС И ЗАМЕТКИ  
О ПОЛОЖЕНИИ ПОДРОДОВЫХ КАТЕГОРИЙ *PLANORBULA*

Б. Б. МИШЛЕР

Более 200 ископаемых раковин *Planorbula campestris* (Dawson) были собраны в двух местах распространения фауны Кьюдехи (Канзан), Мид Каунти, Канзас. Исследование скульптуры раковины и внутренних пластинок в этих и во всех имеющихся современных пробах *P. campestris* и *P. armigera* из коллекции Национального Музея С. Ш. А. и Зоологического Музея Мичиганского Университета показало наличие нескольких ранее неизвестных признаков. Сюда относятся: 1) наличие нескольких наборов зубчиков у *Planorbula campestris* (до 4) и у *P. armigera* (до 2); 2) максимальное количество зубчиков в каждом наборе никогда не превышает 5 у *P. campestris* и 6 у *P. armigera* 3) имеется 2 ясных типа скульптуры, состоящей у *P. campestris* из сильно-развитых непрерывных, вдавленных спиральных линий, прерывающихся слабоприаоднятыми, расположенными на равном расстоянии линиями нарастания. У *P. armigera* скульптура поверхности состоит из менее правильных линий нарастания, перескающих с неравномерно-расположенными, слабо развитыми и непрерывными спиральными линиями и тонкими бороздками. Приведенные характеристики для разделения этих 2 видов (отнесенных Бэкером в 1945 г. к роду *Planorbula* s.s.) являются не менее важными, чем форма нижней полательной пластинки, которую он использовал для разделения двух подродовых категорий рода *Planorbula*: *Planorbula* s.s. и *Haldemanina*. Автор предполагает, что подродовые категории Бэкера для подрода *Planorbula*, возможно являются недействительными.



PHYSIOLOGICAL AND ECOLOGICAL ASPECTS OF PREY SELECTION  
BY THE MARINE GASTROPOD  
*UROSALPINX CINEREA* (PROSOBRANCHIA: MURICIDAE)<sup>1,2</sup>

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ABSTRACT

Behavioral, physiological and ecological relationships between the molluscan predator *Urosalpinx cinerea* (Say) and its intertidal prey on the east coast of the United States have been examined in field and laboratory studies.

In studies of relative attack frequencies in nature and olfactometer responses in the laboratory, barnacles, *Balanus* spp., are shown to be significantly more attractive to *U. cinerea* than either of its other major intertidal prey, oysters and mussels. Field reports of this preference are based upon direct observations of 4,416 snails in 11 intertidal habitats in the continuous east coast range of *U. cinerea* from Massachusetts to northern Florida.

This statistical preference for barnacles is not genetically fixed; indeed, field studies indicate that ecological factors can account for prey selection in intertidal habitats. One of these is intertidal zonation of prey and predator, another is relative abundance of a given prey species within the intertidal zone. The role of external metabolites in bringing the predator to its prey could not be elucidated from my field observations.

Experimental evidence is presented to introduce the concept of *ingestive conditioning*, in which the predator's tendency to respond to effluents from a given prey species is increased after it has ingested living tissues from that species. Ingestive conditioning was partially reversed in juvenile *U. cinerea* by returning them to their original diets. Circumstantial support for the operation of this process in nature was derived from experiments with snails from single- and multiple-prey habitats. Statistical tendencies of *U. cinerea* to select barnacles in preference to oysters was partly confirmed in experiments with young and juvenile stages, which were most easily conditioned to barnacles, but not always in the case of adults, whose diverse natural diets were difficult to reverse.

Evolutionary aspects of this predator-prey relationship are discussed with particular reference to the adaptive value to the predator of ingestive conditioning. Restriction to a single prey species would have disoperative effects, so it is to the predator's advantage to be capable of feeding upon more than one species. However, different attack techniques are utilized for efficient penetration of various prey species, and these are apparently acquired by individual *U. cinerea*. By concentrating upon a single species, *U. cinerea* probably increases its attack efficiency. The mechanism described here as ingestive conditioning provides such a concentrating influence without the irreversibility of genetically fixed prey specificity.

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<sup>1</sup>Adapted from portions of a thesis submitted to Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Contribution Number 229 from the Virginia Institute of Marine Science.

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<b>I. INTRODUCTION</b>	
<p>The marine gastropod <i>Urosalpinx cinerea</i> (Say) is a copredator, with man, of the Virginia oyster, and as such has attracted considerable attention from the shellfish industry and from those biologists whose research is industry oriented. My initial investigation of the feeding habits of this snail began in such a context, but it was not long before several fundamental problems assumed a central importance. The original question "Does this predator have a food preference?" is important to the oyster industry; but the more basic questions which grew from this root have carried the investigation into the fields of ecology, physiology, and biochemistry.</p> <p>The present paper, describing part of this work, will cover field studies of prey selection, and introduce an hypothesis to explain results of both field and laboratory investigations. The other part, "Qualitative and quantitative nature of attractance," will appear separately.</p> <p>Much general information about the species has been compiled by Carriker (1955) for North American populations and by Hancock (1959) for those of the channel coast of England. The following brief review of aspects of the biology of <i>U. cinerea</i> relevant to this report draws heavily from these 2 sources, except where otherwise indicated.</p>	

## 1. Systematics and Morphology

*Urosalpinx cinerea* is a member of the Family Muricidae, Order Neogastropoda, and Subclass Prosobranchia (Thiele, 1931).

The common name of the family is "The Rock Shells," as these are gastropods adapted to, and commonly found in, habitats which offer a firm substrate. *U. cinerea* has a strong, muscular foot that can adhere firmly to a smooth rock or shell surface, but can be retracted fully into the rather heavy shell, and there be protected by a horny operculum.

Adults range from about 17 to an infrequent maximum of 58 mm (total spire height, measured from apex to the tip of the siphonal canal). Typically, discrete populations may vary considerably in size, color, and shell shape. Variations in size are extreme, and Baker (1951) recognized this variation by taxonomic separation. She gave the name *Urosalpinx cinerea* var. *follyensis* to specimens collected from a shell midden on the Atlantic shore of the Delaware-Maryland-Virginia Peninsula at Folly Creek, Accomac, Virginia. Carriker (1961) granted subspecific rank to that population, calling it *Urosalpinx cinerea follyensis*, and referred also to *U. c. cinerea*, which presumably includes all other Atlantic coast populations. But for purposes of the present work, I will regard *U. cinerea* as one species without further taxonomic separation. Specific geographic origins of experimental animals will be given.

## 2. Habitat and Distribution

*Urosalpinx cinerea* is apparently native to the middle Atlantic coast of the United States, but has been inadvertently carried to other parts of the world by man. Its present North American range is from eastern Canada to the northern part of Florida's Atlantic coast; scattered introduced populations have also been reported on the Pacific coast of North America, from northern California to southern Canada. It is dis-

tributed vertically from the middle intertidal zone down to unknown depths, though it probably does not extend beyond the continental shelf.

Within its range, it is found in rock or shell intertidal habitats characterized by intermediate to full oceanic salinities, a wide annual temperature range, good water circulation, and an abundance of sessile epifauna. Under optimal conditions of food and hydrography, populations of this predator (juveniles and adults) can achieve seasonal densities as high as 1000/m<sup>2</sup> (pers. obs.), though this is atypical. On the other hand, there are potential habitats which seem to be ideal but which have not yet been populated by this or any other predatory gastropod. A possible explanation for this distribution is based on the lack of a pelagic larva, though Carriker (1957) has suggested alternative means of transport for the species.

## 3. Feeding Mechanisms

Two distinct capabilities are requisite for successful predation. First, the predator must be able to detect, locate, and move toward its prey. Second, it must possess apparatus with which it can attack and ingest the prey or portions thereof.

It has been amply demonstrated in both field and laboratory studies (Haskin, 1940; Blake, 1960; reviewed by Kohn, 1961) that *Urosalpinx cinerea* detects the presence of, and migrates to, its prey on the basis of chemical stimuli. Other sensory stimuli, such as light, sound, or the sensation of touch, are either not used at all or (as in the case of touch) are probably important only after the predator has located and arrived at the prey. Such a situation can hardly be regarded as extraordinary, since chemoreception is the most primitive and ubiquitous sense in invertebrates and is instrumental in initiating and maintaining numerous inter- and intraspecific relationships (Davenport, 1950 et seq.; Bullock, 1953; Hodgson, 1955; Kohn, 1961).

An animal which preys upon shelled organisms must be able to penetrate the shell. Such an adaptation has been described in the case of *Urosalpinx cinerea* by Carriker (1943; Carriker, Scott & Martin, 1963), and for other prosobranchs by Fretter & Graham (1962). Shell penetration by these gastropods is accomplished in various ways, depending upon the species of predator and prey involved, and upon the individual experience of the predator. In *U. cinerea*, the most commonly occurring method of attack is that of shell perforation. According to Carriker et al. (1963), perforation is accomplished by 2 principal techniques, using different sets of organs. The first of these involves the radula and its associated musculature, ensheathed in a protrusible proboscis. This apparatus rasps shell and soft tissue and conveys these tissues into the esophagus. The other makes use of an accessory boring organ, located in the foot, which weakens the shell at the drilling site for removal by the rasping action of the radula.

The hole produced by the alternating actions of radula and accessory boring organ is slightly conical and just large enough so that the proboscis can be inserted through it to the interior of the prey. There, apparently aided by rapid autolysis of some of the firmer tissues such as the adductor muscle (Carriker, 1955), the radula rasps away bits of dead tissue and carries them to the esophagus.

As suggested above, not all types of prey must be drilled to be successfully attacked. Such behavioral modifications as are employed in attacks upon barnacles, mussels, and other forms will be mentioned later.

#### 4. Responses to Changes in the Environment

Carriker (1955) has reviewed much of the available information concerning the orientation of *Urosalpinx cinerea* to various cues from the environment, such as light, gravity, and current. Briefly, it can be said that at summer tempera-

tures the species is negatively phototactic to bright light but apparently positive to dim light, geonegative and almost uniformly rheopositive.

Experimental evidence has been provided by Carriker (1954) which confirms many previous observations in nature to the effect that, at least in the northern part of its Atlantic Coast range, *U. cinerea* moves downward into the substrate with the onset of winter and then upward into its feeding areas in the intertidal zone as water temperatures begin to increase in the spring. The actual temperatures which initiate these movements vary with latitude (author's unpubl. data).

It is noteworthy also that females move upward more actively and in greater numbers at the respective threshold temperatures than do the males. Carriker (1955) and Hancock (1959) have both reported an upward and inshore movement of spawning females. Two functions of this tendency are apparent. First, by depositing their egg capsules upon elevated topographic features, they reduce danger to their young from suffocation. Second, young snails will be more apt to have access to food of the proper size and kind if they hatch in the richly encrusted middle intertidal zone as opposed to the lower intertidal or subtidal.

The role of temperature in regulating reproductive activity in this species was discussed by both Carriker (1955) and Hancock (1959), who gave threshold temperatures for copulation and oviposition in various populations of *U. cinerea*. Minimal temperatures reported for copulation are uniformly lower than minima reported for ovipositing. This disparity permits the suggestion that fertilization may occur in wintering-over places, during spring when water temperatures are increasing. Afterward, females begin crawling upward, preceding males, until their movement is arrested either by increasing intertidal exposure or by their arrival in an area heavily populated with suitable prey.

*U. cinerea* is only limitedly euryhaline. Carriker (1955) reported the work of several investigators who attempted to establish the tolerance limits of the species: it can survive hyperoceanic conditions of salinity, but is generally not present in waters diluted by more than half. Recently I found (unpubl. data) that variations in the gastropod's nutritive state and thermal history significantly influence its ability to survive salinities below 11 o/oo, and confirmed Carriker's (1955) assertion that the variables of "temperature and time" strongly influence salinity tolerances.

### 5. Prey preference

Several investigators have reported observations of the prey preferences of *Urosalpinx cinerea*. Carriker (1955) summarized the literature up to that time, and reported that this predator fed upon "... its own kind, slipper limpets, edible and ribbed mussels, soft and hard clams, scallops, oysters, small crabs, the carrion of fish, and on such lower invertebrates as encrusting bryozoans ... On the whole its diet appears to consist principally of small oysters, edible mussels, and barnacles when these are available... The effect of the relative abundance and accessibility of food species on the selection of prey is poorly understood, but it may be conjectured that these factors also influence the diet..." (p 48).

Hancock (1959), reporting on populations of *U. cinerea* in the Thames estuary, listed as prey species the bivalves *Ostrea edulis* (L.) and its spat, *Mytilus edulis* L., *Cardium* spp., *Paphia* spp., the American slipper limpet *Crepidula fornicata* (L.), and the barnacles *Balanus* spp. and *Elminius modestus* Darwin. He stated further that "... *Urosalpinx* feeds preferentially on oyster spat. Although barnacles are eaten, tangles [*U. cinerea*] have been observed drilling young oysters, mussels, and even *Crepidula* which were covered by live barnacles." (p 21). Hancock cited

Orton's (1929) counsel that the "influence of environment and habit should be considered" when food preferences are examined.

Of interest is a comparison of food preferences of other boring gastropods. Butler (1953) cited experiments in which muricid snails (*Thais haemastoma floridana* Conrad) attacked almost all of the other sessile animals before attacking large oysters. Under laboratory conditions the order of preference found by Butler was mussels, spat (presumably *Crassostrea virginica* Gmelin), barnacles, clams, hydroids, and finally mature oysters, but he did not describe his experimental methods in detail.

Chew & Eisler (1958) and Chew (1960) investigated the food preferences of another muricid, the Japanese oyster drill "*Ocenebra*" (= *Tritonalia*) *japonica* (Dunker), on the Washington coast. Due mostly to deficient experimental technique, they were unable to report distinct and significant results.

Hancock (1959) reported that "*Ocenebra*" and *Nucella*, especially the latter, seem to prefer mussels to oyster spat and brood oysters.

Kohn (1959), in an experimental study of the food preferences of *Conus* spp. in Hawaii, was able to demonstrate at least a partial correlation between the natural food (based on analysis of stomach contents) and preferences exhibited in a choice chamber.

The crown conch, *Melongena corona* (Gmelin), was reported by Hathaway & Woodburn (1961) to show a preference for live oysters over shelled oyster meats, but was known to feed upon various species both living and as carrion.

In most of the reports of field observations of *Urosalpinx* cited above, little or no quantitative information is available, nor generally are study techniques carefully described.

Such factors as relative access and abundance related to statements concerning prey preferences have not been studied.

There has been recently an increasing

interest in the physiological and ecological factors underlying prey preference. These can be separated for convenience into the following categories:

1. The rate of growth and metabolic rate of the prey.
2. Genetic predispositions for one prey or another on the part of the predator.
3. Factors inherent in the experience of the predator (habit, "olfactory conditioning", etc.).
4. Teleological factors, e.g., a preference based upon some benefit which would accrue to the predator as a result of attack upon a particular prey.

The first experimental demonstration of a relationship between metabolism and attractiveness was provided by Haskin (1940, 1950), who used a simple olfactometer to show that *Urosalpinx cinerea* oriented preferentially to water flowing over young, as opposed to old, oysters. The metabolism concept was extended in work done by Blake (1960), who found a positive correlation between increased oxygen consumption of prey and its attractiveness to *U. cinerea*. It should be pointed out that his respiratory comparisons were made among groups of the same prey species or, in a few cases, different but closely related pelecypod species, so that the effects of metabolic or quantitative differences might be expected to have been given increased prominence, on the grounds that qualitative, interspecific differences were minimal.

Genetic determination has not been considered very seriously as a factor in the prey preferences of *U. cinerea*, principally because it is difficult to imagine a rigid species-specific response by a predator whose prey is so diverse, but Blake (1958) mentioned the genetic factor in connection with the preference phenomenon. In any case, genetic transmission of receptor types specifically sensitive to the odor of only one prey species remains a

theoretical possibility until it is ruled out by convincing evidence.

While it has been demonstrated in several animal phyla, principally in insects, that the experience of individual organisms can modify their preferences for host or prey species (Thorpe & Jones, 1937), no experimental study of gastropods in this regard has been attempted. Nonetheless, several writers have claimed that "habit" or "experience" was responsible for prey preferences in *U. cinerea*. Galtsoff (pers. comm.), in commenting upon the preference for barnacles by Woods Hole, Massachusetts, populations, stated that they attack barnacles out of habit. Orton's (1929) comment on the influence of habit has already been cited. He mentioned further that *Ocenebra's* feeding habits were the product of its habitat, i.e., the food organism(s) to which it had become accustomed. Cole (1942) suggested that his preference results with *Urosalpinx* might be explained in the light of the native food of his experimental animals.

Implied ascription of prey preferences to teleological factors was made by Galtsoff, Prytherch & Engle (1937), who reported that because barnacles were attacked through the opercular aperture, they were more vulnerable than prey whose calcareous valves had to be bored. Engle (1942) stated that *U. cinerea* showed different growth rates when fed upon various prey. In order of decreasing growth rates, the prey were *Mya*, *Ostrea* (= *Crassostrea*), *Balanus* and *Mytilus*. Engle said that laboratory results were partially supported by field observations in which he found that large numbers of young oysters and *Balanus*, but very few *Mytilus*, were killed by *U. cinerea*. Hanks (1957), in a laboratory study of feeding rates of *U. cinerea* at various temperatures, found that predators ate more oyster spat than young mussels at all tested temperatures; but stated that this could have as easily been due to the spatial arrangement of prey in laboratory trays as to a preference.

## 6. Objectives of Present Study

Prey preference is here defined as the tendency of a predator to select a single prey type out of a number of available types, in a statistically significant proportion of given opportunities. A clear demonstration of such a prey preference would give rise to several questions of fundamental physiological and ecological significance. First, indication of a consistent preference would suggest that the predator is capable of selecting one species from an array of other species, or can discriminate. Second, should such a preference come about as the result of the individual predator's experience, and furthermore persist in that individual, it would indicate that a type of learning had occurred. Third, since recognition of prey can be based on nothing but chemical stimulation, it would follow that these stimuli differ from one prey species to another (in kind, amount, or a combination of both) and furthermore that each species would produce, consistently, an odor *characteristic* of that species alone. A characteristic, identifying stimulus must be persistent if its use as a discriminatory cue by the predator is to have adaptive value.

A consistent preference need not be genetic in origin, and is not so defined. The definition of preference given above can be satisfied by a selective tendency growing out of the experience of the individual predator. But the important problems of discrimination, learning, and the nature of stimulus properties, all depend upon demonstration of a statistical preference, regardless of its causes.

A failure to demonstrate significant preference would imply the inverse of statements made above. First, it could mean that the predator lacks sensory or integrative apparatus which permits discrimination. Or it could mean that while discriminatory apparatus is present, each discrimination is made *de novo*, regardless of the predator's previous

experience. Or it could mean that all prey organisms produce the same generalized stimulus, and that no one effluent compound identifies a specific prey. Another alternative is that in each choice situation, the predator's selection is based either on quantitative comparison of attractant concentrations or simply upon the presence of "the" attractant in the odor of one prey (and its absence in all others).

Hence the first objective of the present research is to demonstrate a statistically significant preference. Should it be demonstrated, regardless of cause, the second objective is to describe in as much detail as possible those physiological and ecological conditions under which the preference is exhibited. Third, if the first 2 objectives can be achieved, it should be possible to contribute a better understanding of the ways in which ectocrines (Lucas, 1955) act as carriers of information to bind together the several trophic components of marine habitats, and to make inferences about the ways in which such a predator-prey relationship has evolved.

Two general approaches were followed: field and laboratory. In the first, an attempt was made to carry out a careful census of attacks on prey in nature and to relate this information to other factors in a variety of habitat types. In the second, predators were given an opportunity to make a selection of prey on the basis of effluent discrimination alone, with other factors eliminated (where possible) from the experimental design.

## II. PREY SELECTION UNDER NATURAL CONDITIONS

### 1. Introduction

While it is true that *Urosalpinx cinerea* is found in both intertidal and subtidal habitats, I elected to study it exclusively in the former for rather compelling reasons. The first of these is the relative ease of access to intertidal zones during periods of low water. While

underwater breathing apparatus was available throughout the period of study, long experience with its use had taught me that underwater observations and records could not be as detailed as those made on land. Second, I was interested in the effects of zonation upon prey selection, and this consideration eliminated the subtidal area altogether.

Previous students of preferential feeding habits of *Urosalpinx* have generally reported summary statements without quantitative support, and no systematic study of prey preference in the field is known. To provide acceptable evidence, such a study should include the following categories of information:

1. Relative density of prey species populations versus frequency of attacks per species.
2. Distribution of prey species within predator's intertidal habitat.
3. Distribution of predators in various parts of the intertidal zone as an incidental result of the combined effects of other factors, such as
  - a. seasonal temperature regimes
  - b. exposure: immersion ratios (of time)
  - c. reproductive behavior patterns.

Although such complete information was not obtained from each habitat studied, this outline provided the logical basis for the list given below under "Observational Methods."

## 2. Selection of Field Stations

It would have been highly desirable to obtain observations from as great a diversity of habitats as possible (one source of diversity being wide latitudinal separation), but it was also necessary for some habitats to be examined as intensively as possible with a view toward detecting possible seasonal or annual changes. The following compromise was adopted: several stations were visited only occasionally, and a few received concentrated attention, as will be shown in the observations.

The stations were selected to cover

*Urosalpinx cinerea's* East coast range as shown in Fig. 1. Nobska, West Haven, Ocean City, Shark Shoal, Fort Clinch, and Nassau Sound were studied more intensively than the others. A few had such sparse populations of *U. cinerea* that further study of them was pointless. Others were for various reasons visited only once or twice. The Gloucester Point habitat was examined periodically, but since no deviations from a single-species diet were ever observed, specific examinations are not reported.

## 3. Observational Methods

Field trips were planned so that stations could be visited on ebbing tides. When this was impossible, stations were examined at other stages of tide, with or without diving apparatus, depending upon water depth and temperature.

Notes were taken at the station, by either the writer or an assistant, and when possible these were checked against the observations of other investigators.

With some exceptions, the following records were made when visiting a station:

1. Date, time of day, and state of tide.
2. Bucket sample of water was secured for salinity determination and surface temperature. Air temperature was taken in the shade with a dry, hand-held stem thermometer.
3. Color photograph, including a meter stick or string grid for size and density estimates, of area from which predators were collected.
4. Sketch, with metric measurements, of habitat, with notations of faunal zonation.
5. Estimate of abundance of possible prey species in each intertidal zone.
6. Collection of samples of prey species for later identification.
7. Live collection of all predators in given area of the habitat, with diagnosis of feeding disposition at time of removal from substrate. Ascription of feeding was made only if a close examination (with a hand lens if necessary) showed clear evi-

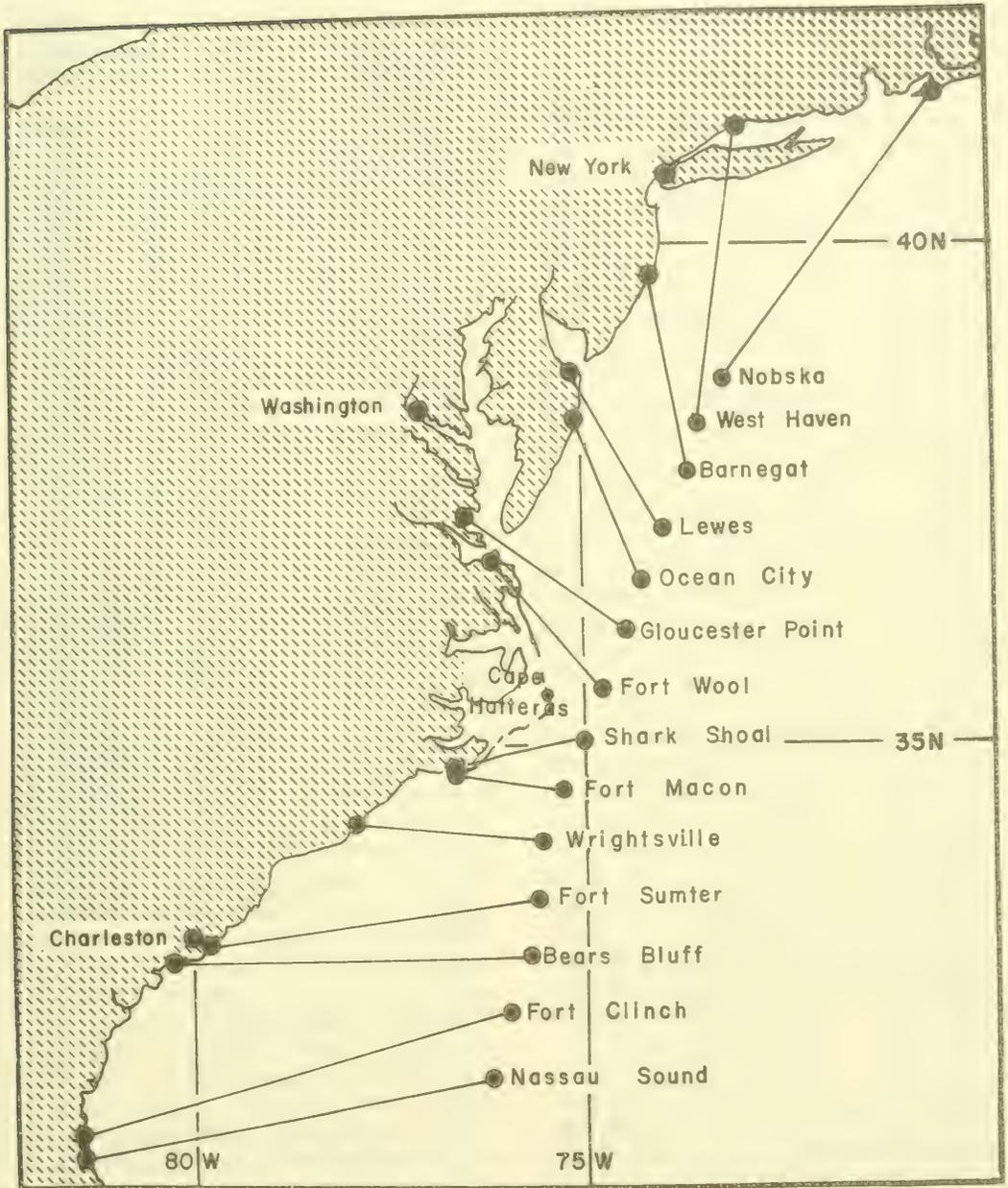


FIG. 1. Locations of 14 field stations on the Atlantic Coast of the U. S. A., from Massachusetts to Northern Florida. The regions north and south of Cape Hatteras correspond to the Virginian and Carolinian faunal regions, respectively.

dence of drilling, or, in the case of predators feeding upon barnacles, that the proboscis was protruded through opercular plates.

8. Records of ovipositional behavior and of "hibernation."
9. Observations of local currents, topographic features, and other

relevant characteristics of the environment.

#### 4. Maintenance of Live Collections

Neither predators nor prey were preserved in the field, but were always carried back to the laboratory alive in polyethylene bags with a small amount of seawater. A large polyethylene bottle of filtered seawater was carried on board the field truck so that seawater changes could be made regularly.

When summer air temperatures were high enough to make such treatment necessary, the plastic bags were placed in a large refrigerator box where a temperature of 10-15° C could be maintained.

Major field trips covering all or part of the east coast range of *U. cinerea* were made in June and October 1959; July, September, and October 1960; April and October 1961; May 1963; and July 1964. The longest duration of these trips, from the time of the first collection to the time of arrival at the laboratory, was 8 days. Shorter local trips were made on numerous occasions from each of the marine laboratories at which work was being done. These field trips, short and long, were made under a variety of weather conditions, and at no time did mortality in transit exceed 3% of the total collection.

In the summary that follows, detailed station descriptions have been omitted. They may be found in Wood (1965b).

#### 5. Summary of Observations

All direct observations of attacks upon the 3 major prey types (barnacles, mussels and oysters) of *Urosalpinx cinerea* are presented in Table 1. The barnacles were represented by *Balanus* spp. and *Chthamalus*, the mussels largely by *Mytilus edulis*, but also by the mytilid *Brachidontes exustus* and the oysters by *Crassostrea virginica*. While it is difficult to compare findings between stations (due mostly to wide variations in conditions of substrate, climate, and prey availability), it seems to me that direct

observations of attacks upon specific prey *in situ* are quite reliable and therefore present an accurate picture of prey selection for any one station.

The sum of 2,451 represents all attacks directly observed during 4 years' field work at various stations between Woods Hole, Massachusetts, and Nassau Sound, Florida. It should be pointed out that if quantitative censuses had been taken during all visits to 2 of these stations (Nobska and Ocean City), this figure would have been increased perhaps by as much as 1,000, but the total relative frequencies of attacks upon barnacles and mussels, respectively, would be changed very little. The reason is that at Nobska, no attacks on mussels were observed, while at Ocean City, *U. cinerea* attacked little else. Thus the summary ratios (58% barnacles, 16% mussels, and 26% oysters) probably reflect with reasonable accuracy observed prey selection tendencies in selected habitats during the period of study.

It will be noted that the selective tendency toward barnacles decreased towards the south, and a corresponding increase was observed in frequency of attacks upon pelecypods. This regional difference is compared to the obvious climatic differences which exist between northern and southern stations. Differences in prey selection patterns were analyzed by the chi-square contingency test of Snedecor (1956) and were significant ( $P < 0.005$ ). The dividing point is Cape Hatteras, following the zoogeography of Hutchins (1947) and Ekman (1953). While Tables 2a, b show what seems to be a correlation between temperature and prey selection, these 2 factors are assuredly not *directly* connected. Rather, to explain this apparent correlation, it is necessary to examine very carefully 2 other factors, namely, differences in intertidal zonation and in relative abundance of prey species within a given intertidal zone, both of which may themselves be influenced by temperature.

TABLE 1. Summary of field observations on prey selection of *Urosalpinx cinerea* on the East Coast of the U. S. A. (1959-1964)

Station (N to S)	Dates	Attacks observed on						Not Feeding N	Total N
		Barnacles		Mussels		Oysters			
		N	%	N	%	N	%		
Nobska	9. VII. 60	110	100	0	0	- <sup>1</sup>	-	87	197
	19. V. 63	187	100	0	0	-	-	94	281
West Haven	7. VII. 60	180	75	60	25	-	-	94	334
	25. IX. 60	49	64	27	36	-	-	115	191
	21. X. 61	1	3	28	97	-	-	106	135
	20. V. 63	178	100	0	0	-	-	nc <sup>2</sup>	178
Lewes	27. IX. 60	91	100	0	0	-	-	83	174
Ocean City	5. X. 59	nc	0	nc	100 <sup>3</sup>	-	-	nc	nc
	11. VI. 60	nc	0	nc	100	-	-	nc	nc
	27. IX. 60	nc	0	nc	100	-	-	nc	nc
	21. VI. 63	("a few")		("many")		-	-	nc	nc
	1. XI. 64	3	9	30	91	-	-	600	633
	6. XII. 64	0	0	0	0	-	-	18	18
Gloucester	29. VII. 62	67	100	-	-	-	-	42	109
Shark Shoal	12. X. 59	8	14	0	0	49	86	nc	57
	18. VII. 60	75	59	20	15	33	26	32	160
Wrightsville	18. X. 59	93	74	0	0	33	26	42	168
	7. X. 60	1	1	27	29	64	70	37	129
Fort Sumter	24. V. 63	9	50	7	39	2	11	2	20
Bears Bluff	8. X. 60	31	23	0	0	104	77	67	202
Fort Clinch	5. III. 61	77	62	18	15	29	23	174	298
	22. IV. 61	1	5	6	28	14	67	150	171
	26. V. 63	21	20	54	51	30	29	12	117
	27. V. 63	55	49	12	11	45	40	nc	112
Nassau	2. VII. 60	37	23	23	14	104	63	81	245
	4. III. 61	5	29	2	12	10	59	32	49
	23. IV. 61	2	14	6	43	6	43	45	59
	25. V. 63	140	43	83	25	104	32	52	379
Total observations:		1,421	58	403	16	627	26	1,965	4,416

Total attacks observed: 2,451

N = Number

<sup>1</sup>(-) = Prey species not present in significant numbers<sup>2</sup>"nc" = Not counted<sup>3</sup>Not counted, but no exceptions seen.

TABLE 2a. Prey selection of *Urosalpinx cinerea* in 2 faunal provinces, East Coast, U. S. A.

Faunal province (north to south)	Observed attacks					All N
	Barnacles		X <sup>2</sup> P <	Pelecypods		
	N	%		N	%	
Virginian	866	85.6	0.005	145	14.3	1,011
Carolinian	855	38.5	0.005	885	61.5	1,440
ALL	1,421	58.0		1,030	42.0	2,451

P= probability

TABLE 2b. Temperature regimes in °C in 2 faunal provinces, East Coast, U.S.A.

Faunal province (north to south)	Annual Regimes <sup>1</sup>			Summer Regimes <sup>2</sup>		
	Min.	Mean	Max.	Min.	Mean	Max.
Virginian	-1.7	12.8	28.0	18.4	21.2	22.8
Carolinian	6.2	20.4	32.2	26.7	27.8	28.3

<sup>1</sup> Five-year means, 1954-58, in °C, converted from values published in Fahrenheit.<sup>2</sup> Including June, July, and August.

Source: U. S. Coast and Geodetic Survey Publ. 31-1 (First Ed.): "Surface water temperature and salinity, Atlantic Coast, North and South America." U. S. Government Printing Office, Washington, D. C., 1960.

### a. Intertidal Zonation

Figure 2 summarizes the observed vertical intertidal distributions of the predator and its prey in selected habitats. Since total intertidal amplitudes vary amongst stations by as much as a meter, it was necessary to express the vertical heights of these zones as a ratio to base 100 of the distance above the mean low water (MLW), as estimated in the field. In this way, the relative juxtaposition of the faunal zones may be compared amongst stations. Such a comparison suggests zonation trends which, despite noted exceptions, seem clear.

First, there is a general decrease from north to south in distinctness of zonation. In the more northerly 5 stations, for example, the barnacle-mussel overlap area is of small amplitude and is quite definite, at least on the outermost, exposed rock surfaces.

But in the more southerly habitats examined, there is extensive overlapping and mixing of barnacle and molluscan prey species.

Second, as one moves from north to south there is a general tendency for the barnacle-mussel overlap zone to move upward in its relative position in the intertidal, reaching its highest observed point at the Wrightsville station (lower part of Fig. 2). South of Wrightsville, the relative vertical positions of barnacles and pelecypods characteristic of the Virginian province, with barnacles above and pelecypods below, undergoes a gradual inversion southward, culminating in the nearly complete position reversal at the Nassau Sound station.

Third, vertical distribution of the predator, *Urosalpinx cinerea*, changes with latitude. In the present context, the most important effects of this shift are two: as shown in the upper part of

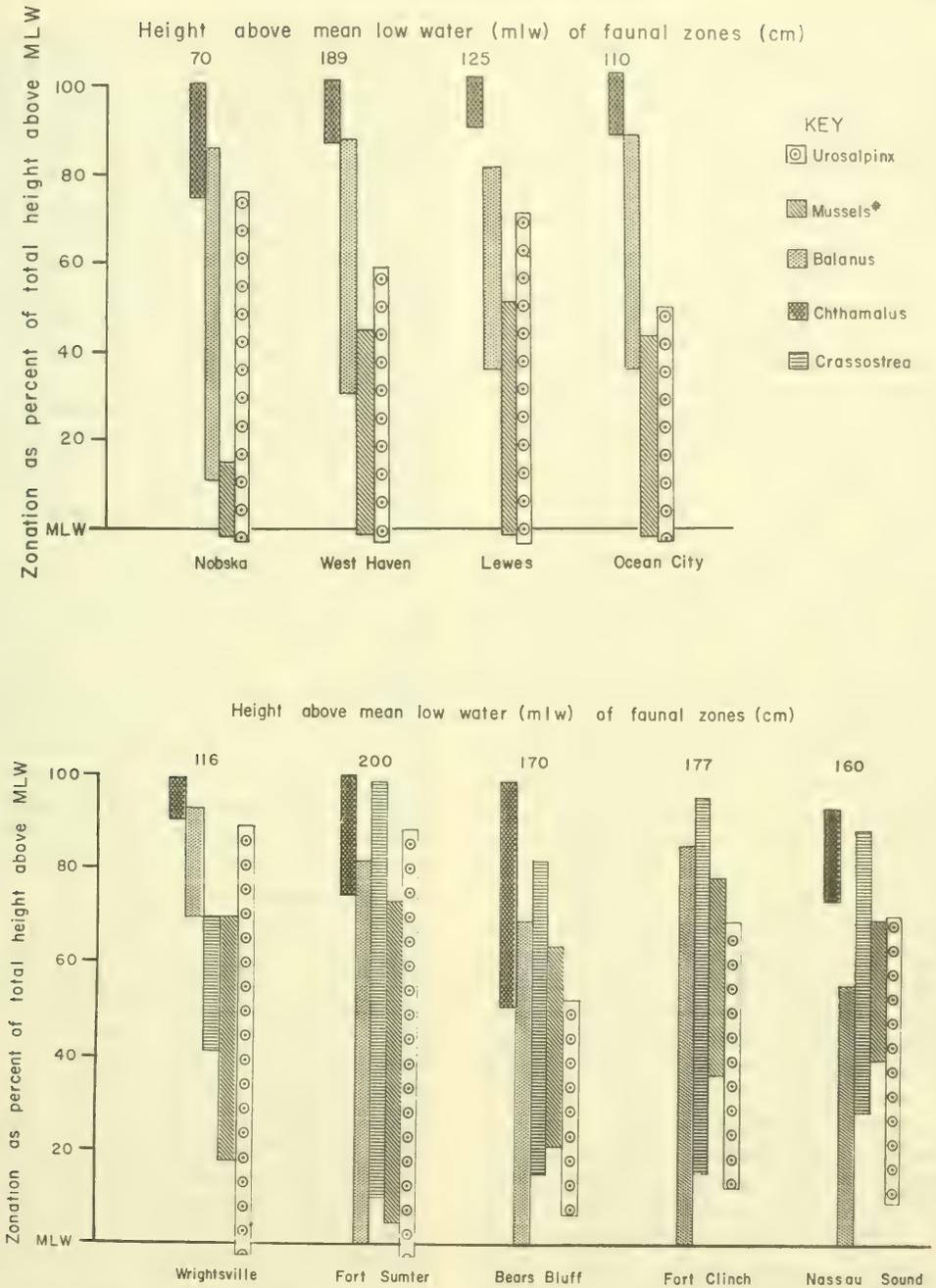


FIG. 2. Relative intertidal zonation patterns of *Urosalpinx cinerea* and its major prey in the northern Virginian province (upper diagram) and the southern Carolinian province (lower diagram). Stations are listed from north to south. For easy comparison of distribution patterns, varying vertical distances between high and low water (70-200 cm) have been converted to a common base (100).

\*Mussel means *Mytilus edulis* in the Virginian province and *Brachidontes exustus* in the Carolinian province.

Fig. 2, the relative vertical positions of the barnacle-mussel overlap and that of *Urosalpinx* differ markedly between the Nobska and Ocean City stations. At Nobska, mussels are few and located in the lowermost intertidal; at Ocean City, mussels are abundant and extend nearly to the middle intertidal. Hence in the northern habitat *Urosalpinx* is conzonal primarily with the barnacle *Balanus balanoides*, while at Ocean City in the south it coexists primarily with the mussel *Mytilus edulis*. Furthermore, at all observed habitats as far south as Fort Sumter in the Carolinian province, there was evidence in favor of the seasonal vertical migration of the predator mentioned on p 270, though at Fort Sumter the evidence was inconclusive and derived from a single station visit. South of Fort Sumter, it was apparent that no seasonal migration occurred. This radical variation in seasonal behavior patterns between northern and southern *Urosalpinx* populations is almost certainly due to climatic differences, shown in Table 2b. Variations on the part of the prey species may also be due to climate, and this thought brings up a rather startling omission in the marine biogeographic literature, for very little work seems to have been done concerning the influence of temperature upon vertical intertidal zonation. Hutchins (1947) discusses in detail the geographic distribution of *Balanus balanoides* and *Mytilus edulis* with respect to temperature regimes, but does not mention latitudinal differences in temperature regime as a factor in subzone variations between cold-temperate and warm-temperate habitats. Doty (1957) notes that temperatures are important in establishing features of vertical zonation, but does not specify the effects of latitudinal differences. Moore (1958) examines the relationship between sea and air temperatures in a single intertidal habitat, but does not examine their influence upon intertidal zonation, nor do Wells & Gray (1960), in a study of subtidal oyster populations immediately north and south of Cape Hatteras, although they cite the temperature gradient which exists there.

While the causes of such latitudinal differences in vertical zonation are only of tangential interest in the present work, the observed fact that the differences exist is central to it. As shown in Fig. 2, there is a progressive rise in the *Balanus-Mytilus* overlap until, finally, at Ocean City, the major part of the *Balanus* population is above the bulk of the predator population, and the predator is essentially conzonal with *M. edulis*. In nearly every habitat studied in the present work, furthermore, it was possible to relate the modal distribution of the predator with the modal distribution of its statistically "preferred" prey. Only the exceptions to the general trend need to be noted here.

At Shark Shoal, in the summer of 1959, an apparently atypical set of the barnacle *Chthamalus fragilis* coated the upper surfaces of the rocks forming the seaward part of the jetty. (While this set is here termed "atypical," it should be noted that a similar set was observed by T. A. & A. Stephenson [1952] at Shark Shoal in 1947.) Thus while *C. fragilis* was numerically the dominant potential prey organism in the upper part of the jetty inhabited by the predator, it was apparently not attacked. In several years of observing the feeding habits of this predator, I have never seen an attack upon *Chthamalus* under natural conditions, and this same observation has been reported (Barnes, pers. comm., 1959; and Crisp, pers. comm., 1959) in the case of the British *Urosalpinx cinerea*. Indeed, *Chthamalus* is usually found so far above the normal intertidal range of the predator that it would seem remarkable if it were attacked. In any case, the fact remains that during the summer of 1959 predators were rarely found on upper surfaces of the rocks with the abundant *C. fragilis*. Hence they were feeding upon numerically secondary *Crassostrea virginica* on the sides and lower surfaces of boulders. In the following summer of 1960, the *Chthamalus fragilis* population at Shark Shoal was overwhelmed by a dense set of *Balanus amphitrite*. With *Chthamalus* gone, predators occurred in abundance upon the upper rock surfaces,

and were feeding largely upon the dominant *Balanus*.

The second noted exception was seen in April 1961 at Fort Clinch. Here, the predator was conzonal with both *Balanus* spp. and *Crassostrea*, and to a lesser extent with the mussel *Brachidontes exustus*. Though barnacles were numerically dominant on the April 1961 visit, most adults seemed to have been recently killed (the suspected cause was industrial pollution of the water by a pulp mill several miles upstream), and only a few *Urosalpinx* were active. Most survivors appeared to be in distress and were clustered within crevices and between encrusting faunal clumps in a manner reminiscent of this snail's wintering behavior in the northern part of its range. Those few which were feeding were in the upper part of the middle intertidal zone, where *Crassostrea virginica* was dominant.

The third exception was observed the following day at Nassau Sound. Here, though the numerous *C. virginica* were dominant in the upper part of the middle intertidal zone, predators were feeding in equal numbers upon the oysters and on *Brachidontes exustus*.

#### b. Relative Prey Density

In no case observed during the field work reported here were predators feeding preferentially upon a prey form not abundant or common in the habitat. The question of effects of relative prey density upon prey selection was difficult to resolve because of irregular prey distribution and the consequent difficulty of quantitative analysis in most mixed-prey habitats. The results of one attempt to secure a rigorous statistical analysis are shown in Fig. 3, and the techniques for it are given below.

The cobbled beach portion of the West Haven habitat in 1960 possessed both major prey species in varying relative abundance. In an attempt to determine the effect of such density variations upon prey selection, I selected 6 quadrats 40 cm on a side and photographed them in color, the field of view coinciding with the quadrat. Three examples were chosen of each of 2 types of relative

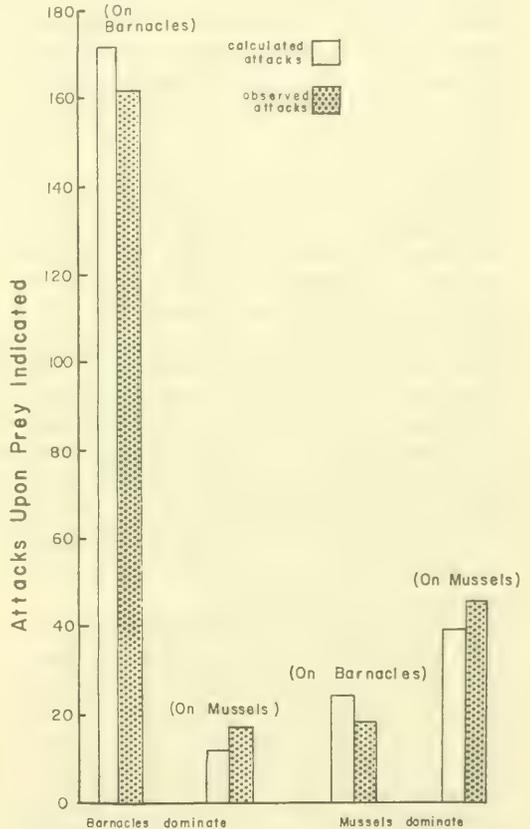


FIG. 3. Correlation of attack frequency (=observed attacks) and prey density (=calculated attacks) in non-zoned, mixed prey habitat at West Haven (Connecticut) field station.

density: *Mytilus*-dominant, and *Balanus*-dominant. As predators from each quadrat were removed, the prey species which they had attacked was noted. In the laboratory, the color slides were placed under a dissecting binocular microscope with transmitted light, and individuals of the 2 prey species were counted. On the basis of these counts, a ratio of expected attacks was calculated, assuming no selectivity.

Figure 3 shows the means of the 3 samples from each of the 2 types of quadrat chosen (*Mytilus*-dominated, *Balanus*-dominated). These "calculated attacks" were compared to the means of the actual attacks observed in the 2 types of quadrats, and the differences between them were analyzed by the chi-square method. No significant difference was found. A similar analysis was performed with the data obtained from a later visit to West Haven with much the same result.

## 6. Discussion and Conclusions

A superficial inspection of the data contained in Tables 1 and 2 would lead one to conclude that (1) there is a significant total preference for the genus *Balanus* over all pelecypods by intertidal *Urosalpinx cinerea* along the east coast of the United States, but that (2) there is a shift from a *Balanus* preference in the northern latitudes to one for pelecypods in the southern part of the predator's range.

A more careful scrutiny of the data, however, has led me to make the following tentative conclusions:

1) In mixed prey habitats, the frequency of attacks upon a specific prey type is a function of the relative density of the prey species present in the habitat.

2) In habitats where prey species are separated from one another as the result of intertidal zonation, prey selection is an incidental product of the co-existence of the predator with whatever prey species is dominant in its zone.

Note that olfaction and olfactory discrimination have not been mentioned in connection with the foregoing field work. The reason for this is simple: no evidence obtained from field studies bears directly upon these concepts. We see upon going into the field that *U. cinerea* has a certain intertidal range and that this range may place it in company with one or more potential prey species. We see further that where the prey species are mixed, it is possible under certain conditions to predict the relative frequencies of attacks upon those species. However, it is still possible that such co-existence of predator and

prey is itself the product of purposeful movement into a specific zone by the predator, and that, further, this movement is elicited by the production of chemical attractants by the prey. Hence one cannot know, on the basis of field information alone, whether a certain ratio of barnacle-preferring predators is present in a barnacle zone fortuitously or as the direct result of attraction to the prey.

Therefore, conclusions based upon field studies must be qualified until the results of experimental inquiry can be presented. Certain specific questions, based partly on field observations and partly on hypothesis, can be asked:

First, can it be demonstrated beyond doubt that *Urosalpinx cinerea* will make an orientational response to the effluents from prey species in the absence of other orientational cues? As much to the point as anything else is whether, in the confused current situations found in nature, such orientations can still be made.

Second, is it possible that the predator's past ingestive experiences influence such orientational responses? This is a question which follows naturally from the observations made at Nobska and Ocean City, where *U. cinerea* exhibits a persistent tendency to continue feeding upon the same prey (different species in each case), despite opportunities to change diets.

These and other questions will be the subject of the chapters which follow.

## III. EXPERIMENTAL METHODS

During the period covered by the field studies described above, experimental investigations of these predator-prey relations were being designed and conducted in a series of marine laboratories. For convenience these studies will be referred to in an abbreviated form by letters followed by numbers, the former indicating the place where they were done and the latter indicating their chronological sequence. The place names designated by the abbreviations and other details are explained below:

FWS. This series was carried out during the summer of 1956 at a temporary

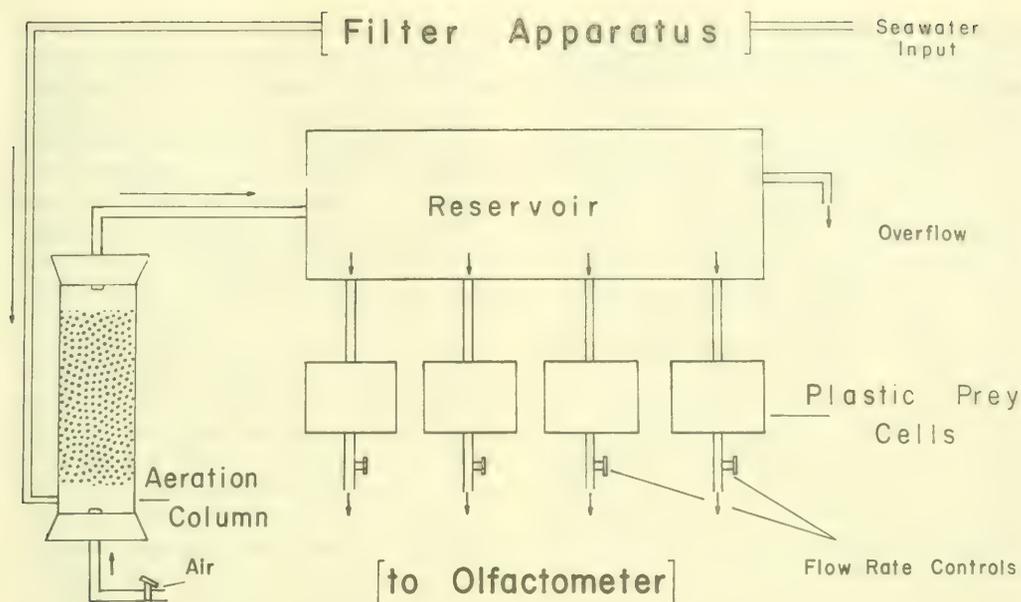


FIG. 4. Simplified diagram showing passage of seawater through filter and aeration column into temperature-controlled reservoir. From the reservoir, seawater flows by gravity through clear plastic prey cells (at least one of which is always kept empty of prey, as a control) and thence through in-line flow meters to the peripheral compartments of the olfactometer shown in FIG. 5. For a diagram of the more complicated controlled conditions system used in the VIMS Series, see Wood, 1965a.

seawater laboratory set up during my brief period of employment by the Clam and Chesapeake Oyster Investigation, Bureau of Commercial Fisheries, Fish and Wildlife Service, Department of Interior. The laboratory was located on Chincoteague Island, Virginia.

IFR. These experiments were carried out in the summer and fall, 1959, and terminated in summer, 1960, while I was working in the laboratory of Dr. M. R. Carriker at the Institute of Fisheries Research of the University of North Carolina at Morehead City, North Carolina.

OI. This series was conducted during the academic year 1960-61 while I was a visiting investigator in the Alligator Harbor Marine Laboratory of the Oceanographic Institute of Florida State University, Tallahassee.

VIMS. These experiments, beginning in the summer of 1961 and continuing through to the present, have been conducted at the Virginia Institute of Marine Science, Gloucester Point, Virginia.

The presentation which follows is organized by topic rather than chronology, but the sequence of the work can be understood from the experiment numbers. Similarly, the section on methods and materials which follows will describe in a general way the experimental techniques employed throughout the investigation. Departures from these general methods will be specified where necessary.

### 1. General Method

The most important criterion for experimental analysis of prey selection is that the predator be allowed to select prey on the basis of a single variable, while other conditions are kept constant. In a system as complex as the marine environment, it is not always easy to satisfy this criterion, as will be seen in descriptions of my successive attempts to attain an ideal experimental procedure.

The procedure used in all olfactometer experiments on prey selection

consisted in placing prey in plastic or glass cells in such a way that incoming seawater had to flow through and around them (Fig. 4) before reaching the "choice" apparatus (Fig. 5) containing the predators. Thus theoretically predators would orient solely on the basis of chemical stimuli emanating from prey organisms. In most cases, one or more of the "prey" cells were used as "control" cells with seawater only from the same source flowing through them to the predators.

Flow rates through prey cells were adjusted until they were equal; prey populations were placed in the cells an hour or 2 beforehand to permit their adjustment to a new environment; temperature and salinity determinations were made; and, finally, predators were placed in the olfactometer's central compartment and a run was started. Duration of a run varied with temperature (Series OI-3), was set arbitrarily at 30-60 minutes (Series VIMS), or the run was declared ended when an arbitrary proportion, usually 3/4, of the predators had made a choice (IFR and OI).

Criteria for "response" (r) were rigid. In the original cross-type olfactometer, snails simply entered a peripheral compartment. Shortly after the beginning of Series IFR, this model was improved (Fig. 5) by adding a smoothed-curve, inclined ramp before each peripheral compartment; responding predators now had to climb the ramp and pass a threshold mark which was *above* the central compartment water level. This meant that, to be counted, individual *U. cinerea* were required to crawl up a ramp from the central compartment and go up a shallow (1-2 mm) stream of odor-laden water into a peripheral compartment. That so many performed this rather unnatural feat speaks for the attractiveness of prey ectocrines.

## 2. Olfactometers

Several olfactometers were employed in this study, and something of their evolution through successive stages of development should be mentioned.

An olfactometer, by definition, is designed to permit one or more subject

organisms to indicate selection of an odorous substance from a neutral background, or to make a choice of one amongst several odors. The indication itself is usually a matter of orientational movement by a free and intact subject, though of course it is possible, and sometimes more convenient, to assay olfactory stimuli by non-orientational behavior, such as proboscidal extension (Carr, 1967). But in the case of *Urosalpinx cinerea* the latter response is not feasible and orientational selection must be employed. From discussions with D. Davenport it appeared that the classical "Y" or "T" maze was not satisfactory on 2 counts. First, depending upon the arrangement of the subject's chemoreceptors versus its dimensions relative to the maze tube or trough, it is possible that the subject could be stimulated by effluents from only 1 of the 2 arms of the maze. Second, only 1 subject can be tested at a time, so that a great deal of time is spent (especially with slower moving forms, such as snails) securing a statistically useful series of tests. To Davenport's objections can be added a point raised by Putnam (1962), who found that *Aleochara* (Coleoptera) tended to repeat initial selections of either right or left arms of a Y-maze, despite the absence of reward or punishment in either arm. Thus non-randomization of successive responses might be based upon cues (either proprioceptive or external) not perceivable by the investigator. At least one way of diminishing errors arising from this possibility is to increase the complexity of responses required by the olfactometer.

Davenport's suggested alternative to the T- or Y-maze was a pie-shaped device consisting of several peripheral compartments surrounding a central drain. Subjects were placed in the center, the water from prey and control cells was run into peripheral compartments, and subjects were to indicate selection by moving from the center to the chosen peripheral cell. This device was used in trials preliminary to Series IFR, but it was not suitable for slowly moving animals and was rejected. Davenport (pers. comm.) had come to a similar

decision independently, though Kleerekoper (1961) successfully employed an elegantly automated variation of the Davenport idea in his study of predator-prey relations of the lamprey *Petromyzon marinus* and the lake trout, and Sastry & Menzel (1962) used yet another adaptation in an investigation of commensal relations between the scallop *Aequipecten* and a pinnotherid crab.

For a recent thorough discussion, see Davenport (1966).

Before Series IFR experiments were finally begun in the fall of 1959, a new olfactometer was designed and constructed. It was used for all Series IFR experiments, and modified only slightly for Series OI and VIMS.

The design was based upon the following criteria:

1. There must be a central compartment, or starting point, large enough for a sample of at least 25 adult predators.
2. Chance of exposure to water from prey or control cells must be the same for all predators, regardless of their initial placement in the central compartment.
3. Water streams from outer compartments must be thoroughly mixed in the center compartment, but there must be a point of choice at which the subject may compare a single "pure" stream with the background mixture.
4. This comparison must result in clearly enumerable selections of prey compartments by subjects.

The design which followed from these criteria is shown in Fig. 5. It consisted of 4 peripheral compartments, with channels leading into a center one in such a way that a circular current pattern is set up around a central floor drain. Water flowing in from the peripheral compartments is thus entrained in a clockwise whirlpool, and makes several rapid circuits before leaving through the drain. This olfactometer has proved to be a sensitive and accurate device for assaying the attractiveness of prey effluents.

### 3. Identification of Individual Predators

*U. cinerea* used in experiments in

Series IFR, OI, and VIMS were marked for identification by means of a 4 color, 5-digit code. Thus a total of  $(4 \times 5)^2 = 400$  identification numbers were available, which exceeded the number of individuals employed in any given series.

For convenience, individual predators were further coded as to their sex, place of origin, and selected prey, if any, at time of capture, through use of additional color dots placed variously upon their shells.

Since it was necessary to dry shells completely prior to application of fast-drying enamel colors, portions of the shells to be colored were scrubbed, rinsed in distilled water, dried, put through 2 rinses of 95% ethanol, and dried again. A few minutes after color dots were applied, the snails were back in seawater.

Apparently no mortality ever resulted directly from this process.

### 4. Predator Maintenance

Predator groups were kept in aquaria supplied with running seawater in all experimental series. Except for studies in which special feeding was required by experimental protocol, predators were not fed while in captivity. Therefore the only food available was that which they could browse from the sides of their containers. *Urosalpinx cinerea* is a species which can endure long periods of food deprivation even at summer temperatures; during the course of this study, some individuals were known not to have had access to prey for about a year. Upon dissection, some of these were found to be apparently normal except for a loss of "non-essential" body tissue.

Individuals of the smaller varieties were not as resistant to starvation as the large snails from the Eastern Shore of Virginia and Maryland, but nonetheless survived periods of gross food deprivation of 3-4 months. In only 1 experiment (OI-3) was mortality a serious problem. It was then primarily restricted to a sample from 1 habitat, Nassau, and could not be ascribed to malnutrition.

In other respects, laboratory populations of *U. cinerea* were apparently

unaffected by captivity. Copulation and oviposition were observed, and the snails added shell. With few exceptions, food-deprived captive predators displayed rheotactic responses comparable to those of controls which had been fed (see discussion of errors below).

Numerous precedents (Haskin, 1950; Carriker, 1955; Blake, 1960) have established the relative ease with which *U. cinerea* can be maintained in captivity.

#### 5. Possible Sources of Experimental Error

Since it has been shown (Carriker, 1955) that *Urosalpinx cinerea* responds to a diversity of external stimuli which could conceivably act as intervening variables in a study of prey selection, considerable care was taken to ensure control of experimental conditions. Measures adopted to this end will be described below.

At least 4 physical factors could influence orientation of predators in the olfactometer: temperature, light, gravity, and current. The first was an experimental variable in the Series OI studies, and detailed discussion of its effects will be saved for a subsequent paper. For the present, it need only be pointed out that ambient seawater temperatures prevailed in Series FWS and IFR, and in the VIMS experiments temperatures were controlled at about 25° C. Water temperatures within the olfactometer did not vary between parts of the apparatus and therefore can be neglected.

#### a. Light

It has been shown that *Urosalpinx cinerea* is sensitive to light. Carriker (1955) summarized reports of other workers in this area (Federighi, 1931; Sizer, unpubl.; Stauber, unpubl.; Cole, 1942): at strong intensities, *U. cinerea* is negatively phototactic; at weaker intensities, it is positively phototactic; and in "dim light" the phototactic response is apparently extinguished.

Two methods of coping with light were employed. In one, the room was darkened, or a black, opaque hood was placed over the olfactometer; in either case, the responses were made in darkness or near darkness. In the second, a broad, diffuse light was placed directly over the olfactometer so that all surfaces were evenly illuminated at an intensity of about 1 foot-candle. The latter method was regarded as the more effective, because in complete darkness, the predators' activity decreased.

In addition, preliminary trials were also run in which a strongly directional light source was placed at one side of the olfactometer to determine maximum effect of such a situation. Regardless of relative orientations of the light *vis-a-vis* prey compartments, there was no significant variation in orientation of predators which could not be ascribed to chemotaxis.

#### b. Gravity

Carriker's (1955) report indicates that *U. cinerea* tends to creep upward (geo-

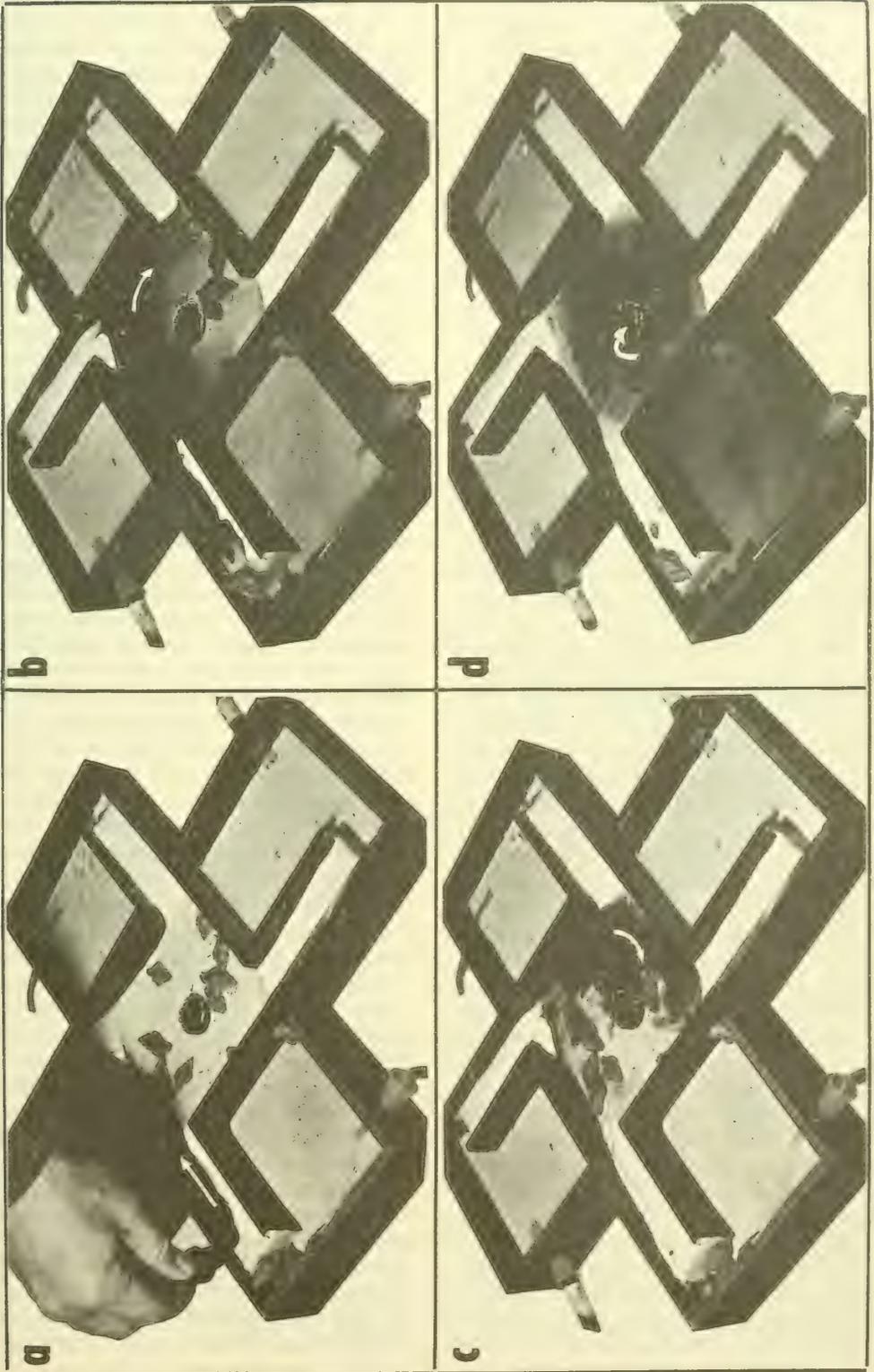
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FIG. 5. Olfactometer used in all but FWS Series of experiments (shown in FIG. 6). Rounded countours were hand molded in plaster of Paris after which the entire inner surface of the device was coated with inert epoxy paint.

Seawater enters square peripheral compartments from corresponding prey cells (or a blank control cell, as shown in FIG. 4) and thence down an inclined ramp where complete mixture takes place in the clockwise gyre of the central compartment before it leaves through central drain. In picture *a*, India ink is placed in the stream going down the ramp and its progress around the central compartment can be followed in pictures *b* through *d*. Passage of materials is so rapid that the olfactometer can clear itself of an ink cloud in about 10 seconds. For this reason, the 4 pictures given here do not represent a sequence from a single dye test, but were selected from several photographed tests in such a way that stages of dye transit through the apparatus are depicted.

The response criterion threshold mentioned on p. 284 is at the point of the arrow in picture *a*. Snails were not counted as responding until after they had crossed this point.

FIGURE 5.



negative) at warmer temperatures and downward when the temperature decreases beyond the "hibernation" threshold. This threshold has been variously reported at between 10 and 15° C.

Presumably, at temperatures employed in the present study (15-about 33° C) predators would have been negatively geotactic. Their tendency to climb up walls of the olfactometer was early recognized, and standard procedure for dealing with this problem was to remove them from the vertical wall and place them at the bottom of it. Since this procedure was applied to all subjects, since, further, the ramp approaches to all 4 compartments were of nearly identical grade, and since finally the apparatus was carefully leveled prior to commencement of each day's trials, it was thought that the geotactic error was minimal.

#### c. Current

It was observed many times in the present study that most predators invariably turned upcurrent in the olfactometer whether or not the current was known to be laden with attractant. A few individuals, however, were consistently rheonegative (i.e., moved in the same direction as the current); none of these individuals ever made a positive prey selection during an experimental run.

While the flow of water into each of the peripheral compartments of the olfactometer was equalized as nearly as possible by volume/time measurements (usually 250 ml/min into each compartment), changes in water pressure and occasional clogging of water lines during a test caused variations in flow rates which were sometimes considerable. Furthermore, even when flow rates into peripheral compartments were identical, there was no effective way of determining differential current velocities amongst separate portions of the central compartment.

Therefore I decided to test the effects of different flow rates upon prey selection, preliminary to Series OI trials. These tests showed that when all cells were *empty* of prey organisms, the input

flow rate difference had to be at least twofold before a significant selective tendency for the higher flow rate compartment could be demonstrated. On the other hand, the outflow rate from cells containing attractive prey organisms could be reduced to nearly zero with little reduction in responses to those cells. I concluded that a marked current velocity difference could alter selection results, but only when prey were relatively unattractive or when differences in attractiveness of 2 or more prey species were very slight.

#### d. Size and Sex of Predators

It has been established (Cole, 1942; Carriker, 1955) that female *Urosalpinx cinerea* grow more quickly than males and reach a larger size. My own observations bear this out. It is reasonable to ask whether a prey preference might change as the predator grows and matures, or whether selective tendencies might differ between males and females.

There is every reason to think that food selected by young predators would differ from that of mature individuals, just on the basis of relative size. Newly hatched *U. cinerea*, with a spire height of about 1.0 mm, could attack a small hydroid or ectoprocot, or a recently set barnacle or oyster, with some degree of expected success (pers. obs.). But on a purely mechanical basis it probably could not successfully attack an adult oyster. On the other hand, would there be similar behavioral differences between a predator with a spire height of 15 mm and a 30 mm predator? While presumably the difference in the size of proboscis would reflect those of the predator's general development, it must be remembered that in these olfactometer tests, we are dealing not with a gastropod's attack and ingestion armament, but with the capability of its chemoreceptors and central nervous system. Hence in order to say that there would be differences between small and large predators we would have to find changes in receptor systems at some point in the snail's development. This in itself constitutes an extended investigation, and therefore it was decided to analyze

existing data for the purpose of determining what correlation there was, if any, between prey selection and size or sex in *U. cinerea*.

During Series IFR experiments, the Wrightsville habitat with its distinctly separated *Balanus* and *Crassostrea* zones was discovered. Since both prey species were about equally accessible and existed in somewhat equivalent densities, information concerning the role of predator size in prey selection could be derived from a study of this population. Therefore every active predator seen was collected, and later, at the laboratory, its spire height was measured and its sex determined. It was found that male-female size differences were greater than size differences between groups feeding upon *Balanus* spp. and *Crassostrea virginica*. Next, samples of 15 of each sex and original prey group (total of 60 animals), were put through 5 prey selection tests in the olfactometer. The results of these tests coincided with those derived from nature: minor differences in spire height cannot account for differences in prey selection. On the other hand, inferences drawn from the VIMS-37 experiments with young snails indicate that orientational ability may develop after they have hatched.

The question of sex-linked differences was solved by an analysis of the data from Series IFR. There were no consistent and significant differences in prey selection which could be attributed to sex.

When Series OI experiments were being planned, therefore, it was decided that since males and females did not differ significantly in selection tendencies, only males would be used. The advantage of using only males was twofold. It has been reported by Peters (1964), but not confirmed by Gibson (1964), that male *Littorina planaxis* Phillipi locate females by chemotactic orientation; in any case a first advantage was gained by denying male *U. cinerea* the diversion of females in breeding condition. Second, it had already been noted that when females were ready to oviposit, they would do so regardless of circumstance. Several times during

Series IFR, at the end of a particularly long run, it was found that a female had not moved from her original position and had deposited an egg case upon the floor of the central compartment during the run.

To summarize: in the experiments of Series FWS, IFR, and OI, only adults were used, while in Series VIMS other stages were employed for specific experiments. As to sex, both were used in Series FWS and VIMS, males and females were run separately in Series IFR, and in Series OI, only males were used.

#### d. Orientation of Predators to one Another

At least one gastropod, *Nassarius obsoletus*, exhibits a type of "schooling" behavior in which aggregations are apparently maintained through the mediation of chemoreception (Jenner, 1959). It was therefore necessary to determine whether or not any such behavior patterns were characteristic of *Urosalpinx cinerea*, since if they were, all experiments in which groups of predators were used would be open to extreme doubt.

One group of 25 *U. cinerea* was placed in the central compartment of the olfactometer. Prey cells were thoroughly cleaned, and in one of them I placed an estimated 2,000 *U. cinerea* from the same laboratory stock. There was no significant response in several repetitions of the test. It was concluded that, at least in the experimental situation adopted in this study, the possibility of a "schooling" tendency based on distant chemoreception could be discounted.

But distant chemoreception is not the only means whereby one predator can follow another. Gastropods characteristically secrete a continuous sheet of mucus from glands in the propodial groove (Peters, 1964). This mucous layer functions as an adhesive and a lubricant, and while relatively soluble in saline solution, it remains applied to hard substrates for several hours. In the olfactometer, its presence could be detected because of the adhesion of minute water-borne particles of debris. It would have been impractical to attempt erasure of this mucous track with a swab

immediately after the passage of each snail from one point to another in the olfactometer, so I decided to determine the effect of the track by means of direct observation: though a succession of predators selected the same peripheral compartments within the space of a few minutes, the tracks of those which followed did not necessarily coincide with those laid down. Nonetheless thorough cleansing of the olfactometer after each test was standard experimental procedure.

#### e. Effects of Food Deprivation Upon Predators

Experimentalists sometimes fall into traps of their own making: in the case of some of my early experiments, it was unavoidable. On the one hand, it was necessary to deprive experimental snails of prey on the grounds that to do otherwise was to risk habit formation or olfactory conditioning (Thorpe & Jones, 1937). On the other hand, there was a chance that food deprivation *per se* might either alter the behavior of the subjects, or impose upon them nutritional stress.

Ideally, some means should have been devised for controlling this factor. However, it was not possible to schedule selection runs at the same stage of food deprivation for each predator, and it was also impossible to obtain a food which was simultaneously palatable to the snails and free of olfactory stimuli.

#### f. Learning During Experiments

When a single subject is used only once, interpretations of results are not hampered by questions of the degree to which the subject was changed by the experiment. For most experiments in the present study, subjects were marked for identification and deliberately re-run several times (up to a maximum of 14 runs in Series OI), precisely for the purpose of determining the extent of individual variations.

Most theories of animal learning depend in some way upon the idea of reinforcement, by reward or punishment,

of a specific behavioral item. It follows from this that non-reinforced behavior tends not to become a permanent part of the animal's behavioral patterns. Application of this idea to the present problem would result in the logical conclusion that because selections were not rewarded, no bias could be placed upon the predator's later selections. (For a review of the theory of reinforcement, see Brogden, 1951.)

At least 2 studies of invertebrate behavior, however, cast some doubt upon this conclusion. Thorpe & Jones (1937), in a study of host selection in parasitic insects, concluded that exposure to a certain chemical environment at some time during an organism's life cycle tended to increase responses to those chemical stimuli in laboratory tests. In this and later investigations, Thorpe (1938, 1939, 1956) explored the nature and function of olfactory conditioning as a type of latent learning.

Though latent learning has been given only limited attention (homing in limpets, Thorpe, 1956) among the molluscs, and no record has been published of olfactory conditioning in this group, it was thought that the possibility could not be ignored. Even without formal reinforcement of a selective response, it was possible that exposure (in a peripheral compartment of the olfactometer) to "pure" prey effluent for several hours might lower response thresholds. Since no way of avoiding this situation was found, experiments were designed for the specific purpose of investigating the effects of prolonged immersion in the chemical environments produced by prey organisms. These will be reported separately below (VIMS-36c, p 305 and Table 5).

The second study which raised the question of learning during experiments is that reported by Putnam (1962) and mentioned briefly above in connection with olfactometer design. Putnam found that 58.9% of his coleopteran subjects, in 20 successive, non-reinforced runs through a Y-maze, chose the same arm

of the Y at least 15 times. If their selective behavior had been random, only 4.14% would have done this. There was no group preference for either arm ( $r$  for left arm = 717;  $r$  for right arm = 743), so the possibility of there being a constant difference between the 2 arms, not detected by the investigator, must be discounted. Analysis of each individual's successive choices suggested that either the initial choice somehow "programmed" the later ones, or there existed in this sample the kind of genetic "right-handedness" or "left-handedness" reported for other organisms, particularly mammals (Morgan, 1951).

Because of the complex design of the olfactometer used in the present investigation, the latter eventuality would present no particular problem. But the possibility of programming is real. Chew & Eisler (1958) and Chew (1960), in studies of prey selection in "*Ocenebra japonica*", reported that snails tended to repeat their initial selections regardless of the prey being attacked at time of capture. Chew (1960) failed to subject this finding to more than a cursory analysis and discussion, but did present his original data.

To detect possible influence of early choices upon later ones, a consistency analysis was devised and applied to the results of experiment OI-3, which will be presented later (p 305-307).

#### g. Contamination of Control "Blanks"

Prey species employed in experiments to be reported here have in each case been the same as, or closely related to, species living outside the laboratories, near or upon the seawater system's pump intake. Hence it can be assumed that "natural" attractants in varying concentrations would be brought in with incoming seawater, and that this background of "chemical noise" in control compartments could easily diminish the significance of experimental results. Steps taken in each series to reduce such interference are described briefly below.

FWS. No preventive measures; seawater pumped directly from Chincoteague Bay into experimental apparatus. No controls run simultaneously with prey experiments.

IFR. Seawater intake pipes and non-return valves cleaned and flushed regularly. Control water run through absorbent cotton and activated charcoal.

OI. Seawater taken from a large reservoir filled at high tide and cleaned as needed. Experimental water passed through aeration column and shell fragment filter.

VIMS. Seawater system cleaned regularly and steamed. Experimental water processed through shell fragment filter, activated charcoal, aeration column, and aeration reservoir, as described elsewhere by Wood (1965a).

## IV. ORIENTATION IN COMPLEX CURRENTS

### 1. Introduction

The question of whether *Urosalpinx cinerea* can orient to its prey in the kinds of confused currents found in nature is of such fundamental importance that it should be presented first. The experiments in series FWS were conducted with a rather primitive olfactometer, later discarded for the very defect that made it suitable for the present question: current patterns were so confused that predator responses were usually quite attenuated.

### 2. Materials and Methods

Figure 6 is a diagram of the olfactometer used in series FWS. Water from a continuously flowing seawater system was introduced through a "T" into 2 prey compartments. (P, Fig. 6). A different prey was placed in each of these compartments. Water flowed out of the prey compartments into the predator compartment (U) by way of 6 acrylic plastic tubes 2.5 cm in diameter arranged to cause water currents to converge at point "X", which was also the center of the starting area for predators at the

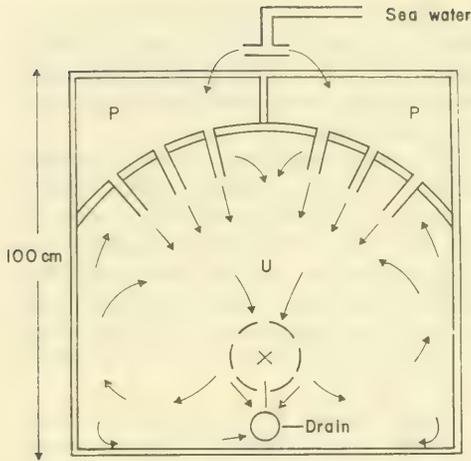


FIG. 6. Olfactometer used in FWS Series. Compartments *P*, separated by a partition, hold groups of prey animals. Seawater flows through prey compartments from "tee" fitting and out into predator compartment *U* through 2.5 cm diameter acrylic plastic tubes. Arrows in large compartment *U* indicate major current patterns as revealed in dye tests. At start of olfactometer test, snails are placed around point *X*, where current streams from the prey compartments converge.

beginning of a test.

The entire apparatus was coated with inert black paint and covered with an opaque lid so that tests were conducted in total darkness. Water flow into compartments "*P*" was equalized by volume/time determinations, and the apparatus was leveled before each test run. Fifty predators, of about equal size, were used each time, a run lasting 1 hour. Seawater temperatures during experiments ranged from 21-29°C. Salinities were determined with a hydrometer calibrated by the U. S. Bureau of Standards, and exhibited a range of 25-28 o/oo.

Predators were collected from sub- and intertidal seed oyster beds and brought to the laboratory within a few hours, where they were maintained without food in running water at ambient temperature and salinity. Prey test samples were young *Mytilus edulis* from

Ocean City and *Crassostrea virginica* from local seed beds (called "rocks" by Chincoteague oystermen). Prey groups were changed after each experiment, and approximately equal amounts of tissue, by whole live weight, were used in each test.

In experiments FWS-1,2, 328 predators were used in groups of approximately 50, with no prey added to the olfactometer. In FWS-5, 120 predators were tested against a mixture of both prey species in the 2 prey compartments. In FWS-6, 2,250 predators were tested in groups of 50 with mussels in one compartment and oysters in the other. As each group finished, those individuals which had successfully crawled through the acrylic tubes and into prey compartments were counted and then kept in a separate container; those which had remained in compartment "*U*" were similarly kept apart. In experiments FWS-7,8, predators were randomly selected from the groups which had made successful orientations in the previous experiments on the one hand, and from non-responders, on the other.

After each test, prey groups were removed from compartments "*P*" and the entire apparatus was scrubbed and flushed with seawater. Then fresh prey groups were so placed that they occupied the prey compartment opposite that the species had occupied in the preceding test. Several minutes' adjustment time was allowed for the prey to open and begin pumping before a new predator group was placed in compartment "*U*" around point "*X*" and a new test run started.

### 3. Results and Discussion

In this experimental series, predators displayed no significant preference for either of the 2 pelecypod species tested. The important question here is whether *Urosalpinx cinerea* can accurately locate prey when olfactory trails are confused by large eddies. Hence orientational behavior in an empty olfactometer was compared to that when prey compartments had mixed prey in them. Re-

TABLE 3. Predator responses in complex currents (series FWS)

Experiment	Predators Responding		X <sup>2</sup> P=	Predators Not Responding	
	N	%		N	%
FWS-1, 2 (no prey)	159	48.5	NS*	169	51.5
FWS-5 (with prey)	91	75.8	< 0.005	29	24.2
FWS-6	1134	50.4	NS*	1116	49.6
FWS-7 (400 responders from FWS-6)	324	81.0	< 0.005	76	19.0
(300 non-responders from FWS-6)	124	41.3	< 0.005	176	58.7
FWS-8 (448 responders from FWS-7)	339	75.7	< 0.005	109	24.3
(252 non-responders from FWS-7)	78	30.9	< 0.005	174	69.1

\*NS = not significant

P = probability

sults are shown at top of Table 3: with no prey, about as many snails moved into prey compartments as stayed outside in compartment "U". With mixed prey, a highly significant majority (75.8%) of the snails moved into a prey compartment.

Despite significant results from experiment FWS-5, I thought conclusions would be on firmer ground if additional tests were carried out with larger samples of predators. Also, it seemed necessary to ask whether failure of nearly 1/4 of the animals in FWS-5 to make successful responses was due to chance or to a characteristic of individual predators and would, in repeated tests, manifest itself in their continued failure.

Therefore a large sample of predators (2,250) was tested against the same 2 prey species, with combined results shown under FWS-6 in Table 3. Ratios of success and non-success were again about one-half, very close to those in FWS-1 and 2 in which no prey was used. The apparently normal distribution of response ratios was analyzed for significance by a t-test, which showed that it could have been due to chance.

I concluded that under the experi-

mental conditions described, and given a large enough sample, only about half the animals could locate prey successfully. This statement must be considered in the light of current patterns in the FWS olfactometer which were quite complex and, as shown in dye tests, were characterized by numerous large and small eddies (some of the more prominent are indicated by arrows in Fig. 6). The net effect of these subsidiary currents was to leave, at the end of the test, aggregations of predators in any or all of 3 primary areas: (1) beneath and around the 6 acrylic tubes (a near miss, presumably), (2) in either or both of the lower right and left corners of the drawing, and (3) against the curved wall of compartment "U," between the 2 sets of acrylic entry tubes. In the case of the 2 latter loci, aggregations could have formed as a result of predators' having followed subsidiary currents upstream (having missed the tubes on first try) to a point at which olfactory trails became multi-directional, totally confusing, and perhaps inhibitory of all directed movement.

In addition to the statistical success with which a large sample of predators could locate prey, there was the question

of whether in successive trials the same predators would continually fail (or succeed). In an attempt to determine this point, 700 predators from FWS-6 were retained, of which 400 had made successful responses. As shown in the lower part of Table 3, a majority of those which had been successful in FWS-6 continued to be so in FWS-7, and still so continued in FWS-8. A similar consistency was observed in the behavior of those which had failed in the first experiment. In all cases, differences were highly significant.

From this experiment it was concluded that the characteristic which attenuates statistically the degree to which predators successfully locate prey is persistent, at least throughout the period of time (about a week) covered by the experiment. Otherwise, re-run groups in FWS-7 and 8 would have tended to split their response ratios evenly in successive trials. The nature of the characteristic(s) involved is unknown, but the possibility of learning on the part of *U. cinerea* cannot be ignored. Snails which made it into the prey compartments were not restrained from predation, and ingestion of prey tissue might have constituted reinforcement. Further discussion of this point will be delayed until a more detailed foundation can be laid.

## V. THE EFFECTS OF PREVIOUS EXPERIENCE

### 1. Introduction

Two problems will be considered in this chapter. First, it will be established that *Urosalpinx cinerea* can in fact exhibit consistent prey preferences under suitable experimental conditions. Second, the effects of individual experience upon the predator's observed behavior will be examined.

Several different approaches to the latter problem have been attempted. (1) Response to a prey species selected in the laboratory was compared with response to that upon which the predator

was feeding at time of capture. (2) Predators in various stages of maturation were maintained upon single-species diets and then given opportunities to make selections in the olfactometer. (3) Chemotactic responses of "naive," newly-hatched *U. cinerea* were examined, prior to and after single-species diets. (4) Attempts were made to determine whether actual ingestion of prey tissue was necessary to establish a preference, or if it could be done by simply exposing predators to effluents from a single prey species, either in aquaria or the olfactometer.

The general purpose of this chapter, then, is to present and discuss evidence concerning plasticity of the predator's prey selection behavior.

### 2. Comparison of Natural and Laboratory Prey Selection

If there are persistent preferences in *Urosalpinx cinerea*, it should be possible to elucidate them at least to some degree by comparing natural and laboratory prey responses, providing it is reasonably certain that the predator was feeding upon only one species for some time prior to capture, and providing further that it does not feed upon other prey species following capture.

Comparisons of natural and laboratory prey selections in an extended series of experiments (IFR and VIMS) were fruitless primarily because predators were maintained too long after capture before being tested and secondarily because of the scarcity of nearby natural habitats in which 2 prey species were equally accessible but were also distinctly separated from one another in the intertidal zone. Both defects were corrected by discovery of the Wrightsville habitat, only an hour's drive from Morehead City.

#### a. Materials and Methods

Owing to unusual local conditions, the *Balanus* and *Crassostrea* zones at Wrightsville were remarkably distinct. About equal numbers of predators were

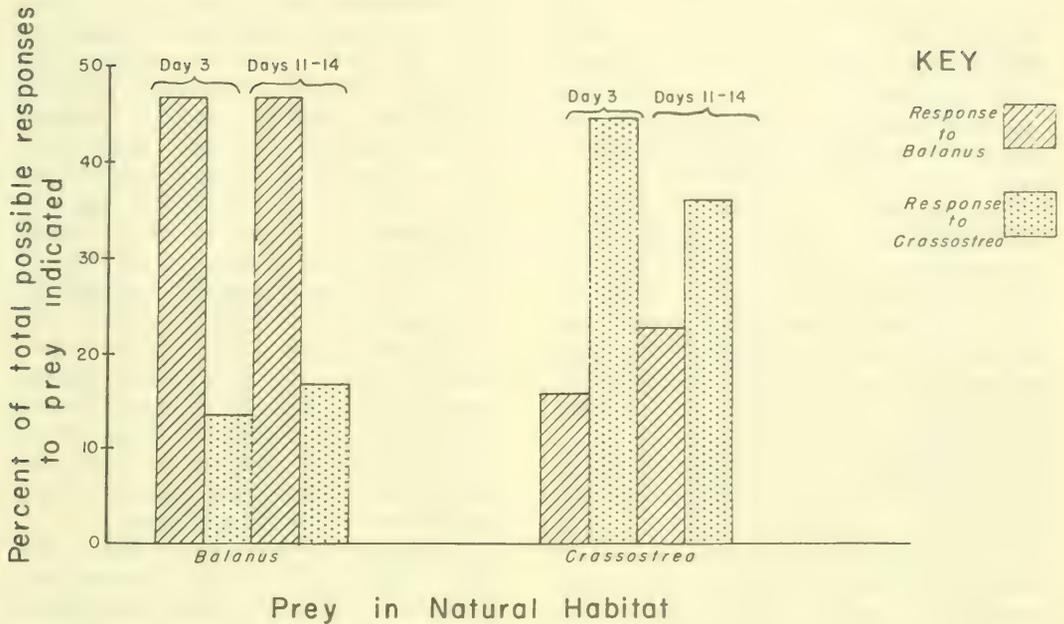


FIG. 7. Experimental prey selection responses of Wrightsville predators from 2 different faunal zones in Series IFR.

collected from each of the 2 prey species in their respective zones, returned to the laboratory on the same afternoon, in separate containers, and tested separately as soon as they could be measured and their sex determined (3 days).

#### b. Results

It has already been mentioned that many of the preference experiments, done with animals whose prey selections in nature were known, gave negative results (in fact, the snails indicated an almost uniform preference for barnacle effluents, regardless of their original natural prey). But the uniquely clear faunal zonation of the Wrightsville habitat permitted rapid collection and testing of 2 predator groups from known prey, the first experiments terminating on the 3rd day after collection. As shown in Fig. 7, responses by predator groups closely reflected their zonal disposition in the habitat. Further, this correspondence persisted through the 14th day

after collection, though there was a tendency, especially in the oyster-feeding group, toward attenuation of the of the difference.

### 3. The Effects of Controlled, Single-species Diets Upon Olfactory Behavior

#### a. Introduction

This section deals with 2 main questions: (1) can "natural" preference of predators be enhanced or intensified by ingestion of only preferred prey, and (2) can "natural" prey preferences be changed by feeding predators a different prey? Should both or either of these questions be answered in the affirmative, it is reasonable to ask next whether such intensification or reversal may vary with the maturity of the predator. In other words, is the selective behavior of an adult predator more or less plastic than that of a younger one? Finally, a crucial question must be asked: do newly hatched, presumably "naive" *Urosalpinx*

*cinerea* demonstrate the same kinds of orientational movements to prey effluents as adults? Are there "innate" prey preferences? If so, can these be demonstrated by olfactometric technique before the young snail has had an opportunity to feed upon specific prey?

These questions have been recognized as fundamental since the inception of the investigation. The present series of experiments represents one of many attempts to secure such information; but all efforts prior to the VIMS-36, 37 series ended in failure, due partly to high mortality rates amongst controlled-diet young predators, and partly to the enormous amount of time-consuming labor required to rear young *U. cinerea* under rigorously controlled dietary conditions. Repeatedly, in successive attempts to execute a basically simple experimental design, I found that it was possible to rear young only on barnacle diets: mortality was excessive amongst groups fed only newly-set mussels or oysters. It is, of course, not difficult to rear the young on natural substrates, but in this investigation experimental protocol required presentation of only one species of potential prey to the young snails.

#### b. Materials and Methods

Adult predators (longer than 15 mm and hatched prior to preceding summer; IFR-3b). Natural rocks from Shark Shoal were placed in running water aquaria together with about 100 Shark Shoal predators. Mixtures of barnacles and oysters were attached to these rocks in a ratio of about 10:1. After prey attacks had begun, the predators were removed and started on controlled diets of the prey species they had selected from the natural rocks. Control groups consisting of half the predators from each "rock" group were maintained without food. After 9 days, the 4 groups were tested in the olfactometer against effluents from the 2 prey species.

Juvenile predators (6-15 mm; hatched in preceding summer; VIMS-36a). Juve-

nile predators were collected from West Haven *Balanus balanoides* populations on 1 July 1963. They were divided into 3 equal groups, one of which was fed nothing but young *B. eburneus* collected on asbestos plates, one upon young *Crassostrea virginica* spat cultured on clean shell in the laboratory, and one maintained as a control in an empty aquarium. First tests were carried out on the 6th day of the diet and upon several irregularly spaced days thereafter up to and including the 16th. On that day, juvenile predators were given reversed diets: those which had been fed oysters were given nothing but barnacles, while those which had been eating barnacles were given nothing but oysters. The control group was discontinued.

Young predators (1-6 mm; VIMS-37). Egg capsules of *Urosalpinx cinerea* collected on 1 November and 6 December 1964 at Ocean City were kept cooled (6-10° C) until they could be sorted by embryonic stage. They were then gradually warmed to 25° C, and allowed to hatch naturally or the capsules were opened under a dissecting microscope. The unfed (except for probable browsing on micro-organisms) young were held for various time periods pending accumulation of sufficient numbers for testing. It was not practicable to maintain the various age groups separately prior to testing and feeding.

The diet prey groups for this experiment consisted of young *Balanus* spp. collected on asbestos plates and cleaned under a dissecting microscope of all other visible material; small *Crassostrea virginica* spat (3-10 mm) either cultured on clean oyster shell in the laboratory or taken alive from barnacle plates; and young (3-6 mm) *Mytilus edulis* collected on 1 November 1964 at Ocean City. All prey animals were maintained in running seawater until controlled feeding periods began. Shell surfaces were always inspected for fouling before being placed with young *U. cinerea*.

Young predators were maintained with

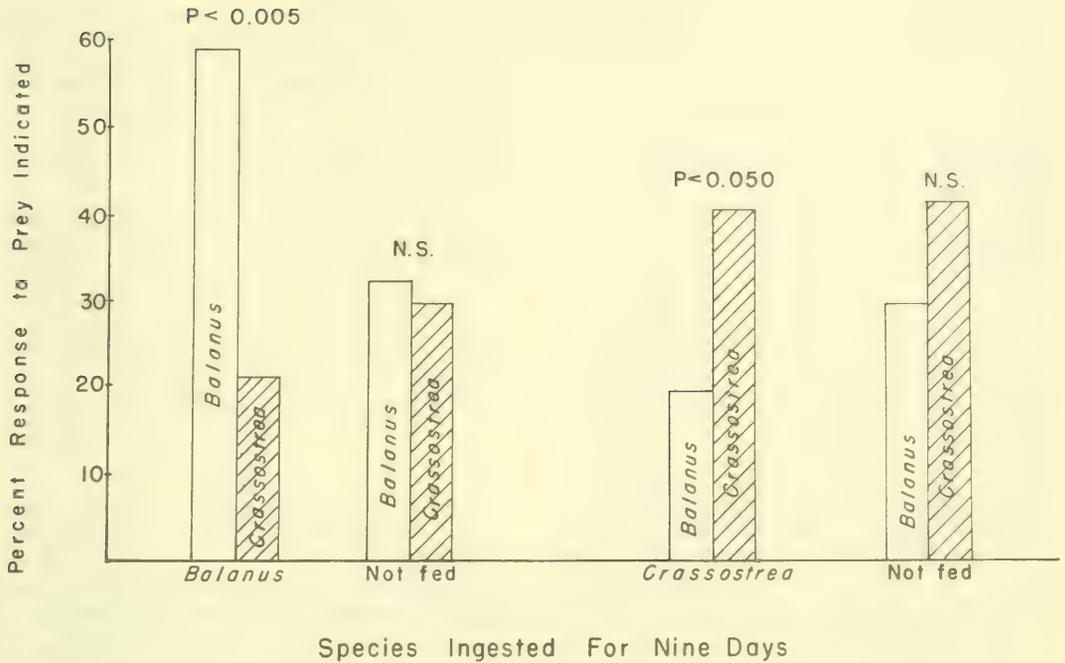


FIG. 8. Experimental prey selection responses of adult predators previously maintained on a single-species diet for 9 days, and of their unfed controls. Series IFR-3b. (P = probability; N.S. = not significant statistically).

their respective prey diets in tightly-covered, 4-liter, polyethylene containers through which flowed filtered seawater at the proper temperature. Except for one early group which was kept for a time at 18° C, temperature for all experimental containers was about 25° C, the temperature used during olfaction experiments. Maintenance temperatures were controlled by mixing cold seawater in a manual valve with heated seawater from an exchange linked to the building's oil-burning hot water system. Failure of this system on a few occasions subjected the animals to temporary temperature changes. In each case, however, they were kept at the stated temperature for several days thereafter before being tested.

As mortality claimed young predators in each of the separate groups, groups were consolidated to save space and to keep experimental numbers as large as possible. Further, individual identifi-

cation of predators so small was not possible, and duration of diet for each group can only be estimated. This change of procedure, however, proved immaterial since there was no noticeable difference between test responses of young predators in early, as contrasted with late, periods of controlled diet.

Throughout these experiments both predators and prey were kept in running seawater at a controlled temperature of about 25° C. During winter, natural planktonic food was augmented by addition of mixed cultures of algae (chiefly diatoms). Both predators and prey showed some growth and suffered little mortality during the experiment.

### c. Results and Discussion

Results of the first feeding experiment, with adults (IFR-3b), are shown in Fig. 8. While the majority of responses of barnacle-feeders were to barnacle effluents, and statistically highly sig-

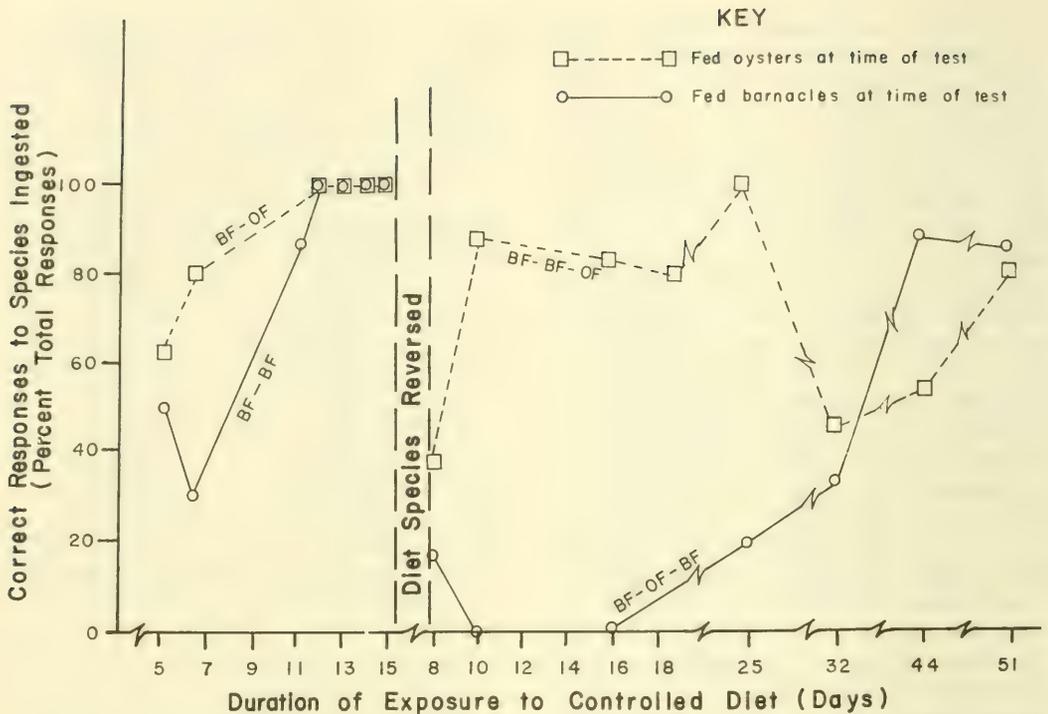


FIG. 9. Prey selection responses after ingestive conditioning of West Haven juveniles (total height 6-15 mm), all originally feeding on barnacles, in 2 controlled diet sequences (VIMS-36a). The sequences are marked to show order of diets: BF = barnacle-fed, OF = oyster-fed. "Correct" prey is that offered last. Note scale discontinuities in time (horizontal axis).

nificant by chi-square test ( $P < 0.005$ ), responses of the barnacle control group were about even. Results of the oyster-feeder tests were comparable: those which had been allowed to feed upon oysters preferred oyster effluents, though by a less significant margin ( $P < 0.050$ ), while the oyster control group's selection was more evenly divided (not significant).

The results of this preliminary experiment indicate that selective tendencies of *Urosalpinx cinerea* are intensified by allowing them to ingest preferred prey. This treatment is designated as *ingestive conditioning* to distinguish it from a similar process, *olfactory conditioning*, described by Thorpe & Jones (1937) for insect larvae. That ingestive conditioning depends upon the actual intake of prey tissue rather than upon ex-

posure to the odor of the prey (as in the case of the Thorpe & Jones investigations) will be shown in a later experiment (VIMS-36c, p 303, Table 5).

The next inquiry concerned the extent to which ingestive conditioning can *reverse* previously demonstrated preferences. The results of experiments with juveniles from West Haven (VIMS-36a, p 296) are shown in Fig. 9.

West Haven juveniles, which in nature had fed upon *Balanus* during spring and early summer of the year in which they were collected, had in pre-diet tests shown a strong preference for the same genus. As already indicated, one group was fed on *Crassostrea*, another on *Balanus*, and a third remained unfed. On days 5 and 6 of the diet, tests showed that *Crassostrea* was becoming highly attractive to the group fed on oysters,

TABLE 4. Effects of single-species ingestion on responses of young<sup>a</sup> *Urosalpinx cinerea* from Ocean City (VIMS-37), to effluents from prey compartments

Diet fed	Duration (days)	Percent total responses to					Total possible responses
		<i>Balanus</i>	<i>Crassostrea</i>	<i>Mytilus</i>	Control	None	
Not fed		9.0	3.7	4.3	1.2	81.7	322
Fed <i>Balanus</i>	15-125	25.4	2.3	4.6	4.9	62.7	263
Fed <i>Crassostrea</i>	15-120	10.8	3.1	1.0	2.1	83.0	194
Fed <i>Mytilus</i>	48-135	4.0	4.0	5.6	13.7	72.6	124

<sup>a</sup>young = hatched, or removed from eggs in protoconch stage in laboratory, length ca. 1 mm, but no greater than 6 mm.

but not at all to the 2 other groups. After about a week the responses of the 2 diet groups to the prey species each was offered did not differ significantly: the tests conducted on days 12-15 showed that ingestive conditioning was apparently complete. Statistical analysis showed that predators fed upon either diet responded to the ingested species significantly ( $P < 0.005$ ); unfed controls, though they continued to select effluents from barnacles, did not do so as pronouncedly ( $P < 0.025$ ) as did the group fed on barnacles.

Having experimentally induced a state of ingestive conditioning in the predators, reversal was attempted by changing diets of both predator groups. The results of olfactory tests conducted during the second controlled diet suggest that there may be a tendency on the part of juvenile *U. cinerea* to resist a secondary reversal. Those which had gone through diet sequence *Balanus-Balanus-Crassostrea* responded to "correct," i.e., last, prey (*Crassostrea*) significantly ( $P < 0.005$ ) on days 8-25, but not on the days 32 and 44. Those which had had diet sequence *Balanus-Crassostrea-Balanus* did not express a significant preference at first, but did ( $P < 0.005$ ) later, and for the "correct" prey, *Balanus*. In both series, predator activity rate was low.

The last of the 3 selected age groups

to be examined were the recently hatched young (maximum size of 6 mm). Results of these experiments (VIMS-37) are presented in Table 4.

Two questions were asked by these experiments: first, do "naive" *Urosalpinx* exhibit positive orientational responses to prey effluents, and if so are these made selectively? Second, are very young snails subject to ingestive conditioning?

Hence in the first examination of VIMS-37 results it should be determined whether distribution of responses amongst the 3 prey and 1 control compartments differed significantly from chance (= 25% to each). Since total responses observed were 59, chance alone would predict 14.75 responses to each of 4 compartments. In the case of 2 of these, responses did not differ significantly from chance (12 to *Crassostrea*, 14 to *Mytilus*). But in the other 2 (29 to *Balanus*, only 4 to control) the difference is highly significant (sum of chi-squares = 21.098, degree of freedom = 3,  $P < 0.001$ ).

In considering the extent to which the response distribution of unfed snails differs from that of diet groups, it is convenient to compare statistically each combination of pairs (NF x BF, NF x OF, NF x MF, where NF = not fed, BF = barnacle-fed, OF = oyster-fed, MF = mussel-fed). Computation of

chi-square contingency tests for each pair revealed that all pairs differed significantly ( $P < 0.001$ ) in the response of their components except one, NF x OF. In other words, these 2 groups made essentially similar responses to the same prey effluents, indicating that the olfactory behavior of the OF group had not been altered by feeding them *Crassostrea*. Both groups responded most frequently to *Balanus* effluents, as did also BF young. Indeed, response frequency of the BF group to preferred prey was greatly increased during the controlled diet. Of all groups, only MF young failed to choose *in majoris* the barnacle effluent compartment; inexplicably, they also did not respond very often to *Mytilus*, but rather to the control compartment.

It will be noted that only a minor fraction of test animals made responses of any kind in these tests. Mean response ratio was 24.8% for the 4 groups, considerably less than those seen in similar experiments with juvenile and adult *Urosalpinx*. In fact, failure of so many of the young snails to respond to any prey odors whatsoever is reminiscent of experiments with unfed control groups (p 303-305). Thus it may be asked whether those predators of the above series that were given opportunities to feed upon a single species did in fact ingest their tissues. Since careful records were kept, where possible, of direct evidence of attack and ingestion, this question can be answered positively for most diet groups whose behavior has been discussed. The exception, for the following reason, is in the case of the barnacle-fed groups. It is true that *Urosalpinx* leaves tangible evidence of its attack upon most prey: a small, slightly conical hole bored through an exposed valve. These holes can be counted and their ratio to total number of dead prey calculated. If a hole is bored through compartmental plates of barnacles, this can also be taken as direct evidence of predation. But if the snail adopts the more efficient

mode of entry through the opercular aperture, it can kill and clean a barnacle without leaving a clue. This can be especially damaging to quantitative analysis when other predators such as the flatworm *Stylochus ellipticus* (Girard), which get into experimental chambers as larvae and which also leave no marks of attack (Wood & Deibel, unpubl.), can account for a considerable but unknown fraction of dead barnacles. It is therefore necessary to go to indirect evidence for ingestion of barnacles. In the present work, growth rates (where available) of barnacle-fed predators were compared to those of unfed controls, and a significant difference between groups was accepted as evidence of feeding.

With these qualifications in mind, evidence of ingestion can be reviewed for each experimental group:

IFR-3b (adults). No growth observations were made of barnacle-feeders; *in situ* observations of both barnacle and oyster feeding groups confirmed that attacks were in progress. At the end of the feeding period, 21 dead oysters were counted, each with at least 1 bored hole, while more than 100 barnacles had been killed and cleaned, probably by *Urosalpinx cinerea*.

VIMS-36a,b (juveniles). Barnacle-feeders showed significant growth during the first 20 days on diet, as compared to that of unfed controls, whose size-class frequency distribution did not change and was indistinguishable therefore from that of the originally collected sample. Bored holes in compartmental plates of barnacles were very rare. Oyster-feeders similarly increased in size during the 20 days, and left behind them many bored valves. During the second reversal of diet, *in situ* observations confirmed feeding, but no quantitative evidence was recorded.

VIMS-37 (young). Though precise information is lacking, repeated microscopic examination of dead pelecypod prey showed that they were being perforated and consumed. Whether the youngest snails perforated the larger

prey organisms is not known, as predators of several size classes were kept together. This much was observed: the higher mortality rates among young predators were in the 2 pelecypod diet groups. These results are consistent with those from my previous attempts to rear *Urosalpinx* upon single-species diets: success has been achieved only by feeding them small *Balanus*. Especially in the case of oysters was great difficulty experienced. While very small *Mytilus* were available in great quantity, young *Crassostrea* spat, produced in the laboratory by artificial fertilization procedures, were harder to obtain at the time of the year when the experiment was performed. Therefore, the oysters frequently seemed too large and thick-shelled for the young predators; many living *C. virginica* were seen with partial holes bored in their shells, suggesting the premature death of young borers.

On the basis of experiments reported here, there is good reason to believe that most predators given opportunities to feed upon single prey species did in fact complete successful attacks. The only group about which serious doubt exists is oyster-fed young, yet some of these were observed consuming prey.

#### 4. Responses of Conditioned Snails to Odor of One Species at a Time

##### a. Introduction

A fundamental question raised by ingestive conditioning experiments concerns the frequency with which olfactometric responses are elicited by effluents from prey species to which predators have *not* been conditioned. We have seen that a predator, given a choice between 2 prey effluents, will with increasing frequency select that to which it has been conditioned. But what if the other, "unconditioned," effluent is the only one present in the olfactometer: will the predator respond at all? While failure to respond cannot be taken directly as evidence of failure *to perceive*, such a conclusion would be

strongly favored.

In previous experiments, it had not been possible to obtain adult or even juvenile predators that had not had access to one or more of the major prey species. In experiment VIMS-36a, for example, barnacles were the native food of both experimental groups. For several years I had searched the U. S. East Coast for a population of *Urosalpinx cinerea* for which neither barnacles nor oysters constituted normal prey, so that this experiment could be done. To my chagrin, such a population was located by a student (Roberts, pers. comm.) in a subtidal *Zostera* bed in front of my own home.

##### b. Materials and Methods

Experimental animals (VIMS-38) were collected in April 1966 from subtidal *Zostera* beds adjacent to Locust Point, Saddlers Neck, Gloucester County, Virginia, in the weakly estuarine Northwest Severn River. They were mostly adults, though their modal size class was 9-10 mm (previous observation had confirmed the small size of adult individuals in this population). Numerous examinations during the summers of 1965 and 1966 convinced me that the primary natural prey of the Locust Point population was the slipper limpet *Crepidula convexa* Say, which occurred there in great abundance. Other potential prey species were the barnacle *Balanus improvisus*, the gastropods *Anomia* (= *Cavolinia*) *simplex* and *Bittium* sp., and a small epiphytic mussel (probably *Amygdalum papyria* Conrad), but these were either (in the case of *Balanus improvisus*) rare in occurrence or there was little or no field evidence of attack by *U. cinerea*.

In brief, the Locust Point predator population had apparently not been conditioned to either of the chief prey species hitherto discussed in this work.

Nearly 400 *U. cinerea* were collected, brought to the laboratory, and tested in the olfactometer within hours. After the initial test, they were randomly assigned

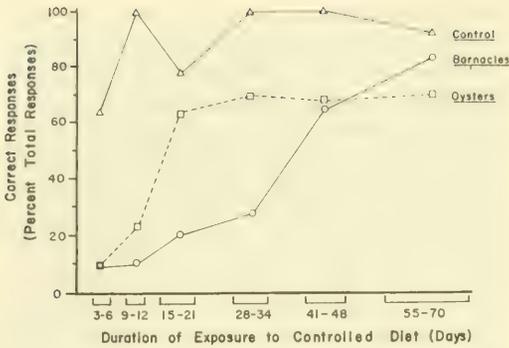


FIG. 10. Prey selection responses during progressive ingestive conditioning of Locust Point adults (VIMS-38) to barnacles and oysters. The predators' natural diet did not include either of these prey animals. The "control" group of predators was maintained throughout the experiment upon the eelgrass *Zostera* and its many accompanying epiphytic invertebrates, chiefly *Crepidula convexa*. The curves showing experimental responses of the barnacle- and oyster-fed snails are reproduced in FIGS. 11 and 12, together with additional corollary information.

to 3 diet groups: young *Balanus* spp., cultured *Crassostrea* spat, and a control consisting of material from the natural habitat (*Zostera* plus epiphytic flora and fauna). Barnacle and oyster diet specimens were obtained and treated as described above in experiment VIMS-36a. Detailed feeding observations were recorded throughout the experiment. In other respects, experimental methods were as already described, except that all 3 predator groups were tested against each of the 2 prey species separately instead of simultaneously.

### c. Results and Discussion

Initial olfactometer tests, made on the day of collection, confirmed suppositions made above about the natural prey of Locust Point predators: of the 384 tested, less than 1% responded to either barnacle or oyster effluents, but 5.5% selected one or another of the "control" compartments. It should be recalled at this point that "control"

water consisted of seawater pumped from the York River estuary and through a modified controlled conditions system (Wood, 1965a) before flowing into the several olfactometer compartments from a common reservoir. The main seawater pump intake was situated about 40 m from the edge of an extensive *Zostera* community, the fauna of which was markedly similar to that at Locust Point. Hence it is not unreasonable to assume that external metabolites produced by the nearby *Zostera* community, and pumped into the laboratory system, were quite similar to those of the predator population's original habitat. Such an interpretation was further supported by subsequent tests of the 3 controlled diet groups, partly shown in Fig. 10. The control group, maintained in an aquarium with *Zostera* and associated biota, did not exhibit a typically dramatic increase in "correct" responses to control compartments since the ratio of "correct" responses in the first time period (3-6 days) was already 63%. In the terminal period (55-70 days) it was 92%, and intervening ratios fluctuated between 78 and 100%. Both oyster-feeders and barnacle-feeders, on the other hand, did exhibit a pronounced incremental tendency, commencing with 9% "correct" responses in the first period and terminating with 70 and 83% "correct" responses, respectively. The "control" responses to seawater (not illustrated) of BF and OF diet groups showed the opposite, decreasing, tendency: barnacle-feeders started with a 90% response ratio and terminated with 12%; oyster-feeders began with 86% and ended with 29%. Of considerable significance, in my opinion, is the fact that in the case of both diet groups, increase in "correctness" of response was primarily at the expense of "control" (*Zostera* community) responses, and not "incorrect" prey effluent responses, again suggesting that barnacles and oysters were not part of the Locust Point snails' natural diet.

Evidence of actual ingestion of prey

during the experiment is offered in Figs. 11 and 12, in the form of dotted vertical bars which denote the ratio between the number of predators observed actually feeding and the total number of predators living in the aquaria. This ratio is corrected for variations in duration of the periods of observation. Curves from Fig. 10 are superimposed upon Figs. 11 (barnacle-feeders) and 12 (oyster-feeders), respectively, so that "correct" olfactory response ratios can be compared to feeding activity and also to rate of oviposition (open vertical bars), the latter process being commonly accepted as an index of health in captive invertebrates. Two factors are apparent in these results. (1) Feeding activity was greater and its onset was quicker in the oyster-feeding (Fig. 12) than in the barnacle-feeding (Fig. 11) group. This is reflected in both the more rapid conditioning rate of oyster-feeders (compare both response curves in Fig. 10) and in their greater fertility. (2) In both groups, feeding and oviposition increased together to a peak in the 3rd time period (days 15-21) and then declined as the summer season waned, a trend widely observed in natural populations of *Urosalpinx cinerea* (Carriker, 1955) and often seen in our laboratory populations.

The more rapid conditioning of *U. cinerea* to oyster effluents, observed in both phases of experiment VIMS-36a (Fig. 9) and in the present experiment (VIMS-38), is thought to be due to a difference in required attack techniques, which resulted in the more rapid commencement of feeding upon young oyster spat than upon barnacles. As has been stated above, an experienced predator can successfully complete an attack upon a barnacle in less than an hour by simply inserting its proboscis between the barnacle's opercular plates. Boring a hole through thickened compartmental plates of *Balanus*, on the other hand,

may require more time and energy than boring a hole through the thin valve of an oyster spat of the same or even slightly greater basal diameter. Examination of attacked barnacles revealed that Locust Point *U. cinerea* apparently did not often employ the opercular entry method, but instead bored holes through opercular or compartmental plates. Frequently these holes were incompletely bored at the time of observation.

Whether or not *U. cinerea* chemoreceptor surfaces are actually sensitized by ingestive conditioning is a question that must await application of electrophysiological techniques (commenced summer 1967), but experiment VIMS-38 supplies circumstantial evidence that some kind of sensitization may occur. The alternative explanation of these results requires that a quasi-rational "decision," in favor of one prey effluent over another, be made as a function of some ganglionic process when the snail arrives at the point-of-choice in the olfactometer.

## 5. Effects of Long-term Exposure to Prey Odors

### a. Introduction

The concept of "olfactory conditioning" was placed in the literature by Thorpe & Jones (1937). It is now reasonable to ask whether ingestive conditioning is not really the same thing as that which Thorpe & Jones described in insects. In addition to theoretical considerations, a quite practical reason exists for making a clear distinction between the 2 types of conditioning: if predatory gastropods are influenced by what they smell, might not their responses to later olfactometer tests be modified by exposure during an earlier test to concentrated prey effluent in the selected compartment? Two experimental approaches were adopted. The first was a straightforward experi-

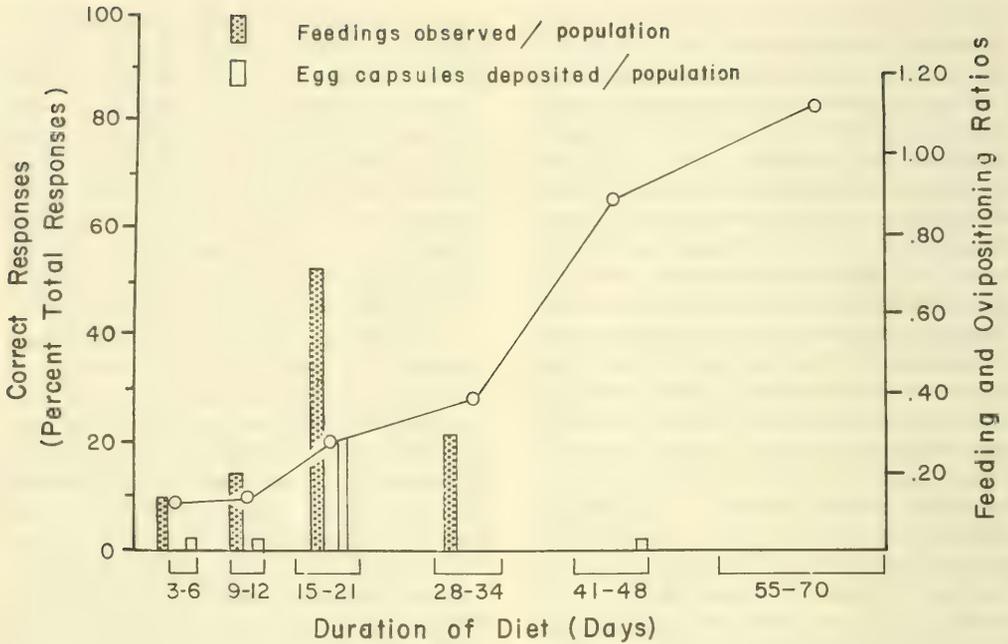


FIG. 11. Feeding and egg-laying activity of Locust Point adults during period of ingestive conditioning to barnacles (VIMS-38). Dotted bars represent the ratio between the number of snails actually feeding and the total number present in the group. Open bars represent a similar ratio for egg capsules deposited. In both cases, figures are corrected for varying time periods covered by the observations. The superimposed curve for "correct" responses is from FIG. 10.

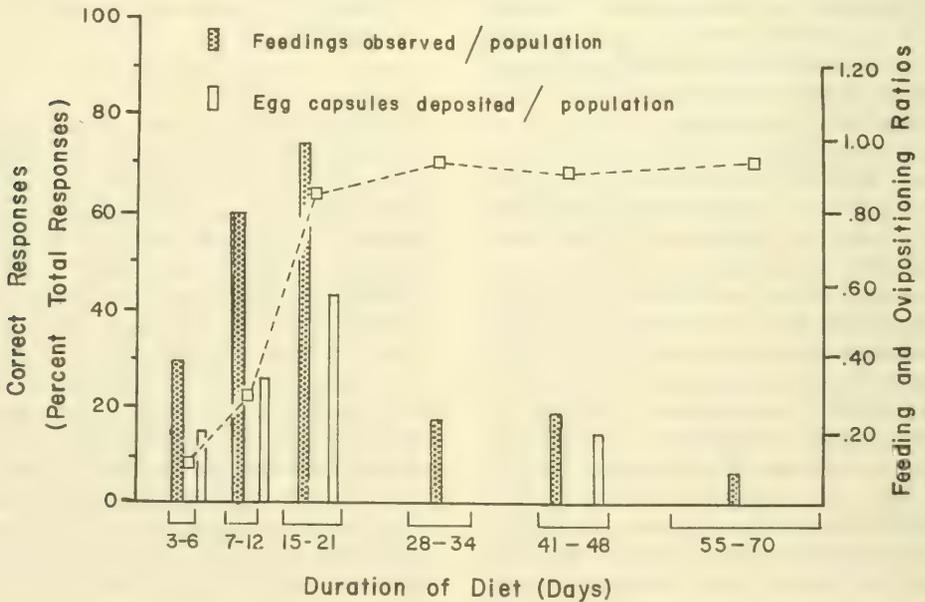


FIG. 12. Feeding and egg-laying activity of Locust Point adults during period of ingestive conditioning to oysters (VIMS-38). Bars represent activities as described in caption for FIG. 11 on the opposite page. The superimposed curve for "correct" response is from FIG. 10. In comparing FIG. 12 to FIG. 11 (opposite), note correspondence in each case between onset of reproductive and feeding activities and the rapidity of ingestive conditioning as indicated by the shape of the response curve.

mental design which will be described immediately below; the second was a *post-hoc* statistical analysis presented later in this section.

## b. Materials and Methods

Experimental subjects for VIMS-36b were Nobska juveniles collected on 30 November 1963 and maintained without visible food and at ambient salinity and temperature until the beginning of the experiment. When controlled diet aquaria for VIMS-36a were set up and that experiment started, perforated polyethylene boxes containing samples of Nobska predators were placed in the aquaria with prey (or in an empty control tank) and with the freely moving West Haven predators. When the latter were tested for response to prey effluents, so were the Nobska animals, under the same conditions.

## c. Results and Discussion

As shown in Table 5, there was no significant difference between those predators exposed to the concentrated odor of *Balanus*, their natural food, and those exposed to seawater alone. Those exposed to odor of *Crassostrea* made slightly fewer (statistically not significant) responses to *Balanus* effluents, but made no responses to oyster effluents or to controls. Comparison of these results with those for unfed control juveniles (pp 298-299) of VIMS-36a indicate that no essential difference exists between responses of that group and any olfactory exposure group: all 3 groups, in fact, displayed a significant preference for barnacle effluents, a consistency which is not surprising since none of

them was known to have fed prior to the experiment upon prey other than *Balanus*.

## 6. Effects of Short-term Exposure in Olfactometer

### a. Introduction

There are at least 2 ways of assessing the influence of early olfactometric choices upon those made later in a continuing series of tests. First, there could be a simple correlation between initial and later choices. But a better method is to determine degrees of choice consistency in a series of tests. Does the choice in Test 1 positively affect the choice expressed in Test 2, etc., or is observed consistency (to be distinguished from *preference*) due entirely to chance? These questions are not just hypothetical, as Chew & Eisler (1958) and Chew (1960) reported without explanation that "*Ocenebra japonica*" tended to repeat its initial experimental prey choice, regardless of the species attacked in its natural habitat, and Putnam (1962) reported that *Aleochara*, a coleopteran insect, repeated its initial choices of either right or left arms of a Y-tube. Should such a tendency be operating in *Urosalpinx cinerea*, it would be best to know about it before interpreting prey selection results.

### b. Materials and Methods

The only test sequence of sufficient length available for the present analysis was OI-3, which will be described in greater detail in another paper. Suffice it here that the experiment was run with standard techniques (described under General Methods, p 283 et seq.), but at a graded series of controlled tempera-

TABLE 5. Effects of exposure on juvenile<sup>a</sup> *Urosalpinx cinerea* from Nobska (VIMS-36c) to effluents of one prey species<sup>b</sup>

Exposed		Percent total responses (pooled) to				Total possible responses
Days	to effluents from	<i>Balanus</i>	<i>Crassostrea</i>	Controls (2 compartm.)	None	
16	<i>Balanus eburneus</i>	21.5	0.7	0.7; 1.3	75.2	149
17	<i>Crassostrea virginica</i>	13.0	0	0; 0	87.0	108
17	seawater alone	26.8	0	0.7; 0	72.5	149

<sup>a</sup> 6-15 mm

<sup>b</sup> Prey in nature = *Balanus balanoides*

tures. The temperature series was carried out in sequential order, the lowest temperature tests being done first, and so on. The controlled temperatures employed were 16, 20, 25, and 30°C. The prey used were *Crassostrea virginica*, *Balanus eburneus* and *Brachidontes exustus*, all from Nassau Sound. Predators were collected at West Haven, Shark Shoal, and Nassau Sound. Samples of 20 animals from each habitat were tested in a series of 14 runs; those which failed to make criterion response in at least 10 of 14 runs were eliminated from the analysis. Since responses to the 2 pelecypod species did not differ significantly, these were lumped together and predator's selections were therefore coded "b" for "barnacle," and "p" for "pelecypod." Individual predators were numbered for identification and their responses recorded at the end of each run.

As the response ration  $b/b+p$  differed significantly between the first 2 (at 16°C) and 12 subsequent runs, it would not be proper to subject the *group* responses to consistency analysis; rather, the consistency of individuals' responses should be examined. The method by which this was accomplished is described below.

An empirical measure of consistency was derived from the series,

bbbb, pppp,  
pbbb, bbbp, bppp, pppb,  
bpbb, bbpb, pbpp, ppbb,  
bbpp, ppbb,  
bppb, pbbp,  
bpbb, pbpb,

where run sequences of 4 selections are arbitrarily ranked from top to bottom in order of decreasing consistency. Consistency has 2 components: (1) predominance of one prey type over another; (2) number of *runs* of either *p* or *b*, i.e., the number of times a prey "preference" is reversed during a sequence. Preference for a prey type can be expressed as a simple ratio,  $P = x/x+y$  where *x* is the predominant choice in a sequence, and *y* is the other. Since consistency decreases as the number of runs (*r*) increases, this element can be introduced as  $C = P/r$ , where *C* is an index of *consistency*.

Now let us apply the index *C* to the ranked series of sequences shown above.

Selection Sequence	P	r	C
bbbb, pppp	1.000	1	1.000
pbbb, bbbp, bppp, pppb	.750	2	.375
bpbb, bbpb, pbpp, ppbb	.750	3	.250
bbpp, ppbb	.500	2	.250
bppb, pbbp	.500	3	.167
bpbb, pbpb	.500	4	.125

P = Preference; r = runs; C = Consistency

In a sequence of 14 selections (resulting from a total of 14 runs in experiment OI-3), the range limits of  $C$  can be shown to be 0.036 and 1.00, the former indicating least consistency: *bbpbpbpbpbpbpb* or *pbpbpbpbpbpbpb*, and the latter indicating perfect consistency: *bbbbbbbbbbbbbb* or *pppppppppppppp*.

As the length of a sequence increases, the lower limit of  $C$  can approach, but never reach, zero.

The index ( $C$ ) was applied to all sequences in experiment OI-3, of length of 10 or greater (41 out of 60 choice sequences, one for each snail), so that values of  $C$  derived therefrom could be compared statistically to the values of  $C$  derived from application of the index to analogous chance sequences.

These chance sequences were obtained from a statistician's "random generator," a small plywood box from which protruded a clear plastic runway just large enough inside to accommodate a single file of marbles. In normal usage, the box is partly filled with marbles of 2 colors (in equal numbers), shaken, and then placed on its side, to permit marbles to roll down the plastic runway, where they are counted.

In the present case, the "population" of marbles was deliberately biased, so that each population contained blue marbles in direct proportion to the number of choices of *preferred* prey selected, and red marbles in proportion to choices of *non-preferred* prey in a given predator sample. Thus the analog for the predator sample from the West Haven contained 88% blue marbles and 12% red marbles, since those predators had made, in experiment OI-3, a total of 162 choices for preferred prey and 23 choices for non-preferred prey. The function of this deliberate bias was to eliminate the effect of *preference* from the analysis of *consistency*. A table of random numbers, which has a roughly even distribution of odd and even numbers, yields sequences whose con-

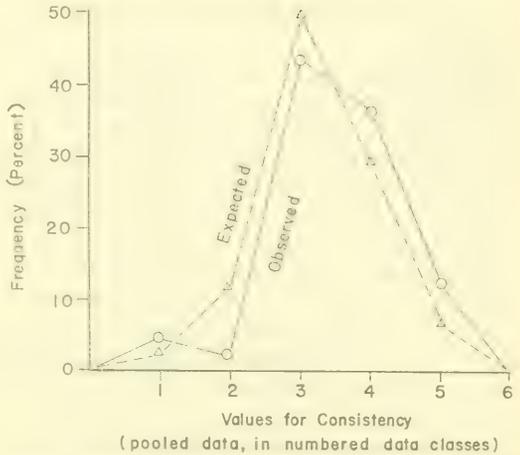


FIG. 13. Correspondence of expected and observed values of the consistency index  $C$ . *Expected* values were derived from chance sequences of marbles of 2 different colors in a statistical "random generator." *Observed* values were derived from prey selection sequences by *Urosalpinx* in Series OI-3. The congruence depicted above suggests strongly that internal consistency within a sequence of prey selection does not differ significantly from chance.

sistency differs significantly from that shown by *Urosalpinx cinerea*, but this difference is not primarily due to predator attributes, but rather to the decrease in the numerator of the expression  $C=P/r$ , produced by the non-preference of random numbers for either odds or evens.

The analogous nature of the marble sequences was carried one step further: the length of each predator's sequence of prey selections (between 10 and 14) was duplicated in its corresponding marble sequence.

In this way, 4 analogous series of marble color sequences were produced to simulate each prey choice sequence. The values of  $C$  for these sequences were compared to the value of  $C$  for the actual prey selection sequences. Significance of difference between the 2 members of each sequence pair (prototype and analog) was determined by  $t$ -test.

### c. Results and Discussion

The distribution of values of the consistency index *C* in the prey selection and marble color sequences showed no significant difference; the frequency distribution curves for data classes can be seen in Fig. 13 and their close correspondence noted.

In other words, consistency of predators' responses in OI-3 did not differ from chance (assuming a biased sample, with barnacles preferable to pelecypods). This, in turn, suggests strongly that no one response, whether in the beginning or near the end of a sequence, necessarily influenced subsequent responses. If this proposition is acceptable, responses to prey effluents should be regarded as an expression of what might be called "latent" preference, either conditioned or natural, and not the result of olfactory conditioning from a previous exposure to the *same* effluents in the olfactometer.

#### 7. Summary of Ingestive Conditioning Experiments

Evidence has been offered in this chapter which confirms the general hypothesis that expressed preferences for specific prey by *Urosalpinx cinerea* are at least partially the result of ingestive conditioning. This process has been defined as a modification of the predator's responses to prey effluents induced by maintenance upon single-species diets.

Results of conditioning experiments with adult Locust Point predators in experiment VIMS-38 were markedly similar to those given in Fig. 10 (VIMS-36a) for juveniles for the period *after* reversal of diets. In both cases, necessary conditioning periods were longer than the time required for initial conditioning of juveniles. Further, snails fed oysters in experiment VIMS-38 were conditioned more rapidly than those fed barnacles which suggests some carry-over from one molluscan prey (*Crepidula*, in native habitat) to another

(*Crassostrea*, in the laboratory). Third, terminal results in both the adult and second-reversal juvenile experiments ranged around 80% correct responses (as contrasted with 100% for the first diet in VIMS-36a, Fig. 10). Finally, responses by both barnacle- and oyster-conditioned *Urosalpinx* to "control" conditions (i.e., odor similar to that of original habitat, which did not include these 2 species) remained disproportionately high in comparison to such responses in previous conditioning experiments, which suggests that, as adults, the Locust Point predators were rather resistant to diet changes.

These considerations lead me to believe that ingestive conditioning is similar to *imprinting*, a term proposed by Lorenz (1935) to explain his adoption by anseriform young as their surrogate mother, and since reported widely by investigators of avian behavior. Two recent reports (Burghardt & Hess, 1966; Burghardt, 1966) described "food imprinting" in vertebrates, a process apparently identical to that described here as ingestive conditioning. The first paper dealt with conditioning of snapping turtles (*Chelydra serpentina*) to a preference for 1 of 3 types of food. The second mentioned the possibility that imprinting might account for differential responses of young garter snakes (*Thamnophis sirtalis sirtalis*) to extracts of food organisms.

Another generalization for which evidence has begun to accumulate concerns the original question of statistical preference: it appears that members of the genus *Balanus* produce an effluent which is, under most conditions, more attractive to *Urosalpinx* than are those of any of the pelecypod species tested to date. *The specific exception to this generalization is provided by the ingestive conditioning experiments.* That is, barnacles are more attractive than pelecypods, *except* to juvenile and adult predators which have been kept on a diet of *Crassostrea virginica*.

It should be kept in mind, in con-

sidering theoretical aspects of this problem, that predators are discriminating the *effluents* from the various prey species, and by olfactory cues alone. Hence it is the olfactory apparatus (in the general sense) which is in some way modified by ingestive experience. The fact of discrimination permits the inference that prey species not only differ in the chemistry of their effluents, but also of their tissues. The very nature of the demonstrated phenomenon of ingestive conditioning, in fact, tends to reject an hypothesis of strictly quantitative response to prey effluents as proposed by Blake (1960), at least when it refers to predators conditioned to prey from differing phyla. Hence the investigator is led to comparisons of the chemical composition of the prey, and of both tissues and effluents. These comparisons have been commenced and will be presented in a later paper. Preliminary results already suggest that ammonia may be a generalized attractant to which unconditioned predators are sensitive. This idea has also been proposed by Blake (1961) and is entirely consonant with both circumstantial and available experimental evidence.

## VI. GENERAL DISCUSSION

### 1. Revision of Preference Concept

The concept of prey preference, discussed in the Introduction (pp 271-272), needs redefinition. First, it has been shown that selection of specific prey, in either natural or experimental situations, varies with a complex of factors. Thus it is pointless to propose a genetically fixed prey preference. It has also been shown that a demonstrated preference can be altered by changing the predator's diet. Therefore, the idea of chemoreceptor "types" specific to effluents of given species is no longer relevant.

It is more fruitful to isolate those factors which influence individual prey selection under known conditions, and from such an analysis to suggest general

hypotheses about the predator's behavior toward its prey.

Evidence offered in this work suggests that the most important factors, all mutually interacting, are as follows:

1. Co-existence of predator and prey within given intertidal zones, either in common response to the same environmental factors, or because the predator is attracted to that zone by the prey.

2. Relative population densities of the prey species within restricted local areas inhabited by the predator.

3. Recent ingestive experience of the predator.

Other factors, shown to affect attraction results in experiments (Blake, 1960), must be regarded as less important in natural habitats. I refer to quantitative aspects of metabolite production, either in time by individual prey organisms (e.g., changes in metabolic rate), or in space by distribution of individuals per unit area. It is likely that metabolic variations *due to local environmental factors* would be common to all prey present. Spatial concentrations of one species would increase probability of attack from a predator, but it is not easy to separate spatial and chemical causes. Analysis of a mixed prey habitat at West Haven (p 281, Fig. 3) showed the number of attacks upon barnacles and mussels, respectively, to be proportional to the *number of individuals* of each species present, and not to their biomass. Another factor not considered before is the area occupied by a prey individual relative to those of its neighbors. Connell (1961, and pers. comm. 1965) suggested that larger prey organisms will cover greater surface areas and therefore be more susceptible to attack, by chance alone. But, had this been the case in the West Haven habitat, observed attacks upon the larger mussels would have significantly exceeded calculated ratios, which they did not. An alternative explanation is now available: ingestive conditioning of predators is in direct proportion to the *number* of prey in-

dividuals present, and which would therefore be capable of affecting the predators' behavior.

Such speculative thoughts do not seem extraordinary when one considers the dilemma imposed by field information, which indicates that hypotheses of olfaction need not be invoked by explain prey selection, and experimental results, which indicate rather elegantly developed olfactory capabilities.

The extent to which olfaction operates in nature as a selection factor must be regarded as problematic, simply because direct information is not available. There is little question that under the conditions of certain types of field experiments, such as those reported by Carriker (1955), *Urosalpinx* can and does orient to food odor sources and can follow current-borne attractants to a source when currents approach rectilinearity and there is a suitable intervening substrate. After all, *U. cinerea* is a bilateral animal belonging to a phylum whose orientational chemosensitivity has been well documented (Blake, 1960; Kohn, 1961; and the present work). But natural situations approximating those in which the field experiments were done are probably rare; as we have seen, most intertidal populations of *U. cinerea* live amongst a multiplicity of prey species (Figs. 2 and 3). Those which do not (Nobska, Ocean City, Wrightsville) have immediate access to dense *substrates* of prey of a single species; in fact, their feet rarely touch solid rock but instead stay nearly always (when on outward, exposed surfaces) upon prey. The snails feed upon prey, move around on it, copulate, and, finally, deposit egg capsules either upon or near it. Why, then, has *U. cinerea* evolved a capacity for olfactory discrimination, demonstrated in the laboratory, if it does not use this capacity in its natural *milieu*?

Of course, there is no evidence that it does not. The crucial question is whether coexistence of predator and a specific prey results from independent

factors (exposure, temperature, turbulence, etc.) or from the predator's directed and purposeful movement toward that prey. The experiment with Wrightsville predators, in which snails from each of 2 clearly separated zones (barnacle and oyster) were tested, failed to distinguish cause and effect. That is, were snails in a zone initially because of an *a priori* preference for its sessile fauna, or had they become conditioned to the fauna through ingestion, having arrived in the zone as the result of other factors? On the basis of field information alone, it is not possible to answer. But ingestive conditioning experiments lead me to choose the latter alternative, at least in interpreting the Wrightsville experiments (p 295).

In support of this choice, several field observations may be cited. While nothing is known of the effect of conditioning in multiple, mixed prey habitats, it is apparently of importance in "single species" habitats such as Nobska and Ocean City, or in the rocky intertidal habitats described by Fischer-Piette (1935), Moore (1938), and Fretter & Graham (1962).

At Nobska, *Balanus balanoides* is overwhelmingly dominant, and *Urosalpinx cinerea* apparently fails to take advantage of seasonal opportunities to feed upon regular but usually sparse sets of *Mytilus edulis*. In spring, 1963, for example, *Balanus* and *Mytilus*, both in great abundance and both recently set, were present in a density ratio of about 3:1. Following analysis of the mixed-prey habitat at West Haven, about a quarter of the attacks at Nobska should have been upon mussels, but none at all were seen; all *Urosalpinx* observed, both adults and juveniles, were feeding upon the dominant barnacles.

At Ocean City, where, because of peculiarities of zonation, barnacles intergrade with mussels only upon undersides of rocks, and where *U. cinerea* is in effect conzonal with *M. edulis*, few attacks upon barnacles have ever been reported, despite the fact that in the

upper intergrade zone, predators have access to numerous *B. balanoides*. Thus we see here the exact inverse of the Nobska situation.

The case cited by Fischer-Piette is one in which separation of prey was in time rather than space. He described a shore community of *Balanus balanoides* located on a point at St. Lunaire (France). The barnacles were preyed upon by a population of "*Purpura*" *lapillus*, a muricid gastropod ecologically analogous to the American *Urosalpinx cinerea* and conspecific with "*Thais*" *lapillus* of the U. S. northeast Coast.\* During a period of 4 years, the predators were never seen attacking the scattered *Mytilus edulis*. At the end of this 4-year period, the mussels began to wax while barnacles correspondingly waned. Eventually, mussels were dominant, but for 2 years (1930-31) "*Purpura*" was not observed to attack mussels at all, and restricted its predations to the diminishing *Balanus* population (note the similarity between this behavior and that of barnacle-conditioned *U. cinerea*). Not until the "*Purpura*" found themselves in small areas which they had cleared of living barnacles, and were surrounded by mussels, did they begin feeding upon the now numerous mussels (near the end of 1931). Fischer-Piette (1935: 167) attributed the reluctance of the gastropods to change prey to the relative ease with which they could obtain nourishment from the barnacles, rather than to differences in ectocrine attractiveness ("*loi du moindre effort ... ayant plus de facilité à sucer des Cirripèdes qu'à percer des Moules. Mais l'explication*

*scientifique reste à trouver.*").\*\*

Another relevant observation was made by Moore (1938) on British "*Purpura*" *lapillus*. He found that young of that species were frequently collected from undersides of rocks in lower intertidal zones, where they were feeding upon the polychaet *Spirorbis*. When he brought young snails in his laboratory, he found they continued to feed upon *Spirorbis*, and not upon small barnacles to which they were also given access. He found, however, that as they matured they changed to a barnacle diet. He did not indicate whether this change was linked directly to maturation.

Fretter & Graham (1962) expressed the opinion that "*Ocenebra*," another predatory muricid, retained its preference for its natural diet after being removed to captivity, citing Orton's (1929) studies of gastropod predation. The retention of a natural preference by *Conus* (Kohn, 1959) has already been mentioned.

## 2. Mechanism of Ingestive Conditioning

There are few papers concerning the effect of experience in modifying gastropod behavior. Fischer-Piette (1935), whose observations of "*Purpura*" *lapillus* have already been examined above, cited a comment by Pelseneer (1924), who spoke of the "case of *Natica* (feeding upon *Donax* and *Tellina*) which had profited (*tiré parti*) from the experience acquired from its initially fruitless efforts, this being the criterion generally accepted for the existence of intelligence." (auth. transl.) Fisher-Piette also recounted the difficulties "*P.*" *lapillus* apparently had in adjusting to the task of penetrating the valves of its new prey (*Mytilus edulis*). Experienced *P. lapillus* entered barnacles through the opercular aperture without boring a hole, but such a technique was not

\* Thiele (1931: 298) lists *Nucella lapillus* as valid name for Linné's *Purpura lapillus*. Clench (1947: 86), however, maintains that "Röding did not intend the species now commonly known as *Thais lapillus* to be included in his subgenus *Nucella*" and that "this latter name should either be abandoned or else associated with *Cantharus*."

\*\*"Law of least effort ... as it is easier for them to suck cirripedes than to pierce mussels. But the scientific explanation remains to be found." (Edit. transl.)

applicable to mussels. Eventually, Fischer-Piette reports, the gastropods made the adjustment successfully, though he seemed reluctant to ascribe this adjustment to individual learning. His comment (p 165) is worth quoting.

"Nous aurions donc sous les yeux une sorte d'*apprentissage* des Pourpres dans leur facon de se nourrir au dépens des Moules. Mais la notion d'éducation *individuelle* ne suffit pas dans le cas présent."\*

He goes on to state that while members of the first generation may have had to learn, individually, how to perforate mussel shells, subsequent generations "know right away how to perforate without error, without passing through the stage of apprenticeship." (auth. transl.)

I have made similar observations of *Urosalpinx cinerea's* patterns of predator behavior in its attacks upon mussels and barnacles, and preliminary results suggest a degree of behavior modification as a result of experience, or a kind of trial and error learning.

Before Fischer-Piette, Garth & Mitchell (1926) used a T-maze to institute a conditioned (= Pavlovian) response in a land snail, *Rumina decollata* L. These investigators stated that the learned response was retained after a 30-day period. They also cited the work of Thompson (1916), who found evidence that another snail, *Physa gyrina* Say, could modify its behavior by forming an association between 2 stimuli. Bullock & Horridge (1965) reviewed briefly the problem of gastropod learning and implied that such early investigations of classical conditioning in gastropods were wide of their mark, a notion with which I thoroughly agree. In Bullock's words (Bullock & Horridge, 1965: 1344): "It

seems probable that we will have no adequate idea of gastropod capacities until tests are used that are natural and meaningful to the species."

The investigators cited above were apparently dealing with *associative learning*, defined by Thorpe (1956) as establishment of bonds between discrete stimuli and units of behavior. Associative learning depends, at least to some degree, upon reinforcement, or reward, a type of which is referred to variously as "drive reduction" or "appetite satisfaction."

Clearly, if ingestion of prey tissue were accompanied simultaneously by olfactory stimulation, and if perceived stimuli were produced only by the species being attacked, criteria for associative learning would be satisfied. Repetition of this experience often enough or long enough (about 2 weeks in the case cited in Fig. 10, p 302) would hypothetically result in a state of ingestive conditioning.

It has been shown that simple exposure to prey effluents will not condition *Urosalpinx cinerea*: ingestion of tissue is required. This implies either reinforcement or the physical transfer of some cue from prey to predator. While I hesitate to ascend to a more speculative plane, it is entirely possible that future studies may show that ingestive conditioning is linked with changes in nucleic acid composition of the predator's chemoreceptor surfaces and these in turn may be influenced by composition of free amino acid pools or patterns of amino acids obtained from metabolic breakdown of ingested prey proteins. A preliminary attempt has already been made (auth. unpubl. data) to compare intracellular free amino acids of 2 groups of predators, one of which had been allowed to feed on barnacles, the other upon oysters, for about 2 weeks. No significant differences were found by chromatographic analysis; nonetheless, comparisons of specific anatomical areas thought to be involved in chemoreception, such as the

\*"We thus seem to have, in *Purpura*, a sort of apprenticeship as to the manner in which it nourishes itself at the expense of the mussels. But the concept of individual learning does not cover the case." (Edit. transl.)

osphradium or propodial groove (anterior pedal gland of Fretter & Graham, 1962) may yield positive results.

In summary, it has been suggested that the phenomenon of ingestive conditioning is plausible in the light of 2 general lines of evidence, one from behavioral studies and the other from field observations.

The next task is to attempt to fit ingestive conditioning into an adaptive context: that is, of what value is such a mechanism to the predator?

### 3. Adaptive Aspects of Ingestive Conditioning

Carriker (1957) points out that newly hatched *Urosalpinx cinerea* must be able to identify, locate, and penetrate food organisms. He demonstrated their ability to perform the second of these functions under his experimental conditions: in a typical experiment, in 1 hour, 72% (67) of the young predators had moved away from their starting places and either to slowly-moving effluents from 10,000 young clams (88%) or to a seawater control (12%). The response ratio of 72% is much greater than that reported from the present experiment with young *U. cinerea*; it is possible that in Carriker's studies the young gastropods were in better condition, because they probably had been feeding in the interim between hatching and testing (pers. comm. from Dr. Carriker). However, this interpretation goes only part way in rationalizing discrepancies between the 2 activity rates (see percentage "none," Table 5). It is possible that differences in response criteria may furnish the remainder of the explanation.

With this qualification, I propose that the low response ratios reported in this paper for very young *U. cinerea* were not an accident but indicated immaturity of behavioral apparatus necessary for their direct orientation to prey effluents. Such an interpretation accords with experimental results and also with the general hypothesis of ingestive con-

ditioning. The adaptive value of such a necessity for maturation should be apparent: when protoconch stage snails emerge from egg capsules, they are probably best equipped for simple radular browsing. In natural habitats (and in the nursery environment provided for his animals by Carriker), the substrate would be covered with hydroids, various matted algae, and other minute encrusting biota, any or all of which could provide nutrition for the young, 1-mm snail. (It is noteworthy that young predators in VIMS-37 were denied such a substrate but were kept in scrubbed plastic containers pending testing; this may have contributed to observed mortality rates.) Eventually, the newly hatched would rasp away at young barnacles or serpulids and for the first time ingest genuine prey tissue, an activity simultaneously accompanied by exposure to effluents from surrounding prey. If initial, and subsequent, contacts with large prey occurred in a single-species habitat, the tendency to respond to that species would be reinforced (viz. Wrightsville, Nobska, Ocean City); if not, it would not (any of the other southern habitats, including Shark Shoal). Meanwhile, responses to a general attractant such as ammonia would be reinforced, regardless of prey species.

The same adaptive mechanism was suggested in the parasitic insect *Nemeritis canescens* studied by Thorpe (Thorpe & Jones, 1937; Thorpe, 1938, 1939): *N. canescens* in Europe parasitizes only larvae of the genus *Ephestia* (meal moth), but could be reared artificially by insertion into larvae of the wax moth *Meliphora grisella*. While *Nemeritis* adults reared upon *Meliphora* still preferred their "normal" host, they gave significant olfactometric responses to odors from the "abnormal" host, *Meliphora*.

To make an analogous comparison, the results of experiment VIMS-37 (Table 4) showed that responses to prey effluents preferred by "naive" *Urosalpinx cine-*

rea were heightened after a diet of that prey (*Balanus*), and so were responses to another prey, second in order of preference in tests (*Crassostrea*).

Such an adaptive mechanism should be of great selective value to a predator (or parasite). Ideally, a predator maintains a condition of stasis with its prey, but occasionally factors extrinsic to the predator-prey coaction will seriously disturb equilibria. Such a condition of disequilibrium was described in the paper by Fischer-Piette (1935). Should the prey population be completely eliminated under such conditions, the predator population must change its diet or it may not survive.

Equilibrium between predator and prey may also be viewed as the interacting evolution of attack and defense mechanisms. The predator will be most successful if it can utilize a variety of food species, but opposed to this is the probability that continued attacks upon a single prey type will permit improvement of attack efficiency. Though at this time quantitative information is not available, impressions suggested by preliminary observations are: (1) attack techniques differ radically from one prey type to another, and (2) individual *U. cinerea* change and improve attack techniques with experience (see Fretter & Graham, 1962, for a discussion of other observations of this kind). Hence on purely deductive grounds, the ideal situation for a predator would be as follows:

1. To possess basic apparatus for efficient attack upon a variety of prey species.

2. To be unrestricted genetically as to prey selection, i.e., to have no innate preference.

3. To possess behavioral mechanisms for concentrating upon one prey type so that attack procedures become more efficient through practice.

But of course prey species are also evolving. To have survived, they must have developed mechanical defenses against attack, resorted to camouflage,

or increased reproductive capacities so much that large resident predator populations can be economically supported.

Let us examine the 3 major prey species of *Urosalpinx cinerea* and determine the degree to which the above adaptations have in reality been effected. As to mechanical defenses, in only 1 case may such have evolved. Carriker (1955) and Hancock (1959) have both reported that heavier-shelled, older oysters are not as attractive to *U. cinerea* as are younger ones, and this may be a "defensive adaptation." In the case of barnacles, it depends upon the experience of the predator. At West Haven or Nobska, where *Balanus balanoides* had been dominant for a long time (longer at Nobska), an adult barnacle may be approached, penetrated (between opercular plates), and cleaned out by native *U. cinerea* within 20 minutes (pers. obs.). Predators inexperienced with barnacles, however, drill a hole through or between compartmental plates, requiring several hours. Mussels (*Mytilus edulis*) can be bored with apparent ease, but are more efficiently penetrated at valve intersections, though this contention is not easy to support with quantitative data.

Camouflage, in this predator-prey relationship, should mean *chemical* undetectability, since it is by chemosensory means that prey is located. In this context, the evolution of *Urosalpinx cinerea* may have outstripped that of its prey. For potential prey to be chemically "invisible" to *U. cinerea*, one or more of several conditions would have to apply, on the basis of available evidence: (1) the prey individual could be already dead; (2) it could be old, slowly growing or both; (3) it could be starved; (4) the species, during its evolutionary development, could "elect" *not* to produce an attractant; but since the probable attractants in this case are waste products, such a course seems unlikely. It is in the light of this point that the irony of the proposed ammonia attractant is most clearly revealed: a material which, if

retained in the prey, would be lethal in dilute concentrations, is excreted, only to be detected, in dilute concentrations, by receptors of a predator, also lethal. To avoid ammonia production, prey species would be required either to excrete all excess nitrogen as amino acids, short-circuiting normal deamination processes, or as urea (an adaptation found in animals not blessed with abundant water). There is no evidence, certainly, that either process is occurring.

A more effective defense, evolved by all of *U. cinerea's* major prey, is high reproductive capacity. The enormous potential of the oyster is well-known: it has been estimated that each pair of mature *Crassostrea virginica* may shed more than a billion gametes into the water during a spawning season. Mussels (*Mytilus edulis*) are similarly adapted. In 8 years of observing Ocean City populations, I have never noted even a slight reduction: the mussel zone is replenished, year after year, by tens of millions of young, despite predations of abundant *Urosalpinx*.

Barnacles may be less able than the other 2 major prey species to repopulate denuded areas under combined pressures of predation or diseases and mechanical destruction from winter ice or storms. The intertidal habitat described by Fischer-Piette (1935) is one example, while Moore (1958) has discussed others. Moore points out that despite low rates of larval production per individual (he estimates a few thousand), there are so many adult individuals that within 1 km of the shore, barnacle larvae may be present in plankton in numbers as high as  $10^8$ - $10^{13}$  per km of shore. Such extremely high numbers seem doubtful, despite a personal observation of an overwhelming set of *Balanus balanoides* along the New England shoreline in May 1963. These early spring sets repopulate rocky surfaces which have been scoured free of barnacles at the time of winter ice. In any case, during the years in which I have monitored

the Nobska populations of *U. cinerea* and *B. balanoides*, in no instance has an entire barnacle population on any boulder been consumed by resident *U. cinerea*.

In conclusion, it appears that *Urosalpinx cinerea* is a highly successful predator, not only because it satisfies ideal criteria proposed in this discussion, but also because its prey has evolved techniques of survival which do not normally thwart its predations. *U. cinerea* can apparently perceive and locate any ammonotelic, sessile invertebrate within its range; it has evolved a variety of attack techniques which in each case are adapted to mechanical defenses evolved by its prey; and while it can and apparently does develop transient predilections for specific prey, it is not held rigidly to such preferences by genetic limitation. Its success as a competitor was noted by Cole (1942), who observed how long established populations of *Nucella lapillus* and "*Ocenebra*" *erinacea* on British east coast oyster beds were unable to compete with recently introduced *Urosalpinx cinerea*. While *U. cinerea* populations increased, *N. lapillus* declined, and apparently *O. erinacea* disappeared entirely from at least one area (Blackwater Estuary).

It is therefore not surprising that, in the United States, *U. cinerea* is the only common, intertidal, predatory gastropod within its continuous middle Atlantic range: "*Thais*" *lapillus* intergrades with it in the north, and *Thais haemostoma floridae* and *Murex fulvescens* Sowerby can be found in limited numbers in isolated habitats to the south, but within its own intertidal domain, from Cape Cod to the north coast of Florida, *Urosalpinx cinerea* (Say) is clearly dominant.

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## RESUMEN

ASPECTOS FISIOLÓGICOS Y ECOLÓGICOS SOBRE SELECCIÓN DE  
PRESA POR EL GASTROPODO MARINA *UROSALPINX CINEREA*  
(PROSOBRANCHIA: MURICIDAE)

Langley Wood

Relaciones fisiológicas, ecológicas y de comportamiento entre el molusco rapaz *Urosalpinx cinerea* (Say) y su presa, en la costa oriental de los Estados Unidos, fueron estudiadas en el laboratorio y en ambiente naturales.

Por la relativa frecuencia de los ataques naturales, y estudios oftalmométricos realizados en laboratorio, se comprobó que los percebes, *Balanus* spp., son preferidos por *U. cinerea* a cualquier otra presa mayor de la zona entre mareas, ostras, mejillones, etc. Esta preferencia se destacó en observaciones sobre 4416 caracoles en 11 habitats diferentes de la zona de mareas, en la continua distribución de la especie de Massachusetts hasta el norte de Florida.

La preferencia estadística no está fijada genéticamente: factores ecológicos pueden contarse para la selección de la presa en tales lugares. Uno de ellos es la mutua zonación de predator y presa, y otro es la abundancia relativa de una específica presa en la zona.

Se introduce, con evidencia experimental, el concepto de *acondicio namiento ingestivo*, por el cual la tendencia de la especie rapaz de responder a las emanaciones de la presa, aumenta después de haber ingerido tejidos vivos de ella. En *U. cinerea* juveniles, se produjo una reversión parcial de tal acondicionamiento, por retorno a sus dietas originales. La operación de este proceso se comprobó experimentalmente en caracoles de habitats con presas únicas y otras múltiples. La tendencia estadística a preferir *Balanus* en lugar de ostras, fue parcialmente confirmada al experimentar con jóvenes, que se acondicionan a *Balanus* con mayor facilidad, lo que no siempre sucede con los adultos, cuyas divergentes dietas naturales fueron difíciles de cambiar.

El aspecto evolutivo de estas relaciones se discute con preferencia particular a el valor adaptivo de la rapacidad de ingestión acondicionada. Restricción a una sola presa podría tener efectos desoperativos, y es ventajoso para la especie rapaz ser capaz de alimentarse con más de una especie de presa. Sin embargo, diferentes técnicas de ataque son usadas para la penetración eficiente de cada presa, y en apariencia son adquiridas individualmente. Concentración en una especie única aumenta la eficiencia del ataque. El mecanismo aquí descrito como acondicionamiento ingestivo produce concentrada influencia, sin la irreversibilidad, o fijación genética de la especificidad de la presa.

## АБСТРАКТ

ФИЗИОЛОГИЧЕСКИЙ И ЭКОЛОГИЧЕСКИЙ АСПЕКТЫ ВЫБОРА ЖЕРТВЫ  
МОРСКИМИ ГАСТРОПОДАМИ *UROSALPINX CINEREA*  
(PROSOBRANCHIA: MURICIDAE)

К. ВУУД

В лабораторных условиях и в поле, на литорали восточного побережья С. Ш. А. изучались поведенческие, физиологические и экологические взаимоотношения между хищными моллюсками *Urosalpinx cinerea* (Say). В природе, при исследовании относительной частоты нападения моллюсков на свою жертву и ответной их обонятельной реакции в лабораторных условиях оказалось, что баянусы (*Balanus* spp.) значительно более привлекательны для *U.*

*cinerea* чем большинство любых других их жертв на литорали, как например, устрицы и мидии. Полевые данные об этом предпочтении основываются на прямых наблюдениях над большим количеством (4, 416 экз.) моллюсков из 11 мест обитания на литорали, в области непрерывного распространения *U. cinerea*, от Массачусетса до северной Флориды. Эти статистические данные показывают, что предпочтение баянусов моллюсками не закреплено наследственно; так, по полевым наблюдениям экологические факторы на литорали могут влиять на выбор жертвы моллюсками. Одним из таких факторов служит степень совпадения зоны обитания жертвы и хищника; другим является относительное обилие на литорали данного вида жертвы. Также нельзя не учитывать при полевых наблюдениях роль внешних метаболитов, приводящих хищника к жертве.

Чтобы ввести представление об условиях заглатывания, при которых у хищника наблюдается тенденция реагировать на влияние данного вида жертвы и которая увеличивается после заглатывания им живых тканей этого вида, -автором приводятся полученные экспериментальные данные.

Условия заглатывания у молоди *U. cinerea* частично меняются в обратном направлении, через возвращение их к исходной диете. Данные о действии этого процесса в природе были получены из экспериментов с улитками из мест обитания одиночных и многих экземпляров особей жертвы.

Статистические данные о тенденции *U. cinerea* выбирать баянусов, предпочитая их устрицам, были частично подтверждены экспериментами с молодью и ювенильными стадиями, наиболее легко приспособляющихся к баянусам; это не всегда бывает со взрослыми формами, разнообразную естественную диету которых бывает трудно восстановить в эксперименте.

В работе обсуждается взаимоотношения хищника и жертвы в эволюционном аспекте, с особым вниманием к адаптивному значению для хищника условий заглатывания. Ограничение лишь одним видом-жертвой было бы невыгодным для моллюска так как хищник получает определенные преимущества, если может питаться более разнообразно - более, чем одним видом жертвы. Хищниками употребляются различные способы нападения на жертву и это ведет к более эффективному захвату различных видов жертв и это, видимо свойственно различным особям *U. cinerea*. Описанный здесь механизм заглатывания обеспечивает такое концентрированное влияние при нарушении наследственно-закрепленной приуроченности (специфичности) жертвы моллюска.

CULTURING *ONCOMELANIA* SNAILS  
(PROSOBRANCHIA: HYDROBIIDAE)  
FOR STUDIES OF ORIENTAL SCHISTOSOMIASIS<sup>1</sup>

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ABSTRACT

Six types of vivaria were tested to determine the ecological conditions in which the 4 subspecies of *Oncomelania hupensis* thrived best in the laboratory. Efficient procedures were established to assure optimal conditions for: adult survivorship, production of young per female per unit time, survival of young and rapid growth of the young.

Guided by an extensive survey of the literature and past experience, aquateraria were established in the following containers: aquaria, cylindrical glass (battery) jars, large and medium sized shallow clay pots, plastic trays and Petri dishes. Success in culturing *Oncomelania* hinged on providing 2 distinct environments: (1) one where adult mortality was minimal and productivity optimal; and (2) another where young would grow rapidly without stunting and with low mortality.

Conditions in the "medium clay pot," with 5 males and 5 females, proved superior for both adult survival and production of young. Its superiority is due to the fact that a few females are highly productive in a limited volume where a soil-filter paper-water ratio apparently is optimal. Because of the small volume of the environment, more productive culture units can be used in place of larger, more cumbersome, less productive culture types. Finite monthly rates of adult mortality were about 2% during the first year; mortality had not reached 50% at close to 2 years and was usually considerably less. For producing young it is recommended that, at 24 months of age, females of all subspecies except *Oncomelania hupensis quadrasi* be replaced by young females. Those of the latter subspecies should be replaced at 12-14 months since they showed a marked increase in mortality in the second year of adult life.

In other vivaria rapidly increasing rates of mortality, or finite rates of 12% or greater per month, indicated unfavorable culture conditions. The poorest cultures were in the aquarium, large clay pot and battery jar. The aquarium was extremely awkward to handle because of its bulk and the snails did not develop well in it. The large clay pot showed excessive mortality of young snails. The battery jar was characterized by high adult mortality rates and extreme erosion of the shell.

The greatest yield of juveniles occurred in the medium clay pots where, depending upon the subspecies, 3-11 young hatched per female per month for the productive months, a rate about twice that of other cultures. Although production was sporadic, all subspecies reproduced each month of the year.

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In medium clay pots, mortality of young in the parental culture was 4-10%, but generally 5%. In other vivaria it was much higher; in large clay pots, for instance, from 42-78%. The deaths occurred within 1-2 weeks of hatching.

As discussed by us (1965), optimal rates of growth occurred in Petri dish cultures with 1-2 snails per dish at the 2.0-2.5 whorl stage. The logarithmic growth phase was over in 5-9 weeks, while full growth was obtained within 8-13 weeks, depending on the subspecies. A maximum of 20% mortality occurred over the 8-9 week growing period.

In culturing *Oncomelania* the following factors are important. The soil, both a source of food and a substrate for depositing eggs, should be fine textured, high in calcium content, and should support a dense flora of diatoms. This flora, along with the attendant decomposers, provides an adequate source of food; the only other supplement is filter paper, the classic food additive.

Water should be neutral to slightly alkaline (pH 7.0-7.6) and devoid of chlorine or other toxic agents, such as copper ions.

Room level light intensity was found adequate for good survival of adults and production of young; constant light tended to increase the rates of mortality over prolonged periods of time (1 1/2 to 2 years), and also caused mortalities on account of excessive proliferation of algae. Optimal rates of growth for young occurred in light (130-160 ft. candles) cycled 10-12 hours per day.

Productivity decreases and mortality rates increase with increased snail density. Daily maintenance is necessary to assure optimal conditions. Biotic factors most destructive to snail cultures were mold, worms (oligochaetes) and mites.

A model is presented on the number of medium clay pots, Petri dishes, space and labor necessary to raise 500 snails per month of each of the 4 subspecies of *Oncomelania hupensis*.

## INTRODUCTION

A number of papers have been published on various aspects of the natural history and laboratory culture of *Oncomelania* Gredler (1881). Many of them are useful in that they give information on such isolated phenomena as rearing and maintaining these snails; the time it takes for eggs to hatch; the optimal temperature for survival or production of young; the most suitable foods, etc. However, data usually are lacking which would enable one to predict what culture conditions are necessary to produce a definite number of snails of uniform size and age within a designated time. Predictions of that kind are possible only when one knows the effects of various culture conditions on the mortality, natality, survival and growth of young.

During the past 4 years it was possible to develop the methods here presented.

These methods are designed to assist those involved in experiments which require large numbers of specimens of each of the 4 so-called species of *Oncomelania*. To meet these demands, the various culture conditions described in the literature were also tested. These tests have been undertaken not only to find the most efficient procedures but also to discover the conditions which will provide large numbers of snails all year round for experimental purposes. The information has been organized to provide in a comparative way the results obtained in the various types of culture and with an emphasis on the following categories:

- (1) mortality rates of field and laboratory-reared snails;
- (2) production of young per female per month;
- (3) the survivorship of newly-hatched snails in various environments;

TABLE 1. Types of vivaria used by various workers for culturing *Oncomelania*

Vivarium Type	Author	Dimensions
1) Large scale reconstruction of the environment	Vogel, 1948	
2) Aquaria	Stunkard, 1946 Ward et al., 1947 DeWitt, 1951, 1952 Pesigan et al., 1958	8" x 28" x 12" 9" x 16" x 10"
3) Battery Jars	Bauman et al., 1948 Moose & Williams, personal communication, 1965 Davis, 1967	12" diam., 18" high 8" diam., 10" high
4) Plastic Trays	Moose & Williams, 1961-62 van der Schalie & Davis, 1965 Davis, 1967	9" x 24" x 3.5" 7.5" x 11" x 2.5"
5) Clay Flower Pots	Sugiura, 1933 Williams, personal communication, (1952) Wagner & Wong, 1956 Wong & Wagner, 1956	32 cm x 24 cm 5" diam., 1.25" high 6" diam., 1.75" high
6) Petri Dishes	McMullen, 1949 Sandground & Moore, 1955 Otori et al., 1956 Komiya et al., 1959 van der Schalie & Davis, 1965	10 cm diam. 15 cm diam. 9 cm diam. 9 cm diam. 9 cm diam.

(4) conditions providing optimal growth and survival of young snails;

and

(5) the culture type which provides minimal mortality as well as maximum production of young.

For those involved in the technological aspects of chemotherapy, etc., a model is presented which shows the type and number of cultures needed to raise 500 snails per month of each of the "species" of *Oncomelania*. The model is designed to assist in procuring the facilities necessary with regard to equipment, space, and the manpower needed for rearing that number of snails. Based on culturing experience the recommended procedures take into account ease of handling as well as yield, both of which are considered to be equally

important.

#### Note on Nomenclature

Success in hybridizing the 4 so-called species of *Oncomelania* and in obtaining fertile hybrids was reviewed by Davis et al. (1965). Burch (1964), after a cytological study of both the parents and the hybrids concluded that the 4 species were no more than geographic populations or races of the same species. After additional morphological studies, Davis (1967) considered these 4 groups as subspecies of *Oncomelania hupensis*. Consequently, this subspecies concept will be applied throughout this work.

#### HISTORICAL

Reports (Table 1) on methods for maintaining *Oncomelania* in the labo-

ratory have appeared over a period of about 30 years. A review of this earlier work is important since those earlier papers lay a foundation upon which successful culture work has been made possible. The procedures that have proven successful in our laboratory were derived in part from the information obtained from many of the published papers. For purposes of review the data can be most easily organized under 2 headings: i.e. the physical and the biotic factors which are involved in culturing *Oncomelania*. A review by Ritchie (1955) should be consulted since his report also pertains, in part, to culture technique. Reports of the 406th Medical Laboratory (1951-64) contain a wealth of isolated facts concerning the biology of *Oncomelania*.

## Physical Factors

### 1. Culture Types

In most cases cultures were designed to simulate as nearly as possible the amphibious natural environment of *Oncomelania* snails. Vogel (1948) referred to such cultures as aquaterraria; they are generally characterized as a container with a bank of soil that slopes into a reservoir of aerated water. The kinds of aquaterraria used by various investigators vary, but usually 6 major types (Table 1) can be recognized.

Active aeration was generally used only where there was a large reservoir of water, such as in aquaria or plastic trays. Battery (cylindrical glass) jars used as vivaria were constructed in various ways; Bauman et al. (1948) had a mud bank with a water reservoir, while Moose and Williams (1965, personal communication) preferred to use these jars for holding large numbers (200 per jar) of snails for 6 to 7 months and covered their bottoms with moist filter paper. Davis (1967) modified this latter arrangement by adding a glass plate which was placed on the bottom with a mound of soil to encourage egg laying. Most of these vivaria were covered with

glass plates with a crack left open for ventilation; battery jars were usually covered with cheesecloth or glass plates. Moose & Williams, (1961-62) covered the plastic trays with snug fitting lids perforated with many small holes, while van der Schalie & Davis (1965) used plexiglass covers which were drilled in several places to permit ventilation. The soil banks were generally arranged to project one to several inches above the water in such a way that the emergent soil accounted for 1/3-1/2 of the area of the container. The reservoirs were generally one to several inches deep.

The use of Petri dish cultures (see Table 1) for maintaining *Oncomelania* was recently discussed by van der Schalie & Davis (1965). Sandground & Moore (1955) used 10-15 cm Petri dishes in which they constructed a sloping soil bank and small reservoirs of water. For food they used filter paper strips impregnated with sodium alginate. Komiya et al. (1959) used 9 cm dishes in which some had a sloping soil bank ("good for adults") while others had a flattened soil mass covered by a sheet of water ("good for young"); they used cultured diatoms and rice powder for food. Van der Schalie & Davis (1965) emphasized that such cultures were best for rearing young snails to maturity and were not suitable as vivaria for maintaining adults or encouraging production of young.

### 2. Substrata

The several types used and mentioned in earlier accounts have been recorded in Table 2. While the substrate is generally described as a soil bank, the nature of the soil has seldom been further characterized. Wagner & Wong (1956) sterilized a mixture of soil made of 2 parts soil, 1 part gravel, and 1 part sand. In our laboratory (van der Schalie & Davis, 1965), it was found unnecessary to sterilize soil and our cultures were seldom plagued by mold or algal contamination. We found that a high incidence of mold accompanied

TABLE 2. Substrates used in vivaria for culturing *Oncomelania*

Author	Substrate
Sugiura, 1933	Soil from the habitat of <i>Oncomelania hupensis nosophora</i> dead leaves and sticks placed on the soil
Stunkard, 1946	Mud
Ward et al., 1947	Mud, moss, small sticks
Bauman et al., 1948	Mud and sand
DeWitt, 1952	Sandy loam sprinkled over with decaying vegetation
Sandground & Moore, 1955	Loam
Wong & Wagner, 1956 Wagner & Wong, 1956	Mixture of 2 parts soil, 1 part gravel, 1 part sand; sterilized; pieces of brick added
Komiya et al., 1959	Clayey-sandy soil
Moose & Williams, 1961-62	Gravel lightly covered with sterile loam that was passed through a U. S. #80 screen. Soil from the habitat of <i>Oncomelania hupensis nosophora</i>
van der Schalie & Davis, 1965	Non-sterile soil from the habitat of <i>Pomatiopsis cincinnatiensis</i> , a North American snail related to <i>Oncomelania</i> . Soil alkaline; sand 40-70%; silt 13-42%; clay 7-24%.

TABLE 3. Soil analysis of 34 colonies of *Oncomelania hupensis quadrasi* as presented by Pesigan et al., 1958

Chemical Radical	Range in ppm	Average ppm
NO <sub>3</sub>	0.8-20	5.9
P	12.5-100	90.8
K	45-185	90.4
Ca	250-5000	1286.8
NH <sub>3</sub>	0.5-2.5	0.85
Mg	0.5-5.0	3.5
Mn	0.5-5.0	3.5
Al	0.5-50	23.0
NO <sub>2</sub>	1-5	1.5
Fe	0.5-25	15.0
SO <sub>4</sub>	50-250	85.2
Cl	25-100	51.0
Soil pH	range: 4.6-7.2	average: 6.6

soil sterilization.

It appeared that soil texture *per se* is not critical for the survival of *Oncomelania hupensis nosophora* (Ishii & Tsuda, 1951). *Oncomelania* were maintained successfully (van der Schalie & Davis, 1965) under conditions where sand varied from 40-69%, silt from 13-42% and clay from 7-24%. Wagner & Wong (1956) had success rearing *Oncomelania* on a mixture of 50% soil, 25% gravel and 25% sand. Hosaka et al. (1953) noted, however, better growth of *O. h. nosophora* on sandy and pebbly soil.

Pesigan et al. (1958) found *Oncomelania hupensis quadrasi* surviving in the field on sandy loam; fine sandy loam; silty clay loam; silt loam; and clay loam. They also found that soil chemistry had "nothing to do with the distribution of *Oncomelania* in the Palo area" in the

TABLE 4. The physical conditions for culturing *Oncomelania*

Author	Type of water used	pH of water	Temperature of culture °C
Stunkard, 1946	NS*	(a) 6-7 (b) 7.3-7.7	NS
Ward et al., 1947	Filtered Potomac River water	7.2-8.0	26°-27°
Abbott, 1948	NS	6.8-7.8	<i>O. h. quadrasi</i> 24°-27° <i>O. h. nosophora</i> <i>O. h. hupensis</i> 16°-24°
DeWitt, 1952	Tap water allowed to stand a few days	NS	26°-28°
Wagner & Moore, 1956	NS	NS	26°
Moose & Williams, 1961-62	Dechlorinated tap water	6.2	22°
van der Schalie & Davis, 1965	Boiled, filtered pond water	7.2	24°±2°

\*Not stated

Philippines. Table 3 contains the results of their chemical analyses of soils from snail habitats. Areas which looked likely to support snails, but in fact did not, were tested and the chemistry was not found to be significantly different. Komiya (1964) simply reiterated their findings.

However, the fine grain soil particles are important since these snails use the substrate for food, sites of egg deposition and for encapsulating the egg with a jacket of fine soil (Sugiura, 1933; Abbott, 1946).

### 3. Water

Water from a wide variety of sources can be used in cultures with success. DeWitt (1952) used tap-water which had stood a few days. Moose & Williams (1961-62) used water dechlorinated by

18 mg sodium thiosulfate per 1 1/2 gallons. Table 4 gives a list of the sources of water used by various workers (when mentioned) as well as the pH ranges.

Stunkard (1946) stated that these snails lived equally well and reproduced in water with a pH range from 6.0-7.0 or 7.3-7.7. Neutral or alkaline water is recommended to avoid undue erosion of the shell. Wagner & Wong (1956) indicated that the water levels in vivaria did affect egg laying. They found that snail production was best in clay pot vivaria filled to 1/3 capacity with water.

### 4. Light

*Oncomelania* avoids direct sunlight or strong, direct light rays (Abbott, 1948; Kawamoto, 1952; Pesigan et al., 1958; Komiya et al., 1959; Moose & Williams, 1961-62). Ward et al. (1947) used

TABLE 5. Foods provided for *Oncomelania* in laboratory cultures

Author	Substances provided as food
Sugiura, 1933	Paper; raw or boiled cucumber; cabbage; decayed leaves; pieces of wood.
Stunkard, 1946	Leaves smeared with yeast and diatoms.
Ward et al., 1947	Coconut shells; fresh maple leaves; palm fronds.
Bauman et al., 1948	<i>Nipa</i> fronds, coconut husks, water plants.
McMullen, 1949	Filter paper.
DeWitt, 1952	Decaying vegetation and powdered commercial fish food.
Sandground & Moore, 1955	Sodium alginate.
Otori et al., 1956	Filter paper, decayed leaves, straw.
Wong & Wagner, 1956	Filter paper and dried maple leaf.
Komiya et al., 1959	Rice powder and diatoms.
Moose et al., 1962	Rice cereal

fluorescent lights to supplement ordinary room lights. Wagner & Moore (1956) and Chi & Wagner (1957) maintained cultures under a single, 20-watt fluorescent tube 9"-10" above the cultures. Wagner (1954-55) reported that there was a definite trend towards greater production of young under constant light.

Van der Schalie & Davis (1965) showed that growth of young was excellent in Petri dishes maintained under 100-150 ft. candles constantly supplied by a 40-watt, white, "cool," fluorescent tube suspended 10" above the cultures. Growth was not appreciably diminished when the exposure to light was halved to 10-12 hours daily, whereas constant light stimulated too great a growth of algae on the non-sterile soil used. Cultures appear to thrive best under cycled artificial light, indirect sunlight or normal room-level daylight.

##### 5. Temperature

Table 4 gives the temperatures at

which *Oncomelania* cultures have been maintained. DeWitt (1952) asserted that the 4 "species" of *Oncomelania* reproduced throughout the year at temperatures between 26° C and 28° C. Wagner & Wong (1956) and Chi & Wagner (1957) reported that the greatest production of young occurred at 26° C for *O. h. nosophora*, *O. h. quadrasi* and *O. h. formosana*. They stated that at this temperature the snail had an intermediate rate of mortality.

Van der Schalie & Getz (1963) tested the response of *Oncomelania* to thermal gradients and found that the average temperature preference was: *O. h. lupensis*, 21° C; *O. h. formosana*, 23° C; *O. h. nosophora*, 25° C; *O. h. quadrasi*, 26° C. Van der Schalie & Davis (1965) found that the growth rates for the 4 subspecies of *Oncomelania lupensis* were greater at 25° C  $\pm$  2° C (under constant or fluctuating light) than those previously reported.

## Biotic Factors

### 1. Food

The materials that have been provided as food for *Oncomelania* are listed in Table 5. Sugiura (1933) stated that fecal material from snails in the field contained vegetable matter such as decayed leaves, roots of water plants and decayed pieces of wood. Mao (1958) found that the foods of *Oncomelania* in the field were Gramineae, diatoms, ferns. Dazo & Moreno (1962) stated that *O. h. quadrasi* "appears to be a herbivore, its diet consists mainly of green algae and diatoms."

In the laboratory, Ward et al. (1947) found that *Oncomelania* ingested dead vegetable matter, e.g. "water-logged maple leaves, twigs, husks and shells of coconuts, and palm fronds." They stated that detritus and mud provide food. McMullen (1949) found that filter paper served as food, and more recent research at the Loma Linda University, Loma Linda, California (Wagner, 1954-1955) showed that snails survived longer on filter paper when compared with other substrates such as leaves, soil, fish food or wood. Winkler & Wagner (1959) discuss the physiological basis for filter paper digestion. Van der Schalie & Davis (1965) maintained *Oncomelania* solely on soil which supported a high level of green algae and diatom production. They found that young snails grew on such a substrate at rates higher than those described anywhere in the literature for laboratory-reared snails.

### 2. Density of Adults per Culture

Abbott (1948) stated that an aquaterarium 12" in diameter should contain no more than 100 specimens. Moose & Williams (1961-62) usually placed 50 specimens in their plastic tray vivaria. Wagner & Wong (1956) and Chi & Wagner (1957) varied snail density in clay saucers from single pairs to 5 females and 3 males per vivarium. Komiya et al. (1959) recommended putting 8-10 adults in Petri dish cultures. The dimensions

for the above vivaria are given in Table 1.

To date, data have been lacking to indicate the optimum density in a given culture or environment at which one could expect least mortality and the greatest production of young. What data are available tend to indicate that cultures perform better when they are maintained with smaller numbers of snails.

### 3. Production of Young per Female

The number of young produced per female varies with time and experimental conditions as the compilation (Table 6) shows. These data provide the order of magnitude one can expect for average production of young per female per unit time. It is known that females mated only once can produce young for 2 years but not in the 3rd year (406th Medical General Laboratory, 1952). This trend was also reported by Chi & Wagner, 1957. Not all females will produce in a uniform manner while some will not produce at all. It is reported by the 406th Medical General Laboratory (1952) that of 114 individually isolated mature females, with 6.5 whorls or larger, only 89% produced young. The varying fecundity of different females was discussed in the above reports. Since young are produced sporadically, it would seem important that observations be made over a 9-11 month period, to obtain more reliable averages. Pesigan et al. (1958) recorded an average of 8.26 days between egg laying for "*Oncomelania quadrasi*," but the most common interval was 4 days. Chi & Wagner (1957) reported that the observed time lapse between 2 periods of egg-laying varied from 1 to 55 days.

Ishii & Tsuda (1951, Japan) stated that egg laying for *Oncomelania hupensis nosophora* began in the laboratory in May and ended in August. Reports at the 406th Medical Laboratory in Japan (1952) indicated that peak hatching in the laboratory (0.33/female/day) for *O. h. nosophora* occurred in April (eggs laid in March) and then declined to nil in September. Vari-

TABLE 6. Production of young under laboratory conditions as reported in the literature

Subspecies of <i>Oncomelania hupensis</i>	Length of observations (months)	Initial No. females observed	Average production of young per female per day	Actual % females not producing	Special conditions	Author
<i>formosana</i>	3	10	1.19	10	single pair of snails, female mated only once	Chi & Wagner, 1957
	4.5	7	0.71	28		
<i>nosophora</i>	5	53	0.16	0	- - - - -	406 Med. Lab. 1952
	6	19	0.37	47	single pair of snails, females mated only once	Chi & Wagner, 1957
	6	17	0.94	41		
<i>quadrasi</i>	11	15	0.51	27	single pair of snails, females mated only once	Chi & Wagner, 1957
	9.5	17	0.83	12		
		3	13	0.39	NS*	Water level fluctuating
	1.7	200	1.85			
	2.5	240	0.12-0.25			

\*NS = Not stated

TABLE 7. Incubation period for eggs of *Oncomelania*

Subspecies of <i>Oncomelania hupensis</i>	Author	Most common time lapse fr. egg laying to hatching Days	Total range of variation	Temperature °C
<i>formosana</i>	Chi & Wagner, 1957	20	9-41	26
<i>nosophora</i>	Sugiura, 1933	11-13	NS	NS
	406 Med. Lab., 1951	peaks at 17.25	13-34	NS
	Ishii & Tsuda, 1951	10-14	NS	NS
	Otori et al., 1956	24-25	12-35	20-25
		18-19	11-33	24-29
	Chi & Wagner, 1957	21	9-43	26
<i>quadrasi</i>	Abbott, 1946	15	NS	NS
	McMullen, 1947	14	NS	NS
	Chi & Wagner, 1957	18	8-40	26

\* NS = Not stated

ous authors have confirmed that most or all "species" of *Oncomelania* produced young in the laboratory the year round (DeWitt, 1952; Wagner & Moore, 1956; Chi & Wagner, 1957; Davis, 1967).

#### 4. Sites for Egg Deposition

The eggs of *Oncomelania* are laid singly, covered in a soil jacket by the snail and deposited on soil or other objects such as sticks or rocks. This type of egg laying is a characteristic of the subfamily Pomatiopsinae (Davis, 1967). Sugiura (1933) found that eggs of *O. h. nosophora* were deposited on the sides of his clay pot vivaria and also on dead leaves or sticks in the pots. Ritchie et al. (1951), noted that *O. h. nosophora* preferred soil as a site for laying eggs. Wagner & Wong (1956) found that in their vivaria *O. h. nosophora* and *O. h. quadrasi* laid over 71% of the eggs above the water line. They noted that eggs of *O. h. nosophora* were laid largely in soil, while those of *O. h. quadrasi* were laid predominantly on objects provided, such as brick and sticks.

#### 5. Hatching of Eggs and Incubation Period

The average time (Table 7) for eggs to hatch tends to vary. Otori et al. (1956) showed that increased temperature shortens this period. As shown in Table 7, the range may vary from 20-30 days; some eggs may not hatch until 40 days after being laid.

Otori et al. (1956) observed that 85-90% of the eggs of *Oncomelania hupensis nosophora* hatched. Pesigan et al. (1958) found that on the whole 88% of the eggs of *O. h. quadrasi* did hatch, although in some experiments as many as 96% hatched.

#### 6. Longevity of Adults

In terms of field conditions, Sugiura (1933) showed that individual *Oncomelania hupensis nosophora* may survive for 5 years. McMullen et al. (1951) did not

recognize any peak mortality for this snail over a 2-year study period in the field. Li (1953) recorded peak mortality for *O. h. formosana* following a time of greatest reproductive activity and also indicated that most *O. h. formosana* live about 1 year. McMullen (1947) stated that *O. h. quadrasi* had a life span of at least 1 year in the field; Pesigan et al. (1958) found that after reaching maturity an average female lives 65.8 days (total age 7-8 months).

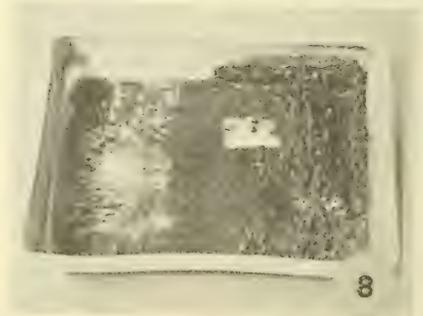
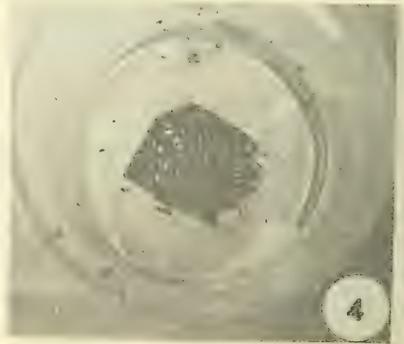
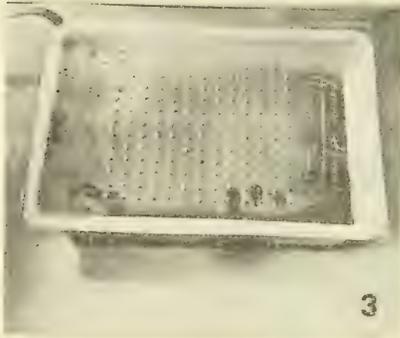
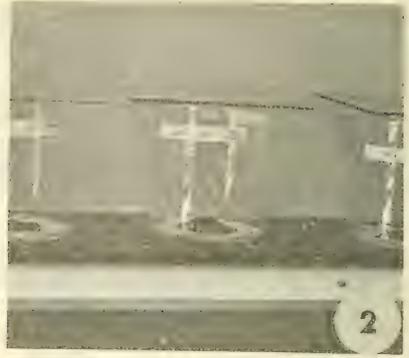
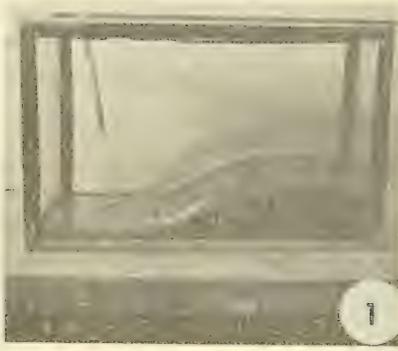
In the laboratory Ritchie (1955) reported that *Oncomelania hupensis nosophora* can survive about 5 years. Wagner & Wong (1956) found an average of 40% mortality of adult snails in 1 year when they maintained them in medium clay pots under varying experimental conditions. Davis (1967) showed that, in the laboratory, mortality rates of field snails, obtained when they were about 1 year old, depended upon the culture conditions to which they were subjected. *O. h. formosana* had a finite death rate of 12% per month when maintained in plastic tray vivaria under 150 ft. candles of light cycled 10-12 hours per day. When *O. h. formosana* was maintained in battery jar vivaria they had significantly greater rates of mortality; their mortality increased with time and 50% of the snails were dead within 4 months.

#### 7. Habits and Survival of Young

Pesigan et al. (1958) stated: "As has long been recognized, the newly hatched snails are aquatic and remain so for some time." These authors presented data for *Oncomelania hupensis quadrasi* showing that during the first week after hatching very few snails are found out of water. After 2 weeks, 75-80% were to be found out of water and this percentage remained fairly constant thereafter; this behaviour also was verified with field observations. They also stated that about 50% of the young snails die by the end of the aquatic stage. However, van der Schalie & Davis (1965) found that among young snails of taxa of

TABLE 8. Vivaria tested in our laboratory for efficient rearing and maintenance of *Oncomelania*

Type of Vivarium	Dimensions			Area of soil in square inches	Depth of soil	Water in Reservoir cc	Vivarium Cover	Remarks
	Le.	Wi.	Diam.					
Aquarium	16" x 8"			72	1.5" average	250	Glass plate; one edge open & screened to permit ventilation	Soil sloped at 30°
Battery Jar		8.25"	10"	6.0 on a glass plate	0.20"	20	Glass plate entirely covers opening	Glass plate with soil rests on double thickness of 20 cm, #500 filter paper
Large Clay Pot		10"		45.0	0.5"-0.75"	100-200	Glass plate as in battery jar	Soil flat except a 2" wide strip sloping into reservoir
Medium Clay Pot		5"	1.5"	about 3	0.75"	35	Glass plate as in battery jar	Soil a mound placed centrally in dish. (2-2.5" diameter)
Petri Dish		9 cm	1.9 cm	about 3	0.65"	35	Regular Petri dish cover	Soil (about 35 gm) smoothed into a mound in center of dish
Plastic Tray	11" x 7.5"			45	0.5"-0.75"	200	Plexiglass drilled at regular intervals for gaseous exchange	Soil flat except 2" strip next to water sloping into reservoir



all "*Oncomelania*" in Petri dish vivaria, at the 2.0-2.5 whorl stage, a mortality of only about 10-20% occurred until the snails reached adult size (60 days).

#### 8. Growth Rates of Young

This topic was reviewed by van der Schalie & Davis (1965) in a paper pertaining to growth and stunting in *Oncomelania*. They concluded that, in the laboratory, optimal rates of growth occur in Petri dish vivaria under fluctuating light (150 ft candles cycled 12 hours per day) with 1 or 2 snails per container. Increasing snail density (i.e., 5 or 10 young per Petri dish or 40-50 young per plastic tray) caused a retardation in growth and also suppressed the development of the sex organs. Growth rates of 0.3 - 0.45 mm per week for the first 8 weeks indicated unfavorable laboratory conditions, while 0.6 - 0.65 mm per week were optimal. A review of the literature pertaining to growth is found in that paper. It was also shown that snails grown under optimal conditions are mature and will commence egg laying in the 9th - 11th week after hatching.

## MATERIALS AND METHODS

### 1. Sources of Snails

*Oncomelania hupensis nosophora* was collected from the Yamanashi Prefecture, Japan, an endemic area for *Schistosoma japonicum*. *O. h. quadrasi* was sent from Palo, Leyte, in the Philippines. *O. h. formosana* was sent from Taiwan, and *O. h. hupensis* was provided by Dr. H. Vogel from his laboratory in the "Institut für Tropenkrankheit," Hamburg, Germany.

### 2. Culture Types Utilized

Six kinds of vivaria were initially tested; aquaria (Fig. 1), a plastic tray (Figs. 3, 7, 8), unglazed shallow large (Figs. 9-11) and medium diameter clay pots (Figs. 5, 6), battery jars (Figs. 2, 4) and Petri dishes (Figs. 12-14); their dimensions and other particulars are given in Table 8. Active aeration was not used where the volume of water in the cultures was small (Table 8) (battery jars, medium clay pots, Petri

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#### FIGS. 1 - 8

FIG. 1. Aquarium with aeration hose, filter paper strip and snails on the soil.

FIG. 2. Battery jar vivaria; snails have climbed up on sides of jar.

FIG. 3. Plastic tray container with aeration tube and plexiglass cover drilled with numerous holes to permit ventilation.

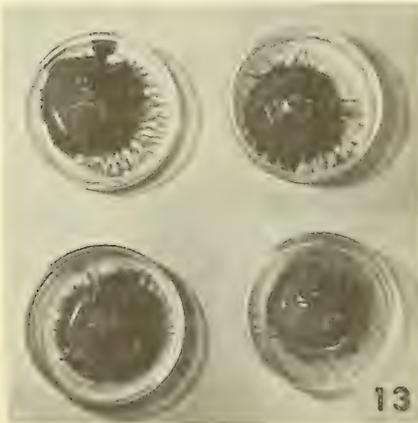
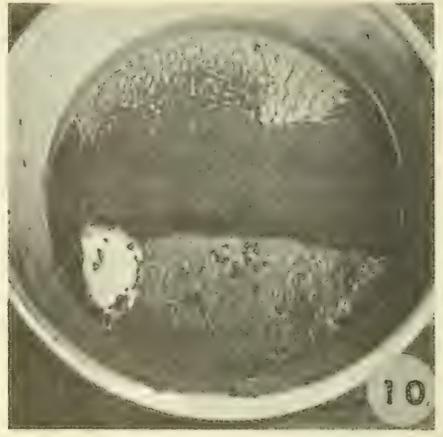
FIG. 4. Top view of battery jar vivarium with a rectangular glass plate supporting a mound of mud; glass plate rests on filter paper. Note that snails are on the sides of the jar.

FIG. 5. Four medium clay pots shown as they are usually housed, in a large plastic tray containing water, which allows handling them as a group and maintains proper water levels for the unglazed clay pots.

FIG. 6. Top view of a medium clay pot with filter paper strips placed in the culture to replace the filter paper which had developed a pronounced mold.

FIG. 7. Plastic tray vivarium just prior to clearing its water reservoir of accumulated silt; snails are on the exposed soil surface.

FIG. 8. Plastic tray not heat-treated for worms; hence soil shows churned up condition due to oligochaete activity. Two snails can be seen on the filter paper.



dishes) or where there was little depth of water in the reservoir (large clay pots) as gaseous exchange was adequate. Active aeration was used where the depth of water in the reservoir was great as in the aquarium and plastic tray types.

The (unglazed) medium clay pots were kept in large plastic trays, 4 per tray, (Fig. 5) that were half filled with water. The dimensions of the trays are 17" x 15" x 3". Glazed pots should work as well, and then the water in the large plastic trays would not be necessary. In our work it was easier to handle 4 of the clay pots as a unit than singly. The large clay pots were also unglazed and maintained on a constant flow water table (Fig. 9) with the water entering the table at 29° C and leaving at 28° C.

### 3. Soil

The unsterilized soil used was obtained from the habitat, in Michigan, U. S. A., of *Pomatiopsis cincinnatiensis*, an amphibious hydrobiid snail related to *On-*

*comelania*. The soil was alkaline and supported high yields of naturally occurring green algae and diatoms. Van der Schalie & Getz (1962: 207-209) indicated that the soil textures in various habitats of *Pomatiopsis* varied widely. The soil used in this study was analyzed chemically by the Cooperative Extension Service of the U. S. Department of Agriculture at Michigan State University (see Table 9).

Qualitative observations indicated that of all the organic matter present in the surface soil, decaying organic material was more abundant than any other organic component; living diatoms were very abundant but did not approach the density of decaying organic substances; green algae were less abundant than diatoms but slightly more prominent than the blue-green algae.

Quantitative estimates of algal density were made in early July and August, 1962, for 36 quadrats (each 0.25 ft. square) of river bank inhabited by *Pomatiopsis cincinnatiensis*. Uniform

### FIGS. 9 - 14

FIG. 9. Water table with numerous large unglazed clay pot vivaria; with glazed pots the water table would not be necessary. A single, large, unglazed clay pot could also be kept in a plastic tray like the one shown in Fig. 5.

FIG. 10. Top view of large clay pot used for temporary storage of 100-200 *Oncomelania*.

FIG. 11. View of a large clay pot which was not heat-treated to kill worms; note piles of worm castings over the surface of the soil.

FIG. 12. Shelving units used to house Petri dish cultures. These cultures are maintained for 8-9 weeks on these shelves under 10-12 hours light daily provided by one fluorescent tube per shelf.

FIG. 13. Four Petri dish cultures with lids removed to show the arrangement of adult snails on the soil.

FIG. 14. Four Petri dish cultures at different stages of algal development; Upper left: fresh and newly established dish; upper right: algal growth moderate (dark cap on soil) and soil spreading out towards side of dish (about 4 weeks); lower left: algal growth moderate and soil almost entirely covered by algae, the algal masses then begin to accumulate in water (5 weeks under constant light); lower right: algae excessive with soil and water clogged with algae (constant light, 5-7 weeks). Snails develop best when the algal growth is maintained at a moderate level.

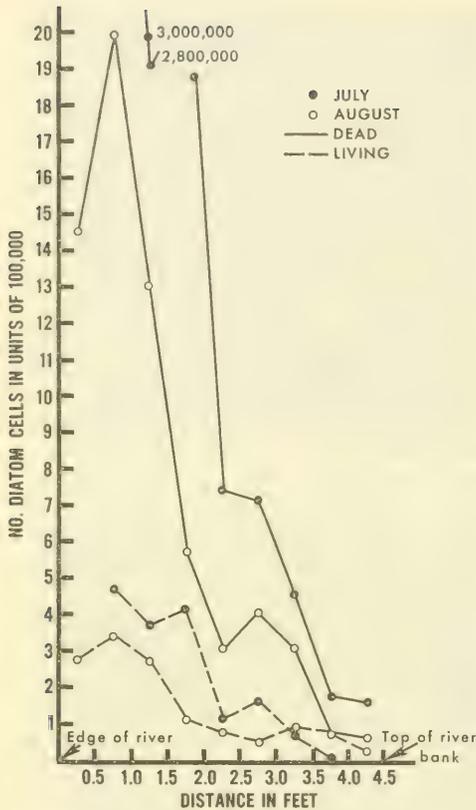


FIG. 15. Mean numbers of living and dead diatoms per gram of dried soil collected from the surface of the bank of the River Raisin. Samples were taken from the top of the bank down to the edge of the river.

soil samples of the surface (1 mm depth) of the river bank were diluted and aliquots placed on slide counting chambers. Only whole diatom frustules were counted; filaments of green and blue-green algae were counted as 1, regardless of whether there were 4, 10, or more cells per filament. Results were recorded in numbers per gram of dried soil.

As shown in Fig. 15 diatom density increased as one sampled from the top of the bank down to the river edge. Typically, there were many more dead than living diatoms (average ratio 7.7:1).

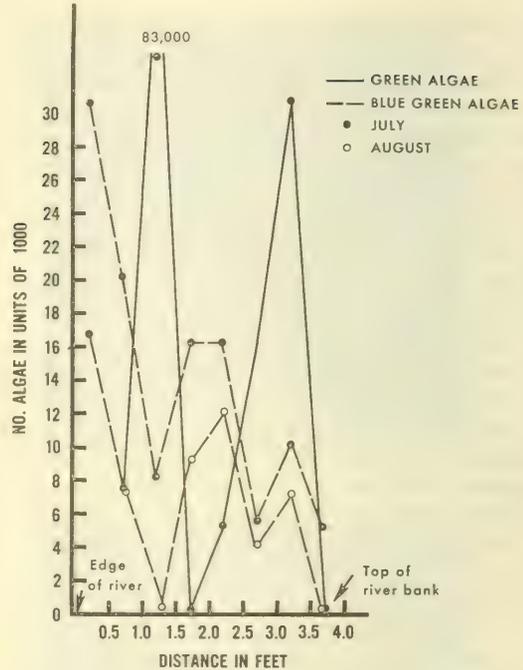


FIG. 16. Mean numbers of green and blue-green algae per gram of dried soil. Samples taken as in Fig. 15: note absence of green algae from samples in August.

TABLE 9. Chemical analysis of air dried surface soil from Bank of River Raisin below Manchester, Mich., U. S. A., used in our cultures.

pH	% organic material	Chemical radical	Range in ppm
7.4-7.5	3.5-6.9	Ca	2540-3470
		Mg	112-312
		K	24-40
		P	1.5-50
		NO <sub>3</sub>	25-75
		SO <sub>4</sub>	< 600
		Chlorides	< 80

Soluble salt (K)\* and total exchange capacity of this soil: K=18-42 (very low to low);\* the exchange capacity (mill equivalents per 100 gms soil) = 14.4-20.0.

\*K=conductivity value expressed in mho  $\times 10^{-5}$  obtained from a 1:2 soil/water mixture.

Living diatoms were as abundant as 100-450 thousand per gram. In other areas studied on the same river bank, densities of 300-600 thousand per gram have been found.

As many as 3 million dead frustules per gram were calculated. Diatoms appeared in every sample studied. In August the numbers of diatoms and other algae were significantly less (Figs. 15, 16). Green and blue-green algae seemed to be scattered irregularly; only half the aliquots studied contained blue-green algae, while 40% contained green. Peak densities of 83,000 per gram for green and 30,000 per gram for blue-greens were uncommon. Densities of both types, where found, were generally below 20,000 per gram. In the August sample no green algae were found and densities of the blue-greens were much less than in July. Although the counting methods are somewhat crude and samples were not extensive in time or space, the results show the order of magnitude of microflora supported by the soil utilized in our experiments and in the routine maintenance of *Oncomelania*.

The cultures when fully prepared were heated in an oven at 60° C for 2 hours. This baking eliminated difficulties often caused by earthworms (oligochaetes) since in unheated cultures the soil usually became totally disrupted with piles of worm castings, and the soil also became badly churned up (Figs. 8, 11). While sterile soil was also tested, more than 80% of the cultures in our laboratory became either heavily infested with mold in a month or the snails suffered excessive rates of mortality. As a result, nonsterile soil has been used continuously and with success.

#### 4. Light

Petri dish cultures were maintained under light (100-150 ft. candles) provided by a 40 watt, white, "cool," fluorescent tube suspended 10" above the cultures (as before, van der Schalie & Davis, 1965). The light was cycled 10-12 hours

per day.

Large clay pots and battery jars were provided with normal daylight combined with regular overhead lights (50-80 ft. candles during the day; room level light).

Medium clay pots were maintained either in room level light, or constant light of 70-100 ft. candles. Plastic tray cultures were placed in room level light, fluctuating artificial light (130-160 ft. candles for 10-12 hours during the day in a windowless room) or constant artificial light of 130-160 ft. candles.

#### 5. Temperature

Temperatures in the Petri dish cultures were 23° - 27° C. In large clay pots the reservoir temperature was 28° while the ambient temperature was 26° ± 1° C. Temperatures in the other cultures varied from 22° to 27° C with an average of 24° C.

#### 6. Water

Boiled filtered pond-water which had a pH of 7.3 was added initially to the cultures; water levels were then subsequently restored with distilled water.

#### 7. Food

It was assumed that the natural soil with its high concentration of algae and decayed organic matter provided food energy in all cultures, especially those under light. Filter paper was used as a food additive in all medium clay pot cultures and in all other cultures maintained in room-level light.

#### 8. Routine Maintenance

Water levels were checked daily in all cultures, at which time snails which had climbed up on the side of the containers were knocked down (*Oncomelania* has a pronounced negative geotropism). Filter paper was added to cultures when needed.

All cultures except medium clay pots were individually serviced every 2 weeks to remove and record dead adults, to record living and dead young snails as well as their whorl counts; further, reservoirs which had become clogged with soil particles were cleaned, and occasional cultures which were badly invaded by mold or in which the soil was much eroded were replaced.

Medium clay pots were checked as above once only each month. If, in daily maintenance, a culture was found to be moldy or infected with mites, that culture was changed immediately. In all cases where cultures were changed, the old culture was also maintained for another month to obtain all young which might hatch from eggs laid prior to the change. Young snails removed from parental culture were immediately placed into Petri dish cultures (2 young per dish), where they would grow to maturity in 8-9 weeks. These cultures were not removed from the shelves for that whole period, though water was added when needed and, after 2 weeks, constant watch was maintained to knock down young which had climbed up to the glass covers.

Large vivaria, such as the large clay pots or plastic trays, were not changed unless excessive amounts of algae or mold had invaded the culture. Such cultures often remained in good condition into their 3rd year. The medium clay pots maintained under both room level and constant light averaged one change per culture every 8 months, although some had to be changed every 3rd month. Under both lighting conditions a prime reason for this rate of change was the utilization of the soil by the snails, as well as the wear on the soil brought about by handling during maintenance, which caused erosion and mechanical soil breakdown. Mites were often a problem and were responsible for 15% of all culture changes. Mites proliferate rapidly and tend to overrun a culture. Excessive mold and algae also necessitated changing cultures. In the

medium clay pots black mold frequently developed on the filter paper. In such cases it was not necessary to change the culture but only the infected portion of the filter paper.

## 9. Adult Density and Sex Ratio

The densities of snails in terms of their culture type are listed in Tables 10 and 11. The medium clay pots usually contained 5 females and 5 males. In other vivaria the females accounted for 45% to 51% of the snails. The sex of the snails was easily and rapidly determined by the method of Williams as adopted by Wong & Wagner (1954). Their technique takes advantage of the fact that *Oncomelania* is dioecious and the verge of the male can readily be observed even at a very young stage.

## EXPERIMENTS

### A. Survivorship

#### 1. Mortality of Stock Snails Initially Received

In order to simplify matters, snails received from the field as well as the *Oncomelania hupensis hupensis* provided by Dr. Vogel from his laboratory are referred to throughout the paper as field snails. Each of the 4 subspecies was received at different times and established in culture as shown in Table 10, where they are listed alphabetically (column 1) and then according to vivarium type (column 2). The age of the snails was unknown but for reasons stated by Davis (1967) it was assumed that the snails had a minimal age of 7-8 months. Only those with varix formation were placed in culture since that condition indicates maturity and cessation of growth. None of the snails that showed extreme wear or shell erosion were used.

The average rates of mortality are shown in Figs. 17-20 plotted on a semi-logarithmic scale. The data here given for *Oncomelania hupensis formosana*

TABLE 10. Set-up of cultures of snails received from outside sources (Field and Laboratory)

Subspecies of <i>Oncomelania hupensis</i>	Culture type	Light	Total snails used	No. of cultures	Average no. of snails per culture	Average life of culture type (months)	Greatest length of culture life and % cultures attaining this (months %)	Cultures at time of writing	50% snails living (months)	10% snails living (months)
<i>formosana</i>	BJ	Room	1680	10	168	18	34 10	T	4	13
	LCP	Room	1000	4	250	38	41 50	Co	3	18
	PT	Alter.	1198	7	171	32	39 50	Co	5	21
<i>hupensis</i>	MCP	Room	10	1	10	20	20 100	Co	*	*
	MCP	Const.	12	1	12	20	20 100	Co	*	*
	PT	Room	53	1	53	20	20 100	Co	5	15
<i>nosophora</i>	A	Room	75	1	75	19	19 100	T	5	16
	BJ	Room	234	2	117	11	18 50	T	4	13
	LCP	Room	310	1	310	17	17 100	T	3	9
	PT	Room	213	3	71	13	18 30	T	2	11
<i>quadrasi</i>	A	Room	105	1	105	12	12 100	T	7	11
	BJ	Room	618	3	206	8	12 30	T	1	6
	LCP	Room	510	3	170	29	37 30	Co	6	17
	PT	Room	472	4	118	25	27 25	T	6	20
TOTAL			6490	42						

Vivarium Type: A = Aquarium  
 BJ = Battery Jar  
 LCP = Large Clay Pot  
 MCP = Medium Clay Pot  
 PT = Plastic Tray

Light: Alter. = Alternating  
 Const. = Constant  
 Room = Room level

Cultures: T = terminated since all snails were dead  
 Co = continuing with some snails still living

\* Mortality had not reached 50%

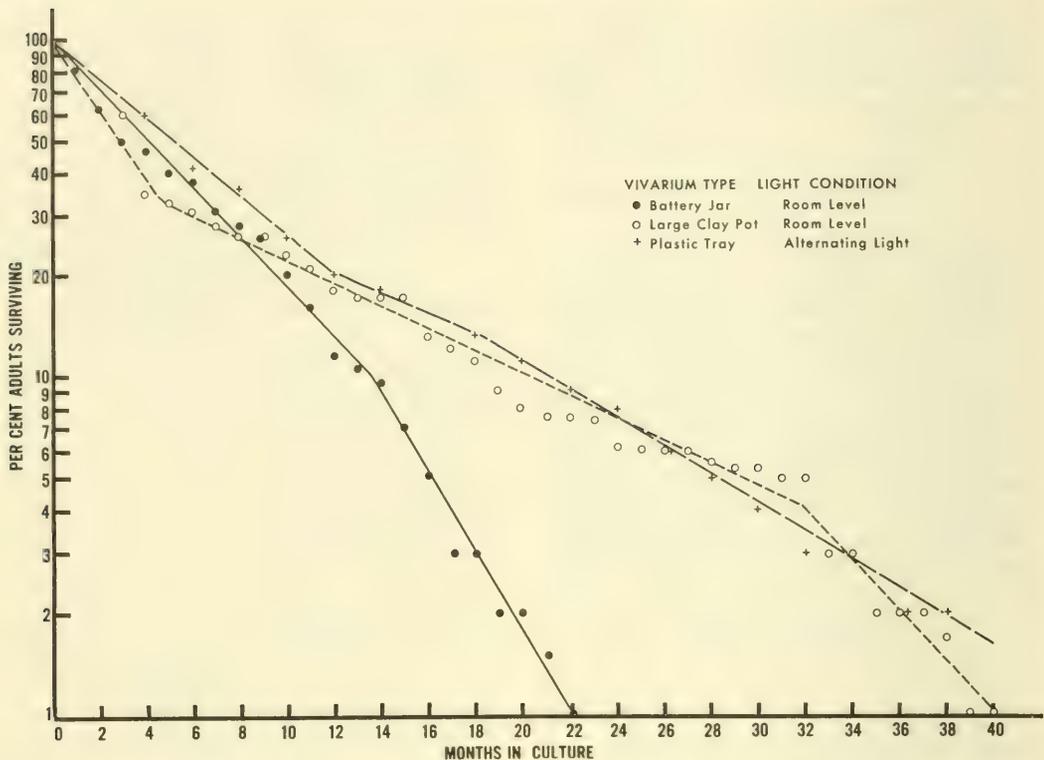


FIG. 17. Survival of field collected *Oncomelania hupensis formosana* in 3 different environments.

maintained in plastic tray vivaria are taken directly from Davis (1967); those for this same subspecies maintained in battery jars were obtained by further expanding those provided by him. A complete survey of mortality is shown graphically in Figs. 17-20, while the basic data are given in Table 10, which presents (1) the average length of life of a culture type, i.e., the period after which all of the snails had died in 50% of the cultures; (2) the greatest length of culture life for a vivarium type; (3) when, on the average, 50% and 10% of the snails were still living in each of the vivarium types.

Exponential death rates over the total culture period are in the minority (linear plottings; Fig. 18, plastic tray; Fig. 20, battery jar); irregularly changing rates were more commonly encountered (Figs. 17, 19, except battery jar, 20). Trends

in mortality are, therefore, more readily grasped from observing the changes in mortality rates recorded in Figs. 17-20.

It is readily seen that *Oncomelania hupensis nosophora* did not survive as well as the other subspecies in the vivaria types tested. Whether this was due to these snails being older than originally supposed or because of the detrimental effects of the vivaria types tested is not known. However, one should note that relatively few cultures were used in establishing this subspecies (Table 10). Whatever the reason, *O. h. nosophora* reacted to the environments in such a way as to call for discussion apart from *O. h. formosana* and *O. h. quadrasi*, which reacted quite similarly to the environments provided. Since comparatively few *O. h. hupensis* were available it was not possible to test the survival of this subspecies in vivaria

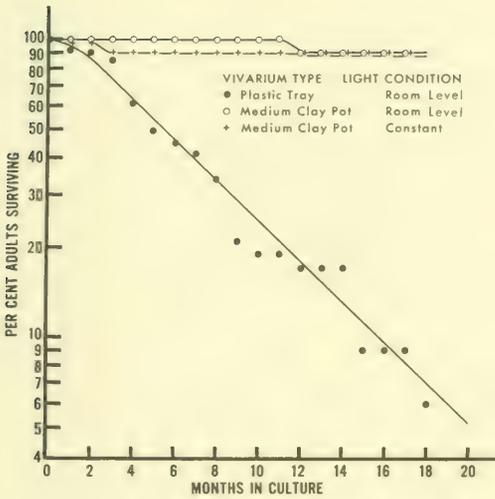


FIG. 18. Survival of *Oncomelania hupensis hupensis* supplied from Dr. Vogel's laboratory in 3 types of environment; note high survival in medium clay pots.

other than those shown. However, its reaction in the plastic tray (Fig. 18) together with the data on productivity in that environment (Table 12) suggested that it responded similarly to *O. h. nosophora*.

Optimal survivorship for *Oncomelania hupensis hupensis* occurred in the medium clay pot vivaria (Fig. 18). When this trend was recognized, 6 *O. h. nosophora*, then 18 months in culture in several plastic tray vivaria, were placed in medium clay pots at room-level light. In their former environment the snails had mortality rates roughly similar to those shown in Fig. 19, and the snails surviving at 18 months represented about 1% of the original population. Data on this small population of *O. h. nosophora* are not included in either Table 10 or Fig. 19 because those cultures had not been uniformly treated and some snails had been removed for various experiments. Consequently, continuous life table data were interrupted.

In the medium clay pots the *Oncomelania hupensis nosophora* suffered no mortality for 8 months (total calculated

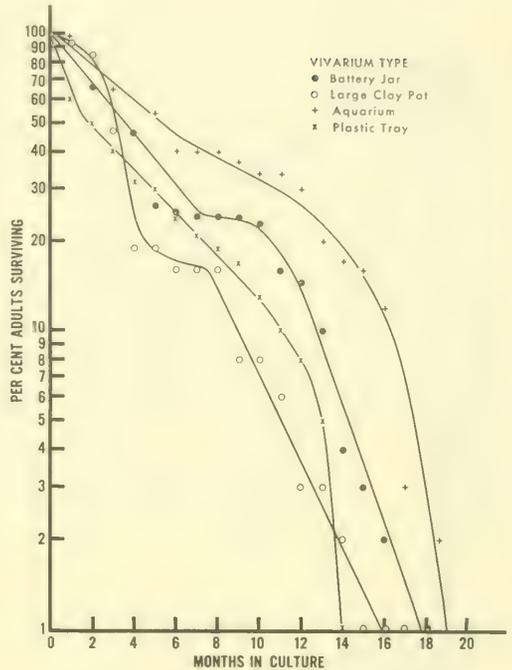


FIG. 19. Survival of field collected *Oncomelania hupensis nosophora* in 4 types of vivaria in room level light.

age of 33 months), dropped below 50% mortality after 18 months (calculated age of 43 months) and were all dead at 20 months or a minimal calculated age of about 3 1/2 years.

In both large clay pot and plastic tray vivaria *Oncomelania hupensis formosana* (Fig. 17) and *O. h. quadrasi* in large clay pots (Fig. 20) still had 1% of their initial population after 3 years in the laboratory; their calculated ages then exceeded 3 1/2 years. In these environments the exponential rates of mortality changed at various times (e.g., 4 months, large clay pot, Fig. 17) yet during the total life of the culture's populations the overall rates of mortality were low with finite rates of 8% or less per month.

From initial data with field snails it appeared that vivaria were unsuitable if rates of mortality increased markedly with time as shown by the curves for all vivaria (Fig. 19) and that for aquaria in Fig. 20. Increasing rates of mor-

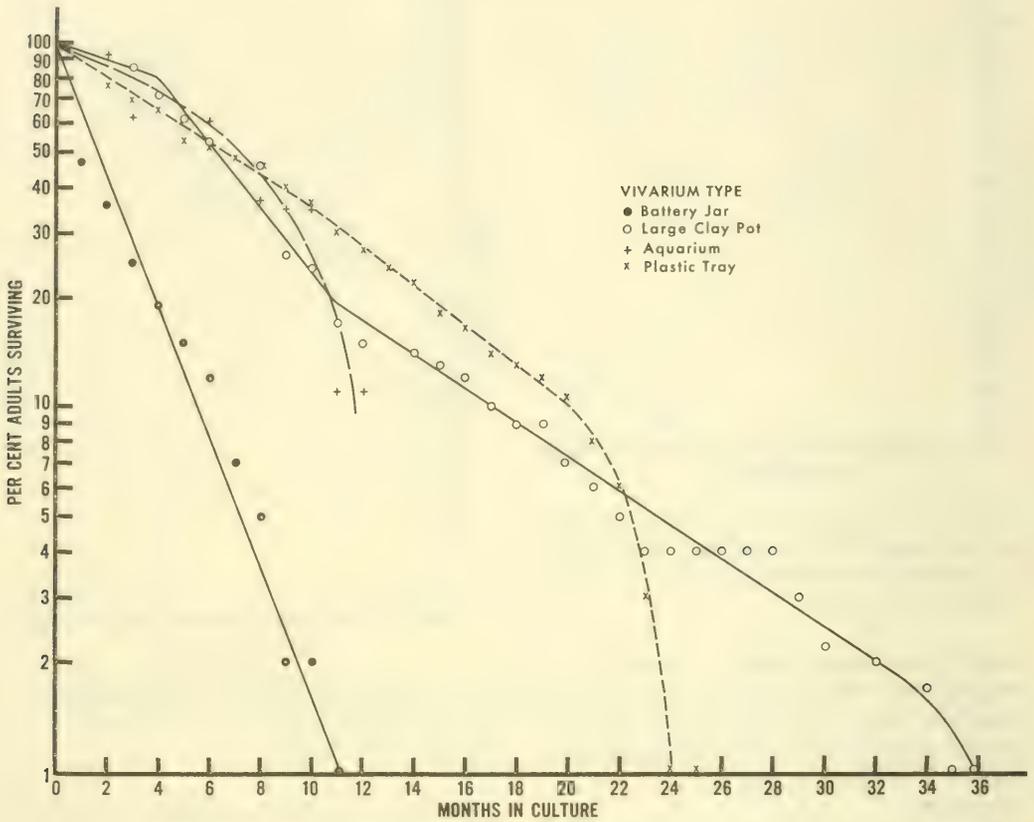


FIG. 20. Survival of field collected *Oncomelania hupensis quadrasi* in the same 4 types of vivaria in room level light.

tality yield curves that are not linear on the semi-log plots but bend toward the time axis. In the experiments with such increasing mortality rates, less than 1% of the field adults survived 24 months in the laboratory. Likewise, vivaria appeared very unsuitable if the finite death rate was 17% per month or greater, e.g. plastic tray, Fig. 18 (17%); battery jar, Fig. 20 (35%).

It was evident that the battery jar vivarium and the aquarium were very poor. In the former, *Oncomelania hupensis formosana* and *O. h. quadrasi* had extremely low survival, in fact the lowest average culture life, as is apparent from Table 10. In this environment, more than in any other, the shells became rapidly eroded.

The aquarium was inefficient, not only because of poor survivorship, but we also found, as did Sandground & Moore (1955), that it was the most cumbersome to maintain: it takes much more space than the other cultures, it is difficult to move, to inspect for young and dead snails, to clean and service.

The greatest length of life attained in this laboratory with field collected snails was, up to the date of writing: *Oncomelania hupensis formosana*, 4 years (some were still living); *O. h. quadrasi*, 3.5 years (all dead); *O. h. nosophora*, 3.5 years (all dead); *O. h. hupensis*, 2 years (and over 50% still living).

Under the conditions tested, the average duration of life in the laboratory

TABLE 11. Set-up of cultures of laboratory reared *Oncomelania* 2.0-2.5 months old

Subspecies of <i>Oncomelania hupensis</i>	Culture type	Light	Total snails used	No. of cultures	Average no. of snails per culture	Average life of culture type (months)	Greatest length of culture life and % cultures attaining this (months)	Cultures at time of writing	50% snails living (months)	10% snails living (months)
<i>formosana</i>	BJ	Room	385	5	77	16	27	T	5	14
	LCP	Room	170	1	170	30	30	Co	5	17
	MCP	Room	50	5	10	18	18	Co	*	*
	MCP	Const.	80	8	10	18	100	Co	*	*
	PT	Room	350	7	50	28	31	Co	12	27
	PT	Alter.	264	6	44	27	29	Co	6	25
<i>hupensis</i>	MCP	Room	40	4	10	14	14	Co	*	*
	MCP	Room	30	3	10	14	14	Co	*	*
<i>nosophora</i>	MCP	Room	20	10	2	18	18	Co	*	*
	MCP	Const.	30	10	3	18	18	Co	*	*
	PT	Const.	33	1	33	21	22	T	12	17
<i>quadrasi</i>	MCP	Room	20	2	10	22	20	Co	*	*
	MCP	Const.	9	1	9	16	20	Co	*	*
	LCP	Room	43	1	43	28	28	Co	16	*
	PT	Alter.	120	4	30	22	25	Co	7	23
	PT	Const.	25	1	25	16	23	Co	6	11
TOTAL			1669	69						

Vivarium Type: BJ = Battery Jar  
 LCP = Large Clay Pot  
 MCP = Medium Clay Pot  
 PT = Plastic Tray

Light: Alter. = Alternating  
 Const. = Constant  
 Room = Room level

Cultures: T = terminated as all snails dead  
 Co = continuing as some snails still living

\* = 50% mortality not reached

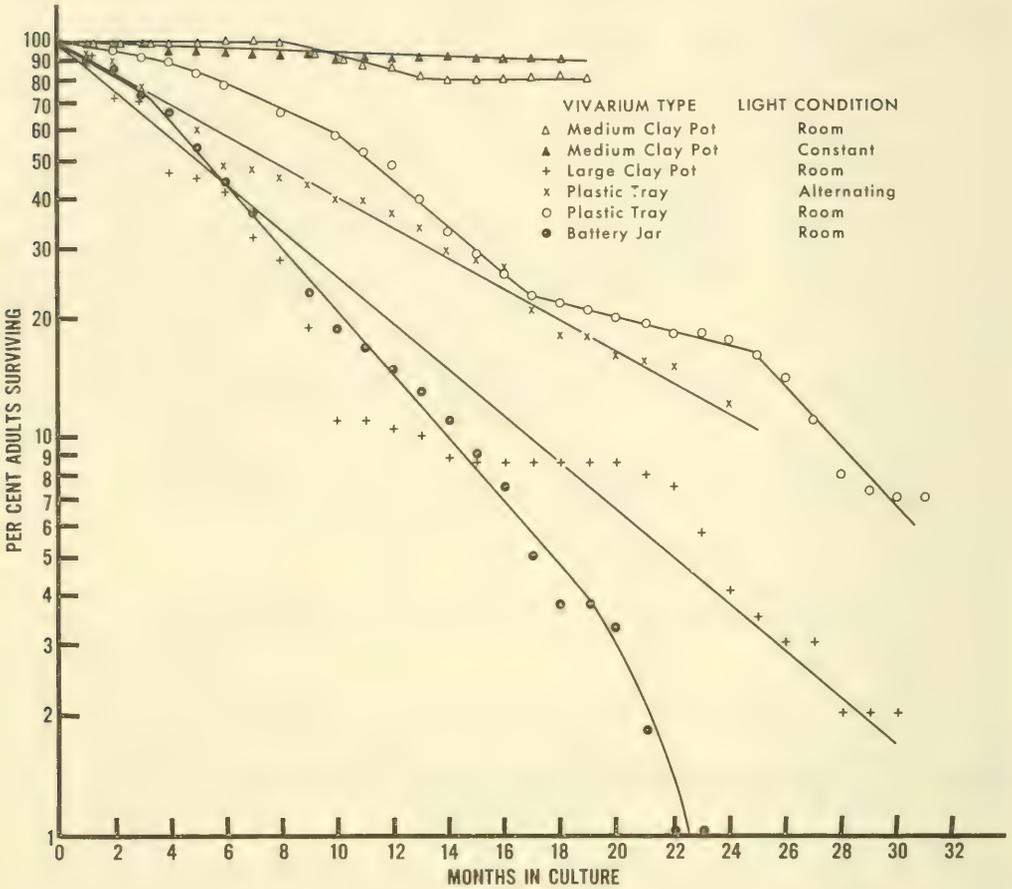


FIG. 21. Survival of laboratory reared *Oncomelania hupensis formosana* in various environmental conditions; note high survival in medium clay pot.

was 5-7 months or minimal ages of 12-14 months for all but *Oncomelania hupensis hupensis*, which had suffered only 10% mortality after 18 months in culture. It is evident from the response of *O. h. hupensis* and the initial results with *O. h. nosophora* that the average duration of life would have been greatly increased, in general, by maintenance in medium clay pots.

## 2. Mortality of Laboratory Reared Snails

First generation laboratory reared snails were placed in culture at a known age of 2.0-2.5 months as listed in Table

11. The average rates of mortality are plotted in Figs. 21-23. With the exception of medium clay pots the density of snails per culture was lower than that of the "field" snails. Table 11 lists: (1) average life of a culture type, (2) greatest length of life for a culture type, and (3) at what month 50% and 10% of the snails were still living.

Optimal survivorship for all species was obtained in the medium clay pots, as was previously suggested on the basis of data from field *Oncomelania hupensis hupensis* and *O. h. nosophora*: 50% mortality had not occurred after 14-18 months in culture (compare with field

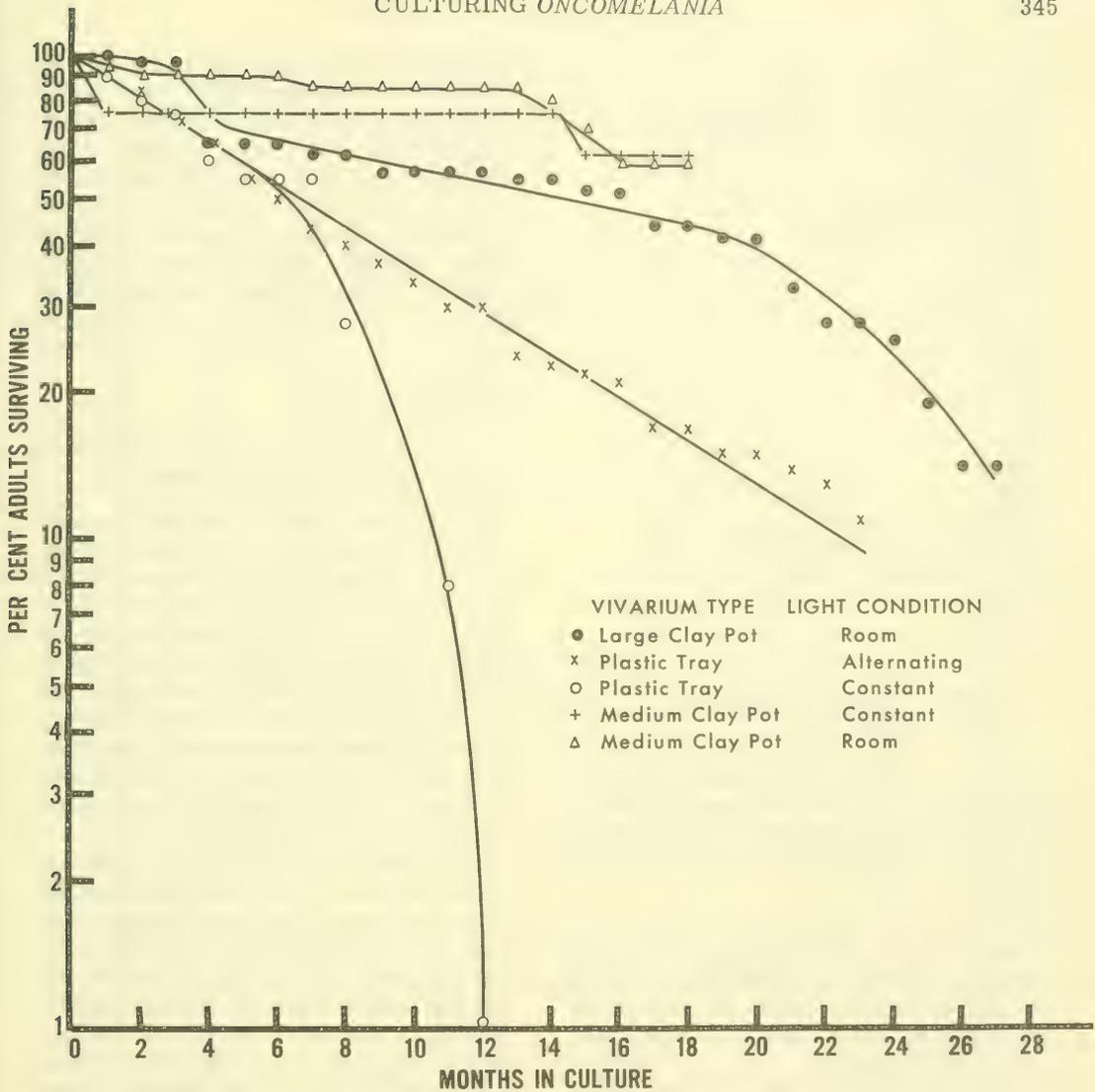


FIG. 22. Survival of laboratory reared *Oncomelania hupensis quadrasi* in 3 types of vivaria under varying light conditions.

snails). In fact, 60% or more of *Oncomelania hupensis quadrasi* were alive at the end of 14-18 months while 80% or more of the other taxa were surviving. The finite rate of mortality was less than 5% per month, generally about 2% per month.

Mortality rates in battery jar vivaria for laboratory reared *Oncomelania hupensis formosana* (Fig. 21) confirm that this environment is not suitable for maintenance when optimal survivorship

is required. Plastic tray and large clay pots provided intermediate survivorship with finite rates of mortality between 5% and 12%. At this point optimal survival is defined in terms of a finite rate of mortality less than 5% per month for at least 2 years, and intermediate survival as a rate between 5% and 12% per month, while in a very unsuitable environment, poor survival rates of 13% or more are found. *O. h. formosana* appeared to have better survival in the

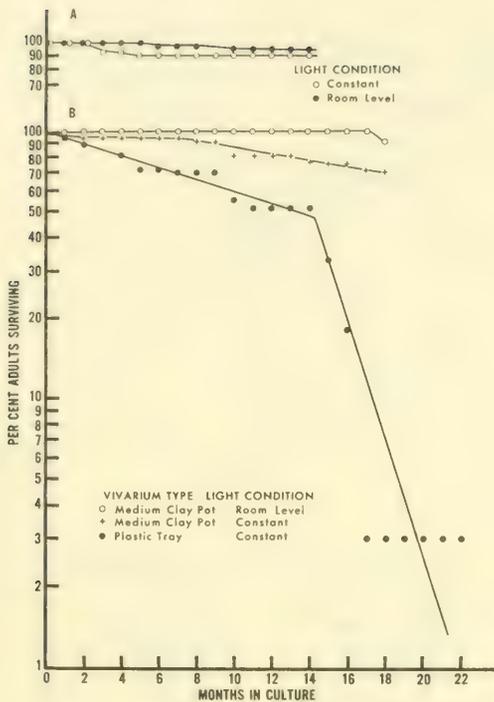


FIG. 23. A. Survival of laboratory reared *Oncomelania hupensis hupensis* in medium clay pots under varying light conditions; B. *Oncomelania hupensis nosophora* under similar environments.

plastic trays than in large clay pots, as also observed for field snails, but not so pronouncedly, while *O. h. quadrasi* survived better in large clay pots than in plastic trays.

It was evident that the increased duration of light was accompanied by increased rates of mortality. This trend can be seen by comparing survivorship curves for *Oncomelania hupensis formosana* maintained under room level or alternating light in plastic tray vivaria (Fig. 21); by the curves for *O. h. quadrasi* in plastic trays under alternating and constant light (Fig. 22); and generally by the survivorship curves for snails in medium clay pots under room level light or constant light (Figs. 6, 22-23).

Although spot checks on male and female mortality in cultures other than

the medium clay pot showed essentially equal rates of mortality, female *Oncomelania hupensis quadrasi* had noticeably greater rates of mortality in the medium clay pots with 50% dead in the 16th or 19th month (Figs. 31, 32). *O. h. hupensis* females in medium clay pots under constant light also had a greater rate of mortality than the males with 20% dead at 14 months and no males dead.

## B. Productivity

### 1. Output of young in relation to subspecies and culture media

Productivity was calculated in terms of young produced per female per month ( $y/f/m$ ) and measured in terms of living and dead young snails removed from parental culture, not of eggs laid. In all cases involving medium clay pots, exact rates of female mortality were recorded. For all other cultures, rates of female mortality were determined by periodic checks on the cultures; it was found that, in these, female mortality rates were similar to those of males.

In Table 12 the average number of young obtained per field-collected female per month is listed for each subspecies, and in decreasing order. Only months when young were hatched are considered (column 6). These figures represent the actual output per female during periods of production. More meaningful to a culture program is the average  $y/f/m$  over a 12 month period, where non-productive months are averaged (Table 12, column 7). Comparison of our figures for the monthly output per female with the average daily output cited from the literature in Table 6 shows that the levels of productivity obtained in our laboratory were far below those recorded by previous authors. These lower levels may be due, in part, to the longer periods of time our snails were in culture and to the greater number of snails in our cultures.

However that may be, our results concerning reproduction involve (1) the

TABLE 12. Reproduction rates of field\* collected females under varying laboratory conditions

Subspecies of <i>Oncomelania hupensis</i>	Culture type	Light	Months during which young were produced		Length of time culture was established (months)	Production (y/f/m)			% young dead in parental culture at 2.0-2.5 whorls
			No.	%		Average for productive months only	for 1st year (months 1-12)	for 2nd year (months 13-24)	
<i>formosana</i>	LCP	Room	39	95	41	3.23	1.04	4.23	60
	BJ	Room	24	70	34	1.29	0.39	1.47	19
	PT	Alter.	25	64	39	0.88	0.63	1.06	10
<i>hupensis</i>	MCP	Room	6	30	20	5.26	2.08	1.65	5
	MCP	Const.	9	45	20	2.91	5.15	0.13	5
	PT	Room	0	0	20	0.00	0.00	0.00	0
<i>nosophora</i>	BJ	Room	4	22	18	0.50	0.17	0.00	2
	A	Room	6	31	19	0.48	0.24	0.00	2.5
	PT	Room	5	28	18	0.23	0.09	0.00	0
	LCP	Room	0	0	17	0.00	0.00	0.00	0
<i>quadrasi</i>	LCP	Room	32	86	37	3.43	0.53	5.14	42
	BJ	Room	7	58	12	0.87	0.51	0.00	5
	PT	Room	18	66	27	0.52	0.31	0.62	10
	A	Room	6	50	12	0.38	0.19	0.00	10

Vivarium type: A = Aquarium  
 BJ = Battery jar  
 LCP = Large clay pot  
 MCP = Medium clay pot  
 PT = Plastic tray

Light: Room = Room level  
 Const. = Constant  
 Alter. = Alternating

\* All snails received from outside sources used for establishing a subspecies in the laboratory will be referred to as field collected.

effect of the environment on reproduction for each subspecies and (2) the different potential of each subspecies for laying eggs in the laboratory.

In column 4 of Table 12 one sees which of the taxa produced more continually than the others in terms of months in which young hatched. In decreasing order of fecundity were *O. h. formosana*, *O. h. quadrasi*, *O. h. nosophora* (especially based on results in the plastic tray cultures) and *O. h. hupensis*.

In column 6 of Table 12, peak reproductive potential is recorded in terms of y/f/m for productive months only. The greatest potential was reached by *O. h. hupensis* in medium clay pots with 5.26 y/f/m. Considering all environments provided, *O. h. formosana* followed by *O. h. quadrasi* showed higher reproductive potential than *O. h. nosophora* (no production in large clay pots) and *O. h. hupensis* (no production in plastic trays). As will be shown later, productivity of all subspecies studied here is heightened in medium clay pots. For *O. h. hupensis* an indication of this situation is given in Table 12 by its relatively high productivity in this culture type, while it produced no young in plastic trays. Reproductive potential in medium clay pots will, therefore, be discussed later under laboratory reared snails, where medium clay pots were tested with each subspecies.

When productivity for each subspecies is considered for 2 years (Table 12, columns 7, 8) in the various environments (medium clay pots excluded), it is evident that again *O. h. formosana* is the most fecund followed by *O. h. quadrasi*, *O. h. nosophora*, *O. h. hupensis*.

The effect of the environment on production of young, when it can be compared, points to peak production in large clay pots; 2nd best productivity was in battery jars and 3rd best in plastic trays.

Corresponding data for laboratory reared females are listed in Table 13.

As already noted for "field" *O. h. hupensis*, the medium clay pot culture was superior, for the other subspecies also, to the other culture types tested. Generally, young were produced in a greater percentage of the months in culture (column 4), except for *O. h. quadrasi* in large clay pots and plastic trays. However, considering the average y/f/m for productive months only, (column 6) it was clear that superior productivity occurred in medium clay pots, at either room level or constant light. Production was generally higher than in field collected snails, but only in medium clay pots was it of the same order of magnitude as that cited by other workers (Table 6).

The gradient in reproductive potential outlined for the various subspecies is further confirmed on the basis of multiplication in the medium clay pots; yield in productive months was highest in *Oncomelania hupensis formosana* (Table 13, column 6) followed by *O. h. quadrasi*, *O. h. nosophora* and *O. h. hupensis* and the same pattern holds true for productivity over a 2 year period (columns 7 and 8) even though, in the first year, *O. h. quadrasi* had the greatest output of young. The lowest productivity in terms of percentage of months where young were produced and of y/f/m occurred in battery jars and plastic trays under constant light.

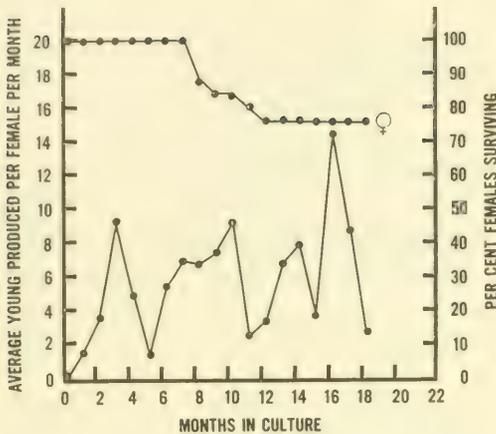
There was no significant difference between the number of juveniles hatched in medium clay pots in room level light or constant light when *Oncomelania hupensis hupensis*, *O. h. nosophora* and *O. h. quadrasi* are compared (Table 13, column 6). Significantly more young were produced, however, by *O. h. formosana* in constant light (11.10 vs. 6.48). Productivity increased in the second year for *O. h. formosana* and declined only in the case of *O. h. quadrasi* and *O. h. hupensis* under constant light. With the latter subspecies, one notes, by comparing Tables 12 and 13, that production for field specimens declined in the 2nd year, when kept under con-

TABLE 13. Reproduction rates of laboratory reared females under varying laboratory conditions

Subspecies of <i>Oncomelania hupensis</i>	Culture type	Light	Months during which young were produced		Length of time culture was established (months)	Production (y/f/m)			% young dead in parental culture at 2.0-2.5 whorls
			No.	%		Average for productive months only	for 1st year (months 1-12)	for 2nd year (months 13-24)	
<i>formosana</i>	MCP	Const.	18	100	18	11.10	9.95	12.30	2
	MCP	Room	18	100	18	6.48	5.17	7.42	5
	LCP	Room	22	73	30	2.65	1.65	3.86	78
	PT	Room	26	84	31	2.18	1.66	2.65	6
	PT	Alter.	24	83	29	1.81	2.38	1.07	5
	BJ	Room	6	22	27	0.97	0.02	0.50	33
<i>hupensis</i>	MCP	Const.	8	57	14	3.18	1.91	0.00	6
	MCP	Room	10	71	14	3.01	0.81	6.79	4
<i>nosophora</i>	MCP	Const.	18	100	18	6.57	7.32	5.06	10
	MCP	Room	15	83	18	5.17	4.78	3.76	8
	PT	Const.	4	18	22	2.53	0.84	0.00	0
<i>quadrasi</i>	MCP	Const.	18	90	20	10.41	13.09	3.80	2
	MCP	Room	12	60	20	8.52	5.28	4.86	5
	LCP	Room	23	82	28	5.09	4.35	5.40	73
	PT	Const.	4	17	23	2.48	0.83	0.00	4
	PT	Alter.	23	92	25	2.36	2.26	2.46	8

Vivarium Type: BJ = Battery jar  
 MCP = Medium clay pot  
 LCP = Large clay pot  
 PT = Plastic tray

Light: Alter. = Alternating  
 Const. = Constant  
 Room = Room level



FIGS. 24-32. The average number of young produced per female each month in medium clay pot cultures; each subspecies is shown under both constant and room level light. Data involve snails reared in our laboratory, excepting Fig. 26 which gives the production by females received from Dr. Vogel's laboratory. The percentage of females surviving each month is also shown. Production in any month is calculated on the basis of the eggs laid by females in the previous month.

FIG. 24. *Oncomelania hupensis nosophora*; room level light.

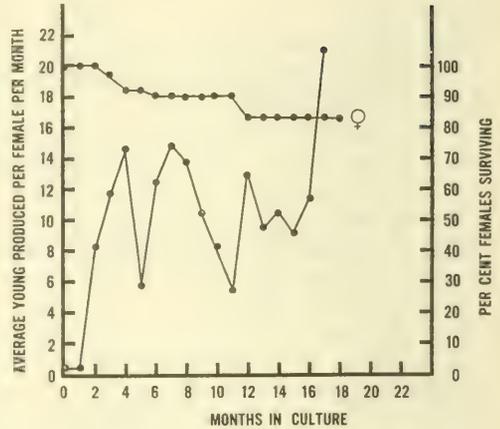


FIG. 25. *Oncomelania hupensis formosana*; constant light.

stant light. The decline in fecundity for *Oncomelania hupensis quadrasi* is probably associated with the increasing rate of female mortality in the 2nd year (Figs. 31-32).

## 2. Sporadic Nature of Reproduction

Young are not produced at a steady rate but sporadically. In any given culture numerous young will appear for 1 or 2 months, followed by a period when few or none hatch. Figures 24-32 show graphically the average number of y/f/m hatched each month in medium clay pots. Also shown are the percentages of females surviving each month. On these graphs, since eggs may take at least 30-40 days to hatch, the production for any given month resulted from eggs layed by females alive in the previous month.

From the data in Figs. 24-32 one can assess that the highest average production reached in any 1 month was 40 y/f/m for *Oncomelania hupensis quadrasi*, 22.5 y/f/m for *O. h. formosana*, 18 y/f/m for *O. h. nosophora* and

11 y/f/m for *O. h. hupensis*.

Since one cannot predict general levels of productivity for a single culture, data for *Oncomelania hupensis quadrasi* in a medium clay pot in constant light are inconclusive. An example for such unpredictability is given by 2 medium clay pots, which were made up in the same way at the same time and maintained side by side. Each received 5 males and 5 females of *O. h. hupensis* chosen at random from a large population of snails sent by Dr. Vogel. After 3 months 1 culture had produced young at 0.5/f/day, while the other produced none.

## 3. Adult Density, Light and the Production of Young

A small scale experiment was set up using 2 sets of 4 medium clay pots and *Oncomelania hupensis formosana*. In one set, a single female was placed in each pot with 3 males; 2 pots were held under constant light and 2 in room level light. The second set of pots was set up similarly except that 2 females and 3 males were established in each

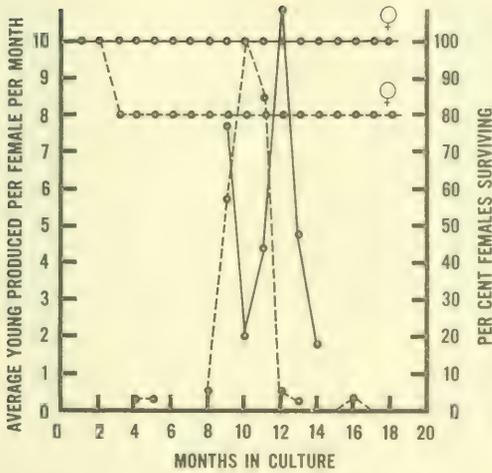


FIG. 26. *Oncomelania hupensis hupensis* from Dr. Vogel's laboratory; room level and constant light.

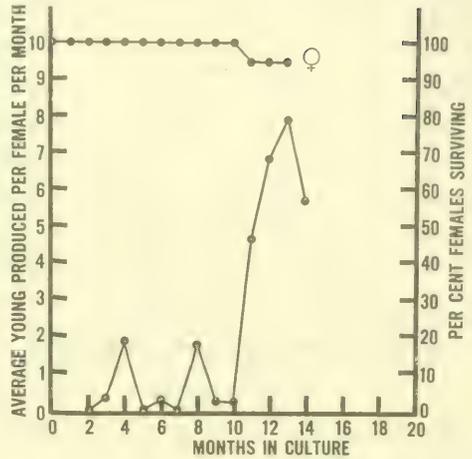


FIG. 27. *Oncomelania hupensis hupensis*; laboratory reared under room level light.

culture.

The cultures were checked every 2 weeks for 12 months; young were removed and recorded, dead females and males were removed and replaced. The results are shown in Table 14. The yield in y/f/m of *O. h. formosana* in this experiment for a 1-year period can be compared with that for 10 snails per pot (5 males and 5 females) for the first year (Table 13, column 7). The production in these latter averaged 7.5 y/f/m (average of productivities at constant and room-level light environment). In this experiment, the overall average was 14 y/f/m for 1 and 2 females in both light and room-level environments. Generally then, productivity is increased when the density of snails is lower in the same given volume and environment.

Too few cultures were used with 1 and with 2 females to provide data that would accurately indicate in which case productivity per female had been greater. With constant light (Table 14) the average output in y/f/m certainly was the same. The difference in output for snails at room-level light (18 y/f/m against 28 y/f/m) may only reflect the difference in an individual snail's capacity to produce young: the greatest potential re-

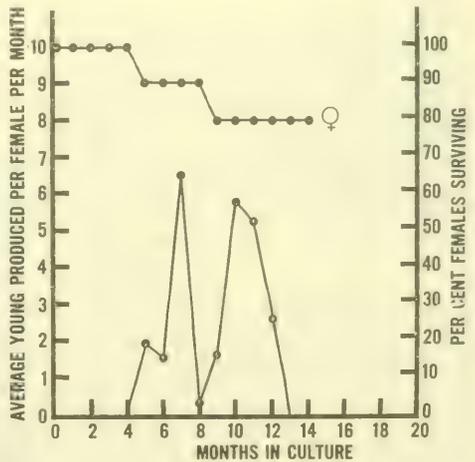


FIG. 28. *Oncomelania hupensis hupensis*; laboratory reared under constant light.

corded so far in this laboratory for a 1-year period was that of 31.45 y/f/m in culture 7 of this experiment.

Observations in this experiment further indicated that greater fecundity and lower rates of mortality occurred in room-level light. The data for cultures 3, 4, 7, 8 show that, in the first year, females at low density (2 females, 3 males) can produce about 18-

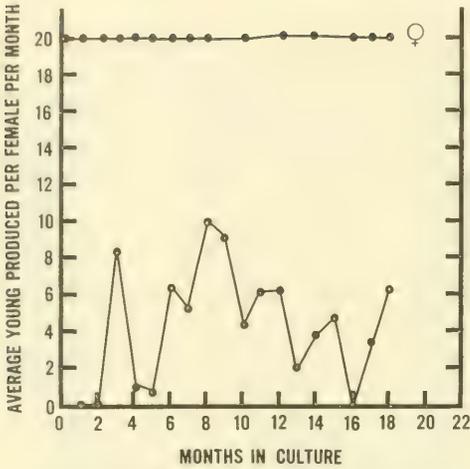


FIG. 29. *Oncomelania hupensis formosana*; room level light.

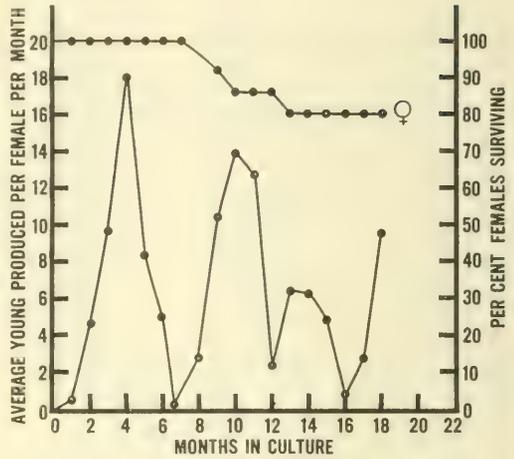


FIG. 30. *Oncomelania hupensis nosophora*; room level light.

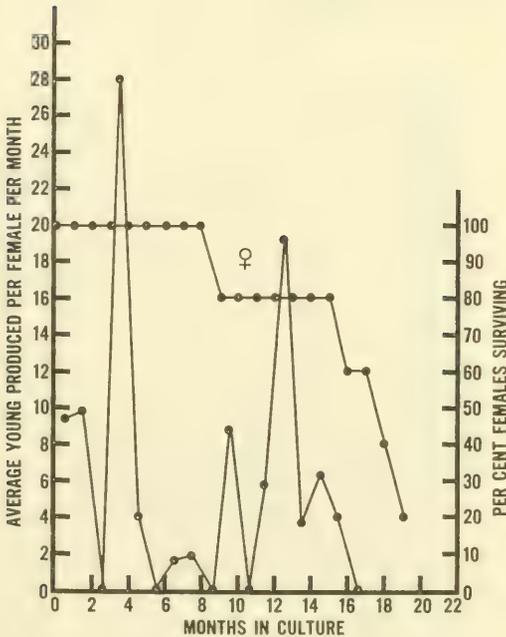


FIG. 31. *Oncomelania hupensis quadrasi*; room level light.

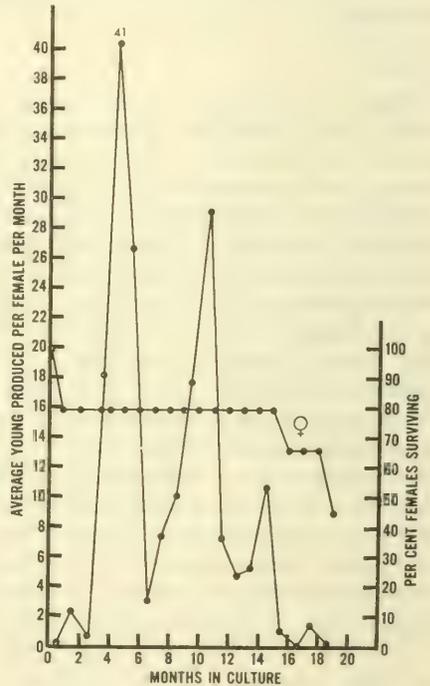


FIG. 32. *Oncomelania hupensis quadrasi*; constant light.

28 y/f/m, i.e., at levels of magnitude corresponding to those reported by Chi & Wagner. However, the role of light is problematic. In the larger scale experiments presented in Table 13, one notes that this same subspecies showed

greater productivity in constant light, when the number of y/f/m was 9.95 in the first year. This figure is comparable to those here obtained for constant light: 5.03-5.33 (Table 14), rather than to the higher values obtained in room level

TABLE 14. Production of young by 1 or 2 female *Oncomelania hupensis formosana* per Medium Clay Pot Vivarium\*, for 12 months, under different conditions of lighting

Constant Light				Room Level Light			
1 female per culture							
Culture	Total Young	Average y/f/m	No. females dying	Culture	Total Young	Average y/f/m	No. females dying
1	59	4.91	1	3	281	23.41	0
2	62	5.16	2	4	149	12.41	0
Average	60.5	5.03		Average	215	17.91	
2 females per culture							
5	84	3.50	0	7	755	31.45	0
6	172	7.16	0	8	572	23.83	0
Average	128	5.33		Average	663	27.64	

\*Each pot also contained 3 males.

light: 18-28.

#### 4. Seasonal Periodicity in Productivity

The percentages of young produced each month of the year in medium clay pots both at room level and constant light with a period of 18 months are shown in Figs. 33 and 34.

One should keep in mind that all cultures were not started simultaneously. Some cultures existed for the whole 18 month period while others were only 4 or 5 months old. No seasonal rhythm was apparent. The marked fluctuations recorded throughout the year mostly reflect culture changes due to culture deterioration, or initiation of new cultures, with the usual month lag before young hatch.

### C. Survivorship and Growth of Young

#### 1. Survivorship

Data on mortality of young snails were derived from both the number of young which died in the parental culture before their routine removal to Petri dish cultures and from the number of young

which died in the Petri dish cultures. The former were just hatched, since about 95% of them had 2.0-2.5 whorls, i.e., hatching size. After hatching, the snails generally added 0.5 whorls per week in the parental culture for a period of at least 4 weeks. Very few dead with 3.0-3.5 whorls were found in the parental culture.

Young snails were maintained in the Petri dish cultures for 8-9 weeks. At the end of that period very few deaths had occurred among snails with 4-6 whorls. Most of the snails which had died in the Petri dish vivaria had but 2.5-3.0 whorls, which indicated that they had died shortly after being placed in culture, i.e., at 2-4 weeks of age.

Tables 12 and 13 show the percentages of young which perished in each vivarium type used for producing young. It is evident that the culture type greatly affects the number of young surviving past the time of hatching. Especially noticeable is the high rate of mortality in large clay pots where 42-78% of the young die upon the soil bank, especially towards the back wall of the bank, away from the water. In other vivaria mor-

tality was 10% or less; in the medium clay pots it was 4-5% with the exception of an average 9% for *Oncomelania hupensis nosophora*.

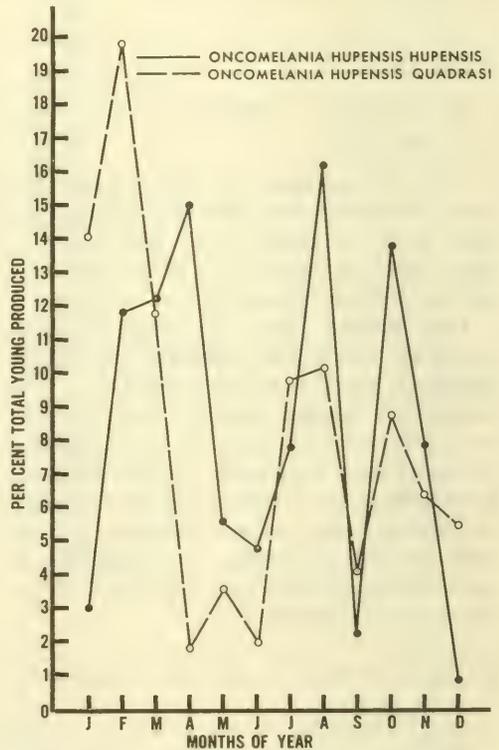
In Petri dish vivaria, van der Schalie & Davis (1965) found that mortality was 10% or less when the algae were kept in check. In the experiments devoted to the study of growth rates, all conditions were closely observed and controlled. One would expect higher mortality rates in the routine handling of large numbers of snails because: (1) in rapid handling of thousands of snails the young tend to be treated a little more roughly in transfer to Petri dishes; (2) in routine maintenance, an individual Petri dish does not usually receive the attention assuring optimal care. Records kept for several years on *Oncomelania hupensis formosana* and *O. h. quadrasi* show that routinely handled snails suffer a 16-20% mortality in Petri dishes when

isolated at 2.0-3.5 whorls but only 8-10% mortality when isolated at 4.0-5.0 whorls.

Considering all sources of mortality from the age of hatching to 10 months, the percentage of snails surviving is shown in Fig. 35. These generalized graphs are based on the use of medium clay pots for producing young, on an assumed 5 or 10% mortality, depending on the subspecies, in the parental culture and on a 20% mortality in the Petri dishes.

2. Growth

In 1965 we have already discussed factors favoring optimal growth for *Oncomelania hupensis formosana* and those which retarded growth. In this



FIGS. 33-34. Monthly percentages of young from all vivaria for each of the subspecies.

FIG. 33. Young produced each month by *Oncomelania hupensis formosana* and *O. h. nosophora*.

FIG. 34. Young produced each month by *Oncomelania hupensis hupensis* and *O. h. quadrasi*.

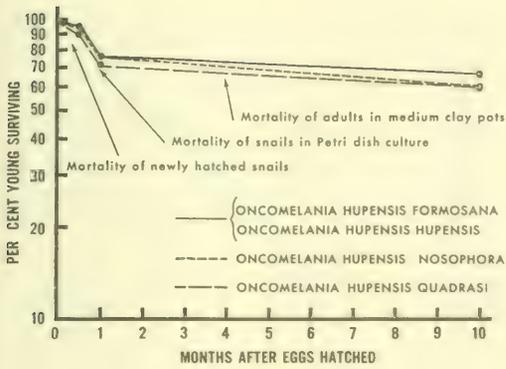


FIG. 35. The percentage of snails surviving from the time of hatching up to 10 months. Mortality of newly hatched snails (0-2 weeks) occurred in parental, medium clay pot cultures; mortality of snails isolated in Petri dish cultures generally occurred from 2 to 4 weeks. After 8-9 weeks in Petri dish culture, snails were placed in medium clay pots.

section, discussion on growth is extended to cover: (1) growth rates for young of that subspecies maintained in plastic trays under constant or room level light; (2) growth rates for the 4 subspecies in Petri dish cultures under constant light.

#### a. Growth in Plastic Trays

Two plastic tray vivaria were established. One culture was placed under constant light (about 150 ft. candles) while the other was maintained in room level light. Forty snails, at the 2.0-2.5 whorl stage, were placed into each culture. Every 3rd day a subsample of 20 snails was chosen from each culture; their lengths were measured to 0.01 mm, using a Nippon Kogaku sliding ocular micrometer, and they were then returned to their respective cultures; the results are shown in Fig. 36. In the initial 8 weeks the snails under constant light grew 0.47 mm per week, while those at room level light grew only 0.22 mm per week.

#### b. Growth in Petri Dishes

Specimens of each of the 4 subspecies studied were placed singly into Petri dish cultures at the 2.0-2.5 whorls stage. The cultures were maintained under

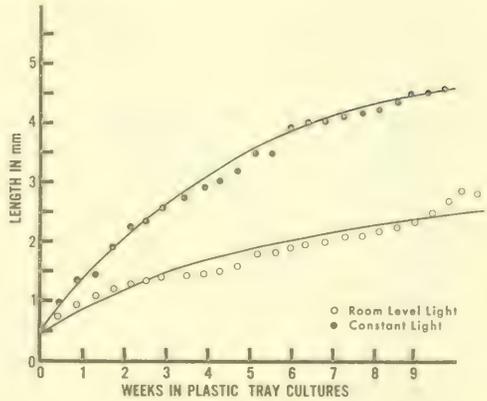
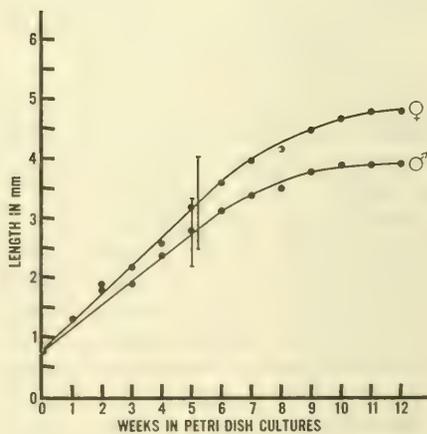
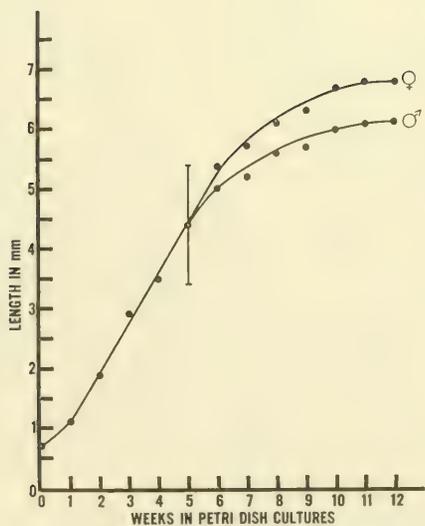
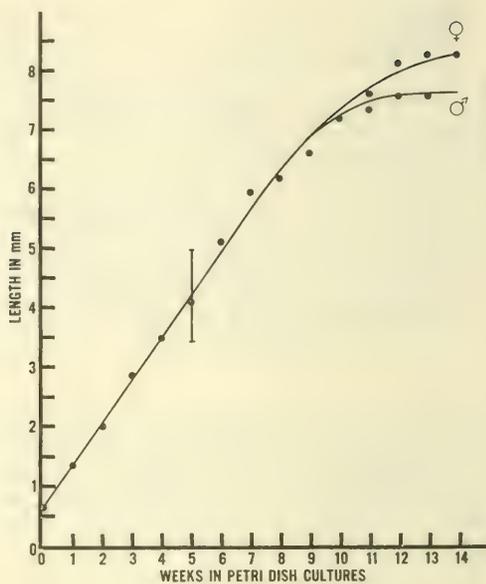
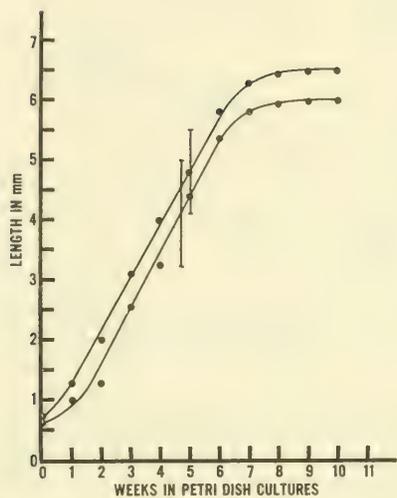


FIG. 36. Growth rates for *Oncomelania hupensis formosana* maintained in plastic trays under constant light and room level light (40 snails each).

constant light and measurements of shell length were made every 3 days; the data are plotted in Figs. 37-40. In all, 25 specimens of each sex of *Oncomelania hupensis formosana* were measured (data from Davis, 1967); 19 females and 18 males of *O. h. hupensis*; 23 females and 30 males of *O. h. nosophora*; and 20 female and male specimens of *O. h. quadrasi*.

Calculations from growth curves (Figs. 37-40 and Table 15) indicate that growth rates for all the subspecies, except *Oncomelania hupensis quadrasi*, exceed 0.60 mm per week for the first 8 weeks. In the logarithmic phase of growth, the length of shell increases at rates greater than 0.40 mm per week for *O. h. quadrasi*, and at about 0.70 mm per week for the other subspecies. *O. h. formosana* grows most rapidly, followed by *O. h. nosophora*, *O. h. hupensis* and *O. h. quadrasi* in that order.

In nature, *Oncomelania hupensis hupensis* has the largest shell followed in decreasing order of size by *O. h. nosophora*, *O. h. formosana* and *O. h. quadrasi*. These differences in size are maintained in laboratory reared snails. In Figs. 37-40 one notes that sexual dimorphism is more pronounced in the smaller subspecies at earlier periods



FIGS. 37-40. Growth rates for the 4 subspecies of *Oncomelania hupensis*, reared singly in Petri dishes under constant light.

FIG. 37. *Oncomelania hupensis formosana* (from Davis, 1967).

FIG. 38. *Oncomelania hupensis hupensis*.

FIG. 39. *Oncomelania hupensis nosophora*.

FIG. 40. *Oncomelania hupensis quadrasi*.

TABLE 15. Comparative growth rates of the subspecies of *Oncomelania hupensis* in Petri Dish Cultures under constant light

Subspecies of <i>Oncomelania hupensis</i>	Growth rate over first 8 weeks in mm per week		Relative growth rate between 3 and 6 weeks in mm per week		Termination of logarithmic phase (weeks)	Maximal growth approached (weeks)
	♂	♀	♂	♀		
<i>formosana</i>	0.63	0.71	0.96	0.86	6	8
<i>hupensis</i>	0.71	0.71	0.70	0.70	9	13
<i>nosophora</i>	0.61	0.68	0.73	0.86	5	10
<i>quadrasii</i>	0.36	0.45	0.40	0.46	6	10

of development than in the larger ones; sexual dimorphism throughout growth is most pronounced in *O. h. quadrasii* (Fig. 40). The graphs start with initial measurements of 0.6-0.7 mm, the size of the young snails generally found in routine isolation from parental cultures. Young snails, measured directly upon hatching, have a minimum length of 0.5 mm and take about a week to attain the size of 0.7 mm.

## DISCUSSION

### 1. Vivaria

Each of the culture conditions discussed in this paper is extremely complex with regard to the interactions of such variables as adult population density, volume of vivarium, soil and water surface area, lighting and temperature, etc. Shifts in these variables markedly affect productivity and survivorship. In the successful culture of *Oncomelania* an environment must be satisfactory on 2 counts, i.e., it must provide conditions (1) where adult survival is optimal and where production of young is the highest, and (2) where the young have rapid growth with low mortality as well.

We have found that no single vivarium type simultaneously provides these 2 points and that the 2 aspects must be separated and handled in different ways.

Our data show that among the vivaria tested for maintaining adults and encouraging the production of young, the medium clay pot provides the best environment. Easy handling must also be a major criterion for rearing large numbers of *Oncomelania*; for efficient manipulation the medium clay pot again proved to be superior to the other types tested since it was most rapidly prepared, maintained and can be most easily surveyed for the recovery of young snails. It takes less space than the bulky aquaria, plastic tray or battery jars. No active aeration is needed as there is adequate gaseous exchange in the little water used in the pot. In the medium clay pot the greatest numbers of young are produced from fewer females, which show exceptionally good survival.

The use of a separate container for the young, the Petri dish, has proved to be an efficient method of rearing. In the parental culture overcrowding usually inhibits the growth of the young snails very markedly. When relatively low numbers of juveniles (40) are placed in plastic tray vivaria, their growth rate is extremely slow, attaining only 1/3 - 2/3 the rate in Petri dish vivaria. If as few as 15-20 young were reared in the plastic trays the problem of increased bulk (i.e., of many trays), the necessity for active aeration and increased time in maintenance would make the process laborious and inefficient.

In any case, data are not available to state whether comparative, i.e., optimal, rates of growth would have occurred with 20 young in a plastic tray.

The aquarium proved to be the poorest of the vivaria types tested, especially in terms of manipulation. The very bulk of an aquarium makes it the hardest container to clean; also, on account of its depth, there is considerable difficulty in handling the adults and finding or removing the young. In the large clay pots, survival of adults was of an intermediate type. The relatively low production of young and their very poor survival after hatching makes this culture unacceptable as a vivarium for the production of young. We have found the large clay pot (Fig. 10) to be an excellent vivarium for the temporary housing of 200-300 snails which are to be used up in experiments. Active aeration is not necessary as only a shallow reservoir of water is used and the culture is easily maintained when one is not concerned with the raising of young.

The battery jar does not encourage production of young and showed excessive rates of adult mortality. This kind of vivarium is best used to keep large numbers of snails (100) for short periods of time (4-5 months) as it is easy to maintain, requires little space, and no soil substrate is necessary.

Survival of adults in the plastic tray was of an intermediate degree and generally the yield of young was low. Aside from the necessity for active aeration because of the depth of water in the reservoir, it can be efficiently maintained but not as quickly and easily handled and used in laboratory studies as the medium clay pot. The use of large numbers of these cultures for any purpose presents the problem of supplying numerous air outlets for active aeration as well as the need for extensive shelf space. This culture type is not suited for holding large numbers of snails for experiments.

## 2. Survivorship

Pesigan et al. (1958), using the aquarium type vivarium, found in laboratory experiments that only 50% of the young of *Oncomelania hupensis quadrasi* survived past the aquatic stage, i.e., 20 days, and only 21% survived 70 days. Over the past 3 years, we found that, by using the medium clay pot, 70% or more of several thousand young survived for 90 days. When the large clay pot vivaria were used the survival dropped to levels of about 20% at the end of 70 days.

The higher mortality of newly hatched snails in the large clay pots appeared to be associated with the areas of egg deposition. Eggs were quite frequently deposited near the soil-brick interface of the retaining wall, an area farthest removed from water. Most of the dead young were found in this area and it is thought that the distance to the water may have been a main factor in the survival of the young.

The good survival of young in our culturing methods is attributed, in part, to thinning them out in Petri dish vivaria when they are about 1-2 weeks old. This type of culture appears to afford a pair of snails an adequate and balanced environment with respect to food energy, volume of container, and soil-water ratio.

When 15 female *Oncomelania hupensis quadrasi* were maintained in medium clay pots (5 per pot) they had an average finite rate of mortality of roughly 3% per month for the first 14 months after maturity (total age 16.5 months) and thereafter an increasing rate of mortality with 50% dead at an average 18 months after maturity (total age 20.5 months). Pesigan et al. (1958) calculated from field data for this subspecies that females, on the average, lived 65.8 days after reaching maturity, i.e., for a total age of about 5 months.

Considering the other subspecies, the

finite rates of female mortality in medium clay pots were about 2% per month for 16-18 months (total age 18.5-20.5 months). In other vivaria such rates were much larger, usually much in excess of 9% per month over a 24 month period.

Generally, any environment where the finite rates of mortality exceed 12% per month is unsuitable for maintenance. Where the finite rates are 2 - 9% per month and the rates begin to noticeably increase before 15 months, one suspects a deteriorating condition in the culture.

Population density affects survival. When 250 or more adults were placed in large clay pots (Tables 10, 11), initial rates of mortality were exceedingly high (Figs. 17, 19). However, when 200 or less were placed in culture, the rates of mortality were less precipitous (Figs. 20, 21, 22). Based on area alone and on the fact that optimal survival was obtained when 10 adults were maintained in the medium clay pot (for all the 4 subspecies), it is estimated that, in the large clay pot, optimal survival would probably occur when 60 adults are maintained in it. As shown in Fig. 22, when only 43 *Oncomelania hupensis quadrasi* were kept in the large clay pot, survivorship, while not as excellent as that in medium clay pots, was extremely good.

Lowering the snail density in battery jars from 168 to 77 per vivarium did not appreciably change the mortality rate of *Oncomelania hupensis formosana* (Tables 10, 11; Figs. 17, 21).

Lighting seemed to affect rates of mortality more than density, as far as survivorship in plastic trays was concerned. Increasing daily exposure to light was correlated with increased rates of mortality.

### 3. Productivity

It is expected that slightly different rates of reproduction and survivorship should occur in different labo-

ratories, even where using the same techniques and similar set-ups, on account of such variables as: different origins of snails, divergencies in temperatures that might be difficult to regulate, and of maintenance care that may vary markedly. Nevertheless, one can expect the same trends to occur. Thus, in experiments involving only 1 or 2 females per medium clay pot (Table 14) our data essentially agree with those of Chi & Wagner (1957), who found that greater production occurred at a low density of females per medium clay pot. Whereas, with 5 males and 5 females per medium clay pot under constant light, *Oncomelania hupensis formosana* produced 10 young/f/m in the first year, i.e., 600 snails per culture in 12 months; with 2 females and 3 males per culture under room level light, production was about 27 y/f/m, or 648 snails per culture in 12 months, as shown in Table 14. Perhaps the yield could be increased above those levels without increasing the number of cultures by having 3 females and 2-3 males per culture.

High levels of production can be expected from the medium clay pots in the 2nd year as well as in the 1st year, except for *Oncomelania hupensis quadrasi*. Because female mortality increases in the 2nd year and production declines, it is advisable to set up new breeding stocks for this subspecies after 14 months.

### 4. Growth

Growth in Petri dish cultures was more rapid and uniform than in any of the culture conditions tested as shown in this paper (Table 15) and previously (van der Schalie & Davis, 1965). Only the growth rates of *Oncomelania hupensis quadrasi* were comparatively slow and more closely equivalent to the rates previously reported for that subspecies, i.e., 0.20-0.25 mm per week (McMullen, 1947; Chi & Wagner, 1957; Pesigan et al., 1958). We recorded average rates of 0.36 and 0.45 mm per week for

male and female *O. h. quadrasi*, respectively, and of about 0.6 and 0.7 mm per week for the males and females of the other subspecies.

According to Chi & Wagner (1957) *O. h. quadrasi* reached up to 6 mm in length. In our laboratory the snail rarely exceeded 5 mm. Data by Pesigan et al. (1958) also show growth culminating at 5 mm. In our laboratory, full growth occurred in 11-12 weeks (Fig. 40), whereas, according to Chi & Wagner and Pesigan et al., it took 20 and 27 weeks, respectively, for full growth to occur.

Pesigan et al. (1958) pointed out that *O. h. quadrasi* males reach sexual maturity at a smaller size than females: 3.5 mm as opposed to 3.7 mm. Hence, as shown in the growth curves for the males and females of that subspecies, (Fig. 40) sexually mature snails can be removed from Petri dish cultures at 7-8 weeks, by which time the snails are about 84% maximum size.

In 1965 we reported that *Oncomelania hupensis formosana* males also reached sexual maturity at a smaller size than females, and that sexual maturity was a function of size, not of age. Our histological data (1965) showed, however, that at 96% growth only 29% of the snails were fully mature, 47% being almost mature. Hence, *O. h. formosana* should be maintained in Petri dish cultures for 9 weeks (about 98% full growth, Fig. 37) to assure full sexual maturity of all the snails.

We suggest that *Oncomelania hupensis hupensis* be left in culture for 10 weeks and *O. h. nosophora* for 9 weeks, when they have attained 90-95% total growth (Figs. 38, 39). We have found that snails of these subspecies at these sizes serve well in establishing new cultures for propagation of stock and are suitable for all types of experimental work.

##### 5. Light as a Factor

"Room level light" as described in this paper is sufficient for maintaining adults with minimal rates of mortality. Young

are produced equally well in room level light or constant light with one exception: *Oncomelania hupensis formosana* appeared to produce more young in constant light (70-100 ft. candles) when 5 males and 5 females are kept per medium clay pot. The pilot experiment with 2 females and 3 males per medium clay pot showed, however, greater production in room level light. As a result the uniform use of room level light is recommended.

Stronger constant light (150 ft. candles) was associated with increased rates of adult mortality. One of the main factors involved is the rapid proliferation of algae on the soil. Algal mats soon cover the soil and, if the culture is not changed, snail mortality increases.

We encountered the same problem when using constant light over the Petri dish cultures. Within 4 weeks algae overran the cultures, causing increased mortality (van der Schalie & Davis, 1965). When light was provided for 10-12 hours per day nearly the same rates of growth were obtained, but algal growth was held in check, and mortality decreased. The rate of algal growth varied markedly with the different soil collections brought in from the river bank. If algal growth was slight (algae covering not more than 1/4 soil area) in 5 weeks, the duration of light was increased. We attempted to maintain a moderate algal growth in the dishes up to the 8th week.

##### 6. A Model for Rearing 500 Snails of each Subspecies to Maturity each Month

To assure 500 living snails of each subspecies in the medium clay pot vivaria at the end of 8 weeks, one needs a total production of 666 newly hatched young of each subspecies, assuming a minimum mortality of 25% of the young up to 8 weeks. Table 16 lists the number of medium clay pots needed (with 5 males and 5 females per culture) to produce this number at the rate of production listed in Table 13 for the first 12 months.

TABLE 16. Medium Clay Pot Cultures needed to produce at least 500 snails per month for each subspecies of *Oncomelania hupensis*

Subspecies of <i>Oncomelania hupensis</i>	Assumed rate of production in y/f/m	Minimum cultures needed with 5 females/culture	Suggested No. of cultures	No. trays needed to house 4 Medium Clay Pots each
<i>formosana</i>	10	14	16	4
<i>hupensis</i>	2	67	68	17
<i>nosophora</i>	6	12	16	4
<i>quadrasii</i>	9	13	16	4
TOTAL		106	116	29

TABLE 17. Shelving space required to house the Medium Clay Pot and Petri Dish Cultures needed to yield 500 young of each subspecies of *Oncomelania hupensis* per month

Purpose of shelving	Dimensions			Capacity per shelf	No. Units needed	Suggested arrangements
	Le.	Wi.	H. betw. shelves			
Holding trays with 4 MCP per tray	38"	15"	10"	2 trays	15	Optional
Holding Petri dish cultures	52"	16"	12.5"	52 Petri dishes	53	9 units each with 6 shelves in a tier (see Fig. 12)

The suggested number of snails and cultures is greater than the minimum requirement because (1) it is better to overshoot in production a little; (2) more cultures help to compensate for the sporadic production that occurs in individual vivaria; (3) the number of cultures was adjusted to multiples of 4 so that each plastic tray, which accommodates 4 medium clay pots, would contain only 1 subspecies.

With 666 young produced monthly by each of the 4 subspecies, facilities must be available to house 2664 young monthly for at least 2 months. As each Petri dish takes 2 young, space is needed to house 1332 Petri dishes in the 1st month, and since the snails take 8 weeks (2 months) to mature, it is necessary to

have an additional 1332 Petri dishes and space to house the production of the second month; thereafter sets of dishes are rotated.

Table 17 shows the shelf space needed to house both plastic trays with the medium clay pots and Petri dishes. Although the plastic tray housing the 4 clay pots is but 3" high, the recommended distance between shelves is 10" to allow for easy inspection of the cultures, for adding water and knocking down the snails daily, and for removing 1 clay pot without having to remove the whole tray.

The length of shelf holding the Petri dishes (52") is determined by the length of the light fixture holding the 47" long fluorescent tube. The width used (16") is recommended for ease of maintenance

as well as for providing adequate illumination for all the dishes from the single, centrally placed tube 8" above the dishes on each shelf. We have found it convenient to use 6-shelf units for rapid maintenance and efficient use of space.

To handle this level of production, 2 full time assistants are needed.

#### 7. Necessity for Routine Maintenance

Daily routine maintenance is absolutely necessary for keeping snail cultures of *Oncomelania* productive and rates of mortality low. Aside from knocking snails from the sides of the vivaria to keep them from dying by desiccation, water levels tend to fluctuate markedly in cultures depending on atmospheric conditions. Mold can appear, spread rapidly, and within a week cause excessive mortality of adults and young.

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- \*Permission to cite this reference in the text and bibliography was given by the authors.

## RESUMEN

CULTIVO DE ONCOMELANIA  
(PROSOBRANCHIA: HYDROBIIDAE)  
PARA ESTUDIOS DE LA ESQUISTOSOMIASIS ORIENTAL

H. van der Schalie & G. M. Davis

Se probaron seis tipos de vivarios para determinar en cuales condiciones las cuatro subespecies de *Oncomelania hupensis* proliferan mejor en laboratorio. Se establecieron los procedimientos eficientes que aseguran las condiciones óptimas para: sobrevivencia de adultos, mayor producción de cría por cada hembra en unidad de tiempo, sobrevivencia de jóvenes y desarrollo rápido de los mismos.

Guiados por nuestra adquirida experiencia y extensiva consulta de la bibliografía, se formaron acua-terrarios en los siguientes recipientes: acuarios, jarros cilíndricos (de baterías), macetas de arcilla grandes y medianas, bandejas plásticas y cápsulas de Petri. Se obtuvo buen resultado de cultivo en dos distintos ambientes: (1) uno en el cual la mortalidad de adultos fue mínima y la producción de crías óptima; y (2) otro en el cual los jóvenes crecieron sin impedimento, rápidamente, y con baja mortalidad.

Las condiciones en macetas medianas, conteniendo 5 hembras y 5 machos, resultaron superiores en cuanto a la sobrevivencia de adultos y producción de cría, debido al

hecho que unas pocas hembras fueron muy productivas en un volúmen limitado, donde la proporción suelo-filtro-papel-agua parece ser óptima. Siendo los ambientes de pequeño volúmen, se pueden usar más unidades productivas, en lugar de los ambientes grandes que son más difíciles de manejar y producen menos. El porcentaje de mortalidad de adultos fue de 2% durante el primer año, al término de dos años no alcanzó al 50% sino que fue menor. Para producir crias se recomienda que a los dos años de edad, las hembras de todas las subespecies, excepto *O. h. quadrasi*, sean reemplazadas por otras más jóvenes; las de la subespecie mencionada deben ser reemplazadas entre los 12 y 14 meses, proque mostraron un aumento en mortalidad en el segundo año de vida adulta.

En otros vivarios, el porcentaje de mortalidad aumentó rápidamente, 12% o más por mes, indicando condiciones de cultivo desfavorables. Más pobres aún fueron los acuarios, las macetas grandes y los jarros cilíndricos. El acuario era muy incomodo de manejar por su mayor tamaño y los caracoles no se desarrollan bien. Las macetas grandes mostraron mortalidad excesiva de las crias. Y los jarros de baterías se caracterizaron por la gran mortalidad de adultos y la corrosión extreme de las conchillas.

El mayor número de crias se produjo en las macetas medianas donde cada mes, dependiendo de la subespecie, la puesta por hembra era de 3-11, casi el doble de la de los otros cultivos. Aunque la producción era esporadica, todas las subespecies se reprodujeron cada mes del año.

Como ya fue discutido por nosotros (1945) proporciones optimas de crecimiento fueron en cápsulas de Petri, con uno o dos caracoles por cápsula de 2 a 2 1/2 anfractos cada uno. La fase del crecimiento logarítmico se completó en 5-9 semanas, mientras que desarrollo completo llevo de 8 a 13 semanas, segun las subespecies. Una mortalidad máxima del 20% ocurrió entre la 8<sup>a</sup> y 9<sup>a</sup> semana.

Para cultivar *Oncomelania*, los siguientes factores son de importancia: el suelo, tanto como fuente de alimento y como sustrato para la deposición de los huevos, debe ser de textura fina, con alto contenido de calcio, y debe mantener una flora densa de diatomeas. Esta flora junto con agentes de descomposición, provee una adecuada fuente alimenticia: el único suplemento es papel de filtro, adimento aditivo ya clásico. El agua debe ser neutral o ligeramente alcalina (pH 70-76) y libre de clorina u otros gases tóxicos como iones de cobre.

La intensidad luminosa de una habitación común fue adecuada para la sobrevivencia de adultos y producción de juvenes: luz constante tiende a aumentar la mortalidad por la excesiva proliferación de algas. Condiciones óptimas de crecimiento para las crias es una intensidad luminosa de 130 a 160 bujías, en ciclo de 10-12 horas diarias. Productividad decrece y mortalidad crece con el aumento de densidad en caracoles en cultivo. Mantenimiento diario es necesario para condiciones óptimas. Factores bióticos más destructivos fueron los mohos, lombrices y gorgojos.

Se ofrece un modelo para trabajo, con el número de macetas medianasm cápsulas de Petri, espacio, y labor necesaria para criar 500 caracoles por mes de cada una de las cuatro subespecies de *O. lupensis*.

#### АБСТРАКТ

#### КУЛЬТИВИРОВАНИЕ УЛИТОК *ONCOMELANIA (PROSOBRANCHIA: HYDROBIIDAE)* ДЛЯ ИЗУЧЕНИЯ ВОСТОЧНОГО ШИСТОЗОМИАЗИСА

Г. ВАН ДЕР ШАЛИ И ДЖ. М. ДЕВИС

Для определения экологических условий, необходимых для наилучшего развития в лабораторных условиях 4 подвидов *Oncomelania lupensis* были испробованы 6 типов различных вивариев для их содержания. Были приняты эффективные меры, чтобы создать оптимальные условия для выживаемости взрослых,

продукции молоди на 1 самку и в определенное время, выживаемости молоди и ее быстрого роста.

На основании изучения обширной литературы и имеющегося опыта, в различных контейнерах были созданы различные акватеррарии: в аквариумах (в стеклянных цилиндрах или батарейных банках), в крупных и средней величины мелких глиняных горшках, на пластиковых подносах и в чашках Петри.

Успех культивирования *Oncomelania* зависел от установления 2 различных сред обитания: 1) где смертность взрослых была бы минимальной, а продуктивность молоди оптимальной, и 2) где молодь росла бы быстро, без нарушений и с малой смертностью.

Условия в "среднем глиняном горшке," где содержались 5 самцов и 5 самок оказались наилучшими, как для выживания взрослых, так и для продукции молоди. Их хорошие качества объяснялись тем, что самки, живущие в малом количестве в ограниченном объеме имеют высокую продуктивность при оптимальном соотношении условий-почва-фильтровальная бумага-вода. Благодаря малому объему места их обитания, можно более успешно употреблять малые ёмкости вместо крупных и громоздких менее продуктивных типов культур. Темп отмирания взрослых моллюсков в течение первого года составлял всего около 2%, не достигая 50% к концу двух лет, обычно же была значительно меньше. Для успешной продукции молоди рекомендуется, чтобы в возрасте 24 месяцев самки всех подвидов, кроме *Oncomelania hupensis quadrasi* заменялись более молодыми самками. Что касается указанного выше подвида, его самки должны заменяться в возрасте 12-14 месяцев, т.к. у них наблюдается заметное увеличение смертности на втором году жизни у взрослых особей.

Наблюдаемое быстрое увеличение скорости утмирания до 12% и больше в месяц в других вивариях указывает на неблагоприятные условия содержания моллюсков. Самые обедненные культуры наблюдались в аквариумах, больших глиняных горшках и стеклянных цилиндрах (батарейных банках). Аквариумы оказались исключительно неудобными для ухода за культурами из-за их большого объёма и моллюски развивались в них плохо. Большие глиняные горшки давали чрезмерную смертность молоди. В стеклянных цилиндрах наблюдалась бóльшая скорость утмирания взрослых и очень сильная эрозия раковин. Наибольший урожай молоди наблюдался в глиняных горшках средней величины, где, в зависимости от подвида, нарождалось 3-11 экз. молоди на 1 самку в месяц, в наиболее продуктивные месяцы т.е. наблюдался вдвое бóльший темп их продукции, чем в других вивариях. Хотя продукция молоди была спорадической, все подвиды размножались каждый месяц года.

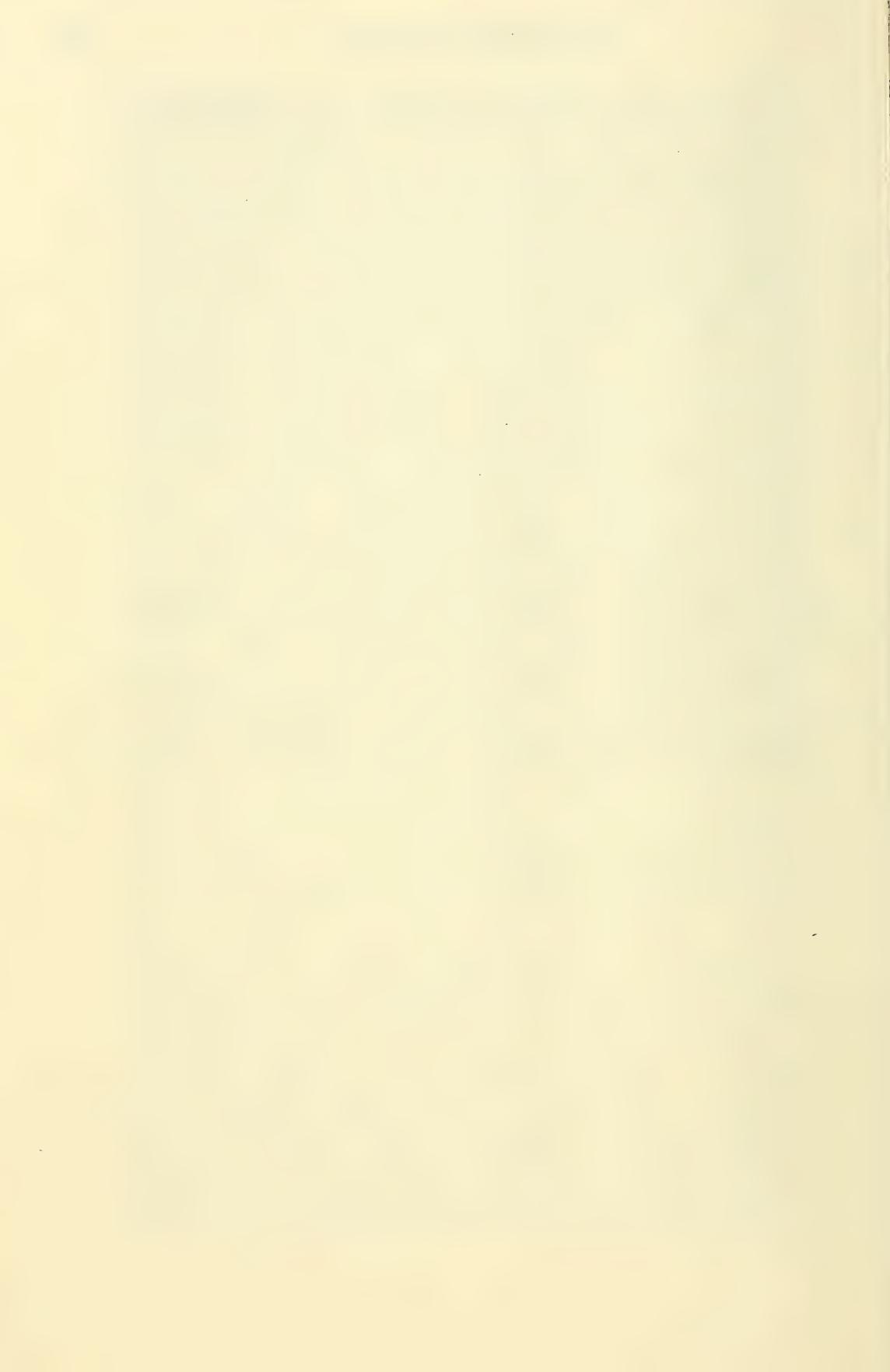
В средних глиняных горшках отмирание молоди в исходных (материнских) культурах была 4-10%, обычно же составляла 5%. В других вивариях отмирание было гораздо выше; в крупных глиняных горшках, например, отмирание составляло 42-78%. Смертность наступала в первые 1-2 недели после отрождения. Как указывалось авторами (1965) оптимальная скорость роста наблюдалась в культурах на чашках Петри, с 1-2 моллюсками в каждой; прирост раковины при этом составлял 2-2.5 оборота.

Начальная фаза роста занимает 5-9 недель, в то время, как весь период роста обнимает 8-13 недель, в зависимости от подвида. Максимальная смертность в 20% наблюдалась после 8-9 недель роста.

При культивировании *Oncomelania* важны следующие факторы: почва, как источник пищи и как субстрат для откладки яиц должна иметь тонкую структуру, высокое содержание кальция и высокую плотность поселения флоры диатомей. Эта флора, вместе с сопровождающими ее потребителями, составляет необходимый источник пищи для моллюсков. Другим единственным классическим добавком к пище служит фильтровальная бумага. Вода должна быть нейтральной или слабо щелочной (рН 7.0-7.6). Вода не должна содержать хлора или других токсических веществ, таких, как ионы меди. Степень (сила) комнатного освещения вполне достаточна для хорошей выживаемости взрослых и для продукции молодежи; вместе с тем постоянная освещенность имеет тенденцию увеличивать скорость отмирания моллюсков в течение длительного периода времени (1.5-2 года), а также способствует смертности из-за чрезмерного развития водорослей. Оптимальная скорость роста молодежи наблюдается при свете, силой 130-160 свечей, действующем 10-12 часов в день.

Продуктивность моллюсков уменьшается, а темп отмирания возрастает по мере увеличения плотности поселений моллюсков. Для создания для них оптимальных условий необходим ежедневный уход. Биотическими факторами, наиболее разрушительными для культур моллюсков служат олигохеты и клещи.

Была выработана типовая модель культуры моллюсков, созданная на целом ряде глиняных горшков средней величины и чашек Петри, определено пространство и количество труда, необходимого для поддержания культуры из 500 улиток в месяц для каждого из четырех подвигов *Oncomelania lupensis*.



STUDIES ON SLUGS OF ARABLE GROUND  
I. SAMPLING METHODS

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ABSTRACT

A number of methods used in the past for estimating slug populations were evaluated on a plot in Northern England. The species involved were *Agriolimax reticulatus* (Müller), *Arion hortensis* Féruccac and *Milax budapestensis* (Hazay).

*Soil Sampling* was the only method to give unbiased information on the species composition and age distribution of populations. Slugs were extracted from soil by: (1) Soil washing, the most efficient method, (with 100% recovery of all stages except for the recently hatched juveniles, which had a recovery rate from 63-86%) that also showed the presence of eggs (91-100% recovery), and by (2) Flooding, a process less laborious and time consuming than soil washing, but less efficient in extracting slugs (88-92% recovery) and not capable of extracting eggs.

A population estimate by the *Marking-release-recapture* method was compared with a simultaneous estimate by soil sampling. The method was less convenient than soil sampling and tended to under-estimate population density.

Duthoit's *baiting* method of estimating populations was evaluated in relation to the actual density of slugs in the area and the elements of weather that affect slug activity (temperature and humidity). Since population density was the only factor significantly influencing the extent of damage to baits, the latter can be taken as a measure of the relative population density.

By the *Night-searching* method, the proportion of the surface dwelling and light coloured *Agriolimax reticulatus* in populations was overestimated. The mean weight of slugs from these samples was greater than that in comparable soil samples.

*Trapping* under sack-shelters also gave a high estimate of the proportion of *Agriolimax reticulatus*. More slugs were trapped in damp cloudy weather than when it was sunny and dry.

It was concluded that, for most purposes, the most efficient sampling method would be a small soil sample to establish species composition and age distribution, plus a large number of baits to measure the relative density of populations.

Slug populations are difficult to estimate since their distribution tends to be aggregated (Hunter, 1966) and many of the commonest species live underground. In previous studies, a number of attempts to estimate the relative density of these animals have been made using baits

(Duthoit, 1961), traps (Getz, 1959), or by counting the number of active individuals at night (Bett, 1960). Their absolute density (i.e., the total number of slugs in a given area) has been estimated indirectly by a system of marking, release and recapture (Johnson, 1964),

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TABLE 1. Recovery of slugs and eggs, previously added to 1 cubic foot of soil, by soil washing

Species	Slugs				Eggs	
	Wt. of over 12.5 mg		Wt. of under 12.5 mg		No. added*	Recovery %
	No. added*	Recovery %	No. added*	Recovery %		
<i>Agriolimax reticulatus</i>	15	100	8	62.5	11	90.9
<i>Arion hortensis</i>	15	100	12	66.7	13	15.4
<i>Milax budapestensis</i>	15	100	13	85.6	15	100.0

\*The operator did not know the number added until after the experiment.

and directly by taking samples of soil and later extracting the slugs from these (South, 1964). In this study the latter direct method was used for 2 1/4 years while taking a series of samples for a study of the biology of *Agriolimax reticulatus* (Müller), *Arion hortensis* Férussac, and *Milax budapestensis* (Hazay) on an arable plot in Northumberland. The opportunity was taken to compare the results from these samples with further estimates from marking-release-recapture, baiting, trapping and night-searching.

## SOIL SAMPLING

### Sampling Technique

The size of sampling unit used and the depth to which sampling is taken will depend on the distribution and abundance of the slugs on the plot. In the present study, soil cores 4 inches in diameter by 1 foot deep (approx. 10 x 30 cm) yielded too few slugs to provide sufficient biological material (a test sample of 20 of these cores produced only 5 slugs, 2 of which were damaged by the sampling tool). Units of 6" and 8" square were difficult to excavate to the required depth, so that the unit finally adopted was of 1 cubic foot of soil. There had been only shallow cultivations on the plot for some time so that slugs were rarely found below 1 foot deep.

### Extraction Technique

Various unsuccessful attempts (South,

1964) have been made to extract slugs from undisturbed soil by using repellants. There is no record of attempts to expel slugs from soil *in situ* by electrical methods, but this technique does not seem entirely satisfactory even for those animals for which it has been used (Satchell, 1955).

The only extraction methods attempted in the present study were therefore those applicable to soil samples brought into the laboratory prior to extraction.

(i) *Soil Washing* (Salt & Hollick, 1944; Raw, 1951). Soil was broken down with a water jet on a bank of sieves (3 meshes, 10 meshes and 30 meshes to the inch). The sieves with their residues were then agitated in magnesium sulphate solution of at least 1.17 specific gravity. All organic material rose to the surface so that both slugs and eggs could be picked off. The recovery rate was tested by adding a number of slugs and eggs to slug-free samples of soil and later extracting them by soil washing. Of the older stages (over 12.5 mg in weight), 100% were recovered but considerably fewer of the newly hatched slugs (63-86%; Table 1). Presumably the small slugs that were not recovered were either missed or destroyed by the force of the water jet. All slugs lost weight during the process, *Agriolimax reticulatus* losing an average of 34% of their weight, *Arion hortensis* 33% and *Milax budapestensis* 27%. This reduction can probably be attributed to loss of slime during the severe washing and ex-

TABLE 2. Recovery of slugs present in 16 cubic feet of soil by flooding

Species	Nos. Extracted	Nos. not extracted*	Nos. damaged**	Success %
<i>Agriolimax reticulatus</i>	78	7	2	91.5
<i>Arion hortensis</i>	49	7	2	87.5
<i>Milax budapestensis</i>	49	6	0	89.1

\* Found later by soil washing.

\*\*Slugs damaged during digging of samples and not included in the calculation of % success.

TABLE 3. Comparison of population estimates for a 1350 sq. ft. plot by the marking-release-recapture and by the soil sampling methods

Species	Marking-Release-Recapture				Soil Sampling
	Nos. marked and released	Nos. recovered unmarked	Nos. recaptured marked	Population estimate	Population estimate
<i>Agriolimax reticulatus</i>	300	285	15	5,364 ± 2,611	6,683 ± 4,374
<i>Arion hortensis</i>	300	17	2	1,800 ± 1,807	10,958 ± 3,645
<i>Milax budapestensis</i>	180	32	1	2,970 ± 3,358	5,693 ± 1,836

traction processes. All *Milax budapestensis* and most *Agriolimax reticulatus* eggs were recovered, but, being much less robust, only a few *Arion hortensis* eggs survived the soil washing process.

(ii) *Flooding*. South (1964) was able to extract slugs by the "cold water process", in which soil is slowly immersed in water, thus forcing slugs inside to creep to the surface. This technique was modified for large scale arable sampling by using plastic bowls of 15" diameter fitting into dustbins of the same circumference. The bowls had holes in the bottom so that soil placed in them could be flooded from below. The bins were filled with water up to the base level of the bowls. The water level was raised 1/2" every 12 hours so as to immerse the soil in 4-5 days. Slugs were forced to move upwards as the water level rose and could be easily picked off from the soil surface or the lid of

the bin. Sixteen sampling units of 1 cubic foot each were tested for recovery rate by this method. After extraction by flooding, the soil was washed (Table 2). A high recovery rate, 92% for *Agriolimax reticulatus*, 88% for *Arion hortensis* and 89% for *Milax budapestensis*, was achieved for all 3 species.

#### MARKING-RELEASE-RECAPTURE

This method was tested in November 1964, and results were compared with those from a soil sample taken at the same time. A collection of 300 *Agriolimax reticulatus*, 300 *Arion hortensis* and 180 *Milax budapestensis* was made from sites adjacent to the area to be sampled. In this type of experiment it is usual to capture animals from the population to be estimated, but this would have disturbed the other observations being made (Hunter, 1968a). The slugs

TABLE 4. Data for the evaluation of the baiting method:\* numbers of wheat grains damaged weekly in relation to slug density, temperature and humidity

Nos. slugs	Temper. in °C	Hours humidity	Grains damaged
98	7.78	12	4
	8.50	20	12
	9.95	44	1
131	11.31	10	10
	12.81	6	8
	13.50	6	9
	12.90	48	6
	13.40	44	1
188	12.50	42	33
	14.90	20	24
	11.37	36	31
178	14.14	22	9
	15.56	20	0
	14.13	15	0
217	17.51	52	3
	10.23	20	1
	16.55	36	16
273	14.53	81	27
	13.01	16	19
	13.10	70	53
277	11.71	42	51
	11.51	58	53
	9.00	36	60
224	8.00	28	36
	7.50	84	50
	8.25	61	54
115	0.75	50	0
	1.50	40	2
	2.25	30	1
107	6.50	22	9
	4.75	22	2
	1.50	70	2
	0.50	72	0
110	4.00	56	4
	4.75	8	2
	4.75	32	8

were marked by feeding them on jelly containing neutral red dye (South, 1964). This dye stains the digestive gland a deep pink colour that can be seen through the skin. Radioactive tracers have been used to mark slugs (Johnson, 1964) but neutral red is more convenient to use and gives satisfactory results. The marked slugs were released at random in an area of 1,350 sq. ft. and the recapture samples taken were collected on the 4th, 5th and 6th nights after release.

The population of each species was estimated (Table 3) using the formula suggested by Bailey (1951, 1952), which was adapted to take into account the unusual capture method, i.e.

$$x = \frac{a(u + 1)}{m + 1}$$

where

a = number captured, marked and released

u = number of unmarked slugs recaptured

m = number of marked recaptured slugs

x = number in the population before the addition of marked slugs

A direct maximum likelihood estimate of the variance of x is given by

$$\frac{a^2 u(u + m + 1)}{(m + 1)^2 (m + 2)}$$

and the 95% confidence limits can be estimated as  $1.96 \sqrt{\text{variance}}$ .

The above population estimate was compared with one from a soil sample taken at the same time. Six units, each of 1 cu. ft. of soil were taken in stratified random fashion and the slugs were extracted by flooding. The number of slugs in each unit was multiplied by 1.1 to approximately correct for loss during extraction. The mean numbers/cubic foot and their 95% confidence limits were then multiplied by 1,350 to give direct population estimates for the total area of release (Table 3). Comparison of the 2 types of estimate indicates that marking-release-recapture was fairly accurate for *Agriolimax reticulatus* but considerably underestimated the num-

\*The partial regression coefficients ( $b^1$ ) are as follows:

$$b^1 Y1.23 = 0.833$$

$$b^1 Y2.13 = -0.213$$

$$b^1 Y3.12 = -0.230$$

TABLE 5. Comparison of the species composition of night-searching and soil samples, November, 1964

Species	Night-searching		Soil Sample		X <sup>2</sup> *	P
	No.	%	No.	%		
<i>Agriolimax reticulatus</i>	300	85.23	27	27.84	121.9	< 0.001
<i>Arion hortensis</i>	19	5.39	46	47.42	106.5	< 0.001
<i>Milax budapestensis</i>	33	9.38	24	24.74	16.0	< 0.001
Totals	352	100.00	97	100.00		

\*2 x 2 contingency tests of the proportion of each species in samples.

bers of *Arion hortensis* and *Milax budapestensis* (presumably because of the low recapture rate of these species - see section on night-searching). Soil sampling was the quickest and most convenient of the 2 methods.

#### BAITING

The amount of damage to various baits has been used as a measure of the density of slug populations (Duthoit, 1961). In the present study Duthoit's method was tested in the field between April 1964 and March 1965.

Each bait consisted of 10 wheat grains laid on a piece of terylene netting measuring 6" x 4" (15 x 10 cm) and covered with a double thickness of sack-ing to prevent birds from eating the grains, the whole being fastened to the ground with wire clamps. Six of these baits were set up on an arable plot and readings were taken for a total of 16 weeks (not all consecutive). The total number of grains damaged was examined in relation to the following factors:

- (i) Direct estimates of the actual density of slugs on the plot. It was only possible to take a soil sample every 4 weeks, i.e. there was not one for each week of the baiting experiment; therefore the baiting estimates were compared with estimates from the most recently taken soil sample.
- (ii) Mean weekly air temperature.

- (iii) Total number of hours per week when the relative humidity of the atmosphere exceeded 90%.

The data (Table 4) were analysed by multiple regression. The partial regression coefficients of numbers of damaged grains on temperature and humidity were not significant ( $b' = -0.21$  and  $-0.23$  respectively) but the coefficient for regression on the actual slug density was significant ( $b' = 0.83$ ). Thus in this case the population density was more important in determining the amount of damage to baits than factors affecting activity.

#### NIGHT SEARCHING

Barnes & Weil (1944a, b) investigated the activity of slugs by searching at night for a specified period (30 minutes) and counting the number of active slugs they observed. Bett (1960) used this method of collecting slugs for work on their life cycles. In the present study the method was tested by comparing the species composition and age distribution (measured in terms of weight) in the catches made by night searching and in soil samples of the same test area. The proportions of each of the 3 species in the 2 samples were subjected to 2 x 2 contingency tests. These tests were designed to show whether there was any significant difference between the ratio of the numbers of each species to the numbers of the other 2 species com-

bined. Thus if *Agriolimax reticulatus* is represented by a, *Arion hortensis* by b and *Milax budapestensis* by c, tests were carried out as follows:

	Night- Searching	Soil Sampling
For <i>Agriolimax reticulatus</i>	$\frac{a}{b+c}$	$\frac{a}{b+c}$
For <i>Arion hortensis</i>	$\frac{b}{a+c}$	$\frac{b}{a+c}$
For <i>Milax budapestensis</i>	$\frac{c}{a+b}$	$\frac{c}{a+b}$

Significant differences were obtained for all 3 species (Table 5), there being a higher proportion of *Agriolimax reticulatus* and a lower proportion of *Arion hortensis* and *Milax budapestensis* in the night-searching sample than in the soil sample. *Agriolimax reticulatus*, being light coloured and surface dwelling, is clearly more likely than the other 2 species to be found by searching at night.

The mean weight of the slugs caught at night was 189.7 mg for *Agriolimax reticulatus*, 150.5 mg for *Arion hortensis* and 401.1 mg for *Milax budapestensis*. Mean weights of slugs in the comparable soil sample (extracted by flooding) were 86.2 mg for *Agriolimax reticulatus*, 53.1 mg for *Arion hortensis* and 257.0 mg for *Milax budapestensis*. Clearly, collecting at night is more likely to yield large slugs than small ones.

### TRAPPING

Getz (1959) has used traps to collect

TABLE 6. Number of slugs trapped under sacking under different weather conditions during a 10-day period in April 1963

Species	Sunny	Cloudy
<i>Agriolimax reticulatus</i>	138	190
<i>Arion hortensis</i>	22	113
<i>Milax budapestensis</i>	23	45

information on the biology and ecology of slugs. South (1964) has shown that this method is not reliable for collecting ecological data for slugs on grassland. For this reason a test was made of the efficiency of this method in producing data for slugs on arable land.

Six sacks were laid on the ground at fixed points adjacent to the routine sampling plot, and slugs were collected from beneath them on 4 occasions during a 10-day period in April, 1963. On 2 of these occasions the weather was sunny and dry, and on the other 2 it was cloudy and damp (Table 6). More slugs were trapped when it was cloudy and damp. Analysis of variance showed that the difference was significant ( $P < 0.001$ ).

The species composition in trap catches was compared with that in the routine April soil sample by contingency tests (Table 7). The proportion of *Agriolimax reticulatus* to other species in the trap catches was significantly greater than in the soil sample. The proportion of both *Arion hortensis* and of *Milax budapestensis* to the other 2

TABLE 7. Comparison of the species composition of trap samples and soil samples, April, 1963

Species	Traps		Soil Sample		X <sup>2</sup> *	P
	No.	%	No.	%		
<i>Agriolimax reticulatus</i>	328	61.8	14	17.3	55.7	< 0.001
<i>Arion hortensis</i>	135	25.4	40	49.4	24.0	< 0.001
<i>Milax budapestensis</i>	68	12.8	27	33.3	23.1	< 0.001
Totals	531	100.0	81	100.0		

\*2 x 2 contingency tests of the proportion of each species in samples.

species in the trap catches was significantly lower than in the soil sample. These differences were probably due to the fact that the surface-dwelling *Agriolimax reticulatus* was more likely to seek shelter under traps than the 2 subterranean species.

#### DISCUSSION AND CONCLUSIONS

The above data suggest that soil sampling is the only method giving accurate estimates of the total numbers, the species-composition and the age distribution of slugs in a given area. However, it is a time consuming and laborious technique and is probably not suitable for workers who need to make frequent checks on the density of many populations. Marking-release-recapture is also a time consuming technique without being as efficient as soil sampling. Night-searching and trapping have the disadvantage of yielding slugs that are not representative of the total population and also give no data during very dry or frosty weather when slugs are not active (Barnes & Weil, 1944a, b; Getz, 1959). While baiting also fails to give information on the structure of the population, it has an advantage over the latter 2 methods in that each reading covers several days, i.e. even in weather that does not favour slug activity some record is more likely to be produced. In spite of the dependence of slug activity on temperature and humidity (Hunter, 1968b), baiting gives a good indication of the relative population density.

It would therefore appear that, for most purposes, greatest efficiency could be attained by (a) a small soil sample, with extraction of slugs by flooding, to give the species composition and age distribution of the population, and (b) a large number of baits to give the relative density of the population.

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## RESUMEN

ESTUDIOS SOBRE "BABOSAS" DEL SUELO ARABLE  
I. METODOS PARA SACAR MUESTRAS

P. J. Hunter

Se probaron varios métodos conocidos para estimar las poblaciones de "babosas", en un solar del norte de Inglaterra. Las especies observadas fueron *Agriolimax reticulatus* (Muller), *Arion hortensis* (Fer.) y *Milax budapestensis* (Hazay).

El único método que dió información imparcial sobre las especies y distribución de las poblaciones según la edad, fue el de las *Muestras de Suelo*. Las babosas fueron extraídas del suelo por: (1) lavado del suelo, el método más eficiente (prácticamente se recobraron el 100% de ejemplares, excepto de los juveniles de reciente eclosión que fue sólo en un 63-86%), el cual también indicó la presencia de huevos (91-100%), y por (2) inundación, proceso que consume menos tiempo y trabajo, pero que es menos eficiente (recobrandose sólo 88-92% de ejemplares) y que no permite la extracción de huevos.

El cómputo de población por *marcado-suelta-recaptura*, se comparó con el tomado simultáneamente de muestras de suelo; el primero fue menos conveniente y tendió a reducir el cálculo de densidad de población.

El método de Duthoit, usando un cebo para captura, se estimó en relación a la densidad de babosas en el área y su influencia en la cantidad de cebo utilizado, así como los elementos climáticos que afectan las actividades.

En otro método, el de búsqueda nocturna, la proporción de habitantes de superficie y los *Agriolimax reticulatus*, de color claro fue sobrestimada, y el peso medio de las babosas en tales muestras resultó mayor al compararse con el calculado por muestras de suelo. También el uso de *Trampas* dio un cálculo muy alto para *Agriolimax*, y mayor número de individuos fueron cazados así en tiempo nublado y húmedo que en días claros y secos.

Puede concluirse que el método más eficiente sería una pequeña muestra de suelo, más una serie de capturas por cebo, para medir la densidad de la población y las edades.

## АБСТРАКТ

ИЗУЧЕНИЕ СЛИЗНЕЙ НА ПАХОТНЫХ ЗЕМЛЯХ  
I. МЕТОД СБОРА ПРОБ

П. ДЖ. ХАНТЕР

Сбор проб. Ряд методов, употреблявшихся в прошлом для определения популяций слизней были опробованы и оценены на участке земли в районе Новой Англии. Были исследованы следующие виды: *Agriolimax reticulatus* (Muller), *Arion hortensis*

*Ferussac* и *Milax budapestensis* (Hazay). Пробы почвы служили единственным методом получения объективной информации о видовом составе и распределении популяций слизней в почве. Моллюски извлекались из почвы различными способами: путем ее промывки, что является наиболее эффективным методом, который практически охватывает 100% особей, исключая только что народившуюся молодежь; последняя охватывалась на 63-86%. Яйца учитываются этим методом на 91-100%. Метод отмучивания почв - менее трудоемкий и длительный, чем промывка, но менее эффективный в смысле извлечения слизней (88-92% охвата), не дающий яиц совсем.

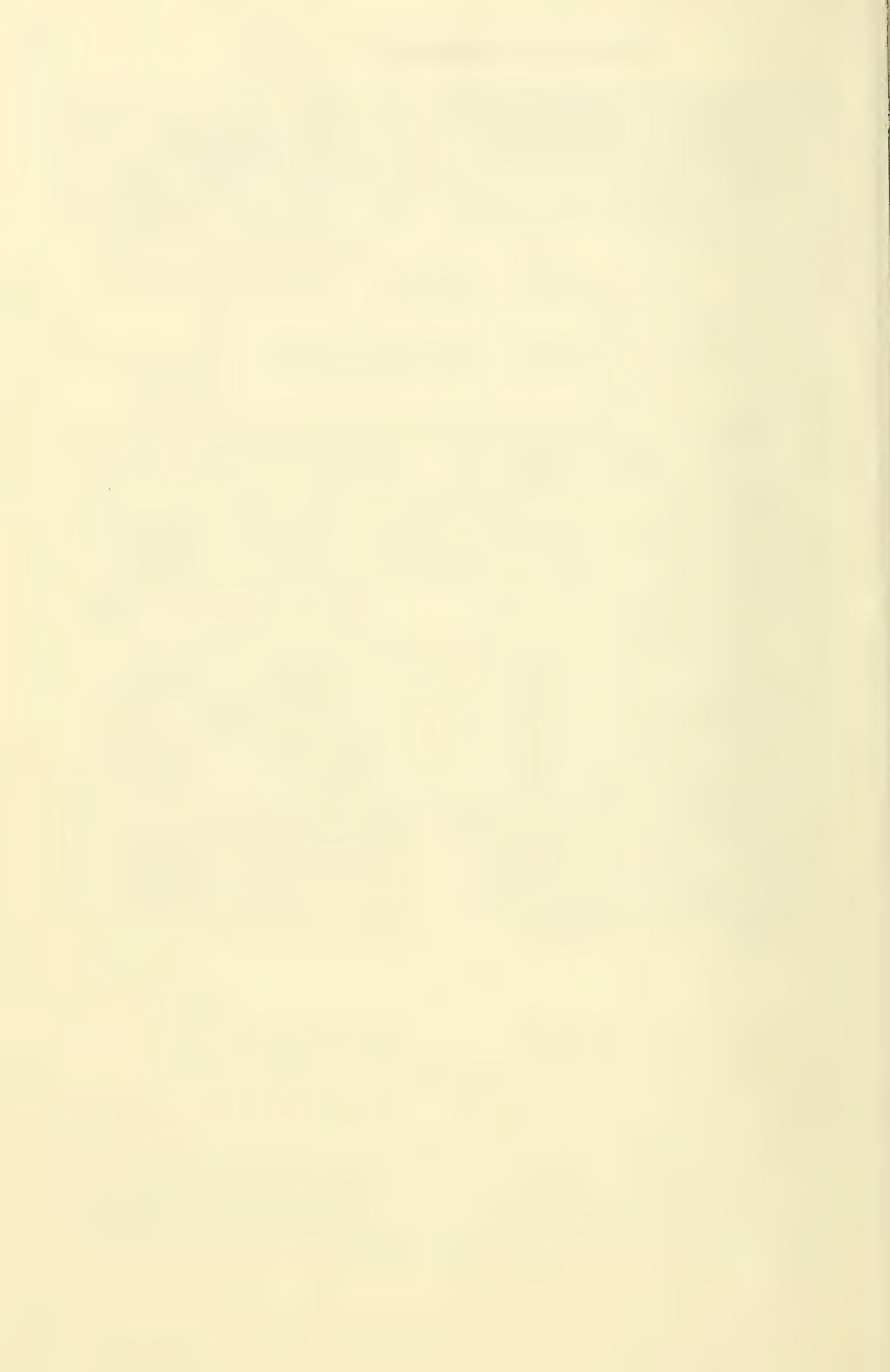
Популяция, оцененная методом сбора, пометок и повторного отлова, сравнивалась с той, которая была одновременно оценена почвенным методом. Этот метод оказался менее удобным, чем почвенный метод и видимо дает заниженные результаты оценки плотности популяции.

Метод ирманок Дютуа для оценки популяций слизней сравнивался по полученным результатам с существующей в почве плотностью их поселений, с учетом условий погоды (температуры и влажности), влияющих на жизнедеятельность моллюсков. Поскольку плотность их популяций была единственным фактором, значительно влияющим на степень повреждения приманок, последние могли служить для измерения относительной плотности популяций слизней в почве.

При помощи метода ночных поисков относительное количество в популяции обитающего на поверхности грунта и светло-окрашенного *Agriolimax reticulatus* была переоценена. Средний вес слизней в этих пробах был больше, чем в сравнимых с ними пробах почвы.

Метод ловушек также дал завышенное относительное количество *Agriolimax reticulatus*. В сырую облачную погоду слизи ловились в большем количестве, чем в сухую и солнечную.

Из всего указанного выше было сделано заключение, что для многих целей наиболее эффективным методом учета слизней служит метод небольших почвенных проб, дающий возможность установить их видовой и возрастной состав и распределение. Чтобы оценить относительную плотность популяций необходимо параллельно употреблять большое количество приманок.



STUDIES ON SLUGS OF ARABLE GROUND  
II. LIFE CYCLES

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ABSTRACT

The life-cycles of *Agriolimax reticulatus* (Müller), *Arion hortensis* Férussac and *Milax budapestensis* (Hazay) were investigated in a study based on routine sampling from an arable plot in Northern England.

*Agriolimax reticulatus* had 2 generations a year, a spring generation hatching about May and an autumn generation hatching about late September. The latter generation took longer (7 months) to complete its life-cycle than the former (5 months).

Most *Arion hortensis* had an annual life-cycle. They hatched about July, grew during the following 11 months, matured and laid eggs when about a year old. A few, however, hatched later and were not ready to lay eggs until after their 2nd winter, thus taking almost 2 years to complete their cycle. The developmental rate of all stages was found to depend on environmental conditions. Individuals laid an average of 64.5 eggs and died shortly after breeding.

Most *Milax budapestensis* had a biennial life-cycle. They hatched between May and August and matured during their 2nd autumn and winter. A few, however, hatched as early as April and laid eggs during their 1st winter. Again the rate of development depended on the environment. Slugs collected from the field shortly before breeding laid an average of 23.5 eggs/individual and died soon afterwards. Slugs kept in field cultures from their young stages laid an average of 32.5 eggs/individual.

A sampling study of the slugs of an arable plot at Close House, Northumberland, U. K. (Grid reference NZ127658), was conducted to determine their reproductive capacities, breeding seasons and generation intervals. The dominant species of slugs present were *Agriolimax reticulatus* (Müller), *Arion hortensis* Férussac and *Milax budapestensis* (Hazay). The sampling plot measured 15 x 25 yards (14 x 23 m) and was on a loam soil in a south-facing walled garden of about 2 1/2 acres. The plot remained uncultivated except for ploughing after the first year of sampling. Samples were taken every 4 weeks between January 1963 and March 1965.

Most sampling methods such as baiting, trapping and searching at night do not give representative data on species and age distribution of slug populations (Hunter, 1968) and since the latter were required in this study, only soil sampling was used to obtain slugs, and their eggs, for examination. Sampling units were of 1 cu. ft. of soil; 9 of these were taken in each sample during 1963 and 12 were taken thereafter. Between January 1963 and February 1964, slugs were extracted from samples by soil-washing and from February 1964 onwards, by flooding.

Eggs were classified into 3 groups: no development, partly developed (when the rudimentary parts of the body were

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TABLE 1. The total numbers of eggs, recently hatched juveniles and adult slugs from routine soil samples, 1, 2 from an uncultivated arable plot in Northern England.

Species and stage	1963											
	i	ii	iii	iv	vv	vi	vii	viii	ix	x	xi	xii
<i>Agriolimax reticulatus</i>												
Eggs - no development	11	18	13	58	39	0	2	25	0	3	29	57
partly developed <sup>3</sup>	14	21	92	4	49	20	7	9	3	5	0	25
well developed <sup>3</sup>	47	69	0	0	12	44	8	12	34	57	13	50
Total	72	108	105	62	100	64	17	46	37	65	42	132
Juveniles <sup>4</sup>	0	0	1	0	0	0	1	5	2	8	5	4
Adults <sup>5</sup>	7	10	7	4	12	0	12	10	14	19	32	16
<i>Arion hortensis</i>												
Eggs - no development	0	0	0	0	0	8	21	0	0	0	5	0
partly developed	0	0	0	0	0	3	0	3	7	0	0	0
well developed	0	0	0	0	0	2	1	6	0	0	0	0
Total	0	0	0	0	0	13	22	9	7	0	5	0
Juveniles	4	4	7	4	0	0	3	13	15	14	13	6
Adults	10	10	12	7	11	3	11	10	6	15	23	24
<i>Milax budapestensis</i>												
Eggs - no development	12	0	2	4	7	0	0	0	0	0	0	34
partly developed	3	7	0	14	24	8	0	0	0	0	0	0
well developed	0	0	6	2	19	0	0	0	11	4	0	0
Total	15	7	8	20	50	8	0	0	11	4	0	34
Juveniles	0	0	0	0	0	6	9	8	3	1	0	0
Adults	7	4	5	5	2	0	0	0	5	4	9	20

<sup>1</sup>Samples were of 9 cubic ft. of soil during 1963 and 12 cubic ft. during 1964/65.

<sup>2</sup>Samples were taken every 4 weeks, i. e. there were 13 per year; however sample xiii of 1963 was omitted.

<sup>3</sup>"Partly developed" eggs refer to stages I-III as described by Carrick (1938) "Well developed" eggs to stages IV-VI.

<sup>4</sup>"Juveniles" here refer to *Agriolimax reticulatus* and *Arion hortensis* of under 12.5 mg and *Milax budapestensis* of under 25 mg.

<sup>5</sup>"Adults" refers to mature slugs with sperm and ova in hermaphrodite duct (Bett, 1960).

becoming differentiated: stages I-III of Carrick, 1938) and well developed (stages IV - VI of Carrick). Any young *Agriolimax reticulatus* or *Arion hortensis* of under 12.5 mg and any *Milax budapestensis* of under 25 mg was regarded as having hatched "recently". The weights of slugs extracted by soil-washing were adjusted to take into account the loss during that process. Slugs were considered

"mature" if the hermaphrodite duct contained sperm or eggs (Bett, 1960).

A number of experimental studies on *Arion hortensis* and *Milax budapestensis* are presented together with the data from soil samples. Such studies were not made for *Agriolimax reticulatus* since an investigation of this species had been undertaken by South (1964, 1965).

Table 1 (continued)

1964													1965		
i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	i	ii	iii
16	8	8													
4	13	30													
41	22	25													
61	43	63													
3	2	4	10	39	23	18	19	20	28	24	10	3	4	5	1
9	4	8	7	16	14	2	7	16	15	15	8	8	9	1	6
0	0	0													
0	0	0													
0	0	0													
0	0	0													
4	3	4	3	3	0	28	33	44	18	12	1	2	2	0	2
13	10	6	8	9	13	4	3	4	11	14	5	5	12	9	6
18	10	6													
0	5	16													
0	3	5													
18	18	27													
0	0	0	1	0	47	28	4	4	2	0	0	0	0	0	0
3	6	3	4	4	2	0	0	2	5	10	11	3	6	4	5

## LIFE CYCLES

*Agriolimax reticulatus*

Eggs were found in samples at all times of the year (Table 1). Numbers fluctuated widely: very few eggs were found in July (sample vii of Tables and Figures) following a low density of adults in June and a very large number were found in November (sample xii, following a high density of adults in October). There were distinct peaks in numbers of recently hatched slugs (< 12.5 mg) in May and late September (samples v and x) of 1964 but no such peaks were apparent in 1963, probably because the soil-washing technique was not extracting all slugs of the very young stages (Hunter, 1968). Smaller numbers of newly-hatched slugs continued to be

found during the summer of 1964, but very few during winter. There were also peaks in the numbers of mature slugs around May and October of each year, while very few were found during June and February. The absence of mature slugs shortly after the breeding season suggests that, as in many other Mollusca (Comfort, 1957), the mature individuals lay their eggs and die within a short period of time.

*Arion hortensis*

## Eggs

Eggs were recovered from the routine samples between June and October (Table 1) but, since the eggs of this species are particularly fragile (Hunter, 1968), some may have been present at other times and destroyed during extraction.

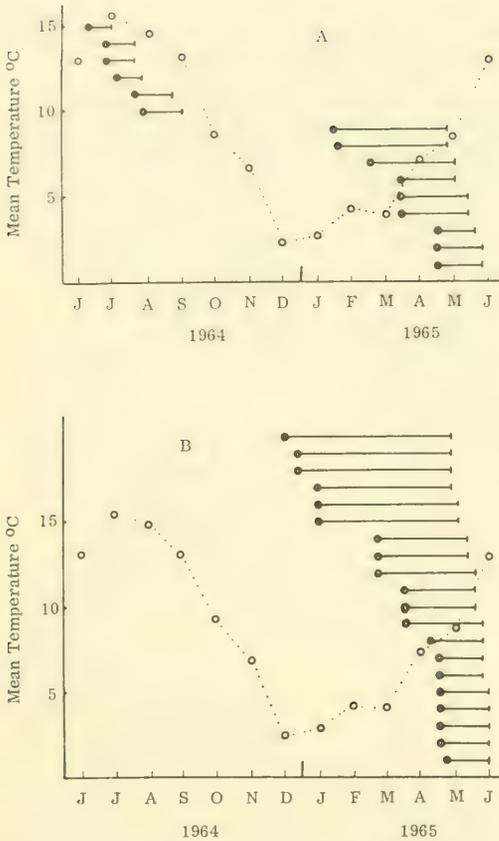


FIG. 1. Development rate of eggs at various times of the year. A. *Arion hortensis*; B. *Milax budapestensis*. Solid points: batch of eggs laid.

End points of lines: first eggs in batch hatched.

Open circles: mean monthly air temperature.

In order to ascertain the developmental rate of these eggs under field conditions, newly-laid batches were kept on damp filter paper in plastic pots out of doors. Whereas development took over 3 months during the winter, eggs laid during the summer hatched within a month (Fig. 1A). At constant temperatures in the laboratory, eggs required a minimum of 2 weeks at 20° C, 3 weeks at 15° C, 4 1/2 weeks at 10° C and 14 weeks at 5° C to hatch.

## Juveniles

There was a peak in numbers of recently hatched individuals (<12.5 mg) around sample ix (early September) of both 1963 and 1964 (Table 1). However, the rise to this peak began later in 1963 (sample viii) than in 1964 (sample vii), probably because the protracted winter of 1962/63 delayed development. Small numbers of juveniles continued to be found during the autumn, winter and spring, suggesting that some breeding occurs throughout the year.

The growth rate of these slugs was determined from 2 groups of juveniles confined in the field as follows:

a) In August 1963, 25 young slugs (mean weight 14 mg) were collected from the field and confined in cultures out of doors. Five plastic pots, 5" in diameter by 2 1/2" deep (approx. 13 x 6 1/2 cm), were filled to a depth of 2" with sifted soil. Gauze tops and bottoms prevented the slugs from escaping while allowing relatively free drainage. The pots were sunk into the soil and 5 slugs together with food (wheat grains, green and rotting vegetation) were added to each. Sacks were placed over the pots to prevent excessive evaporation and protect them from frost. During the winter of 1964/65 a layer of straw was added as further protection from frost. The slugs were weighed every 4 weeks (Fig. 2). They grew very quickly during their first autumn (mean weight 50 mg in November). The growth rate decreased during the winter (mean weight 60 mg in March) but increased again during the following spring (mean weight 170 mg in May). The first eggs were laid in June 1964 and, as in *Agriolimax reticulatus*, individuals died shortly after breeding. The mean weight of the group began to fluctuate at that time because a sharp decrease in the weight of individuals occurred just before death. Some of the slugs continued laying until November and a further laying season began in the following March when they were

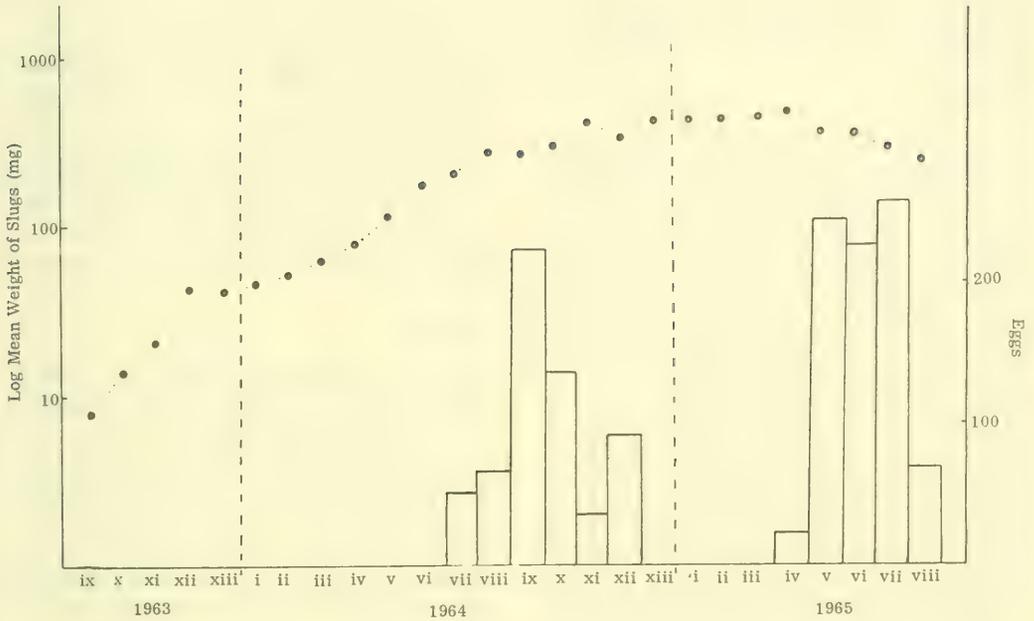


FIG. 2. Rate of growth and egg-laying of *Arion hortensis* in plastic pots. Mean growth rate is represented by curve, total eggs per month for all individuals by columns.

TABLE 2. Mean weights, in mg, of batches of 10 slugs kept in terylene bags

Date 1964- 1965	<i>Arion hortensis</i>		<i>Milax budapestensis</i>	
	Batch 1	Batch 2	Batch 1	Batch 2
June 22nd	-	-	15.0	-
Aug. 17th	8.8	6.2	25.6	62.6
Nov. 5th	83.1	54.1	65.3	104.1
Feb. 8th	154.0	89.3	115.5	129.7

about 18 months old. On the average, the slugs laid 64.5 eggs per individual.

b) In August 1964, 20 young slugs (mean weight 7.1 mg) were collected from the field and kept in terylene net bags (10 slugs per bag) 4" in diameter by 12" deep (10 x 30 cm). The bags were sunk into the ground to a depth of 9" and filled with slug-free soil. Growing vegetation and wheat grains were

TABLE 3. Copulating pairs of slugs observed (January 1963 - December 1964)

Months	<i>Arion hortensis</i>	<i>Milax budapestensis</i>
January	0	2
February	0	4
March	0	1
April	2	3
May	1	0
June	0	0
July	0	0
August	0	0
September	0	6
October	1	15
November	1	11
December	2	5
Totals	7	47

added as food and the slugs were weighed after 3 and 6 months. On the average, these slugs grew slightly faster (approx. 120 mg by February - Table 2) than

those in plastic pots (approx. 50 mg by February). This may have been due to differences in weather conditions in 1963 and 1964.

#### Adults

The abundance of mature individuals in the routine samples is shown in Table 1. Both in 1963 and 1964 there was a decrease in numbers during and after the peak egg-laying period of late June to August as slugs died after laying their eggs. Thereafter numbers rose again, suggesting that some slugs matured later and did not lay eggs until the following spring (as in the cultures above). In the field, copulating pairs were found in April, May, October, November and December (Table 3), providing further evidence that some breeding occurred over most of the year. Copulation was not observed to last longer than 1 hour (in contrast to *Milax budapestensis*, which was seen in copula for over 24 hours). Three pairs of copulating *Arion hortensis* were kept in cultures until they laid their first eggs. The time between copulation and egg-laying varied considerably (3-13 weeks) probably because not all of the pairs were taken during their first copulation.

#### *Milax budapestensis*

#### Eggs

Mainly newly-laid eggs were found in samples between December and January (Table 1) but by May many were well developed. The sharp fall in total numbers by June suggests that many hatched at that time. The presence of a few partly-developed eggs in winter indicates that some had been laid early enough to undergo a little development before the temperature fell. The development rate of eggs in the field was tested by placing newly-laid eggs in plastic pots out of doors, at intervals, between December 1964 and April 1965. The eggs laid at the beginning of winter took longer to develop (Fig. 1B), so that

all of the eggs hatched in the following May and June. Eggs at constant temperatures hatched in 3 weeks at 20° C, 4 weeks at 15° C, 10 weeks at 10° C and 18 weeks at 7.5° C. Very little development occurred at 5° C and field soil temperatures are lower than that level from late November until March. All eggs laid during winter therefore tend to begin development together and most hatching is confined to a peak in spring.

#### Juveniles

Recently hatched juveniles (< 25 mg) were present in the routine samples between April and September. The peak in numbers was later in 1963 (July/August) than in 1964 (June/July), probably due to the long winter of 1962/1963 (Table 1).

The growth rate of these slugs was determined from various groups of confined slugs:

a) Three groups (A, B and C) of young *Milax budapestensis* were collected from the field and kept in plastic pot cultures out of doors (as *Arion hortensis*). They were weighed every 4 weeks and the logarithms of the mean weights of these groups were plotted against time (Fig. 3).

Group A. In August 1963, 20 young slugs (mean weight 33 mg) were collected. By November these had reached an average weight of 138 mg. A very severe frost during late December killed all but one, which by mid-July weighed 396 mg.

Group B. Between January and March 1964, 20 slugs, of approximately the same weight as Group A in December, were confined in cultures which were covered with additional sacks and straw to give further protection from frost. These slugs grew steadily through the spring and summer, and by November 1964 had an average weight of 793 mg. They began to lay eggs in December and lost weight, falling to a mean weight of 652 mg by early March 1965. Eggs continued to be found until July and a few individuals (in poor condition) were still

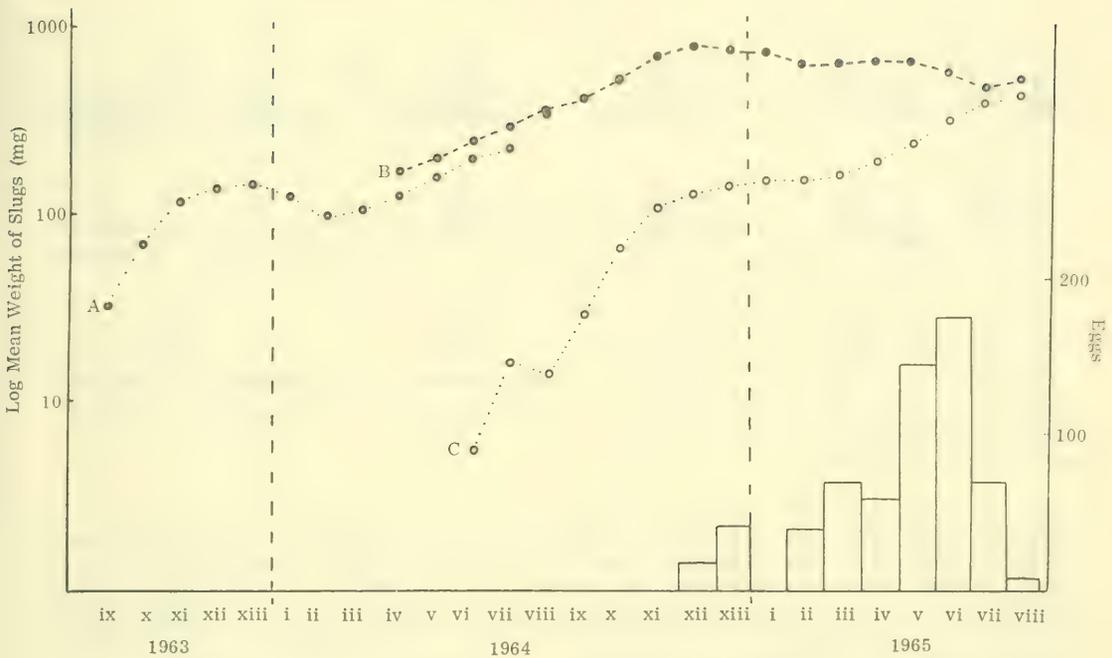


FIG. 3. Rate of growth and egg-laying of 3 groups of *Milax budapestensis* in plastic pots. Mean growth rates are represented by curves, total eggs per month for group B (20 slugs) by columns.

alive in August. An average of 32.5 eggs per individual were laid.

Group C. Five newly-hatched slugs, weighing 5-6 mg, were collected in May 1964. They reached a weight of 13-20 mg by June, but lost weight during their next month, probably due to a fall in the moisture content of the substrate in the culture. They then grew to 36-50 mg by mid-August, when 20 more slugs were collected. Five of these died during the next month, the remainder weighing an average of 128 mg in November and 163 mg in early March 1965. These were dissected in July and, although some weighed over 600 mg, none were mature.

b) Two batches, each of 10 slugs, were collected from the field in June 1964 and kept in terylene bags (as used for *Arion hortensis*). The slugs were weighed after 2, 5 and 8 months (Table 2) and were found to grow at a slightly lower rate than those in plastic pots (Fig. 3).

#### Adults

The abundance of mature slugs in the routine samples is shown in Table 1. None of these slugs were found in July or early August (samples vii and viii) although some of the immature individuals in these samples (not listed in table) weighed over 700 mg. In September, October and November, the numbers of mature slugs (some of which were under 300 mg) built up rapidly and then fell again during the winter and following spring, i.e. they did not live long after laying eggs.

While collecting slugs at regular intervals throughout the year (both at night and during the day) for laboratory experiments, numerous pairs of copulating *Milax budapestensis* were noted (Table 3). Most of these were seen between September and December with occasional pairs between January and April, while none were detected between

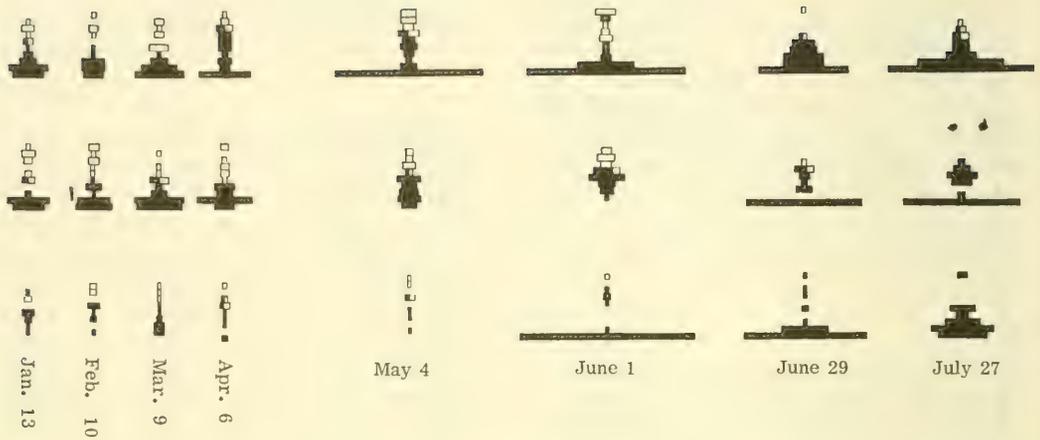


FIG. 4. Weight frequency histograms of slugs from routine samples in 1964. Weight classes in mgs starting from base line as follows: *Agriolimax reticulatus* and *Arion hortensis*; 0-24, 25-49, 50-74, 75-99, 100-149, 150-199, 200-249, 250-299, 300-399, < 400. *Milax budapestensis*; 0-49, 50-99, 100-149, 150-199, 200-299, 300-399, 400-499, 500-599, 600-699, < 700.

May and August. Copulation in the field was often observed to last longer than 12 hours and at 5° C, in the laboratory, 1 pair remained *in copula* for over 36 hours. Six pairs were dissected immediately after copulating and the spermatheca of each slug contained a spermatophore. Three slugs contained the disintegrating remains of a second spermatophore indicating that they had copulated twice. Two pairs, taken from the field *in copula* were observed to copulate again, one pair a week and the other a month after the first pairing. Pairs of slugs, collected at various times of year, were kept in field cultures, and periods of up to 15 weeks occurred between copulation and egg-laying. Pairs taken in the spring did not take as long to lay their first batch of eggs (as little as 5 weeks) as those taken in the autumn, probably because the spring copulating pairs were less likely to have been taken during their first copulation.

A further estimate of the egg-laying capacity of *Milax budapestensis* was made from 10 groups of 4 mature slugs kept in field cultures from mid-October 1964 until they died. Green vegetation and wheat grains were provided as food

and the culture boxes (5" x 5" x 2 1/2"; approx. 13 x 13 x 6 1/2 cm) were covered with sacks to protect them from frost. The soil in the boxes was searched each fortnight for eggs and these were counted and removed. On the average these mature slugs laid 23.7 eggs/individual - considerably fewer than those in the growth rate cultures (Juveniles, Group B). Although the slugs for this observation were collected before the field egg-laying season and no eggs were laid in the boxes until 6 weeks after they were confined, it is possible that they had deposited some eggs before being caught. However, the lower egg-laying rate in the boxes may also have been due to less favourable conditions of food or microclimate than in the bags.

#### DISCUSSION AND CONCLUSIONS

The generalised life cycles of the 3 species were summarised by making weight-frequency histograms of data from samples; 1964 was taken as typical and data for that year are presented in Fig. 4. These histograms, together with the above sections on separate

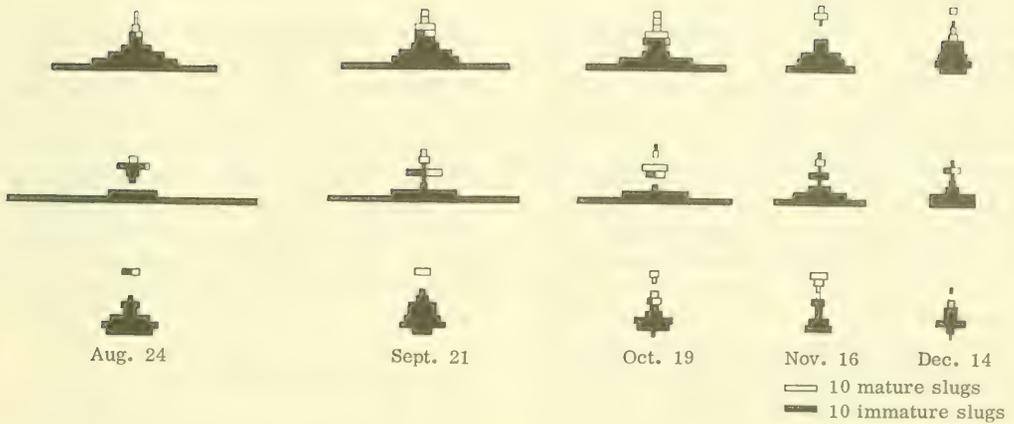


FIG. 4. continued.

stages, suggested the following:

*Agriolimax reticulatus*: There was some breeding activity throughout the year, but a distinct peak was apparent during spring and autumn. The slugs hatching in spring laid their eggs and died during the autumn and winter, and those hatching in autumn matured and died during the spring and summer of the following year. The spring generation developed more quickly (May to late September) than the autumn generation (late September to May).

*Arion hortensis*: In the second half of the year there were 2 distinct generations represented in the samples, (a) an older generation maturing during summer and autumn to die out in the first part of the following year, and (b) a new generation hatching from late June (sample vii) onwards and gradually growing in weight during the rest of the year. Most slugs of this generation continued to grow in the spring of the following year to mature at about 1 year old, but a few which were late in hatching were not ready to mature before their second winter, i.e. they were almost 2 years old before breeding.

*Milax budapestensis*: In the samples after May (sample v) 2 generations were

apparent, (a) an old generation represented by a few slugs (over 700 mg by early August, sample viii) which matured in early autumn and died out in the winter, and (b) a new generation mostly hatching about May and growing in weight during the summer and autumn. Some of these matured during the winter and spring of their first year; but those that were unable to reach maturity before their first spring did not seem to mature until the autumn of their second year (in spite of reaching weights of over 700 mg some months earlier). Since development of the eggs laid by the latter slugs was halted during the winter, it was 2 years before their cycle was completed.

In none of the 3 species was there a rigid life cycle followed by all individuals. In *Agriolimax reticulatus* and *Arion hortensis* there seemed to be no intrinsic barrier to breeding at any time of year; the periods of high breeding activity were probably regulated by the weather. However, there seemed to be some kind of barrier to the maturation of *Milax budapestensis* during the summer months: during June and July large individuals (over 700 mg) remained immature, but by October most *Milax*

of over 400 mg were mature.

Previous published work on the life cycles of slugs depended on night-searching (Barnes & Weil, 1944a, b; Bett, 1960) or trapping (Getz, 1959). These sampling methods probably gave unreliable life-cycle data since they tend to overestimate the proportion of large slugs in the population (Hunter, 1968). Thus the breeding seasons were possibly not recognised until some time after they occurred. However, it is still apparent that the life cycles of slugs vary considerably under different climatic conditions. Bett (1960) claimed that in Hertfordshire, central England, *Agriolimax reticulatus* has 2 generations a year, *Arion hortensis* 1 a year (with hatching in January-February) and *Milax budapestensis* 1 a year (with hatching during the Autumn). Getz (1959) reported that in Michigan, U.S.A., *Deroceras reticulatum* (= *Agriolimax reticulatus*) was an annual species with only a few adults surviving the winter to lay eggs the next spring.

Since the length of generation interval is the most important factor influencing the capacity of a population to increase in numbers, the density of a particular generation of slugs will depend mainly on the proportion of the previous generation that matured during their first breeding season. Thus, the severe winter of 1962/63 impeded the development of all 3 species and the 1963 generations were small. A much greater proportion of slugs was able to continue development during the mild winter of 1963/64 and the subsequent generations were larger.

Further research is required to establish the reproductive controlling mechanisms of all 3 species and the effect on the life-cycles of the various climates in different regions of the world.

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## RESUMEN

ESTUDIOS SOBRE "BABOSAS" DEL SUELO ARABLE  
II. CICLO DE VIDA

P. J. Hunter

*A. reticulatus* tiene dos generaciones anuales, una primaveral haciendo eclosión para Mayo, y otra en Otoño, hacia Septiembre. La segunda toma más tiempo para completar el ciclo (7 meses), que la primera (5 meses).

*A. hortensis* en su mayoría tiene un ciclo anual. Nacen alrededor de Julio y crecen durante los once meses siguientes para desovar cuando llegan a tener un año; algunos nacidos más tarde no fueron capaces de desovar hasta el segundo invierno, así que tomaron 2 años en completar el ciclo. Cada individuo pone un promedio de 64,5 huevos y mueren pronto después de la puesta.

La mayoría de *M. budapestensis* tienen ciclo bienal; hacen eclosión entre Mayo y Agosto y maduran durante el segundo otoño e invierno. Los pocos nacidos temprano, en Abril, pueden desovar el primer invierno. Como en las otras especies, la rapidez de desarrollo depende de las condiciones ambientales. Depositán un promedio de 23,5 huevos por individuo, muriendo después. Individuos cultivados en el campo desde los primeros estados, llegaron a depositar un promedio de 32,5 huevos.

## АБСТРАКТ

ИЗУЧЕНИЕ СЛИЗНЕЙ НА ПАХОТНЫХ ЗЕМЛЯХ  
II. ЖИЗНЕННЫЙ ЦИКЛ

П. ДЖ. ХАНТЕР

Автором исследовался жизненный цикл у *Agriolimax reticulatus* (Muller), *Arion hortensis* Ferussac и *Milax budapestensis* (Hazay); работа проводилась на основании изучения обычных проб, собранных на участках пахотной земли в Северной Англии. *Agriolimax reticulatus* имел 2 генерации в год, -весенняя, в мае, и осенняя - конце сентября. Последним особям требовалось больше времени (7 месяцев), чтобы завершить свой жизненный цикл, чем первым (5 месяцев).

Большая часть *Arion hortensis* имели годовой жизненный цикл; они отрождались в икле, росли в течение последующих 11 месяцев, созревали и откладывали яйца в возрасте около 1 года. Некоторые, однако, рождались позже и до 2ой зимы своей жизни еще не были готовы откладывать яйца, т.е. употребляли для своего цикла около 2 лет. Было найдено, что скорость их развития на всех стадиях зависит от условий среды. Особи откладывали в среднем 64.5 яйца и погибали вскоре после размножения.

Большинство *Milax budapestensis* имели двухлетний жизненный цикл. Они отрождались между маем и августом и созревали в течение второй осени своей жизни и зимой. Меньшая часть, однако отрождалась в апреле и откладывала яйца в первую зиму своей жизни. Но скорость их развития опять таки зависела от условий существования. Слизни, собранные в поле перед самым размножением, откладывали, в среднем по 23.5 яйца на 1 особь,

вскоре после чего погибали. Слизни, содержащиеся в полевых культурах, начиная с их ранних стадий, откладывали, в среднем по 32.5 яйца каждый.

STUDIES ON SLUGS OF ARABLE GROUND  
III. FEEDING HABITS

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ABSTRACT

Observations and experiments were made to establish the feeding activity and preferences of the slugs *Agriolimax reticulatus* (Müller), *Arion hortensis* Férussac and *Milax budapestensis* (Hazay).

Feeding activity, which has a bearing on control by baiting, was measured by the number of wheat grains damaged per unit time. A preliminary test showed that *Arion hortensis* fed more often than *Agriolimax reticulatus* and the latter more often than *Milax budapestensis*. The total weight of wheat eaten per unit time, however, was similar in all 3 species; i.e., the species that fed more frequently consumed less at each feed. Experiments in laboratory and field showed that feeding was dependent on temperature, being maximal at 20° C, although some feeding occurred even at very low temperatures: at just above 0° C *Agriolimax reticulatus* was most and *Milax budapestensis* least active. It was also shown that activity was greater at 100% than at 95% relative humidity but did not depend on day length, and that a distinct nocturnal rhythm occurs, slugs feeding most in the early part of the night.

Dissection of slugs from the field showed that the surface-dwelling *Agriolimax reticulatus* has a greater tendency to feed on green vegetation than the underground-dwelling *Arion hortensis* and *Milax budapestensis*.

The ecology of slugs on an arable plot in Northumberland was investigated by a routine sampling study between January 1963 and March 1965 (Hunter, 1966, 1967, 1968a, b). At the same time a number of observations and experiments on the feeding habits of these slugs were made to establish the relationship between their feeding and their general biology and ecology. Feeding habits are also important in that they have a direct bearing on the control of these pests by baiting. Work was confined to the 3 commonest species in the area, *Agriolimax reticulatus* (Müller), *Arion hortensis* Férussac and *Milax budapestensis* (Hazay).

FEEDING ACTIVITY

The effect on feeding of the major environmental factors was studied in laboratory and field experiments. Activity was measured as the number of wheat grains damaged per unit time. This method gives an easily visible record of activity, so that accurate measurements can be quickly taken. A preliminary test was conducted to establish the amount of each grain that each of the 3 species consumed at each feed and this test was followed by assessments of the effect of temperature, humidity, day-length and the nocturnal rhythm on feeding activity. *Arion hor-*

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TABLE 1. Relationship between number of grains damaged<sup>+</sup>, amount eaten from each grain and total weight of wheat eaten<sup>+</sup>

Species	Mean No. damaged per slug*	Mean Wt. eaten per grain (mg)*	Mean total Wt. eaten (mg)
<i>Arion hortensis</i>	6.8	16.1	109.5
<i>Agriolimax reticulatus</i>	4.9	28.0	137.4
<i>Milax budapestensis</i>	3.7	33.6	124.5

\* These means are significantly different ( $P = < 0.001$ ) from each other (analysis of variance and Tukey's test).

+ Over a total of 10 days.

TABLE 2. Mean numbers of grains damaged at various constant temperatures by 10 slugs over 10 days

Species	Temperature in °C								Overall Mean
	0.5	2.0	4.0	5.0	10.0	15.0	20.0	25.0	
<i>Arion hortensis</i>	0.50	<u>2.50</u>	<u>2.80</u>	4.35	7.60	<u>9.20</u>	<u>9.60</u>	1.65	4.78
<i>Agriolimax reticulatus</i>	0.80	<u>1.65</u>	<u>1.85</u>	<u>2.80</u>	3.15	3.20	<u>3.50</u>	1.50	2.31
<i>Milax budapestensis</i>	<u>0.05</u>	<u>0.85</u>	0.60	<u>0.70</u>	<u>1.90</u>	2.05	2.25	1.70	1.26

-- Means that do not differ significantly from each other ( $P = > 0.05$  by analysis of variance and Tukey's test) are linked by underlining.

-- The overall species means differ significantly from each other.

*tensis* and *Agriolimax reticulatus* of over 200 mg and *Milax budapestensis* of over 400 mg were used in experiments.

#### Preliminary Test

Slugs were confined individually in glass tubes 2" high x 0.9" diameter. The bases of these tubes were sunk in damp vermiculite, an inert, granulate material, and the tops enclosed by gauze caps. Ten slugs of each species were subjected for 10 days to one of 5 sets of alternating temperatures, namely 25 and 20° C, 20 and 15° C, 15 and 10° C, 10 and 5° C, or 5 and 0° C, the higher temperature in each case obtaining from 09:00 to 21:00 hrs Greenwich Mean Time and the lower from 21:00 to 09:00

hrs. The temperatures were maintained in cooled incubators which were accurate to approximately  $\pm 1^{\circ}$  C (checked by maximum - minimum thermometers throughout the study). Each slug was given 3 wheat grains weighing  $65 \pm 2.5$  mg. Damaged grains were counted, air-dried and weighed after each 12-hour period.

The data from this experiment provided a number of points of information on slug activity:

1. *Arion hortensis* damaged more wheat grains than *Agriolimax reticulatus*, which, in turn, damaged more than *Milax budapestensis* (Table 1). The data were subjected to analysis of variance which showed that there were significant differences in numbers of grains damaged by the 3 species; further

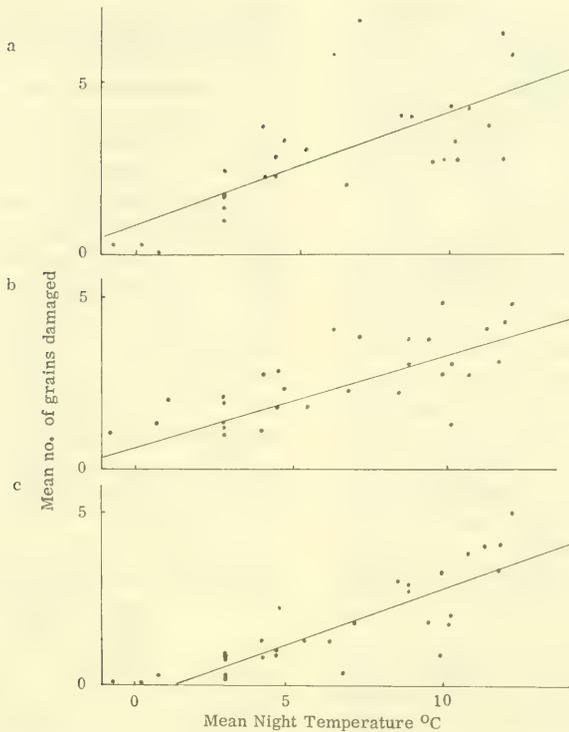


FIG. 1. Relationship between mean night temperature and the mean number of grains damaged by 30 slugs (10 of each species) on 30 different occasions.

a. *Arion hortensis*; b. *Agriolimax reticulatus*; c. *Milax budapestensis*.

analysis of data by Tukey's Test (cf. Snedecor, 1956) showed that each of the above differences was significant.

2. *Milax budapestensis* consumed more of each damaged grain than did *Agriolimax reticulatus* and the latter more than *Arion hortensis*. Again, by analysis variance and Tukey's Test, these differences were significant.

3. The average weight of wheat eaten by individuals of the 3 species over 10 days was similar (Table 1) and analysis of variance showed that there were no significant differences present. It would therefore seem that, although the 3 species feed at different frequencies, the actual amount eaten does not differ significantly.

4. There were no significant differences in the amount of grain eaten at each feed at the various temperatures. Thus, although temperature affects the

frequency of feeding (see next section) it did not affect the extent of each feed.

#### Effect of Temperature

(a) The numbers of wheat grains damaged at constant temperatures of 0.5, 2, 4, 5, 10, 15, 20 and 25°C (all  $\pm$  approx. 1°C) were compared. For this purpose, slugs were confined in plastic boxes (5" x 5" x 2 1/2"), with 10 slugs in each box. The boxes, with gauze tops and bases, were half filled with sand and were standing in 1/4" of water to keep the sand wet. The experiment ran for 20 days. Fifteen wheat grains were added to each box; damaged grains were counted and replaced daily. All slugs were renewed on the 10th day of the experiment, and any that died during the experiment were replaced immediately.

Feeding activity of all 3 species increased as temperature rose to 20° C but showed a decrease at 25° C (Table 2). At low temperature (just above freezing point) *Agriolimax reticulatus* was the most active of the 3 species (as established by Mellanby, 1961) and *Milax budapestensis* the least active.

(b) Nine terylene-net bags (4" in diameter x 12" deep) were half-filled with damp vermiculite and sunk (to the depth of the vermiculite surface) in the soil of an outdoor plot. There were 3 bags for each species and 10 slugs in each bag. Six sets of observations were taken between July and December 1963. Slugs were freshly collected for each experiment and kept in the bags for at least 2 days prior to the observations. Ten grains were added to each bag and during the following 5 days damaged grains were counted and replaced daily.

The mean number of grains damaged daily by each species was plotted against the average night air temperature (18:00 - 06:00 hrs; Fig. 1). Significant regressions ( $P = 0.001$ ) of feeding activity on temperature were obtained for the 3 species, i.e. under the above conditions, feeding was directly related to temperature. However, even at a mean night temperature of 12° C, the feeding proportion of the population was only 50-60%.

#### Effect of Humidity

Controlled humidities (Winston &

TABLE 3. Establishment of controlled humidity

Added to lower chamber of desiccator	Relative humidity produced
Distilled water	100%
Saturated soln. of potassium permanganate	98%
Saturated soln. of sodium sulphite	95%

Bates, 1960) at 20° C were created in the upper chambers of 3 desiccators as shown in Table 3.

The humidities were checked with hair hygrometers previously calibrated against a Gregory hygrometer. Three each of *Arion hortensis*, *Agriolimax reticulatus* and *Milax budapestensis* were placed in each desiccator at any one time, each individual being confined in a glass tube with gauze caps at either end. Three wheat grains were put in each tube and damaged grains were counted and replaced daily. The experiment comprised three 5-day 'trials'; new slugs were used for each trial.

Fewer grains were damaged at low humidities than high ones (Table 4).

TABLE 4. Mean numbers of grains damaged under varying conditions of humidity at 20° C

Species	Relative Humidity		
	100%	98%	95%
<i>Arion hortensis</i>	1.22	1.00	0.89
<i>Agriolimax reticulatus</i>	2.22	1.67	1.00
<i>Milax budapestensis</i>	1.56	1.00	0.67
All species	1.63	1.26	0.85

The data were subjected to analysis of variance which showed that some significance ( $P < 0.05$ ) can be attached to these differences. The restriction of slug activity by low humidity has been noted by Hughes & Kerkut (1956) and Kerkut & Taylor (1956), who suggested that activity is regulated by the haemolymph concentration, the latter being affected by humidity. Rozsa (1962) did not support this view in her claim that activity can be stimulated directly from "osmoreceptors" (humidity receptors) in the foot. It is not known how often the humidity of the environment in the field is low enough to limit feeding while not low enough to kill the animal.

Slugs lost weight in all experiments,

TABLE 5. Influence of Humidity on Weight loss\* of slugs during a 5-day period

Species	Relative Humidity (at 20° C)		
	100%	98%	95%
	%	%	%
<i>Arion hortensis</i>	24.72	36.50	43.22
<i>Agriolimax reticulatus</i>	25.47	27.33	42.50
<i>Milax budapestensis</i>	16.46	22.58	25.60

\*Mean losses calculated from 9 slugs each.

the loss being the greater, the lower the humidity (Table 5). However, there was considerable variation between replicates, the weight loss decreasing and the number of damaged grains increasing in the 2nd and 3rd trials.

#### Effect of Length of Daylight

The effect of day length on the feeding activity of slugs was tested over a period of one week. For this purpose, 40 adults of each of the 3 species were confined individually in glass tubes out of doors, with 3 wheat grains placed in each tube. Damaged grains were counted and replaced at 22:00 and 10:00 hrs British Summer Time (B. S. T.). During the experiment natural darkness lasted from approximately 22:00 hrs - 04:00 hrs. Total darkness for this period and longer was contrived by covering the tubes with opaque boxes as shown in Table 6.

TABLE 6. Establishment of controlled length of darkness

Time of day covered hrs. B. S. T.	Resulting hours of darkness	No. of slugs of each species
22:00-04:00	6	10
22:00-10:00	12	10
16:00-10:00	18	10
continuously	24	10

The numbers of damaged grains are given in Table 7. Length of daylight had no significant effect ( $P = < 0.05$ ) on the numbers of grains damaged. Slugs damaged significantly more grains during the 22:00 - 10:00 period than during the day ( $P = < 0.001$ ) irrespective of when they were covered. These results confirm the work of Dainton (1954) who established that the nocturnal rhythm is maintained by falling temperature at night.

#### Effect of Nocturnal Rhythm

Four groups of each of the 3 species (each group containing 10 slugs) were confined in plastic boxes outdoors. Ten wheat grains were added to each box and those damaged were counted and replaced every 6 hours, at 01:00, 07:00, 13:00 and 19:00 hrs., B. S. T. The experiment took place over 5 consecutive days in October when darkness lasted from 19:00 - 07:00 hrs. Slugs were feeding most actively in the early part of the night and least actively during the day (Table 8).

#### FEEDING PREFERENCES

Between July and November 1963, slugs were extracted from 4-weekly routine samples by soil washing (Hunter, 1968a). The extraction was carried out immediately after taking each sampling unit and was completed within 2 hours of digging: since sampling took place during the day and most slugs feed at night (see above), it is unlikely that they would have fed while awaiting extraction in the laboratory. Slugs were dissected immediately and their gut contents were examined under a low power microscope. It was not possible to classify plants that had been eaten, but the type of vegetation (leafy green material, white stems and brown roots and soil) could be distinguished.

For the purpose of statistical analysis, vegetation in the gut was classified into dominantly green or non-green material

TABLE 7. The number of grains damaged by 10 slugs over 1 week under varying conditions of light and darkness

Species	No. of slugs used	Hours exposed to daylight							
		18		12		6		0	
		Night	Day	Night	Day	Night	Day	Night	Day
<i>Arion hortensis</i>	4 x 10	23	5	27	8	29	8	24	8
<i>Agriolimax reticulatus</i>	4 x 10	25	2	29	5	19	11	21	8
<i>Milax budapestensis</i>	4 x 10	8	1	7	3	8	5	6	5
Totals	120	56	8	63	16	56	24	51	21
		64		79		80		72	

Night = total grains damaged between 22:00 and 10:00 hrs. B.S.T.

Day = total grains damaged between 10:00 and 22:00 hrs. B.S.T.

TABLE 8. Mean number of grains damaged\* by 10 slugs throughout a 24-hr. period

Species (40 each)	Night		Day	
	19:00 to 01:00	01:00 to 07:00	07:00 to 13:00	13:00 to 19:00
<i>Arion hortensis</i>	3.75	3.05	0.60	0.00
<i>Agriolimax reticulatus</i>	2.60	2.30	0.40	0.30
<i>Milax budapestensis</i>	2.25	1.30	0.50	0.00
Overall Mean	2.87	2.18	0.50	0.10

\* Out of 10 grains provided for each 6 hr. period

TABLE 9. Numbers of slugs containing green and non-green material

Month (1963)	<i>Arion hortensis</i>		<i>Agriolimax reticulatus</i>		<i>Milax budapestensis</i>	
	Green	Non-Green	Green	Non-Green	Green	Non-Green
July	15	44	50	27	6	38
August	29	38	48	10	9	21
September	1	56	50	26	2	20
October	8	53	52	19	1	14
November	4	58	43	7	4	24
Totals	57	249	243	89	22	117

Table 9). Analysis of variance showed that a significantly larger proportion of *Agriolimax reticulatus* (73.2%) contained dominantly green material than *Arion hortensis* (18.6%) or *Milax budapestensis* (15.8%). There was no significant variation in the proportion of slugs eating green material during the 5 months of sampling, i.e. slugs were not obliged to eat more non-green vegetation when much of the surface vegetation died during autumn.

#### DISCUSSION AND CONCLUSIONS

The experiments on feeding activity have relevance to baiting as a method of slug control and as a method of estimating populations. There is clearly a close relationship between slug activity and the temperature and humidity of the environment. The greatest efficiency in control by baiting can be expected on warm, humid nights (as established by Webley, 1964, 1965). However, even at a mean night temperature of 12°C, when the substrate was wet (see experiment in terylene bags: points at highest temperature on Fig. 1), only a little over half the population was feeding. Thus, to be effective, poison baits must be applied over a considerable period of time.

The effect of environment on activity, however, does not invalidate the results of bait trapping for population estimation (Hunter, 1968a). Although the amount of damage to baits cannot give accurate estimates of the size of populations, they can indicate the time and direction of changes of density.

The observations on feeding preferences showed that under normal conditions there was little competition for food. The 3 species ate a wide variety of food that was commonly available, so it would seem that there will be few situations in which an absolute shortage occurs. The same has been previously demonstrated by Boycott (1934) who concluded "that food has no influence either by its quality or quantity on the re-

currence of our land Mollusca, excepting Testacellidae and such meagre habitats as shifting sand dunes". Getz (1959) demonstrated in laboratory experiments that 3 species of slugs including *Agriolimax reticulatus* accepted a wide range of plants as food. The present observations further show that slugs do not move far to feed, i.e. species that live underground tend to feed underground and those that live on the surface are surface feeders. *Agriolimax reticulatus* which eats mostly green material, is mainly surface dwelling (Hunter, 1966); *Arion hortensis*, which eats less green food, is more often found under soil level; and *Milax budapestensis*, which eats still less green food, is found deeper underground.

#### ACKNOWLEDGEMENTS

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## RESUMEN

ESTUDIOS SOBRE "BABOSAS" DEL SUELO ARABLE  
III. HABITOS ALIMENTICIOS

P. J. Hunter

Las funciones alimenticias en las mismas especies fueron medidas, y sus preferencias controladas, por la cantidad de granos de trigo, usados como cebo, que averiaron por unidad de tiempo. Una prueba preliminar demostró que *Arion hortensis* come con más frecuencia que *Agriolimax reticulatus* y la segunda más que *M. budapestensis*, pero el total de trigo consumido fue similar en las 3 especies, desde que las que comen con más frecuencia consumen menos por vez. Experimentos en el campo, así como en el laboratorio, indicaron que la actividad depende de la temperatura, siendo máxima a los 20° C, aunque pueden alimentarse hasta en muy bajas temperaturas: justo a 0° C *Agriolimax reticulatus* fue un poco más activo que *M. budapestensis*. Se notó también que la actividad alimenticia es mayor a 100% que a 95% de humedad relativa, pero que no depende de la duración diurna, sino que ocurre también en ritmos nocturnos, la mayoría alimentándose en las primeras horas de la noche.

Disectando individuos silvestres se encontró que los que viven en superficie como *A. reticulatus* prefieren alimentarse de vegetación verde en una mayor proporción que los que viven enterrados como *Arion hortensis* y *Milax budapestensis*.

## АБСТРАКТ

ИЗУЧЕНИЕ СЛИЗНЕЙ НА ПАХОТНЫХ ЗЕМЛЯХ  
III. ПИТАНИЕ

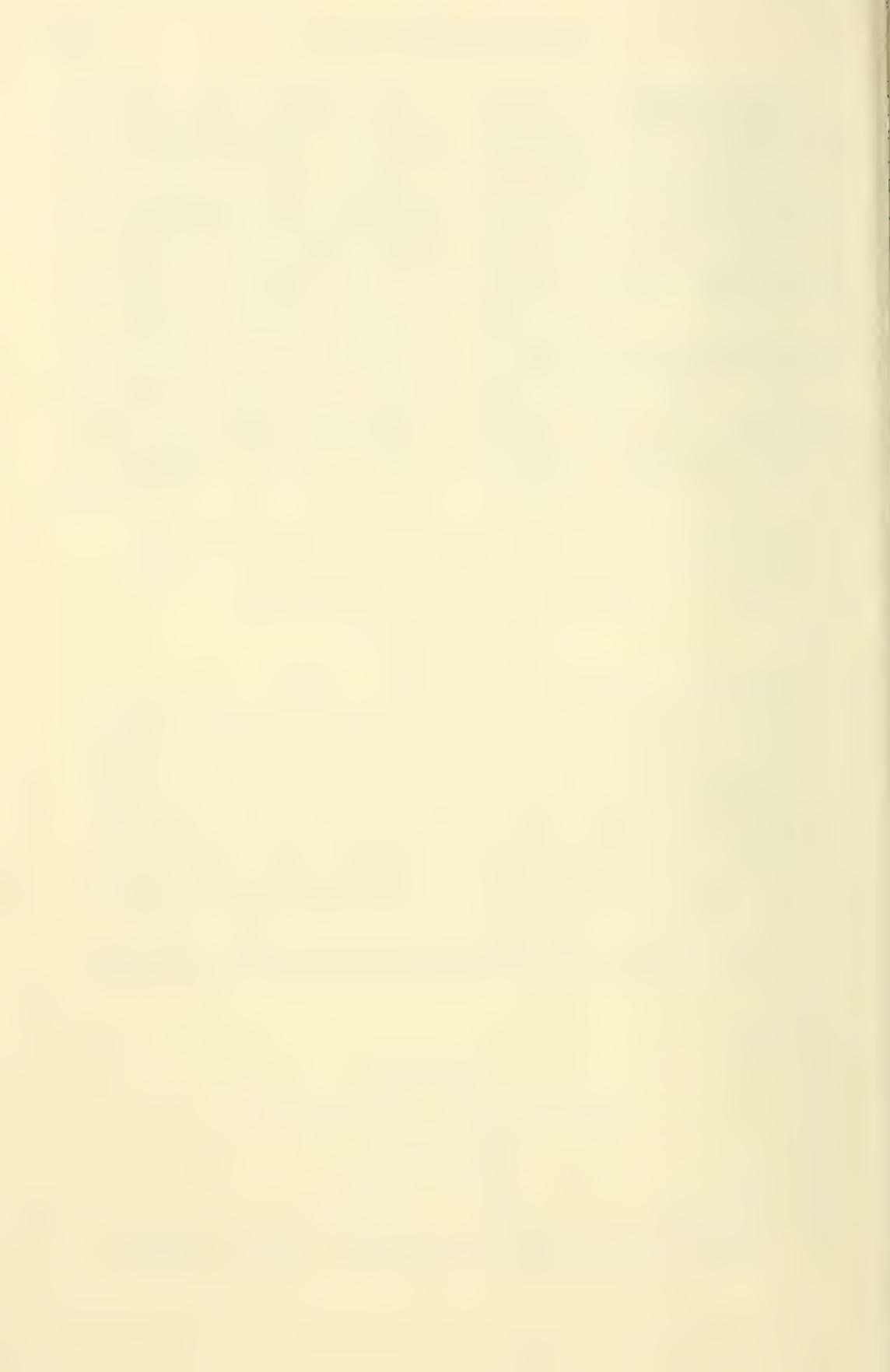
П. ДЖ. ХАНТЕР

Были проведены наблюдения и эксперименты для установления активности питания и предпочтения той или иной пищи слизнями

*Agriolimax reticulatus* (Muller), *Arion hortensis* Ferussac и *Milax budapestensis* (Hazay).

Активность питания, контроль над которой осуществлялся при помощи приманок, измерялся количеством зерен пшеницы, поврежденных слизнями за единицу времени. Предварительные пробы показали, что *Arion hortensis* питается чаще, чем *Agriolimax reticulatus*, а последний — чаще, чем *Milax budapestensis*. Общий вес съеденных зерен пшеницы за единицу времени был, однако сходным для всех трех видов, т.е. виды, питавшиеся чаще потребляли меньше пищи за каждую еду. Эксперименты в лаборатории и в поле показали, что питание слизней зависело от температуры, будучи максимальным при 20°C, хотя некоторое питание наблюдалось даже при очень низкой температуре: почти при 0°C *Agriolimax reticulatus* был более активен, а *Milax budapestensis* несолько менее активен. Было также показано, что активность питания была выше при 100%, чем при 95% относительной влажности, но не зависела от продолжительности дня, и что у них имеется хорошо-выраженный ночной ритм: слизи питаются больше в раннюю пору ночи.

Вскрытия слизней, собранных в поле показали, что живущие на поверхности *Agriolimax reticulatus* имеют большую тенденцию питаться зеленой растительностью, чем живущие в под-поверхностном слое *Arion hortensis* и *Milax budapestensis*.



THE LOCOMOTION OF THE FRESHWATER CLAM  
*MARGARITIFERA MARGARITIFERA* (UNIONACEA: MARGARITANIDAE)

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ABSTRACT

The locomotion of *Margaritifera margaritifera* (L.) was investigated by use of electronic recording devices and its habits are described with brief reference to the effect of shell shape (e.g., the presence of a pronounced pedal gape in fast water forms) on burrowing. The series of burrowing activities, termed the 'digging cycle', are very similar to those of other bivalves and are used both in burrowing and, with little modification, in locomotion over the surface of sand. The most obvious differences involve the more anterior orientation of the foot and the relative magnitude of contraction of the anterior and posterior retractor muscles.

During the digging cycle the valves adduct to loosen the adjacent sand and to obtain a pedal anchorage through dilation of the foot by means of blood pressure. When on the surface of the sand, the valves are reopened exclusively by the hinge ligament; but, with more than 1/3 of the shell buried, the pressures derived from pedal retraction are used to supplement the ligament. Burrowing activity involves the integration of adduction and opening of the valves with protraction and retraction of the foot. Adduction produces a pedal anchorage, allowing the shell to be drawn down at retraction, while the shell is held firm in the sand by the opening thrust of the ligament (secondary or shell anchor) at pedal protraction.

Bivalves living on a hard substrate have a basically similar locomotory pattern but without the occurrence of adduction, possibly because the pedal anchorage is not obtained in the same manner.

INTRODUCTION

The process of burrowing in the Bivalvia has been recently studied in common British littoral species using electronic recording techniques for detailed analysis of their activities (Trueman, Brand & Davis, 1966a). Previous work, summarized by Morton (1964), who gives a full bibliography, in general lacks the precision which the more modern techniques can provide. Nevertheless it indicates, together with the present observations, that burrowing by bivalves follows a common pattern of activity. Burrowing consists essentially of a series of step-like movements into the substrate, termed the 'digging sequence' by Ansell (1962) and

more recently the 'digging cycle' (Trueman et al., 1966a). Digging generally commences with the bivalve lying on its side extending its foot into the substrate to lift the shell erect before pulling it down into the sand in a succession of digging cycles. The term 'digging period' may be applied to describe this activity from the start of burrowing until the final position in the substrate is reached.

The attention of the author was drawn to the freshwater clam *Margaritifera margaritifera* (L.) by Dr. R. M. C. Eagar, who pointed out that the form of its shell was similar to that of certain Carboniferous bivalves, e.g. the Antracosidae. In some forms of *Margaritifera* the shells have curved dorsal margins and inflected lower borders (IN,

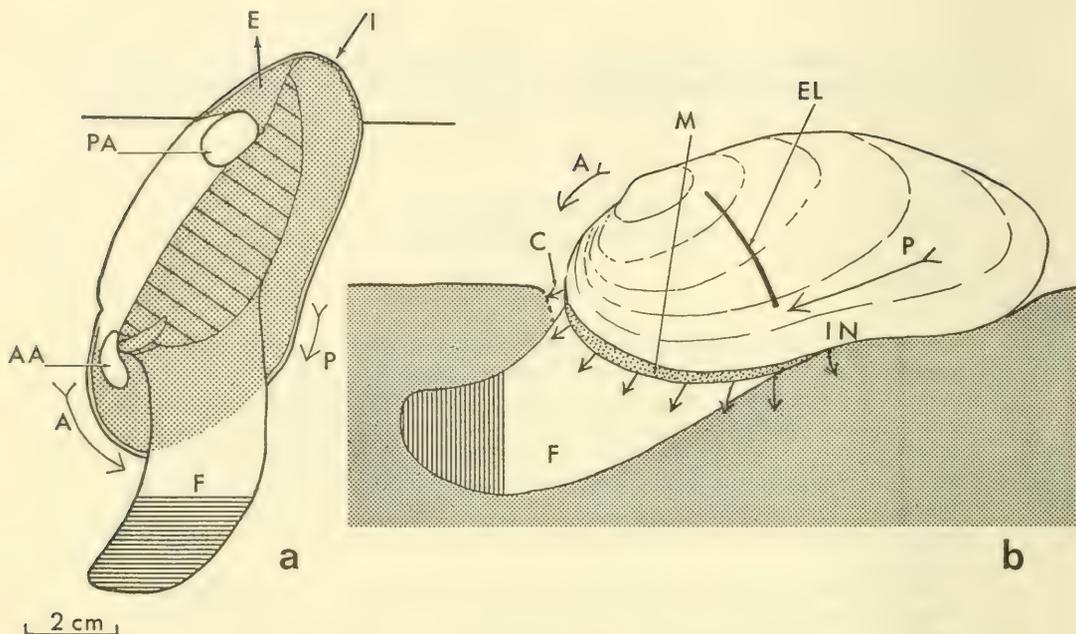


FIG. 1. Diagrams of *Margaritifera margaritifera*, reconstructed from filmed sequences of burrowing, (a) when buried and (b) moving over the surface of the sand.

a: shows a sagittal section with the foot (F) extended in the active digging position, the surface of the sand (horizontal line), the extent of the mantle cavity (stippled) with gills hatched, and the inhalent (I) and exhalent (E) siphons. AA, PA, anterior and posterior adductor muscles.

b: shows the position of the foot in the sand during locomotion across the surface of the sand (stippled), water ejection currents from the mantle cavity at adduction of the valves with the siphons closed producing a cavity (C) in the sand in front of the shell and over the upper surface of the foot. EL, position of electrode to record valve movement; IN, inflected ventral margin; M, outer mantle folds around pedal aperture.

In both figures the direction and relative movement of the shell by anterior (A) and posterior (P) retraction is indicated ( $\leftarrow\leftarrow$ ) and the region of the foot forming the pedal anchor is shown by hatching.

Fig. 1b) which are characteristically associated with relatively swiftly flowing water. In contrast, forms with straight hinge lines and more rounded lower borders are generally found in slowly moving water (Eagar, 1947, 1948). Although a comprehensive study of *Margaritifera* in relation to its ecology has been made by Hendelberg (1960), the current investigation of locomotion in this genus was initially carried out so as to attempt further elucidation of the relationship between shell shape and habitat. The findings concerning the latter were limited, but the work led on to a more complete understanding of the

mechanics of burrowing in Bivalvia, which are described below principally in terms of *Margaritifera*.

#### METHODS

Observations of the digging period have been made by filming the activity of *Margaritifera* in a glass tank for subsequent analysis and by recording simultaneously valve movements and the pressures developed in the substrate. Recording valve movement involves the placing of a pair of fine wire electrodes (EL, Fig. 1b), one on each valve, between the umbone and the ventral margin.

These are of very light wire and, with a loop stretching between the electrodes and the recording device, proved to be of little hindrance since the bivalve burrowed quite normally. A small oscillatory current ( $2 \mu$  amp, 25 kc/sec) is passed between the electrodes and any movement of the valves affected the impedance between them. A voltage, proportional to the change in impedance, was fed to a pen recorder by A.C. coupling, which allowed any change of impedance to be recorded about a preset level. Thus opening or closing of the valves gave positive or negative swings of the pen respectively, while, if the valves remained still at any angle of gape, the preset level would be recorded (Fig. 3).

The method of recording pressure involved the connection of a sensitive Statham pressure transducer to a tube opening beneath the sand near the foot of the burrowing bivalve. Interpretation of these recordings must always be related to direct observations of the animal's activity since negative pressures are recorded either by the application of pressure or by withdrawal of the foot, while the ejection of water from the mantle cavity of a bivalve beneath the sand at adduction causes positive pressures. Once the recordings have been interpreted by observation they afford a ready means of continually recording the digging of bivalves even when completely invisible beneath the sand. A full account of these techniques is given by Hoggarth & Trueman (1967).

#### HABITS

Specimens of *Margaritifera*, of the arched morphological variety, were collected from the River Lune (at Crook of Lune, Lancashire, England), a fairly fast running salmon river, and were taken to the laboratories of the Zoology Department of the University of Hull for immediate investigation of burrowing habits. The clams were found, during a dry spell, in water 3 feet deep, almost

completely buried in fine sand as in Fig. 1a, leaving about 2 cm of the posterior valve margin with the inhalent and exhalent siphonal apertures exposed above the substrate. The same position was also taken up after burrowing in an aquarium. The angle at which *Margaritifera* burrows allows an uninterrupted exhalent current to flow from the postero-dorsal exhalent siphon. When specimens were taken to the laboratory and placed in an aquarium containing sand from the River Lune, a few burrowed immediately, but the majority actively moved across the surface of the sand for a day or so before digging downwards.

The position of the foot and its extension from the valves anteriorly is shown in Fig. 1b when the clam was moving over the surface of sand. The foot was never observed to extend posteriorly of the inflection (IN) of the ventral margin of the valves at any stage of digging. In *Margaritifera* from a different habitat with more rounded ventral valve margins the foot was similarly extended, never being observed behind the mid-ventral region of the shell. The location of the extended foot in *Margaritifera* is similar to that observed in other bivalves with elongated shells, e.g. *Donax*, whereas with more rounded valves, e.g. *Cardium*, the foot may extend much more posteriorly. In the inflected forms there was also a pronounced anterior pedal gape even when the valves were completely closed mid-ventrally, through which the outer mantle fold (M) protruded at pedal extension. This pedal gape may possibly be associated with an extension of the foot for longer periods in those animals that live in faster waters, so as to ensure a better anchorage. For the other features of the shell associated with fast flowing water no function was apparent from these studies of burrowing.

Movement over the sand took place by a series of digging cycles, identical in respect of sequence of activities with those observed when burrowing into the

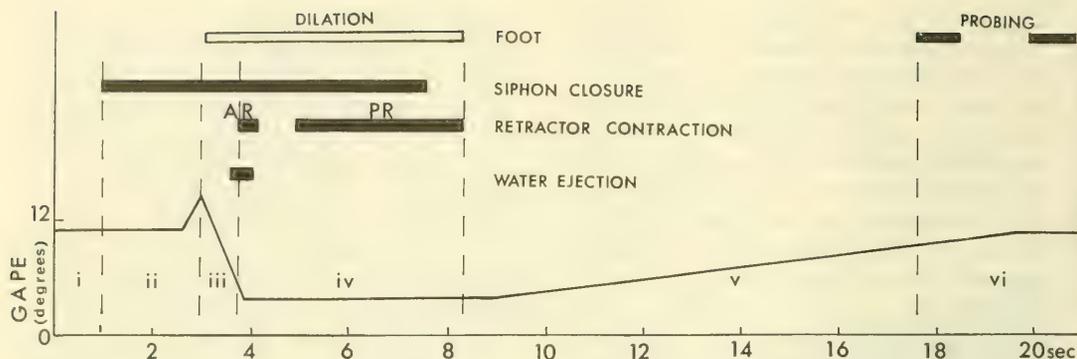


FIG. 2. Diagram of the analysis of a single digging cycle of *Margaritifera margaritifera* from film and recordings. The change of gape of the valves is taken from a single cycle when the bivalve was moving over the surface of the sand and the probing action of the foot (■), its period of maximum dilation (□), the period of closure of the siphons, the contraction of the retractor muscles (AR, anterior; PR, posterior), and the period of water ejection are placed in the correct time sequence by observation of many digging cycles. The stages of the cycle (i-vi) are indicated. See text below for further information.

sand, at intervals of about 1 1/2 minutes. Each cycle gave a forward movement of about 1/2 cm (in a specimen of 10 cm length). This surface locomotion continued, sometimes rather spasmodically, until the clam began to dig deeply. There was no apparent stimulation or other explanation for this change in behaviour, but in the natural habitat locomotion over the surface of the sand, after being dislodged from the buried position, may be the means of finding more suitable conditions.

#### EXPERIMENTAL RECORDINGS

The detail of movements of *Margaritifera* during the digging cycle have been elucidated by means of impedance and pressure recordings and analysis of film taken of complete digging cycles both from lateral and frontal aspects. The results, summarised in Fig. 2, indicate that the digging cycle is a closely integrated series of movements of different regions of the body. This series is the same for all digging cycles of *Margaritifera* and resembles those found in other species of bivalves, e.g. *Glycymeris*, *Mercenaria*, *Ensis*, *Tellina*, *Donax* (Trueman, 1966; Trueman et al.,

1966a). The cycle, which is best understood by reference to Fig. 2, comprises the following 6 stages from left to right:

(i) The foot makes a major probe downwards tending to raise the shell if pedal penetration is not easily achieved. This probe occurs in many bivalves, e.g. *Tellina*, *Macra* and *Ensis* (Trueman, 1966) and, although it has not been observed in *Margaritifera*, the stage is marked in the figure. Pedal probing appears to cease for approximately 5 seconds before adduction of the valves.

(ii) Siphons close, preventing water passing out through their apertures during the next 2 stages.

(iii) Adduction of the valves occurs rapidly within 0.25 seconds and corresponds to the onset of pedal dilation and the ejection of water from the mantle cavity. Pressure recordings of *Margaritifera* and other genera, e.g. *Ensis*, *Donax*, show that these are respectively brought about at adduction by the increase of fluid pressure in the haemocoel and in the mantle cavity (Trueman, 1966). Dilation of the distal part of the foot secures the pedal anchorage.

(iv) Contraction of the pedal retractor muscles, that of the anterior being followed by the posterior, imparts a

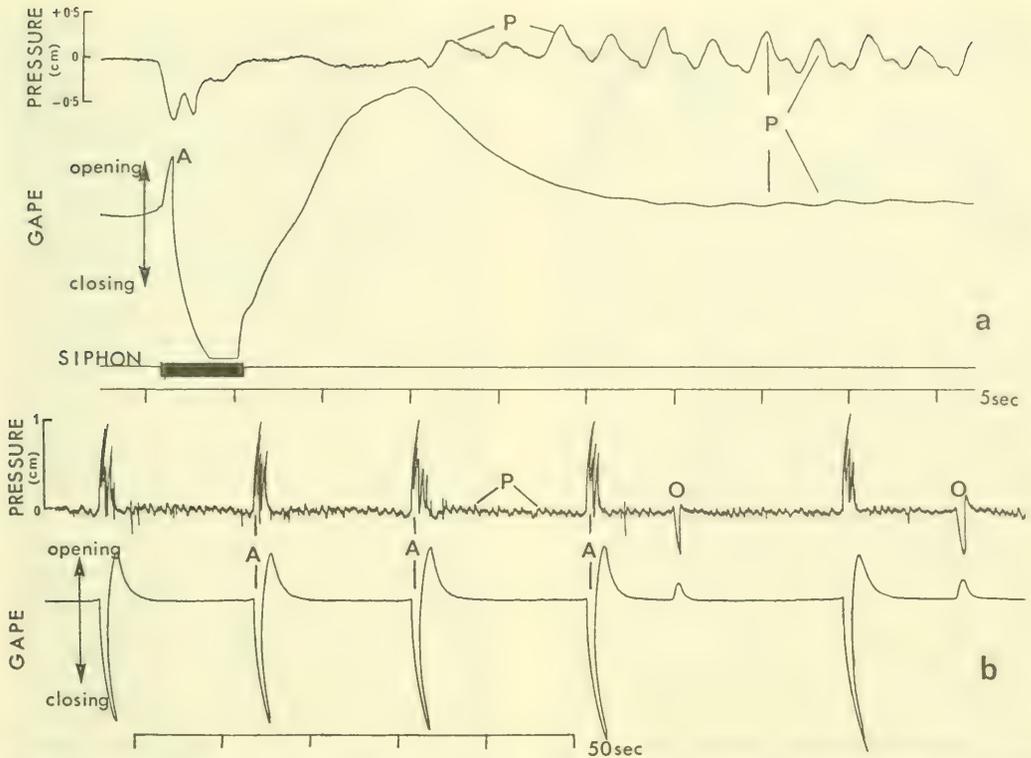


FIG. 3. Recordings of the pressure changes (upper records) in the sand and valve movements (Gape, lower records) during (a) locomotion over the surface of the sand and (b) burrowing into sand with 1/3 the shell under the surface. A pressure transducer is connected by tubing to near the foot of the *Margaritifera* beneath the sand, and the valve movements are recorded by means of electrodes attached to the valves connected to an impedance pneumograph, which was coupled (A. C.) to a pen recorder.

a: shows adduction at A, corresponding to negative pressure in the sand caused by dilation and retraction of the foot. Successive pedal probes (P) commence after the valves have reopened, the thick line indicates period of closure of the siphons.

b: with the specimen more deeply buried, negative pressure at adduction (A) becomes positive, due to the expulsion of water into the sand and many pedal probes (P) are recorded between the adductions of the valves. Negative pressure during the period of probing (O) is due to the retraction of the foot associated with the further opening of the valves. Further information in the text below.

rocking motion to the valves as indicated by the arrows (A and P) on Fig. 1. This sequence of contractions results in movement along the surface or into the sand according to their relative magnitude and the orientation of the foot. During this phase of pedal retraction the siphons reopen and pedal dilation is reduced.

(v) Relaxation of the adductor muscles, slow reopening of the valves, together

with loss of pedal anchorage.

(vi) Pedal probing recommences and continues until the next cycle. This stage has been previously termed the 'static period' (Trueman et al., 1966a) since the shell does not move except in response to the downward pressure of the foot during probing.

Recordings of digging cycles when the clam was either on the surface or partially under the sand (Fig. 3a, b) show

negative or positive pressure peaks (A) according to the depth of burial at stages iii and iv. The latter are probably caused by the ejection of water from the mantle cavity into the sand, whereas, when the animal is on the surface, this ejection is much more superficial so that the effect of pedal dilation is then seen as a negative pressure. The effect of water ejection is to loosen the sand in front of the shell immediately before pedal retraction takes place so as to facilitate shell movement (C, Fig. 1b). This loosening extends to the upper part of the foot as indicated by the arrows, but it does not affect its lower part, which must act as an anchor (Fig. 1, hatching). Work on *Margaritifera* and other bivalves (Trueman, 1966; Trueman et al., 1966a) has demonstrated that pedal anchorage is obtained by the dilation of the foot brought about by a sudden increase in blood pressure in the pedal haemocoel that is derived from adduction of the valves. This pressure and anchorage are sustained by pedal retraction. Without an anchorage the foot would be pulled into the shell on contraction of the retractor muscles instead of the shell being pulled down. It is important that the loosening of the sand should only extend around the proximal part of the foot and not affect the pedal anchorage.

When *Margaritifera* is moving over the surface, the valves show much more movement than when it is beneath the sand, which has a damping effect. The movements are noticeable when probing (P, Fig. 3a) and immediately prior to adduction (A). A similar sharp increase in gape has been observed in other bivalves at the surface of the substrate, e.g. in *Cardium*, and may possibly be due to the relaxation of the 'slow' adductor muscle fibres before the 'fast' fibres contract. When the clam was buried more deeply, the surrounding sand prevented this additional gape.

The cycle represented in Fig. 2 is of a *Margaritifera* moving over the surface of the sand and, apart from the relative

magnitude of anterior and posterior retraction, only differs materially from deeper burrowing by the increasing duration of the digging cycle with depth (Fig. 3b). Digging involves a greater interval between successive adductions, a longer static period, and consequently more probes by the foot. The longer time per cycle is probably related to the increasing difficulty that the foot may have to penetrate the substrate at greater depth (Trueman, Brand & Davis, 1966b). The rapid succession of probes (P) being made by the foot is clearly shown in Fig. 3a, where they may be observed to recommence only after the reopening of the valves. When burrowing on or near the surface, reopening occurs in about 10 seconds, but after about 1/2 of the shell is beneath the sand a secondary opening movement (O) of the valves is necessary to achieve the full gape. Direct observations of this secondary opening movement indicate that it coincides with the closure of the siphons and the retraction of the foot. The negative pressure recorded (Fig. 3b) is probably caused by the withdrawal of the foot. Similar observations made on *Mercenaria* (Ansell & Trueman, 1967) suggest that the contraction of the pedal retractor muscles increases the hydrostatic pressure in the haemocoel and that this pressure contributes to forcing the valves open.

The function of the hinge ligament is to open the valves when the adductor muscles relax. When moving over the surface, the strength of the ligament (opening moment, Trueman, 1964) is sufficient to open the valves adequately; but, as the shell lies progressively deeper and the resistance to the opening of the valves becomes greater, the ligament requires supplementation.

It appeared that the ligament ceases to be adequate for the full opening of the valves when more than 1/3 of the shell is buried in fine sand. Determinations of the opening moment of the ligament, using the method described by Trueman (1954) show that a moment of

27,600 g mm is available to open the valves of a *Margaritifera* 9.4 cm in length. This force is equivalent to a moment of 8.8 g mm/mm<sup>2</sup> in relation to the projected surface area of a valve. Since the ligament is inadequate for the opening of the valves when more than 1/3 is buried, the resistance of fine sand to opening is approximately 3 times this figure (26 g mm/mm<sup>2</sup>). The shell of *Mya* was used to determine the resistance of marine substrates to the opening of the valves (Trueman, 1954) and it was shown that in fine sand the application of a moment of 20 g mm/mm<sup>2</sup> would produce a gape of about 4°. Allowing that *Mya* is more deeply buried than *Margaritifera*, these figures are comparable and suggest that the latter species may well need to supplement the ligament when more than 1/3 of the shell is buried. The additional hydrostatic pressure would probably not be required if the valves reopened more rapidly after adduction, while the sand was still loosened by the water ejected from the mantle cavity, as occurs in *Tellina* and *Ensis* (Trueman et al., 1966a).

#### DISCUSSION

The digging cycles consist essentially of the repeated adduction and reopening of the valves, integrated with the retraction and protraction of the foot. Adduction accomplishes 3 things: a) pedal anchorage, by dilation of the distal part of the foot; b) water ejection from the mantle cavity, thus loosening the sand adjacent to the shell; c) reduction in effective width of the shell (Fig. 4a). Functions b and c both facilitate movement of the shell into the substrate at retraction but a pedal anchor (P) is an essential prerequisite. After retraction the foot must be protracted before a further digging cycle can take place. Protraction is achieved during the static period (vi) largely by the intrinsic pedal musculature; it involves the contraction of the transverse pedal muscles and the relaxation of the retractor muscles (TM,

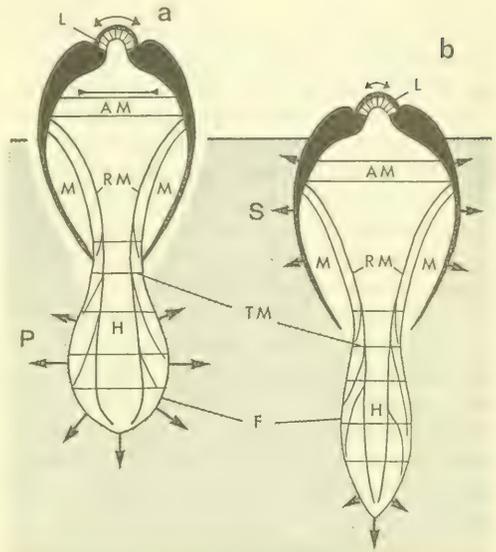


FIG. 4. Diagrammatic transverse sections of a bivalve during burrowing into sand (stipple) to show the conditions (a) of pedal anchorage (P) after adduction and before retraction (stages iii-iv) and (b) of shell anchorage (S) during static period (vi). In (a) pedal anchorage is established by the dilation of the foot (F; arrows) due to the increase of haemocoel (H) pressure at adduction (▶◀) by tension in adductor muscles (AM). Adduction also causes the loosening of the sand adjacent to the valves (unstippled area) and places the ligament under maximal strain (◀▶). In (b) the flattened foot probes downwards (arrows), the adductors (AM) are relaxed, and the ligament (L) presses the valves (black) open against the adjacent sand (arrows). M, mantle cavity; RM, retractor muscles; TM, transverse pedal muscles.

Fig. 4) in a manner similar to that described for *Ensis* and for members of the Tellinacea (Morton, 1964; Trueman et al., 1966a). When the shell is opened by the ligament, it is pressed against the sand forming a shell or secondary anchorage (S, Fig. 4b). This anchorage

tends to prevent the animal from being pushed upwards as the foot pushes down. Drew (1907) and Pohlo (1963) have previously observed that the valves of members of the Solenidae may grip the walls of the burrow during pedal protraction. The force with which the foot can probe is a function of the effectiveness of the shell anchorage, which in turn is related to the strength of the ligament. Probing forces in excess of the secondary anchorage cause the shell to be pushed upwards during probing as has been demonstrated in *Cardium edule* (Trueman et al., 1966a, b). When *Margaritifera* moves over the surface of the sand there can be no shell anchorage and the strength of probing is limited to the weight of the animal.

Whilst discussing the burrowing of worms, Clark (1964: 93) suggests that the fundamental method of burrowing used by all soft-bodied animals is the same. Part of the bodywall is dilated to form an anchor while the head is forced into the substrate by contraction of the circular muscles. The anterior end of the worm then dilates forming a new anchor while the body is drawn downwards by the contraction of the longitudinal muscles. These 2 anchorages correspond to the shell and pedal anchors of bivalves respectively, while the circular muscles are represented by the transverse pedal muscles and the longitudinal muscles by the retractors. Thus the burrowing movements of a bivalve conform to Clark's description. Bivalves have the advantage, however, of an additional fluid muscle system, the pallial system, by means of which powerful water jets assist penetration.

Bivalvia are primitively adapted to shallow burrowing in soft, often unstable, substrates (Morton, 1964). An important adaptation to a burrowing mode of life is the bivalved shell and the fluid-muscle system of the foot which permits the strength of adduction to be used in digging to anchor the foot. The pattern of the digging cycle is similar in all genera in which burrowing has

been examined by the use of modern recording techniques (Trueman, 1966), and is retained by *Margaritifera* when moving over the surface of sand. Those bivalves, which have changed from the primitive infaunal to an epifaunal mode of life and can progress over a hard substrate, retain the rhythm of extension and foreshortening of the foot (Morton, 1964). Observations on the surface locomotion of *Mytilus* (by the author) and of *Lasaea* (Morton, 1960) indicate, however, that pedal movement in those genera is carried out without adduction of the valves. Extension of the foot involves only the intrinsic musculature, as does pedal probing during digging, and retraction is not preceded by dilation since pedal anchorage is obtained in a different manner on a hard substrate. It would appear from the present work that elimination of adduction from the locomotory cycle may be a consequence of movement over a hard rather than a soft substrate. Further detailed observations of the locomotion of bivalves over hard surfaces, using the methods described in this article, would be of interest.

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## RESUMEN

LA LOCOMOCION DE LA ALMEJA DE AGUA DULCE  
*MARGARITIFERA MARGARITIFERA*  
 (UNIONACEA: MARGARITIFERIDAE)

E. R. Trueman

Esta investigación se efectuó mediante registros electrónicos, y el comportamiento de *Margaritifera margaritifera* (L.) se describe con breve referencia a el efecto que la forma de las valvas ejerce en la actividad excavadora (por ejemplo, la presencia de una pronunciada brecha pedal en formas de aguas rápidas). La serie de funciones llamada "ciclo excavador" es muy similar a las de otros bivalvos (sus movimientos sirven tanto para la excavación propiamente dicha como para la locomoción sobre la superficie arenosa), y las diferencias más obvias se presentan en la orientación, más anterior, del pié y la magnitud relativa de contracción de los músculos retractores, anteriores y posteriores.

Durante el ciclo excavador, las valvas se contraen para que la arena circundante quede más suelta en el agua, y obtener así anclaje pedal dilatando el pié por presión circulatoria. Cuando la almeja está sobre la arena las valvas se entreabren sólo por acción del ligamento, pero cuando están enterradas hasta un tercio, entonces la presión de la retracción pedal reemplaza a la del ligamento. La actividad excavadora se integra con el entreabrir y cerrar de las valvas y protracción y retracción del pié. La aducción produce un anclaje pedal, permitiendo así a la concha ser arrastrada en la retracción, hacia abajo, mientras está firme en la arena por el empuje apertural del ligamento durante la protracción pedal.

Bivalvos que viven en substratos duros tienen básicamente una locomoción similar, pero sin que se produzca aducción, posiblemente porque el anclaje pedal no se obtiene en la misma forma.

## АБСТРАКТ

ДВИЖЕНИЕ ПРЭСНОВОДНЫХ МОЛЛЮСКОВ *MARGARITIFERA MARGARITIFERA*  
(UNIONACEA: MARGARITANIDAE)

Е. Р. ТРУМЕН

Движение у *Margaritifera margaritifera* (L.) исследовалось при помощи электронных самописцев; в работе рассматривается также влияние формы раковины моллюска на его закапывание (при этом имеется ввиду наличие выдающегося ножного выступа у форм, обитающих в водах с быстрым движением). Ряд движений моллюска при его зарывании, названные автором "циклом закапывания", в общем сходны с теми движениями, которые производят другие двустворчатые как при закапывании, так и при движении по поверхности песчаного грунта. Наибольшие различия заключаются в положении ноги, направленной больше вперед и в относительной силе сокращения переднего и заднего мускулов-ретракторов.

В течение цикла закапывания створки смыкаются, чтобы освободиться от окружающего песка и чтобы образовать заякоривание моллюска при помощи расширения его ноги благодаря увеличению давления крови в ней. Если моллюск находится на поверхности грунта, то его створки открываются исключительно при помощи лигамента; но когда более 1/3 раковины уже погрузилось в грунт, давление, полученное при помощи сокращения ноги служит для усиления действия лигамента. Акт закапывания включает совместное действие смыкания и размыкания створок и вытягивание и сокращение ноги. Смыкание створок создает заякоривание при помощи ноги, позволяющее раковине быть втянутой вниз при сокращении, в то время как раковина крепко удерживается в песке при открывающем действии лигамента при вытягивании ноги (вторичное или раковинное заякоривание).

Двустворчатые, живущие на жестком грунте имеют сходные характеристики движения, но без смыкания створок, вследствие чего ножное заякоривание происходит иначе.

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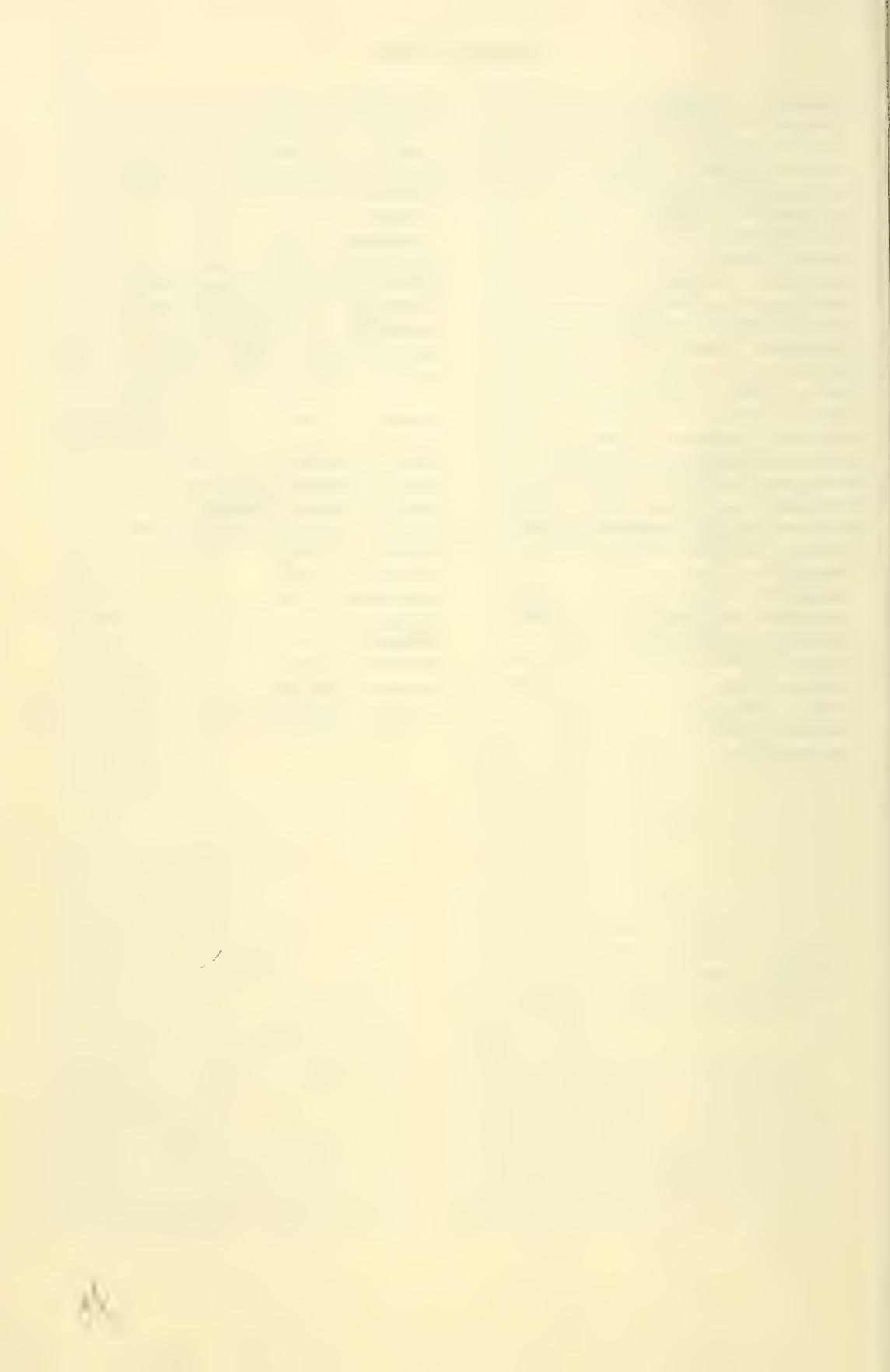
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