ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 41 • 1956 • NUMBERS 1 TO 19

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

Part 1. April 23, 1956

Part 2. September 17, 1956

Part 3. November 23, 1956

Part 4. December 31, 1956

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 41 • PART 1 • APRIL 23, 1956 • NUMBERS 1 TO 6

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

1

The Embryological Origin of Pigment Cells in Platyfish-Swordtail Hybrids[1]

Douglas G. Humm & Richard S. Young

Department of Zoology, University of North Carolina, Chapel Hill, N. C.

(Plates I & II; Text-figure 1)

Introduction

THE first evidence concerning the embryological origin of pigment cells in vertebrates was supplied by Borcea (1909) in observations made on the development of several species of marine fishes. He asserted that teleost chromatophores developed from ectodermal cells in the dorsal portion of the nervous system. Later, Weidenreich (1912) discussed the ontogeny and phylogeny of vertebrate pigmentation and suggested that a positive correlation existed between the central nervous system and the primary distribution of pigment cells in animals representing several vertebrate classes.

DuShane (1935), who worked with salamander embryos, was the first to demonstrate experimentally in any vertebrate that the primary pigmentation was derived from cells of the neural crest which arose in close association with the nervous system. Likewise Dorris (1938, 1939) showed, by transplanting neural crest tissue from chicken embryos of a black strain to embryos of a white strain, that feather pigmentation in these animals was produced by cells that arose from the neural crest. Ris (1941) extended this observation and showed that the neural crest cells were the only source of pigmentation in birds.

The embryology of pigment cells in fishes differs somewhat from those forms already mentioned, in that fishes do not possess a group of cells that arises as does the neural crest in the amphibian and the chick. In fishes the nervous system arises as a solid rod and becomes hollow by cavitation. The cells in fishes that would be analogous to the neural crest are found in and along the length of the dorsal portion of the nervous system. These cells might be expected to be determined as pro-pigment cells (or melanoblasts) during the formation of the nerve cord and to migrate at a later stage. They transform first into melanocytes and then melanophores.

Lopashov (1944) investigated the origin of pigment cells in the perch (*Perca*) and two species of loach, (*Misgurnus* and *Nemacheilus*). Pieces of nervous system, when transplanted under the yolk sac epithelium of another embryo, gave rise to pigment cells. In most cases mesoderm alone failed to produce pigment cells. Similarly Newth (1951), who investigated the derivatives of the neural tissue in the lamprey (*Lampetra planeri*), showed that melanophores arose from this source.

It has long been considered possible that the etiology of pigmented tumors in mammals might be traced back to neural crest cells. There is evidence indicative of this. For example, Rawles (1953) showed that the pigment cells of mice have a neural crest origin. Neural crest cells, at least that portion represented by the pro-pigment cells, are intensively migratory, moving to all portions of the embryos, along almost any cell surface. Similarly, the melanoma has been recognized as one of the most readily metastasizing atypical growths (Raven, 1953), and this was believed to be due to an inherent tendency of tumorous melanocytes to become detached, singly or in groups, and escape into the blood and lymph system. The capacity to develop melanin is another characteristic that has been recognized as being possessed in common by neural crest cells and melanoma cells. Since melanomas have been studied metabolically (Greenstein & Algire, 1944; Greenstein, Werne,

[1] This research was supported in part by grants from the Damon Runyon Memorial Cancer Fund and grant #1717 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

Eschenbrenner & Lewthardt, 1944; Burke, Algire, Hesselbach, Fischer & Legallais, 1948; DuBuy, Woods, Burke & Lackey, 1949; Humm & Clark, 1955) with respect to enzyme activity patterns, direct evidence indicating the metabolic similarity between melanoma and neural crest cells would also be highly desirable. Technical difficulties, however, have stood in the way. A significant contribution to this problem has been made by Flickinger (1949), who studied the metabolism of the neural crest of salamanders and was able to observe tyrosinase activity in these cells.

An ideal approach to a study of the total etiology of the melanotic tumor would be achieved were it possible to characterize a unique type of cell in the tumor of an adult whose embryo lends itself to microsurgical technique, and to trace this cell type back to its site of origin in the embryo. Regrettably, no melanomas are known in urodeles or chickens, although one was reported in a salamander and then lost (Sheremetieva-Brunst & Brunst, 1948). Fortunately melanoma may be produced at will in platyfish-swordtail hybrids, and it has been shown by Gordon (1937) that the melanosis and subsequent melanoma are always associated with macromelanophores. These unique cells are easily identified.

Gordon, in a series of papers which he reviewed in 1951, showed that the black pigmentation of the platyfish, *Xiphophorus maculatus,* is the result of the distribution of two different types of melanophores. Gordon (1927) showed that the first, and by far the most abundant of the platyfish's pigment cells are the *micromelanophores,* which are fairly heavily pigmented, highly dendritic and almost round in shape, with a diameter up to 100μ. These cells appear to have little or nothing to do with the production of melanomas. Micromelanophores are under the control of an autosomal gene. The second type of melanophore found in *X. maculatus* is also heavily pigmented and highly dendritic, but differs from the first type in size. These *macromelanophores* range from 100 to 300μ. Their presence, distribution and pattern of growth in the fish have been shown by Gordon (1948) to be under the control of a series of sex-linked genes.

The background color of the wild-type swordtail, *Xiphophorus helleri,* as in *X. maculatus,* is produced by many micromelanophores.

If a hybrid is produced by mating a swordtail with a platyfish that carries the sex-linked gene, *Sd* (for macromelanophore-spotting of the dorsal fin), the genic balance controlling the growth and differentiation of macromelanophores in the dorsal fin of the hybrid is disturbed. The result is a heavy overgrowth of these large black cells in the hybrid. The eventual result of such hypertrophic macromelanophore growth in these fish, as recently retraced by Ermin & Gordon (1955), is the gradual appearance of a distinctly invasive pigmented tumor.

Since the macromelanophore must be present before a hybrid fish can develop a melanoma (Gordon, 1937), this cell type represents an identifiable and essential component of the tumor. Therefore the embryological origin of the *macromelanophores* becomes of particular importance in a study of the etiology of melanomas.

The purpose of this study is to determine whether or not the potentially tumor-producing macromelanophores arise in the embryo of these hybrid fish from the same embryonic anlage as do the normal pigment cells. If it were possible to show that these cells arise as derivatives of the anterior-dorsal nervous system, and that these cells give rise first to melanosis and then to melanoma, this would constitute clear-cut experimental support for the neural origin of melanoma in at least one type of vertebrate.

The present experiments were designed to test the effect of transplanting presumptive melanoma-producing tissue of platyfish-swordtail hybrids into swordtail hosts which, although possessing genetic growth intensifier factors, could not develop melanomas themselves. The experiments reported in this paper verify Lopashov (1944) as to the neural site of pigment cell production in teleost fishes, and they show that both micromelanophores and macromelanophores appear when the dorsal portion of the nerve cord of a hybrid fish is grafted into a swordtail embryo.

Materials and Methods

The fish used in these experiments were obtained through the courtesy of Dr. Myron Gordon from the Genetics Laboratory of the New York Aquarium, New York Zoological Society. Platyfish (*Xiphophorus maculatus*), bearing the dominant sex-linked gene (*Sd*), for the occurrence of macromelanophores in the dorsal fin, in the homozygous condition, were crossed with recessive wild-type swordtails (*Xiphophorus helleri*). The swordtail carries certain modifying genes which act as intensifiers of macromelanophore growth (Gordon, 1937). The F_1 offspring were all heterozygous for the gene *Sd,* and all of them showed considerable melanosis in the dorsal fin.

The heterozygous melanotic F_1 hybrids (*Sd*+) were then back-crossed to recessive wild-type swordtails (++) and as a result, 50% of the backcross offspring had the macromelanophore

Sd gene; half were normal, homozygous recessives and had micromelanophores only. Those bearing the *Sd* gene showed advanced melanosis as small adults, owing to the atypical growth of macromelanophores. Many of them died from the invasive effects of the melanoma before becoming mature. Since the platyfish, the swordtails and their hybrids all have large numbers of micromelanophores uniformly distributed on the body surface, all donor and host embryos would be expected to produce micromelanophores during their development.

In accord with observations made by Hopper (1943) and Tavolga (1949) on the embrology of platyfish, swordtails and their hybrids, the timing of the stage of the embryos used was accomplished at first by making the assumption that the new complement of eggs in the female is fertilized and begins development seven days after the last brood of young are born. This assumption was experimentally verified in this laboratory. The normal embryological stages described by Tavolga (1949) have been used as a guide in the selection of appropriate stages of donor and host embryos.

The donor embryos used were backcross offspring obtained from the mating of *Sd* platyfish-swordtail hybrids back to wild-type swordtails, as described above. The most frequently used embryo donor stage, Tavolga Stage 10, may be summarized as follows: The blastopore is still open, although becoming narrow, 2 to 4 somites have made their appearance, the extra-embryonic membranes have grown posteriorly and are approaching the otic capsule.

The swordtail embryos which were used as hosts in the experiments were selected at Stage 20 for several reasons. At this stage the embryo is large enough to work with easily, but it has not yet become too active. Accordingly, it is somewhat easier to prepare the graft site and introduce the tissue. Also, at this stage the normal host pigmentation is confined to the retinal pigment and a few plate-like pigment cells in the cranial region, apparently on the meninges, as illustrated by Gordon (1931, fig. 37). The typical pigment cells are quite small, bi- or tri-polar cells and are oriented in the direction of their migration to the rear. One receives the distinct impression that the melanocytes advance from the head region posteriorly, since a mechanical obstacle will often appreciably delay pigmentation on the caudal side of an obstruction. Because of this delay in movement along the anterior-posterior pathway, it is possible to detect the early-appearing pigment cells on the graft before the host's pigment cells become too abundant to obscure the site of the graft. (Plate I, Figs. 1 and 2).

The grafting was carried out in Columbia watch glasses containing sterile 2% agar in which a slight depression had been made to hold the embryos. Donor embryos were removed from the yolk and carefully divided into pieces about 0.8 cu. mm. In order to avoid confusion concerning the origin of the graft, each embryo fragment was placed in a separate watch glass until used.

Host embryos were removed from the ovary of a gravid female under sterile conditions, washed 6 times with 3× Niu's (1954) solution[2], decapsulated, washed twice more and left in 3× Niu's solution until used. Decapsulation or removal of the chorion in these embryos is quite simple. Apparently the capsule is osmotically active since, when an embryo is placed in 3× Niu solution for a very short time, the capsule lifts sufficiently from the yolk to permit the insertion of the points of well sharpened Dumont watch-maker's forceps. Grasping the membrane over the embryo with a second pair of forceps breaks the chorion and the embryo comes out of the membrane with considerable force.

The operation on the host embryo was carried out using glass needles. A notch was cut in the back in the region anterior to the dorsal fin. Every precaution was taken to avoid injuring the nervous system and the underlying notochord. As fast as possible after the host had been prepared, the donor tissue from a platyfish-swordtail hybrid was picked up on the point of the glass needle and laid on the wound. No bridges were needed since the donor tissue was usually somewhat sticky, and when the two areas were contacted, attachment was instantaneous and healing very rapid. The watch glass was usually set aside for 5 to 10 minutes so that healing was well advanced before the embryos were transferred, by means of a wide mouth pipette, to a Stender dish containing 3× Niu's solution.

Maintainance of the experimental animals proved to be difficult. The early xiphophorin embryo develops a heavily vascularized serosa with which, it is presumed, it receives oxygen and nutrient from the mother and through which it eliminates waste. Scrimshaw (1944a, b, 1945) has shown that the xiphophorin embryo does not lose weight during its development, indicating that it is truly viviparous. It evidently obtains materials from the maternal circulation that are at least equivalent in weight to what is

[2] Niu's Solution (3×): Solutions A and B mixed 1:1.

A		B	
NaCl	–10.2 gm.	Na_2HPO_4	–0.33 gm.
KCl	– 0.15	KH_2PO_4	–0.06
$Ca(NO_3)_2 . 4H_2O$	– 0.24		
$MgSO_4$	– 0.30		

lost through metabolic end-products. Moreover, the presence of the serosa makes the removed embryo particularly vulnerable to differences in osmotic pressure and to traces of deleterious substances in the medium.

It was found that 3× Niu's solution was isosmotic with the embryonic circulation, but survival of the embryos in sufficient numbers to make the xiphophorin fish embryo a good experimental animal was not achieved until "conditioned" tank water, that is, water in which adult fish had lived for a time, was substituted for the double glass-distilled water. The results of this substitution were truly dramatic. For example, in 3× Niu's solution in double glass-distilled water that had been sterilized by autoclaving, a mortality of 50% after 4 days and 100% before 10 days was the rule. In Niu's solution made with "conditioned" tank water and sterilized by Seitz filtration, the post-operative mortality fell to zero. More than 80% of the embryos were successfully hatched and grown into young fish.

Tavolga & Rugh (1947) suspected that the pericardial serosa had a nutritive and excretory function in addition to the respiratory function suggested by Turner (1937). With this in mind, the post-operative embryos were placed in a medium of Niu's solution to which had been added 25 mgm% of glucose, 25 mgm% of casein hydrolysate, and a 2% solution of embryonic extract. Penicillin (100 units/100ml.) and streptomycin (20 mgm./100 ml.) were added as antibacterial agents. The air above the nutrient medium was replaced by acid-washed oxygen. The results of the added nutrient were not spectacular, although they were sufficiently marked to warrant the inclusion of these additions as part of the standard treatment. Over a period of two weeks the embryos' development in glucose, casein hydrolysate and embryonic extract was accelerated about one to two days over untreated controls.

Hatched young fish were acclimatized to "conditioned" aquarium water by progressive dilutions of 3× Niu's solution. It was found advisable to delay acclimatization to tank water until the yolk sac had almost disappeared. Apparently the intact yolk sac and yolk were in equilibrium with a concentration of 3× Niu's solution, and any decrease in this concentration before their resorption resulted in the appearance of fluid in the sac.

As soon as the yolk material was closed over by the body wall, the small fish were transferred to a finger bowl containing tank water and fed *Paramecium* and *Daphnia* until they were large enough to accept *Enchytraeus* and prepared fish food .

Results

The results reported here are concerned with the embryonic source of micro- and macromelanophores in the potentially melanomatous platyfish-swordtail hybrid (Text-figure 1). The possible effect of any of the modifying growth-stimulating factors in the normal swordtail host tissues as well as the possibility of inducing melanoma development in the host fish as a result of grafting neural tissue fragments will be considered in subsequent publications.

Origin of Macromelanophores

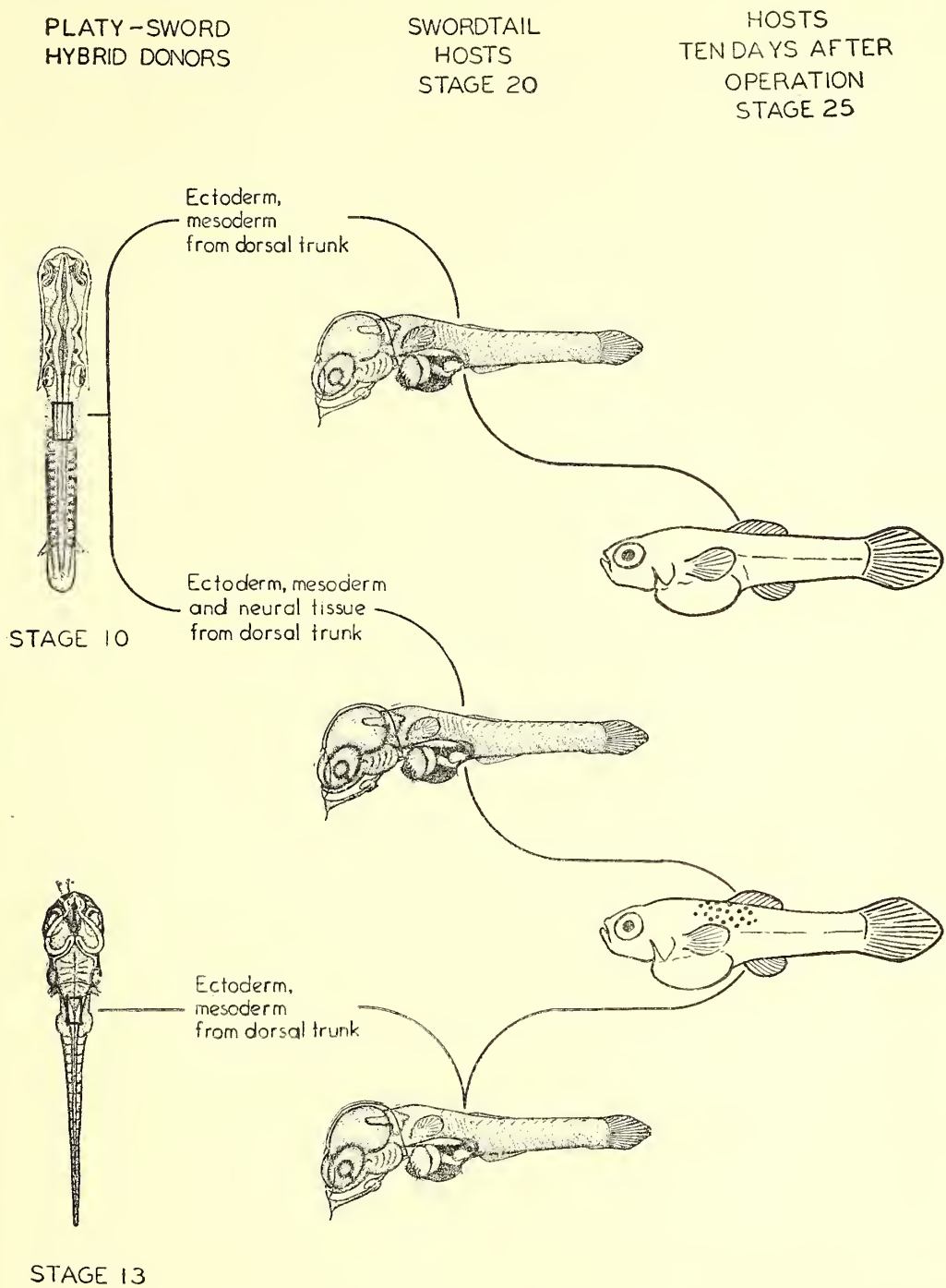
In two cases it was possible to demonstrate macromelanophores in the graft region of a host swordtail embryo (see Plate II, Figs. 3 & 4). The graft tissue had been taken from one *Sd* platyfish-swordtail hybrid from the trunk region just posterior to the hindbrain. From another *Sd* hybrid the tissue was taken from the same region just anterior to the dorsal fin. Both grafts from Stage 10 donors contained ectoderm, somite mesoderm and a portion of the nervous system.

The normal time for the appearance of macromelanophores in platyfish hybrids is considerably later than for the appearance of micromelanophores (Tavolga, 1949). Since at the time these experiments were performed, some difficulty was encountered in maintaining the embryos over long periods of time, many of the embryos unquestionably died before differentiation of macromelanophores could take place. Since macromelanophores do not occur in the genetic strain of swordtail employed by us, the presence of large pigment cells in these two swordtail embryo hosts indicates that the macromelanophores originated from the grafted tissue. They were definitely not normal host swordtail micromelanophores.

Origin of Micromelanophores

A word of explanation is required regarding the method used in making a decision for or against small pigment cell formation in a particular graft. The pigment cells of the swordtail host embryo appear first in the neck region, then rapidly populate the head and finally spread as an advancing wave down the trunk to cover the whole animal. Under the conditions used in these experiments, there was no way to distinguish directly between host and donor micromelanophores, except in those cases where the donor tissue also differentiated into macromelanophores. Accordingly the following criteria for identfication of donor melanophores were established and adhered to:

1. If, prior to the appearance of any host melanophores in the region of the graft, mel-



TEXT-FIG. 1. Diagrammatic summary of the grafting experiments showing the effect of the age of donor tissue and the germ layers used in the production of melanophores. (Drawings of embryo after Tavolga). Donor tissue from various regions of Stage 10 hybrid embryos was isolated by means of glass needles and transferred by means of a sterile mouth pipette to an agar-lined dish containing the host embryo. A slit was prepared in the host epidermis in the dorsal region just anterior to the dorsal fin region and the graft was quickly inserted. Stage 10 donor tissue containing ectoderm, mesoderm and neural tube gave rise to both micro- and macromelanophores; when neural tube was lacking, pigment was not produced. At a later stage, ectoderm and mesoderm gave rise to pigment cells in grafts.

anophores appeared in the host at the site of the graft, these cases were regarded as positive.

2. If, after the host melanophores had populated the graft area, pigment cells appeared *inside* the graft and upon sectioning could be shown to be within the graft, these also were regarded as of donor origin.

3. Any graft which became populated with melanophores at about the same time the host cells migrated into the grafted region was considered negative, since by observation it was not possible to distinguish between host and donor micromelanophores.

1. *Grafts Containing Neural Tissue.*—In the first series of operations, totalling 25 animals, the tissue obtained from the region of the first somite of the hybrid donor embryo at Stage 10 contained ectoderm, somite mesoderm and part of the nerve cord (see Table 1). In 17 out of 25 swordtail hosts, micromelanophores were present in the graft, with one case in doubt. The seven remaining cases were regarded as negative although these, too, may actually represent grafts which differentiated their hybrid donor melanophores too late to be clearly distinguished from host pigment cells.

In addition to pigment cells, the grafted tissue in this series was found to contain well differentiated muscle and nerve tissue. In some of the embryos that were permitted to develop for a longer period of time, cartilagenous skeletal elements were found, Plate II, Fig. 4.

2. *Grafts without Nervous Tissue.*—In the second series of 14 grafted embryos, the donor tissue from a platyfish-swordtail hybrid was taken with particular care to exclude nervous tissue. This was done with iridectomy scissors by first removing from a Stage 10 donor a piece of tissue that usually contained ectoderm, somite mesoderm and neural tissue. The neural tissue was then carefully dissected away by means of glass needles and discarded. The remaining piece was trimmed to an appropriate size and implanted in a swordtail host embryo. Of the 14 experiments done in this way, 3 host embryos gave rise to micromelanophores of donor origin. In 11 host embryos, no donor pigment cells appeared before host pigment cells had surrounded and covered the graft area.

3. *Time of Migration.*—The last series of experiments was carried out using tissue from platyfish-swordtail embryo donors at Stage 13. Grafts containing ectoderm and mesoderm were placed dorsally in Stage 20 swordtail embryo hosts. Micromelanophores appeared in the graft in 7 of the 8 swordtail embryos, while one embryo failed to produce pigment cells. These experiments suggest that during the period between Stage 10 and Stage 13 (from 3½ to 7 days; or from the 13th somite to the 25th somite stage), the pro-pigment cells have migrated from the point of origin in the nervous system and have moved into the more superficial tissues.

Discussion

The evidence presented in this paper confirms the observations of Lopashov (1944), who found that in three species of teleost fishes black pigment cells appeared in the host after pieces of brain were grafted under the epithelium of the yolk sac. When grafts of ectoderm and mesoderm, with and without neural tube, from platyfish-swordtail embryos in comparable stages of development to those used by Lopashov, were

Table 1. Appearance of Pigment Cells in Transplants of Various Tissues from Swordtail-Platyfish Hybrid Embryos into Swordtail Embryos[1]

Donor Stage	Donor Tissue	Number of Cases	Pigment Cells Found in Graft in Host[2]	No Pigment Cells in Graft in Host[2]
10	Neural Tube Ectoderm Mesoderm	25	17 1 doubtful	7
10	Ectoderm Mesoderm	14	3	11
13	Ectoderm Mesoderm	8	7	1

[1] For criteria see text, pages 4 and 6.
[2] Swordtail embryo Stage 22.

implanted into swordtail embryos, good evidence was found that the presence of neural tissue is required for the differentiation of black pigment cells. Lopashov was also able to show that the introduction of mesoderm alone in most cases did not lead to the production of pigment cells. Although one series of experiments reported in this paper indicated that pro-pigment cells were present in the mesoderm, this was undoubtedly due to the fact that the donors used for these grafts were somewhat older than those used for the other experiments, or those used by Lopashov. Accordingly, it was concluded that between Tavolga Stages 10 and 13, the migration of pro-pigment cells probably occurs in embryonic xiphophorin fishes. Further experiments are in progress to check this point.

Of perhaps more interest than the appearance of pigment cells from the neural tissue grafts were the types of pigment cells that made their appearance. Micromelanophores were abundant in the graft, which is in keeping with the picture in the normal fish. The presence of macromelanophores in the tissue graft is of particular interest. Since the normal experimental swordtail host embryos never produce macromelanophores, the only possible source of these cells is from the donor tissue of potentially melanomatous platyfish-swordtail hybrids. The donor embryos from which these cells were taken were at Stage 10 or younger, and the tissue taken included nerve cord, somite mesoderm and ectoderm. If the evidence of Lopashov concerning the non-appearance of melanocytes from purely mesodermal grafts may be assumed to hold in these forms, it seems likely that the macromelanophores arose from the same region of the nervous system as the micromelanophores. This region of the nervous system of the fish embryo is presumably homologous with the neural crest in higher forms.

The relative infrequency of macromelanophores in our experimental embryos is due to two factors. First, the expected incidence of the larger pigment cells in the grafts made was only 50% because only 50% of the backcross platyfish-swordtail embryo donors carried *Sd* gene for macromelanophores. Second, the larger black pigment cells normally appear in genotypically *Sd* fish only postnatally; rarely do they appear just prior to birth. Therefore, the macromelanophores in the grafts observed in the swordtail embryo hosts would not represent the entire possible population of these cells, but rather only those that had made their appearance at the time of death, by fixation, of the host.

It has been an open question as to whether melanoma cells are of primary (embryological) or secondary (derived) origin. It was previously impossible to determine by direct observation whether definitive melanoma cells, particularly the macromelanophores, were embryologically-derived normal body constituents which in their ontogeny become malignant in response to their genotype, or whether these and other pigment-containing cells in the melanoma had become pigmented and malignant as a result of influences external to the cells. In the platyfish-swordtail hybrid the theoretical problem of true pigment cell origin can be resolved because it now appears that macromelanophores, at least, are normal products of the embryonic nervous system. To complete the picture, Gordon (1937) has shown that macromelanophores, in their development in hybrids of appropriate genotype, initiate the growth of melanomas. Marcus & Gordon (1954) and Ermin & Gordon (1955) presented evidence that some melanocytes, which are melanin-synthesizing cells, transform into macromelanophores; pigment-containing hypertrophic fibroblasts, giant cells and connective tissue stroma cells, however, are secondary pigmented cells that acquire their melanin from contact with macromelanophores and melanocytes.

The neural theory of melanoma development in hybrid xiphophorin fish seems to be supported by direct evidence derived from the application of genetic and experimental embryological techniques. The extension of this type of experimental approach to the problem of the embryological origin of mammalian melanomas has as yet not been achieved. Therefore the conclusions that may be drawn concerning the origin of mammalian melanomas must be tentative. However, the observations that pigmentation in the mouse originates from cells derived from the neural crest (Rawles, 1953) and the morphological resemblance of the primary pigment cell types (melanocytes) found in fish and mouse and human melanomas (Grand, Gordon & Cameron, 1941) argues in favor of a similarity in the final tumors and in their etiology.

Summary

The origin of pigment cells in xiphophorin fish was studied by grafting tissue from platyfish-swordtail hybrid embryos (50% of which were genetically destined to develop a melanosis or a melanoma) into wild-type swordtail embryos.

When the grafts from a Stage 10 hybrid donor embryo contained ectoderm, mesoderm and nervous tissue they gave rise in the swordtail host embryo first to micromelanophores, and later to macromelanophores, as well as to muscle, nervous tissue, and in some cases to cartilagenous elements.

When the grafted tissue from Stage 10 hybrid

donors contained only ectoderm and somite mesoderm, micromelanophores appeared in only three of the 14 swordtail host embryos prior to the appearance of host pigmentation; macromelanophores appeared in none.

Using only ectoderm and somite mesoderm tissue grafts from a Stage 13 hybrid donor, pigment cells appeared in 7 out of 8 swordtail embryo hosts; this indicated that pro-pigment cells migrate from the nervous system to the more superficial layers of the hybrid embryo between Stages 10 and 13.

The presence of macromelanophores in the grafted tissue, and therefore of donor nervous system origin, permits a re-evaluation of the neural crest theory of melanoma cell origin. From the evidence presented in this paper, it seems likely that the genetically determined macromelanophores that give rise to the melanomas of platyfish-swordtail hybrids are normal derivatives of the nervous system. This in part confirms the conclusion reached by geneticists that heredity is primarily responsible for the growth of melanomas in these animals.

Literature Cited

Borcea, I.

1909. Sur l'origine du coeur, des cellules vasculaires migratrices et des cellules pigmentaires chez les Téléostéens. C. R. Acad. Sci. Paris, 149: 688-689.

Burke, Dean, G. H. Algire, M. L. Hesselbach, C. E. Fischer & F. Y. Legallais

1948. Tissue metabolism of transplanted mouse melanomas, with special reference to characterization by paraphenylenediamine. *In* Biology of Melanomas (R. W. Miner and Myron Gordon, eds.), Spec. Publ. N. Y. Acad. Sci., 4: 437-446.

Dorris, F. S.

1938. The production of pigment in vitro by chick neural crest. Roux' arch., 138: 323-334.

1939. The production of pigment by chick neural crest grafts to the 3-day limb bud. J. Exp. Zool., 80: 315-345.

DuBuy, H. G., M. W. Woods, D. Burke & M. D. Lackey

1949. Enzymatic activities of isolated amelanotic and melanotic granules of mouse melanomas and a suggested relationship to mitochondria. Jour. Natl. Cancer Inst., 9: 325-336.

DuShane, G. P.

1935. An experimental study of the origin of pigment cells in Amphibia. J. Exp. Zool., 72: 1-31.

Ermin, R., & M. Gordon

1955. Regeneration of melanomas in fishes. Zoologica, 40: 53-83.

Flickinger, R. A.

1949. A study of the metabolism of amphibian neural crest cells during their migration and pigmentation in vitro. J. Exp. Zool., 112: 465-484.

Gordon, M.

1927. The genetics of a viviparous top-minnow *Platypoecilus;* the inheritance of two kinds of melanophores. Genetics, 12: 253-283.

1931. Morphology of the heritable color patterns in the Mexican killifish, Platypoecilus. Amer. Jour. Cancer, 15: 732-787.

1937. The production of spontaneous melanotic neoplasms in fishes by selective matings. II. Neoplasms with macromelanophores only. III. Neoplasms in day old fishes. Amer. Jour. Cancer, 30: 362-375.

1948. Effects of five primary genes on the site of melanomas in fishes and the influence of two color genes on their pigmentation. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 216-268.

1951. Genetic and correlated studies of normal and atypical pigment cell growth. Growth (Symposium), 10: 153-219.

Grand, C. G., Myron Gordon & Gladys Cameron

1941. Cell types in tissue culture of fish melanotic tumors compared with mammalian melanomas. Cancer Research, 1: 660-666.

Greenstein, J. P., & G. H. Algire

1944. Comparative oxidase activity of melanotic and amelanotic melanomas. Jour Natl. Cancer Inst., 5: 35-38.

Greenstein, J. P., J. Werne, A. B. Eschenbrenner & F. M. Lewthardt

1944. Chemical studies on human cancer. I. Cytochrome oxidase, cytochrome C and copper in normal and neoplastic tissues. Jour. Natl. Cancer Inst., 5: 55-76.

Hopper, A. F., Jr.

1943. The early embryology of *Platypoecilus maculatus*. Copeia: 218-224.

Humm, D. G. & E. E. Clark

1955. The metabolism of melanomas in platyfish-swordtail hybrids. J. Natl. Cancer Inst., 16: 741-761.

Lopashov, G. V.

1944. Origin of pigment cells and visceral cartilage in teleosts. C. R. Acad. Sci. Moscow, n.s., 44 (4): 169-172.

Marcus, T. R. & Myron Gordon

1954. Transplantation of the *Sc* melanoma in fishes. Zoologica, 39: 123-131.

NEWTH, D. R.

1951. Experiments on the neural crest of the lamprey embryo. J. Exp. Biol., 28: 247.

NIU, M. C.

1954. Further studies on the origin of amphibian pigment cells. J. Exp. Zool., 125: 199-220.

RAVEN, R. W.

1953. Problems concerning melanoma in man. *In* Pigment Cell Growth, 121-137, New York, Academic Press, Inc.

RAWLES, MARY

1953. Origin of the mammalian pigment cell and its role in the pigmentation of hair. Growth (Symposium), 1: 1-12.

RIS, H.

1941. An experimental study of the origin of melanophores in birds. Physiol. Zool., 14: 48-66.

SCRIMSHAW, N. S.

1944a. Superfetation in poeciliid fishes. Copeia: 180-183.

1944b. Embryonic growth in the viviparous poeciliid fish, *Heterandria formosa*. Biol. Bull., 87: 37-51.

1945. Embryonic development in poeciliid fishes. *Ibid.*, 88: 233-246.

SHEREMETIEVA-BRUNST, E. A. & V. V. BRUNST

1948. Origin and transplantation of a melanotic tumor in the axolotl. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 269-287.

TAVOLGA, W. N.

1949. Embryonic development of the platyfish *(Platypoecilus)*, the swordtail *(Xiphophorus)* and their hybrids. Bull. Amer. Mus. Nat. Hist., 94: 165-229.

TAVOLGA, W. N. & R. RUGH

1947. Development of the platyfish, *Platypoecilus maculatus*. Zoologica, 32: 1-15.

TURNER, C. L.

1937. Reproductive cycle and superfetation in poeciliid fishes. Biol. Bull., 72: 145-164.

WEIDENREICH, FRANZ

1912. Die Lokalisation des Pigmentes und ihre Bedeutung in Ontogenie u. Phylogenie der Wirbeltiere. Zts. f. Morph. u. Anthr., 2: 59-140.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. Swordtail host embryo 24 hours after the implantation of a graft (containing ectoderm, mesoderm and neural tube from a stage 10 hybrid donor) into a notch just anterior to the dorsal fin region. The graft appears as a small lump. × 50

Fig. 2. The same host 3 weeks after grafting. Note the considerable growth and differentiation of the graft and the appearance of a macromelanophore at its base. × 20

Plate II

Fig. 3. Cross-section of transplanted ectoderm, mesoderm and neural tube. Transplant taken from a Stage 10 platyfish-swordtail hybrid donor and placed in a swordtail host at Stage 20. Host killed after 10 days. mm = macromelanophore.

Fig. 4. Section as above, showing differentiated muscle (m); macromelanophores (mm); nervous tissue (n); and cartilage (c) of graft origin.

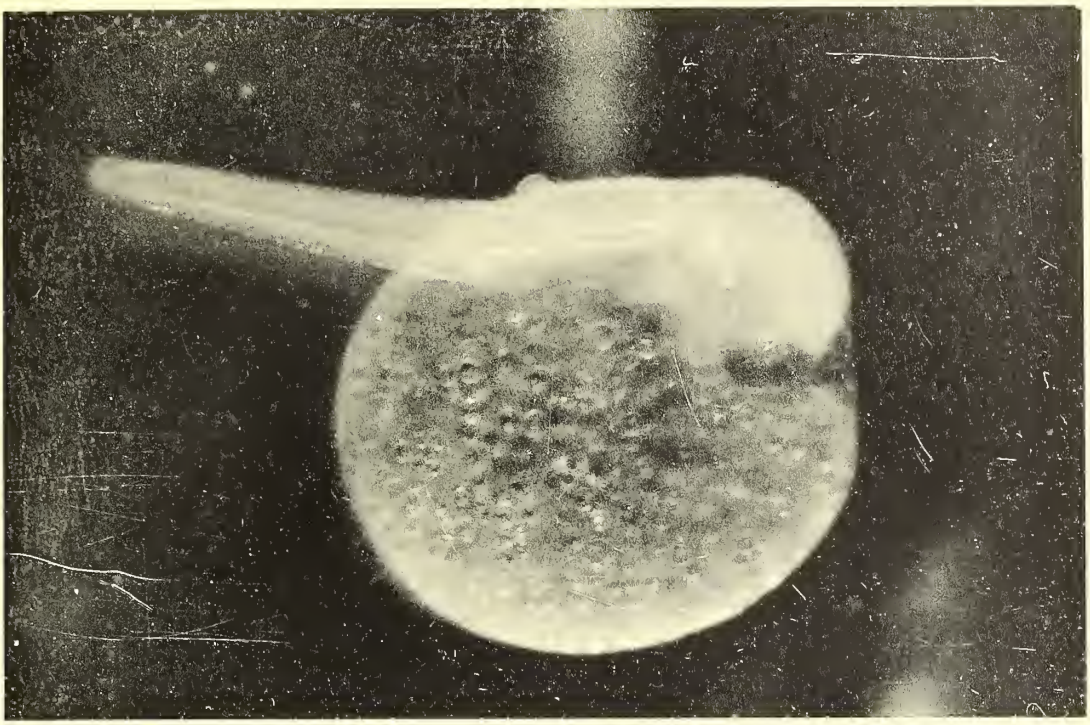


FIG. 1

FIG. 2

THE EMBRYOLOGICAL ORIGIN OF PIGMENT CELLS
IN PLATYFISH-SWORDTAIL HYBRIDS

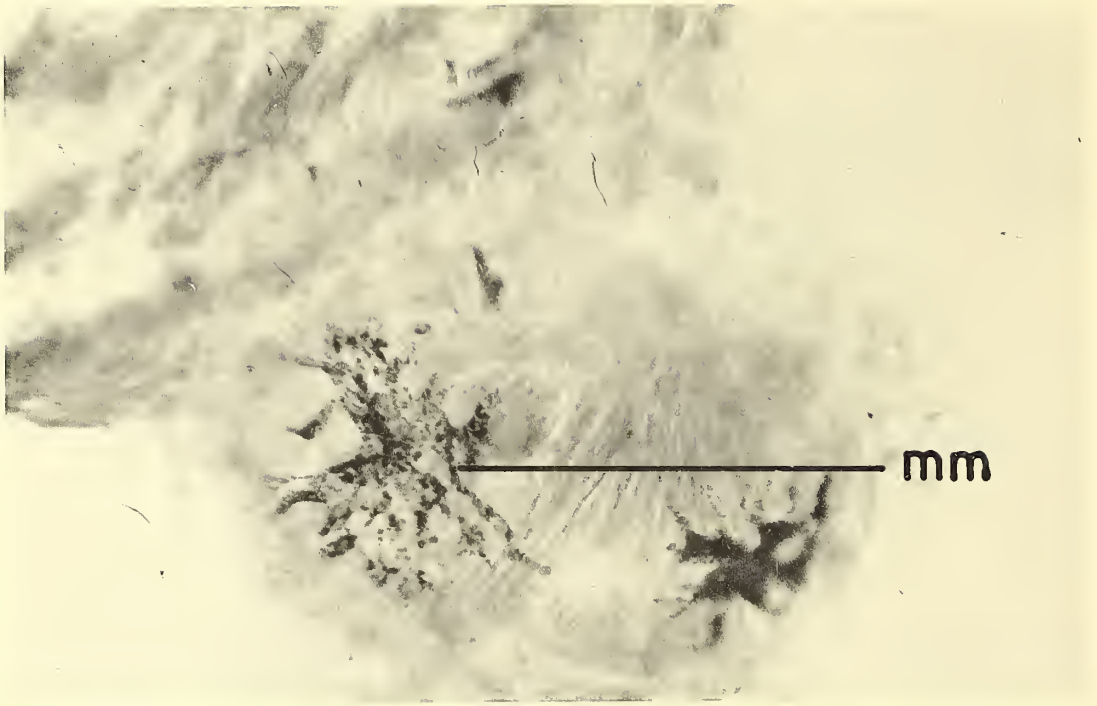


FIG. 3

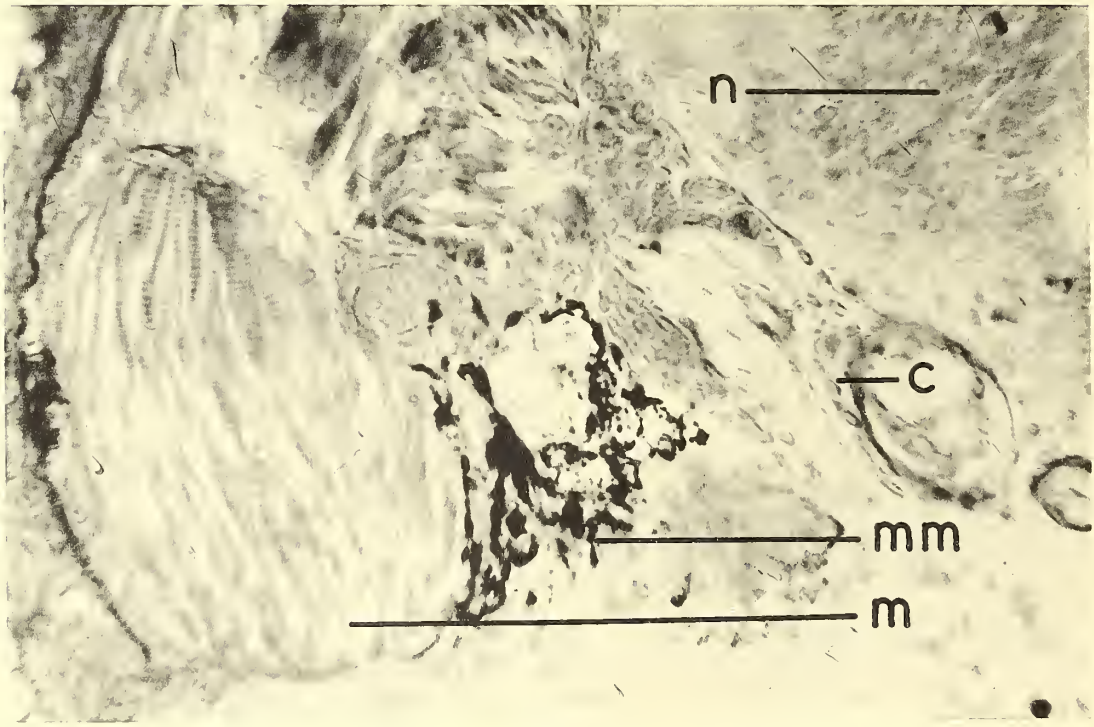


FIG. 4

THE EMBRYOLOGICAL ORIGIN OF PIGMENT CELLS
IN PLATYFISH-SWORDTAIL HYBRIDS

2

A New Species of Frog from Kartabo, British Guiana[1]

Doris M. Cochran
United States National Museum

(Text-figure 1)

AMONG some amphibians given to the United States National Museum by the New York Zoological Society is a large frog from British Guiana, collected by Dr. William Beebe, which seems to represent a new species. I take pleasure in naming it for its collector.

Eleutherodactylus beebei, new species

Diagnosis.—Head large, with smooth bony ridges forming a "U" between and behind the eyes; upper eyelid with a dermal horn; head a little broader than body; tympanum ⅔ the diameter of the eye; vomerine teeth in two heavy, slanting, narrowly separated patches behind the choanae; adpressed heel reaching beyond tip of snout; dermal appendages on elbow, knee and heel.

Description of the Type.—USNM 129526 (Beebe 3568), an adult male from Kartabo, British Guiana, collected on June 22, 1924, by Dr. William Beebe. Head large, with heavy, smooth bony ridges forming a "U" between and behind the eyes; body stout, in post-axillary region a little narrower than head. Vomerine teeth in two heavy, slanting, narrowly separated patches behind the choanae, not projecting beyond the outer rims of the choanae; tongue long, slightly notched behind, about ⅓ the width of mouth-opening. Snout rounded when seen from above and in profile, 1⅓ times the diameter of eye; canthus rostralis rounded but distinct; loreal region slightly concave and sloping to upper lip; nostrils supralateral, their distance from tip of snout ⅓ their distance from eye; interval between nostrils twice their distance from tip of snout. Eye large, prominent, its length ⅔ its distance from tip of snout; upper eyelids ⅔ as wide as the interorbital diameter; tympanum very distinct, ⅔ the diameter of eye, separated from eye by a distance equal to ¾ its own diameter. Fingers free, long, 1st longer than 2nd; all the fingers with enlarged rounded disks, that of 3rd finger covering about ¼ the tympanic area; metacarpal tubercles well developed; a heart-shaped palmar pad, and an elongate one on base of 1st finger. Toes with a faint trace of web, long, 5th longer than 3rd, which does not quite reach base of antepenultimate phalanx of 4th, the disk of which covers a little less than ¼ the tympanic area; a large elongate inner and a very small outer metatarsal tubercle. Hind legs long; when adpressed, heel reaches beyond tip of snout; when limbs are laid along the sides, knee and elbow touch; when legs are laid at right angles to body, heels overlap considerably. Skin glandular above; a horn-like tubercle on upper eyelid, surrounded by several granular tubercles; a crescentic glandular ridge above each shoulder; a dorsolateral fold on each side of back, with numerous scattered glands and tubercles on the sides; a pair of small tubercles above anus; a heavy supratympanic ridge; throat and chest smooth; belly smooth anteriorly, covered with small granules posteriorly; lower surface of thigh minutely granular. A long slit on each side of tongue in the male.

Dimensions.—Head and body 85.5 mm.; head length from posterior edge of tympanum to tip of snout 30 mm.; head width 34.5 mm.; femur 41.5 mm.; tibia 47 mm.; foot from base of inner metatarsal tubercle to tip of 3rd finger 23.5 mm.

Color.—Dorsum seal brown, lightening to cinnamon on snout, hands and feet; a clove brown patch in front of and behind tympanum; dorsolateral fold and ridge above shoulder outlined in clove brown; three or four indistinct dark bands across center of back; sides indistinctly marbled with dark and light suffusions. Legs with indistinct dark bars above; femur with a coarsely

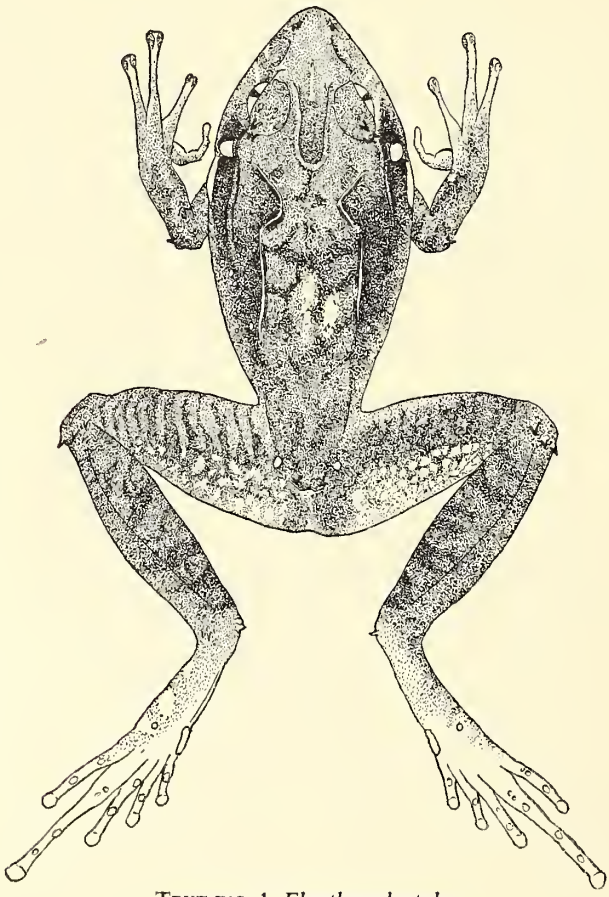
[1] Contribution No. 964, Department of Tropical Research, New York Zoological Society.

reticulate pattern of light spots on a dark ground posteriorly. Venter immaculate cream-buff, darkening to vinaceous on palms and soles.

Remarks.—A note was made by Dr. Beebe at the time of collection: "Leaped off fallen log to the ground, where I caught it among the leaves of the jungle floor. There were two, apparently mating, but the second escaped."

The Haitian *Eleutherodactylus inoptatus* closely resembles the new form. Both attain a large size compared to most members of the genus, and both have a dermal horn on the upper eyelid.

Some frogs from the mainland of South America may resemble it also, such as *E. cornutus* Jimenez de la Espada from Ecuador and Colombia, and *E. devillei* (Boulenger) from Ecuador.



TEXT-FIG. 1. *Eleutherodactylus beebei*, new species.

3

Notes on the Behavior and Habits of the Medusa, *Olindias phosphorica tenuis* Fewkes

C. M. Breder, Jr.

The American Museum of Natural History, New York 24, N. Y.

(Plates I & II)

INDIVIDUALS of *Olindias phosphorica tenuis* Fewkes are occasionally seen drifting past a submerged light at the head of the dock at the Lerner Marine Laboratory at Bimini, Bahamas. They do not appear to be influenced by the light but seem rather merely to drift in the tidal flow. Nevertheless, they have always been noted to be in active locomotion, which is rather vigorous in this form. They have never been seen at this place in the daytime, a fact that is probably more than accidental, as some of the following observations suggest.

Unlike so many medusae, if these are transferred to an aquarium they live well and appear to be extraordinarily hardy. Two individuals taken at the submerged light on November 22, 1953, were maintained in an aquarium which was supplied with running sea water and which measured 2′ x 1′ x 1′. They were still living on December 18 when the visit to the laboratory was terminated.

It was clearly demonstrated each day that these animals are strictly nocturnal. Shortly after nightfall they became very active, moving with considerable energy throughout the confines of the aquarium. The turning on of lights did not cause them to stop swimming but did seem to reduce their activity to some extent. Plate I, Figure 1, was made by photo-flash at night in the darkened laboratory. This photograph of one of the animals in active locomotion was made just after a vigorous pulsation of the umbrella, as the animal slowly sank slightly before it was again lifted by the next pulsation. The upwardly-bent tentacles are, of course, indicative of this stage of the locomotor cycle.

Their behavior is markedly different with the coming of morning light. Then they sink to the bottom and wedge themselves into some out-of-the-way place where they remain until the following nightfall. If routed out of their resting place they swim about for a time and then settle back to their quiescent state. At this time they bring their tentacles into tight helical coils and, unlike *Cassiopeia,* settle with the oral surface downward. In the aquarium they usually nestled in some filamentous green algae which grew on rocks and shells. In these situations they became very inconspicuous, as can be seen from Plate I, Figure 2. Here the coiled tentacles may be seen drawn up close to the inner surface of the umbrella. The outline of the exumbrella may be seen above them partly hidden by the algal filaments, the tentacles showing through its transparent substance. It is this behavior which leads us to suppose that in the sea they are only active at night.

Supplied with small fishes such as *Atherina* or *Eucinostomus,* which they probably encounter in their normal travels, they fed, one might almost say, voraciously. Contact with a tentacle quickly killed these small, fragile fishes of about one-half to three-quarters of an inch in length, and these were drawn up within reach of the rather actively exploring manubrium. The size of the fish, actually rather large for the mouth of the medusa, made necessary a considerable oral stretching. There the fish lodged for a long time, as feeding proceeded. No favored orientation was noted, but probably for fully mechanical reasons most fish were found either head or tail first in the tube-like opening. So far as could be determined, one fish per night was sufficient. Numerous others were killed by being stung but this was presumably incidental to the confined space of the aquarium.

Ordinarily these two medusae avoided each other and at no time were they seen to collide randomly. It was quite evident that the introduction of fishes to the aquarium caused them to

accelerate their activity, a process which would seem to insure a greater chance of stinging a fish. At one time when only a few fishes were presented, one of the medusae quickly caught one and soon had more than half of it in the tube-like mouth. The other and now more active medusa evidently sensed the presence of the fish which its companion was consuming. The two came together in a tangle of tentacles and the still-exposed portion of the fish was ingulfed by the second medusa. For perhaps half an hour both mouths held on to the fish, when finally the second medusa succeeded in taking it away from the first. There was no difficulty in distinguishing the two as one had a nick in its umbrella.

As an illustration of the resistance of these creatures to mechanical injury, the one mentioned above as showing a nick in the umbrella may be taken as an example. This medusa had been sucked into the small glass standpipe which served as a drain for the aquarium. In order to clear the overflow pipe, this was blown into with considerable force, causing the medusa to shoot out with some violence. The animal was so mutilated that there was no thought of its possible recovery and the seeming bit of pulp was left in the tank and forgotten. Nevertheless, a few days later the creature was moving around and about two weeks later, when the previously-described feeding episode took place, there was only a nick left to distinguish it from the other.

The following year the preceding observations were confirmed. Again only two individuals were obtained. They were caught as before, on October 27 and 28, and maintained in an aquarium until December 3, when they were preserved. This time it was convenient to feed them *Gambusia,* on which they thrived just as well as on the other species used the preceding year. In addition to swimming actively when ready to feed, these individuals frequently stationed themselves and extended their tentacles fully. Sometimes they adhered to the aquarium wall or other support near the surface and permitted their tentacles to fall to the bottom, as shown in Plate II, Figure 3. At other times they floated passively with the tentacles extended. At such times they sank slowly through the water until a certain amount of the trailing tentacles would be resting on the bottom. Evidently a definite point of equilibrium was reached because the medusae then floated motionless in mid-water, where they rested for long periods. Such a posture is shown in Plate II, Figure 5. It would seem that at such times the tentacles, which are clearly heavier than water, counter-balanced the umbrella or some portion of it, which was evidently somewhat lighter. This would seem to be a condition essential to the observed behavior. On the other hand, during the daytime behavior, the whole animal was evidently heavier than the water, as they sank rapidly. All this suggests that in a state of nature this form not only swims about actively for feeding purposes but "fishes" in the manner described by attaching itself to some shell or other support and resting quietly until some fish or other animal strikes a tentacle. Under aquarium conditions it was noted that fish were caught by both means. Usually animals were quiescent for about twenty-four hours after feeding, and neither the active swimming nor the quiescent "fishing" were ever seen during that period. Most frequently the remains of the fish were disgorged two to four hours after catching.

The aquarium in which these medusae were kept, unlike the earlier one, was devoid of anything except the hard-rubber drain pipe and a flooring of sand. Consequently it was impossible for the medusae to hide. A typical daytime resting position is shown in Plate II, Figure 4, where one is attached to the drain pipe. The tightly coiled and pendant tentacles, typical of this resting condition, are especially evident here. This photograph is life size.

It is perhaps noteworthy that the form under discussion and *Cassiopeia,* although in different classes, Hydrozoa and Scyphozoa respectively, agree in being hardy in captivity and in having regular contacts with solids.

The present usage follows Bigelow (1938)[1] in considering the western Atlantic *O. tenuis* a subspecies of the Mediterranean *O. phosphorica.* Dr. P. L. Kramp, who was kind enough to check the identity of one of the specimens collected the second year, wrote in a letter to Dr. Libbie Hyman that *tenuis* is ". . . distinguished by its smaller number of tentacles, marginal clubs and centripetal canals, and, moreover, by its gonads occupying only the distal halves of the radial canals. The present specimen is peculiar in so far as its gonads are almost as long as the radial canals; it has 6-7 centripetal canals per quadranth, like *tenuis;* in the number of secondary tentacles (41) it likewise agrees with *tenuis;* but it has a much larger number of primary tentacles (97) and marginal clubs (130). Thus it seems to bridge the gap between *phosphorica* and *tenuis.*" Dr. Hyman kindly read and criticized the present manuscript.

[1] Zoologica, Vol. 23, pp. 112-113.

EXPLANATION OF THE PLATES

Plate I

Olindias phosphorica tenuis Fewkes

Fig. 1. An individual in active nocturnal swimming, taken by photo-flash.

Fig. 2. The same individual in diurnal rest in the shelter of algae.

Plate II

Olindias phosphorica tenuis Fewkes

Fig. 3. An individual in its quiescent "fishing" position, attached to the back wall of the aquarium.

Fig. 4. A resting daytime pose, attached to the aquarium drain pipe. Life size.

Fig. 5. Two medusae, one in the free-floating "fishing" position and the other in active locomotion. The fish is *Gambusia.*

FIG. 1

FIG. 2

NOTES ON THE BEHAVIOR AND HABITS OF THE MEDUSA,
OLINDIAS PHOSPHORICA TENUIS FEWKES

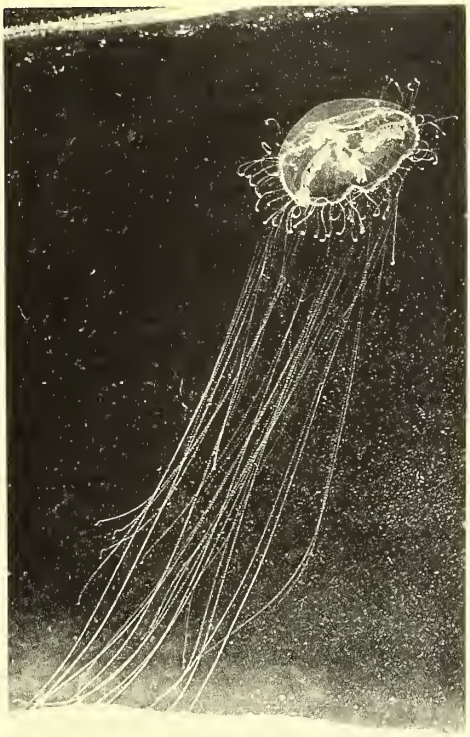


FIG. 3

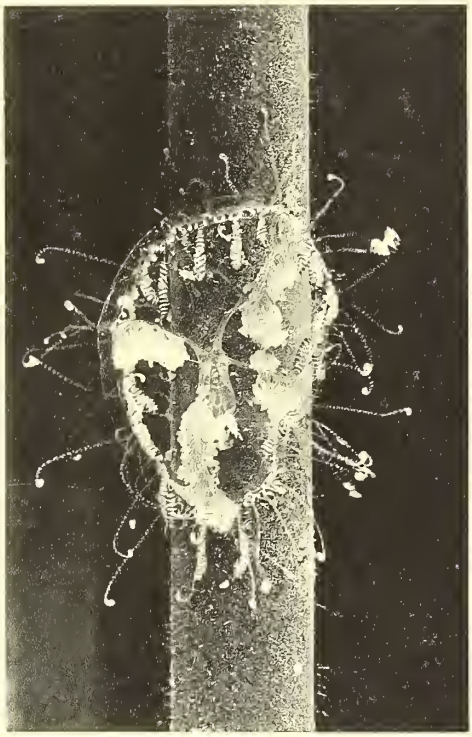


FIG. 4

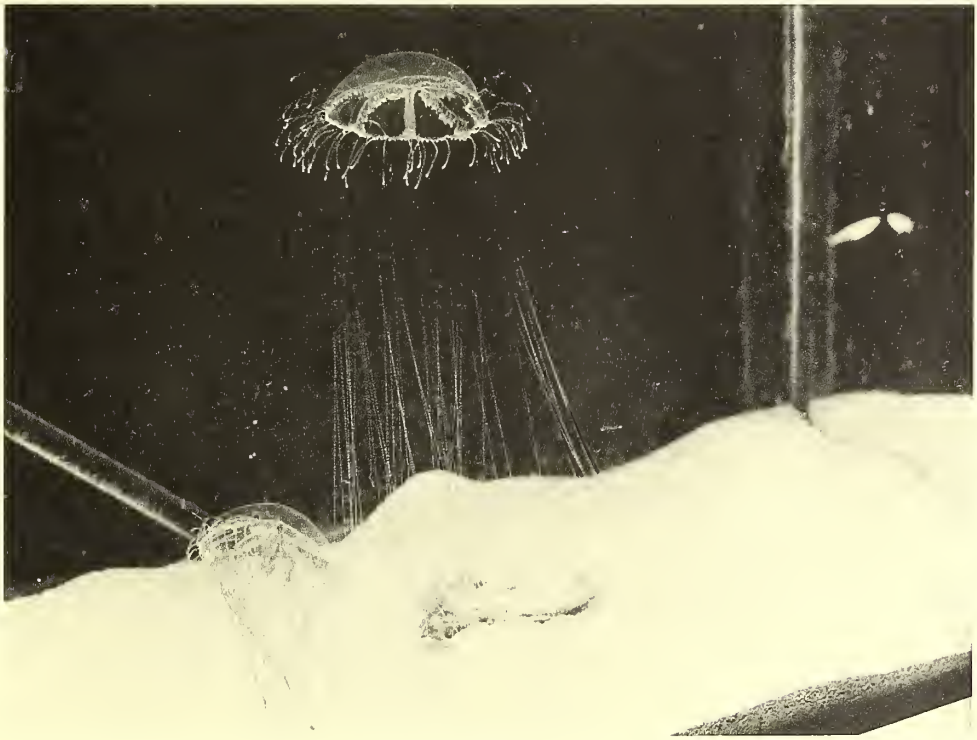


FIG. 5

NOTES ON THE BEHAVIOR AND HABITS OF THE MEDUSA,
OLINDIAS PHOSPHORICA TENUIS FEWKES

4

Observations on Evisceration and Visceral Regeneration in the Sea-cucumber, *Actinopyga agassizi* Selenka

Carol Mosher

The American Museum of Natural History, New York 24, N. Y.

(Plates I & II; Text-figures 1 & 2)

Introduction

It is well known that a number of holothurians autotomize their viscera under various conditions, and it is generally believed that complete regeneration takes place until the reformed organs closely resemble their normal counterparts. The general interpretation of the process has been that this is an action of defense, the expelled viscera entangling a potential enemy, and also that it may permit the survival of the holothurian by reducing the need for oxygen under adverse, suffocating conditions. The aspidochirote holothurian, *Actinopyga agassizi* Selenka, is one of the commonest shallow water species found in the Bahama Islands area. This sea-cucumber can be relatively easily induced to eviscerate and it contains a large and conspicuous organ of Cuvier. A study of this animal has been made in order to determine the gross pattern and mechanics of the reformation of the intestine, together with some of its histological features. A number of preliminary experiments were performed in an attempt to discover the factors stimulating evisceration and to elucidate the role of the organ of Cuvier in the light of recent information on its toxicity, reported by Nigrelli (1952) and Nigrelli & Zahl (1952). I wish to acknowledge the kind support and assistance of Mr. William S. Glazier who made it possible for a large portion of this work to be carried out. My thanks are due Mrs. Louise Palmer Wilson for her advice and suggestions towards the completion of the histological studies made at Wellesley College. A portion of this work was carried out at the Lerner Marine Laboratory, Bimini, Bahama Islands. I wish to acknowledge the great assistance of Dr. Charles M. Breder, Jr., in the development of the diagrammatic presentations included herein.

Evisceration

Experimental Induction

The striking phenomenon of self-mutilation which occurs widely throughout the orders of Holothuroidea has drawn the attention of many investigators. The manner and extent of self-mutilation appear to be similar among all of the large aspidochirote holothurians in which it has been studied. Evisceration is effected by a rupture through the cloaca of almost the entire alimentary canal with its associated glands, some of the gonad tubules, and in some species, one or both of the respiratory trees. The organ of Cuvier is associated with the respiratory trees of some species and is apparently linked in some way with the phenomenon of evisceration. The occurrence of this organ, however, is extremely variable, even within a single genus (Deichman, 1930), so that its role is not one indispensable to total evisceration. A number of species of the genus *Holothuria* have a large, conspicuous organ of Cuvier which becomes a mass of sticky threads on contact with sea water. The general interpretation is that the expulsion of this organ is associated in some special way with evisceration and precedes the more radical defense response of autotomy of the digestive system. In his studies of *Holothuria nigra*, Hérouard (1889) reported that the expulsion and elongation of the tubules of the organ of Cuvier were results of water being forced into their lumina from the left respiratory tree. Mines (1912) supported these observations and like Hérouard, expressed the view that the Cuvierian organ played no specific role in evisceration, but rather that it was expelled before the viscera simply as a result of its anatomical proximity to the cloaca. On the other hand, Minchin (1892) reported an intrinsic extending apparatus in the Cuvierian

organ of *H. nigra* and expressed his view that the organ was specialized for expulsion, probably in the role of a defense organ. Crozier (1915) reported a similar intrinsic extending apparatus in the organ of *H. parvula* (=*H. captiva*). The interpretation of the response of total evisceration in different species varies widely. Bertolini (1930) believed that in *Stichopus regalis* it is a naturally occurring seasonal phenomenon. Minchin (1892) took a similar view of *Holothuria nigra.* In contrast to this, it has been reported that evisceration of *H. parvula* is a result of adverse conditions rarely occurring in nature (Crozier, 1915).

The Cuvierian organ of *Actinopyga agassizi* seems to be in some way involved in total evisceration. As described for species of the genus *Holothuria*, the organ of *Actinopyga agassizi* may be everted but not eliminated without evisceration occurring, but evisceration never occurs without the previous everting of the organ of Cuvier. It appears to react to the same stimulus which induces autotomy, or to be a factor leading up to expulsion of the viscera. In the majority of cases, the organ is expelled within the first half hour under experimental conditions, although generally it is not completely eliminated, maintaining its attachment to the respiratory trunk while hanging free outside the body cavity. It is not an immediate preliminary to total evisceration, for in many cases the animal, having thrown out the organ of Cuvier within the first half hour, will not eviscerate totally for five or six hours. At the time of evisceration, if the Cuvierian organ still maintains its attachment to the respiratory trunk, it appears to be a matter of chance whether or not it is torn free by the expulsion of the alimentary tract.

A variety of methods was tried for stimulating evisceration in *Actinopyga agassizi.* With all of them the length of time before evisceration did not seem a significant factor for comparison, because within any given method the time before evisceration varied to a great extent from one animal to another. Freshly collected animals were used in all cases, rather than those which had been kept in stock tanks.

The most effective method appeared to be to allow a number of animals to remain in standing water in a galvanized container exposed to the sun, so that the water was heated to a relatively high temperature. The majority of such animals eviscerated within one-half to seven hours and there was almost 100% survival during the experimental regeneration period. In an attempt to single out a particular condition in this general method responsible for stimulating evisceration, three controllable factors were tested: temperature, trace metallic ions and oxygen deficiency.

To avoid contamination with trace metallic ions present in galvanized containers, a number of animals were kept in enamel pails or glass bowls. Three were allowed to stand in enamel pails at a temperature varying from 20° to 25° C. One of these holothurians showed no sign of evisceration in six hours. However, when it was removed to fresh running sea water, it eviscerated immediately. Two animals were left in enamel pails for 24 hours and showed no indication of evisceration at the end of this period. Subsequently they were put together in a galvanized tub, at about 20° C., and they eviscerated in four to ten hours. One animal, maintained in an enamel pail at a temperature which was raised to 40° C. and allowed to range down to 33° C., eviscerated in the first three hours. Two others were placed in glass bowls in about two liters of water at a temperature of 35° C. They eviscerated in five hours. During the same period of time, four animals in water of 20°-22° C. in a galvanized tub did not eviscerate. One of these subsequently eviscerated upon being moved to an aquarium with a supply of running sea water.

Freshly collected animals were placed in large glass bowls in two liters of water at about 22° C. and to each a metallic chloride was added —zinc to the first, copper to the second and iron to the third. A precipitate formed when each salt was added to sea water. The animal treated with zinc eviscerated after five hours, whereas the others, including a control to which no salt was added, did not eviscerate for a much longer period. After 17 hours, both the control animal and the one treated with copper ion had eviscerated, but the one treated with iron showed no effect other than expulsion of the Cuvierian organ in the first half hour of treatment, as did the other experimentals and the control.

The results of these tests are somewhat contradictory and puzzling. In addition, casual observation indicates that many animals eviscerate for no readily apparent reason. If they are kept undisturbed in aquaria with no detectable change in environment, after about a week one may eviscerate while another in the same aquarium will not.

It appears, however, that evisceration of this sea-cucumber occurs but rarely, if at all, in nature. Expelled viscera are never observed and animals found with incomplete digestive systems have not been reported. Such factors as the presence of metallic ions, especially zinc, and high temperature evidently play an important role in the stimulation of evisceration, but no clear-cut pattern can be established by these experiments.

It is possible that some adverse condition was common to all the methods used, or that the same reaction by the animal can be brought on by several different factors. It is interesting to note here that Kille (1936) reported being able to induce evisceration by means of electrical stimulation.

Role of the Organ of Cuvier

The exact role of the organ of Cuvier in evisceration remains a problem. Whether its behavior is only a part of the response to conditions which induce autotomy, or whether it is the total response, in its turn creating conditions inducing autotomy, remains obscure. Recent investigations by both Nigrelli and Zahl on the toxic factors of *Actinopyga agassizi* have presented a rather different view. The organ of this species is not extremely sticky, although it is ejected in much the same way when the animal is disturbed as has been described for species of *Holothuria*. In his analysis of the various organs of the sea-cucumber, Nigrelli (1952) determined that the organ of Cuvier contained an active toxic agent. The water soluble factor extracted from this organ proved intensely toxic to both fish and mice. The organ of Cuvier is generally considered a modification of the respiratory system, an outgrowth at the junction of the left respiratory tree with the main trunk. The organ in certain species has been described as tubular in structure, although this has not been found to be the case in *Actinopyga agassizi*. In many species of holothurians in which a large Cuvierian organ is present, these tubules, when expelled, become excessively sticky. It seems to be because of this property, that authors have concluded that the organ is specialized for defense, being thrown out to entangle a potential enemy.

To determine the nature of the organ of Cuvier, a variety of tests with living animals and a histological study of the organ itself were made. For testing the toxic factor, *Cyprinodon baconi* Breder was the fish used. It takes but little stimulation of the sea-cucumbers to cause the release of the toxic substance. Merely by picking one of the animals out of an aquarium, rubbing it slightly and replacing it, enough poison is released in the water of a 15-gallon tank to kill fish within less than half an hour. The most apparent observable result of rubbing a live animal in this way is the secretion of a quantity of mucus all over the surface of the body.

In order to determine the source of the released toxin, sea-cucumbers were kept in aquaria with running sea water until there was no contamination in the tank—usually a matter of 24 hours. Fish were put in these tanks several hours before the test to check the absence of toxicity in the water. Subsequently, the holothurians were picked out of the tanks and samples of the skin mucus were collected in beakers as it accumulated and dripped off the body wall. After the animal was held for several minutes, there was a strong expulsion of water from the cloaca. This water was collected in beakers held at a distance from the anal opening to prevent possible contamination by the skin mucus dripping down from the test. To determine the toxic potency of these samples, test fish were established in glass bowls in two liters of sea water. It was found that when a sample as large as 10 cc., collected from the cloaca as described above, was added to the two liters of sea water in a glass bowl, the test fish survived for a period of at least four hours, at which time the test was discontinued. In contrast to this, only an hour after addition of a few drops of the collected mucus secretion, the test fish died in the violent spasms typical of fish poisoned with holothurin.

To determine the effect of removal of the Cuvierian organ on toxin production, two animals were induced to eviscerate, with an attempt to eliminate the whole organ. Following evisceration each was maintained for five days in a 15-gallon tank supplied with running sea water. Throughout this period, the animals were disturbed once a day until mucus skin secretion clouded the tank. As standard procedure, fish were introduced into the tanks before the holothurians were disturbed to check the absence of residual poison from previous tests. At the end of five days, the sea-cucumbers whenever disturbed proved as effective in killing the test fish as animals retaining the Cuvierian organ. Dissection showed no trace of this organ in either of the two test animals.

A normal sea-cucumber was maintained in a 15-gallon aquarium with running water for a period of six days, during which it was daily disturbed and handled until sufficient skin mucus was secreted to cloud the tank. Test fish were introduced into the tank an hour or more before the animal was disturbed each day to confirm the absence of contamination from the previous day. It was found that, although the tank water was toxic to these fish up to 12 hours after contamination, it was generally non-toxic at the end of 24 hours. The sea-cucumber showed no adverse effects of being handled so extensively, although generally an animal when disturbed to such an extent will eviscerate readily. When it was killed and dissected at the end of six days, a pearl fish, *Carapus bermudensis*, was found in one of the respiratory trees. The fish showed no deterioration whatsoever and it is probable that it was killed only on fixation of the sea-cucumber. The obvious question arises as to how

this fish survived the week of constant poisoning, in which the host was pumping highly toxic aquarium water into its respiratory system. In preliminary tests, Dr. L. Aronson (*personal communication*) found that pearl fish exposed with several *Cyprinodon* to the toxin in a tank clouded with mucus skin secretion of *Actinopyga agassizi* were affected immediately, although they survived about three times as long as the *Cyprinodon*. Nigrelli (1952) reports that a dilution of 1:100,000 of a standard stock solution of Cuvierian organ extract killed *Cyprinodon* in 23 minutes; whereas a dilution of 1:1,000 killed pearl fish in eight minutes. This single instance of survival of a pearl fish within the respiratory system suggests the possibility of some mechanism which protects the sea-cucumber itself from the highly toxic substance released by the skin glands, and which, incidentally in this case, evidently protected the pearl fish.

Sections made of the Cuvierian "tubules" do not present the picture described in such detail for the organ of Cuvier of other holothurian species. Although Jourdan (1883), Cuénot (1891), Guislain (1953), Hérouard (1889), Minchin (1892) and Mines (1912) have described these structures as tubules in *H. nigra* and *H. impatiens*, they are clearly not tubules in *A. agassizi*. In sections of the cloaca and the base of the respiratory trees, no lumen could be traced out from these structures other than the typically constructed branches of the respiratory system. In preparations of the Cuvierian organ itself (Pl. I, Fig. 1), there is no central canal, and no evidence of epithelial cells in the central area of the rod which might indicate a collapsed lumen. On the contrary, the central area appears to be a core of connective tissue. The tissue surrounding the central core is not of a secretory nature, but rather gives the appearance of a storage tissue. It is highly vacuolated, the vacuoles being surrounded by fine cytoplasmic strands containing two or three nuclei. Halfway out to the periphery from the central connective tissue core, there are both circular and longitudinal muscle strands. In the sections studied, prepared by standard alcohol techniques, the vacuoles appeared empty.

Regeneration

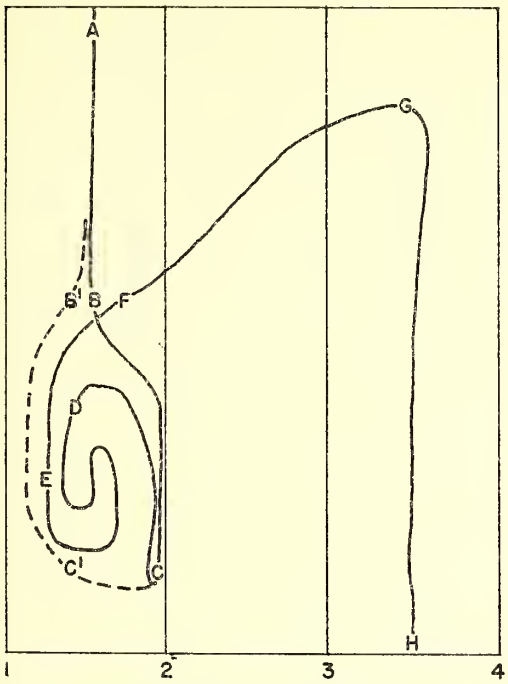
Morphological Aspects

In the study of regeneration of tissue that has been either mutilated or completely discarded in evisceration, an effort was made both to discern the gross plan together with the mechanics of the reformation and to determine the histology of the newly differentiated tissues and organs. Following evisceration, the animals were maintained either in the sea in wire cages permitting free flow of water, or in aquaria with a supply of running sea water. In these two environments, the temperature ranges differed to some extent: in the cage the range was approximately 30°-33° C., while in aquaria it was 20°-24° C. The two sets of experimental animals kept in different environments present somewhat different regeneration schedules, but these schedules vary in time rather than in sequence. Following evisceration, animals were sacrificed at intervals of 1 to 31 days. They were killed by injecting into the body cavity either formalin or Bouin's fluid, followed by immersion of the whole animal in the fixing fluid. No dissection was done until the specimen had been thoroughly fixed and the tissues hardened to some extent. Bouin's fluid was found to be by far the more successful fixative.

The general plan of the alimentary tract is diagrammatically represented in Text-fig. 1, with labelled reference points which will be used to relate the regenerating structures. The essential course of the intestine is shown, omitting the numerous convolutions into which it falls, especially from B to H. The lettered points relate both to the course of the intestine and to the base of the supporting mesentery where it is attached to the coelomic wall. In the area B-C, the intestine and attachment to the coelomic wall do not follow similar paths. The path followed by the latter is indicated by the broken line, the connecting mesentery conforming in a complicated manner between the region C-F.

In specimens killed very shortly after evisceration, the coelomic cavity appeared to be almost an empty shell, containing only extreme anterior and posterior fragments of the intestine, the respiratory trees and the cloaca. The variation of extent of evisceration affected only the gonad tubules and the organ of Cuvier. The evisceration of the gonad tubules was extremely variable and apparently depended solely on mechanical conditions, the extent of their entanglement with the intestine and its associated glands seeming to determine their fate, *i. e*, either evisceration or retention within the body cavity. To some extent, the retention or evisceration of the organ of Cuvier appeared to depend on a similar mechanical condition. However, it seems that this organ plays a more active role in evisceration, a role that is not yet adequately understood. Whereas it was the usual occurrence for this organ to be thrown out through the cloaca long before total evisceration, it appeared to be a matter of chance whether it was torn free from its attachment to the respiratory system or whether it maintained this attachment and was withdrawn into the body cavity.

Despite the total elimination of the intestine and its mass of associated glands, the track of



TEXT-FIG. 1. Diagram of part of the coelomic wall cut and spread to form a flat surface. The perpendicular guide lines represent four of the five radia. 1, right dorsal radius; 2, left dorsal radius; 3, left radius; 4, ventral radius.

The curved solid line represents the path of the intestine from its junction with the stomach, **A**, to the point at which it empties into the cloaca, **H**. This line also represents the path of the attachment to the coelomic wall of the supporting mesentery, except from **B-C**. There the broken line **B'-C'** represents the base of the mesentery where it is attached to the coelomic wall.

the original alimentary canal could be traced and the reference points readily identified by the torn supporting mesentery. In the first three days following evisceration, it was the growth and reformation of the mesentery alone that could be followed by gross observation. Following the autotomy of the viscera, this appeared only as a very narrow flap of tissue, much torn at its free edge. The following stages were established arbitrarily on the basis of observation of gross changes of the reforming mesentery and intestine. The letters denoting the area of the mesentery and intestine rudiment refer throughout to those reference points marked in Text- fig. 1.

STAGE 1: The stomach, broken at its junction with the intestine during evisceration, appears as a tube opening into the coelom. Just posterior to the end of this tube, the dorsal mesentery A-C is much reduced in width, presenting only a narrow flap of tissue, with a very irregular free edge. The course of this mesentery may be traced through its convolutions, C-F, posteriorly to the level of the cloaca, H, and it presents a picture identical with that of the mesentery base found in normal animals. Because the mesentery has been sheared off close to its attachment to the coelomic wall, the portion B'-C' does not extend across D-F, but lies as a narrow fringe to the left of this area. In no area does the mesentery appear to have been completely discarded during evisceration, but merely torn close to its attached edge. At the level of the cloaca, H, there may be a short segment of the posterior intestine, varying in length, which appears as an open tube into the coelom very similar to the anterior tube of the stomach.

STAGE 2: The open end of the stomach tube has a gathered appearance about its circumference, reducing the opening. In specimens in which a remnant of the posterior intestine is retained through evisceration, the anterior extremity of this remnant is closed. The most striking feature of early regeneration is the great extension in width of the supporting mesentery, especially along its length from A to G. Because of its spiral course, B-F, parts of the widening mesentery overlap other parts. There is not such extreme widening of the mesentery from G to H, but here the free edge of the mesentery is longer than its base so that the narrow fringe becomes much folded upon itself laterally, giving a ruffled appearance.

STAGE 3: The posterior tip of the stomach tube is closed. There is further widening of the mesenteries. The length of the mesentery G-H is extremely folded upon itself laterally, and its free edge gives the appearance of having been pulled together by a drawstring. There is a very fine, rod-like thickening along the free edge of this part of the mesentery.

STAGE 4: A fine, thread-like thickening is apparent along the free edge of the mesentery posterior to the stomach, and the broad part of the mesentery B-F supports a relatively short, thick intestine rudiment. The part of the mesentery originating along B'-C' has widened sufficiently to permit the rudiment which it supports to lie almost directly over the area D-E. This enlarged rudiment, lying midway between B'-C' and B-C, curves towards the left dorsal radius (Guide line no. 2, Text-fig. 1), continuing a curving path until it almost completes a circle, then turns back on itself towards F as it tapers to a fine thread. There is a lumen present in this enlarged curving segment of intestine rudiment although in both its anterior and distal extremities the rudiment consists only of a fine solid rod.

STAGE 5: Immediately following the formation and enlargement of the rudiment in the dorsal interradius, there is a further widening of the mesentery B′-F, permitting the formed rudiment to follow a more simplified path. The enlarged curved portion of the rudiment now turns sharply, forming a cusp, and passes under the more proximal part. There is a general increase in diameter of the rod-like rudiment along the mesentery A-B, tapered so that the anterior part of the rudiment is of smaller diameter than the enlarged curved part of the rudiment B-F. The diameter of the rudiment F-G has also increased, tapering to a fine thread at G. There is little observable change in the area G-H, except for a small increase in width along the length of the mesentery and some slight increase in the diameter of the intestine rudiment.

STAGE 6: There is a continuous lumen from the stomach, through the intestine rudiment of the curve, but not extending beyond F. Distal to F, the rudiment remains as a solid rod along the edge of the mesentery.

STAGE 7: There is now a period in which there does not appear to be any great extension in total length of the rudimentary intestine. Rather there is continuation of the development of the existing rudiment, which increases in its diameter along its entire length, and the extension of a lumen through the posterior intestine until the lumen is continuous from stomach to cloaca.

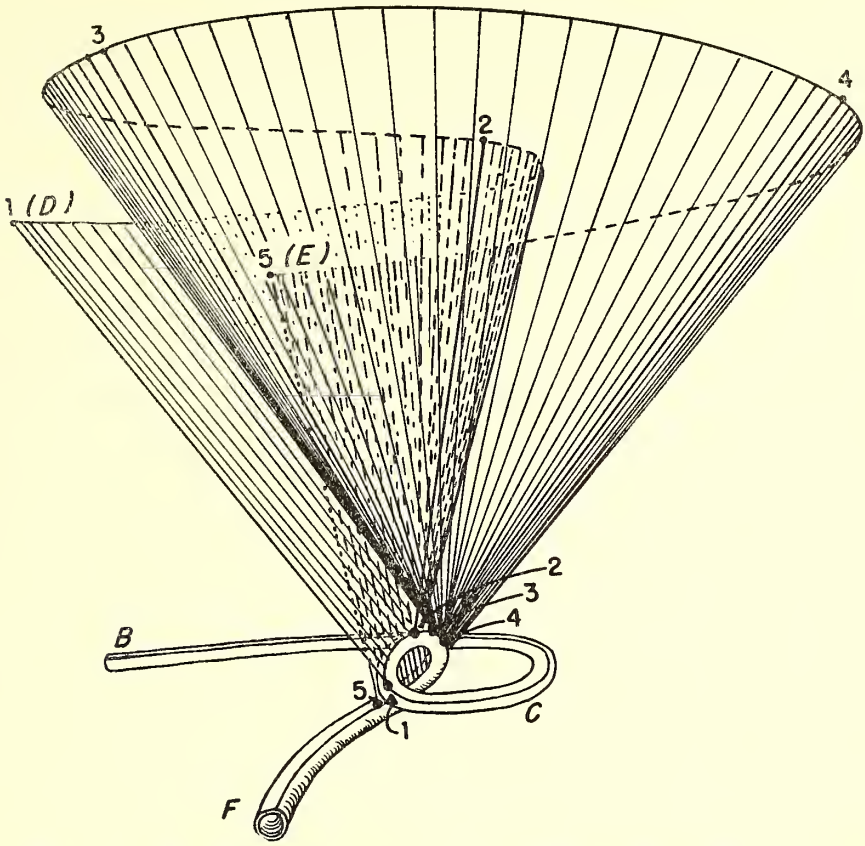
STAGE 8: Once the lumen is continuous throughout the rudiment, there follows a period of growth in which the intestine increases extensively in length. There is extensive lengthening in the curved portion of the rudiment B-F, forming a loop of intestine which extends posteriorly almost to the level of the cloaca before curving anteriorly and ventrally towards F. This loop does not follow the double spiral, B-F, but simply has a descending and ascending limb. The descending limb depends from a wide part of the mesentery, B-C, and thus is permitted to extend across and obscures the ascending limb as far as F.

The posterior intestine, G-H, is extensively lengthened, having carried its previously folded mesentery out into a smooth sheet which follows the convolutions into which the intestine rudiment falls. This longitudinal growth of the intestine continued through 31 days of regeneration, the period at which the last specimen was sacrificed. At this time, the reformed intestine was still considerably shorter than in the normal animal, especially in the curving area B-F, and in the posterior area G-H.

In the group of animals regenerating at higher temperature, the initial development was extremely rapid. In this group, 4-, 5- and 6-day specimens correspond to Stages 4, 5 and 6. One specimen, having regenerated for only 18 days, had developed a continuous lumen from stomach to cloaca that was, at the time of fixation, at least mechanically functional. The group of animals maintained at lower temperature did not develop so rapidly; a 10-day specimen was only at Stage 5 of regeneration.

The general plan of regeneration is the formation of an intestine rudiment along all of the ragged free edge of the remaining mesentery. However, because of the double spiral which the mesentery forms, B-F, and because of differential growth rates of areas of this part of the mesentery, the convolutions of the developing intestine rudiment in this area do not retain a fixed course, but go through a gradual change as development proceeds. Text-fig. 2 shows the relationship between the mesentery and the rudiment found at Stage 4. Whereas the mesentery represented corresponds to D-E the regenerating tube shown corresponds to the area B-F of Text-fig. 1. This illustrates very well one of the basic complicating factors in the mesentery-rudiment relationship. In the beginning stages of regeneration, the rudiment formed is very much shorter than the normal intestine, yet it is supported by the existing mesentery. The formed rudiment does not bypass any part of this existing mesentery but follows its free edge throughout. In the early stages of regeneration, the mesentery widens extensively, but at the same time its free edge becomes very much shorter than its base on the coelomic wall, permitting attachment to the short rudiment.

In order to understand the relation between the curving path of the formed rudiment and that of the normal intestine and to grasp the progressive development which permits the path of the former to approach the path of the latter, it is necessary to compare closely the two diagrams of Text-figs. 1 and 2. The rudiment B-C in Text-fig. 2 does not follow the path of the normal intestine but rather lies close to broken line B′-C′ in Text-fig. 1. Because its supporting mesentery is still relatively narrow, it is not extended over C-F, from left to right in Text-fig. 1, but only covers over a small portion between D-E. As may be seen in Text-fig. 2, the mesentery D-E is shortened to a very great extent at its attachment on the rudiment D-E, and differential growth in width of this part of the mesentery results in the first half of the rudiment D-E being directly above the second half, *i. e.*, closer to the coelomic wall at which the mesentery originates. The attachment of the mesentery D-E rotates through an arc of 180° on the circumference of the rudiment, thus permitting the rudiment 1-3



TEXT-FIG. 2. Diagrammatic detail of portion of the regenerating tube at Stage 4, showing the manner in which it is suspended by the mesentery. The curved line marked **1-5** represents the course of attachment of the mesentery sheet to the dorsal surface of the coelom, and corresponds to the line **D-E**, Text-fig. 1. The regenerating tube **B-F** corresponds to the intestine path **B-F** of Text-fig. 1. The attachment of the sheet to the tube is indicated by the line along the upper surface of the tube. Numbers **1** to **5** along this line correspond to those on the body wall, and the lines of attachment are indicated, together with a phantom suggestion of the sheet of mesentery. The line of attachment of the sheet to the tube passes to the back of the tube in negotiating the loop so that point **4** is on the far wall of the tube. See text for full explanation.

to follow the free edge of the mesentery without passing through the sheet of mesentery supporting the rudiment 4-5 in Text-fig. 2.

As development proceeds, the mesentery B′-C′, Text-fig. 1, widens to a great extent, allowing the rudiment which it supports to approach B-C, overlapping C-F. In Text-fig. 2, this development may be visualized if it be imagined that the rudiment B-C comes towards the viewer. The result at this angle of observation will be one of B-C in the foreground passing beneath E-F. Concomitant with this shift in spatial relationships, the mesentery 1-3 in Text-fig. 2 increases in width until it corresponds closely to the mesentery 4-5. This results in the attached rudiment 1-3 approaching the plane of the rudiment 4-5 so that these two parts now lie side by side instead of one on top of the other.

Another factor further complicates the shift of the rudiment from its path as shown in Text-fig. 2 to the normal course as represented in Text-fig. 1. The tubular rudiment shown in Text-fig. 2 does not extend along the mesentery as such beyond points B and F, but rather tapers abruptly between B-A and F-G, Text-fig. 1, to a fine solid rod, as described above in Stage 4. Concomitant with the development of the mesenteries allowing a shift in position of the rudiment, the development of the rudiment and its lumen progresses towards A from B and towards G from F. The effect is that of points B and F, Text-fig. 2, being pulled out along their axes,

shortening the rudiment C-E until rudiment D-E forms a sharp cusp where the rudiment changes direction, as described above in Stage 5. As both the rudiment and its supporting mesentery lengthen during further development, this cusp expands until it is a loop, as described in Stage 8. Not until regeneration is far advanced does the rudiment of this loop attain a length sufficient to follow the double spiral course of the normal intestine.

Histology

Although a rod-like intestine rudiment arises along the entire free edge of the mesentery, this rudiment develops a lumen betwen points B-F before there is any indication of a lumen at either extremity B-A or F-G. Histological cross-sections of this rudiment B-F support this observation and demonstrate the source of the lumen within the rod. At the extremities of this enlarged curved portion, the rudiment appears as a solid rod of connective tissue surrounded by a narrow band of epithelium only a few cells thick. However, in many places about the periphery of the rudiment in area B-F, a sequence of events resulting in the formation of a definitive lumen within the connective tissue of the rod may be followed in serial sections such as those shown in Plate I, Figs. 2, 3 and 4. A thickening of the epithelium results in an infolding of epithelial cells at the periphery of the rod. These folds deepen and are closed over at the periphery, leaving rings of epithelial cells surrounding a lumen within the rod itself, as seen in Pl. I, Fig. 4. A similar series of ridges and furrows runs lengthwise along the rod-like rudiment, the epithelium of the ridges thickening until two adjacent ridges close over a furrow, leaving a blind lumen lined with epithelial cells derived from the external epithelium of the rudiment. The change in size of this lumen through the rod of connective tissue may be traced in serial sections taken from area A-B of the regenerating intestine of an animal at Stage 5 (see Pl. II, Figs. 5, 6, 7 and 8). In Fig. 5 there are three individual lumina in the connective tissue core of the rudiment. It will be seen by following these lumina in the succeeding sections, Pl. II, Figs. 6, 7 and 8, that the right and left central ones are enlarging in a direction parallel to the long axis through the cord in one direction, while the lumen at the top of the section is enlarging in the opposite direction. The lumen at the top of the section in Pl. II, Fig. 5, may be seen to be reduced in size with thickened epithelial lining in Fig. 6, and in Fig. 7 there is no longer a lumen present, only a solid patch of epithelial cells. The opposite sequence may be seen in the left and right central lumen. In Fig. 6 there is a streak of epithelial cells running from the lumen lining out into the surrounding connective tissue, and in Fig. 7 the lumen extends up into this streak. The sequence of events is clear. There are present in the rudiment a number of blind lumina running through the connective tissue of the rod formed as demonstrated in Pl. I, Figs. 2, 3 and 4. These extend in both directions along the axis of the rudiment, preceded by a solid core of actively multiplying epithelial cells. Thus numerous small lumina arise in this primary solid rod of tissue, destined to fuse into a continuous tube.

Sections of the rudiment A-B at Stage 6 (not illustrated) show it to be a simple tube with a single lumen running throughout. The walls of this tube are simply constructed, being of three layers: external, epithelium, generally one cell thick, surrounding the tube; connective tissue; and internal epithelium, one cell thick, lining the lumen. The internal epithelium closely resembles the external.

Sections made of the rudiment at G in a Stage 6 specimen show it to be largely a solid rod similar to that in the area B-F in Stage 4. There are some places at which there is a proliferation of epithelium at the periphery of the rod with subsequent folding and inclusion of a lumen but no well-defined, centrally located lumen is present.

To determine the extent of differentiation of the intestine wall, sections were made of the anterior dorsal intestine in area A-B from an animal which had regenerated for 25 days at the higher temperature range and whose gut was functional, at least mechanically, in ingesting and passing food matter. The histological picture of this animal was compared with sections made of the same area of the intestine of a normal animal. The normal picture corresponds closely to that described in detail by Jourdan (1883) Ludwig (1889-1892) and Bordas (1899). The lumen is lined by columnar epithelium capped by a hyaline cuticle. This epithelial layer projects into the lumen in numerous slender villi. Below the epithelial layer is a solid layer of connective tissue surrounded by a muscle band largely made up of circular fibers. On the outside of this muscular layer is another layer of connective tissue surrounded by a one-cell-thick sheath of cuboidal epithelium. The intestine wall of the 25-day-regeneration specimen appears strikingly similar to this. The columnar epithelium with its hyaline cuticle is raised in a few slender villi although these are not nearly as numerous as in the normal intestine. The greatest difference between the normal and regenerated intestine is the lack of a well-defined muscle layer. Only a few circular fibers can be discerned running through the connective tissue in the regenerated intestine.

Discussion

It is apparent that, although the organ of Cuvier in *Actinopyga agassizi* contains the toxic factor holothurin, as reported by Nigrelli (1952), it plays no direct part in the secretion of this substance when the living animal is stimulated by rubbing. Further experimentation is necessary to determine whether or not there is some relationship between storage of the toxin by the organ of Cuvier and its secretion by the skin glands, although it has been demonstrated that there is no apparent effect on toxin secreted from the test of the animal over a period of five days following the removal of the Cuvierian organ. The single case of the survival of a pearl fish living inside a sea-cucumber after extensive poisoning indicates the possibility of a rather different role of the Cuvierian organ. It is conceivable that this organ, which contains the active toxic factor yet is not glandular in structure and apparently does not serve as a temporary storage reservoir in relation to the secretory cells of the test, acts in some way as a protection either on the respiratory system of the animal or on the coelomic fluid, possibly preventing poisoning of the sea-cucumber by its own toxin. If this were the case, a more direct relationship between the Cuvierian organ and evisceration might be hypothesized. The autotomy of the viscera would then be a reaction caused by the loss of the animal's protective mechanism, since the organ of Cuvier is eliminated prior to evisceration. Evisceration would be caused by exposure of the holothurian to its own toxin. On the basis of these preliminary studies, such a role of the Cuvierian organ can only be suggested as a possibility worthy of further investigation.

Although as described for *Stichopus regalis* (Bertolini, 1930), the regenerating intestine of *Actinopyga agassizi* arises along the entire free edge of the supporting mesentery, it has been found that in *A. agassizi* there is considerable variation in growth and development in different areas. The area B-F leads growth in widening of the mesentery, enlargement of the intestine rudiment and development of a lumen within the connective tissue core of the rudiment. This demonstrates initiation of regeneration and some control in differentiation derived locally from the mesentery rather than from the anterior broken tube of the alimentary canal. Not only is the early, solid, rod-like rudiment derived from the mesentery, but also the epithelial elements which invade the connective tissue core in the formation of a lumen originate locally from the simple external epithelium, rather than migrating into the formed rudiment from the lumen of the retained anterior fragment of the alimentary tract. This anterior fragment apparently has no influence on the regeneration of the intestine. Its end opens into the coelom, closes in the early stages of regeneration, and opens again only after the rudiment has been developed into a tubular structure.

The factors which influence the higher speed of development in the area B-F cannot be clearly discerned at this time. Because of the important role of the mesentery, it is conceivable that in this area, in which a very large portion of the mesentery is gathered to support a relatively short portion of regenerating rudiment, one factor is simply that of quantitative influence of the large area of mesentery. In no other area throughout the track of the regenerating system is there even an approach to the ratio of mesentery to rudiment that exists in this area. It is in the initial stages of regeneration that the area B-F shows such marked advance over other areas. As the rudiment in this area lengthens, thereby reducing the mesentery-to-rudiment ratio, the areas A-B and F-H of the rudiment gain and eventually parallel the development of B-F.

Although the regeneration of the intestine has not been studied through to a condition closely resembling the normal one, regeneration appears to be sufficiently complete in the later specimens to permit ingestion and digestion of food matter. The histological picture of the regenerated intestine closely resembles that of the normal specimen in all but muscular development. It appears that further development consists chiefly of increasing length of the intestine. In addition, there must be further differentiation of musculature in the intestine walls and an increase in diameter before the regenerated organ closely resembles the normal.

Summary

1. As has been found to be true of other aspidochirote holothurians, stimulation of evisceration can be easily effected in *Actinopyga agassizi* Selenka by a variety of conditions including high temperature and the presence of various metallic ions.

2. Preliminary experiments indicate no readily apparent relation between the organ of Cuvier and the ability of the living animal to release a potent quantity of the toxin, holothurin.

3. Histological sections of the organ of Cuvier show that it is not of tubular structure as described for other species, and bears no resemblance to a secretory organ. It gives the appearance rather of a storage tissue.

4. Subsequent to evisceration, the formation of an intestine rudiment takes place along the entire free edge of the remaining mesentery, but one area of growth proceeds at a faster rate than

all other areas. The greater length of the original mesentery and differential growth rates result in position shifts of the short, developing intestine rudiment.

5. A lumen is formed by proliferation of simple external epithelium at the periphery of the rudiment, followed by folding of the surface of the rudiment resulting in the inclusion of blind lumina within its connective tissue core. These lumina progress in both directions parallel to the axis of the rudiment, preceded by a mass of epithelial cells.

6. After 25 days of regeneration at 30° -32° C., the intestine is functional and histologically resembles closely the normal organ, although it is shorter and less convoluted and the walls lack normal musculature.

Bibliography

Bertolini, F.

1930. Rigenerazione dell'apparato digerente nello *Stichopus regalis*. Pubb. Staz. Zool. Napoli, 10: 439-447.

Bordas, L.

1899. Étude sur l'anatomie et les functions physiologiques des poumons aquatiques des Holothuries. Ann. Mus. d'Hist. Nat. Marseille, Zool., tom. 5, mém. 3: 5-15.

Crozier, W. J.

1915. A note on the physiology of the Cuvierian organs of *Holothuria captiva* Ludw. Amer. Jour. Physiol., 36: 196-202.

Cuénot, L.

1891. Études morphologiques sur les Echinodermes. Archives de Biologie, 11: 369-374.

Deichman, Elisabeth

1930. The Holothurians of the western part of the Atlantic Ocean. Bull. Mus. Comp. Zool., Harvard, 71: no. 3.

Guislain, R.

1953. Recherches histochimiques sur les canaux de Cuvier de *Holothuria impatiens* Forskal. C. R. Soc. Biol. (Paris), 147 (13-14): 1254-1256.

Hérouard, E.

1889. Recherches sur les holothuries des côtes de France. Arch. Zool. Exp. et Gén., sér. 2, tom. 7: 535-704.

Jourdan, Etienne

1883. Recherches sur l'histologie des Holothuries. Ann. Mus. d'Hist. Nat. Marseille, Zool., tom. 1 mém. 6: 5-64.

Kille, F. R.

1936. Regeneration in Holothurians. In Ann. Rept. of the Tortugas Laboratory. Carnegie Instit. Wash. Year Book no. 35: 85-86.

Ludwig, Hubert

1889-1892. Echinodermen. In Bronn, H. G., Klassen und Ordnungen des Tier-Reichs.

Minchin, E. A.

1892. Notes of the Cuvierian organs of Holothuria nigra. Ann. Mag. Nat. Hist., ser. 6, 10: 273-284.

Mines, George Ralph

1912. Note on the mechanism of discharge of the Cuvierian organs of *Holothuria nigra*. Quart. Jour. Micr. Sci., N. S., 57: 301-304.

Nigrelli, R. F.

1952. The effects of Holothurin on fish, and mice with Sarcoma 180. Zoologica, 37: 89-90.

Nigrelli, R. F. & P. A. Zahl

1952. Some biological characteristics of Holothurin. Proc. Soc. Exper. Biol. and Med., 81: 379-380.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. Single strand ("tubule") of organ of Cuvier, in cross-section. 70×.

Fig. 2. Cross-section of intestine rudiment showing proliferation of external epithelium at the top of the section, left of center. 70×.

Fig. 3. The same area in the following section showing the proliferating epithelium thrown into deep folds. 70×.

Fig. 4. A subsequent section showing the area of thickened epithelium cut off from the surface and surrounding a small lumen.

Plate II

Fig. 5. Transverse section of intestine rudiment A-B, showing three lumina. 16×.

Fig. 6. A following section showing the same three lumina. The one at the top of the section narrows as it nears the blind end. the other two becoming larger. A cord of epithelial cells is seen proliferating from the left central lumen. 16×.

Fig. 7. A following section showing closure of the lumen at the top of the section, only a core of epithelial cells remaining. The epithelial cord of the left central lumen now possesses a lumen of its own. 16×.

Fig. 8. This section shows complete obliteration of the lumen at the top, while the two central lumina are larger still. This is interpreted as the two central lumina progressing lengthwise of the intestine in a direction toward the viewer, the top lumen progressing in the opposite direction. Top of Section: A few epithelial cells. Center: Right: Single-cell boundary of epithelial lining bulges into lumen.

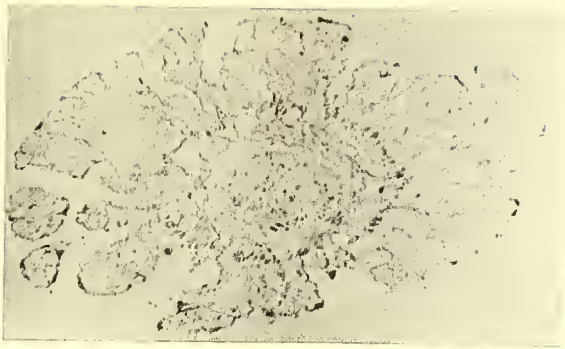


FIG. 1

FIG. 2

FIG. 3

FIG. 4

OBSERVATIONS ON EVISCERATION AND VISCERAL REGENERATION
IN THE SEA-CUCUMBER, ACTINOPYGA AGASSIZI SELENKA

FIG. 5

FIG. 6

FIG. 7

FIG. 8

OBSERVATIONS ON EVISCERATION AND VISCERAL REGENERATION
IN THE SEA-CUCUMBER, ACTINOPYGA AGASSIZI SELENKA

5

Hematology of Healthy and Virus-diseased Sockeye Salmon, *Onchorhynchus nerka*[1]

MARGARET E. WATSON,[2] RAY W. GUENTHER[3] & RODNEY D. ROYCE[3]

(Plates I-III; Text-figures 1-4)

INTRODUCTION

Epizootiological investigations have been routine in this laboratory[4] ever since a virus disease began depleting the Federal hatchery stocks of fingerling sockeye salmon in 1951 (Rucker *et al.*, 1953; Watson, 1954; Watson, Guenther & Rucker, 1954). Only cursory observation of stained blood films had been made from diseased fish. More detailed hematological studies were instituted by a short term budget allotment to determine the characteristic blood picture of the virus disease. It was also of interest to find a rapid diagnosis of the disease from either live or stained blood films. In addition, determination of hematocrits was undertaken.

LITERATURE REVIEW

Hematology of teleosts is still a pioneering field of research. Reports on morphology of normal fish blood cells are not scarce but are contradictory. The best reviews of this subject are found in the doctoral dissertations of Yokoyama (1947) and Katz (1949). Dombrowski (1953) has described the normal blood picture of 15 species of fresh water fish.

The very scant literature on hematological characteristics of diseased fish is described by Katz (1950). The hematology of carp afflicted with stomach dropsy has been discussed by Lyman & Shpolanskaia (1949), Schäperclaus (1953) and Dombrowski (1954). Glucksman & Gordon (1953) have studied the hematology of normal and melanomatous fish.

No reports have been found on the observation of blood cells of fish with a phase-contrast microscope although exploratory and also significant hematological studies of higher vertebrates have been undertaken with this equipment (Bennett *et al.*, 1951, p. 213).

Values for percent. hematocrit (the volume of packed red blood cells) have been found for 10 species of fresh water fish and 4 species of marine fish (Young, 1949; Aserinsky, 1954; Lepkovsky, 1930; Hall, Gray & Lepkovsky, 1924; Wilson & Adolph, 1917; Vars, 1934; Field, Elvehjem & Juday, 1943; Benditt, Morrison & Irving, 1941). The average hematocrit values for fresh water fish varied from 24.8 to 46.1 percent.; for marine fish from 13.5 to 29.5 percent. Methods used for obtaining blood were cardiac or bulbous puncture, severance of tail and pricking of caudal artery above the posterior margin of the anal fin.

It is believed that this study will report the first account of a micro-hematocrit method for small fish (30-60 mm. in fork length). These fish could deliver only one or two drops of blood after their tails had been severed.

MATERIALS AND METHODS

The sockeye salmon fingerlings used in this study were reared at the Federal hatchery in Leavenworth, Washington, 130 miles from Seattle, Washington, and were trucked to the laboratory for use in experimental infections. In Seattle, 200 of these fish were kept in running spring water in a baked enamel trough, 4′×1′×6″, held at 50-54° F. The water level averaged 5 ½″. Sockeye fingerlings which contracted the virus disease by natural infection were kept at the Leavenworth hatchery in a trough which

[1] Work done under Chief of Western Fish Disease Laboratory, Fish and Wildlife Service, United States Department of the Interior.

[2] Present address: Department of Pathology, School of Medicine, University of Wisconsin.

[3] Fish and Wildlife Service, United States Department of the Interior.

[4] Western Fish Disease Laboratory, Fish and Wildlife Service, United States Department of the Interior, Fisheries Center, University of Washington, Seattle, Washington.

contained 13,000 fish at the onset of the disease. These diseased fish were brought to Seattle in lots of 400 for three successive weekends. At Seattle hematological studies were made on fish naturally infected with the virus disease and on fish which had earlier been experimentally exposed to the virus.

Experimental Procedures for the Study of the Virus Disease

Preparation of virus material for experimental infection of fish.—The original source of the virus for this research was virus-diseased fish naturally infected in hatcheries in the state of Washington. This virus material was passed through at least four serial transfers prior to the making of a frozen stock suspension. The suspension consisted of a frozen (−40° C.) emulsion of infected fish which had been diluted 1:3 with tap water and sealed in glass vials. After six months, this culture was again transmitted through 13 serial transfers before use in the experiment. For this study, fish from the 13th serial transfer were blended in an "Osterizer" for 30 seconds and centrifuged for 3 minutes at approximately 2,000 rpm. The supernatant was then filtered through a 7 lb. Mandler filter. The filtrate was diluted with sterile tap water to a concentration of 1:100. For the artificial infection, 200 fish were suspended for 15 minutes in a gallon of Mandler filtered suspension diluted 1:100. Control fish were given the same treatment as the infected fish except for the exposure to the virus agent.

Protocol.—Antecedent to the experiments recorded in this study, four trial infection experiments were carried out in order that hematological characteristics of the virus disease could be determined. Following these, three lots of 200 fish each were used. One lot comprised the control or uninfected fish. On May 11, 1954, the two remaining lots were suspended in 1:100 dilution of infected material for 15 minutes. Beginning on May 12 and daily thereafter through May 20, then again on May 23 and June 7, the following techniques were practised. An average of 6 infected fish and 6 control fish were used daily, and for each a Wright-stained and a peroxidase-stained blood film was prepared. One live film was studied with a phase-contrast microscope, either unstained or supra-vitally stained with brilliant cresyl blue, and one hematocrit was made. Lack of blood or time sometimes prevented observation of a live film on all 12 fish. Blood samples from 73 infected and 61 control fish were analyzed during the experiment.

On May 15, naturally infected fish were brought into the Seattle laboratory from the virus-infected trough at the Leavenworth hatchery. The protocol described above for the experimentally infected fish was followed with these naturally infected fish on May 16, 21, 22, 23, 29, 30, June 1 and 9. Controls were likewise employed with these fish. Sixty-four infected and 28 control fish were studied.

Preparation of equipment and fish for hemic studies.—Microscope slides and coverslips were soaked in concentrated nitric acid for a minimum of 4 hours, then washed in running tap water for 4 or more hours, then rinsed in distilled water and finally stored in 95 percent. ethyl alcohol. Slides and coverslips were dried with clean gauze just prior to use.

Fork length, head length, general external appearance and activity were recorded for each fish. The fish was then placed between blotters or covered with gauze in such a way that only the portion of the body immediately posterior to the anus was exposed. This exposed portion was dipped into 95 percent. ethyl alcohol, dried and wiped quickly with gauze. The tail was then severed from the body with scissors by a quick firm snip in the region immediately posterior to the anal and adipose fins. The drop of blood which oozed from the severed caudal vein and artery was touched to a capillary hematocrit tube. After sufficient blood had entered the tube, the remainder of the drop of blood on the fish was first touched to one coverslip from which a stained film would be made and then touched to a second coverslip from which a live wet film or a supra-vitally stained film would be prepared. For these small fish, cover slips used were 15 mm. square, No. 1 thickness.

Stained blood films.—All blood films were routinely prepared by the standard coverslip technique (Wintrobe, 1949). One coverslip of the pair used was stained with Wright's stain and the other with peroxidase stain (Hughes, 1953). The freshly prepared and dried films were not fixed in methyl alcohol prior to staining. The peroxidase stain was made with absolute methyl alcohol instead of 95 percent. alcohol, hence fixation was unnecessary.

Live unstained and supra-vitally stained blood films.—A freshly cleaned slide was lowered carefully toward a drop of blood on a coverslip until the blood touched the slide. The slide was then upturned and the edges of the coverslip ringed with petroleum jelly. For supra-vital staining, a freshly cleaned coverslip was touched by a very small drop of brilliant cresyl blue (0.1 percent. in absolute ethyl alcohol, freshly filtered). A second clean coverslip was placed over the slip containing the stain, and the two were pulled apart immediately. As soon as the stain on each coverslip dried (a few seconds),

the stained coverslip was ready to receive a drop of blood.

Micro-hematocrits.—A self-filling capillary tube[5] with a capacity of 5 or 10 lambda or the narrow stem of a red blood cell diluting pipette was used as a hematocrit tube. After a tube was filled with blood, the length of the column of blood was measured with a micrometer calipers which was read to tenths of a millimeter. The end of the tube at which the blood had been introduced was closed with sealing wax. The hematocrit tube was inserted into a piece of thick-walled rubber tubing before it was placed in a centrifuge, thus cushioning it from damage by the sides of the metal centrifuge tube holder.

All samples were centrifuged in a clinical model centrifuge at 3,000 rpm. for 35 minutes, and micrometer measurements of red blood cell volume and total volume were recorded within half an hour after centrifuging. If the volume of blood recorded before centrifuging varied more than 1 mm. from the volume measurement of total blood after centrifuging, the sample was discarded.

The use of an anticoagulant was necessary. The hematocrit tubes were rinsed in a solution of heparin (0.25 mg./cc. of sterile distilled water) and blown dry with a jet of air, rinsed a second time with a fresh drop of heparin solution and again blown dry.

Hematocrit tubes were cleaned first by ejecting the wax plug with a fine wire and then by rinsing several times in normal saline and 95 percent. ethyl alcohol. Occasionally a rinse in concentrated nitric acid was required to clean the tube thoroughly.

Phase-contrast microscopy photographs.—An American Optical phase-contrast microscope was used with an American Optical (No. 735) spherical microscope illuminator or a Wilcox Strobolite with or without ground glass interference. A 1.8 mm. medium dark-contrast objective was used. The camera was a 4×5 Speed Graphic adapted for photomicrography.

Morphology of Normal Blood Cells

Time permitted only a few blood counts on normal and diseased fish. Hence, the following account can only be qualitative. Normal, apparently healthy fingerlings provide a very uniform blood film for study. Mature erythrocytes are so numerous that one must examine the blood film carefully to detect the presence of the few lymphocytes and clusters of thrombocytes. Occasionally a neutrophil is seen as the only representative of a granulocyte.

[5] Obtained from Wakefield Industries Corporation, Skokie, Ill.

One to 2 percent. of the erythrocytes are orthochromatophil or polychromatophil normoblasts. They can be distinguished from the mature erythrocytes by their chromatin pattern, characteristics of the mitochondria and the size of the nucleus relative to the size of the cytoplasm.

In the nucleus of a mature erythrocyte (Plate I, Fig. 4, left of center), the nuclear membrane is very thin, the chromatin particles are small and form a tightly meshed network. Two examples of an orthochromatophil normoblast seen in Plate III, Fig. 13, show that the nuclear membrane is more distinct than that in a mature erythrocyte and that the chromatin particles are slightly larger and less tightly meshed. In a polychromatophil normoblast (3 cells in Plate III, Fig. 14), the chromatin particles are large and form a coarse-stranded meshwork; the nuclear membrane is definitely thicker than that seen in the mature erythrocytes.

Mitochondria in a mature erythrocyte are few in number (8-12) and appear as tiny dots or short rods. In an orthochromatophil normoblast the number of mitochondria is roughly twice that found in a mature erythrocyte, and the majority of them are short, fat rods, although a few may be long, thin rods. In a polychromatophil normoblast, the majority of the mitochondria are long, very thin rods which sometimes appear tangled or interwined. The cytoplasm of the polychromatophil normoblast is crowded with mitochondria. In a live blood film, stained supra-vitally with brilliant cresyl blue, the mitochondria in all erythrocytes become a light shade of blue when examined under bright-field microscopy.

The cytoplasmic area in a polychromatophil normoblast is less than that in either an orthochromatophil normoblast or a mature erythrocyte. The cytoplasm of a mature erythrocyte appears to cover a large surface area because the nucleus is slightly smaller than that of the polychromatophil or orthochromatophil normoblast. The nucleus in both the mature erythrocyte and in the orthochromatophil normoblast is oval, while that in the polychromatophil normoblast is usually more round than oval.

The size of the 3 erythrocytes just described is variable and is directly related to the proximity of either an impending or recent mitotic division. Cells which have recently divided can be as small as 17 microns on their long axis. Cells near division can be as long as 22 microns. The average dimensions of the majority of mature erythrocytes are 19×11 microns. The average nucleus measures from 7-9×4-6 microns.

A basophil normoblast, rarely seen in the

blood films of healthy fish, is labeled *BN* in Plate I, Fig. 4. The nucleus and cytoplasm have almost the same index of refraction, and the nuclear chromatin appears homogenized but does contain indistinct areas of chromatin agglomerations. A thin, but definite, nuclear membrane is present, and occasionally the chromatin forms small thickenings on the inner surface of the nuclear membrane. Mitochondria are long and so thin that they are difficult to discern. Occasionally the mitochondria are arranged like small beads on a string instead of being thin, smooth rods.

The leukocytes seen in normal sockeye salmon fingerlings are lymphocytes, thrombocytes (the nucleated homologue of the human platelet) and neutrophils (heterophils). A small active lymphocyte, labeled *L*, is shown in Plate III, Fig. 15, and a small quiescent lymphocyte occupies the center of Plate III, Fig. 16. The active lymphocyte has a round nucleus which occupies almost the entire cell, and that part of the cytoplasm which is visible forms a pseudopod containing short rod-like or small dot-like mitochondria. In Plate III, Fig. 16, the mitochondria point inward toward the indented part of the nucleus. These mitochondria could not be resolved photographically. An example of a large lymphocyte, which is seldom seen in healthy fish, is pictured at the lower right of Plate II, Fig. 9. The mitochondria in this large lymphocyte are larger than those in the small lymphocyte and form either long rods or spindles. In both large and small lymphocytes, the nuclear membrane is etched sharply. The chromatin in the small lymphocyte is coarsely clumped, and the open meshwork between chromatin strands is refractile (Plate III, Fig. 16). The chromatin in the large lymphocyte consists of coarsely meshed strands, but the open meshwork is less refractile than that in the small lymphocyte. Neither the small lymphocytes (5 to 10 microns in diameter) nor the large lymphocytes (18 to 25 microns in diameter) display movement other than a very slow oozing or rearrangement of the cytoplasm.

Small lymphocytes and thrombocytes appear quite similar when a live blood film is examined for the first minute after its preparation. Following the first minute, the thrombocytes bleb or balloon out, thus performing their natural function of clotting. A blebbed thrombocyte occupies the right center of Plate I, Fig. 1. The white circular dot near the upper border of the thrombocyte is seen only in virus-diseased fish. The fine threads of fibrin can be seen radiating from the periphery of the cell. An unblebbed thrombocyte in a fingerling sockeye salmon would resemble the lymphocyte pictured in Plate III, Fig. 16, except that the nucleus of the cell would have only a small crevice instead of a deep indentation or the nucleus would completely fill the cell. Only 3 or 4 tiny round granules may be seen in the cytoplasm of a thrombocyte. As a fingerling sockeye salmon approaches a length of 50 mm., a few mature thrombocytes can be seen. These are similar in appearance to the size and shape of the nucleus in a mature red blood cell. A thin, almost imperceptible, rim of cytoplasm encircles the nucleus. In live or stained films from healthy fish, the thrombocytes are usually clumped in groups of 2, 3 or 4.

The most motile cell in a live blood film is the neutrophil or heterophil. As a rule the nucleus trails while the cytoplasm leads the direction of motion. A nonfilamented neutrophil is pictured at the center right of Plate I, Fig. 3. The white refractile dot in the cytoplasm is only characteristic of neutrophils in fish afflicted with the virus disease. This non-filament neutrophil is considered a metamyelocyte which can be recognized by the appearance of the nucleus in an active cell; the nucleus alternately has a hump (as in Plate I, Fig. 3) or a depression midway along its length, as if it performed an undulating motion. The cytoplasm also contains a few tiny round granules which appear slightly darker than the mitochondria. Metamyelocytes are not common in the blood of healthy fish.

The bi-lobed neutrophil is normally the predominant stage seen in the peripheral blood, although highly polymorphic nuclei with 3, 4 and 5 lobes are also occasionally seen. No promyelocytes or myelocytes could be identified as such in any live films examined. After the peroxidase test, the cytoplasm of all neutrophils, regardless of their maturity, was replete with purplish black granules.

Basophils and eosinophils are easily identifiable with phase-contrast microscopy. The eosinophil is a small round cell (8-12 microns) which is entirely filled with minute refractile granules (1-2 microns). These granules are as luminous as a mass of tiny, very bright, light bulbs. The nucleus was rarely seen. When visible it was thin and flattened at one edge of the cell membrane. An eosinophil has never been seen in motion.

Though eosinophils and basophils are uncommon in healthy fish, from 5 to 10 of each have been seen on a single blood film from starved fish, virus-diseased fish and fish infected with bacterial gill disease. Plate II, Fig. 8, depicts one type of basophil which is the same size and shape as the eosinophil just described. Instead of very refractile granules, the basophils have very dark granules which are encircled by a bright halo when viewed under a phase-contrast micro-

scope. The nucleus of the basophil in Fig. 8 is not visible. All of the granules, which really fill the entire cell, are impossible to focus in the same plane; thus the pictured cell appears to be only partially filled with granules. On the other hand, the majority of the granules filling the cytoplasm are visible in the basophil pictured in Plate II, Fig. 9. This cell is flattened, while the basophil in Fig. 8 is rounded. In a flattened basophil, the nucleus is eccentric and has a coarse network of chromatin which is disposed in thick clumps at points of anastomoses. No instance of a polymorphic nucleus has ever been seen in live or stained films. The basophils in Figs. 8 and 9 showed no signs of movement; however, a third type of basophil illustrated in Plate II, Fig. 7, glided slowly with the cytoplasm trailing the nucleus. This is an immature cell, there being fewer and smaller cytoplasmic granules. The diffuse chromatin reveals a faint network.

The granules in all three types of basophils just described have in common an unusual internal characteristic. Each granule contains 2 or 3 parallel striae which appear darker than the granule itself. In addition these granules are specifically stained a deep bluish-purple by the supra-vital dye, brilliant cresyl blue. No other cellular component in the blood of sockeye salmon is so colored by this stain. When one is searching for the presence of basophils in a supra-vitally stained blood film, they can be easily detected by their purplish color during examination with bright-field microscopy and 100 magnification. Corroborating evidence for the identification of basophils of teleosts by their supra-vitally stained granules may be found in Michels (1938, p. 327).

Morphology of Blood Cells Associated with the Virus Disease

When viewing either a stained or live blood film from a fish heavily infected with the virus disease, one is immediately impressed with the tremendous amount of cellular debris and unidentifiable particles present in almost every field of observation. From a study of a stained film alone, it would have been impossible to determine the origin of this debris, examples of which are shown in Plate I, Figs. 2, 4 and 5, and Plate II, Figs. 10 and 11.

Besides the cellular debris in the blood, the next most obvious characteristic is the delayed clotting time. Ordinarily, with healthy fish, one must work with utmost speed when preparing live or stained blood films in order to prevent clotting, which occurs in about 15 or 20 seconds. Blood from a virus-diseased fish will remain fluid as long as 3 minutes after exposure to air.

At the first sign of decreased clotting time, the thrombocytes in a Wright-stained blood film have a smudged, unevenly faded blue appearance, instead of the normal, homogeneously dark purple. They may or may not have a tiny, dark brown, round pigment spot in their cytoplasm. This pigment spot is about 1 micron in diameter when it is round, but it also may appear as a short fat rod (2×1 microns). With phase microscopy, this included pigment spot appears as a refractile dot. The thrombocyte in Plate I, Fig. 1, is in its earliest stage of degeneration and possesses an intracytoplasmic refractile dot. Plate I, Fig. 2, shows a totally degenerated thrombocyte with 3 pairs of pigment granules. If a live film is examined with bright-field microscopy, these refractile dots appear dark brown to black. These pigment granules have no affinities for stain in a Wright-stained film but do retain the dark brown color which is seen in a live film under bright-field observation.

A totally degenerated thrombocyte, as seen in Plate I, Fig. 2, with phase-contrast microscopy in a live mount, cannot be recognized as such in a Wright-stained film. One can only see the pigment granules in a stained film with bright-field microscopy; however, if the stained film is examined with a phase-contrast microscope, the outline of the degenerated thrombocyte with its included pigment granules is made visible. Variously diseased salmonoids other than sockeyes have been examined and no live or stained blood film has disclosed degenerated or degenerating thrombocytes with included pigment granules. Pigment granules free in the serum are occasionally seen in healthy fish but their presence has been attributed to leaching of pigment from the incised tissues of the tail region during the preparation of the blood film.

During one phase of the virus disease there exists a true basophilia which reveals the existence of the three types of basophils described earlier and pictured in Plate II, Figs. 7, 8 and 9. The majority of the basophils are of the type seen in Fig. 9. Gradually the large numbers of normal-appearing basophils are replaced by degenerating basophils, an example of which is illustrated by Plate II, Fig. 10. The degenerating basophil usually does not have pigment granules, but in a few instances they have been seen. Fig. 10 indicates the pigment granule as a white circle with a gray center dot. The granules in a degenerated basophil are irregular in shape rather than round and do not take a brilliant bluish-purple stain but rather a faded blue color in a film supra-vitally stained with brilliant cresyl blue. The fine striae seen in the granules of normal basophils no longer exist in the irregularly-shaped granules of the degenerating basophil.

Another characteristic of the blood picture in a virus-diseased fish is clasmatosis—the dissemination or scattering of basophil granules, and to a far lesser degree, the dissemination of eosinophil granules in the blood stream. The extracellular granules of basophils are usually enclosed in bits of cytoplasm and appear singly, paired or as many as four in a group (Plate II, Fig.11). In a Wright-stained film there have been only single, isolated instances when these extracellular granules have been located adjacent to or in the immediate vicinity of mature basophils. It is not believed that the granules represent an artifact produced by the mechanical rupture of the basophil. In a live film, they do not possess the fine striae which exist in the intracellular granules. Also, these extracellular granules take on either no color or only a faded blue color in a blood film supra-vitally stained with brilliant cresyl blue. Macrophages have been observed with faded blue granules recently included in their cytoplasm (Plate I, Fig. 6) Phagocytized eosinophil granules have not been observed.

After the peak of mortalities has passed, engorged and degenerating macrophages outnumber all other leukocytes. The nuclear chromatin forms a diffuse spongy network; the cytoplasm has long thin mitochondria and a few dark round granules (Fig. 6) Macrophages are monstrously large cells—one occupying the space which three normal erythrocytes would fill if placed side by side. Little or no ameboid movement was witnessed in macrophages. As many as 12 pigment granules have been seen in an engorged macrophage. Plate I, Fig. 5, shows a macrophage which had recently ingested a large degenerated lymphocyte. The nucleus of this macrophage is a thin lunate structure pushed toward the periphery of the cell.

The presence of many large lymphocytes is also characteristic of the blood of fish severely infected by the virus (Plate II, Fig. 9, center right). Degeneration in the large lymphocyte is manifested by an included pigment granule or cytoplasmic vacuolization, or both. The vacuoles are not refractile. In a Wright-stained film, a degenerated lymphocyte has a thin rim of pale blue cytoplasm and a gray to grayish-blue nucleus. A large normal lymphocyte has a reddish-purple nucleus.

The large leukocyte seen in Plate II, Fig. 12, is as yet unidentifiable. It has rarely been found in the blood of healthy fish. Its cytoplasm differs from that of all other leukocytes in that it contains only small spherical granules which are dark and do not resemble the mitochondria seen in other leukocytes. These granules are about one-third the size of those found in a basophil. Some overlie the nucleus, which appears as a diffuse mass of chromatin circumscribed by a thin membrane. The cell has a slow creeping motion with the cytoplasm leading the nucleus. No counterpart for this cell in a stained film could be found. Further study on larger numbers of these cells is necessary before a definite identification can be made. At this time it will be referred to as a monocyte.

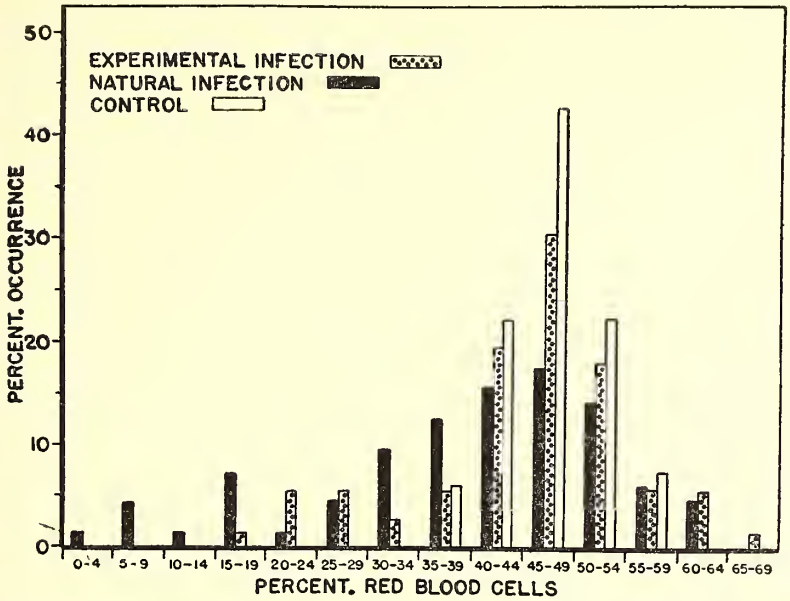
In fish which are recovering from the virus disease there is an extremely high increase in the number of immature erythrocytes (basophil normoblasts particularly). Many of the immature erythrocytes are undergoing mitosis, which is rarely seen in the blood of healthy fish.

Hematocrits

Healthy fingerling sockeye salmon.—The average percent. hematocrit of 68 control fish was 47, representing a range of 35.8 to 57.9 and a variation of 38 percent. (Text-fig. 1). The fish whose hematocrits were in the range of 35 to 39 and 55 to 59 percent. (8 fish) did not present a truly healthy blood picture upon analysis of their Wright-stained films. A variety of abnormalities was represented, neutrophilia and anemia being the most prevalent. They did not show any evidence of the virus disease.

The hematocrits of the control fish used in conjunction with the experimentally virus-infected fish are plotted in the upper part of Text-fig. 2. Beginning the seventh day after artificial infection, the controls also showed evidence of infection not only from the analysis of the live and stained blood films but also from the rise of the average percent. hematocrit. It is not possible to ascertain how the accidental contamination of the control trough occurred. Both the controls and the infected fish were kept in the same hatchery; however, separate equipment was assigned for each trough. The main stock of healthy fish, from which all experimental fish were obtained, was kept in a different hatchery. These stock controls did not acquire the virus disease; hence, accidental contamination was presumed to have occurred in the controls used. A new group of control fish was selected from the stock of healthy controls for obtaining the data listed under the twelfth and twenty-seventh day after infection.

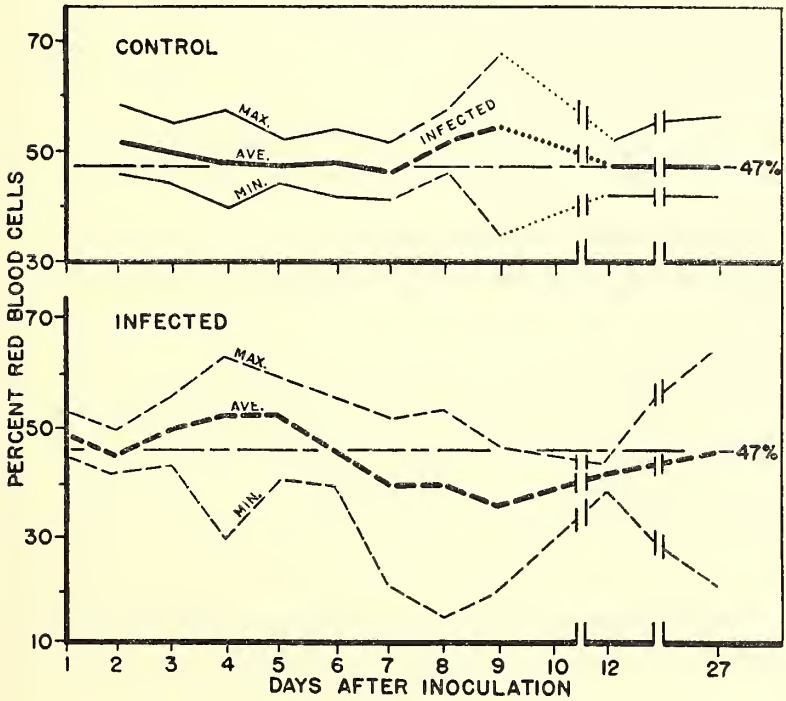
Fingerlings experimentally infected with the virus.—The average peak hematocrit was 54.8 (maximum 68) for the accidentally infected controls and 53 (maximum 64) for the experimentally infected fish. For the latter group this peak occurred on the fourth day after infection. From Text-fig. 3 it is possible to correlate the average hematocrit with the percentage of daily mortality. Two days following the peak hematocrit, the maximum number of mortalities oc-



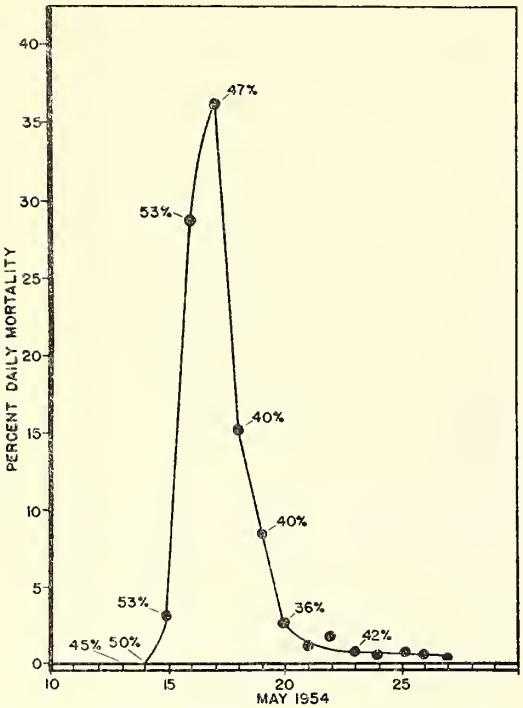
TEXT-FIG. 1. Hematocrit values of fingerling sockeye salmon – control and virus-diseased.

curred—35 percent. of the total fish inoculated with the virus. The explanation for this may be that, because of the shock incurred from the infection, there is a loss of plasma which accounts for the rise of average percent. hematocrit; following the effects of shock, the plasma level and the normal percent. hematocrit (47) are restored in the surviving fish by the sixth day after exposure to the virus.

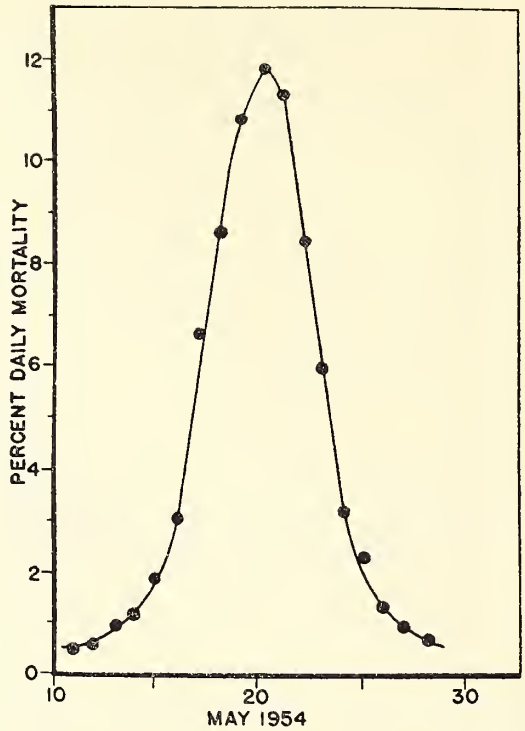
Fingerlings naturally infected with the virus.—On May 12, 1954, only 4 fish survived transport to Seattle from the disease-infected trough at the Leavenworth hatchery. It was possible by the examination of live blood films of these fish with a phase-contrast microscope to detect the typical blood cells associated with the virus disease heretofore studied only in experimental infections. The next lots of diseased fish were trans-



TEXT-FIG. 2. Maximum, minimum and average hematocrit values of control and experimentally virus-infected fingerling sockeye salmon.



TEXT-FIG. 3. Percentage of daily mortality of fingerling sockeye salmon experimentally infected with the virus disease, and average hematocrit values of moribund fish delineated on the mortality curve.



TEXT-FIG. 4. Percentage of daily mortality for the duration of the virus infection in a trough containing 13,000 fingerling sockeye salmon naturally infected at the hatchery in Leavenworth, Kansas.

ported to Seattle on May 16, 21 and 28. Since an insufficient number of samples was studied from the onset of infection up to the day of peak mortalities (May 11-May 20), complete hematological data for the entire course of the disease could not be tabulated as was done for the experimentally infected fish (Text-fig. 2).

From May 1 to May 10 the daily mortalities in the diseased trough at Leavenworth ranged from 8 to 24 fish a day. On May 11 there were 65 dead and on May 12 there were 89. A cumulative mortality of 50 percent (6,500 fish) was recorded on May 20, on which day also occurred the peak mortality (1,620 fish) (Text-fig. 4). By May 28 the cumulative mortality had reached 80 percent. These mortalities were comparable both in total magnitude and daily incidence to those which had occurred during the virus epidemic at this same hatchery the preceding year (Watson, Guenther & Rucker, 1954).

The highest hematocrit recorded for the naturally infected fish was 64 percent. on May 21. The lowest hematocrits were 16 percent. for the experimentally infected fish and 1 percent. for the naturally infected fish. Beginning May 22 and continuing through June 7, hematocrits below 35 percent. were recorded for 30 percent. of the fish examined. By contrast, only 15 percent. of the experimentally infected fish registered hematocrits below 35 percent. Infected fish in both groups registering hematocrits below 35 percent. displayed erythema at one or more of the following areas: base of any or all of the fins, at the anus and the isthmus. These low hematocrit values were probably caused not only by the erythema but also by the necrosis seen in hemapoietic areas of kidney tissue sections.[6]

Microscopic findings related to the hematocritis.—The hematological schedule on page 35 was compiled, presupposing one single fish could have been bled every day from the onset of exposure to the virus infection. These data are based on the results obtained from both experimentally and naturally infected fish.

DISCUSSION

This study has demonstrated that phase microscopy provides more useful visibility of live blood cells than does bright-field microscopy of stained blood cells. During the course of the virus disease, certain blood cells were difficult to differentiate with certainty by their staining characteristics because they were undergoing de-

Days after Infection	Percent. Hematocrit	Microscopic Blood Findings
1	45-50	No change from the normal.
2 3	45-50 50-55	A few thrombocytes possess one pigment spot; increase in number of metamyelocyte neutrophils.
4	55-65	Majority of thrombocytes and neutrophils contain one to four pigment spots; delayed clotting time; a few basophils; first mortalities occur.
5 6	65-55 55-45	Degenerated thrombocytes and neutrophils; a large number of basophils (10-12 on each blood film); a few large lymphoctyes, eosinophils and macrophages; extracellular granules of basophils; by the end of the sixth day maximum daily mortalities occur.
7	45-35	Large number of macrophages; pigmented degenerated thrombocytes, neutrophils; basophils and large lymphocytes; extracellular granules of basophils.
8 9	35-10 10-30	Immature erythrocytes outnumber mature erythrocytes; mitotic division seen in many of immature erythrocytes; macrophages; many unidentifiable particles of degenerated cells; clotting time still prolonged.
12	30-40	Polychromatophil and orthochromatophil normoblasts in minority, no basophil normoblasts; a few normal neutrophils and thrombocytes.
27	40-50	Clotting time normal; blood picture normal; first evidence of scoliosis in recovered fish.

[6] Unpublished data.

generation or were completely degenerated. The possible vagaries and variations in stains and staining techniques make it difficult to reproduce results. The characteristic striae seen on each basophil granule were not seen in a stained blood film, nor were they reproducible photographically. Thus, they could only be observed by phase microscopy.

Basophilia has not been recorded in published reports as a diagnostic feature of any other fish disease. The hematology of stomach dropsy in carp has been described in two separate studies. Dombrowski (1954) reported a decrease in lymphocytes and an increase in leukocytes, followed by degeneration of the leukocytes (a term which Dombrowski used without qualification). Lyman & Shpolanskaia (1949) also reported a decrease in lymphocytes, but were specific in naming the types of increased leukocytes, although they did not describe them. At the severest stage of infection, monocytes increased from the normal 5.7 to 38 percent., neutrophils from 0.2 to 7.0 percent., polymorphonuclear cells from 2.1 to 11.2 percent. The type of cells included in the term *polymorphonuclear cells* was not stated. Eosinophils were not found in the peripheral blood until the third year; the term *basophil* was not mentioned.

Besides basophilia, other notable characteristics of the virus disease are prolonged clotting time, the existence of pigment particles in the degenerating thrombocytes and neutrophils, and extracellular granules of the basophils. None of these characteristics has been delineated for any other fish disease described in the literature.

Beside microscopic blood findings, a sharp rise in percent. hematocrit values indicates the onset of the virus disease. However, future studies may prove that high hematocrit values are also corollary to other infections in fish. In two other instances, hematocrit values of fish have risen above the normal. Vars (1934) found that the toxicity of ammonium salts resulted in raising the mean percent. hematocrit of carp from 32 to 39. Menhaden have been exposed for various intervals of time to an asphyxiating environment by Hall, Gray & Lepkovsky (1924). The increase in hematocrit values from the normal (29.5) to 68.9 percent. was roughly proportional to the length of time of asphyxiation. These high hematocrit values of asphyxiated menhaden were attributed to the effect of the release of water from the blood to the tissues.

For the naturally infected fish, the successive number of days in which mortalities occurred ran twice as long (17 days) as it did for the experimentally infected fish (8 days). The longer duration of the disease could be the result of (1) residence in water cooler by 10° F. than that in experimentally infected fish troughs; (2) spread of the infection rather than simultaneous exposure of all fish; (3) difference in route of infection.

Acknowledgements

Physical facilities for this study were provided by the School of Fisheries, University of Washington. Sincere appreciation is accorded Dr. Q. B. DeMarsh, Department of Anatomy, Universi-

ty of Washington, for his guidance and criticism; Mr. E. F. Marten, Supervisor of Still Photography Production Unit, University of Washington, for his expert assistance with the photomicrographs; and Mr. Vladimir Lipski for his Russian translations.

Summary

Observations of cell types found in the peripheral blood of healthy and virus-diseased sockeye salmon fingerlings (*Onchorhynchus nerka*) are made with a phase-contrast microscope and reproduced photographically. Cells described include erythrocytes, thrombocytes, lymphocytes, neutrophils, eosinophils, basophils and macrophages.

Two diagnostic characteristics of the virus-diseased fish are: (1) before mortalities occur, degeneration in leukocytes and thrombocytes is made evident by the presence of an intracytoplasmic, highly refractile body which appears as a dark brown pigment granule when viewed with bright-field microscopy; (2) during the severest stage of the infection, extracellular granules of basophils appear, as well as totally degenerated leukocytes and thrombocytes which are only visible with phase-contrast microscopy.

The average percent. hematocrit of 68 control fingerlings (30-60 mm. in fork length) was 47. Hematocrit values were determined for fish which had been experimentally and naturally infected with the virus disease. At four days after experimental infection, the percent. hematocrit rose to an average of 53 (maximum 64); at 6 days, peak mortalities occurred and moribund fish registered a normal percent. hematocrit. Hematocrit values fell to a low of 16 percent. by the eighth day after exposure to the virus and then gradually rose to normal by the 27th day. Associated with below normal hematocrit values were erythematous areas at the base of the fins, at the anus or the isthmus. Microscopic blood findings and corresponding hematocrit values were related. Microhematocrit tubes used were capillary pipettes (5-10 lambda).

Literature Cited

Aserinsky, Eugene

1954. The effect of electroshock upon survival time of young salmon under conditions of osmotic stress. Tech. Rep. No. 6, U. S. Corps of Engrs., N. Pacif. Div., School of Fisheries, Univ. of Washington, 12 pp., 5 tables (typewritten).

Benditt, Earl, Peter Morrison & Laurence Irving

1941. The blood of the Atlantic salmon during migration. Biol. Bull., Wood's Hole, 80-(3): 429-440.

Bennett, Alva H., Harold Osterberg, Helen Jupnik & Oscar W. Richards

1951. Phase microscopy. John Wiley & Sons, Inc., N. Y., 320 pp.

Dombrowski, Heinz

1953. Untersuchungen über das Blut des Karpfens (*Cyprinus carpio* L.) und einiger anderer Süsswasserfischarten. Biol. Zbl., 72 (3/4): 182-195.

1954. Das Blut des bauchwassersuchtkranken Karpfens. Der Fischwirt, Nr. 1:9-11.

Field, John B., C. A. Elvehjem & Chancey Juday

1943. A study of the blood constituents of carp and trout. J. Biol. Chem., 148: 261-269.

Glucksman, Michel A. & Myron Gordon

1953. Hematology of normal and melanomatous fishes: a preliminary report. Zoologica, 38 (3): 151-154.

Hall, F. G., I. E. Gray & S. Lepkovsky

1924. The influence of asphyxiation on the blood constituents of marine fishes. J. Biol. Chem., 61(3): 549-554.

Hughes, Margaret E.

1953. A review of some basic principles and techniques in hematology. Amer. J. Med. Tech., 19(2): 62-72.

Katz, Max

1949. The hematology of the silver salmon, *Oncorhynchus kisutch* (Walbaum). PhD. thesis, Univ. of Wash., 110 pp. (typewritten).

1950. Some interesting cells in the blood of a diseased silver salmon fingerling. Copeia, No. 4: 255-298.

Lepkovsky, Samuel

1930. The distribution of serum and plasma proteins in fish. J. Biol. Chem., 85: 667-674.

Lyman, E. M. & A. Iu. Shpolanskaia

1949. Some new facts on the treatment and epizootiology of stomach dropsy in carp. In Russian. Rybnoe chozjasjstvo, 4: 38-41.

Michels, Nicholas A.

1938. The mast cells. *In* "Handbook of Hematology." Ed. Hal Downey. Section IV, 1: 231-372, Paul B. Hoeber, N. Y.

Rucker, R. R., W. J. Whipple, J. R. Parvin & C. A. Evans

1953. A contagious disease of salmon possibly of virus origin. Fish. Bull. U.S., 54: 35-46.

Schäperclaus, W.

1953. Infecktionsablauf und natürliche Infektionsabwehr bei der ansteckenden Bauchwassersucht des Karpfens. Z. Fisch., Band I, N.F. Heft 3/4, seite 273-294.

VARS, HARRY M.

1934. Blood studies on fish and turtles. J. Biol. Chem., 105: 135-137.

WATSON, STANLEY W.

1954. Virus diseases of fish. Trans. Amer. Fish. Soc., 83: 331-341.

WATSON, STANLEY W., RAYMOND W. GUENTHER & ROBERT R. RUCKER

1954. A virus disease of sockeye salmon: interim report. U. S. Fish and Wildlife Serv., Spec. Sci. Rep. No. 138, 36 pp.

WINTROBE, MAXWELL M.

1949. Clinical hematology. Lea & Febiger, Philadelphia, 862 pp.

WILSON, D. WRIGHT, & EDWARD F. ADOLPH

1917. The partition of non-protein nitrogen in the blood of fresh water fish. J. Biol. Chem., 29: 405-411.

YOUNG, R. T.

1949. Variations in the blood cell volume of individual fish. Copeia, No. 3: 213-218.

YOKOYAMA, HISAKO OGAWA

1947. Studies on the origin, development, and seasonal variations in the blood cells of the perch, *Perca flavescens*. PhD. thesis, U. of Wisc. 150 pp. (typewritten).

EXPLANATION OF THE PLATES

All figures are photomicrographs of blood cells found in live wet films of peripheral blood which was viewed with a phase-contrast microscope.

Plate I

Fig. 1. Cell in upper right is a blebbed thrombocyte. The colorless circular area near the upper margin of this cell is a refractile body which is a pigment granule whose presence is the first evidence of cellular degeneration and denotes a virus infection.

Fig. 2. In the upper half of the field is a totally degenerated thrombocyte containing 3 pairs of pigment granules which appear as refractile bodies, each outlined by a thin dark line. In the lower half of the field is a senile erythrocyte containing a pycnotic nucleus.

Fig. 3. A non-filamented neutrophil (metamyelocyte) occupies the center and right portions of the field. Its cytoplasm contains a refractile body common in a virus infection.

Fig. 4. A normal mature erythrocyte is located left of center. Basophil normoblast is labeled *BN*. Center of the field is occupied by a totally degenerated neutrophil found in a virus-diseased fish.

Fig. 5. Macrophage which has recently ingested a large degenerated lymphocyte is characteristic of virus-diseased blood. Nucleus of macrophage is compressed at the upper right margin of the cell.

Fig. 6. Macrophage containing phagocytized granules of basophils which are numerous in a virus-diseased fish.

Plate II

Cells characteristic of blood from a virus-diseased fish.

Fig. 7. Immature basophil with only a few small, dark granules and a large nucleus.

Fig. 8. Small, round basophil filled with dark granules; nucleus is not visible.

Fig. 9. Large lymphocyte at lower right, above and to the left of which is a basophil.

Fig. 10. Degenerating basophil.

Fig. 11. Three groups of extracellular granules of basophils: upper left is a pair of granules, extreme lower right is a single granule, above which is a pair of granules unequal in size.

Fig. 12. Large leukocyte not positively identifiable but called a monocyte.

Plate III

Fig. 13. Polychromatophil normoblast between two orthochromatophil normoblasts from a virus-diseased fish.

Fig. 14. Three polychromatophil normoblasts from virus-infected blood.

Fig. 15. *L* is an active small lymphocyte with a pseudopod. A polychromatophil normoblast is to the right of the lymphocyte. All other cells are mature erythrocytes (healthy fish).

Fig. 16. Small quiescent lymphocyte (healthy fish).

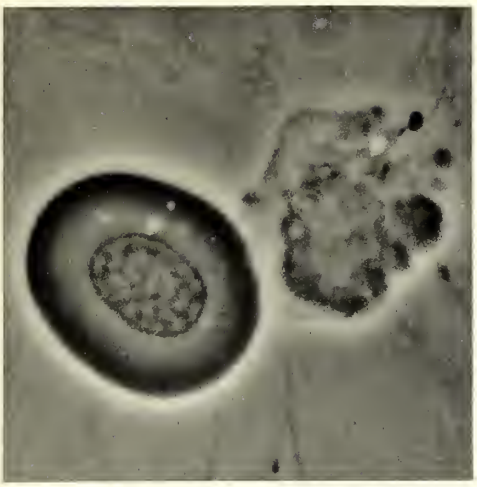


FIG. 1

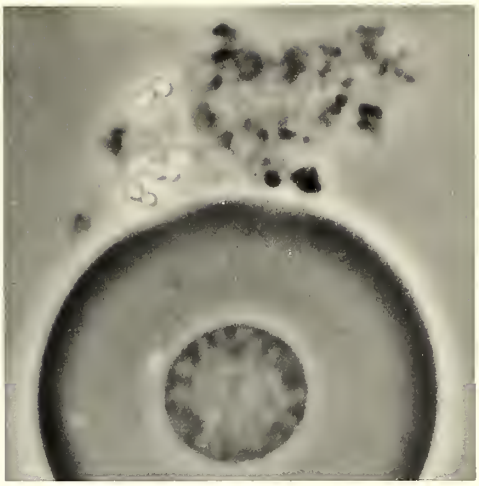


FIG. 2

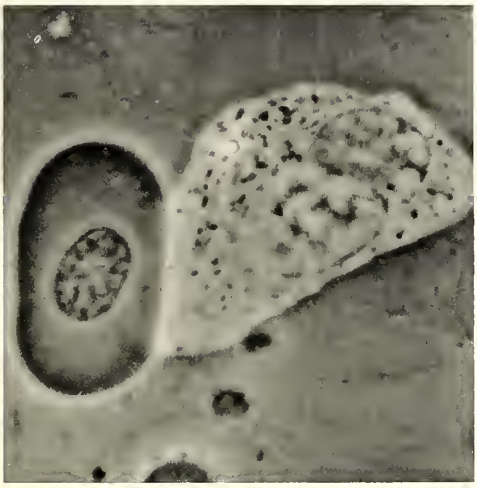


FIG. 3

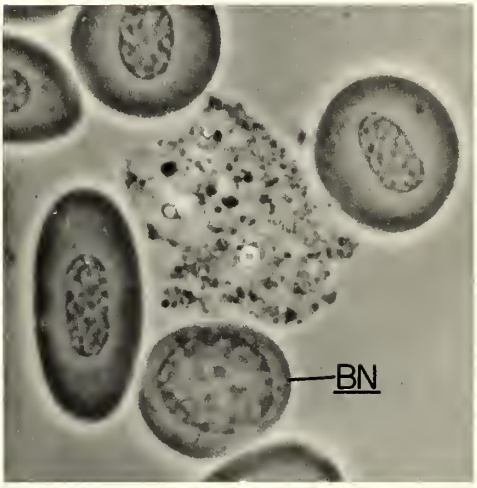


FIG. 4

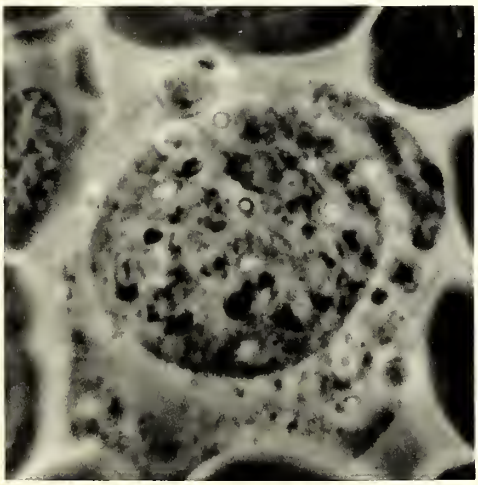


FIG. 5

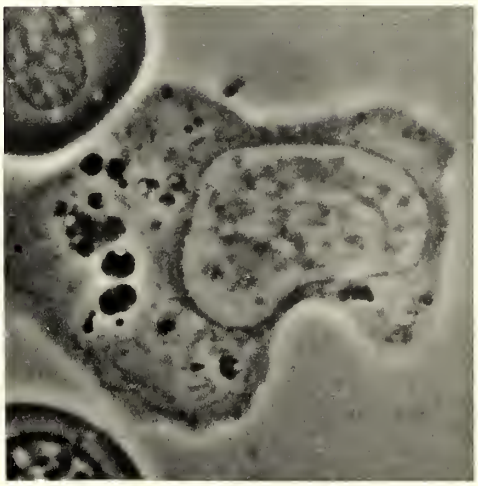


FIG. 6

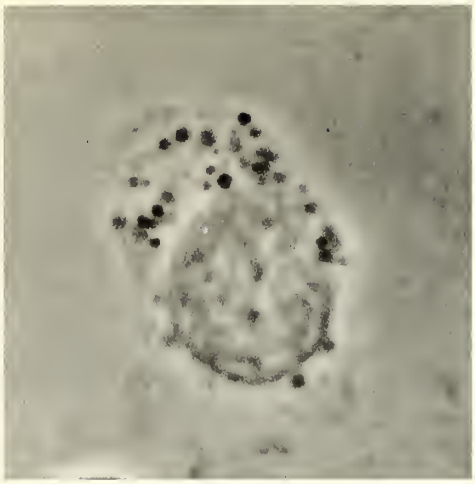


FIG. 7

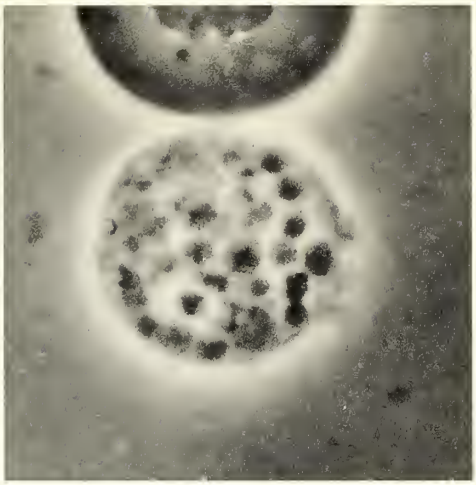


FIG. 8

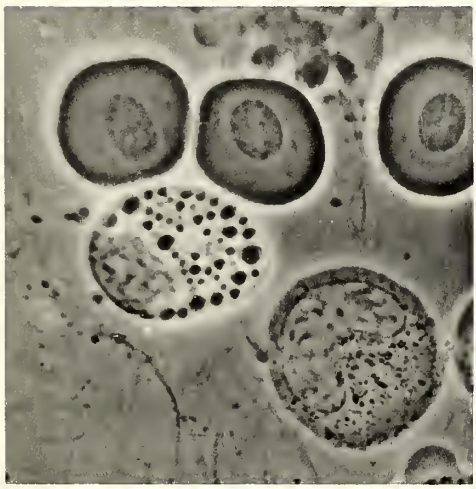


FIG. 9

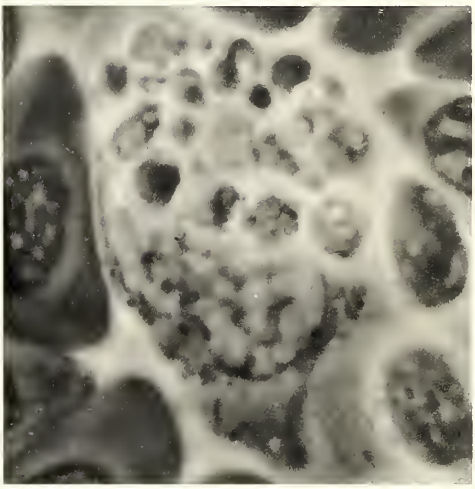


FIG. 10

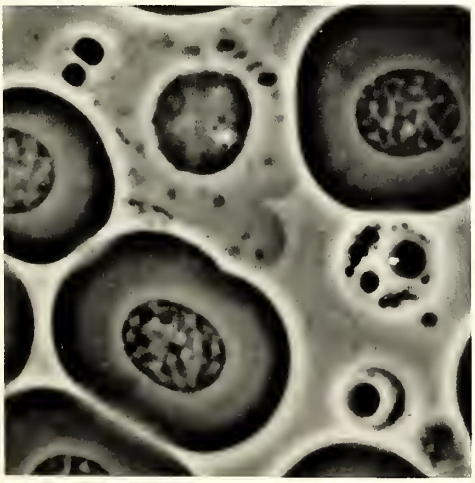


FIG. 11

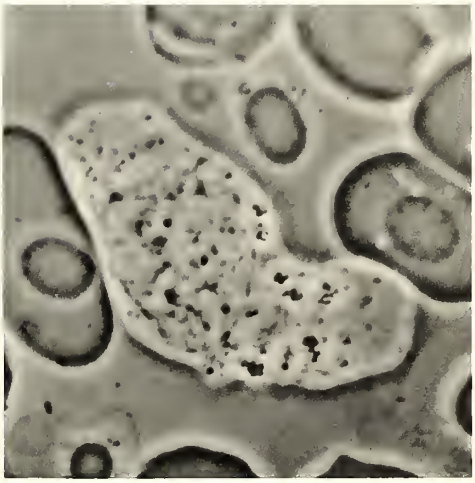


FIG. 12

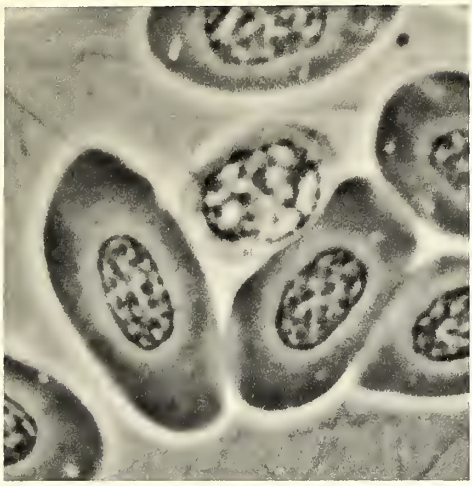


FIG. 13

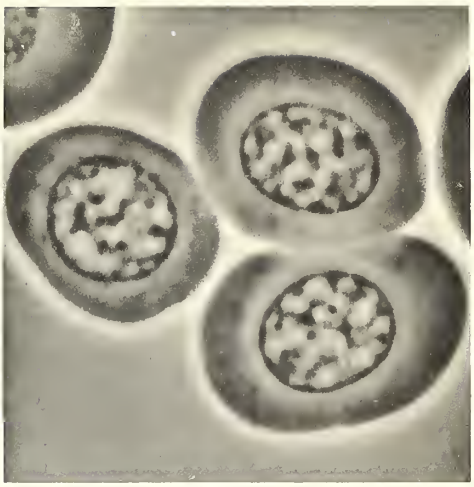


FIG. 14

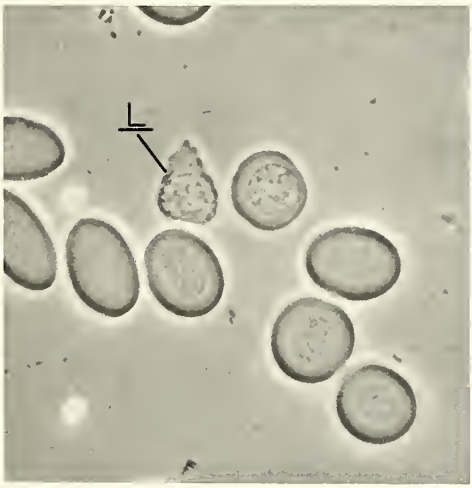


FIG. 15

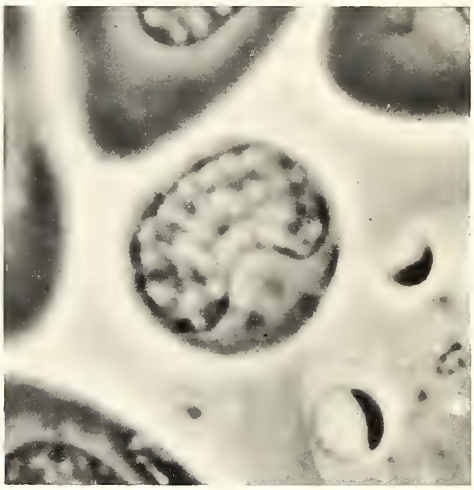


FIG. 16

6

Geographic Variation in the Respiratory Metabolism and Temperature Coefficient in Tropical and Temperate Forms of the Fiddler Crab, *Uca pugnax*[1]

RICHARD E. TASHIAN

Department of Tropical Research,
New York Zoological Society, New York 60, N. Y.

(Text-figures 1-6)

INTRODUCTION

RECENTLY Rao & Bullock (1953) and Dehnel (1955), in re-evaluating and making rather complete reviews of the literature on climatic adaptation in marine poikilotherms, have brought together convincing evidence in support of the following conclusions: (1) rate functions within a given species are usually similar in individuals from northern and southern regions when measured at their normal environmental temperatures; (2) at any given temperature, activity rates are greater in forms from the northern latitudes; (3) cold-adapted or northern individuals have lower temperature coefficients than related warm-adapted species; (4) larger animals of the same species are generally more sensitive to temperature changes than are smaller ones. The purpose of the present investigation is to examine these concepts on one species having an especially great north-south distribution, for in so doing it would seem that these important basic concepts relating to climatic adaptation in cold-blooded animals could be further clarified.

The fiddler crab, *Uca pugnax,* was considered particularly well suited as the experimental animal, ranging as it does from Cape Cod, Massachusetts, south to Rio de Janeiro, Brazil. This semiterrestrial crab lives in the intertidal zone and is most often found in a muddy rather than a sandy habitat. Two races have been described: the nominate form extending from Massachusetts to east-central Florida, and the southern subspecies (*rapax*) from southern Florida to Brazil. The tropical forms average larger in size than those from the temperate regions. *Uca pugnax rapax* was studied from material collected in Trinidad, B.W.I., and southern Florida, and *Uca pugnax pugnax* from New York and North Carolina. Comparisons were made not only of the oxygen and temperature coefficients of representative populations throughout most of the range of this species, but also of their tolerance to low temperature.

The experimental work was carried out at the field station of the Department of Tropical Research of the New York Zoological Society, Arima Valley, Trinidad, B.W.I.; the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas, B.W.I.; the Duke University Marine Laboratory, Beaufort, North Carolina; and the zoological laboratories of Columbia University, New York. I wish to thank Dr. Charles M. Breder, Jr., directer of the Lerner Laboratory, and Dr. Arthur W. Pollister, executive officer of the Department of Zoology, Columbia University, for making facilities available to me as a guest investigator.

I am especially grateful to Dr. William Beebe, director emeritus, and Miss Jocelyn Crane, assistant director, of the Department of Tropical Research of the New York Zoological Society for their support and encouragement of this project. I am likewise much indebted to Dr. F. John Vernberg, Department of Zoology, Duke University, for the loan of apparatus used in these experiments as well as his valuable assistance in many ways during the course of this study. I also wish to thank Mr. David H. McDermott, formerly of the Biology Department of Long Island University, for making some of the respirometers used in this investigation. Identification of much of the critical ani-

[1] Contribution No. 965, Department of Tropical Research, New York Zoological Society.

mal material from Florida was made by Miss Crane. Mr. Ludolf Wehekind, hydrologist for the Works and Hydraulics Department, Port-of-Spain, Trinidad, kindly supplied the climatic data for Trinidad.

Materials and Methods

In comparing animals from different latitudes, populations were selected having reasonably comparable environmental temperatures at the time of collecting (Text-figure 1). *Uca pugnax* was collected from the vicinity of the following localities on the inclusive dates: Port-of-Spain, Trinidad (10° 40′ N), March 29 to April 4; Jamaica Bay, New York (40° 35′ N), June 13 to July 12; Beaufort, North Carolina (34° 42′ N), July 22 to August 4; and Key Biscayne, Florida (25° 47′ N), August 15. Specimens of *Uca leptodactyla* were taken from Bimini, Bahamas (25° 44′ N), August 23 to 29. Only males were used. In those crabs from New York, North Carolina and Florida, the males were noted to be displaying (waving the claws) during the collecting periods, implying that they were in breeding condition (Crane, 1943). The one exception was in the New York area where waving was not observed until June 18. The animals were maintained in terraria and fed regularly. It was always sought to collect fresh material weekly; however, in some cases where accessibility was a problem, they were maintained as long as two weeks.

Oxygen consumption was tested in a water-saturated atmosphere at two temperature levels: the upper ranging from 23.3 ±0.5 to 24.6 ±0.6° C. and the lower from 14.0 ±0.1 to 14.9 ±0.4° C. For large specimens, volumetric respirometers were employed of a type described by Flemister & Flemister (1951), and for the smaller animals a volumetric plastic micro-respirometer designed after that developed by Scholander (1950) was used. In these determinations 30 minutes were allowed for thermal equilibrium, and the test was then run for four and a half hours, the first half hour's reading being discarded. The results are corrected to standard temperature and pressure. The crabs were not acclimated in any way but in all cases were taken directly from room temperature where they had been maintained to the experimental temperature.

When the oxygen coefficient or Qo_2 [total O_2 consumption (cc.)/wet body weight /(gms.)/time (hrs.)] was plotted against the observed weight a curve was obtained of the type:

$$Y = aX^b$$

where Y represents the Qo_2, X the observed weight, and a and b are the regression coefficients of the intercept and slope. These data were plotted double logarithmically. The constants a and b were determined from the following formulae (deviation from the means of X and Y are represented by x and y, and the number of determinations by N):

$$b = \frac{\Sigma(\log X \cdot \log Y) - N(\Sigma\log x \cdot \Sigma\log y)}{\Sigma\log^2 X - N\log x^2}$$

$$\log a = \log y - b(\log x)$$

Further statistical data: the standard deviation from regression or standard error of the estimate ($S_{y \cdot x}$ or $S_{\log y \cdot \log x}$) and the coefficient of correlation (r) were calculated from the equations:

$$S_{y \cdot x} = \sqrt{\frac{\Sigma\log^2 y - b(\Sigma\log x \cdot \log y)}{N-2}}$$

$$r = \sqrt{\frac{b(\Sigma\log x \cdot \log y)}{\Sigma\log^2 y}}$$

In determining the tolerance to low temperatures the crabs from all localities but New York were placed in water-saturated containers (precooled to the experimental temperature) and then immersed in a constant temperature water bath kept at 5° C. The time of entry was recorded and the animals then periodically examined to determine the lethal point. The specimens from New York were kept in moist bowls in a constant temperature (5° C.) cold room and examined daily.

The climatological figures for New York, North Carolina and Florida were taken from the records for New York, Hatteras and Miami Beach in the Climatological Data (U. S. Dept. of Commerce) for 1954-55. The data for Trinidad are from the St. Clair Experimental Station, Port-of-Spain.

Results and Disscussion

Respiratory Metabolism and Latitude

The comparative data on respiration rates are summarized in Table 1. At the upper temperature testing levels (23.3–24.6° C.) *Uca pugnax* from Trinidad showed the highest oxygen consumption. This becomes even more significant when we realize that not only do the Trinidad forms average larger in size, but also that they were not in breeding condition. At the lower temperatures (14.0–14.9° C.) the rates were relatively similar for all populations. Since the warmer testing temperatures were reasonably close to the environmental temperatures during the collecting periods (Text-figure 1), it can be seen that at their normal habitat temperatures

TABLE 1. SUMMARY OF METABOLISM DATA FOR *Uca pugnax*

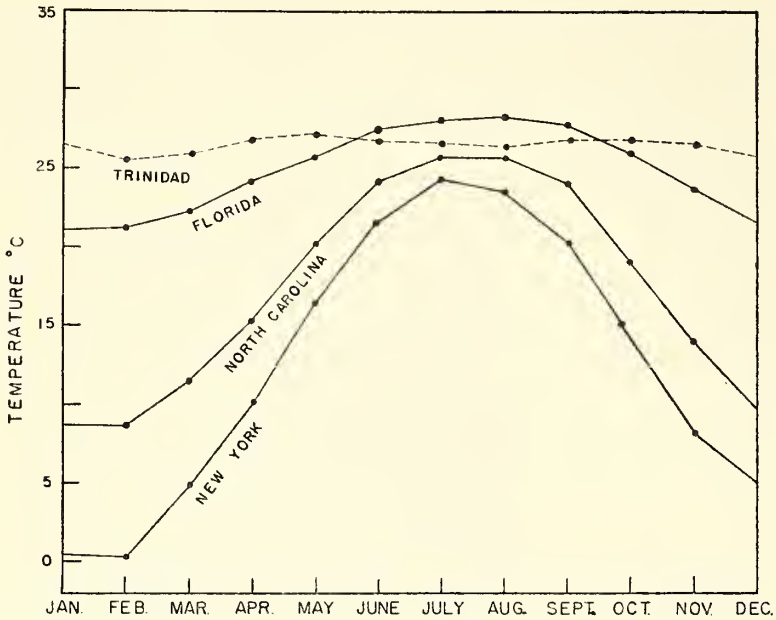
Locality	Temperature °C.	Size of Sample	Wet Body Weight (gms.)		Q_{O_2} (cc./gm./hr.)		Statistical Analysis			
			Range	Mean	Range	Mean	a	b	$S_{y \cdot x}$	r
New York	23.8 ±0.7	33	.10-4.26	2.09	.070-.575	.154	.140	—.321	.189	—.68
	14.1 ±0.2	31	.02-4.12	1.54	.038-.269	.107	.091	—.167	.246	—.45
North Carolina	23.3 ±0.5	41	.09-5.58	2.41	.035-.344	.114	.109	—.305	.162	—.74
	14.0 ±0.1	33	.07-4.71	1.93	.019-.144	.051	.045	—.269	.131	—.79
Florida	24.4 ±0.2	24	.50-3.17	1.94	.073-.274	.156	.158	—.148	.174	—.18
	14.9 ±0.4	23	.19-2.69	1.45	.022-.140	.060	.055	—.110	.213	—.17
Trinidad	24.6 ±0.6	41	.09-8.51	3.20	.060-.553	.215	.217	—.329	.130	—.84
	14.6 ±0.2	21	.10-8.32	3.51	.028-.245	.080	.082	—.359	.115	—.89

the warm-adapted crabs had the higher rates of metabolism. This holds true, however, only if we consider the overall data on the temperate populations, as the New York specimens show a higher rate than those from North Carolina. These results are not in accord with the concept that northern and southern forms have similar rates at the same habitat temperatures.

Two studies (Takatsuki, 1928, and Scholander *et al.*, 1953), in which related organisms from truly tropical (Palau, West Caroline Islands? and Panama) and colder waters are compared, give opposite results. Scholander *et al.* (1953) demonstate that at their respective habitat temperatures the tropical forms have higher metabolic rates than those from the arctic. These results are in general agreement with the findings of the present study. From the data of Takatsuki (1928), who compared the heart rates of two species of oyster, the picture is one in which similar rates are shown for both species at their normal environmental temperatures. Mayer (1914) found that at their respective temperatures the pulsation rates of the medusa, *Aurellia aurita,* from Nova Scotia and Florida, were similar. If we compare the Florida and New York data on *Uca pugnax,* the rates are also quite similar. Material from the middle of the range (North Carolina), however, shows a lower metabolism than either of the other two localities.

It should be kept in mind that previous workers have dealt almost entirely with aquatic organisms. The fact that the fiddler crab is semiterrestrial may have some bearing on the results. In the study on climatic adaptation in truly terrestrial animals (insects and spiders) by Scholander *et al.* (1953), the correlation with their other findings is not pronounced.

If winter animals from the temperate regions were tested, these relations would undoubtedly be modified. Edwards & Irving (1943), in their seasonal study of the sand crab, *Emerita,* found the winter oxygen consumption greater than that of the summer by 50 to 100% at temperatures below 20° C. Roberts (1952) reported that the thermal acclimation of the semiterrestrial crab, *Pachygrapsus crassipes,* from southern California, reflected short term fluctuations in intertidal temperatures during the winter rather than any seasonal trends. He also found that the metabolism of crabs from different localities was adjusted to their respective habitat temperatures, for after 6 weeks of acclimation to 16° C. the original metabolic differences were doubtfully significant. Vernberg (1955), in comparing warm- and cold-acclimated (7 and 27° C.) *Uca pugilator,* showed that the summer warm-acclimated forms



TEXT-FIG. 1. Mean monthly temperatures for the collecting localities.

had a lower Q_{O_2} than the warm-acclimated winter crabs. The cold-acclimated forms showed the opposite picture.

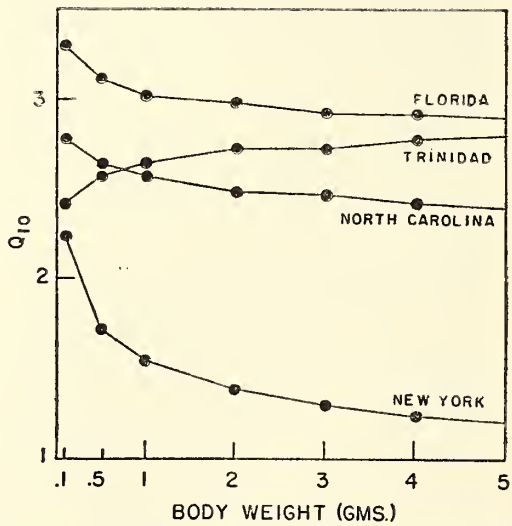
Respiratory Metabolism and Size

The coefficient of correlation of Q_{O_2} to weight was significant in all but the animals from Florida. Although there was no correlation between slope of regression and latitude at the upper testing temperatures, the slopes at the colder temperatures reflected a flattening with increasing latitude. Between the normal ranges in habitat temperature, Roberts (1952) found that in *Pachygrapsus crassipes* the coefficient of regression (weight to specific respiration) remained at –.336. This slope compares favorably with the mean of the significant regression coefficients (–.318) in the present study at the warmer testing temperatures.

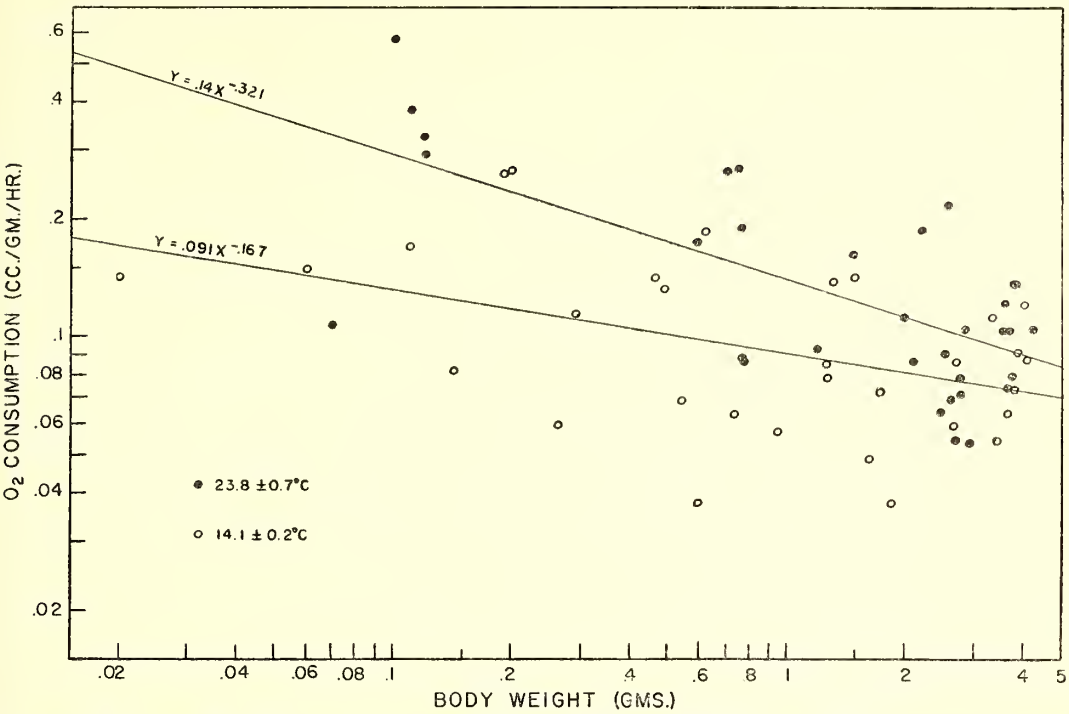
Temperature Coefficient and Latitude

The temperature coefficients of *Uca pugnax* between the two temperature levels tested show a decrease with increasing latitude (Text-figure 2). The Q_{10}'s were calculated directly from the slopes in Text-figures 3-6. Since the temperature coefficients reflect size differences, comparisons were made within weight levels (2-5 gms.) that showed the least change.

Rao & Bullock (1953) have compiled good evidence in favor of decreasing Q_{10}'s with increasing latitude and comment that results to the contrary (Scholander *et al.*, 1953) possibly were due to comparing different tropical and arctic species and to the scatter resulting from size differences. Where the same species are taken into account (Mayer, 1914; Moore, 1949; Rao, 1953; and Dehnel, 1955) the weight of evidence is strongly in agreement with the idea that northern or cold-adapted poikilotherms are less sensitive to temperature changes than are warm-adapted animals. If we compare the rates between 15 and 25° C. from the data of Takatsuki (1928), we find a Q_{10} of about 1.5 for the temperate species of oyster and 2.6 for the tropical species. No weights are given.



TEXT-FIG. 2. The relation of temperature coefficient to size in four populations of *Uca pugnax*. See Table 2 for temperature levels.



TEXT-FIG. 3. The relation of oxygen consumption to size in *Uca pugnax pugnax* from New York at two temperature levels.

From the metabolism data of Scholander *et al.* (1953, Fig. 10) of *Uca mordax* from Panama we find a Q_{10} of about 2.5 between 15 and 25° C. (1.1.–2.4 gms., 8 determinations). In the present investigation, *Uca leptodactyla* from Bimini showed a temperature coefficient of 2.3 when measured between 14.8 and 24.6° C. (.2–.6 gms., 18 determinations) and *Uca speciosa* from Key Biscayne, Florida, a Q_{10} of 2.6 between 14.8 and 24.5° C. (.3–1.4 gms., 7 determinations). All of these species of *Uca* range from southern Florida or the Bahamas south to Brazil with the exception of *Uca speciosa,* which is limited to the West Indies. The temperature coefficients of the tropical subspecies of *Uca pugnax* seem to be in general agreement with those of other tropical species within the family at similar temperature and weight levels.

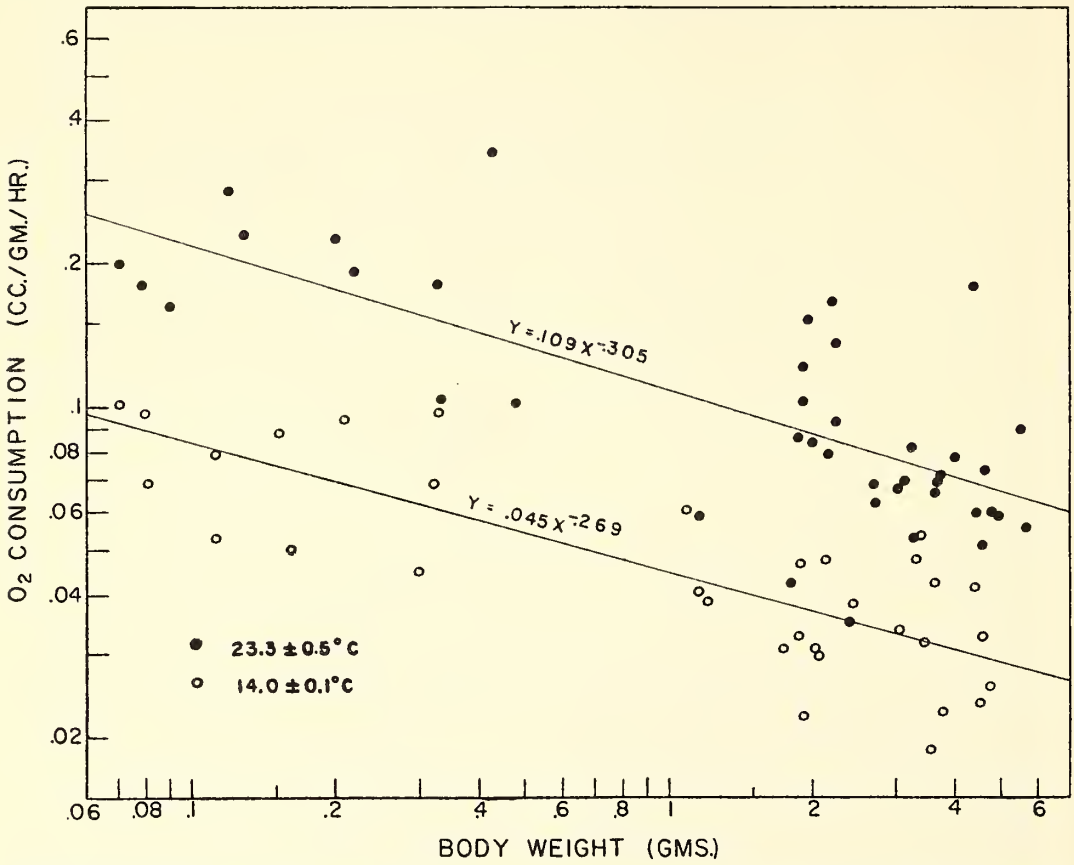
Temperature Coefficient and Size

Three of the weight-temperature coefficient curves (New York, North Carolina and Florida) in Text-figure 2 show decreasing Q_{10} values with increasing weight. In the case of the Trinidad material the opposite is true. We have here, then, in a single species, examples of two trends. Rao & Bullock (1953) believe that the tendency for larger animals to be more sensitive to temperature change is the general rule.

Tolerance to Low Temperature

As might have been expected, the results in this phase of the study revealed an increasing tolerance to low temperatures at the higher latitudes (Table 2). Intrasubspecifically, the southern populations responded in a similar fashion to cold stress. There was, however, a significant gap in the data from Florida and North Carolina, and an even greater one between North Carolina and New York. The only other species of *Uca* tested, *Uca leptodactyla* from Bimini, showed a lower tolerance than *Uca pugnax rapax.* This may have been partially due to the small size (.2–.6 gms.) of the *Uca leptodactyla* tested. There is good correlation between low temperature tolerance and the respective temperature coefficient, the picture being one of increasing temperature tolerance with decreasing sensitivity to temperature change.

The crabs from Trinidad were, in addition, subjected to a series of low temperatures ranging from 2 to 9.5° C. The time between the first death and last recovery was seen to increase proportionately with an increase in temperature. For example, at 2° C. the interval was only 1.5 (1–2.5) minutes; at 5°, 5 (5–10) minutes; at 7°, 45 (15–60) minutes; and at 9.5°, over 150 minutes. This general curve probably does not vary significantly in other species of this family



TEXT-FIG. 4. The relation of oxygen consumption to size in *Uca pugnax pugnax* from North Carolina at two temperature levels.

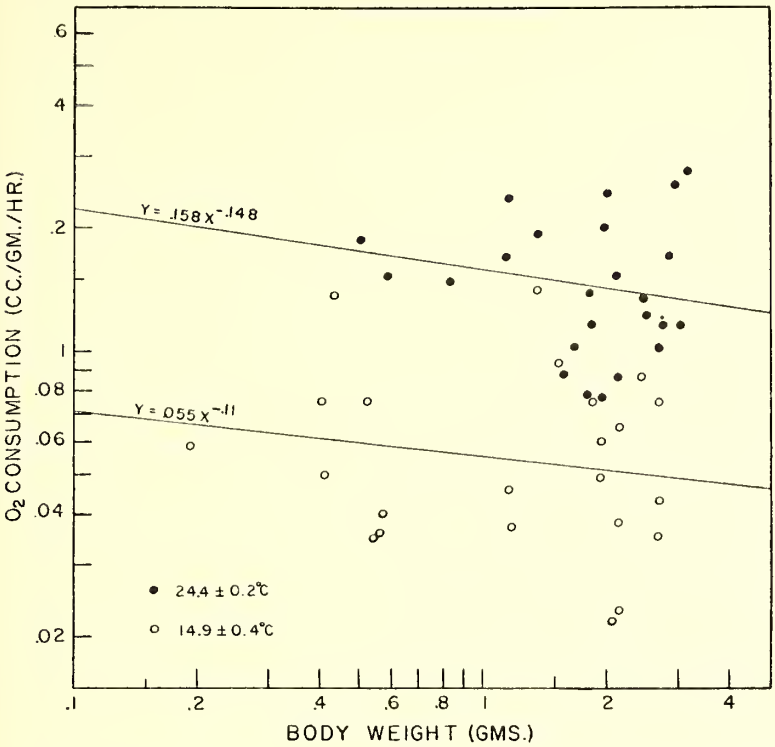
from tropical regions. In cold-adapted species, however, one would expect the curve to be considerably lower, with some toleration of freezing or sub-freezing temperatures for short periods.

Phylogenetic Significance

The two races of *Uca pugnax* appear to be morphologically sound. In addition, Crane (1943) found that the display patterns of the males from the northern subspecies differ significantly from that of the southern form. With both criteria the separation between the races seems to occur in the region of east-central Florida. Physiologically, in the only values that give a good clinal trend (temperature tolerance and Q_{10}), the break appears to take place north of southern Florida. The forms from Trinidad and Florida have very similar values, indicating homogeneity within the southern race, at least for these characters. In the northern subspecies, however, there is considerable variation.

Although comparative data are sparse, the responses to temperature change in *Uca pugnax*, which has successfully adapted to wide thermal fluctuations, does not seem to differ significantly with related species of more limited distribution at similar latitudes. This is essentially what was observed in *Rana pipiens* by Moore (1949), who suggests that in view of the variations in embryonic growth rates, temperature tolerance, Q_{10}, etc., in a single species (*Rana pipiens*) of wide distribution, and the lack of variation in these factors in other species of *Rana* with more limited range, this very lack of adaptive embryological factors might be responsible for the inability of these species to radiate adaptivity in space. He later found (Moore, 1952) that a frog (*Rana septentrionalis*) may have a restricted southern distribution even though it possesses adaptations which had been previously shown to be successful in the southern distribution of other species of *Rana*. Obviously factors in addition to physiological ones are responsible for the successful encroachment in time and space of ecological niches.

During its evolution and distribution from



TEXT-FIG. 5. The relation of oxygen consumption to size in *Uca pugnax rapax* from southern Florida at two temperature levels.

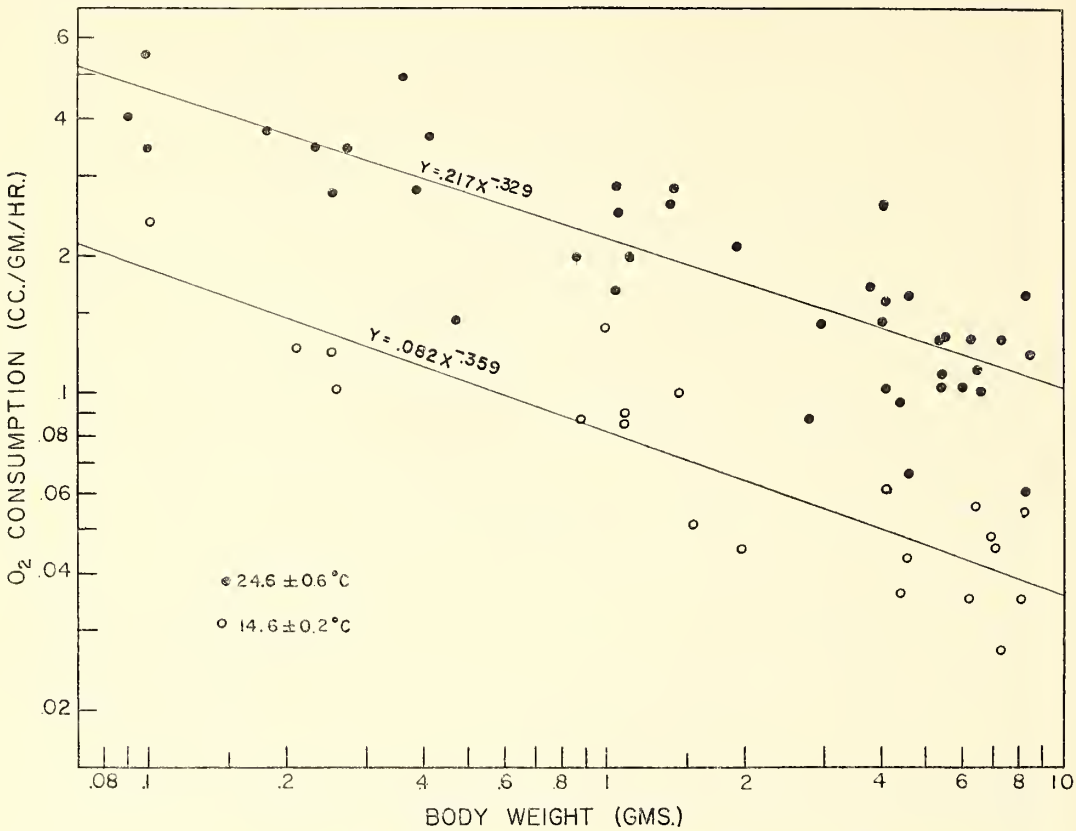
tropical regions, *Uca pugnax* succeeded in migrating to the temperate zone without being accompanied by any great morphological changes. The fact that it was successful physiologically is more important, evolutionally speaking, than any structural features that may have been related to these adaptive processes.

Although non-embryonic factors were tested in the study of *Uca pugnax*, the lower thermal sensitivity of the northern populations as well as the differences in their courtship displays indicate that northern and southern individuals could be separated on the basis of these factors alone. The subspecies of *Uca pugnax*, therefore,

TABLE 2. TOLERANCE OF *Uca pugnax* AND *Uca leptodactyla* TO 5°C

Locality	Annual Temperature °C.			Size of Sample	First Death	Last Recovery
	Monthly Range	Mean	Q_{10}*			
			Uca pugnax pugnax			
New York	0.3-24.3	12.4	1.21	5	28 days	30 days
North Carolina	8.6-25.8	17.2	2.40	12	150 mins.	240 mins.
			Uca pugnax rapax			
Florida	21.1-28.2	24.7	2.89	14	10 mins.	15 mins.
Trinidad	25.6-27.3	26.5	2.80	8	5 mins.	10 mins.
			Uca leptodactyla			
Bimini				12	2 mins.	3 mins.

*Taken from Text-figure 2 for weight of 5 gms.



TEXT-FIG. 6. The relation of oxygen consumption to size in *Uca pugnax rapax* from Trinidad, B.W.I., at two temperature levels.

appear to be as sound behavioristically and physiologically as they are morphologically.

Careful studies on the growth rates and breeding cycles throughout the range of *Uca pugnax* would contribute much to the further understanding of its evolution and distribution.

SUMMARY

1. Determinations were made of the respiratory rates in *Uca pugnax* from New York, North Carolina, Florida and Trinidad, B.W.I., at two temperature levels.

2. At their normal habitat temperatures the Trinidad forms showed a higher metabolic rate than those from higher latitudes.

3. Although there was no apparent correlation between slope of regression (Qo_2 to size) and latitude at the warmer testing temperatures, the slopes at the colder temperatures reflected a flattening with increasing latitude.

4. A decrease in sensitivity to temperature change from southern to northern populations is shown along with an increase in tolerance to low temperature.

5. Individuals from New York, North Carolina and Florida showed a decrease in temperature coefficient with increasing size, whereas the opposite was true in the Trinidad forms.

6. The phylogenetic significance of thermal adaptation in temperate forms is discussed.

LITERATURE CITED

CRANE, J.

1943. Display, breeding and relationships of fiddler crabs (Brachyura, Genus *Uca*) in the northeastern United States. Zoologica, 28: 217-223.

DEHNEL, P. A.

1955. Rates of growth of gastropods as a function of latitude. Physiol. Zool., 28: 115-144.

EDWARDS, G. A. & L. IRVING

1951. The influence of temperature and season upon oxygen consumption of the crab, *Emerita talpoida* Say. Jour. Cell. & Comp. Physiol., 21: 169-182.

FLEMISTER, L. J. & S. C. FLEMISTER

1951. Chloride ion regulation and oxygen consumption in the crab *Ocypode albicans* (Bosc). Biol. Bull., 101: 259-273.

MAYER, A. G.

1914. The effects of temperature upon tropical marine animals. Papers from Tortugas Lab., 6: 3-24.

MOORE, J. A.

1949. Geographic variation of adaptive characters in *Rana pipiens* Schreber. Evolution, 3: 1-24.

1952. An analytical study of the geographic distribution of *Rana septentrionalis.* Amer. Nat., 86: 5-22.

RAO, K. P.

1953. Rate of water propulsion in *Mytilus californianus* as a function of latitude. Biol. Bull., 104: 171-181.

RAO, K. P. & T. H. BULLOCK

1954. Q_{10} as a function of size and habitat temperature in poikilotherms. Amer. Nat., 88: 33-44.

ROBERTS, J. L.

1952. Studies on acclimatization of respiration to temperature in the lined shore crab, *Pachygrapsus crassipes* Randall. Ph.D. thesis, Univ. Calif., Los Angeles.

SCHOLANDER, P. F.

1950. Volumetric plastic micro respirometer. Rev. Sci. Instr., 21: 378-380.

SCHOLANDER, P. F., W. FLAGG, V. WALTERS & L. IRVING

1953. Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zool., 26: 67-92.

TAKATSUKI, S.

1928. The heart pulsation of oysters in tropical seas compared with that of those living in seas of the temperate zone. Records of Oceanographic Works in Japan, 1: 102.

VERNBERG, F. J.

1955. Studies on the oxygen consumption of *Uca pugilator* (Bosc) as influenced by season and thermal acclimatization. (Abstract). Anat. Rec., 122: 457-458.

ADDENDUM

The following important review articles bearing on the present study have come to the author's attention:

PROSSER, C. L.

1955. Physiological variation in animals. Biol. Rev., 30: 229-262.

BULLOCK, T. H.

1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev., 30: 311-342.

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 41 • PART 2 • SEPTEMBER 17, 1956 • NUMBERS 7 TO 11

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

7

Visual, Chemical and Sound Stimuli as Cues in the Sex Discriminatory Behavior of the Gobiid Fish *Bathygobius soporator*

WILLIAM N. TAVOLGA[1]

Department of Animal Behavior, The American Museum of Natural History, N. Y.

(Plate I)

INTRODUCTION

SEX discrimination, in its broad sense, may be defined as a pattern of activity exhibited by one animal toward another of the same species, which activity varies according to the sex, maturity and stage of gonadal cycle of the other. The probability of successful pairing and reproduction is thus increased. Such behavior can also maintain a pair until both members attain the physiological condition necessary to consummate mating. Indeed, the behavior pattern itself can stimulate and synchronize the achievement of this physiological state.

Among fishes, the territorial and nesting species are particularly useful in studies of sex discrimination, since the behavior of a territory-holding male is well differentiated with respect to intrusion by females and other males. Such sexually dimorphic behavior (as well as structure, coloration, etc.) is present in the Centrarchidae (Noble, 1934; Breder, 1936), some of the Cichlidae (Breder, 1934; Noble & Curtis, 1939), Gobiidae (Guitel, 1892, 1895; Tavolga, 1954), Blenniidae (Guitel, 1893; Breder, 1939, 1941), and numerous other groups of teleosts. Among some species, the behavior of the two sexes is differentiated to a lower degree, and the distinctions between male and female activity may be entirely quantitative, as in the cichlid fish *Tilapia macrocephala* (Aronson, 1949).

Two behavior patterns are commonly observed among territorial fishes (and in many other animals as well). On the one hand the encounters between a territory-holding male and an intruding male consist of color flashing, nipping or pecking, and the display of similar identifying devices. Terms such as "fighting," "combat," "territorial aggression," "intimidating" or "warning" devices, have been commonly employed in descriptions of this behavior. Morris (1954), and others, have used the term "agonistic behavior" for this and all other activities not directly associated with mating. On the other hand, there is the activity of a male which attracts and stimulates a female toward eventual mating. "Courtship" is the almost universally used term for this behavior. The two behavior patterns are not mutually exclusive, however, and one frequently contains many of the elements of the other. Thus, a given component act can lead to very different results depending upon the context within which the movement occurs. The terms "courtship" and "combat," as used by some authors, frequently imply the presence of two separate neural mechanisms, centers or drives. As used in this report, however, these terms describe two patterns of behavior which occur as part of the general reproductive activity, and the emphasis here is upon the external stimuli which control the direction of response.

The majority of analytical studies on fish behavior have been concerned only with the visual cues which lead to sex discrimination, and within these investigations various areas of visual perception have been included, i.e. shape, shade, color and movement. Artificial models have been used in the work of ter Pelkwijk & Tinbergen (1937) and Tinbergen & Van Iersel (1947) on the stickleback *(Gasterosteus)*, and by Baerends & Baerends-Van Roon (1950) on the Cichlidae. Most of these studies were done within the theoretical framework of the releaser hypothesis of innate behavior (Baerends, 1950; Lorenz, 1950; Tinbergen, 1951).

Other sensory mechanisms have been only infrequently investigated in connection with reproductive behavior. The role of tactile and chemical factors was described in the courtship

[1]Public Health Service Research Fellow of the National Institute of Mental Health.

behavior of the catfish *(Ameiurus)* by Breder (1935), and the blind goby *(Typhlogobius)* by MacGinitie (1939). Sounds produced by the purring gourami *(Ctenops vittatus)* are known to form a part of the pre-spawning behavior pattern (Stampehl, 1931). It is conceivable, then, following the general view of Lashley (1938), that a number of sense modalities may be simultaneously responsible in eliciting and modulating the sex discriminatory behavior among teleosts.

Males of the gobiid species, *Bathygobius soporator* (Cuvier & Valenciennes), possess a highly differentiated behavior pattern, particularly during their territorial and pre-spawning activities (Tavolga, 1954, 1955a). In addition to visual cues, chemical and sound stimuli were found to elicit and orient the behavior of both males and females. The following report presents data on the role of these factors in the pre-spawning behavior pattern.

Acknowledgements

The author is indebted to the staff and facilities of Marine Studios, Marineland, Florida, for making this project possible, and to Mr. F. G. Wood, Jr., Curator of the Marineland Research Laboratory, for his cooperation and hospitality. Dr. Lester R. Aronson of The American Museum of Natural History and Dr. Myron Gordon of the New York Zoological Society were kind enough to offer their comments and criticisms of the manuscript. The author is also grateful to Dr. Edward R. Baylor of the University of Michigan for his aid and advice in recording the sounds made by the fish. The analysis of the sound recordings was done with the generous help of Mr. Robert Laupheimer of The City College, N. Y., and Mr. Louis Gersten of the Haskins Laboratories.

Source and Maintenance of Material

The specimens of *B. soporator* were collected from tide pools and shallows along the Intracoastal Waterway, in the vicinity of Marineland, Florida. All the collections and observations were made during the summer months, when the spawning season of this species is at its height.

Individuals used here were all sexually mature and varied in size from 40 mm. to 90 mm. in standard length. The males which were used as test animals in the observations were chosen from the largest size group, ranging from 80 mm. to 90 mm. Females ranged in size from 40 mm. to 75 mm. Some smaller males, below 75 mm. in length, were also used.

Large males were isolated in five-gallon aquaria, and the rest were kept as stock (sexes separate) in ten-gallon tanks. All aquaria were supplied with running, filtered sea water, and the animals were fed daily on minced shrimp and mullet.

The resident males were provided with shelters consisting of unglazed flooring tiles (approximately 4″×4″×½″), one of which was inclined against the front glass of the aquarium. These triangular shelters were readily used by the gobies for hiding and spawning.

Summary of Reproductive Behavior Pattern

The reproductive behavior in *Bathygobius* has been reported in detail elsewhere (Tavolga, 1954, 1955a), but a brief summary is appropriate here.

The male enters a shell or crevice and establishes a territory in the vicinity, and cleans loose sand from the interior of the shelter. Other males intruding into the territory are chased or fought. The combat behavior between males is characterized by a sideward approach and an intense darkening of the coloration, accompanied by fin erection, gaping, quivering, butting, tail slapping and biting. Much of this type of behavior is included within the term "lateral display" as described by Baerends & Baerends-Van Roon (1950) for the Cichlidae.

In the presence of a gravid or pre-gravid female, the coloration of the resident male changes to a light, patternless tan with a blackened chin and throat. The female is approached with rapid fanning and gasping movements. If ovulation is imminent, the gravid female follows the male into the shelter and there deposits adherent eggs on the inner surfaces. After spawning, the male guards and fans the eggs until they hatch four to five days later.

It must be emphasized that although in summary the pattern may appear sterotyped, numerous variations of both qualitative and quantitative nature occur (Tavolga, 1955a).

Observations

Analysis of Visual Stimuli

Twenty mature male *Bathygobius* were isolated in small 5-gallon tanks with shelters for three days prior to testing. The tests were made once a day, thus allowing sufficient time to elapse between tests to reduce the amount of overlap of responses (Tavolga, 1955a). In the majority of the tests, the stimulus-animal was placed in a one-liter Erlenmeyer flask filled with sea water, and the flask was slowly lowered into the tank. The actions of the resident male were observed for a five-minute period, the flask removed and then dipped into the next tank. One side of each tank was constructed of slate (containing an overflow); thus the resident was

unable to see into neighboring aquaria. In some of the tests, the stimulus-animal was confined in a screw-top vial, filled with sea water, into which the fish fitted tightly. The vial was lowered into each tank on a thread. In these tests, the stimulus-animal was unable to make any movements other than sporadic respiratory ones. In another series of tests, the stimulus-animals were anesthetized in 0.08% MS-222 (Tricaine methanesulfonate – Sandoz Pharmaceuticals) dissolved in sea water. This concentration stopped all except slow opercular and pectoral fin movements. The anesthetic induced an expansion of all melanophores, thus presenting a totally blackened stimulus-animal. In contrast, some of the stimulus-animals were injected with 0.05 ml. of 1/1000 adrenalin (Parke-Davis) in normal saline. This drug induced a contraction of melanophores and the animals became a light tan color with slight evidences of crossbands. The adrenalin-treated fishes were quiescent and exhibited no more movement during the tests than did the anesthetized animals. These drugged fishes were placed in a flask and used as stimulus-animals as described above.

In most cases, non-anesthetized stimulus-animals exhibited little movement other than respiratory and pectoral fin pulsations, and certainly no reactions which could be correlated with the behavior of the resident. In some cases, notably among males, the stimulus-animals exhibited combat behavior shortly after the initial reaction of the resident.

The stimulus-animals were all closely matched as to size, ranging from 60 to 65 mm. in standard length. In test series I, four types of stimulus-animals were used as follows:

(a). Gravid females—mature eggs easily extrusible; abdomen distinctly swollen.

(b). Non-gravid females – eggs not extrusible; abdomen flat as in males.

(c). Non-gravid females – eggs not extrusible; recently fed so that abdomen is swollen to same extent as in gravid females.

(d). Males—young, but sexually mature.

The type of stimulus-animal was alternated from day to day (as shown in Table 1). Each resident male was tested on 20 successive days, making 400 tests in all. The instances in which the stimulus-animals exhibited combat behavior are marked with an asterisk in the table. The symbols indicate the type of reaction by the resident male, as described below.

In all cases, the resident males approached the stimulus-animal within a few seconds. The types of reactions were classified and roughly quantified as follows:

C4—An immediate change to courtship coloration accompanied by courtship movements, followed by an approach to the stimulus-animal and continuation of the courting for the full five-minute test period.

C3—Initially an approach to the stimulus-animal, followed by courtship for the full test period.

C2—A vigorous courtship beginning immediately after the approach; courtship continued for less than one minute; male then became quiescent and often returned to the shelter.

C1—A slight lightening of the coloration shortly after the approach; no courtship movements, followed by return to shelter.

N—An approach to the stimulus-animal; no color change; quiescence or return to shelter within less than 30 seconds.

F1—An approach accompanied or followed by a slight darkening of the coloration; no actual combat; shortly followed by quiescence or return to the shelter.

F2—An approach followed by a definite darkening toward the combat coloration; some combat movements and sideward approaches which last for not more than one or two minutes.

F3—An approach followed by a complete exhibition of the combat coloration and behavior; continued for full test period.

F4—Darkening to combat coloration, followed by approach to stimulus-animal; combat behavior continued for full test period.

Table 1 shows that in some cases the behavior of the resident began as courtship but subsequently changed to combat. In all such cases, the stimulus-animal exhibited darkening and sideward approach. In one instance, the response changed from a darkened combat type (F1) to a lightened coloration of incipient courtship.

On the basis of the observations listed in Table 1, it is apparent that the types of reactions of the resident males were not correlated with the sex or gravidity of the stimulus-animals, except in the cases where the stimulus-animals exhibited combat behavior. Males nos. 1 and 16 reacted with courtship toward all stimulus-animals; males nos. 2, 11 and 15 courted in all cases, except where the introduced animals showed combat, in which tests the residents' behavior shifted toward the combat type. Males nos. 10, 12, 18, 19 and 20 showed frequent combat type responses, mixed with some C and N reactions. The remainder gave variable responses, consistent only in that frequent neutral and weak responses were observed.

Series II (Table 2) represents a continuation of the tests in series I, using the same resident

TABLE 1. RESPONSES OF RESIDENT MALES TO VISUAL STIMULUS OF GOBY IN FLASK (TEST SERIES I).

Type of Stimulus-animal in Flask	Resident Males & Their Responses																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Female—gravid	C4	C4	N	N	C1	C4	F1	F1	F4	C2	C4	F1	N	F1	C4	C4	C3	N	C2	C1
Female—gravid	C4	C4	N	N	C1	C4	F1	C1	N	C2	C3	F1	C1	C1	C4	C4	C2	F1	C3	C3
Female—non-gravid (fat)	C4	C3	N	N	C1	C3	C1	C1	N	C2	C4	F1	C1	C2	C4	C3	C2	N	C2	C2
Female—non-gravid (thin)	C4	C3	F1	C2	C2	C3	C1	C1	N	C2	C3	F1	N	C1	C4	C3	C3	N	C1	C2
Male	C4	C3	F1	C1	C2	C3	C1	C1	C1	C3	C4	N	N	N	C4	C4	C2	F3*	C1-F3*	C2-F3*
Male	C4*	C2-N*	F3*	C1-F3*	C1-F3*	C4-F3*	C1-F3*	C1-F3*	F3*	C1-F3*	C1-F3*	F3*	F3*	F3*	C2-F3*	C4*	C2-F3*	F3*	F3*	F3*
Female—gravid	C4	C3	F1	F1	C1	C3	F1-C2	C1	F1	F1	C4	F1	F1	N	C3	C4	N	F3	F1	N
Female—non-gravid (thin)	C4	C3	F3*	F3*	C1	C3	N	C2	N	C1	C4	F1	F3*	N	C3	C4	N	F3*	F3*	N*
Female—non-gravid (thin)	C4	C3	F1	F1	C2	C2	C1	C2	N	C1	C4	F1-C2	F1	C1	C3	C4	N	F2	F3	F1
Female—non-gravid (fat)	C4	C3	F1	C1	C1	C1	C1	C1	C1	C1	C4	F1	N	N	C3	C4	C1	F2	F2	F2
Female—gravid	C4	C3	N	N	N	C2	N	N	C2	C1	C4	F1	N	C1	C3	C4	C1	F1	F2	F3
Male	C4	C3	N	F3*	N	C1	F3*	N	C2	F3*	C4	F3*	N	C1	C3	C4	C1	F3*	F3*	F3*
Female—gravid	C4	C3	N	N	N	C2	F2	N	C1	F4	C4	F3	N	C1	C3	C4	N	F1	N	F3
Male	C4	C3	N	N	N	C2	F2	N	C1	F4	C4	F3	N	C1	C3	C4	N	F2	F2	F4
Female—non-gravid (thin)	C4	C4	F1	C1	N	C2	F1	N	C2	F4	C3	F3	F1	C1	C3	C4	N	F2	F2	F4
Female—non-gravid (thin)	C4	C4	N	C1	N	C2	F1	N	C2	F3	C3	F3	F1	C1	C3	C4	N	F3*	F3*	F4*
Female—gravid	C4	C4	C1	C1	C1	C2	N	N	N	F1	C3	F2	F2	N	C3	C4	N	F2	F1	F3
Female—gravid	C4	C3	C1	N	N	N	C1	C1	C1	N	C3	F1	F1	C1	C3	C4	C1	F1	F1	F2
Male	C4*	C2-F3*	C1-F3*	F3*	F3*	F2*	F2*	F3*	F3*	F3*	C2-N*	F3*	F3*	F3*	C2-F3*	C4*	C2-F3*	F3*	F3*	F3*
Female—gravid	C4	C3	C1	C1	N	F1	F1	N	N	N	C3	F1	N	N	C3	C4	C3	N	F1	F3

* Cases in which stimulus-animal exhibited combat behavior.

males. The first five tests used stimulus-animals that were immobilized in small vials. The rest of the tests used anesthetized and adrenalin-treated males. Table 2 shows that in this series, as before, males nos. 1, 2, 11 and 15 gave strong courtship responses. No. 17 gave stronger courtship responses here than in series I. No. 16 also showed strong courtship, but on the 15th test, and those following, changed to a neutral reaction type. Males nos. 6, 7, 10, 18 and 20 exhibited frequent combat responses. The remainder gave weak and neutral reactions. In some cases, there appeared to be a correlation of the reaction type with the coloration of the stimulus-animal, i.e. nos. 6 and 18 showed combat more consistently towards the dark anesthetized animals.

In comparing the two series, disregarding differences in the types of stimulus-animals, some of the males remained consistent in their responses. Some, however, changed their predominant response types, viz. nos. 6, 10, 17 and 18.

Test series III was set up to determine if the predominant response types of resident males could be altered by a strong combat or courtship experience. The same resident males were used as in series I and II. A large mature male was introduced into the aquaria of half of the residents—nos. 1, 2, 3, 8, 11, 12, 14, 15, 16 and 17. In each case, mutual combat began within a few seconds and was allowed to continue for one or two hours. In two instances, the introduced animal had to be removed in slightly less than an hour when it became evident that the resident would inflict serious damage. Gravid females were placed with resident males nos. 4, 5, 6, 7, 9, 10, 13, 18, 19 and 20. Courtship began immediately, and was permitted to continue for two hours.

Half an hour after the introduced animals were removed, the residents were tested in their reactions toward anesthetized and adrenalin-treated males confined in a flask (as in series II), and thereafter the tests were repeated nine additional times at daily intervals. Half these tests (resident nos. 1-10) used adrenalin-treated stimulus-animals, the remainder (resident nos. 11-20) used the darkened MS-222-treated animals.

Table 3 shows that the combat or courtship experience persisted in its effect on the first and, in many cases, subsequent tests. Males nos. 1, 2, 11, 15, 16 and 17 were, in series I and II, strong in their courtship responses, and the combat experience changed their subsequent responses to combat or neutral types. Nos. 8 and 14, after previous neutral responses (in series II), changed to strong combat reactions as a result of the extended combat experience. Nos. 3 and 12 appeared to be only slightly, if at all, affected by the combat experience.

Seven of the ten males given a courtship experience persisted in strong courtship responses in subsequent tests. This change to courtship was particularly striking in nos. 7, 10 and 18, which showed frequent combat responses in previous series. Nos. 6, 19 and 20 exhibited only a brief change to courtship response as a result of the courtship experience.

The observations summarized in Table 3 do not indicate any correlation between the responses of the residents and the color phase of the stimulus-animals.

Series IV comprises a group of miscellaneous visual stimulus tests that were performed on resident males other than the 20 used in series I-III.

In three instances, males that were brooding eggs were given the visual stimulus tests. In no case were any courtship responses observed, and, in 8 out of 9 tests, combat took place.

In preliminary tests with stimulus-animals in a flask, it was found that courtship-oriented residents reacted toward very small stimulus-animals. For example, a 25 mm. goby in a flask elicited a strong courtship response from a 75 mm. resident male. This same stimulus-animal without the protection of a flask would otherwise be chased about and even nipped to death in a few minutes by the male. Stimulus-animals below this size elicited a courtship response only in combination with olfactory stimuli, as will be described later.

Attempts to elicit courtship responses with simplified models were uniformly unsuccessful. Similarly, no courtship or combat responses could be elicited using dead or anesthetized gobies manipulated with threads or wire. It appeared that the reactions of the residents to these and to the models were similar. In most cases, the resident darted out of his shelter toward the stimulus object, and immediately returned to the shelter. In several tests, the residents nipped at the dead gobies as they would at a piece of food.

Other species of fish were used in flasks as test objects, *e.g.* gobies *(Gobiosoma bosci* and *Gobionellus boleosoma),* blennies *(Hypsoblennius ionthas* and *Hypleurochilus geminatus),* and others *(Fundulus heteroclitus* and *Mugil cephalus).* The specimens used were selected to fall into the size range 60-65 mm. In a few cases, the resident males showed a few quick approaches, but most of the time the stimulus-animal was not reacted to in any way.

Demonstration and Analysis of Chemical Stimulus

The initial demonstration of a chemical stimu-

TABLE 2. RESPONSES OF RESIDENT MALES TO VISUAL STIMULI (TEST SERIES II).

Type of Stimulus-animal	Resident Males & Their Responses																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(Immobilized in vial)																				
Female—gravid	C4	C2	N	C1	C1	N	N	N	C1	F2	C3	N	N	C1	C3	C4	C3	F1	F1	F1
Female—non-gravid (fat)	C4	C2	N	C1	C1	N	N	N	C1	F2	C3	N	F1	C2	C4	C4	C3	F3	F1	N
Female—non-gravid (thin)	C4	C3	C1	C2	N	F1	N	N	N	F2	C3	N	F1	C3	C4	C3	C3	F2	N	N
Male	C4	C2	C1	C3	N	F1	F1	N	C1	F2	C3	N	N	C1	C3	C4	C4	F2	N	N
Female—gravid	C4	C3	C1	C2	C1	F1	F1	N	C1	F2	C3	N	N	N	C3	C4	C4	F2	N	N
(Drugged males in flask)																				
Adrenalin	C4	C3	N	C1	C1	F1	F1	C1	N	F2	C3	N	N	C3	C3	C4	C4	F3	F2	F4
MS–222	C4	C3	N	N	C1	F2	F1	N	N	F2	C2	F2	N	N	C3	C4	C4	F3	F2	F4
Adrenalin	C4	C4	N	N	C2	N	F1	N	N	F2	C2	N	F1	N	C3	C3	C4	F3	F2	F3
MS–222	C4	C3	C1	C1	C1	F2	F1	N	N	F2	C3	F2	F1	N	C4	C4	C4	F4	F2	N
Adrenalin	C4	C3	C1	C1	C1	N	F1	N	N	F2	C3	N	F1	C1	C4	C4	C4	F4	F1	N
MS–222	C4	C2	F1	F1	N	F2	F2	N	C1	F3	C4	N	F2	C1	C4	C4	C4	F4	F2	N
Adrenalin	C4	C3	C1	C1	N	N	F1	N	C1	F2	C4	N	N	C1	C3	C4	C4	F1	F1	N
MS–222	C4	C2	C1	N	N	F2	F3	N	N	F3	C4	N	N	C1	C3	C4	C4	F4	N	F2
Adrenalin	C4	C3	N	N	N	N	F1	N	N	N	C2	N	N	N	C3	C4	C4	F1	N	F2
MS–222	C4	C3	N	F1	N	F2	F3	N	F1	F3	C3	F3	N	N	C3	N	C4	F4	N	F1
Adrenalin	C4	C3	N	N	N	N	N	N	N	N	C3	N	N	N	C3	N	C4	F1	N	F1
MS–222	C4	C3	N	F1	F1	F2	F3	N	N	N	C3	F2	F1	C1	C3	N	C4	F4	N	F2
Adrenalin	C4	C2	N	N	N	N	N	N	F1	N	N	F1	N	N	C3	N	C4	F1	F1	N
MS–222	C4	C3	N	F1	N	F2	F1	N	F1	N	C3	F1	N	N	C4	N	C4	F4	N	N
Adrenalin	C4	C3	N	N	N	N	F1	N	F1	N	C3	F1	N	C1	C3	N	C4	F1	N	N

TABLE 3. EFFECTS OF PRIOR EXPERIENCE UPON RESPONSES OF RESIDENT MALES TO VISUAL STIMULUS (TEST SERIES III).

	Resident Males & Their Responses*																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Type of prior experience—Courtship (*C*); Combat (*F*)	F	F	F	C	C	C	C	F	C	C	F	F	C	F	F	F	F	C	C	C
First test 30 minutes after above experience	F4	F4	F4	C4	C4	C4	C4	F3	C4	C4	F4	F4	C4	F4	F3	F4	F4	C4	C4	C4
Subsequent daily tests using drugged males in flasks as stimulus-animals	F1	F4	F3	C4	C4	C1	C4	F2	C4	C3	F4	N	C3	F4	N	F4	N	C4	N	C1
	F1	F4	N	C4	C3	N	C4	F2	C4	C3	F4	N	C4	F4	N	F4	N	C4	N	C1
	N	F1	N	C4	C3	N	C4	F2	C4	C3	F3	N	C3	F4	N	F3	N	C4	N	C2
	N	N	N	C4	C4	N	C4	F3	C4	C2	F1	N	C3	F4	N	F1	F1	C4	N	C1
	N	F1	F1	C4	C4	N	C4	F3	C4	C4	F4	N	C3	F4	C1	F3	F1	C4	N	C1
	N	N	N	C4	C4	N	C4	F3	C4	C4	F1	N	C3	F4	N	N	F1	C4	N	N
	N	N	N	C4	C3	N	C4	F3	C4	C3	N	N	N	F4	N	F1	F1	C4	N	N
	N	N	N	C4	C3	N	C4	F3	C4	C3	N	N	N	F4	N	F2	F1	C4	N	N
	N	N	N	C4	C3	N	C4	F2	C4	C3	N	N	N	F4	N	F1	N	C4	N	N
	Stimulus-animals adrenalin-treated											Stimulus-animals anesthetized with MS-222								

* Same Resident Males as in Series I and II.

lus to courtship behavior in *Bathygobius* males was performed as follows: a resident male was selected which, on the basis of previous tests, showed a strong tendency toward courtship reactions; a gravid female was placed in a 50 ml. beaker filled with sea water for two minutes; the water from the beaker was then pipetted into the tank with the resident male. Within five to ten seconds, the male exploded into courtship. He displayed the courtship color phase and went into vigorous courting movements, swimming randomly all about the aquarium. If the running sea water supply to that tank was turned off, this courtship kept up for about an hour. If the sea water was kept running, however, the male lapsed into quiescence within 10 to 15 minutes.

In further testing the potency and site of origin of this stimulus, the resident males were all previously tested and given a strong courtship experience with a gravid female. This was to insure uniformity in their responses and, possibly, to increase their sensitivity toward these stimuli.

Obtaining the gravid-female-water as described above, it was found that as little as 0.5 ml. was effective in eliciting a courtship response. Smaller quantities produced either no response or a slight lightening of the male's coloration for a few seconds.

In order to determine the source of this stimulating factor, gravid females were wrapped closely in absorbent tissue paper. Care was taken that the tissue was not permitted to touch the anal-genital area. The females were first rinsed in a strong stream of sea water. Thus the tissue became soaked with the mucus secreted by the skin. In addition, small fragments of absorbent tissue paper (about 2-3 mm. square) were touched to the anus and to the tip of the urogenital papilla of gravid females. A small piece of the mucus-soaked tissue was dropped a few centimeters in front of a resident male. The other fragments of tissue were similarly tested.

Courtship responses were elicited only from the tissue that had been in contact with the urogenital papillae. The reactions of the males took place within 10 to 20 seconds and lasted for as long as 15 to 30 minutes. A full, vigorous courtship response was obtained. The mucus-soaked and anus-touched tissue fragments did not elicit a courtship response at any time, although in a few cases the male picked up the tissue in his mouth and spit it out as with a piece of food.

Various internal body fluids from gravid females were tested as possible sources of this chemical stimulus by either pipetting a few drops into the aquarium with a male, or by soaking the liquid up in a small bit of absorbent tissue paper and dropping the fragment near the resident.

The following fluids were tested: ovarian fluid; urine; coelomic fluid; blood; feces; stomach fluid. Of these, only the ovarian fluid elicited a courtship response. As little as 0.1 ml. in a five-gallon tank was sufficient to evoke a response lasting up to 30 minutes. The ovarian fluid was obtained by removing an ovary (enlarged and turgid with mature eggs), rinsing it off in 0.65 saline solution, cutting it open in a watch glass containing about 5 ml. of normal saline and squeezing out the eggs. After a few moments, the eggs settled to the bottom and the solution was pipetted off. Only a substance from the interior of the ovary was effective. Mucus and other fluids from males were negative in their effects.

It is not known whether this stimulating ovarian substance is secreted by the eggs or the ovary itself. Freshly extruded eggs are effective in eliciting courtship, but they lose the effectiveness within 10 or 15 minutes, whether or not they are fertilized. At the present time, no further data are available on the source and nature of the stimulating ovarian substance.

In order to determine the sensory organ involved in the response of males to this ovarian substance, the nostrils of the males were plugged by inserting a hot needle into each aperture (both incurrent and excurrent nostrils were plugged). The heat produced a plug of coagulated mucus at each aperture. This plug lasted for about two days. Permanently anosmic males were produced by cauterizing the entire nasal epithelium with a hot wire inserted into the nostrils.

These anosmic males did not respond to any amounts of the courtship-stimulating substance. The temporarily anosmic animals regained their sensitivity to the substance after two days, when the mucus plugs disappeared.

In comparing the efficacy of ovarian substance from animals at various stages in their ovarian cycle, the ovaries of non-gravid, recently spawned, pre-gravid and gravid females were used. Both the non-gravid and recently spawned females contained small, white, immature eggs. The ovaries of pre-gravid females (2-3 days prior to spawning) had eggs that could be extruded, but were not fertilizable. The latter ovaries elicited courtship responses in resident males, whereas the ovarian material from non-gravid and recently spawned females was only slightly effective or not at all. A slight reaction was one in which the male swam out of his shelter briefly, turning lighter as he did so.

It may be added that none of these ovarian substances had any perceptible effect on the behavior of isolated female gobies. Testicular material and male mucus secretions had no observable effect on either male or female behavior.

In connection with these experiments with chemical stimuli, a striking example of a conditioned response was observed in two of the resident males. In some of the preliminary tests, these two males were tested several times a day in their reactions to various fluids pipetted into their aquaria. After about a dozen tests, it became evident that they were responding with courtship behavior toward control substances, such as normal saline and sea water. It was further found that the simple act of holding an object or a hand a few inches above the tank was sufficient to elicit a courtship response. This conditioning was short-lived. After six to eight hours, the response to the conditioned stimulus was slight or absent, and the conditioning was no longer evident after 12 hours. In subsequent tests, intervals of at least 24 hours were allowed in order to avoid this complicating factor as much as possible.

Responses to Combination of Visual and Chemical Stimuli

On the basis of the results in test series I-III, resident males were classified into three reactive types:

A—Strong courtship reaction toward visual stimuli.

B—Frequent combat reactions toward visual stimuli.

C—Responses toward visual stimuli varied but never consistently strong; most frequent responses of the C1, F1 and N types.

Using resident males so classified, a series of tests were made in which the visual and olfactory stimuli were combined.

Type A Resident Males

An olfactory stimulus alone is sufficient to elicit a courtship response in these animals, but the addition of a visual stimulus of another goby in a flask orients the behavior. Without this visual stimulus, the courting behavior involves random movements all about the aquarium. With the visual stimulus, the male directs his attention toward the stimulus-animal, circling about the flask and butting against it. This courtship response continues for several hours, i.e. longer than with the olfactory stimulus alone. Furthermore, the courtship response is elicited and is continued toward all types of stimulus-animals: male or female, gravid or non-gravid, dark or light-colored, anesthetized or not, dead or alive, etc. The courtship continues even if the stimulus-animal exhibits combat behavior. The size limit of an adequate stimulus-animal is well below normal behavioral levels; a strong courtship will take place toward a 15 mm. goby in a flask (this was the smallest size available).

However, models and fishes of other species were ineffective in inducing an orientation of the courtship elicited by the olfactory stimulus.

Anosmic males, previously determined as highly reactive in terms of courtship response, never showed any courtship behavior. Their reaction toward gobies introduced into the aquarium was invariably a combat, even toward gravid females. Their responses toward a stimulus-animal in a flask were either of the combat or neutral type.

Type B Resident Males

The olfactory stimulus alone elicited a short courtship response in males. However, the combination of this with a visual stimulus of a goby in a flask usually produced a prolonged and vigorous courtship. In several cases, males so tested were found in subsequent tests to have changed to a type A reactivity.

In several tests, the visual stimulus was presented first, using a male as the stimulus-animal. A mutual combat began and was allowed to continue for a few minutes, then the ovarian fluid from a gravid female was poured into the tank. Within 10 or 15 seconds, the combat behavior of the male started to change to courtship, although the combat attempts of the stimulus-male continued for several minutes.

In a few cases, the resident males, when given a simultaneous olfactory stimulus and visual stimulus of a combating male, exhibited an alternation of combat behavior directed toward the stimulus-animal and of courtship behavior randomly oriented about the tank.

Type C Resident Males

An olfactory stimulus alone had little or no effect. Sometimes a brief lightening of the coloration took place and the male left his shelter, darted about the tank a few times, then returned to the shelter. In two cases, out of seven males tested, the olfactory stimulus alone elicited a strong courtship response. One of these two males subsequently changed to a type A reactivity.

A combined olfactory and visual stimulus elicited strong courtship responses and the resident males became highly responsive in that they then reacted with courtship toward visual and olfactory stimuli separately—i.e. type A reactivity. In a few cases, the courtship reaction toward the combined stimuli was temporarily changed to combat when the stimulus-animal exhibited darkening and sideward approach. In these cases, the courtship of the resident became random and disoriented, whereas spells of combat were distinctly oriented toward the stimulus-animal.

Sound Production

On the supposition that sound production might be involved in either the courtship or the combat behavior in *Bathygobius,* sound detection equipment of a preliminary nature was assembled. A waterproofed microphone was lowered into an aquarium containing the fish. The microphone was connected to a 10-watt amplifier, which, in turn fed the signal to the input of a tape recorder. The recordings were later played back into the aquarium with the same equipment, but using the microphone as a transducer.

The tape recordings were analyzed by means of oscilloscope observations, recording galvanometer traces and sound spectrograms. Unfortunately, the recording equipment was of low fidelity and the recordings contained some interfering background noise. The following descriptions of the sounds should be regarded as preliminary approximations.

In *Bathygobius,* only males were found to produce detectable sounds, and these were made only during the courtship behavior. No sounds could be detected during combat behavior between males. The courtship sounds consisted of short, low-pitched grunts, synchronized with the downward head-snapping or gasping movement which occurs frequently during vigorous courtship.

The intensity of these grunts could not be measured with the present equipment. The sounds were below audible level, even with the ear held up against the tank.

The frequency with which the sounds were produced was extremely varied. When the male was courting at some distance from the female, the grunts and head snaps occurred at the rate of only one or two during the course of a 30-second courting spell. When the male was vigorously courting a nearby female, or when the female was following the male into the nest, the grunts were produced at two- to four-second intervals, and sometimes four or five in quick succession.

The duration of each grunt varied from 0.15 to 0.20 of a second. Spectrograms of three sounds of about average duration are shown in Plate I. The fundamental pitch frequency of each grunt was a broad band from about 100 to 200 cycles per second. This value was determined from oscilloscope observations in which the fish sound was matched against sine waves of known frequency. The recording galvanometer showed a fundamental frequency at about 150 cps. Sound spectrograms in the 200 cycle/inch range showed a broad band about 100 cycles in width. The top of this band was at about 200 cps. at the beginning of the sound,

dropping to below 100 cps. toward the end. The resolution of the lower frequencies on the spectrogram was poor as a result of excessive low frequency noise in the recording.

Plate I shows that frequencies up to 4800 cps. were detected during the initial 0.05 second of the sound. Toward the end of the sound burst, the peak frequency dropped logarithmically. The pattern on the first 0.17 seconds of the spectrogram represents low frequency background noise in the recording.

When the sounds of a courting male were played back to an isolated gravid female, she began to dart quickly about the tank. Her swimming movements were similar to the short hops which are characteristic of a female that follows a courting male into the nest. After one or two minutes, she subsided into quiescence. This same behavior could be stimulated again but only if the playback were turned off for a half an hour or more. After three or four exposures to these stimulations, the gravid female no longer reacted to the sounds. Non-gravid females showed no discernable response to the sounds.

When the playback of the sounds was combined with the visual stimulus of another goby in a flask, the gravid female oriented some of her darting movements toward the stimulus-animal. A visual stimulus alone had no effect. Attempts to enhance the value of the visual stimulus by manipulating anesthetized, adrenalin-treated males into a simulation of the courting movements did not appear to increase the response of the female to the sounds, nor did models colored to mimic the light body and black chin of a courting male have an effect on orienting the response of a gravid female to the sound playback. There was no effect on the female's response by the addition of a chemical stimulus, i.e. water in which a male goby had been previously soaked.

The female's orientation toward a visual stimulus was unaffected by the relative position of the transducer. If the flask containing the stimulus-animal were moved, she followed it with short darts.

Isolated males responded to the playback of the courtship grunts. The male immediately swam up to the source of the sound, darted quickly about the tank and returned to the nest. He left his nest again and approached the transducer several times before he became quiescent. The same response was elicited 15 to 30 minutes later. After four or five trials with the same male, he no longer responded to the sounds. During the response, the male's coloration became a light tan (as in the courtship phase), but was unusual in that the chin and throat were almost white. If a visual stimulus of another goby in a flask were provided at the same time or slightly after the playback began, the male approached the stimulus-animal, turned a dark color, and exhibited the movements typical of combat behavior.

Discussion

Visual Cues

In fishes, vision has always been considered a major factor in sex discriminatory and territorial behavior. Noble (1934) and Noble & Curtis (1939) reported that the coloration and behavior of an intruding animal are of prime importance in sex discrimination in sunfish and jewel fish *(Eupomotis* and *Hemichromis).* The size and shape of the stimulating object appear to function in the response of male guppies *(Lebistes)* toward females (Breder & Coates, 1935), while color plays a role in male-to-male encounters (Noble, 1938). Noble (1938) concluded that the bright colors of males of many teleost species function primarily as warning devices in territorial aggressions. The function of these colors in courtship behavior evolved subsequently. The work of ter Pelkwijk & Tinbergen (1937) and Baerends & Baerends-Van Roon (1950) on the stickleback *(Gasterosteus)* and the Cichlidae revealed that specific colors, patterns and shapes function as stimuli ("releasers") for particular actions on the part of both sexes during reproductive behavior. Tinbergen's interpretations (1951) of stickleback behavior have been applied in studies on *Cottus gobio* and *Gobius microps* by Morris (1954) and Nyman (1953), respectively. Extensive and rapid changes in color and pattern in *Bathygobius* are associated with environmental conditions (Tavolga, 1950), and the specific color phases that are associated with combat and courtship have been described earlier (Tavolga, 1954).

The behavior of the introduced animal is also important in determining the resident male goby's reaction (Tavolga, 1955a). A quickly retreating animal frequently elicits a nip-chase or combat response, whereas a slow retreat or none at all may stimulate courtship. Noble (1938) discussed a similar behavior among many species of fishes, and Baerends & Baerends-Van Roon (1950) describe the "submissive" behavior of females of many cichlids as one that stimulates courtship behavior. The general idea in most of these interpretations has been that initially the territory-holding male exhibits a generalized "challenge" to an intruder; then, a male intruder may return the "challenge" and a combat ensues. A fleeing intruder will be chased, whereas a gravid female holds her ground. The latter behavior stimulates courtship. This interpretation is inadequate for the be-

havior situation in *Bathygobius,* which appears to be more complex, as shown by some preliminary quantitative studies (Tavolga, 1955a). No distinctive colors or shapes serve to identify a female or her gonadal condition, yet a gravid female is courted vigorously regardless of her behavior, since the male is strongly stimulated by her ovarian secretions. The darkened coloration of a fighting male, together with the attendant fin-bristling, gaping, quivering and biting, form a visual stimulus-complex which elicits combat behavior on the part of the territory-holding male. Here there are two well-differentiated stimulus patterns which, in general, elicit a discriminatory response, i.e. courtship versus combat behavior, on the part of the resident male. The presence of intermediate and variable stimulus and response conditions deserves closer attention, however. Smaller males and non-gravid females react to the initial approach of a resident male in a variety of ways ranging from flight to quiescence. There is a tendency for the resident to court those individuals that hold their ground and to nip and chase those that flee, but the male's response is extremely variable (Tavolga, 1955a). The present work shows that the visual stimulus of the appearance and behavior of the intruder is only part of the configuration to which the resident responds. Low levels of olfactory stimulation as well as the recent experiences of the male contribute to the channeling of the response.

Olfactory Cues

The earlier work of Parker & Sheldon (1913) and Olmstead (1918) clearly separated olfactory sensitivity as distinct from the contact chemical sense of taste in fishes. More recently, the function of olfaction has been demonstrated in schooling (Wrede, 1932), fright reactions (von Frisch, 1941a and 1941b), and the detection and discrimination of plant odors (Walker & Hasler, 1949). There is ample evidence of a high level of sensitivity and discrimination of chemical stimuli present in the olfactory apparatus of many fishes. The role of olfaction in reproductive behavior is poorly known. Jaski (1939) postulated the existence of a hormone-like substance ("copulin") which is secreted by males of the guppy *(Lebistes)* and stimulates the receptivity of the female. However, both Jaski's evidence and interpretations could not be confirmed by Breder (as cited by Clark & Aronson, 1951). Chemical sense appears to be involved in sex discrimination in the catfish *(Ameiurus)* during courtship, and the "barbel-play" probably includes tactile and gustatory stimuli as well as olfactory (Breder, 1935; Hoagland, 1933). Chemical factors are clearly involved in the pairing of the blind goby *(Typhlogobius),* as described by MacGinitie (1939), but here too the chemical stimuli were not experimentally separated into olfactory and gustatory.

The nature of the stimulating substance in *Bathygobius* is not known, and the present evidence demonstrates only that it is rapidly diffusible and present exclusively in the ovaries and female genital tract. Whether it is secreted by the ovarian tissue or from the eggs, it is present in higher concentration or potency in ovaries with mature eggs. Ovaries of non-gravid or recently spawned females possess the courtship stimulating substance in small amounts or low potency.

The absence of courtship behavior in anosmic males demonstrates that the stimulus is an olfactory one rather than gustatory. Furthermore, the tendency toward combat behavior in these males may indicate that the olfactory stimulus not only elicits courtship but inhibits combat. This inhibition is not necessarily complete, as illustrated by the cases in which a conflicting situation was set up—an olfactory stimulus together with a visual stimulus of a male in combat. The actions of the resident then alternated from combat display toward the animal in the flask to random, non-oriented courtship.

Castrated male *Bathygobius* do not exhibit combat behavior. However, such operated males vigorously court gravid and non-gravid females, as well as other males in the same manner and to the same degree (Tavolga, 1955b). If, as demonstrated here, the courtship response is in the main elicited by an olfactory stimulus, it is possible that the male sex hormone affects the sensitivity of the olfactory organs in some way that renders these organs differentially sensitive to one or more substances. Castration may lower the sensitivity threshold and permit chemicals other than the ovarian fluid to stimulate courtship behavior.

Set

In an earlier study (Tavolga, 1955a), it was found that tests of responses by resident males had to be spaced several hours apart to avoid a persistence of the response from a previous test. In practice, a 24-hour spacing together with an alternation of sex of the test animal was found to be sufficient. This persistence may last for several days when the stimulus is purely visual and the stimulus-animal confined in a flask. It may be that the latter method is a more sensitive test for the existence of a predisposition on the part of the male, since the behavior of the stimulus-animal is restricted and plays less of a role in determining the response of the resident. The term *set* is one that is used

by psychologists for this type of predisposition or tendency towards a particular kind of response.

Series I of the visual tests indicated that a prior set of the male determined his type of response. This set was strong enough, in some cases, to overcome a visual stimulus towards another type of behavior. Specifically, a strong courtship orientation would overcome a visual combat stimulus, and courtship would continue. In cases where the combat display on the part of the stimulus-animal altered the response of the resident from courtship to combat, the set can be interpreted as weak. A set can also exist toward combat behavior, although this is never as strong as that toward courtship. Whether the neutral response of the resident indicates a third type of set or a lack of set, is a matter of nomenclature.

Series II showed that the appearance of the stimulus animals often has little effect upon the type of response if the resident is in a strong set. The only purely visual stimulus that can overcome a set is the color and behavior of another male in combat.

Series III showed that the set could be changed by a prolonged experience of either a vigorous courtship toward an unconfined gravid female or a mutual combat with another male. A courtship set, once established, was of longer duration than a combat set. Similarly, a courtship set was much easier to establish and harder to alter than a combat set.

The above method of establishing a courtship set included, of course, an olfactory stimulus. The olfactory stimulus is of prime importance, then, not only in stimulating courtship but in establishing the set. In situations of conflicting olfactory and visual stimuli, the courtship response usually predominated, although sometimes an alternating response occurred. In no case did the visual stimulus override the olfactory.

A male in courtship set will react with courtship toward the olfactory stimulus alone or toward a visual stimulus alone. The latter may even be subnormal in size.

A visual stimulus of a combating male can, at times, override a courtship set. The cues involved in a combat stimulus include not only a dark-colored adversary but the movements of sideward approach as well. A dark-colored but unmoving stimulus animal rarely elicits combat.

The possibility was considered that this apparent set could have resulted from the conditioning of the resident animals toward the act of presenting them with a stimulus situation. The type of stimulus-animal was varied as much as possible to prevent any conditioning, but the possibility exists nevertheless. The set, then, may be a situation induced by the testing procedure. Even so, the speed with which a set can be established, whether by conditioning or by some internal change, is of significance. Fishes are capable of forming remarkably rapid and complex associations. Topographic learning appears to be well-developed in many species (Goldsmith, 1905, 1912, 1914), and especially in littoral and tidal zone species such as *Bathygobius* (Aronson, 1951). Visual, tactile and kinesthetic factors are involved in maze-learning in fishes (Benuic, 1938; Churchill, 1916). Many investigators have used complex conditioning techniques in studies on visual perception (Herter, 1929; Perkins & Wheeler, 1930; Rowley, 1934). Hager (1938) showed that *Phoxinus* was capable of several types of associations simultaneously, and that retrained animals learn more quickly. Combinations of two or more sense modalities in the learning of configurations was demonstrated by Herter (1929) and Sanders (1940). The studies of Breder (1950, 1954) demonstrated the interdependence of visual and tactile senses among shelter-seeking fishes such as *Bathygobius* and *Pomacentrus.*

Fishes frequently form associations that may be surprising and unexpected. For example, males have "courted" a pipette or the observer's hand. A similar observation was made by Berghe (1929) during the course of his studies on the olfactory sense of *Cottus* and *Blennius.* Many home aquarists know how quickly their fishes learn to come to a specific feeding corner when the light is turned on, or the cover moved, or even when a shadow moves across the tank.

In the case described here, the response comprised a complete change to a courtship color phase and the courting movements for several minutes. Such a situation deserves further study, especially with a view toward finding out how quickly fishes learn to respond with specific behaviors toward models possessing elements of a normal stimulus pattern. On this basis, it could be possible to reinterpret the experimental evidence involving the reactions of fishes to specific stimuli, e.g. "sign stimuli" or "releasers," tested by means of abstracted models. The prespawning and spawning behavior in the stickleback (ter Pelkwijk & Tinbergen, 1937; Tinbergen & Van Iersel, 1947) was analyzed by this method, and the interpretation of the behavior as a series of sequentially dependant innate responses to releasing stimuli is extensively given by Tinbergen (1951). A similar line of thought is followed by Baerends & Baerends-Van Roon (1950) in their investigations of behavior in the Cichlidae. In all of this, and related, work, the innate nature of these responses

is assumed and there is little direct evidence given. Indeed, the very definition of an "innate behavior" as given by Tinbergen (1951) is subjective and intuitive. It is equally possible, therefore, that the responses of fishes, as well as of other vertebrates, to sign stimuli are conditioned or learned (in the broad sense of the word). Certainly fishes of many species can be quickly conditioned to respond to highly specific stimulus patterns, which can be purely artificial as well as being abstractions from their normal enviroment.

Sound Production

The production of sound by fishes is well-known, especially for groups such as the grunts (Haemulidae), and croakers and drumfishes (Sciaenidae). The early investigations of Agassiz (1850), Smith (1905) and Tower (1908) described various swim bladder mechanisms as the sound producing organs. Kellog (1953) summarized much of the literature available on noises made by marine organisms. There have been extensive studies, supported by the U. S. Navy and carried out by Fish (1954), wherein the sounds were analyzed as to frequency and intensity and the sound-producing species identified.

Some of the sounds made by marine fishes have accompanied feeding and schooling behavior, and some have been associated with spawning migrations, especially in the case of the "drumming" of croakers and drumfishes (Fish, Kelsey & Mowbray, 1952). The high frequency clicking in the sea horse (*Hippocampus*) may be involved in some phase of reproductive activity (Fish, 1953). The only specific case of sound production correlated with sexual behavior has been in the so-called croaking or purring gourami, *Trichopsis* (*Ctenops*) *vittatus*, as described by Stampehl (1931), Beyer (1931) and Reickel (1936). Both sexes in this species have been described as producing an audible purring noise during pre-spawning behavior.

In *Bathygobius*, the grunting noises produced by a courting male are distinctly stimulating to the female. Her response appears to be oriented by the visual stimulus. The olfactory and auditory stimuli comprise a set of distance cues leading toward successful pairing. The ovarian substance stimulates a non-oriented courtship behavior on the part of the male. This includes the production of sounds by the male. The sounds, in turn, stimulate generalized activity on the part of the female. The visual stimuli which occur subsequently, orient and re-enforce the pre-spawning behavior of both sexes. This, however, cannot preclude the possibility that under other conditions, both the chemical and sound stimuli may also be of orienting and directional value. Observations in larger tanks and in the field will be necessary to determine the extent to which these fishes can orient themselves toward the source of a chemical or sound stimulus.

Although there has been a considerable amount of investigation on the auditory apparatus and function in fishes, the lowest frequency response limits have not been adequately established. The early work of Parker (1902, 1910, 1918) indicated that a number of fishes are sensitive to auditory stimuli at least as low as 100 cps. There is no evidence that the teleost ear may not be sensitive to even lower frequencies.

Rode (1929) and Dykgraaf (1933) performed some of the definitive experiments which showed the lateral line system of teleosts is sensitive to low frequency vibrations, including the detection of sharp blows, shocks and currents in the water. Rode (1929) obtained behavioral responses via lateral line stimulation with frequencies up to 60 cps. Schriever (1935, 1936) was able to detect action potentials from the lateral line nerve with stimuli of up to 150 cps. The uppermost reactive limit of the lateral line was placed at between 200 and 300 cps. with a tonal reproduction limit of 180 cps. (Suckling & Suckling, 1950).

On the basis of the above evidence, obtained from several species of teleosts, the fundamental frequency of the sounds produced by the male *Bathygobius* would fall into the reactive range of both the auditory and lateral line sensory systems, and at the present time it is not known which of the two senses, if not both, are involved in the reaction of the female. The lashing undulations of the body and tail of the male during courtship certainly produce oscillations that could be detected by the lateral line. These courtship undulations have been estimated as varying from five to twelve oscillations per second (Tavolga, 1954).

General Conclusions

It is evident that a number of sensory mechanisms function in concert to elict, channel and modulate the responses of at least some fishes toward each other. Territorial and reproductive behavior is differentiated into its various forms by stimuli acting through the olfactory, auditory (including perhaps the lateral line system) and visual organs. Indeed, the visual cues involve a number of perceptual mechanisms such as discrimination of form, shade, color, movement, etc.

This interdependence of sense modalities has been widely studied in the higher vertebrates, including mammals, and the conclusions have been admirably stated by Beach (1951):

"Sexual arousal and mating behavior in all or nearly all animals appear to involve the activation of several different afferent systems. Orientation to, and movement toward a potential mate may occur in response to visual, auditory, or chemical cues. Any or all of these modalities may continue to influence the degree of excitment and to guide the bodily reactions of male and female during precoital courtship."

Summary and Conclusions

1. Males of *Bathygobius soporator* (Cuvier & Valenciennes) possess a highly differentiated territorial and pre-spawning behavior pattern. Sex discrimination is achieved by a differential response on the part of a resident male according to sex and gonadal condition of the intruding animal.

2. *Bathygobius* males respond readily to members of the same species, but do not react with either courtship or combat toward models and other fishes of similar shapes and sizes.

3. The courtship behavior of a male can be elicited by non-specific visual stimuli, i.e., differentiated sexually dimorphic cues are not present in this species. The appearance of another *Bathygobius* alone is often sufficient to elicit courtship, without regard to the behavior, coloration or sex of the stimulus-animal.

4. A distinct exception to the above is the reaction of a male to the appearance of a goby in combat, i.e. in cases where the stimulus-animal darkens and exhibits sideward approach. Visual stimuli comprising combat behavior are of primary value in eliciting a combat response on the part of a territory-holding male.

5. In the absence of a visually stimulating animal, the male responds with courtship to the presence of a chemical substance produced by a gravid female. The source of the stimulating material is the fluid of the ovary, and the male detects this rapidly diffusing substance by olfaction.

6. Males do not respond to visual stimuli in a stereotyped and predictable manner, and their type and intensity of response is governed, in part, by previous recent experiences. A strong predisposition (set) towards courtship response can be induced by a prolonged courtship experience. A similar, but less distinct, predisposition toward combat can also be induced. Repeated tests show that this predisposition can change from one type to the other, or toward a neutral responsiveness. Such changes are apparently "spontaneous" and cannot be correlated with changes in external conditions.

7. During courtship, the male produces frequent grunting sounds. Gravid females respond to these sounds with short quick darting movements, as would be characteristic of their pre-spawning behavior. The female's reactions to the sounds alone appear unoriented, but the presence of a visual stimulus of another goby serves to direct her movements.

Literature Cited

Agassiz, J. L.

1850. Manner of producing sounds in catfish and drumfish. Proc. Amer. Acad. Arts Sci., 2:238.

Aronson, L. R.

1949. An analysis of reproductive behavior in the mouthbreeding cichlid fish, *Tilapia macrocephala* (Bleeker). Zoologica, 34: 133-158.

1951. Orientation and jumping behavior in the gobiid fish *Bathygobius soporator*. Amer. Mus. Novitates, no. 1486:1-22.

Baerends, G. P.

1950. Specialization in organs and movements with a releasing function. In: Physiological mechanisms in animal behaviour. Symp. Soc. Exp. Biol., no. 4:337-360.

Baerends, G. P. & J. M. Baerends-Van Roon

1950. An introduction to the study of the ethology of cichlid fishes. Behaviour, suppl. 1:1-242.

Beach, F. A.

1951. Instinctive behavior: Reproductive activity. In: Handbook of experimental psychology. Editor, S. S. Stevens. John Wiley & Sons, N. Y., pp. 387-434.

Benuic, M.

1938. The roundabout path of the fighting fish (*Betta splendens* Regan). Proc. Zool. Soc. London, ser. A, 108:403-422.

Berghe, L. van den

1929. Observations sur l'olfaction et sur le méchanisme des courants olfactifs chez quelques téléostéens. Bull. Class. Sci. Acad. Roy. Belgique, sér. 5, 15:278-305.

Beyer, L.

1931. *Ctenops vittatus* Cuv. Aquatic Life, 15:61.

Breder, C. M., Jr.

1934. An experimental study of the reproductive habits and life history of the cichlid fish, *Aequidens latifrons* (Steindachner). Zoologica, 18:1-42.

1935. The reproductive habits of the common catfish *Ameiurus nebulosus* (Le Sueur) with a discussion of their significance in

ontogeny and phylogeny. Zoologica, 19:143-185.

1936. The reproductive habits of the North American sunfishes (Family Centrarchidae). Zoologica, 21:1-50.

1939. On the life history and development of the sponge blenny, *Paraclinus marmoratus* (Steindachner). Zoologica, 24:487-496.

1941. On the reproductive behavior of the sponge blenny, *Paraclinus marmoratus* (Steindachner). Zoologica, 26:233-236.

1950. Factors influencing the establishment of residence in shells by tropical shore fishes. Zoologica, 35:153-158.

1954. Further studies on factors influencing the reactions of tropical shore fishes to shells. Zoologica, 39:79-84.

BREDER, C. M., JR. & C. W. COATES

1935. Sex recognition in the guppy, *Lebistes reticulatus* Peters. Zoologica, 19:187-207.

CHURCHILL, E. P.

1916. The learning of a maze by goldfish. J. Anim. Behavior, 6:247-255.

CLARK, E. & L. R. ARONSON

1951. Sexual behavior in the guppy, *Lebistes reticulatus* (Peters). Zoologica, 36:49-66.

CLARK, E., L. R. ARONSON & M. GORDON

1954. Mating behavior patterns in two sympatric species of xiphophorin fishes: their inheritance and significance in sexual isolation. Bull. Amer. Mus. Nat. Hist., 103:135-226.

DYKGRAAF, S.

1933. Untersuchungen über die Funktion der Seitenorgane an Fischen. Ztschr. vergl. Physiol., 20:162-214.

FISH, M. P.

1953. The production of underwater sound by the northern seahorse, *Hippocampus hudsonius*. Copeia: 98-99.

1954. The character and significance of sound production among fishes of the Western North Atlantic. Bull. Bingham Oceanogr. Coll., 14:1-109.

FISH, M. P., A. S. KELSEY, JR. & W. H. MOWBRAY

1952. Studies on the production of underwater sound by North Atlantic coastal fishes. J. Marine Res., 11:180-193.

VON FRISCH, K.

1941a. Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. Ztschr. vergl. Physiol., 29:46-145.

1941b. Die Bedeutung des Geruchsinnes im Leben der Fisches. Naturwiss., 29:321-333.

GOLDSMITH, M.

1905. Recherches sur la psychologie de quelques poissons littoraux. Bull. Inst. Gén Psychol., 5:51-67.

1912. Contribution à l'étude de la mémoire chez les poissons. Bull. Inst. Gén. Psychol., 12:161-176.

1914. Les reactions physiologiques et psychiques des poissons. Bull Inst. Gén. Psychol., 14:97-228.

GUITEL, F.

1892. Observations sur les moeurs du *Gobius minutus*. Arch. Zool. Exp. Gén., sér. 2, 10:499-555.

1893. Observations sur les moeurs de trois blenniides, *Clinus argentatus, Blennius montagui,* et *Blennius sphynx*. Arch. Zool. Exp. Gén., sér. 3, 1:325-384.

1895. Observations sur les moeurs du *Gobius ruthensparri*. Arch. Zool. Exp. Gén., sér. 3, 3:263-288.

HAGER, H. J.

1938. Untersuchungen über das optische Differenzierungsvermögen der Fische. Ztschr. vergl. Physiol., 26:282-302.

HERTER, K.

1929. Dressurversuche an Fischen. Ztschr. vergl. Physiol., 10:688-711.

HOAGLAND, H.

1933. Specific nerve impulses from gustatory and tactile receptors in catfish. J. Gen. Physiol., 16:685-693.

JASKI, C. J.

1939. Ein Oestruszyklus bei *Lebistes reticulatus* (Peters). Proc. Konin. Nederl. Akad. Wetensch., 42:201-207.

KELLOG, W. N.

1953. Bibliography of the noises made by marine organisms. Amer Mus. Novitates, no. 1611:1-5.

LASHLEY, K. S.

1938. Experimental analysis of instinctive behavior. Psychol. Rev., 45:445-471.

LORENZ, K. Z.

1950. The comparative method in studying innate behavior patterns. In: Physiological mechanisms in animal behavior., Symp. Soc. Exp. Biol., no. 4:221-268.

MACGINITIE, G. E.

1939. The natural history of the blind goby, *Typhlogobius californiensis* Steindachner. Amer. Midland Naturalist, 21:489-505.

MORRIS, D.

1954. The reproductive behavior of the river bullhead (*Cottus gobio* L.), with special reference to the fanning activity. Behaviour, 7:1-32.

NOBLE, G. K.
1934. Sex recognition in the sunfish, *Eupomotis gibbosus* (Linné). Copeia:151-155.
1938. Sexual selection among fishes. Biol. Rev. Cambr. Philo. Soc., 13:133-155.

NOBLE, G. K. & B. CURTIS
1939. The social behavior of the jewel fish, *Hemichromis bimaculatus* Gill. Bull. Amer. Mus. Nat. Hist., 76:1-46.

NYMAN, K-J.
1953. Observations on the behavior of *Gobius microps*. Acta Soc. Fauna Flora Fennica, 69 (5):1-11.

OLMSTED, J. M. D.
1918. Experiments on the nature of the sense of smell in the common catfish, *Ameiurus nebulosus* (Le Sueur). Amer. J. Physiol., 46:443-458.

PARKER, G. H.
1902. Hearing and allied senses in fishes. Bull. U. S. Fish Comm., 22:45-64.
1910. Sound as a directing influence in the movements of fishes. Bull. Bur. Fisher., 30:97-104.
1918. Hearing in fishes. Copeia:11-12.

PARKER, G. H. & R. E. SHELDON
1913. The sense of smell in fishes. Bull. Bur. Fisher., 32:33-46.

TER PELKWIJK, J. J. & N. TINBERGEN
1937. Eine reizbiologische Analyse einiger Verhaltenweisen von *Gasterosteus aculeatus* (L.). Ztschr. Tierpsychol., 1:193-204.

PERKINS, F. T. & R. H. WHEELER
1930. Configurational learning in the goldfish. Comp. Psychol. Monogr., 7:1-50.

REICKEL, A.
1936. *Trichopsis vittatus* (Cuvier & Valenciennes) (Croaking or purring gourami). The Aquarium, 4:257-259.

RODE, P.
1929. Recherches sur l'organe sensoriel latéral des téléostéens. Bull. Biol., 63:1-84.

ROWLEY, J. B.
1934. Discrimination limens of pattern and size in the goldfish, *Carassius auratus*. Genet. Psychol. Monogr., 15:245-300.

SANDERS, F. K.
1940. Second-order olfactory and visual learning in the optic tectum of the goldfish. J. Exp. Biol., 17:416-433.

SCHRIEVER, H.
1935. Aktionspotentiale des N. lateralis bei Reizung der Seitenorgane von Fischen. Plüg. Arch. Gesamm. Physiol., 235:771-784.
1936. Über die Funktion der Seitenorgane der Fische. Verhandl. Physiol.-med. Geselsch. Würzburg, 59:67-68.

SMITH, H. M.
1905. The drumming of drum fishes (Sciaenidae). Science, 22:376-378.

STAMPEHL, H.
1931. *Ctenops vittatus* (der Knurrende Gurami). Blatt. Aquar.-Terrarienk., 42:394-395.

SUCKLING, E. E. & J. A. SUCKLING
1950. The electrical response of the lateral line system of fish to tone and other stimuli. J. Gen. Physiol., 34:1-8.

TAVOLGA, W. N.
1950. Pattern variability among populations of the gobiid fish, *Bathygobius soporator*. Copeia:182-194.
1954. Reproductive behavior in the gobiid fish, *Bathygobius soporator*. Bull. Amer. Mus. Nat. Hist., 103:427-460.
1955a. Pre-spawning behavior in the gobiid fish, *Bathygobius soporator*. Behaviour, 9:53-74.
1955b. The effects of gonadectomy and hypophysectomy on the pre-spawning behavior in males of the gobiid fish, *Bathygobius soporator*. Physiol. Zool., 28:218-233.

TINBERGEN, N.
1951. The study of instinct. Clarendon Press, Oxford.

TINBERGEN, N. & J. J. VAN IERSEL
1947. Displacement reactions in the three-spined stickleback. Behavior, 1:56-63.

TOWER, R. W.
1908. The production of sound in the drumfishes, the sea-robin and the toadfish. Ann. N. Y. Acad. Sci., 18:149-180.

WALKER, T. J. & A. D. HASLER
1949. Detection and discrimination of odors of aquatic plants by the bluntnose minnow (*Hyborhynchus notatus*). Physiol. Zool., 22:45-63.

WREDE, W. L.
1932. Versuche über den Artduft der Elritzen. Ztschr. vergl. Physiol., 17:510-519.

EXPLANATION OF THE PLATE

PLATE I

FIG. 1. Sound spectrogram of three bursts produced by a male *Bathygobius* during courtship. The low frequency trace at the beginning constitutes background noise and amplifier hum, and it is continuous throughout the recording.

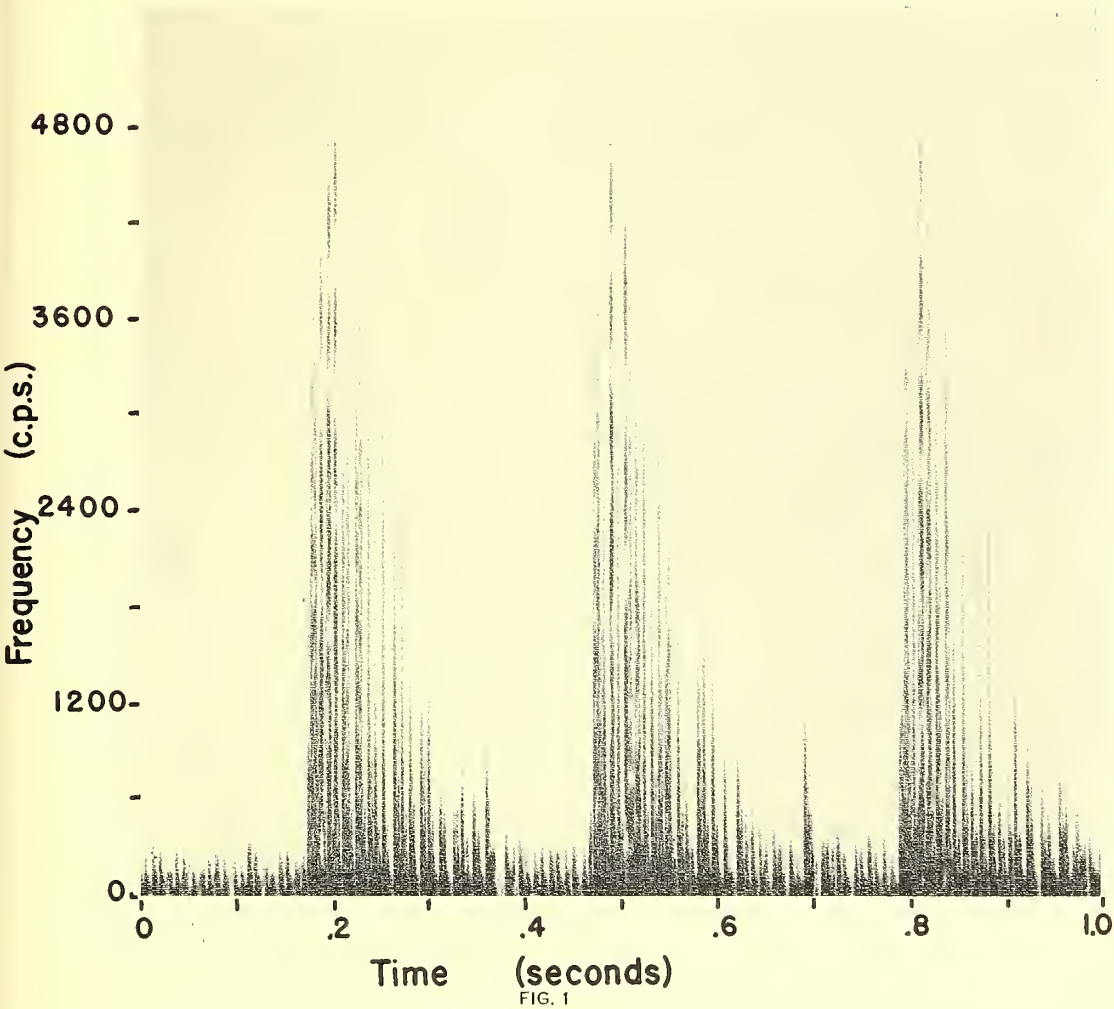


FIG. 1

VISUAL, CHEMICAL AND SOUND STIMULI AS CUES IN THE SEX DISCRIMINATORY BEHAVIOR OF THE GOBIID FISH, BATHYGOBIUS SOPORATOR

8

Patterns of Herd Behavior in Free-ranging Elk of Wyoming, *Cervus canadensis nelsoni*

MARGARET ALTMANN
Jackson Hole Biological Research Station
Moran, Wyoming

(Text-figures 1-5)

IN A long-range study[1] continuing over seven years, an attempt was made to analyze the social patterns observed in groups of elk, or wapiti *(Cervus canadensis nelsoni)*, of the Jackson Hole herd in the upper drainages of the Snake and Buffalo rivers and the adjacent slopes and drainages in Wyoming.[2] Some of the results are presented in this paper.

Elk live in herds, have migratory habits and are relatively free-ranging in their struggle for food and safety. Their group and individual interaction is mostly by signals rather than by physical contact enforcement, a matter of particular interest because little work in this area of study has been reported.

From the numerous observations made, some definite patterns of herd behavior and structure emerged. They varied with the season and were studied at spring migration, during the period the elk spent on summer range and during the rutting season. Within the elk herd forms of communication are vocal and by means of gestures rather than by contact as in buffalo *(Bison bison)*. For example, threats concerning behavior within the group are usually at first gesture signals, such as lifting the front leg or lifting the head and folding the ears back. The members of a group seem to react to such signals over distances up to approximately 250 feet and members of other groups will retaliate with the same gesture. In meeting animals of other species, the elk's signals or gestures are at times not respected or understood. On one occasion a Hereford calf accidentally joined an elk nursery herd and although it was threatened with lifted front legs by the nearest cow elk, it did not retreat until it was actually hit by the elk.

Among vocal communications of the groups, bugling in the nursery herd in spring, as reported by Murie (1951) and by Altmann (1950), is an unusual sound. It was found that in cases of concern, but when no direct scent of danger is noticed, cow elk occasionally bugle in spring, apparently as a warning and challenge, in combination with head shaking up and down and stamping of the front feet. Bugling is otherwise strictly reserved for bull elk during the rutting season. Uncertainty about a visible intruder is frequently expressed by a short snort or bark which startles the rest of the group into attention. Vocal and gesture signals are often coordinated and expressed simultaneously. Vocal expression, however, does not always serve as a signal of danger or challenge in the elk herd. At times, for instance in the nursery herd, a cow-to-calf and calf-to-cow call serves as a bond of security when the movements of the herd temporarily widen the distance between dam and calf.

I. SPRING MIGRATION

The patterns at spring migration time were difficult to establish. Many years of consecutive observations were required because of the complexity and variation of the situation. The giant group of elk, more than 10,000 in number, containing males and females in random association, assembles for winter feeding in the National Elk Refuge at an altitude of 6,500 feet. With the beginning of early spring and the snow-melt, the big aggregate gradually breaks into subgroups of smaller and smaller numbers. These subgroups drift away from the flat pastures to hillsides and valleys upstream and begin to migrate close on the receding snowline or on slopes still covered with snow.

[1]See previous Altmann publications in Bibliography.
[2]This research is being sponsored by the New York Zoological Society and is carried out from headquarters at the Jackson Hole Biological Research Station of the University of Wyoming at Moran, Wyoming.

At the approach of the calving season in late May and early June, the pregnant cows drop out of the formation and seek separate trails to secluded calving areas. They usually take the yearlings with them.

The rest of the groups, now predominantly males, migrates toward higher altitudes. These bull groups remain separate during most of the summer until the beginning of the rutting season in September. The bull groups are usually small to medium in number, ranging from two to sixteen head. There is great tolerance toward the spike bulls, extending up to velvet-rubbing time between sparring partners.

With the segregation of the cows on the calving grounds the pattern of the nursery herd begins to be shaped. The herd size is usually medium, twelve to thirty cows. A typical pattern in the nursery herd is described below. At the time of giving birth the cow stays near the herd, but seeks a secluded corner or sheltering clump of trees. The newborn calf is usually hidden for one to two weeks. It is left by the cow for various lengths of time while she is grazing.

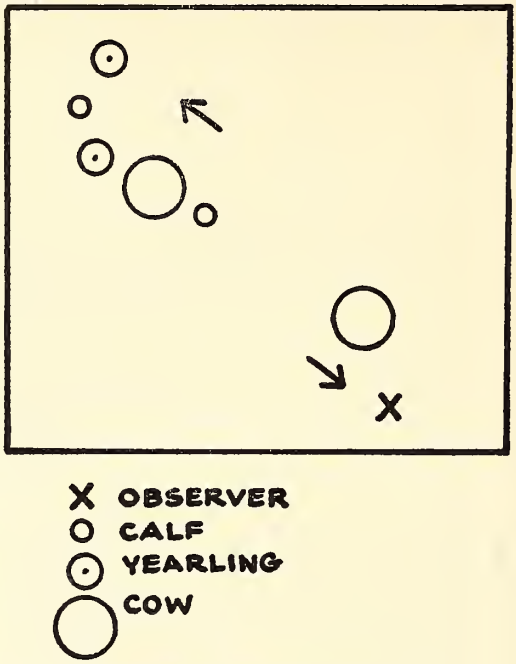
After the first nursing, the calf either drops down to a resting position or is pushed down by the cow. While its mother grazes it remains in quiet position until the next nursing, and any attempts to get up and stagger around are cut short by the cow. The calf may stay in this position from twenty minutes to six hours, or even longer.

It was noted that a push with a front foot was at times necessary to subdue the calf, but in most cases the mere approach or threatening gesture of the cow seemed sufficient to make the calf lie down and stay put.

Rigidity in this down position is the rule when the calf is disturbed by an intruder; not even the ears are moved. Occasionally it was observed that the calf's eyes were moving while following an intruder. When the disturbance had passed the calf relaxed and the ears moved freely to drive off insects.

Little vocalization was observed in the very young elk calf up to two weeks of age.

Cooperative protection of young calves was found to occur in guarded "calf pools." One or two cows serve as guardians and stay with the young while the others graze and return at intervals for nursing and licking their calves. The location selected for such pools is usually a meadow with aspen stands, favorably sloping to the morning sun. When a calf is about three weeks old it is able to follow the herd, and it leaves the calf pool and in general joins the herd activities. It takes longer rest periods than the adults, however.



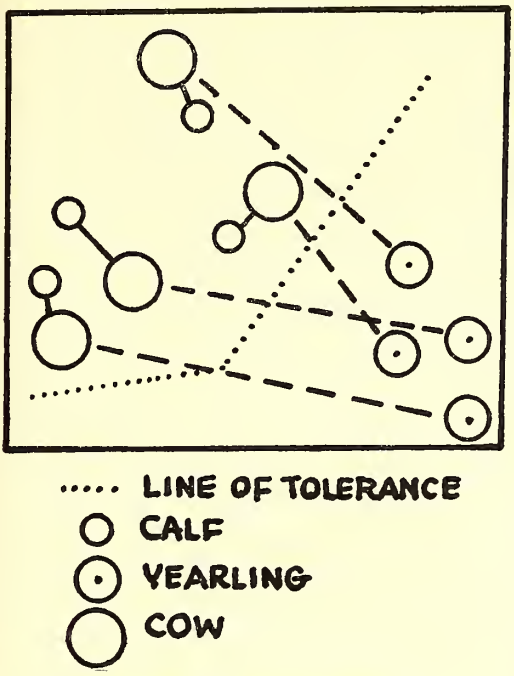
TEXT-FIG. 1. Shielding pattern.

A typical pattern of shielding the calf in case of disturbance by human beings (Altmann, 1953) is depicted in Text-fig. 1. Cow elk with calves and yearlings moved slowly through a meadow. Grazing, they got scent of the observer and the horse. One of the cows, apparently the leading individual, turned and approached the observer slowly in high-stepping gait. The others, shielding the calves, disappeared in the opposite direction in rapidly moving single file.

As soon as calves are born, the yearlings are driven into an outer or marginal zone by their mothers. The rejection of last year's calf, the yearling, is shown in Text-fig. 2. If the yearlings venture too close to the cows they are struck with the fore feet. In most cases this enforcement is not necessary, because the yearlings yield to a gesture of the cow which consists of folding the ears back and raising the head and one front foot.

Migration of the nursery groups continues as soon as the calves have rejoined the herd formation and are able to keep up in speed, cross streams and follow herd signals. The increasing intervals of time between nursings allow for longer stages on the migratory route.

Some period of training the calves for water crossings was repeatedly observed. The cows sought out quiet, shallow side arms of the rivers and by splashing games they made the calves familiar with the water. The youngsters enjoyed the games. Squealing calves jumping and run-



TEXT-FIG. 2. Rejection pattern.

ning through the water were regularly observed on the days before major crossings through high water were undertaken by the herd.

Initiative is exhibited by mature cows when crossing obstacles during migration to the high summer ranges. Highways with traffic, fences and streams and feedless areas are the normal obstacles on this route. If part of a herd during migration is delayed because of a disturbance, the split-off group usually searches, sometimes for hours or even days, for the others, or simply waits. Such a group will smell and look intensely at other elk passing by but still wait for the original group to which it belonged.

Dangerous, swift streams are crossed by elk groups in protective formation. A typical example of this is shown in Text-fig. 3. Cows swam below the calves in the current, thus preventing their being swept away downstream. In entering the stream the calves sometimes were pushed into the upstream position by the dams. Stream crossings took place most frequently in the morning hours when usually the streams were less swollen than later. When fatigue or obstacles on the trail bring the group to a halt, the calves, frequently in unison, begin to nurse. Sometimes the nursing is initiated by one calf and then the whole group takes it up.

Weaning is very gradual and extends up to the fall season. There were, in general, no nursing elk calves seen after the onset of the rutting season. In a few cases where a calf had been dropped exceptionally late, the elk cow was still lactating while the rutting season was under way.

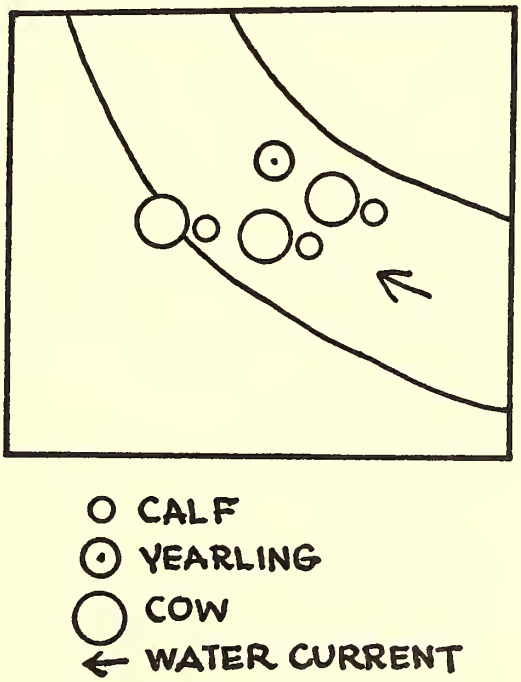
II. On Summer Ranges

Four summer range plateaus were under observation. They comprised about ten to sixteen square miles each and ranged in altitude from 8,700 to 10,000 feet.

The cool temperature and freedom from insects and other disturbances on these summer ranges bring a characteristic change in the elk routine as observed at all other seasons. On the summer range the elk rest at night and sleep until after sunrise. They graze during a large portion of the day.

Considerable uniformity of behavior patterns was found to exist in the elk on high summer ranges in successive years (1948-1954). Some of the swiftly migrating groups of bulls reached the summer range when it was still in its snow-covered stage. Sunny south slopes allowed for early summer grazing, but feed was often scarce.

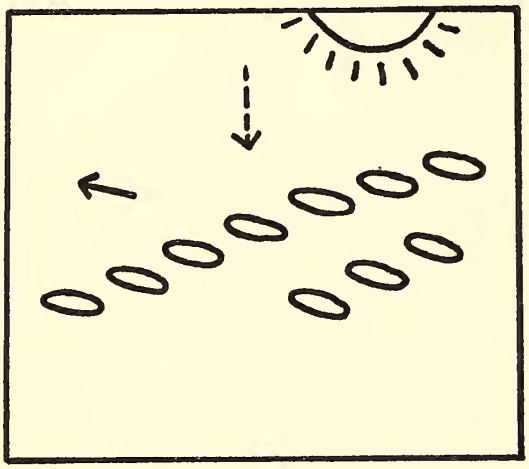
The small size of the bull groups (two to seven) made it possible for them to utilize small patches of exposed grass vegetation. The bull groups, once on the high range, were bold and exposed themselves on the ridges as well as on the slopes. The flight distance to human beings was very low and the bulls could be observed with little difficulty even when sleeping or resting.



TEXT-FIG. 3. Crossing a river.

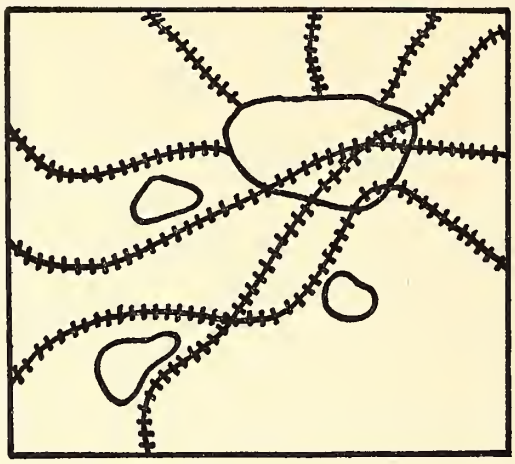
Tolerance for the younger bulls, two and three years old, by the big-antlered, mature bulls was complete in this phase. Because of the soft velvet on their growing antlers, tussling or mock fighting was rare. Most of the time was spent in grazing the short vegetation, but baths in snowdrifts and evening expeditions to salt or alkali licks were also the rule. One can spot the dominance order of the group only then, when obstacles or limitation give the opportunity. Examples are the passage on a rock-bound trail or the most desired place at the lick. No leadership in respect to safety is displayed in case of disturbance. A surprised bull group will break away in a disorganized way, and sometimes a younger, sometimes an older, group member may run first.

The bands of cows with calves and yearlings begin to arrive in increasing numbers following the arrival of the bulls. At first these migratory units keep to themselves but soon the groups melt into larger units of twenty to forty animals until a very large herd up to a thousand head is formed. Grazing is then done in spread out fashion, and many calves congregate to play and rest together rather than heel their mothers. An often-seen grazing pattern on the summer range is the "windrow formation" which at first puzzled me very much. This formation (Text-fig. 4) is in evidence when a fresh cool wind and sunshine coincide. In bad weather the elk graze in cluster formation or stop eating and seek shelter behind and in the dense fir aggregates.



ELK
DIRECTION OF GRAZING
DIRECTION OF WIND

TEXT-FIG. 4. Windrow formation.



ELK TRAIL
MINOR LICK
MAJOR LICK

TEXT-FIG. 5. Salt lick.

At times of sudden storms, the elk quickly entered the shelter places and the younger (yearling) and lower-ranking individuals were chased from the best locations while calves were taken in by the cows. Such dominance tests occurred only when the number of elk in the group was larger than could be accommodated by the available space under cover. The rejected minor elk moved to more distant places of shelter or stayed nearby under wind cover. Actual contact and fighting were not observed, and displacement was accomplished by mere gesture. In cows, the gesture of threat consisted of empty beats with the front feet. In bulls a shaking of the antlers with lowered head was the gesture of threat.

Big nursery herds into which the bull groups only rarely or temporarily were incorporated, rested in the bottom of the plateaus or slopes on cold days, on the upper ridges or wind-blown top slopes in hot weather. In a typical nursery herd on summer range, one or several mature cows usually located themselves in a place where approach ways could be watched. This was the general rule, but did not always hold. It was sometimes observed that smaller branch-groups, grazing on side slopes or on expeditions to nearby alkali licks, also served as safety scouts against intruders. Their flight reaction or signal alarmed the big herd and cleared the whole summer range plateau. In the case of such a general alarm, the herds on summer range were

found to move to a number of "secondary" slopes, which were frequented mainly in cases of disturbance. These secondary slopes permitted a view of the main plateau, to which the elk usually came back after the disturbance had disappeared. There was, however, a time margin of safety, which lasted from several hours to several days after the disturbance.

On the summer range, time devoted to play activity was related to the amount of food available to the herd. In early and late summer, most time was spent in search of food. The games of calves (Altmann, 1952) were also seen on the summer range, but included the whole herd or at least most of the group on the high plateau. Sometimes the snowdrifts[3] formed the playground, sometimes a green meadow or a pond. Splashing and squealing in the sun, the calves went through the ritual with noisy unconcern. A comparison with elk groups living in especially poor habitat showed that elk calves did not play under the latter conditions and that the whole herd was more nervous, had a high flight distance and fewer vocal expressions.

At dusk and occasionally on moonlit nights the herd descended from the summer range to the big alkali licks on the steeper slopes below the plateau. A widely branched system of trails was discovered (Text-fig. 5) leading to and from the salt licks. Observation revealed the almost formal procedure which elk groups use in entering and using the lick. Definite social dominance of the mature elk keeps the younger and the disabled animals in the marginal area so that small secondary licks become established. Other species such as moose, mule deer and porcupines are driven out of the lick, usually not by individual fighting but by the massed approach of the group.

Trail patterns worked by the elk in the higher mountain ranges indicate their most elaborate way of commuting. Elk trails follow the contour of the hillside and do not climb extremely steep places as deer trails do. In meadows and grassland the trails often vanish because the elk groups "fan out." On slopes or in timber passages they reappear. Elk in general do not avoid wet, swampy places and many trails lead through such locations. Under pressure of a disturbance, however, the elk attempt to stay on firm ground, circumvent swampy areas and often endeavor to escape uphill.

III. Rutting Season

A seemingly unorganized group of animals is apt to reveal a completely different picture when disturbed. In the present study, natural or man-made stresses were utilized and studies were made of the effect of the rutting and hunting season on herd structure. As control groups, the elk in protected National Park habitats were contrasted with those in hunted areas.

The Period of Unrest

The transition from the peaceful summer range pattern to the rutting season pattern is extremely gradual. The first changes in behavior are detected in the bull groups and in the spikes. This period is designated as the pre-rutting season. The velvet on the fully-grown antlers begins to shrink and apparently to itch, and velvet-rubbing begins. What at first looks like a harmless tussle becomes more and more a practice sparring and even a short battle. Spikes and younger bulls which were previously members of the bull groups are now driven off one by one to a respectful distance or they are beaten off entirely. A few very young spikes may be tolerated a while longer. In early August spike bulls in the nursery cow group have occasionally been seen in a pre-rutting game. They playfully run to single out a cow, and bucking and kicking, drive her around. They may even bugle. The juvenile bugle is a little higher in pitch than the mature one and the other elk do not seem to pay much attention to it.

In the early stage of the rutting time unrest comes to the cow nursery herds. The weaning of calves is under way. Some cows kick the young or drive them off by other means; others continue nursing but with long intervals in between.

Summer range plateaus are grazed to the bare soil late in August when the snowdrifts have melted and the water-runs are scarce. The big herds first break into smaller groups and these graze the side slopes and creek bottoms. The summer range becomes deserted and the hitherto neglected secondary pastures come into use.

Here and there an early-rutting bull bugles and attempts to join a band of cows, but the confusion and apparent unreadiness of the cows keep him off. The general trend of the elk is to the lower country. Colder nights and the absence of food drive them to the lower stream beds. While this picture was true for all four elk habitats under observation in this study, another factor enters in two of the areas outside Yellowstone National Park. Here, the hunting-season opens just as the rutting season gets under way. This pressure creates a severe conflict situation.

The conflict pattern and the way it affected the different elk groups and ages will be described after the observations during a normal undisturbed rutting situation have been recounted.

[3]Snow games of the Olympic elk (*Cervus canadensis roosevelti* Merriam) were beautifully shown in the "Olympic Elk" film of the Crislers (Disney Prod.).

Rutting Time in an Undisturbed Herd in Protected Area (Yellowstone Park)

The stage of unrest gradually develops into the "rutting season" proper. Harem elk groups consisting in general of ten to fifteen cows with their calves can be observed in timber borders and meadows. The bull of the group circles the harem and often drives a particular cow. While the cows, yearlings and calves are grazing, the bull rarely takes time to graze. Bugling, roaring and watching the harem keep him fully occupied. A bugle made by a rival puts the bull into a rage and starts him on a widespread search for the intruder. One bull, seen tramping and sniffing through the underbrush, broke into a group of fir trees and after discovering a hiding bull, drove his rival away with sharp-sounding blows of his antlers.

Another factor contributing to instability of elk cows in harems was found to be the size of the harem, the large ones being most subject to unrest. Aggregates of twenty and more cows seemed too widespread when grazing. Often the bull, while rounding up cows at one end of a meadow, lost control of the other end of the group. Minor unattached bulls without harems, roaming the vicinity of the harem groups and looking for a chance to mate, then found that chance.

During the peak of the rutting season, the spike bull groups are completely by themselves and are driven away whenever they approach a harem group or meet a mature bull. Fear of the harem bulls brings them quite often out of the timbered slopes into dangerous situations facing the hunters rather than being beaten by a big bull elk.

The Rutting Season in the Hunting Area

For observations on the rutting season in the hunting area, test areas of approximately eighteen square miles each were selected on two summer ranges, at an altitude of 9,000-10,000 feet. Only four hunting camps were located here, and the remote wilderness area with a small number of camps was chosen because excessive pressure by large numbers of hunters on foot or in trucks and jeeps would have complicated and obscured the observation of events.

The area was visited at least three times a week for several weeks preceding the opening of the hunting season. Elk groups grazing and resting were noted. From the beginning of the hunting season, observations were made from overlook points daily for six days. Thereafter, three visits were made each week for three weeks.

It was found that the preparations for the hunting season "spook" a considerable number of harems out of the hunting region into the safety of Yellowstone Park. Pack-trains with equipment, wood-chopping and tent-setting noises as well as grazing groups of horses with bells and hobbles ringing through the night contributed to the disturbance of the elk. The cows, not the bulls, gave the signal, or initiated the exodus into the safety zone.

The morning of the first hunting day, after the first volley of shots sounded (8:00 A.M.), the simultaneous flight of four different cow groups, three of which contained a bull, was spotted in the border zone of the Upper Snake River valley. The elk came down the slopes of the Big Game Ridge where the shots were fired. The escape, in quiet single and double file, led through the Snake River bed into the Crooked Creek slopes within the Yellowstone Park area. Elk groups resident in the borderline area were found grazing on three different slopes at the onset of the hunting season. Upon the sound of shots, some raised their heads, but the group continued to graze. Two of the groups, on their exodus from the hunting area, came upon these grazing groups, and mutual looking and sniffing took place. After a few minutes, the two retreating groups continued to the safety of the Park. The borderline elk stayed in their habitat despite further volleys of shots in the distance. Very few harems were found in the hunting areas after the second day following the opening of the hunting season. Erratic and transient elk bands or single animals were spotted frequently, however. Many confused spikes were encountered. This seems to indicate that in their state of intimidation by the bulls, the spikes disregarded the shooting and the commotion created by the hunting camps.

The fact that sight of the peacefully grazing elk bands did not stop the escaping groups was surprising, since elk, in general, will be quieted and "stabilized" by the sight of other grazing elk, or even by other grazing and undisturbed animals (wild geese, moose, deer).

The evasive migration of the groups after the opening of the shooting was characterized by a relatively long range, the animals fleeing from three to eight miles until they reached the safety areas. Mature cows were the leaders, whereas the bulls were not too eager to move. In fact, the appearance of the groups lost the "harem character" temporarily. No rounding up by the bull was observed. Rutting activity was resumed after the evasive migration was completed and after the group had quieted down for at least one day. Perhaps the bugling from elk established in the area of retreat contributed to resumption of the rutting activity.

The following pattern was found: In a situa-

tion of persecution, which was one of severe stress and strain, the elk reacted by evasion and rutting activity was curtailed, or perhaps even suppressed. The cows were the first to yield and to initiate the evasion; the bulls yielded more reluctantly. The bulls maintained certain aspects of the rutting behavior; they kept spikes at a distance and kept restless vigilance against other bull elk.

Summary

In a long range study (7 years) an attempt was made to analyze the pattern of social behavior in groups of elk, or wapiti, of the Jackson Hole herd of Wyoming.

Elk were chosen because they are herd-living, have migratory habits and are still relatively free ranging. Consistent elk herd patterns were established in comparable habitats, seasons and situations. The group structure and its reorganization were analyzed from the spring migration to the rutting season. Natural and man-made disturbances provided test situations suitable for study of individual and group interaction.

Patterns of social behavior observed and analyzed dealt with the break-up toward spring of the huge elk aggregate in the winter refuge, into the swifter moving bull-spike bands and the cow-nursery herds.

Bull groups comprised several mature elk and a few spikes (yearlings). Tolerance to young bulls prevailed until the pre-rutting stage.

In the elk nursery herds the problem of leadership, the formation of calf pools, the shielding pattern, the training for migration and the displacement of the yearlings by the new calf were studied.

On summer range the merger of migratory groups into the large summer herd, the change in daily routine and in reaction to disturbances were observed. Favorable habitat increased play activity and social interaction. Social test situations yielded information on trailing patterns, shelter use, resting and flight patterns. Salt licks revealed extremely strict enforcement of social domination within species and conflict with other species.

In the pre-rutting and rutting season, the position of the spike bulls provided insight into conflict patterns. Group behavior during rutting season in undisturbed areas was compared with areas under hunting pressure. Evasive migration took place, over-ruling and counteracting the natural migration during this season.

Interaction of elk groups under stress with elk free from stress was observed.

References

Altmann, Margaret

1949. The Group Life of Wapiti Elk. Paper presented at the Virginia Academy of Science meeting, May 7.

1950. Social Behavior of Wapiti. Paper presented at the American Society of Mammalogists, July 29, at Yellowstone Park, Wyoming.

1951. Patterns of herd structure in free-ranging elk. Paper presented at the Phil. Meeting of the Am. Society of Zoologists. Abstr. Anat. Record, V. III, No. 3, Nov., p. 74.

1952. Social Behavior of Elk, *Cervus canadensis nelsoni,* in the Jackson Hole Area of Wyoming. Behavior, Vol. IV, 2.

1953. Social Graces in Elk Society. Animal Kingdom, (N. Y. Zool. Soc.), Vol. LVI, No. 3, pp. 66-72.

Boulière, Francois

1954. The Natural History of Mammals. A. Knopf, New York.

Darling, F. F.

1937. A Herd of Red Deer. Oxford University Press.

1952. Social Life in Ungulates. Colloques Internationaux, Centre Nat. de la Rech. Sci., XXXIV, Structure et Phys. des Soc. Animales. Paris, March, 1950. CIVRS: 221-226.

Hediger, H.

1952. Beiträge zur Säugetier-Soziologie. Colloques Internationaux, Centre Nat. de la Rech. Sci., XXXIV, Structure et Phys. des Soc. Animales, Paris, March, 1950. CIVRS: 297-321.

Murie, Olaus

1951. The Elk of North America. Stackpole Co., Harrisburg, Pa.

Schenkel, R.

1947. Ausdrucks-Studien an Wölfen. Behaviour, 1:81-129.

Tinbergen, Niko

1951. The Study of Instinct. Oxford, Clarendon Press.

9

New Frogs from Ecuador and Southwestern Colombia

John W. Funkhouser

Natural History Museum, Stanford University

(Plate I)

In the course of collecting in Ecuador in 1950, several undescribed frogs were discovered. Descriptions of two of these new forms are presented here, along with field observations on them. In addition, two more new forms from collections sent to me by Mr. Rolf Blomberg and Dr. Gustavo Orcés V. of Quito are described. These frogs represent three genera and two families.

* * *

Much confusion exists in the classification of the *tinctorius* group of *Dendrobates* from Central America and northern South America, and I am greatly indebted to Dr. E. R. Dunn for information concerning the relationships and ranges of some of the members of this group. Most of the information on these points is taken from his letters.

Dendrobates histrionicus sylvaticus, n. ssp.
(Plate I, Fig. 1)

Diagnosis.—A form of *Dendrobates histrionicus* Berthold (1845a, b) most closely related to *Dendrobates h. wittei* Laurent (1942) (the latter figured by Boulenger [1913: pl. CIV, fig. 4] as *Dendrobates tinctorius coctaei*), from which it differs in having strikingly smaller and more closely spaced markings, those of the dorsum having very irregular and frequently poorly defined margins so that in some cases the color of the markings shades into the background. The markings of the venter tend to be slightly larger than those of the dorsum and have well defined, regular margins. In life all markings are bright vermilion upon a shiny, jet-black ground, in preservative becoming much less distinct and changing to blue-gray upon a gray-black background.

Holotype—SU[1]10568, an adult, 27 mm. snout to vent, from the Hacienda Espinosa, elevation about 1,000 ft., 9 km. west of Santo Domingo de los Colorados, Province of Pichincha, northwestern Ecuador. Collected by John W. Funkhouser on Oct. 2, 1950.

Paratypes.—SU 10566-10567, 10569-10576, same locality data, collected between Oct. 2 and Dec. 2, 1950, by the author; SU 10383, vicinity of Santo Domingo de los Colorados, May, 1950, by Sr. Muñoz; SU 10380-10381, banks of rivers Pupusa and Quila, near Santo Domingo de los Colorados, by Sr. Muñoz; SU 10615, Hacienda Lelia, ±3,000 ft., about 35 km. east of Santo Domingo de los Colorados in the Andean foothills on the road to Quito, Sept. 3, 1950, by the author; and SU 10360-10361, ±1,500 ft., on the Río Mulante, an affluent of the Río Blanco, Esmeraldas drainage, Province of Pichincha, by Sr. Antonio Proaño.

Description of Holotype.—Snout truncate, almost vertical in profile, the corners angular. Nostril to front corner of eye about three-fourths length of exposed part of eye. Internareal distance equal to exposed part of eye. Canthus rostralis indistinct. Loreal region vertical, slightly or not at all concave. Width of upper eyelid equal to two-thirds interorbital space, which is equal to exposed part of eye. Tympanum indistinct, equal to about half exposed part of eye. Width of head behind eyes about equal to length of head.

Finger I reaches to base of disk of II; finger II reaches to second joint of finger III; finger IV reaches very slightly farther on finger III than does II. The width of the disks of II, III and IV about equal and about twice the width of their digits; disk of I narrower, about one

[1]The following abbreviations are used throughout this paper: AMNH, The American Museum of Natural History; ANS, Academy of Natural Sciences of Philadelphia; OV, private collection of Gustavo Orcés V.; SU, Natural History Museum, Stanford University; USNM, United States National Museum.

and a half times the width of its digit. Fingers free of webbing. A single oval palmar tubercle; subarticular tubercles readily visible, but not strongly developed.

Toe I marks the first joint of II; disk of I only slightly wider than toe, about half as wide as disk of II, which is almost twice as wide as toe II. Toe II reaches midpoint of first joint of III; disk of II about equal to that of III. Toe III reaches to last subarticular tubercle of IV and is about two-thirds the length of IV; disk of IV about equal to that of III. Toe V reaches to just below second subarticular tubercle of IV; disk of V about one and a half times as wide as toe and about two-thirds as wide as disk of IV. Widths of toes I and V about equal and equal to two-thirds the widths of II, III and IV. Subarticular tubercles readily visible, but not strongly developed; a round outer and an elliptic inner metatarsal tubercle; an indistinct tarsal tubercle. Heels overlap slightly when legs are folded at right angles to the body. When hind limbs are carried forward along the body, the tibiotarsal articulation marks the hind part of the eye. Snout to vent length slightly more than four times width of sacrum. Omosternum rudimentary.

Skin smooth on all surfaces. Dorsal markings closely spaced, small, irregular in outline, and inclined to run together in places or to shade into the background. Ventral markings larger and smoother outlined, but irregular in shape and size. On the limbs the markings may be present or absent, but when present are usually large with smooth margins. *In preservative* the background is gray-black and the markings a lighter gray. *In life* the background is shiny black and the markings bright vermilion.

Measurements of Holotype, in Millimeters.—Snout to vent 26; width of head (behind eyes) 8; length of head 8; length of orbit 4; length of exposed part of eye 3; interorbital distance 3; internareal distance 3; subocular distance 1.5; eye to tip of snout 4; eye to nostril 2.3; greatest diameter of tympanum 1.5; length of femur (vent to knee) 8; length of tibia (knee to heel) 10; length of longest toe (measured from far side of metatarsal tubercle) 12; length of foot (heel to tip of longest toe) 19.

Variation.—The coloration was the same in all specimens which I saw in the field. The size and spacing of the markings of the dorsum and venter vary minutely, but on the limbs vary greatly in size and number, or are totally absent. In no specimen examined are the limb markings as profuse as those of the dorsum and venter.

Distribution.— This frog occurs in the humid tropical forests of the coastal plain and Andean foothills of northwestern Ecuador. At present it is impossible to establish its northern and southern limits, though it is reasonable to assume that the latter would coincide with the zone of transition to the drier regions of southwestern Ecuador. Specimens from San Javier (AMNH 10607) and Salidero (AMNH 10601-10603), northwestern Ecuador, appear to be of this type, but are in such a bad state of preservation that determining their original pattern with certainty is virtually impossible. Two specimens (USNM 20603-20604) from Playa de Oro, Province of Esmeraldas, have larger and fewer spots, one of these (USNM 20603) closely approaching *Dendrobates h. wittei* as figured by Boulenger (1913); the other has more and smaller spots. This may prove to be a zone of intergradation between the two subspecies.

Ecology and Behavior Notes.—I found these frogs to be diurnal and to inhabit the jungle floor. (The only exception to the latter which I saw occurred on the afternoon of October 14, 1950, when I took one and saw another crossing a trail through a grassy pasture. These were at least 50 yards from the edge of the jungle.) When not active, they remain hidden under leaves, logs and debris.

As far as I could determine, their activity increases markedly during and immediately after rain. I counted five in sight at once on the afternoon of October 22, 1950, after it had been raining for perhaps half an hour. These were in a spot in the jungle where a dense growth of large-leaved plants about two feet high covered the floor. The frogs were moving busily about, traveling in short walks or runs, with now and then a short hop. Occasionally one would climb to the top of a leaf as though to survey the situation. They seemed to be exploring every crevice, every stem, every leaf. Upon my approach, they became alarmed and moved back among the plants, adeptly hiding under debris. Capturing this species always presented a problem, and I was able to catch only one at this place.

Their locomation is not rapid; their hops never cover more than three inches. In spite of their brilliant red and black coloration, they blend so well with spots of red fungus which grow on the jungle floor and with small flecks of light that they can easily be overlooked.

When captured they do not go through the kicking characteristic of most frogs, but try to wriggle away, using a walking or running motion with both hands and feet. Occasionally they lie perfectly still for a while and then suddenly become active.

They are extremely nervous and alert. In a specimen jar, they revolve this way and that looking for an avenue of escape. The throat

characteristically pulsates very rapidly, seeming to flicker. Occasionally this movement is stopped for a second or two.

As to voice, I heard only one specimen make a sound, and this individual kept up a steady clamor of annoyance while in the collecting bag and when handled. The sound was identical with that made by a wood borer in dead wood.

DENDROBATES HISTRIONICUS CONFLUENS, n. ssp.

Diagnosis.—A form of Dendrobates histrionicus very close *to D. h. sylvaticus* in every way except color pattern. The markings of the dorsal, and usually the lateral, surfaces are what would result if the vermilion spots of *sylvaticus* were to enlarge and merge until only small spots and flecks of the black background remained. Hands and feet black with light (presumably red in life) spots. Venter varying from all black to marbled to mostly light with large black spots (especially pronounced on the throat region). The collector reports these frogs "red" in life.

Holotype.— SU 13151, an adult 27 mm. snout to vent, from La Ciudad (de Madrigar), lying in the pass through the western Cordillera of the Río Patia, Department of Nariño, southwestern Colombia (approximately Long. 77° 30′ W. × Lat. 1° 46′ N.), at an elevation of ±600 mtr., collected by Rolf Blomberg on June 22, 1951.

Paratypes.— SU 13246-13267 (22 specimens), same locality data, collected Nov. 18, 1953, by Rolf Blomberg.

Description of Holotype.—Snout slightly rounded; slightly sloping in profile. Nostril to front corner of eye about three-fourths length of exposed part of eye. Canthus rostralis indistinct. Loreal region vertical; more or less plane. Width of upper eyelid two-thirds interorbital space, which is equal to exposed part of eye. Tympanum indistinct, its greatest diameter about half the length of the exposed part of the eye. Width of head behind eyes about equal to length of head.

Finger I reaches to base of disk of II; II reaches to second joint of III; IV reaches very slightly farther up on III than does II. Disks of II, III and IV about equal and almost twice the widths of their digits; of I narrower, not greatly wider than its digit. Fingers free of webbing. A single oval palmar tubercle; subarticular tubercles readily visible, but not strongly developed.

Toe I marks first joint of II. Disk of I only slightly wider than its toe, about half as wide as the disk of II, which is almost twice as wide as toe II. Toe II marks the middle of the first joint of III; disk of III slightly larger than disk of II. Toe III marks the middle of the penultimate phalanx of IV; disk of IV about equal to that of III. Toe V reaches to base of second subarticular tubercle of IV; disk of V about half as wide as that of IV, intermediate in width between those of I and II. Subarticular tubercles readily visible, but not strongly developed. A round outer and an elliptic inner metatarsal tubercle; an indistinct tarsal tubercle. Heels overlap slightly when legs are folded at right angles to the body. The hind limb being carried forward along the body, the tibiotarsal articulation marks the eye. Snout to vent distance four and a half times the width of the sacrum. Omosternum rudimentary.

Skin appears smooth when viewed without magnification, but with magnification it appears, for the most part, punctate on all surfaces—the dark areas, however, much less strongly than the light. The dorsal pattern is made up of a light background on which are sparsely scattered, mostly small, irregularly margined dark spots and flecks. The ventral pattern is similar (though inclined to be marbled) except for the lower surfaces of the thighs, which are dark. Hands and feet dark with light spots. Color *in preservative* light pink (red-orange at time of receipt, six months preserved in *aguardiente*) with black or very dark gray markings. According to the collector, the background color *in life* is red.

Measurements of Holotype, in Millimeters.— Snout to vent 27; width of head (behind eyes) 8.2; length of head 8.2; length of orbit 4; length of exposed part of eye 3.5; interorbital distance 3.5; internareal distance 3; subocular distance 1.7; eye to tip of snout 4; eye to nostril 2.8; greatest diameter of tympanum 1.9; length of femur (vent to knee) 13; length of tibia (knee to heel) 14; length of longest toe (measured from far side of metatarsal tubercle) 12; length of foot (heel to tip of longest toe) 19.

Variation.— The paratypes vary in snout to vent length from 27 to 30 mm. The dorsal marking of all specimens is more or less the same, but on the venter the markings vary from all dark to marbled to mostly light with large dark spots (especially pronounced on the throat region).

Disribution.—I know of only the type locality (where, according to Blomberg, the collector, these frogs are quite abundant), but I believe that they very probably range throughout the tropical and even into the subtropical reaches of the Río Patia pass. Only future collecting can show how localized this form is and whether it ranges into the actual coastal foothills of the western Cordillera. Four specimens (ANS 25349-52) from La Guayacana, Department of Nariño, appear to be intermediate between *confluens* and *sylvaticus,* though closer to *confluens.* (La Guayacana lies in the pass through

the western Cordillera of the Río Guiza, approximately Long. 78° 19′ W. × Lat. 1° 15′ N. According to data with the specimens, its elevation is 3,000 ft. To judge from maps of the region, it is more likely very close to 1,000 ft.)

* * *

As indicated above, Dr. Dunn has given me considerable information on this group of frogs. He plans to publish a report outlining their relationships and nomenclature at a future date, but I take the liberty of quoting briefly some of his conclusions as a basis for my own. *Dendrobates histrionicus* Berthold has long been relegated to the synonomy of *D. tinctorius,* but according to Dr. Dunn it has no close relationship to the latter, which comprises a group of tiny frogs with light stripes known from Panama to eastern Brazil and Guiana. *Dendrobates histrionicus* is made up of a group of larger, spotted forms occuring along the west coast of northwestern South America.

Dendrobates h. sylvaticus is replaced in the Pacific coastal plain of Colombia by *D. h. wittei* Laurent. In the western Andean foothill country of Nariño it is apparently replaced by *confluens,* or forms similar to *confluens* if the latter proves to be confined to the Río Patia pass. The previously mentioned specimens from La Guayacana lend credence to this view. These specimens show less confluence of the spots, especially on the sides of the body and upper surfaces of the limbs. However, their ventral markings tend strongly toward the marbling common to *confluens* rather than the individual ventral spots of *sylvaticus.* Their locality falls in a line between the known territories of *sylvaticus* and *confluens,* but closer in distance to the latter.

In view of the above findings, it appears that at least two lines diverge to the north from *sylvaticus,* one of these in the coastal plain and characterized by the enlargement and reduction in number of spots, and the other in the Andean foothills and characterized by the confluence of the spots so as almost (if not totally) to obscure the black background.

Phyllobates espinosai, n. sp.[2]
(Plate I, Figs. 2 & 3)

Diagnosis.—A *Phyllobates* without light lateral stripes except for an irregular light line beginning in the groin and extending cephalad for about one-fourth of the groin-to-eye distance. Ventral surfaces strikingly marked by well defined, clear, light spots and vermiform markings on a dark brown background. Light markings are blue in life, white in preservative. Tadpoles with normal mouth-parts.

Holotype.—SU 10577, an adult male from the Hacienda Espinosa, elevation about 1,000 ft., 9 km. west of Santo Domingo de los Colorados, Province of Pichincha, northwestern Ecuador. Collected by John W. Funkhouser, Oct. 6, 1950.

Paratypes.—SU 10578, 10585 (adults) and SU 10579-10584 (juveniles), same locality data, collected between Oct. 6 and Nov. 14, 1950, by the author; and SU 10375, Santo Domingo de los Colorados, May, 1950, by Sr. Muñoz.

Distribution.—I have knowledge only of the types listed above. A very similar *Phyllobates* from eastern Ecuador is discussed later in this paper.

Description of Holotype.—Snout truncate. Tip of snout to corner of eye about equal to orbit. Canthus indistinct; lores almost vertical. Nostrils nearer to tip of snout than to eye. Interorbital space equal to width of upper eyelid. Tympanum distinct, equal to almost one-half length of exposed part of eye.

Finger I longer than II, disk of II marking the middle of the ultimate phalanx of I. Tip of II reaches almost to end of penultimate phalanx of III. IV very slightly shorter than II. Disks of fingers only slightly wider than their respective penultimate phalanges except for III, which is swollen, its width being almost equal to disk. Fingers entirely free of webbing. A round central and a smaller oval inner palmar tubercle.

Toes II, III and IV slightly webbed at base. I reaches almost to first joint of II. Disks of I and II about equal, slightly smaller than disk of V, and about half of those of III and IV. Tip of II reaches first subarticular tubercle of III. III reaches second subarticular tubercle of IV. Tip of V falls about midway between first and second subarticular tubercles of IV. Diameter of toes I, II and V about equal, these being about one-half the diameter of IV, which is slightly larger than III. Subarticular tubercles not overly distinct. An oval inner and a more or less round outer metatarsal tubercle. An inner tarsal tubercle located at about the midpoint of the tarsus. Heels meet at midline when legs are folded at right angles to body. The hind limb being carried forward along the body, the tibiotarsal articulation marks the rear part of the eye.

Skin of dorsal surfaces finely granular; below smooth. *In preservative,* dorsal surfaces dark red-brown with slightly raised spots of darker brown, as are the lateral surfaces. Venter beautifully and distinctly marked by vermiform markings of clear blue-white on a deep brown

[2]To Sr. don Alfredo Espinosa P., of Quito, my host at the Hacienda Espinosa, I owe much of the success of the field work in Ecuador. I take pleasure in naming this species in his honor.

ground; throat has proportionately fewer markings, there being only a strong median and two lateral vermiform markings. An elongated white spot below eye, followed by a similar spot below tympanum (this may be continuous), which is followed by another spot leading to and then above, behind, and under juncture of forelimb to body, sending two branches down limb toward the elbow, one in front and one behind. A narrow, irregular stripe extending from groin about one-fourth of the distance between it and the hind-margin of the eye. Upper surfaces of thighs irregularly marked with light, there tending to be three diagonal markings. *In life,* dorsum deep red; sides black. Femur black; tibia deep red. All parts described as light in preservative, turquoise. Background color of venter, deep chocolate.

Measurements of Holotype, in Millimeters.—Snout to vent 17; width of head (behind eyes) 5.5; length of head 6; length of orbit 2.5; length of exposed part of eye 2; interorbital distance 2; internareal distance 2.5; subocular distance 1; eye to tip of snout 3; eye to nostril 2; greatest diameter of tympanum 1.2; length of femur (vent to knee) 7; length of tibia (knee to heel) 8; length of longest toe (measured from far side of outer metatarsal tubercle) 7; length of foot (heel to tip of longest toe) 12.

Variation.—In the four adult specimens dorsal variation is minute, the most notable being stronger or weaker markings on the upper surfaces of the thighs. Ventrally, the holotype possesses stronger markings (i. e., larger and proportionally more included area) than any of the adult paratypes. This is especially noticeable on the throats of the paratypes (that of SU 10585 being almost free of markings), though the tendency toward a median and two lateral throat markings remains. The abdominal markings of SU 10375 are mostly composed of round and oblong spots, the vermiform markings being greatly minimized. The ventral markings of the juveniles are poorly developed, the abdomens being mostly light and the throats dark, though in some the adult markings have begun to become defined. Snout to vent length of adult paratypes: 16-17 mm.

A specimen of *Phyllobates* (SU 10340) collected in the Río Suno Region, eastern Ecuador (Lat. 0° 40′-0° 44′ × Long. 77° 10′-77° 20′; elevation 380-420 mtr.), by Jorge Olalla, was presented to me by Dr. Gustavo Orcés V. of the Escuela Politécnica Nacional, Quito. In preservation this specimen so closely resembles *P. espinosai* that I should be strongly inclined to place it in this species if its locality were not separated from the locality of *espinosai* by such a barrier as the Andes. The only morphological difference between this and the adult specimens of *P. espinosai* which I have at hand is that it possesses a round, light blur covering the caudad two-thirds of the throat (possibly due to injury or preservation), has a white line bordering the upper lip and is slightly larger (19 mm. snout to vent).

Tadpole (Based on 3 tadpoles, SU 10586, collected from the back of paratype SU 10585). —Body 12 mm. long, somewhat depressed, four-fifths as broad as long; ventral profile slightly convex; no abdominal bulge. Eyes large, their longest diameter equal to almost one-fifth of body length, dorso-lateral in position and located in the second one-fifth of body length. Nostrils widely spaced, about half way between eye and tip of snout; internareal distance somewhat less than interorbital. Anus medial. Spiracle sinistral, slightly below the lateral axis and slightly nearer to base of tail than to tip of snout, its margin tubular and slightly projecting upward. Tail approximately twice body length. Fins well developed, the dorsal being distinctly keeled and having a maximum width of twice the ventral. Tip bluntly pointed.

Pigmentation in an even, close stippling of chromatophores on top of head and body. This extends about two-thirds of the way down the sides of the body and is continued on to the ventral side of the head where the stipples are spaced farther apart. Chromatophores of tail grouped into irregular spots with the individual chromatophores becoming smaller cauded. Chromatophores of ventral fin very sparse and small.

Rows of labial teeth 2/3, the teeth being of moderate size and crowding except for the third lower row where the teeth are minute and scarcely discernible. Second upper row divided, the middle one-third being vacant. The first lower row equal to or very slightly shorter than the two upper rows; the second lower row very slightly shorter than the first; and the third very slightly shorter than the second. Beaks narrowly edged with black; serrations comparable in size and spacing with the labial teeth; linear measurement from one side to the other equal to one-half of the first lower row of labial teeth. Upper beak broadly triangular; starting at the center the two sides first bow inward slightly, then outward. Lower beak also triangular with slightly narrower angle and the sides bowed very slightly inward. Single rows of papillae on lower and lateral labial margins forming three distinct series. The lower row is a continuous series below and lateral to the three lower tooth rows. Here the papillae number 22-28, are linearly arranged, and radiate outward. They are approximately twice as long as broad, are of fairly

uniform diameter—though tapering slightly to a blunt point—and are crowded closely together. A marked emargination separates this series from the series which lie one on either side, opposite and slightly overlapping the ends of the two upper rows of teeth. These series, each composed of five backwardly projecting papillae (the first and outer papilla difficult to see because of lying under the second) whose size and shape approximate those of the lower row, are connected by the labial margin which overlies the upper row of teeth to a depth of about ½ their length.

Ecology and Behavior Notes.—My observations indicate that *Phyllobates espinosai* is a diurnal frog which inhabits damp, shaded areas of the jungle floor. Most of my specimens came from the banks of the small Río Chila, and I presume that the juveniles which I saw nowhere but in this location had spent their larval life in the stream and had metamorphosed just recently. Two adult males (SU 10578 and 10585) collected here were carrying tadpoles on their backs—two and three respectively. These were not arranged in any particular fashion on the back of the carrier.

The behavior of this species coincides almost exactly with that of other dendrobatids which I saw. They give the impression of extreme alertness, and all their movements are nervous and quick. They usually walk or run, or when greatly excited vigorously kick both hind legs out and back together in a swimming movement, at times projecting themselves an inch or two through the air (this is as close to a typical frog jump as they ever come). The throat pulsates rapidly, seeming to flicker; this movement occasionally is stopped for a second or two. Even when sitting the little creatures seem tense and alert. The head is elevated and the hind legs are tensed and not drawn in close to the body. When disturbed and seeking an avenue of escape, they squat in this position and revolve jerkily this way and that. In the field they scurry under debris for safety; they do not take to water even when it is close by.

The tadpoles share the adults' nervousness and are prone to sudden fits of movement. While I was collecting one of the adults carrying tadpoles, one of these slithered over the carrier's eye. With a quick movement of the forefoot he brushed it away, and at the same moment it projected itself some six inches across my hand. When these tadpoles were placed in a glass of water, they would lie quietly on the bottom and then suddenly take off in a frenzied dash around the glass. Their movements were always in darts, never a leisurely swim.

Hyla orcesi, n. sp.[3]

Diagnosis.—Superficially similar to *Hyla leprieurii britti* Melin, from which it most notably differs in having an internal vocal sac instead of two external sacs behind the angles of the jaw. Upper surfaces densely covered with small conical tubercles, each having one or more spinose tips and densely covered with minute spines; a strong swelling or pad on the inside of the forearm (possibly a sexual character); uniform dark grayish-tan above.

Holotype.—SU 13150, an adult male from Pacayacu, a stream that flows into the Cotapino, drainage of the Suno, Río Napo region, eastern Ecuador; alt. 600-650 mtr. Collector not known.

Paratype.—O. V.—A. 101, personal collection of Dr. Gustavo Orcés V., Escuela Politécnica Nacional, Quito; an adult male from the same locality as the holotype.

Description of Holotype.—Derm of head not involved in cranial ossification. Omosternum a large cartilaginous disk. Choanae large. Vomerine teeth in two strong series, forming together a ⌐¬, the transverse portions being at the level of the hind edge of the choanae and extending from choana to choana, while the diagonal portions extend behind the level of the choanae. Tongue subcircular, slightly free and slightly nicked behind. Head large, depressed, as broad as long. Lores oblique, concave; canthus rostralis distinct, slightly incurved. Snout rounded, one and a half times diameter of eye. Interorbital space a little broader than upper eyelid, equal to diameter of eye. Nostrils much nearer to tip of snout than to eye. Tympanum very distinct, two-thirds diameter of eye. A fold above tympanum and a noticeable concavity just dorsocaudad to it. Three outer fingers slightly webbed, a strong swelling on the inner side of first finger. Disks of three outer fingers about two-thirds diameter of tympanum, disk of first finger about half this size. Two palmar tubercles. A strong swelling, or pad on the inner side of the forearm, beginning at the elbow and extending two-thirds of the distance to the hand. Tibiotarsal articulation reaches between eye and tip of snout; length of femur one-half snout to anus. Heel without dermal appendage; a very indistinct tarsal fold. Feet three-fourths webbed; disks of toes 2-5 slightly smaller than those of outer three fingers; disk of first toe about two-

[3]Dr. Gustavo Orcés V. of the Escuela Politécnica Nacional, Quito, sent for my inspection two specimens of this very unusual *Hyla* from eastern Ecuador. These appear to be of an undescribed species, and Dr. Orcés has kindly donated the holotype to the Stanford Natural History Museum. In recognition of Dr. Orcés's many courtesies and favors, I take pleasure in naming the species in his honor.

thirds diameter of outer four. Subarticular tubercles well developed. A strongly developed inner, ellyptic metatarsal tubercle. Male with internal vocal sac. Upper surfaces, with the exception of the forelimbs, densely covered with conical tubercles, each having one, or sometimes more, spinose tips and covered with small, usually dark spines; the skin between tubercles of a lichenaceous texture and bearing scattered small spines. Belly and lower surfaces of thighs coarsely granular. Coloration in preservative: dorsum brownish-gray with tips of tubercles whitish; tympanum yellowish-brown; venter light yellowish-brown studded with dark brown stipples visible under magnification. (Dr. Orcés states that he believes the color in life to be little different from that here described).

Measurements of Holotype, in Millimeters.—Snout to vent 52; width of head (behind eyes) 16; length of head 16; length of orbit 6.5; length of exposed part of eye 5; interorbital distance 5; internareal distance 4.5; subocular distance 2; eye to tip of snout 8; eye to nostril 5; width of upper eyelid 4; greatest diameter of tympanum 3.5; length of femur (vent to knee) 27; length of tibia (knee to heel) 26; length of longest toe (from far side of metatarsal tubercle) 32; length of foot (heel to tip of longest toe) 36.

Variation.—The paratype, measuring 50 mm. snout to vent, is more yellowish-brown on the dorsal surfaces, and the tips of its tubercles are more nearly the color of the background.

Acknowledgements

Indebtedness is acknowledged to E. R. Dunn for the information which he has furnished on the *Dendrobates histrionicus-tinctorius* complex and for the loan of specimens in the Academy of Natural Sciences of Philadelphia. Thanks also go to The American Museum of Natural History and to the United States National Museum for their loans of specimens. Especial thanks go to Gustavo Orcés V. of the Escuela Politécnica Nacional, Quito, and to Rolf Blomberg of Quito for their donations of specimens, including two of the holotypes described in this paper.

References

Berthold, Arnold Adolph

1845a. Nachrichten Georg-Augusts Universität, Göttingen, pp. 43-44.

1845b. Abhandl., Göttingen, Bd. 3, pp. 15-16, pl. 1, fig. 8.

Boulenger, George Albert

1913. On a collection of batrachians and reptiles made by Dr. H. G. F. Spurrell, F. Z. S., in the Choco, Colombia. Proc. Zool. Soc. London, pp. 1019-1029, pls. 102-108.

Laurent, Raymond

1942. Note sur les procoellens firmisternes (Batrachia Anura). Bul. Mus. Roy. d'Hist. Nat. Belgique, v. 18, no. 43, pp. 1-20, figs. 1-30.

EXPLANATION OF THE PLATE

Plate I

Fig. 1. Dorsal view of *Dendrobates histrionicus sylvaticus* Funkhouser. (Charcoal drawing by Anne Funkhouser).

Fig. 2. Ventral view of holotype of *Phyllobates espinosai* Funkhouser. (Charcoal drawing by Anne Funkhouser).

Fig. 3. Mouthparts of tadpole of *Phyllobates espinosai* Funkhouser.

FIG. 1

FIG. 2

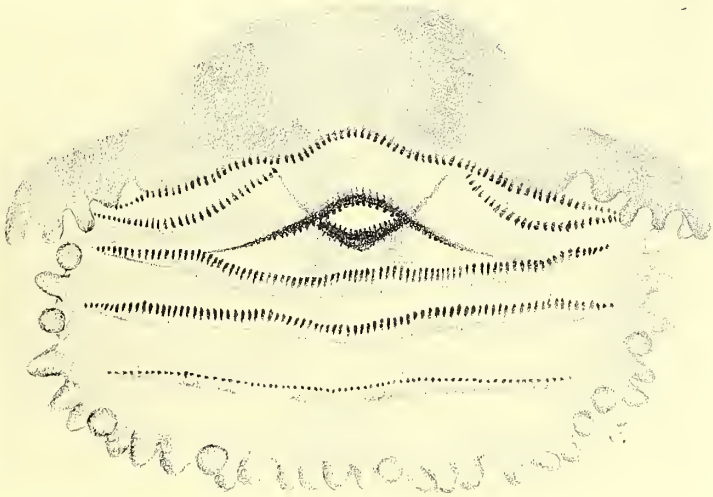


FIG. 3

NEW FROGS FROM ECUADOR AND SOUTHWESTERN COLOMBIA

10

Neonesthes gnathoprora, a New Species of Astronesthid Fish from the Atlantic Ocean[1]

Daniel M. Cohen

Natural History Museum, Stanford University, California

(Text-figures 1-3)

THE specimen to be described, the sole known representative of a new species of astronesthid fish, was discovered among the deep-sea fishes collected by the Bermuda Oceanographic Expeditions of the New York Zoological Society and subsequently deposited in the Natural History Museum of Stanford University.

Neonesthes gnathoprora, n. sp.

Holotype: S. U. 46381, a female, 127 mm. in standard length; collected from the *Gladisfen* by the Bermuda Oceanographic Expeditions, 1929-1930, of the New York Zoological Society; meter net no. 388 at a depth of 1,646 meters. Locality, an eight-mile cylinder, the center at 32° 12′N. Lat. and 64° 36′W. Long. off Bermuda (see Beebe, 1931, for further details concerning locality and collecting data).

Diagnosis: Neonesthes gnathoprora differs from all three of the previously described species of the genus in its lack of a ventral adipose fin and lack of large photophores in well-defined series, and by having the anterior projection of the subocular luminous gland completely covered by a pigmented layer. In fin counts and proportions it seems closest to *N. macrolychnus,* differing only in a slightly more slender body and short head. *N. gnathoprora* differs from *N. microcephalus* in having a larger head and a higher dorsal and anal count and from *N. nicholsi* in having a larger head, smaller eye, shorter barbel and higher dorsal and anal count.

Description: Body elongate, the greatest depth immediately posterior to the head, tapering to the least depth at the caudal peduncle. Pelvic, dorsal and anal fins elevated from the body. Base of dorsal adipose fin not elevated. Ventral adipose fin absent. Scales absent. Origin of dorsal closer to caudal than snout; dorsal origin closer to base of pelvics than to origin of anal; origin of pelvic fins closer to origin of pectoral fins than to origin of anal fin.

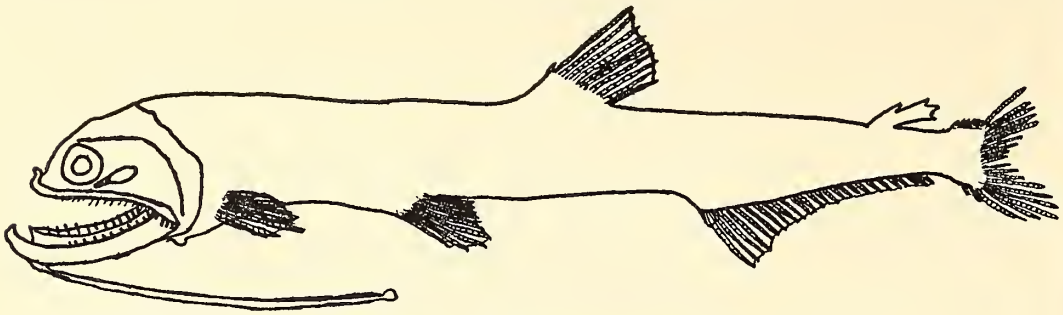
Head relatively short; maxillary extending far backwards, its posterior end 20 mm. from tip of snout. Anterior end of premaxillary retrorsely rounded at the symphysis, forming a notch for the reception of the prow-shaped lower jaw. Snout shorter than the eye. Needle-like teeth present on the maxillaries, premaxillaries, mandibles, palatines and ceratohyals. One tooth present on the vomer. Gill rakers stronger than the jaw teeth, resembling the ceratohyal teeth.

Barbel present, reaching slightly past the origin of the last pelvic ray. The core is light brown and covered by an outer transparent sheath. A darkly pigmented area partially encircles the barbel at a distance of 4 mm. from its origin. The barbel terminates in a brown bulb approximately 1 mm. in length, which is inclosed in a continuation of the transparent sheath which covers the barbel. Proximal to the bulb is a darkly pigmented area which terminates the light brown core of the barbel. A filament originates at the proximal end of the bulb and extends for almost its entire length. Two small white areas are present lateral to the origin of the filament on the sides of the bulb. These may be the bases of other (lost?) filaments, or they may be minute photophores.

The color of the body in alcohol is a uniform dark brown, the head lighter colored, fins pale.

The large photophores which make up the series commonly used in the classification of astronesthid fishes are absent; however, smaller light organs are present. A somewhat similar situation has been recorded by Zugmayer (1913) in his description of *Astronesthes myriaster.* In *N. gnathoprora* the small light organs are sparsely distributed over the body with a greater

[1]Contribution No. 969, Department of Tropical Research, New York Zoological Society.



TEXT-FIG. 1. Holotype of *Neonesthes gnathoprora* × 1.

concentration below the midline than above. Eleven pairs of small photophores form a pattern corresponding to the I-P series. Although small light organs are present in the other areas where counts are usually made, they are not given for this specimen because of the difficulty in identification of the series. In the head region light organs are present between the branchiostegal rays at both margins of the membranes, on the mandible, the maxillaries, the snout and the opercle, with fewer on the preopercle. A postocular luminous gland is present with a narrow subocular extension which is entirely covered by a black pigmented layer. Light organs are present mesad of the bases of the paired fins and at the base of the anal. They are sparsely distributed at the base of the caudal and absent from the base of the dorsal.

Counts and Measurements: Dorsal fin, 11; anal fin, 27; pelvic fins, 7-7; pectoral fins, 8-8; gill rakers on lower arm of 1st arch, 27; branchiostegal rays, 18-17. In the following measurements the first figure represents the measurement in mm.; the second figure is the percentage of the standard length. Greatest body depth, 19.0, 15.0; depth of caudal peduncle, 5.5, 4.3; head length, 23.2, 18.3; head depth, 18.5, 14.6; base of pectoral to origin of pelvic, 26.5, 20.8; base of pelvic to origin of anal, 36.2, 28.5; length of anal, 32.9, 25.9; last dorsal ray to insertion of adipose, 37.9, 29.8; length of barbel, 51.0, 40.2. In the following measurements the first figure represents the measurement in mm. while the second figure represents the percentage of head length. Eye, 5.3, 22.8; snout, 4.6, 19.8; postocular luminous gland (including anterior extension), 7.0, 30.2.

A slit present in the body wall slightly anterior to the right pelvic fin shows the body cavity to be filled with eggs. They are spherical in shape, orange and slightly less than 0.5 mm. in diameter.

The name *gnathoprora* refers to the resemblance of the lower jaw to the prow of a ship.

Discussion: The following artificial key should serve to separate the known species of *Neonesthes*.

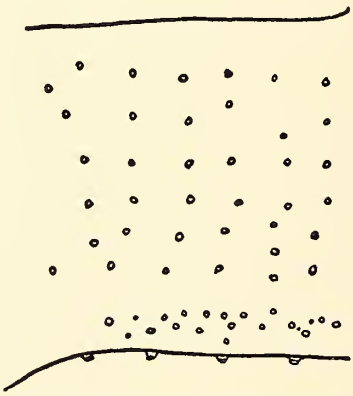
1a. Large photophores in well-defined series; ventral adipose fin present.

2a. Anal rays 23 or fewer; head into body length 6 times or more.
N. microcephalus Norman, 1930, p. 307, fig. 16.

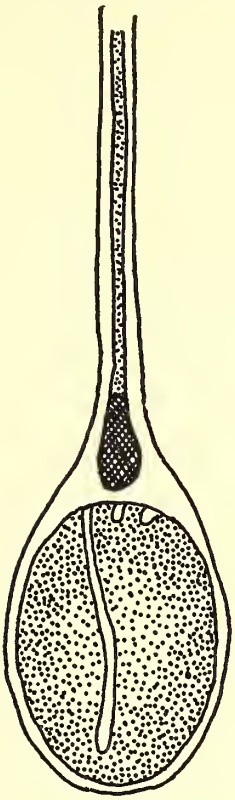
2b. Anal rays 25 or more; head into body fewer than 6 times.

3a. Barbel more than 3 times head length; filament present on bulb at end of barbel; eye into head more than 5 times.
N. nicholsi Beebe, 1933, p. 161, fig. 1.

3b. Barbel less than 2 times head length; no filament present on bulb at end of barbel. Eye into head 5 times or less.
N. macrolychnus Regan & Trewavas, 1929, p. 30, pl. 6, fig. 2.



TEXT-FIG. 2. Distribution of light organs on side of *Neonesthes gnathoprora*. × 4. (Between base of dorsal and origin of anal).



TEXT-FIG. 3. Bulbular distal end of barbel of *Neonesthes gnathoprora*. × 40.

1b. No large photophores in well-defined series; ventral adipose fin absent; anal rays 27; head into body 5.6 times; barbel 1.4-2.2 times head length; filament present on bulb at end of barbel; eye into head 4.4 times.
N. gnathoprora n. sp.

Distribution of the Genus: Neonesthes is, at the present, known only from the Atlantic. Three of the four species have been taken near Bermuda, probably because of the extensive deep-sea collecting in this area. *N. nicholsi* and *N. gnathoprora* are known only from the Bermuda area. *N. microcephalus* has been captured off of Angola in the South Atlantic. *N. macrolychnus* appears to be the most widely distributed species. It has the most southerly occurrence, at 32° 45′ S. in the South Atlantic, and in the North Atlantic it has been taken near the Bahamas in the west, near the Cape Verde Islands in the east, and as far north as 33° 51′.

Following is a summary of the distribution of the species of *Neonesthes*.

Neonesthes macrolychnus

15° 50′ N. 26° 30′ W. Regan & Trewavas, 1929
21° 04′ N. 73° 48′ W. Regan & Trewavas, 1929
28° 15′ N. 56° 00′ W. Regan & Trewavas, 1929
33° 51′ N. 66° 43′ W. Regan & Trewavas, 1929
31° 47′ N. 41° 41′ W. Regan & Trewavas, 1929
32° 45′ S. 08° 47′ E. Norman, 1930
32° 07′ N. 64° 37′ W. Grey, 1955
32° 09′ N. 64° 36′ W. Grey, 1955
32° 12′ N. 64° 36′ W. S.U. 45423

Neonesthes microcephalus

15° 55′ S. 10° 35′ E. Norman, 1930

Neonesthes nicholsi

32° 12′ N. 64° 36′ W. Beebe, 1933

Neonesthes gnathoprora

32° 12′ N. 64° 36′ W.
32° 12.7′ N. 64° 35.2 W. Grey, 1955[1]

Acknowledgements: The author wishes to thank Prof. George S. Myers for advice concerning the preparation of this paper.

LITERATURE CITED

BEEBE, WILLIAM

1931. Bermuda oceanographic expeditions 1929-1930. No. 1—introduction. No. 2—list of nets and data. Zoologica, vol. 13, nos. 1, 2. 36 pp. 7 figs.

1933. Deep-sea stomiatoid fishes, one new genus and eight new species. Copeia, no. 4, pp. 160-175, 8 figs.

GREY, MARION

1955. Notes on a collection of Bermuda deep-sea fishes. Fieldiana: Zoology, vol. 37, pp. 265-302, figs. 45-56.

NORMAN, J. R.

1930. Oceanic fishes and flatfishes collected in 1925-1927. Discovery Rpts., vol. 2, pp. 261-370, pl. 2, text figs. 1-47.

REGAN, C. TATE, & ETHELWYNN TREWAVAS

1929. The fishes of the families Astronesthidae and Chauliodontidae. The Danish "Dana" —Expeditions 1920-22. Oceanographical Rpts., no. 5, 39 pp., 7 pl., 25 text-figs.

ZUGMAYER, ERICH

1913. Diagnoses des Stomiatides nouveaux provenant des campagnes du yacht "Hirondelle" (1911 et 1912). Bull. de l'Institut Oceanographique, no. 253, 7 pp.

[1]Since this paper was written, Mrs. Marion Grey of the Chicago Natural History Museum kindly forwarded to the author the specimen which she listed (1955, p. 278) as *Neonesthes microcephalus*. Examination of her fish has shown it to be a specimen of *N. gnathoprora*, agreeing in most respects with the description given above. Her record of *N. microcephalus* from Bermuda should therefore stand as corrected.

11

A Sand Launce, *Ammodytes*, in the Coelom of a Weakfish, *Cynoscion*, with Earlier Records of Similar Phenomena

C. M. Breder, Jr.

The American Museum of Natural History

(Plate I)

A CONSIDERABLE number of reports have been made on the occurrence of ophichthids in the body cavities of various fishes. These have been found in a mummified condition and invested in membranes which evidently developed as part of the fishes' reaction to foreign bodies. A summary of the reports on such cases has been given by Walters (1955.1) together with a history of thoughts expressed by various persons on the possible methods of entry. Such coelomic entry, it is believed, is accomplished after the eels have been swallowed, by means of their sharp, hard-pointed tails, which are normally used to burrow into sand and similar materials. This specialization is a boring tool of considerable power.

There are also a considerable number of reports on similar behavior on the part of *Ammodytes*. Here it is the sharp anterior end which is evidently used for such penetration. Actually the hard anterior point in these fishes is formed by the projecting sharp chin, and not the snout as is sometimes stated. All such reports till the present have involved the three commonest North Atlantic gadoids, the pollack, cod and haddock, either from New England waters or the North Sea.

The present case differs from the previous reports in that it concerns the sciaenid, *Cynoscion regalis* (Bloch & Schneider), and was taken in Delaware Bay, N. J.[1] The *Ammodytes americanus* De Kay measured 105 mm. and was found during the process of cleaning a weakfish weighing three-quarters of a pound, which had been caught commercially in Delaware Bay during July, 1955. The photograph and radiograph, Plate I, make possible the certain identification. The sand launce was dry, shriveled, odorless and brownish in color, not unlike the ophichthid reported by Breder (1953). The preservation is not as good as in the latter, which may be merely a matter of longer entombment. This would be entirely possible because of the relatively small size of the *Ammodytes*. When the weakfish was less than half its size at capture, it could have swallowed the *Ammodytes*. The ophichthid noted above, in contrast, was very large in respect to the fish from which it was removed.

The only other fishes that have been reported to have found their way into the coeloms of larger fishes involved *Syngnathus* on two occasions. These accounts are general and rather vague as to details. Both evidently refer to the common *Syngnathus fuscus* Storer, although the North Carolinian case (see below) may involve one of its more southernly, but basically similar, relatives. While these fishes are certainly hard-bodied and angular they would not ordinarily be thought of as being "sharp" enough to puncture the wall of a gut. Evidently under exceptional circumstances this is possible. It it notable, nonetheless, that all three types of fishes could, *a priori*, be expected to present a hazard to a thin or damaged part of the intestinal tract. A consideration of the following annotated list of all such occurrences emphasizes this situation, as does the absence of hard-bodied but not slender fishes, and slender but not hard-bodied kinds.

The records of these fishes now stand as follows:

Ophichthidae.

Omochelys cruentifer (Goode & Bean).

?In a *Pollachius virens* (Linnaeus). Massachusetts. Atwood (1859). This was merely described as an eel. Since Atwood

[1]Dr. Florence Wood, to whom we are indebted for this specimen, obtained it from a retail fish market in Avalon, N. J. The proprietor, Mr. George Heits, is also the source of the ophichthid reported by Breder (1953).

knew of *Ammodytes* in such situations it would seem most likely that this occurrence was an *Omochelys*.

In unidentified fish. North Atlantic. Goode & Bean (1895).

In *Centropristes striatus* (Linnaeus). New Jersey. Breder (1953). A.M.N.H. No. 20300.

Ophichthus apicalis (Bennett).

In *Serranus pantherinus* (Bleeker).
Letherinus nebulosus (Forskål).
L. miniatus (Schneider).
Pristipomoides pristipoma (Bleeker).
Lutianus dodecacanthus (Bleeker).
Unnamed lutianids. Ceylon. Deraniyagala (1932).

Myrichthys acuminatus (Gronow).

In *Promicrops itaiara* (Lichtenstein). Florida. Breder & Nigrelli (1934).

Ophichthus and *Apterichthys*.

In *Lophius piscatorius* Linnaeus. Eastern North Atlantic. It is questionable whether this is properly referable to present considerations. See Walters (1955.1). Suvorov (1948).

Ophichthus ocellatus (LeSueur).

In *Alphestes* sp. Bahamas. Walters (1955.1). A.M.N.H. No. 19902.

Syngnathidae.

Syngnathus sp.

In *Gadus callarias* Linnaeus. Massachusetts. Atwood (1857). Unidentified fish. North Carolina. Gudger (1922).

Ammodytidae.

Because of the confused status of the species and subspecies in this genus, it is impossible to be absolutely certain of the names applied below. See, for example, the remarks of Walters (1955.2). Hence the question marks, although the probability is great that the names are properly applied.

Ammodytes americanus DeKay ?

In *Gadus callarias*. Massachusetts. Atwood (1857 and 1868).

Bigelow & Welsh (1925) wrote, "Sand eels' noses are so sharp that when swallowed by cod, and perhaps other fish, they sometimes work right through the stomachs and into the body cavities of their captors, to become encysted in the body wall, but this must be an exceptional event for none of the fishermen of whom we have inquired have seen it, nor have we." Bigelow & Schroeder (1952) repeat the statement but drop the phrase following the last comma.

In *Cynoscion regalis* (Block & Schneider). New Jersey. A.M.N.H. No. 20301.

Ammodytes tobianus Linnaeus ?

In *Melanogrammus aeglefinus* (Linnaeus). Scotland. Barrett (1885).
Three *Ammodytes* in a single fish in one instance. Williamson (1911).
Gadus callarias. Scotland.
Pollachius virens. Scotland. Williamson (1911).

Bibliography

Atwood, N. E.

1857. [Notes on the habits of some marine fishes.] Proc. Boston Soc. Nat. Hist. 1856-59 (1859), 6: 176.

1861. Remarks on a specimen of Naucrates and on the natural history of the cod. Proc. Boston Soc. Nat. Hist. 1859-61 (1861), 7: 4.

1868. Exhibition of a cod fish which had a number of sand-eels in the walls of the abdominal cavity [with remarks by Jeffries Wyman]. Proc. Boston Soc. Nat. Hist. 1866-68 (1868), 11: 364.

Barrett, W. H.

1885. Note on the liver of a haddock in which a sand-eel was partly imbedded. Third Annual Rept. Fish. Bd. Scotland, for 1884. Appendix F, No. 5, pp. 70-72.

Bigelow, H. B. & W. C. Schroeder

1953. Fishes of the Gulf of Maine. Fish. Bull., Fish and Wildlife Serv., vol. 53. Fish. Bull. 74, pp. 1-577.

Bigelow, H. B. & W. W. Welsh

1925. Fishes of the Gulf of Maine. Bull. U. S. Bur. Fish., 40, pt. 1: 1-567. Bur. Fish. Doc. No. 965.

Breder, C. M., Jr.

1953. An ophichthid eel in the coelom of a sea bass. Zoologica, 38: 201-202.

Breder, C. M., Jr. & R. F. Nigrelli

1934. The penetration of a grouper's digestive tract by a sharp-tailed eel. Copeia (4): 162-164.

Deraniyagala, P. E. P.

1932. A curious association between *Ophichthus apicalis* and percoid fishes. Ceylon Jour. Sci. (Spolia Zeylanica), sect. B, 16 (3): 355-356.

Goode, G. B. & T. H. Bean

1895. Oceanic ichthyology. Special Bull. U. S. Nat. Mus., xxxv + 529 pp.

GUDGER, E. W.

1922. Foreign bodies found embedded in the tissues of fishes. Natural History, 22 (5): 452-457.

SUVOROV, E. K.

1948. Osnovy ikhtiologii. Second ed., augmented. Leningrad. pp. 312, 512. [In Russian].

WALTERS, V.

1955.1 Snake-eels as pseudoparasites of fishes. Copeia (2): 146-147.

1955.2 Fishes of western Arctic America and eastern Arctic Siberia. Bull. Amer. Mus. Nat. Hist., 106, art. 5: 255-368.

WILLIAMSON, H. C.

1911. Sand-eels (*Ammodytes* sp.) and an hermit crab (*Eupagurus bernhardus*) encysted in the abdominal cavity of the Haddock (*Gadus aeglefinus*), Cod (*Gadus callarias*), and Saithe (*Gadus virens*). 28th Annual Rept. Fish. Bd. Scotland, for 1909, pt. 3, Scientific Investigations, pp. 62-63.

EXPLANATION OF THE PLATE

Plate I.

Ammodytes from the coelom of a *Cynoscion*.

Fig. 1. The specimen as removed from the body cavity. A.M.N.H. No. 20301.

Fig. 2. Radiograph of the above specimen.

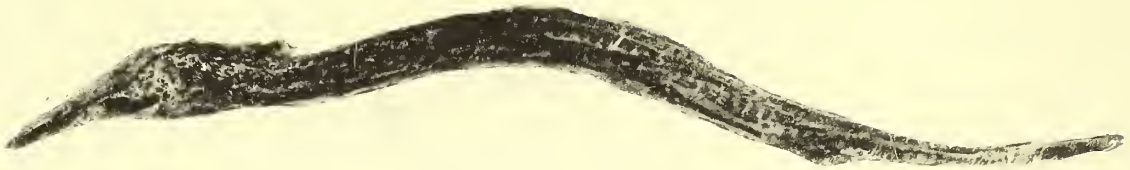


FIG. 1

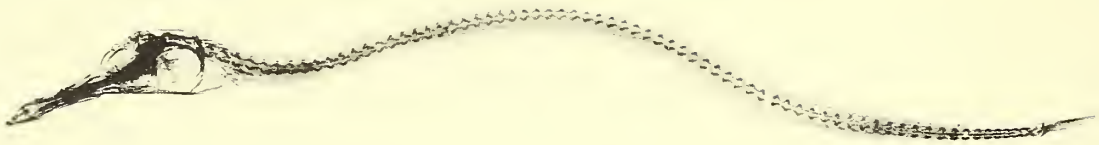


FIG. 2

A SAND LAUNCE, AMMODYTES, IN THE COELOM OF A WEAKFISH, CYNOSCION, WITH EARLIER RECORDS OF SIMILAR PHENOMENA

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 41 • PART 3 • NOVEMBER 23, 1956 • NUMBERS 12 TO 15

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

12

The Behavior and Social Relations of the Gibbon (*Hylobates lar*) Observed under Restricted Free-range Conditions

BERNARD F. RIESS[1]

(Text-figures 1 & 2)

FIELD studies of animal behavior have a long and honorable history in comparative psychology. There have been, however, relatively few attempts to correlate data gathered in natural habitats with laboratory studies of behavior in the same species. The activity of laboratory animals has been assumed to be characteristic of a species despite the restrictions of the laboratory and the effects of selective breeding. Until Calhoun's (1950) research on the behavior and demology of the free-ranging albino rat, little was known of the social behavior of this rodent under simulated natural conditions. The significance of the fact that all Syrian hamsters, *Cricetus aureus,* derive from a few pairs captured in Syria, has not been apparent in the laboratory studies of the behavior of this mammal. The influence of selective breeding and long years of laboratory life can be appreciated and evaluated only by matched field and laboratory investigation. Richter (1954) has shown that there is structural and glandular change in rats after domestication. Riess (1950) has pointed to other areas in which comparative field and laboratory research would be helpful.

When interest centers on social psychological investigations, it is generally agreed that such studies should be undertaken under conditions optimal for the development of inter-individual behavior. The naturalist Hediger (1942), among others, has emphasized the important influence of "territoriality" on the social groupings of infra-human species. So, too, Colias (1950) has shown the relationship between dominance and environment to be one determinant of social behavior. Despite the growing awareness among comparative psychologists of the importance of the processes in the concept of territoriality, we still lack basic studies of social behavior in systematically varied levels of environmental restriction. For instance, the effect of sound-induced seizures in rats may be a function of the size of the auditory enclosure within which the rat lives. By and large, for most laboratory subjects in comparative psychology, ecological-psychological description of their behavior under non-laboratory or natural conditions is very sparsely represented in the literature.

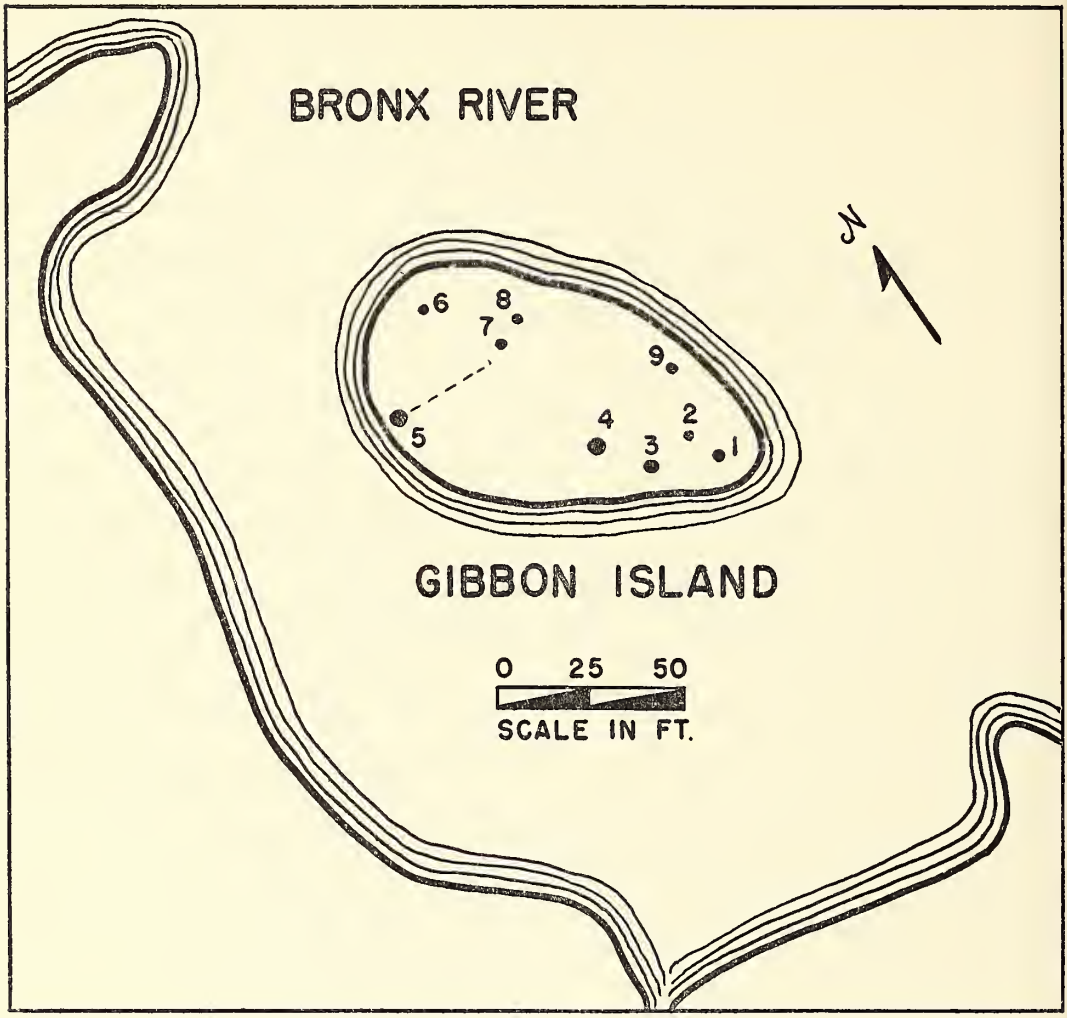
The author participated for several years in a program designed to evaluate the research possibilities in the zoological collections of living material at the New York Zoological Park (Bronx Zoo). Here, for certain species, it was possible to obtain data based upon observations of animals in environments of variable size, ranging from large compounds of several acres to small cages. Several studies based on the work at the Bronx Zoo have already appeared to validate the usefulness of the project.[2]

The present paper is a study of a family of gibbons of a species whose behavior in its natural habitat has been systematically described by Carpenter (1940). Comparison of free-range behavior with that in a relatively restricted captive territory was felt to offer opportunities for the evaluation of the effect of territoriality and other factors on social activity. Furthermore, since the family of gibbons to be studied was exhibited over a number of years under similar conditions, long-range observations could be made.

Subjects and Habitat.—The animals observed were a family of gibbons, *Hylobates lar,* native to the Burma region. The gibbon is usually placed by taxonomists within the anthropoid ape group. According to Carpenter (1940), their

[1] Behavior Research Fellow, New York Zoological Society, 1947-1948.

[2] Success of the project was largely owing to the splendid cooperation of the staff and keepers at the Park. Special appreciation is extended to Drs. Fairfield Osborn and John Tee-Van and Messrs. Lee S. Crandall, William Bridges, Gordon Cuyler and Quentin Schubert of the staff of the Zoological Park.



TEXT-FIG. 1. Map of Gibbon Island in Lake Agassiz, Bronx River, New York Zoological Park.

social organization is characterized by small family groupings ranging in size from two to eight animals. The group under observation at the New York Zoological Park originated from a male and female, fully adult at the time of this study, which were obtained from a dealer in 1942. A mating occurred and produced a male offspring in June, 1946. Subsequently, in 1948 and 1950, young were again produced, male and female respectively. During both summers of my tenancy of the research fellowship, the family consisted of the parents and first one and then two young animals.

During the winter the family lived as a unit in a large cage in the Primate House. It was on exhibition from 10 A.M. to 5 P.M. during the week and somewhat later during week-ends. During the summer months, from June to September, the gibbons were exhibited on Gibbon Island, a small, circular body of land in Lake Agassiz at the north end of the Park. Text-figure 1 shows the general topography of the island. It is separated from the mainland by a narrow channel approximately 30 feet wide. Vegetation consisted of nine trees of various heights, maples, oaks and plane trees, growing amid grass and weed. The only artifact on the island was a guy wire running from Tree #5 to the ground. At the tree, it was about 15 feet above ground level and ran 20 feet to its termination in a stake in the ground.

The gibbon family was the only regular mammalian exhibit on the island. Various aquatic birds made their homes in and around the lake and shore and came to the island for remnants of food dropped by the apes. Flocks of wild Canada geese and some ducks also dropped in on the island in their fall migratory flights.

Routine feeding of the apes took place between 11:30 A.M. and 12:30 P.M. daily. The keepers tossed food over the water gap to the foot of Tree #5 where the gibbons would pick it up and carry it to the limbs of the trees. Diet consisted of half loaves of bread, whole cabbages, heads of lettuce, carrots, beets, turnips, oranges, apples and bananas.

Observational Procedures.—The gibbons were studied by the author for varying periods of time each day for a total of 115 days during June, July, August and early September of 1947 and 1948. The periods ranged from one to five hours. Summer observations were systematically scattered so that each hour of the day between 6:00 A.M. and 9:00 P.M. was covered at least twelve times in the two summers. In addition, five full days were devoted to dawn-to-dusk study in order to obtain continuous activity data. Observations were also made irregularly while the animals were in winter quarters in the Primate House.

All observations were made from a rocky eminence on the west shore of the lake, opposite the island at the narrowest part of the water gap. The elevation of the observation post was approximately equal to the height of the mid-section of Tree #5 in which the animals spent most of their time. From the vantage point of the rock, the entire island could be observed and with a little practice in the detection of concealed members of the family, the behavior of the apes could be brought under close scrutiny. A pair of field-glasses (8×30), a stop watch and an automatic counter were used to obtain the data of this report.

Results.—The quantitative data obtained from the gibbon family on Gibbon Island will be presented at a later point. While meaningful in themselves, the present results take on additional significance when interpreted against the background of Carpenter's (1940) observations on the same species in the wild in Thailand. From the latter's monograph, the following statements have been abstracted so as to give a concise picture of *Hylobates lar* under free-ranging conditions.

A. Family Structure.—Gibbons exhibit a pattern of family grouping consisting of parents and two to four young ranging in age estimated at two to six years. Solitary animals of both sexes are found on the periphery of the family group. Semi-solitary individuals of early adult status may pair to form the nucleus of a new family organization. Within the family, the sexes are equi-dominant where dominance is defined in terms of access to incentives involved in feeding, sexual activity, grooming positions and group-coordinating behavior.

B. Inter-individual Behavior.—Copulation was infrequently observed and was seen on only two of several hundred observation periods. In both instances, the female was the more active of the pair. Presentation and genital inspection were rarely observed.

Male-young relationships were characterized by inspection and grooming by the father while the young were in the infant stage of development. As the young animals matured, some play with the parent was observed and males were seen to "guard" the offspring when alarm calls were sounded. Further maturation of the young led to overt hostility between the older male and the developing young.

Female-young interaction starts with the nursing and carrying of the young by the mother. Infants were almost invariably placed on the mother's belly where the female could cover them with her arms and legs. Riding pick-a-back was never observed. Considerable inspection and grooming were found. It is Carpenter's impression that the relationship between mother and young depicted above is commoner among gibbons than among other observed groups of simians or apes.

Young-young relations are somewhat overshadowed by the dominant familial pattern of parental interaction with the young. Infant animals give evidence of a tremendous amount of play by themselves. Play was maximal during the early morning and during the afternoon period of parental quiet.

C. Territoriality.—The amount of territory covered by a group in its native habitat depended on (1) the number of animals in the group; (2) size and kind of forest; (3) competition from other groups; (4) availability of nutrition; (5) disturbance by human beings or "enemies"; (6) optimal conditions of environment such as humidity and temperature; (7) previous adaptation; (8) pressure from other gibbon groups. It was roughly estimated that the territory ranged from 30 to 100 acres for various groups of gibbons in Thailand. It should be kept in mind that extent of territory is related to the three-dimensional structure of the life-space of the species. Where trees are tall and dense, area tends to be more circumscribed than otherwise. Territory is fairly rigorously defended and defined, sometimes by overt aggression but more frequently by vocalization. Within the territory, gibbons preferred the midportions of trees rather than the tops or bases. They were strictly arboreal and rarely came to the ground except during the dry season for water.

D. Social Behavior.—Gestural activity was found to be an important factor in the social life of the gibbon in the wild. Grimaces, lip and eye

TABLE 1. OCCURRENCE OF INTER-INDIVIDUAL BEHAVIOR

Type of Behavior	1947						1948									
	Male with		Female with		Young$_1$ with		Male with		Female with		Young$_1$ with		Young$_2$ with			
	Fe	Y_1	M	Y_1	Fe	M	Fe	Y_1	Y_2	M	Y_1	Y_2	Fe	M	Fe	Y_1
Sexual																
Genital Inspection	1	..	2	..	..	..	3	..	..	2	..	..	..	..	..	..
Presentation	..	..	1	..	..	..	..	..	..	4	..	..	..	..	..	..
Grooming																
Frequency	25	8	37	11	5	6	32	18	6	43	15	59	10	16	2	..
M'n. Duration (in secs.)	80	12	30	110	24	14	106	58	30	45	96	240	38	21	6	..
Fighting																
Frequency	..	2	..	..	4	..	..	..	..	..	..	..	..	..	..	..
Play-chasing																
Frequency	85	19	101	26	12	16	97	42	..	118	36	..	59	48	..	..
M'n. Duration (in secs.)	80	65	150	105	57	76	82	70	..	175	90	..	120	80	..	..

movements and gross motor manifestations tended to fall into patterns which were specific to certain situations. Greeting after separation or upon the inception of group action, angry gestures and fighting positions constituted the most commonly observed patterns.

Vocalization is a mode of social interaction and such communication serves coordination among gibbon groups to an extent rarely found in other primates. Through the use of parabolic reflectors and disk recorders, Carpenter was able to differentiate nine types of calls and to describe the situations eliciting them. The two most frequent were (1) a series of hoots of rising pitch and intensity, and (2) single, discrete notes, sometimes in repeated series. Type 1 seemed associated with exploration, defensive action and protection of territory. It was more frequently emitted by females and never by the young. Type II served to localize territory and to avoid inter-group conflict over the range. It was predominantly an adult male call. The peak of vocalization fell in the early morning hours between 7:30 and 9:00 A.M. with a secondary rise in late afternoon. Both of these periods coincided with the maximum movement behavior of the group.

Aggressive behavior seemed to be a minor factor as observed in the life of the organized gibbon group. Vocalization represented a kind of substitutive activity for fighting. Unlike captive animals, wild gibbons were never seen in actual combat. This may be the result of pre-established patterns of dominance status. Inter-species hostility was less than intra-species.

Dominance and grooming are usually found to be concomitant variables in studies of primate and simian groups. In the gibbon, where equi-dominance of male and female seems established, grooming by others and by self were almost equally frequent. Grooming was relatively common and definite evidence of consistent initiation by a given animal was wanting.

With this background, the material presented in Tables 1, 2 and 3 offers a basis for certain limited generalizations about the effect of captivity, restricted range and lack of pressure from other groups on social behavior. Table 1 lists the frequencies for behavioral items involving inter-individual contacts, such as sexual activity, grooming, fighting and playing. One exception has been made in the inclusion of inter-individual items in Table 1; eating, whether individually or in groups, has been located in Table 2, together with other forms of individual activity. Frequencies in all cases are expressed in absolute terms and represent the cumulative occurrences of the behavior in question in approximately 100 hours of observation per summer.

TABLE 2. OCCURRENCE OF INDIVIDUAL BEHAVIORAL ITEMS

Behavior	1947			1948			
	Male	Female	$Young_1$	Male	Female	$Young_1$	$Young_2$
Eating							
When alone	148	196	205	120	115	170	2
With one or more of family	60	75	39	105	127	162	104
On ground	19	24	6	22	19	16	..
On trunk	86	102	151	54	80	87	49
On limbs	103	145	87	149	143	229	57
Food taken from							
male by	..	14	18	..	42	40	..
female by	12	..	66	26	..	3	86
$young_1$ by	..	20	..	15	37	..	..
Resting Position							
On wire	86	72	101	90	70	88	..
Tree #1	4	6	..	6	10	2	..
#2	3	10	2	2	4	4	..
#3	2	1	0	2	1	1	..
#4	12	5	4	10	7	16	..
#5	572	521	516	566	626	582	16
#6	202	198	175	187	115	110	..
#7	212	201	121	156	132	187	..
#8	101	142	262	181	108	190	..
#9	18	26	31	20	15	28	..
Position when in Tree #5							
At top	16	46	318	15	66	253	..
In middle	156	288	90	212	389	180	16
On side limbs	214	82	34	157	69	61	..
On trunk	186	105	74	182	102	88	..
Brachiation							
M'n. time/hr.	18′	31′	9′	16′	34′	21′	..

No attempt has been made to plot frequencies against diurnal variations. Where conclusions as to distribution over certain hours of the day can be drawn, they will be stated textually.

It will be seen from the data in Table 1 that the family on Gibbon Island in the Bronx Zoo has certain specific resemblances to the behavior of animals in Thailand as reported by Carpenter. Sexual activity is infrequent, an observation which was made for gibbons as long ago as 1771 by Turpin. Since the gestation period is variously estimated at seven months (Yerkes, 1929) and births have been noted from May through June, it may be that the summer season is not propitious for the observation of sexual behavior. What activity did occur was exclusively between the two adult members of the group and was equally divided as to initiation between the male and the female. The increase in frequency of both inspection and presentation during the second summer as compared with 1947 is noteworthy, but its etiology is unknown.

Grooming has usually been assigned a place as a determinant of that vague construct known as dominance. In the gibbon, as comparison of Table 1 and 2 shows, "self" and "social" grooming occur with almost equal frequency. However, when inter-individual grooming is considered alone, family relationships seem to stand out as important determinants of the frequency of occurrence of this type of behavior. Both the absolute amount and the duration of grooming of family members by the male adult increased as the size of the family became greater. This increment holds true also of the female and to a lesser extent of the older offspring. The absence of self or social grooming by the most recent family member may be related to the prolonged period of biological dependence of the gibbon infant upon its mother.

The distinction made in the next two categories, fighting and play-chasing, is somewhat subjective. Real aggression with injury to one or more gibbons was never seen during either summer period of observation.

From Carpenter's data on collected speci-

TABLE 3. TYPE AND FREQUENCY OF VOCALIZATION PER HOUR OF THE DAY

Time of Day		Male I	Male II	Male III	Male IV	Female I	Female II	Female III	Female IV	Young$_1$ I	Young$_1$ II	Young$_1$ III	Young$_1$ IV
6-7	'47 Mn.	2.4	2.1	..	2.2	2.5	1.0	..	1.6	1.0	..	..	3.0
A.M.	'48 Mn.	2.8	3.0	..	2.1	2.2	1.5	..	1.4	2.3	1.8	..	1.9
7-8	'47 Mn.	2.8	3.6	..	2.4	2.4	2.1	..	1.8	1.0	1.0	..	2.4
A.M.	'48 Mn.	2.6	3.5	..	1.9	2.7	2.0	..	1.7	1.1	2.0	..	1.7
8-9	'47 Mn.	2.6	3.5	..	1.5	3.0	2.8	..	..	3.5	1.8	..	1.0
A.M.	'48 Mn.	2.5	3.3	..	1.0	3.4	2.1	..	.5	4.2	1.7	..	1.9
9-10	'47 Mn.	2.0	1.5	..	..	2.6	1.4	..	..	..	..	..	..
A.M.	'48 Mn.	1.5	2.1	..	.7	3.1	1.3	..	..	2.6	1.0	..	..
10-11	'47 Mn.	1.6	1.2	1.0	..	2.0	1.0	1.0	..	..	..	..	..
A.M.	'48 Mn.	1.8	1.9	1.1	..	1.9	1.4	1.0	..	.5	1.8	1.0	..
11-12	'47 Mn.	1.4	4.2	2.8	..	4.5	2.0	1.7	..	2.8	2.0	..	..
A.M.	'48 Mn.	1.9	4.3	2.5	..	4.1	2.3	1.2	..	2.0	4.0	1.1	..
12-1	'47 Mn.	1.4	3.9	1.0	..	4.5	1.5	1.1	..	2.1	1.9	..	..
P.M.	'48 Mn.	1.2	4.0	1.0	..	4.7	1.3	1.0	..	1.6	3.9	1.0	..
1-2	'47 Mn.	..	..	..	..	..	1.0	..	..	..	..	..	..
P.M.	'48 Mn.	..	..	..	..	..	..	..	..	..	..	..	..
2-3	'47 Mn.	..	..	..	..	..	..	..	..	..	..	..	..
P.M.	'48 Mn.	..	..	..	..	..	..	..	..	..	..	..	..
3-4	'47 Mn.	1.0	2.1	..	..	2.0	1.8	..	..	..	..	..	..
P.M.	'48 Mn.	1.3	3.0	..	..	3.7	2.3	..	..	..	3.0	..	..
4-5	'47 Mn.	..	..	1.4	1.8	..	..	1.7	1.6	..	..	1.6	..
P.M.	'48 Mn.	..	..	1.3	1.0	..	..	1.5	1.7	..	..	1.7	1.4
5-6	'47 Mn.	..	..	1.0	1.0	..	..	1.0	1.0	..	..	..	..
P.M.	'48 Mn.	..	..	1.1	1.7	..	..	1.3	1.6	..	..	.9	1.2
6-7	'47 Mn.	1.5	3.2	..	..	3.1	1.5	2.0	..	2.2	2.0	..	3.0
P.M.	'48 Mn.	1.8	3.8	..	1.0	4.0	1.7	1.9	..	1.6	3.4	..	..
7-8	'47 Mn.	1.0	2.6	..	..	2.6	1.0	1.5	..	1.8	2.5	..	2.5
P.M.	'48 Mn.	1.4	4.3	..	..	4.6	1.1	1.0	..	1.5	3.5	..	..
8-9	'47 Mn.	..	..	..	2.0	..	..	..	2.0	..	..	..	1.8
P.M.	'48 Mn.	..	..	..	3.0	..	..	..	2.5	..	..	..	1.1
Total Mean	'47	1.2	1.8	.5	1.0	2.0	1.1	.6	.6	.9	.7	.1	.9
of Means	'48	1.2	2.3	.4	.8	2.3	1.0	.6	.6	1.1	1.6	.4	.6

mens shot in the wild, it would appear highly doubtful that the gibbon is as non-aggressive as our observations would imply. Examination of these specimens shot in Thailand showed numerous scars, wounds and fractures despite the failure of the author to see any actual fights. That the gibbons on Gibbon Island can be aggressive to human intervention is attested by many incidents related by their keepers. However, what the animals do relative to human beings cannot be directly carried over to intra-species behavior.

All in all, only six instances of fighting were observed at the Zoo. In these, the most outstanding fact was vocalization seemingly of Carpenter's Class III. Overt aggressive movement was present but not actual physical contact. All the six incidents involved the older of the two young animals and were relatively mild in nature and short in duration. The mother was attacked four times, while fighting was initiated by the father on two occasions. Competition for food was the precipitating stimulus for each behavior.

The activity called play-chasing describes a sequence of brachiations initiated by a member of the family and then taken up by one or more additional members. Although the paths of the initiator and the other animals were in general alike, there was by no means use of identical branches in swinging through the trees. However, the direction of progression was always the same. Typically such behavior ended with the initiator coming to a halt on one of its preferred resting places and the followers then seeking theirs. Here, too, there was an increase in fre-

quency during the second summer, as the second youngster became a member of the group. Whether the increase represents the effect of increased maturity of the young or social facilitation by a larger group is not clear. One final note on play-chasing is worth recording. The duration of each play-chasing sequence is relatively short but the distance covered during the behavior was considerable. A rough estimate of space covered would certainly set it at several hundred feet.

Table 2 indicates the growing socialization of familial patterns during the second summer. For instance, the number of times that eating in groups was observed was substantially greater in 1948 than in 1947. So, too, the locus of feeding activity shifted from the trunk to the favorite resting place in the limbs where groupings were possible. Increase in food exchange also marked the development and growth of the family.

Tree #5 was used most often as a resting place. This was undoubtedly the result of the fact that it was the place where food was thrown across the water gap by the keepers. Foci of sociality were thus established within the territory. In general, the trend of the two summers was to bring the family units closer together spatially as well as behaviorally. This was illustrated by the frequency of the positions assumed by the young ape during the summer. There was an increase in the use of Tree # 5 and a decrease in the use of isolated positions elsewhere with the exception of Tree # 8. This latter resting place was favored by offspring I as indicated by the fact that this animal's aggressive calls of Type III occurred when the parent animals brachiated into the tree. Tree # 8 was also frequently approached by the first offspring when walking upright on the ground.

Territoriality in the gibbon is a three-dimensional affair in which elevation from the ground operates as a selective factor. As can be seen in next to the last part of Table 2, the top branches were almost exclusively favorite resting places for the young animal, whereas the middle limbs in the center of the tree were used by the mother, and the peripheral, middle-height limbs were frequented by the father. The capacity of the branches to sustain the weight of the animals was not the decisive factor in the selection since the resting places of the father and offspring were about equally thick.

Vocalization is an activity of gibbons which universally excites curiosity and speculation among observers of the species. As Carpenter remarks "the coordination and control of gibbon families is carried out by complex systems of gestures and patterns of vocalization." The variety of calls, their recurrence in well-marked patterns as well as their carrying power, make an important area of research. In our observation of vocalization, Carpenter's classification system has been used. However several types of calls noted by him were so rarely found in our animals that they have been omitted. Indeed, this may be one of the marked effects of the living situation out of the total biotic context, especially away from other families. Four patterns composed the great majority of calls at the Bronx Zoo. These can be described as follows:

Type I (Carpenter and Riess)—A series of hoots with rising inflection, rising pitch and increasing tempo with a climax followed by two or three notes of lower pitch. Duration was usually between 12 and 30 seconds.

Type II (Carpenter and Riess)—Single, discrete calls in series, which may be repeated over and over.

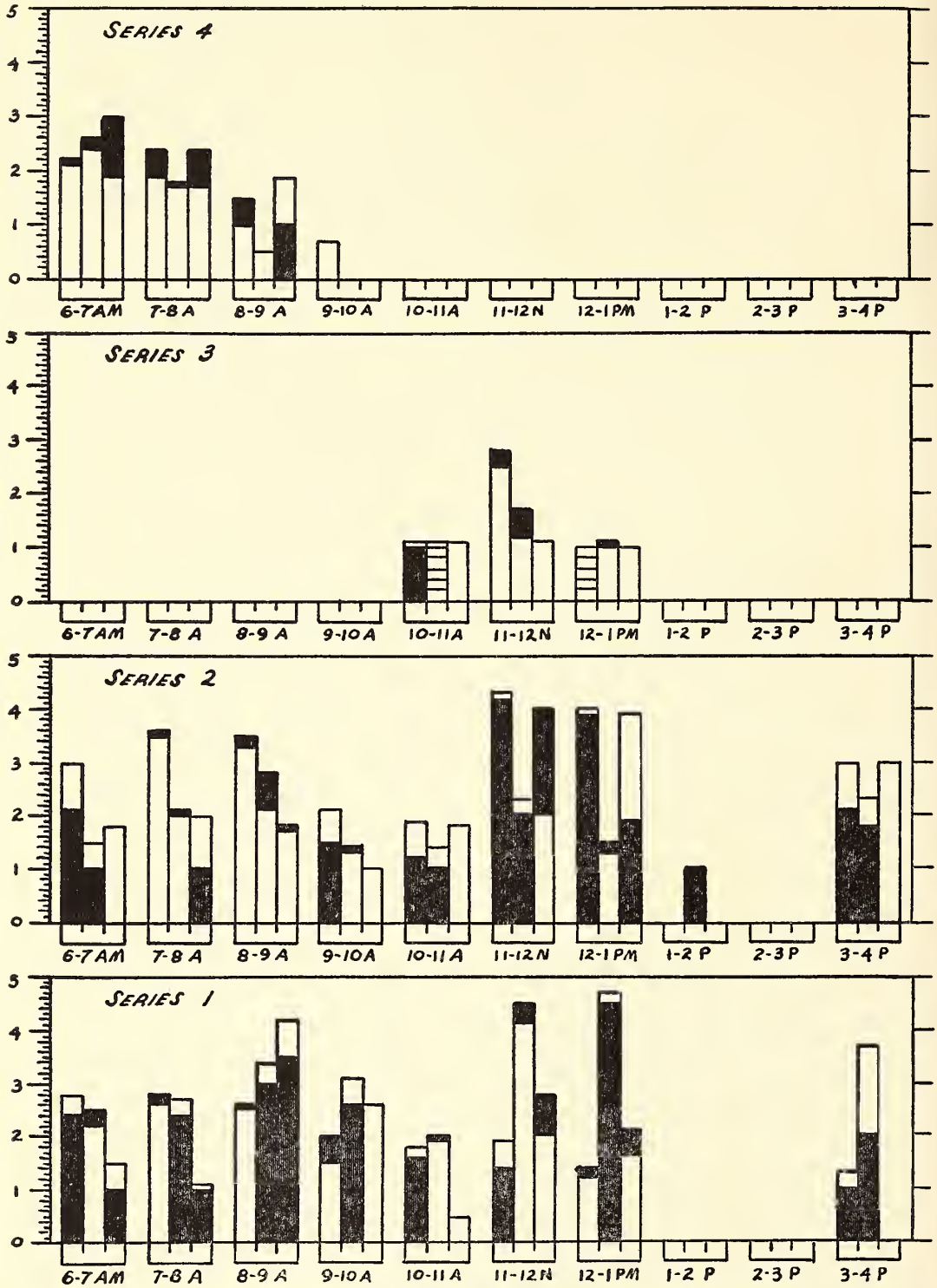
Type III (Carpenter and Riess)—Loud, high-pitched single note.

Type IV (Carpenter's Type VIII)—A fretting cry.

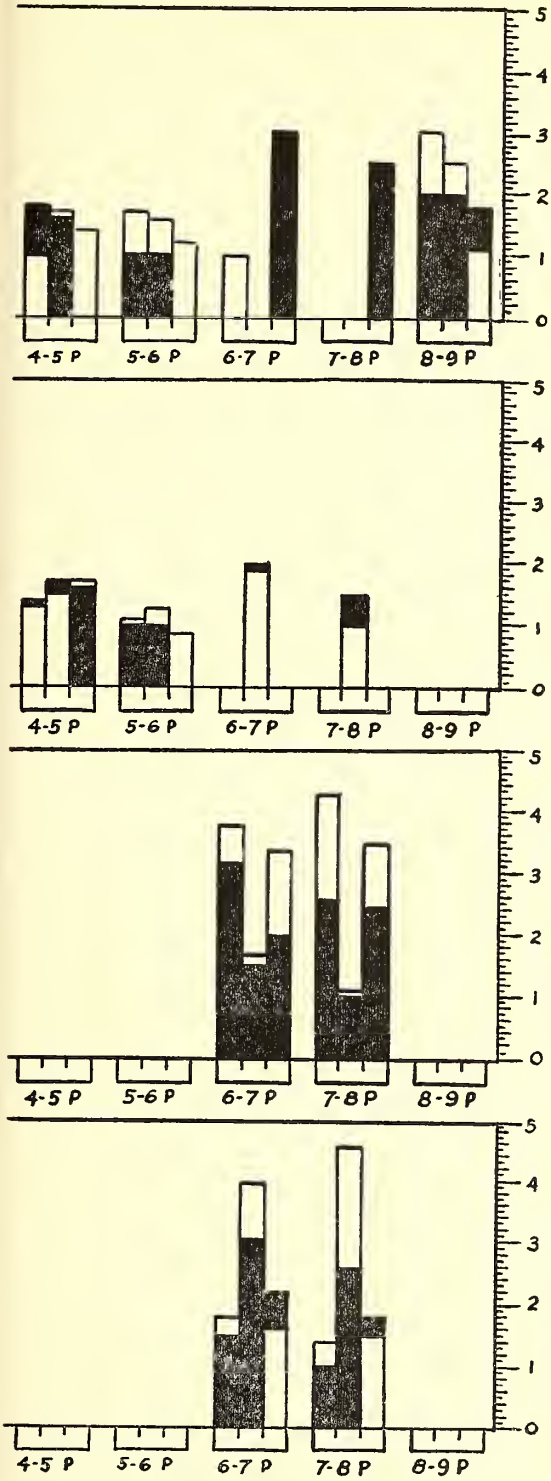
In studying this aspect of social behavior, several questions presented themselves. First, how did the various types of calls distribute themselves over the diurnal activity cycle of the gibbon? Second, were the differences in the types of calls given by various members of the family specific to the individual caller? Third, as increasing familialization took place, were there changes in a given type of call? The objective measurement of calls was difficult and it was decided to use Carpenter's lead in setting up a frequency count. During each period of observation, the observer tried to indicate the frequency of each type of call on a tally sheet previously prepared. At the end of each session, these frequencies were converted into points on a five-point scale in which 1 denoted maximum frequency.

Table 3 and the accompanying graphs (Text-fig. 2) summarize the findings. In terms of over-all ranks, it is clear from the last two rows of the table that there were evident differences in the use of various calls by the gibbons under observation. The male adult in both summers used Type II predominantly, whereas the mother used Type I. Offspring # 2 shifted from Type I in the summer of 1947 to Type II in 1948. What this represented was not clear from any data available.

Each of the four graphs in Text-fig. 2 represents one type of call. Within each type, the first column for any hour of observation records the frequency of the responses by the adult male,



TEXT-FIG. 2. Types of vocalization related to hour of the day, the animal emitting the call, and to each summer of observation. Each series describes one type of vocalization. The first column in each group of bars refers to the male adult, the second to the female adult, and the third to the first-born offspring. Bars in solid black refer to observations made in 1947, and those in outline to observations made in 1948.



the second by the female and the third column by the older offspring. This presentation, in a way, illustrates the value of comparison of field and restricted range data since it is possible in the latter to correlate behavior with known individuals, a technique difficult if not impossible in the field. Black areas in the figure show the data for 1947 and light areas for 1948. Where the scores for the first summer are higher than for the second, the column will be light at the base and dark on the upper part. The cycles in diurnal frequency stand out clearly in the graph. All types of calls diminish to the vanishing point after 1:00 P.M. This is generally also a time of motor inactivity, following as it does upon the noon feeding period.

Considering the calls of Type I alone, with the exception of the session from 7:00 to 8:00 A.M. in 1947, the female showed more of this type of vocalization than did the adult male. Offspring # 1 tended to follow the female pattern. The trend to female preponderance in calls of Type I was intensified in the summer of 1948. This observation is in agreement with Carpenter's opinion of relative frequency in Thailand. This investigator believed that the function of these calls was exploratory and defensive as well as protective of territory. In our experience they seemed to be a function of general activity as well as a stimulus to activity of a play-chasing kind.

Type II calls originated more frequently with the male than the female or young in both summers. There was also a tendency for the young to resemble the father more during the second summer than in the earlier period. Carpenter's hypothesis as to function posits a territorial localization purpose. From our data there is little to support such a theory. Type II calls were given by the male and elicited both Type II and I calls from the other animals. Little evidence was obtained of specific situations stimulating the calls. However, with reference to both types, it should be stated that these calls in the wild may be related to the pressure of other neighboring groups.

The feeding period at noon seemed to be the precipitating situation for calls of the third type. According to Carpenter, in the field these single, isolated notes were heard when the group of animals was under observation and served the function of alarm or defensive reactions. The case at the Zoo is not clear, since the feeding hours were also generally the periods of heaviest visitor observation. It is probable that this type of call is most readily conditioned by experience and so comes to be the most circumscribed in point of time of occurence.

In contrast to calls of Type III, Type IV (VIII

in Carpenter's enumeration) seemed to be an early morning vocalization. It was also found before the evening activity cycle. All three animals gave it in equal amount although in the very late evening the younger animal was more often engaged in these vocalizations.

Other than the above translations from the graph and table, few conclusions could be drawn regarding vocalization. The reliability of the observations is a question that requires an answer. From preliminary work with a second observer, Dr. Daniel Lehrman, the estimated correspondence between 10 observations by each experimenter was above 90%. Automatic recording of vocalizations would have been a more objective technique but was not possible under the conditions of this study.

Comparison with Carpenter's field data shows that there is some difference of significance as a result of restriction of range and population. In the first place, the vocalizations were not as varied as those found in the native habitat. Whereas Carpenter differentiated nine types of calls, we were able to identify only four patterns. In the second place, there was little evidence of defensive and group organizational vocalization at the Zoo. This difference may be explained by the absence of competing gibbon groups in the same habitat. More significant than the differences, was the existence of similarity in observed data. Such resemblances bear on the pervasive effect of biological determinants within the framework of different environmental complexes.

Discussion.— Although the major purpose of the work on which this paper is based was to determine the feasibility of research at a zoological park, especially as it served as a basis of comparison of free-ranging animals with members of the same biological type living in a more restricted territory, some comments can be tentatively advanced along a broader comparative line. The study of mammalian, infra-human sociology has been increasingly developed as field methodology has advanced and the twin restrictive errors of teleology and anthropomorphism have diminished in frequency. Sociometry is supplementary to qualitative description. The main trend in the area has been the insistence of workers that mammalian sociology is basically a matter of ecology, demology and reproductive physiology and psychology. Theories about the origins of group activity in the infrahuman primates range from Briffault's dictum that "the causes which give rise to the formation of a group among animals and the bonds which hold such a group together are in every instance manifestations of the reproductive instincts" to the use of territoriality by Hediger and others as a concept with group cohesive and binding force. In contrast to this theoretical demand for a single etiological factor are the elements elicited by the comparative surveys of primate behavior. Carpenter (1942) stated that "valid investigation of some problems in comparative behavior makes it imperative to study not only animals as wholes but whole animals in a species-adaptive and species-selected environment."

For this type of sociological study, data are now available on the following species: howler monkeys (*Alouatta palliata*), red spider monkeys (*Ateles geoffroyi*), macaques (*Macacus rhesus, M. assamensis coolidgei, M. mulattus*), gibbons (*Hylobates lar*), orang-utan (*Pongo*), chimpazee (*Pan*) and gorilla (*G. gorilla gorilla* and *G. gorilla berengei*). For our purposes, five factors have been selected for emphasis: population density, male-female ratio, extent of range, level of sexual excitability and dominance as exhibited in feeding, grooming and sexual behavior. These elements represent the most common items in the field descriptions of the groups cited above. The independent variable under consideration is group organization. Of the species of organisms listed, the range of social organization is from the larger "tribal" to the smaller "familial" relationship, or from the relatively loose aggregation-like mass to the small, fairly well-integrated group. At the tribal end are the baboons, spider and rhesus monkeys while the gibbon typifies the familial organization. Of the five factors that have been held causal by one or more authorities, none seems to be directly related to the continuum of group organizational forms. Species of high population density are found at both extremes, male dominance is exhibited by all species except the gibbon, females predominate in all organized groups except again that of the gibbon. The two most fertile areas for further research seem to be the range or territoriality and sexual excitability. In the gibbon, at least, the relative absence of presenting, mounting and sexual play stands out as a differentiating possibility. Here also the altitude of the range or territory may be important since both the howler and the gibbon use the upper levels of their arboreal environment, in contrast to other species, and are closer in organizational type than the other groups.

Comparison between the free-ranging gibbons and those on Gibbon Island also produces some hypotheses for future exploration. The animals at the Zoo, living relatively free from pressure of other competitive groups of species-mates, seem to be less familial in their group organiza-

tion than those in Thailand, as measured by the amount of inter-individual contacts. With the addition of a new member in the person of a biologically dependent infant, all types of inter-individual contacts increased. This gain in cohesiveness is intensified by the absence of polarization in the form of dominance relationships. Here the restrictive and bond-forming area of sexual interest seems to play a major part.

Summary and Conclusions.—A group of gibbons living under simulated natural conditions was observed over a period of two summers. Initially the group consisted of an adult male and female and an independent young offspring. During the second summer a newly born young was added to the group, the result of second pregnancy of the female. Observations were compared with those which Carpenter made on free-ranging gibbons in Thailand. The following conclusions were drawn:

(1) Inter-individual contacts of all types increased in frequency during the second summer of observation when the population increased.

(2) No evidence of polarization (dominance) was observed during either summer.

(3) No evidence of sexual play was observed.

(4) Aggression was manifested on few occasions and seems to be at a low level in the group.

(5) Carpenter's observations on dominance, sex-related vocalization and the absence of sexual play were verified.

Literature Cited

Calhoun, J.

1950. The Study of Wild Animals Under Controlled Conditions. Annals N. Y. Acad. Sci., 51, Art. 6, 1113-1122.

Carpenter, C. R.

1940. A Field Study in Siam of the Behavior and Social Relations of the Gibbon, *Hylobates lar*. Comp. Psych. Monogr., 16, No. 5: 1-212.

1942. Societies of Monkeys and Apes. Biolog. Symposium, VIII: 177-204.

Colias, N. E.

1950. Social Life and the Individual among Vertebrate Animals. Annals N. Y. Acad. Sci., 51, Art. 6: 1074-1092.

Hediger, H.

1942. Wildtiere in Gefangenschaft. Benno Schwabe and Co. Verlag, Basel.

Richter, C. P.

1954. The Effects of Domestication and Selection on the Behavior of the Norway Rat. Jour. Nat'l. Cancer Inst., 15 (3): 727-738.

Riess, B. F.

1950. The Isolation of Factors of Learning and Native Behavior in Field and Laboratory Studies. Annals N. Y. Acad. Sci., 51, Art. 6: 1093-1102.

Turpin, F. R.

1771. Histoire civile et Naturelle du Royaume de Siam. Vol. 1: 305-309.

Yerkes, R. M.

1929. The Great Apes. Yale Univ. Press.

13

Thyroid Follicles in the Head Kidney of the Goldfish, *Carassius auratus* (Linnaeus)

WALTER CHAVIN

The American Museum of Natural History, New York City, and Wayne State University, Detroit, Michigan[1]

(Plate I)

SINCE the positive identification of the thyroid gland in the conger eel, *Conger conger*, by Baber in 1881, this endocrine organ has been identified and described in many species of teleosts. The teleostean gland corresponds closely to that of the Amphibia and amniotes in structure, but is anatomically diffuse and lacks a connective tissue capsule. The individual follicles are more or less scattered in the throat region, most commonly from the first to the fourth aortic arches (Maurer, 1886; Gudernatsch, 1911). In only three groups of teleosts, the swordfish *Xiphias gladius* (Addison & Richter, 1932), the parrotfishes *Pseudoscarus guacamaia, Sparisoma* sp. and *Scarus* sp. (Matthews, 1948), and the mormyrid *Gymnarchus niloticus* (Thomopoulos, 1950), are the follicles grouped into a compact gland with a connective tissue capsule, but even in these species a number of subsidiary follicles may occur outside the main thyroid mass. It is not surprising, therefore, that teleostean tumors of the thyroid usually consist of a tumor body in the throat with invasive growth occurring into the neighboring gill and pericardial regions (Schlumberger & Lucké, 1948; Schlumberger, 1955). Surprisingly, these tumors have only rarely been found to metastasize even when malignant (Nigrelli, 1952; Berg *et al*, 1953; Schlumberger, 1955). Recently, however, Baker *et al* (1955) reported aberrantly-located thyroid follicles and tumors in the platyfish, *Xiphophorus maculatus*. The present study describes the presence of thyroid follicles in the lymphoidal pronephric remnants, the head kidneys, of the goldfish, *Carassius auratus* (Linnaeus), and the responses of these atypically-located follicles to various physiological stimuli.

[1]Present address, Wayne State University.

MATERIALS AND METHODS

One hundred and fifty-three common, commercially hatched, xanthic goldfish were used in this study, the primary purpose of which concerned melanogenesis (Chavin, 1956). The fish were one to two inches in standard length and less than one year old. They were given dried food (Aronson, 1949) with an occasional supplement of living tubificid worms. During the course of experimental treatment the water temperature ranged from 30° C. in the summer to 22° C. in the winter.

Thirty normal fish were immersed in 0.7% sodium chloride in conditioned aquarium water, and two animals were sacrificed at intervals ranging from one hour to 19 days after initiation of treatment. Thirty control animals were sacrificed at similar intervals. Five hypophysectomized goldfish exposed to 0.7% saline for 14 days and eight hypophysectomized fish maintained under control conditions were also studied. Other experimental groups and controls are indicated in Table 1. The implanted tissues had been freshly removed from large goldfish 8-10″ in standard length. The animals were sacrificed after 10 days of treatment.

The fish were killed and fixed in Bouin's fluid, imbedded in paraffin and sectioned serially at seven microns. The sections were stained with hematoxylin-eosin or Masson's trichrome stain. The epithelial height of the follicles in the head kidney was not measured, because the changes in height were obvious and the follicles were usually too variable in number for such figures to be of statistical significance.

The author is deeply grateful to Dr. Charles M. Breder, Jr., of the American Museum of Natural History for his generosity in providing both laboratory quarters and materials. Thanks

TABLE 1. VARIATION IN THYROID EPITHELIAL HEIGHT IN THROAT AND HEAD KIDNEY REGIONS OF THE GOLDFISH, *Carassius auratus* L., WITH EXPERIMENTAL TREATMENT

Treatment	Number of Animals[1]	Number of Animals with Head Kidney Thyroid	Mean Thyroid (Throat) Epithelial Height[2] *Microns*	Reaction of Thyroid (Head Kidney) Epithelium[3]
Untreated	30N	23	2.29 ± 0.0096	Normal
	8H	7	1.12 ± 0.0124	Atrophy
Immersion in 0.7% Saline	30N	26	1.90 ± 0.0196[4]	Depressed
	5H	4	1.12 ± 0.0145	Atrophy
Pituitary Implant	4N	4	8.33 ± 0.1348	Hypertrophy
	4H	3	8.76 ± 0.1543	Hypertrophy
Optic Lobe Implant	4N	4	1.82 ± 0.0233	Depressed
	4H	2	1.16 ± 0.0207	Atrophy
Head Kidney Implant	4N	4	1.83 ± 0.0215	Depressed
	4H	4	1.15 ± 0.0182	Atrophy
Opisthonephros Implant	4N	4	1.89 ± 0.0240	Depressed
	4H	4	1.15 ± 0.0188	Atrophy
Isotonic Saline Vehicle (0.05 cc. daily)	4N	3	1.90 ± 0.0163	Depressed
	4H	3	1.13 ± 0.0165	Atrophy
ACTH (1 I.U. daily)	4N	4	1.74 ± 0.0192	Depressed
	4H	4	1.12 ± 0.0165	Atrophy
ACTH (1 I.U.) and TSH (0.44 USP unit daily)	4N	3	6.03 ± 0.1045	Hypertrophy
	4H	4	5.90 ± 0.0872	Hypertrophy
ACTH (1 I.U.) and Intermedin (133.3 Phoxinus units daily)	4N	3	1.86 ± 0.0213	Depressed
	4H	4	1.10 ± 0.0192	Atrophy
Intermedin (133.3 Phoxinus units daily)	4N	4	1.89 ± 0.0245	Depressed
	4H	3	1.16 ± 0.0156	Atrophy
Adrenal Cortical Extract (=2.5 g. beef cortical tissue daily)	4N	3	1.79 ± 0.0211	Depressed
	4H	3	1.12 ± 0.0159	Atrophy

[1]N: Normal goldfish; H: Hypophysectomized goldfish.

[2]Based upon data presented by Chavin (1956).

[3]For definition of terminology see text.

[4]The first statistically significant depression in epithelial height occurs two days after saline immersion to the figure indicated. The mean height gradually increases over a period of seven days to the normal range on the ninth day.

are also due Dr. Irby Bunding of the Armour Laboratories, Chicago, for supplying ACTH Lot 146-RS3 and ACTH-TSH Lot 84-5U2S2; Dr. Henri Choay of the Laboratoire Choay, Paris, for the intermedin; and Dr. David Klein of the Wilson Company, Chicago, for the adrenal cortical extract.

RESULTS

In 85% of the 153 goldfish examined, irregularly round to oval follicles filled with colloid were found in the lymphoid tissue of the head kidney. The number of follicles varied from two to several hundred per animal, and was not correlated with experimental treatment, sex or level of gonadal maturity. The scattered and separate thyroid follicles of the goldfish were found in the throat, clustered for the most part about the origin of the first and second afferent branchial arteries (Chavin, 1956). In 77% of the control animals, follicles were present in the head kidney. Such follicles closely resembled those of the thyroid in histological structure, but were unencapsulated. The slightly basophilic cuboidal epithelial cells varied somewhat in size and shape, and their lateral cytoplasmic limits were indistinct (Plate I, Fig. 1). Nuclear shape was variable. The follicular colloid was homogeneous and stained with eosin and fast green.

Follicles in the head kidney occurred in 87% of the experimental fish. The number of animals containing such follicles in each group is indicated in Table 1. The epithelial heights of these follicles varied with the experimental procedure

to which the animals were subjected, Table 1. Immersion in hypertonic saline produced a temporary depression of epithelial cell height after two days. At this time, there was increased cytoplasmic basophilia with little or no vacuolation. The nuclei were oval and deeply basophilic (Plate I, Fig. 2). Epithelial height and cell morphology returned to the normal state by the ninth day of treatment. Hypophysectomy produced atrophy of the follicular epithelium. The basophilic nuclei appeared as flattened bulges in the attenuate cells, (Plate I, Fig. 3). The epithelium of hypophysectomized fish was not altered by the administration of isotonic saline, ACTH, optic lobe of the brain, head kidney or opisthonephros or by saline immersion. These treatments, however, depressed the follicular epithelial height in normal fish. In both the hypophysectomized and normal animals, implantation of pituitary tissue or injection of purified ACTH with TSH elicited epithelial hypertrophy. The now deeply basophilic and columnar epithelial cells contained many coarse granules and several large chromophobic vacuoles (Plate I, Fig. 4). The large, vesicular nuclei each contained a prominent nucleolus. The follicular colloid was usually vacuolated.

Discussion

The histologic findings indicate that the follicles in the head kidney of the goldfish are structurally similar to the thyroid tissue in the throat. In addition, the responses of the head kidney follicles to various physiologic alterations are identical with those of the throat thyroid. It is reasonable, therefore, to conclude that the above-described structures in the head kidney are functional thyroid follicles.

The origin of the thyroid tissue in the head kidney is an interesting problem. The presence of follicles in a lymphoid organ a distance from the normally located gland suggests their metastatic origin. As the first lymphoid organ in the venous drainage of the head, the head kidney is a filter in which the blood-borne cells may lodge. In addition, the absence of the connective tissue membrane (normally found around each thyroid follicle) about the head kidney follicles may be an indication of their migratory origin. On the other hand, the ontogeny of the thyroid suggests an alternate means by which the follicles may appear in the head kidney. The teleost thyroid originates as a median ventral outgrowth from the pharygeal floor in the region of the second pair of gill pouches. This diverticulum grows posteriorly and eventually comes to lie anterior to the heart, but with further development it spreads anteriorly until the adult condition is attained (Maurer, 1886; Gudernatsch, 1911). If some of the cellular precursors of the thyroid deviate slightly to proliferate in a dorsal direction, the eventual appearance of thyroid follicles in the head kidney may result. In the discussion following the paper of Gorbman (1955), it was suggested that the atypically-located thyroid tissue in the platyfish is neither of teratological origin nor derived from mesonephric blastema. This suggestion is supported by the results of the present study, for the majority of goldfish, normal in all observed histological aspects, contained thyroid tissue a considerable distance from the normal locus in material derived from the pronephros. Thus the concept of metastatic or ontogenetic origin of the aberrantly located thyroid follicles, presented by Baker *et al* (1955) and Gorbman (1955), is favored at this time, although no direct evidence is on hand to support or deny any suggestion as to the origin of the described follicles.

Cellular proliferation may not always remain under the control of the mechanisms normally directing growth in fishes or other organisms. Such independent or random multiplication of cells which is later controlled, not at the cellular but at the tissue level, has been suggested to be part of a normal development pattern (Breder, 1952). The subsequent integration of the results of such atypical growth processes into the economy of the goldfish is indicated by the follicular organization of thyroid cells in the head kidney and the similar reaction of these and normally located cells to various stimuli. Because of this developmental pattern, the goldfish thyroid may prove to be an invaluable tool in the morphological, physiological and biochemical study of normal and atypical growth processes.

Summary

Thyroid follicles have been found in the lymphoidal pronephric remnants, the head kidneys, in 85% of 153 goldfish studied microscopically. These follicles are similar to those of the normally-located thyroid in structure and in reaction to various stimuli (hypophysectomy; saline immersion; implantation of pituitary, brain, head kidney or opisthonephros; injection of isotonic saline, ACTH, ACTH and TSH, ACTH and intermedin, intermedin, or adrenal cortical extract).

The origin of the aberrantly-located thyroid tissue is suggested to be a metastatic or ontogenetic phenomenon.

The utility of the goldfish thyroid in the study of normal and atypical growth processes is indicated.

Literature Cited

Addison, W. H. F., & M. N. Richter
1932. A note on the thyroid gland of the swordfish, *(Xiphias gladius)*. Biol. Bull., 62: 472-476.

Aronson, L. R.
1949. An analysis of reproductive behavior in the mouthbreeding cichlid fish, *Tilapia macrocephala* (Bleeker). Zoologica, 34: 33-42.

Baber, S. C.
1881. Researches on the minute structure of the thyroid gland. Phil. Trans. Roy Soc. London, 172: 577.

Baker, K. F., O. Berg, R. F. Nigrelli, A. Gorbman & M. Gordon
1955. Functional thyroid tumors in kidneys of platyfish. Cancer Res., 15: 118-123.

Berg, O., M. Edgar & M. Gordon
1953. Progressive growth stages in the development of spontaneous thyroid tumors in inbred swordtails, *Xiphophorus montezumae*. Cancer Res., 13: 1-8.

Breder, C. M., Jr.
1952. The problem of directives to cellular proliferation as illustrated by ontogenetic processes in certain fishes. Growth, 16: 189-198.

Chavin, W.
1956. Pituitary-adrenal control of melanization in xanthic goldfish, *Carassius auratus* L. J. Exp. Zool., 133: 1-46.

Gorbman, A.
1955. Phylogenetic and developmental aspects of the thyroid. Brookhaven Symp. Biol., 7: 3-8.

Gudernatsch, J. F.
1911. The thyroid gland of teleosts. J. Morph., 21: 709-782.

Matthews, S. A.
1948. The thyroid gland of the Bermuda parrotfish, *Pseudoscarus guacamaia*. Anat. Rec., 101: 251-263.

Maurer, F.
1886. Schilddruse and Thymus der Teleostier. Morph. Jb., 11: 129-175.

Nigrelli, R. F.
1952. Spontaneous neoplasms in fishes. VI. Thyroid tumors in marine fishes. Zoologica, 37: 185-189.

Schlumberger, H. G.
1955. Spontaneous hyperplasia and neoplasia in the thyroid of animals. Brookhaven Symp. Biol., 7: 169-191.

Schlumberger, H. G. & B. Lucké
1948. Tumors of fishes, amphibians, and reptiles. Cancer Res., 8: 657-754.

Thomopoulos, T.
1910. Sur la thyroide de *Gymnarchus niloticus* Cuv. (Téléostéen Mormyridae). Bull. Soc. Zool., France, 71: 293-306.

EXPLANATION OF THE PLATE

Plate I

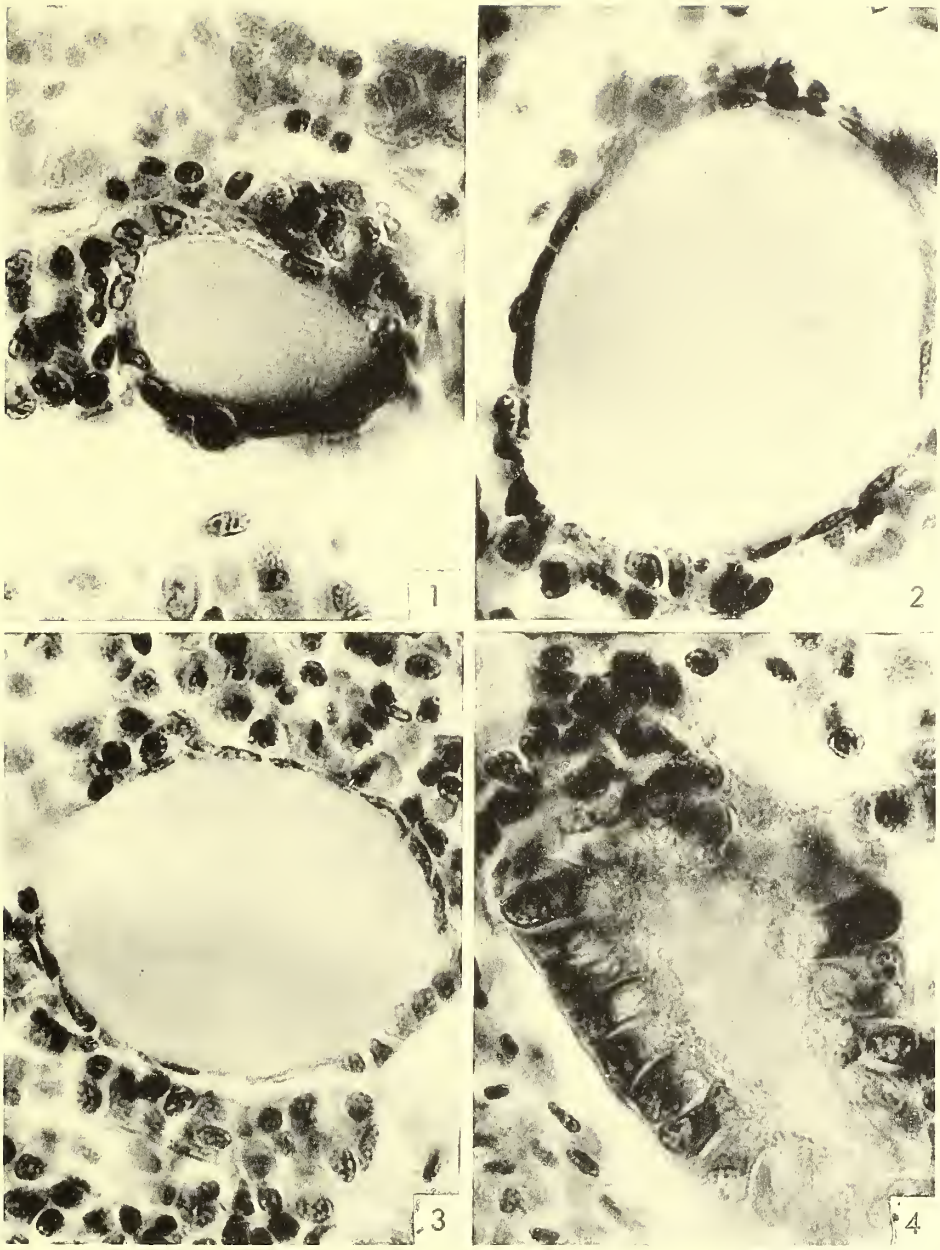
Thyroid follicles in the head kidney of the goldfish, *Carassius auratus* (L.) Hematoxylin-eosin. 980×.

Fig. 1. Control goldfish.

Fig. 2. Depressed epithelial height in a normal goldfish immersed in 0.7% saline for two days.

Fig. 3. Atrophy of epithelium in a three-week postoperative, hypophysectomized goldfish.

Fig. 4. Epithelial hypertrophy in a hypophysectomized goldfish after administration of ACTH-TSH.



THYROID FOLLICLES IN THE HEAD KIDNEY OF THE GOLDFISH, CARASSIUS AURATUS (LINNAEUS)

14

Ecology, Behavior and Population Dynamics of the Wyoming or Rocky Mountain Moose, *Alces alces shirasi*

Rollin H. Denniston, II

University of Wyoming, Laramie, Wyoming

(Text-figures 1 & 2)

I. Ecology

WITHIN recent years the Wyoming moose, *Alces alces shirasi,* has markedly increased its numbers and range. A study of its ecology, behavior and population dynamics, besides its intrinsic interest, may have some practical application in terms of the light it throws on reasons for the subspecies' success.

The studies reported here are the results of work during the late springs, summers and early autumns of 1947, 1948, 1949, 1950 and 1952, and the winter of 1947-48. This work was supported by the New York Zoological Society and the University of Wyoming. The author is indebted to these institutions and to numerous individuals for help and information. Mr. James Simon, director of the Jackson Hole Research Station at the time of these observations, gave constant friendly interest and assistance without which the work would have been impossible.

The method used was close personal observation, aided by a pair of 10 × 40 binoculars, a 20-power scope and still and motion picture cameras. Moose were approached on foot, on horseback or in a jeep. On-the-spot notes were made of all observations except those at night. Nocturnal notes were recorded within an hour of the end of the observation period. Approximately eighteen hundred hours were spent in actual observation. In the census and distribution work, personal observation was supplemented by interviews with and questionnaires from personnel of Federal and State agencies, as well as numerous proprietors of guest ranches, who have been most helpful as a group.

Moose and Their Environment

The moose is not only a solitary deer, but is the deer most dependent on water for its livelihood. Much of its summer food is aquatic vegetation and willow browse in the littoral zone of both streams and ponds. In addition to supplying food, ponds and lakes offer protection from insect pests. Non-feeding moose are often found partially submerged at the height of the fly and mosquito seasons. In 1950 A. D. Cannavina, at that time Assistant Chief Ranger in Glacier National Park, called my attention to the bright green color of the hair between the moose's toes. The pigment was extracted by a method suggested by L. W. Roberts (1953) and is believed to be chlorophyll. Possibly moose acquire this pigment when pond bottom algae are squeezed between their splayed toes. This might explain why moose show the coloring, while other large members of the deer family sharing their range do not. In addition to water and suitable food, moose seem to require a low average daily temperature. Thus they are found at relatively high elevations at the latitude of Wyoming, but not so high as to remove them from the proximtiy of small bodies of water. Merrill (1916) says "The moose swims well, but not rapidly. Like the caribou, his shoulders are well above the water when swimming. Stone relates how an Alaska bull two or three years old swam eight miles without showing evidence of exhaustion." Many of the ponds utilized by moose are beaver ponds. This is true to such an extent that in a good part of its range the moose is dependent on the beaver. Moose and beaver areas of distribution show a significant overlap. Hosley (1949) refers briefly to beaver-moose relationships, indicating a possible adverse effect of over-flooding of poplar stands on moose food. We have never observed such effects in the Rocky Mountain area.

Foods and Feeding

The moose food during the observation periods consisted principally of three types: willow,

algae and pond weeds. There was an abundant growth of the last two in the ponds west of the town of Moose, Wyoming, and several hundred hours of close observation were made of moose grazing these plants.

A note made on August 5: ". . . female entered ponds, leaving calf sampling sedge, willow and aspen. Female ate algae for one hour, keeping head under 10 to 25 seconds; ears were always above the water and erect, and twitching off flies. Then she went down on her knees and submerged completely except for her hump. She took large mouthfuls of algae, and when she came up she held her ears 'lop-eared.' She now stayed under about 30 to 45 seconds at a time." There are three points worthy of comment here: (1), the persistent feeding on algae; (2), the sampling, intermittent feeding of the calf, which was typically different from the consistent, steady feeding of the adult; and (3), the position of the ears, which was later observed whenever a moose submerged these organs and which suggested the possibility that the external ear might be used as a cover to prevent water from running into the external auditory meatus and canal. This seemed particularly likely as moose were observed to lower their ears before submerging them. This response was so constant in this stimulus situation as to resemble an anticipating reflex to submersion. Similar responses are known in other aquatic mammals. Algae seemed to be a preferred food, even in the presence of an abundant supply of a reputed favorite, the water lily. Moose were never observed to take water lily stems or leaves, although they grazed algae at the border of a large clump of them. Isolated instances of adult moose browsing on other plants such as sedges, sage brush and aspen were noted from time to time. Although many observations have been made of moose feeding on roots of the pond lily, *Nymphea*, only one instance of moose eating other parts of these plants has been noted in five summers of observation. When moose eat the lily roots, the plants drift to shore and show no evidence of tooth marks on other parts.

The sampling proclivities of the first-year calves are further illustrated by the following note made on August 14: ". . . saw female and calf at east end of ponds. Cow was grazing algae, calf playing on edge of water at first, then started eating yellowed leaves and fruits of cow parsnip, *Heracleum*, then alternated with nibbles from meadow rue, *Thalicutrum*, and willow, *Salix*."

In winter, moose have two obvious food sources. The first is the twigs of willow and aspen along the open river bottoms. Moose are found in their greatest aggregations in winter in such locations. It is also this winter browse which seems to represent a limiting factor in the moose habitat. Evidence of over-browsing has been observed in many areas of the range. A second source of winter food is hay. This may be either some rancher's hay spread in a corral for cattle or stacked in a field, or the hay distributed by the State elk-feeding stations. When the snow starts to melt in late April, moose gather in numbers on the first patches of grass to show through. They kneel and graze in this position for as long as two hours at a time. Shortly after this grazing period they may be found browsing sage in the middle of the day.

Where there are several kinds of willow, moose often show a preference. In the Roaring Fork area northeast of Yellowstone Park, a short, yellow-stemmed variety, *Salix wolfii*, is almost untouched, while a tall, red-stemmed willow, *Salix geyeriana*, growing intermixed with it, is over-browsed.

Where moose concentrate in winter, as in certain eastern areas of Yellowstone Park and the upper Wind River near Du Bois, Wyoming, very obvious over-browsing has affected the willow. This over-browse is made more evident by exclusion plots established in these areas by Federal and State authorities. The Yellowstone Park area is especially dramatic. Within the exclusion fence there is a dense growth of willow, while in the immediate neighborhood hardly a single willow is to be seen. As Hosley (1949) points out in his excellent survey of moose literature, the Rocky Mountain moose is much more dependent on willow than its eastern relatives. Willow and aspen make up the major part of its diet winter and summer. In summer it takes mainly the leaves but in winter it uses twigs of both genera up to a half-inch in diameter. Sage, *Artemesia*, forms a possibly unexpected part of the Wyoming moose's diet during late spring. Although these animals take conifer tips occasionally, mostly fir, they do so to a much lesser extent than in the east, where Aldous & Krefting (1946) found balsam to comprise approximately 15 per cent. of the diet. The absence of birch, *Betula*, from the Rocky Mountains excludes from local moose diet a plant which is a favorite in other areas.

In general, moose seem to have very definite preferences for certain food plants but nevertheless are able to exist on a remarkably wide variety of browse if they have to.

Locomotion and Other Characteristic Activities

Moose have as characteristic gaits a fast walk, a very smooth trot and a rather ungainly crashing canter. The first two are the modes of almost all movements by adults. Yearlings seem

to canter much more, even when not running with an adult. From the circumstances in which the young canter, the observer is led to consider the possibility that this may represent a form of play. Several checks on the speed of moose trotting gave an average of slightly more than 20 miles an hour.

The moose is an excellent swimmer. It crosses turbulent rivers such as the Snake and swims far out in large lakes quite frequently. So confident does it seem to be of its swimming ability that several moose drown in the Snake river each winter when they break through thin ice and are unable to climb out on the thicker shore ice.

Moose do considerable feeding underwater—that is, they gather food underwater and swallow it on emerging. Periods of submersion are quite constant and usually vary between 20 and 60 seconds, depending partly on the depth of the water and the length of time the moose has been feeding. Intervals above water are usually shorter than those below when the moose is actively feeding.

In common with other herbivores, moose frequent natural salt licks and salt blocks put out for stock. One heavily-utilized lick lies at the western end of the "Thoroughfare" just southeast of Yellowstone Park.

Beds and Forms

Everywhere that moose were encountered more than once, I found several beds. These were simply depressions where the grass was matted down. In the fall one "wallow" was found similar to those described by Seton (1928). This wallow smelled of urine and feces. Several beds were under willows, and the willows showed low browsing in such places. In one instance I observed a young bull browsing while lying down for about twenty minutes at a distance of 25 paces from me.

Adaptation to Human Structures and Influences

One reason for the success of moose in the Rocky Mountain area may well be their ability to adapt to human influences. In Yellowstone Park and those areas of what is now the Grand Teton Park where moose are in almost daily contact with human beings during the tourist season, the animals soon learn to continue their feeding and other activities apparently undisturbed. Such tolerance does have it limits, however. For example, in 1947 and 1948, 23 different moose were seen along a four-mile stretch of U. S. highway 287 just west of Togwotee Pass. Extensive road construction was undertaken in this area early the following year, and frequent checks showed only three moose at this time. Where moose have less frequent contact with human beings they show more marked avoidance. Under the latter conditions their flight distance averages between 90 and 140 yards, compared with 20 to 50 yards where frequent contact has brought about adaptation.

During the winter, moose utilize two sorts of man-provided facilities and often become nuisances in the process. The first is the cleared highway. Moose get on to these and block traffic, failing to use the "moose turnoffs" plowed out by the highway department. They often trot ahead of an automobile for a mile or more and then stop and refuse to move. Attempts to get them to do so result in mutual irritation so that either the moose may charge the car or the driver may ram the moose. Close to one-tenth of one per cent. of the Jackson Hole moose population is killed on highways each winter.

Another facility used by moose in winter is stored hay. Ranchers often complain of moose taking hay from field stacks. Moose also come into feeding corrals and feed side by side with cattle. In the smaller elk-feeding stations, such as that at Black Rock, Wyoming, moose come in for the hay put out for the elk. Occasionally they even enter a hay barn. Under any of these circumstances they are in an approach-avoidance conflict situation. It is consequently at these times that most moose "attacks" on human beings are recorded.

Predators

Man is the only significant predator affecting the moose. Accounts of other predation are so rare as to be memorable. In the vicinity of Glacier National Park there have been two reports of bear molesting moose in the last twenty years. The calf moose would be vulnerable to coyotes if it were not for the constant close protection afforded the calf by its mother.

Parasites

Ectoparasites

During late July and August moose exhibit behavior which might indicate that they are being disturbed by ectoparasites. The ear-twiching, skin-shaking activity is apparently often a response to stable flies, *Stomoxys calcitrans*. Although an observer may often be annoyed by mosquitoes while within 40 feet of a moose, the latter rarely seems to respond to these pests. In the late winter and early spring moose are often heavily infected with the "winter tick" *Dermacentor albipectus*. If other weakening conditions are present, these ticks may be a contributing cause of "winter kills."

Endoparasites

Since the author was aware of no studies on the endoparasites of the Shiras moose, he undertook an examination of the viscera of two moose killed by hunters in September, 1947. A heavy infestation in the upper small intestine of both specimens with *Nematodirella longispiculata longispiculata* was found. A few individuals of *Trichiuris* (probably closely related to *Trichiuris ovis*) were present in the viscera of one animal. Identification was made by R. Honess of the Wyoming Game and Fish Department. Shiras moose do not aggregate to any extent and the danger of transmission of internal parasites in food and drink is therefore apparently small.

Life History

Breeding and Gestation

Mating of moose in Wyoming takes place from mid-September through early November. The young are born in late April and May after a gestation period of approximately 240 days. This is similar to the duration of gestation quoted from Lydekker by Asdell (1946) for the European moose. Asdell further quotes Lydekker and, for the Alaskan moose, Cahalane, to the effect that two calves is the usual number at birth. With regard to this, Merrill (1916) says (p. 83), "A cow moose usually has one or two calves at a time—very rarely three." Adolph Murie (1934) states that, "From my observations, it seems that cows have, as a rule, but one calf each. Forty-four cows were seen, in 1929, followed by one calf each, and only one cow with twins. Schierbeck states that only twenty-one, or eight per cent. of the 252 pregnant cows carried twins." Hosley (1949) suggests that the ratio of twin to single births may vary with range condition. Our observations would tend to confirm this. In the Jackson Hole area in 1948, 4 cows were observed to be accompanied by twin calves and 22 by single calves, or about 27 per cent. twin births. These data, it should be noted, were selected to preclude the possibility of duplicate counts. Reproductive success of the moose is indicated by the percentage of all cows that were accompanied by at least one calf. A. Murie (*op. cit.*) found approximately 50 per cent. of all cows to bear a calf each year. In 1948 we observed 26 cows with calf and 21 dry ones. Range and browse were good in Jackson Hole that year.

In 1948 we were able to follow the development of an orphaned calf moose from the time she was two weeks old until she died at the age of seven months. For the first 12 weeks it was possible to weigh, measure and photograph the calf. This moose calf came in for feeding of condensed milk and water which she received at four-hour intervals throughout the 24 for the first three weeks, and then drank in increasing amounts at less frequent intervals. At five weeks of age she was consuming two quarts at each of three daily feedings. At one month she began browsing willow. During this period she was completely unrestrained except for the time of her weekly weighing and measuring.

Because of the constant close protection afforded her calf by the cow, the weights and measurements in Table 1 are practically unique. Only six items are recorded under chest girth, for two reasons. The calf was measured while she was drinking her milk and she often finished the bottle before this last measurement in the series could be made; also, she grew more restless while the tape was passed around her chest than during any other measurement. These data are in agreement with those of Kellum as quoted by Hosley (1949), but are somewhat lower than some earlier estimates. The increase in face length between the seventh and ninth week marks a turning point in heterogonic growth from the typical short-faced calf to the more adult type. This sudden change has been confirmed by field observation and should be useful for the experienced observer in determining whether a calf is more or less than two months of age. At three months the calf's coat color starts to change from a fuscous red-brown to the typical adult agouti color.

Suckling is very difficult to observe, since it usually takes place in secluded locations. The

Table 1. Growth of a Moose Calf. (All Linear Measurement in Millimeters)

Weeks of age	2	3	4	5	6	7	9	11	13
Shoulder height	813	838	864	876	876	940	1092	1118	1143
Total length	914	991	1067	1092	1181	1181	1346	1448	1626
Occiput-muzzle	279	279	305	305	305	305	368	381	381
Ear length	127	152	152	152	152	178	190	203	203
Chest girth			686	737	813	864		965	1072
Weight in pounds	39	46	52	57	72	84	110	128	

moose udder is not large and is located high between the thighs. The calf starts supplementing its milk diet with browse at about one month of age. My field notes for August 23 describe one of the rare occasions that we have observed suckling: "A buckskin colored cow walked by us into the lake and grazed submerged vegetation for more than an hour. She then called softly. A calf on the opposite shore replied, and ran to meet the cow as she emerged from the water. The calf nursed for three and one-half minutes, butting vigorously at the udder all the while. Then the female and calf disappeared into the woods."

Weaning seems to occur early in September, although the calf remains close to its mother for some months more. By October the calf is more than half as tall as its mother and its coat has taken on an adult shade.

Relative development of the antlers is almost the only way of estimating the age of moose older than yearlings. The yearling has knobbed spikes not much longer than the ears. The next year the antlers are about twice the length of the ear and show a bilobed condition distally. From the third year on, a palm appears and grows, probably reaching a maximum between seven and ten years. Evidence of tooth wear seems to indicate that very old bulls have lighter antlers, possibly with more points. Also correlated with age is the time of shedding of velvet. The author has observed old bulls with whitened antlers in early September, at which time the smaller-antlered bulls are still in velvet. There is a corresponding difference in the time of dropping the antlers, the older bulls losing theirs first.

II. Behavior

Unlike the red deer studied by Darling (1937) and the elk or wapiti as studied by O. Murie (1951), the moose is almost unique among deer in being a solitary form without definite herd organization. This trait has several possible causes and several probable results of interest to the student of animal behavior. It means that the bull moose, unlike the bull elk, collects no "harem;" that the cow moose is solely responsible for the care of the calf without being able to turn it over to a nursery herd, and that the problems of "peck-order," leadership and social hierarchy would appear in their most elementary and primitive state in moose. In an attempt to gain some understanding of the ways by which a moose meets the exigencies of its environment alone and successfully, the author has spent approximately eighteen hundred hours in the actual observation of moose behavior.

Cyclic Phenomena

Diurnal Cycles

During the summer months moose become active at about 4:00 or 4:30 A. M., or about an hour before dawn, moving from aspen or conifer thickets where they have spent the night into more open areas and ponds. They feed actively until the middle of the morning, or for about five hours. At about 9:30 A. M. they retire to beds or forms usually close to ponds and often in willow thickets. Here they may continue to browse occasionally while lying down, moving only if disturbed. Calf moose often doze at this time. In mid-afternoon the moose re-enter ponds or begin to browse once more. At full dark they may leave the places where they have been feeding and move into thickets, sometimes at a distance of more than a mile. A cow with a calf less than six weeks of age does not move as far at night and may simply use the midday rest area again. As day length decreases, the inception of activity is delayed and the midday "siesta" is shortened. This daily movement of moose is quite similar to that for elk but somewhat more restricted.

Annual Cycle

Moose spend their winters where food in the form of willow or aspen browse or hay is available and where the snow is not too deep. Windswept stretches on the river bottoms fill these requirements, as do certain south-facing slopes. The available niches are far fewer in winter so that at this time moose are found in their greatest aggregations on the relatively few favorable feeding areas. On a census flight in March, 1948, in Jackson Hole, we counted 65 moose. They were grouped along the Snake river bottoms as follows: two, four, six and five per group in one feeding area; two singles and a group of four on one aspen slope; two groups of two, two groups of three and one group of five in another area; two, five, three and one around a series of beaver ponds. This gives an average group size of two and eight-tenths individuals for the 23 groups. Five areas were observed with an average of four and eight-tenths groups per area. Where there is one rather isolated favorable area, 20 to 30 moose may often be found in it in the winter. As mentioned before, it is at this time that the moose come into closest contact with human beings.

Brown & Simon (1947) point out that, as is the case with other deer, winter is the critical period for moose. They say that those acquainted with the haystack manners of moose favor this species over the elk, because it feeds more cleanly, without scattering the hay over

the ground or trampling and mixing it with filth. Many moose, unlike elk, do not come in to take man-provided food every day, even though it is readily available. Of 37 moose wintering near Black Rock ranger station, only 22 were known to come in for hay, and those not constantly. These authors comment further on the status of orphan calves in winter. These were observed lying alone and in the same position for many days, until it seemed that they would never rise to browse willow again. However, close examination of their bed grounds often gave evidence that the young had been actively feeding, to such an extent that dense willow thickets were reduced to mere stumps.

This observation suggests caution in the interpretation of the significance of local areas of apparent over-browse. Brown & Simon mention that in the spring moose move onto sage flats and browse sage to a considerable extent. This plant has been observed by us to be a part of moose diet at other times of the year as well.

Still later in the spring the bulls and some barren cows follow the receding snow line to higher and higher elevations in a sort of altitudinal migration. Cows with calf apparently do not go so far or so high, but move back into very secluded areas for calving which takes place usually in May. Yearling calves usually remain near the cow throughout their second summer. Such cows with first-year calves move very little unless disturbed and may be observed in the same area of a few acres throughout the summer. These summer grounds are not held to the exclusion of other moose, which may drift in and out, forming loose temporary groupings of up to ten animals. During the summer months at least some bulls and dry cows wander as much as 30 or 40 miles, often moving across passes from one drainage to another. In late August and early September the older bulls attach themselves to cow-calf groups. At this time a typical group consists of the mature bull and cow and either a first year calf or a yearling calf, or sometimes both. My field notes describe the typical behavior of such a group (September 16, when the hunting season had been open about ten days): "At the mouth of Spread Creek where it enters the Snake, I noted a cow which stood perfectly still and looked in my direction for three or four minutes. A four-year-old bull, quite black, with white antlers moved out from behind willow bush between me and the cow. As the cow turned and entered the Snake, a this year's calf and a yearling got up from where she had been standing and followed her across the river. The bull followed the cow and calves." I watched this group for three more hours and saw it again the next day. There was no sign of sexual behavior during this time. The bull stays with the cow (and calf or calves) for ten days to two weeks. Apparently he does not attempt to drive the calves away.

(September 17, Pelican Creek): "12:30 P.M. –Three-year-old male, female and this year's calf. The bull followed the cow quite closely as she moved, sniffing at her hindquarters but making no actual contact. At 5:45 P.M. still in the same area, the bull swings his head from side to side, beating his antlers against the brush, and runs around and around in a circle." (Next day, same place): "10:30 A.M.–The same group as yesterday moves slowly into sight, browsing on willow. Then the male moves out onto a sedge flat followed by the calf, which touches noses with him and stays very close to him as he crosses the stream. The cow crosses about fifteen minutes later and runs in a tight circle. The male now spends about one half hour rubbing his antlers on a spruce. Erection of the penis was intermittently apparent at this time."

Darling (1937) has noted the association of antler rubbing with erection and has referred to the situation as "masturbatory." It seems to this observer that the antler rubbing may be one stimulus-producing response in the sex drive-response complex, while erection represents a later similar response. In other words, these may be two results of the same complex of causes, and not have a simple cause and effect relationship between themselves, (Denniston, 1954).

Actual breeding apparently follows several days or even weeks of association of the bull with the cow. (September 23–Pelican Creek): "6:00 A.M.–One big bull with white-polished antler tips chases two younger bulls away from his wallow. He then squats, urinates in it, and paws the ground. He chases away a younger bull which comes up to paw at the edge of the wallow, then lies down and rolls in the wallow. He approaches a female standing within five yards of the wallow, and sniffs her hindquarters. Next the bull throws his head back, opens his mouth, and retracts his upper lip, holding this posture for three minutes. (This posture is exactly like that of a bull elk when bugling, but no sound was discernable to the observer at a distance of about sixty yards.) The bull now approaches the front of the female and shoulders her toward the wallow. At 7:30 A.M. he mounts her and thrusts four or five times. The female runs out from under him and runs in a circle, returning to the wallow. The bull mounts again and stays mounted for three minutes. They move out of sight of the observer behind dense willow, but the male's head and shoulders appear above them as he apparently mounts the cow a third time."

Wallow production is probably closer to the consummatory response in the time series of stimulus-producing responses in this sexual situation, and therefore possibly more of an essential component in the drive-arousing response series. Moose have been observed to breed in shallow ponds, but whenever circumstances permitted, a wallow was observed on the bank.

Individual Behavior

Development of Behavior

Since it was rather rare to see two calves together, most of the apparent play observed was solitary gamboling and exploration. The author is well aware of the potential pitfalls in characterizing a given activity as play, which have been pointed out by Beach (1945). Nevertheless, the term perhaps serves as a useful descriptive label for certain aspects of juvenile behavior which have no apparent immediate utility.

The orphan calf moose observed regularly during the summer of 1948 afforded a unique opportunity for the study of the play patterns of *Alces*. For the first month of her life no play-like activity was apparent. At about one month of age she would push back when pushed gently on the forehead and would push gently at the observer on her own initiative. When two months old she would "play" with a lawn sprinkler, striking at it with her forefeet, biting it and running back and forth through the spray. A little later she would run around and around any fair-sized structure such as a shed or barn. This running in circles was also shown by mature females in situations of a sexual nature. At about the same age she would strike at the whiffletree of a carriage with her head or forefeet, dodging back quickly to avoid the rebound of the other end. A little later she had to be confined to a corral, as she would repeatedly run and butt at some of the smaller children on the ranch where she was fed. At no time during such activities, or even when being restrained for her weekly weighing and measuring, did she show any component of the adult "rage" pattern.

Several times in late August three-months-old calves were observed to beat with their heads at low brush, a pattern otherwise shown by males in rut.

Play-like activities by more adult moose were noted. A young barren cow feeding in the same large pond as a mature bull reared several times and splashed down, then cantered through the water past the bull, sending great sheets of water over him. Then she turned around and bleated softly. The bull moved slowly out of the pond, in the opposite direction. A sixteen-months-old bull with antlers still in velvet was observed to run several times towards a small willow clump and veer off just as he reached it. After several such runs he put one antler under a small bunch of twigs and tossed it, repeating this action several times.

Other differences between juvenile and adult behavior were somewhat similar to those seen in many species. A two-months-old calf was much more restless than the cow, moving about constantly while the latter stayed in one place or moved along slowly, browsing as she went. The calf showed apparent curiosity or interest by interruption of other activities and orientation of the receptors to many natural sounds to which its mother gave no outward sign of attention. The calf was much more vocal than its mother, often emitting a two-note blatt repeatedly. The calf fed much more intermittently than did its mother and sampled many things not included in the adult diet. (See section on Foods and Feeding.)

Behavior Associated with Aggression

Just as it is impossible to state definitely that certain behavior represents play, so, technically, one cannot label another type of behavior as "anger." There are nevertheless changes in moose behavior associated with certain situations that impress the observer with the necessity for cautious procedure on his part. In other words, although the laboratory psychologist might object to the field observer's characterization of a moose's behavior as anger, the latter is often in no doubt that he had better climb a tree when he sees such behavior. This "rage" pattern of moose was most frequently observed in cows with young calves at the side. It was not shown by bulls in rut except when driving other bulls from a wallow. It was seen in many moose cornered by snow in winter. In this pattern the mane is erected, the ears flattened back against the neck, the lips retracted, the tongue protruded and curled up over the upper lip and nose and repeatedly darted in and out, licking the upper lip. The animal usually rears on its hind legs, pawing the air with the forefeet, if the stimulus object is at a distance of less than about forty feet. In a more distant encounter the animal usually lowers the head while showing the rest of the pattern. That this pattern may represent a bluff or threat is apparent from several encounters the author has had with moose. A representative experience is quoted from a note made in the field: "July 22, Canyon southwest of Brooks Lake. Surprised a cow moose and small calf at less than forty feet as I topped a rise on the trail. The horse shied and trembled as the cow whirled toward me, pivoting on her hind feet. Her mane was erected and her ears flattened back. She licked her nose very rapidly.

I wheeled the horse, tied him out of sight, and returned on foot. The moose were feeding quietly on willow at the bottom of the canyon about 100 feet below me. They showed no sign of emotional disturbance." That this behavior pattern serves the function of warning animals encountered by moose so that actual conflict seldom ensues, and that moose will not charge until ample warning of this sort has been given, is borne out by a statement made by D. E. Hess, Supervisory Park Ranger of Yellowstone National Park (1951). Speaking of moose gathered about a salt block in the autumn, he says he was able to approach within eight to ten feet before the warning signs mentioned above were followed by a slow advance. He says further that every moose he encountered face to face or at a distance, in timber or open country, yielded the trail or gave a choice of going around it. The moose would not turn tail, but neither would it advance.

"Attention" Attitude

Another recognizable attitude or posture was one that impressed the observer as indicating something like attention or interest. By these terms we wish to imply no hypothetical autonomous central process of Hebb (1949), but rather a notable orientation of the receptors toward the source of an unusual change in the environment. In this condition the head is raised, the ears erected and turned forward, the nostrils dilated; "testing" type motions of the head were also in evidence. Further remarks on this posture will be found below under "Moose-Man Reactions."

Social Behavior

Intra-specific Relations

A. Mother-Young Relations

Because of the solitary nature of the moose, the juvenile individual is particularly dependent on its mother not only for sustenance but also for a sort of psychological guidance and protection. The young suckle for about two months, weaning being a gradual process. During the latter part of this time the calf samples many types of vegetation, including several not utilized by the adult, to an ever increasing extent. The young are reported to remain with their mother through their second year. Although close associations of mature females and second year calves were often observed, there was no way of proving the familial relationship. The female exerted a guardianship over her calf which varied from individual to individual and from time to time. The protection ranged from threats, if the observer approached too closely or surprised the animals, to apparent temporary desertion. The calf played and explored fairly close to its mother and often lay down and rested in partial concealment near her while she browsed.

July 13, three miles south of Togwotee Pass: " . . . the female apparently alone. She watched us three to four minutes, then went over to willow screen and a young calf got up and followed the cow. After traveling about three yards this way the female waited and calf went ahead slowly." Less solicitude was shown by a cow at the ponds: " . . . the female walked off into the brush leaving the calf at the edge of the pond. The calf bleated quite loud and repeatedly (Myah—Myah—Myah) but the cow continued to move away. The calf, now bleating constantly, attempted to cross the mouth of a small feeder stream, became mired twice, finally struggled out and ran after the cow who was at the edge of a pine grove about 200 yards away and still moving."

The cows seemed completely indifferent to the near approach of bulls and other cows and were only mildly affected by calves other than their own.

The only evidence of communication involving vocalization in the maternal-young relationship, in addition to the instance already mentioned, was the following: "I was watching a mother and calf in a pond when suddenly there was repeated plaintive bleating and crashing of the brush and a grayish dogie calf approached the female. She blatted very softly, and the calf ran to her and licked her nose, and then ran on still calling."

A so-far unexplained dependence of the weaned young on the mother has been reported to me several times by competent observers. Daniels (1953) has commented on this situation. According to these reports, if the calf loses its mother as late as November, when it is seven or eight months old, it often does not survive, succumbing to exposure or malnutrition. There are at least two possible explanations for this situation. One is that the cow initiates movement from one locale to another. In the crucial winter period an inexperienced calf is likely to stay in an area of deep snow and poor browse until too weak to escape from it. (See Brown & Simon *op. cit.*). Secondly, if the calf is fortunate enough to find a good browse area, there will be other moose, elk and possibly deer using it. In these areas competition for browse is quite keen, both intra-specifically and inter-specifically. Without the protection afforded by the cow, a calf is truly at the bottom of the "peck-order," and suffers the fate of similar unfortunate individuals of other species.

B. Male-Young Relations

The mature bull moose appears to be very tolerant of first year calves even during rut. We have never observed a bull attempt to drive a young calf away from the cow's side, even during the height of sexual activity. The calf seems to reciprocate and often follows and associates with a bull that has joined the family group early in the breeding season. This could be an effect of "imprinting," with transfer from the mother to any large-size moose. The bull seems to become somewhat less tolerant on the winter feed grounds, however.

C. Male-Male Relations

As mentioned in other sections of this report, during much of the year bulls may associate in loose groups of as many as half a dozen individuals, although the most common grouping is a pair of bulls. Very little social interaction is observable in such groups throughout the year, with two exceptions. A mature bull will defend his "breeding wallow" against other males. This is the only form of territory defense noted in almost two thousand hours of observation. Males will "threaten" other males on a winter feed ground, but this is true of all mature moose in such a situation. There is some indication that the largest and most mature bull takes the initiative in group action. He often seems to occupy the first postiion when a group of males moves from forest out to feed on willow flats or in other similar movements. This may represent a generalization from the juvenile situation on the part of the smaller bulls. They are early dependent on a larger moose (the mother) and learn the habit of following it. The cue of larger size may then become associated with following such an individual and be transferred to the largest bull in an all-male group.

This observer has noted "mock combat" between two- and three-year-old bulls on several occasions. They run toward each other and then "wrestle" by means of their antlers, even when these are still in velvet. James Simon, at the time he was Director of the Jackson Hole Research Station, reported a pair of young bulls "very carefully rubbing each other's antlers." In view of the possible erogenous nature of deer antlers, as indicated by Darling (*op. cit.*), such observations are suggestive of possible homosexual activity.

On two occasions we observed the largest bull in the group walk in between such a pair of antagonists, and once a mature bull walked between the observer and a two-year-old bull which had adopted a "threatening" posture in relation to the observer.

The fact that more mature bulls come into rut, i.e., under maximum sex hormone influence, before the younger males, may well contribute to this age dominance pattern, as well as insuring that most of the breeding will be done by bulls that have been successful in reaching maturity—an obviously adaptive situation from the point of heredity.

Actually, the observer is struck by the lack of social interaction between moose during most of the year. Moose of either sex may approach individuals or groups, feed as close as ten feet from them for hours, and then leave without a single individual changing its behavior in any way apparent to the observer. A rare exception to this lack of social response is recorded in our field notes: "August 6, 7:00 A.M. Two males were feeding on the near side of the ponds. One appeared disturbed by me as I attempted a closer approach, and ran across the pond splashing water in great sheets higher than his head. The second moose could not have discerned me but followed the first much more slowly."

Inter-specific Reactions

Because the moose is almost exclusively a browsing animal it is in minimum competition with other large mammals and with domestic stock, which are grazers. Also, since it is solitary, it does not have to compete with other members of a herd. As a possible result of this lack of competition, the moose seems not to have built up effective social habits that would enable it to obtain its share of food in a competitive situation. When a moose encounters other species on a winter feed ground it is at a definite disadvantage.

A. Moose-Elk

In the Rocky Mountain region the species most frequently co-inhabiting the moose's territory is the elk, a form with highly developed herd organization. When individuals of these two species come into conflict the elk is almost always successful. Daniels (*op. cit.*) has this to say: "I have seen a huge bull moose completely bullied by a cow elk, and sometimes even an elk calf will run a moose from a pile of hay. While the two species will mingle and feed together, the elk are antagonistic toward the moose and abuse them constantly."

B. Moose-Stock

Where moose and horses or cattle come into contact there seems to be very little open conflict. Many of the meadows where horses graze are bordered by willow on which moose browse. It is not uncommon to find moose mixed in with a herd of horses after a night's grazing and it is sometimes difficult to cut the moose out of

the herd when the horses are rounded up and brought into a corral.

If a large group of cattle is moved onto summer range, moose seem to move out of the bordering willows and never associate as closely with the cattle as they do with horses. However, under the hardships of winter moose will come into corrals where hay has been put out for cattle. At such times they are perforce in fairly close contact with cattle, but nevertheless little overt friction is observed.

C. Moose-Predator

In the territory of the Shiras moose, almost the only effective predator is man. Coyotes and bob-cats would represent some danger to calves if it were not for the effective protection afforded them by their mothers well into their second year. Mountain lions and bears, both black and grizzly, possibly could prey on two- or three-year-old moose. There are at least two recent confirmed reports of such predation by bears in the Glacier National Park area. One of these was by a grizzly and one by a very large black bear.

Possibly because of the lack of predation, moose have the habit of standing and orienting their receptors toward the source of any disturbance. This pattern is in contrast with that of elk and deer under similar circumstances. These animals go bounding off immediately. The moose's delay in taking flight makes it extremely vulnerable to hunters.

D. Moose-Man

Like many animals, moose tolerate a quiet, unobtrusive observer who makes no loud noises or quick motions. If he remains downwind and motionless, moose may approach very close or they may be observed for hours without being disturbed. August 12, 5:00 P.M.: " . . . while sitting on a log in an alder thicket, three paces from a moose trail, I observed a very large female walk slowly up the trail toward me, approaching with the wind. Just previously I had heard the loud voices of a surveying crew. When the female was barely past me, she stopped and looked hard at me for about thirty seconds then went on silently. Her tracks looked as though she had been walking on tip-toe."

The next day I watched and photographed a two-year-old bull from 9:30-11:00 A.M. When some tourists stopped their car nearby and approached the ponds, talking in fairly loud tones, the moose dashed into the cover of the brush.

Moose will often circle a quiet observer, moving very silently and swiftly even in rather dry brush. July 10: "I crept through brush till I came opposite the bull without being seen. He browsed willow at end of growth on island, then crossed seventy yards downstream to my side; this from 7:45-8:10 A.M. I heard a few twigs snap and then saw movement about thirty yards behind and above me. The moose then recrossed to the island at 8:45, having made a complete circle around me."

The "attention" of moose (meaning preexisting set in the Hebbian sense) often does not seem to depend on the quantity of environmental change. Thus, when the observer's efforts at quiet stalking fail, they seem to disturb the moose more than much stronger, but less stealthy, stimuli. A somewhat amusing incident from my field notes illustrates this point. September 7, Pelican Creek: "A two-year-old bull with a bloody antler tip partly in velvet was browsing on a willow flat. Later he was joined by a female and a large white-antlered bull. Still later tourists yelled and whistled at them without much apparent effect. I walked about a half mile down to the end of the ridge. I tried to walk very quietly and to keep out of sight in returning. The old white-antlered bull was somewhat nearer the ridge and a little separated from the others. He looked toward me with ears turned forward, then shook his head and trotted away." Evidently on this occasion my efforts at stalking disturbed this moose more than all the noise made by the tourists.

Moose can be provoked into aggression or threats even during the summer but, as Hess (1951) aptly remarks, "anyone attacked by a moose must really have asked for it."

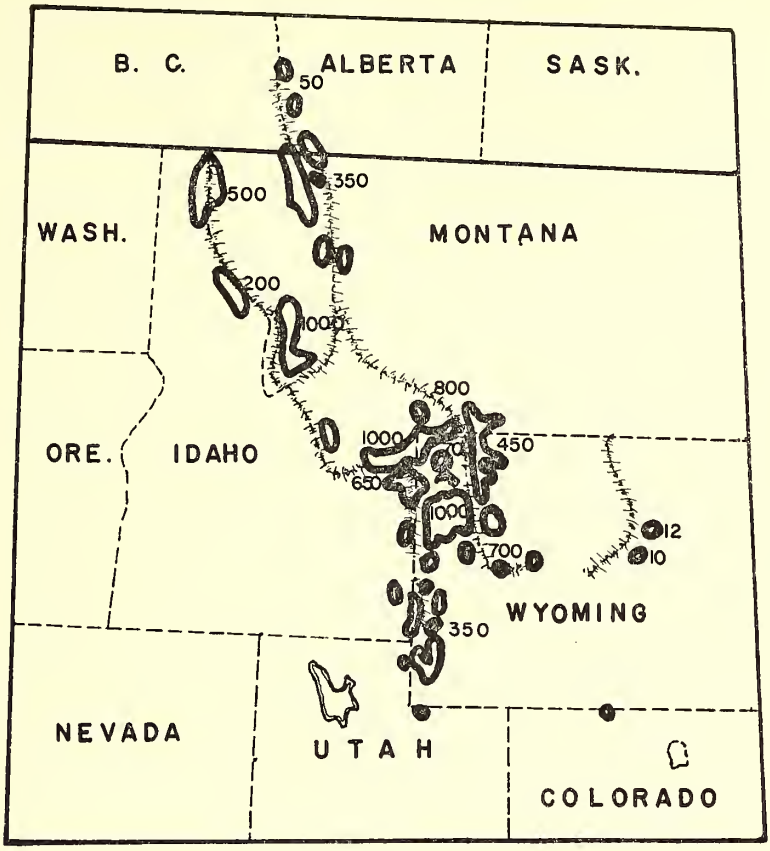
Dr. Robert Rausch, a co-worker at the Jackson Hole Research Station, did "ask for it" on one occasion. In early June he encountered a cow with young twin calves and in order to test their reactions he yelled and threw sticks until the female trotted toward him in a "rage" attitude. He climbed a small tree, carrying his collecting shotgun. While treed he noticed a raven that he wished for a specimen, so he shot it down. The cow moose ran to the raven and completely macerated it with the hooves of her forefeet.

It is much less difficult to get moose to attack in the winter and most moose-human encounters occur at this time. Daniels (1953) tells of being charged repeatedly by a moose in a large hay barn in winter. Brown & Simon (1947) reported being chased through the snow and brush by a young moose they were trying to feed. Attacks on automobiles by moose trapped on the highways by high banks of snow have been mentioned previously.

III. Population Dynamics

Present Distribution

The area studied most intensively in this paper



TEXT-FIG. 1. Present distribution of *Alces alces shirasi.*

is the type location for *Alces alces shirasi* and also one of the areas of the greatest abundance of the subspecies. (See Text-figure 1). At the present time there are between 3,000 and 4,000 moose in and around Yellowstone National Park. The protection afforded by the Park to an unmolested breeding nucleus has played some role in this concentration, but the naturally favorable habitat is probably of almost equal importance. An interesting aspect of this abundance is its rather recent occurrence. The moose population of the Park itself is holding steady at about 700 animals and there are now between 1,000 and 1,100 moose in Jackson Hole just south of Yellowstone Park. From Jackson Hole proper the animals seem to be spreading south across the Gros Ventre River to the upper Green River, where they have increased to 700, and southeast onto the upper Wind River, where there are only a few less. The latter region is one of the few where Shiras moose may be overcrowding their range. The Forest Service and the Wyoming Game and Fish Department have established special exclusion plots north of DuBois to study this situation. A few moose have appeared as far south as Lander on both sides of the Wind River Range.

To the west lies a second area which comprises several rather isolated groups of moose on both sides of the Salt River Range. There may be as many as 350 animals here. These appear to have come rather recently almost straight south from the Hoback, although Robert Patterson of the Wyoming Game and Fish Department tells me of seeing a few moose well out in the Red Desert, so that some animals could have come across from the south end of the Wind River Range.

In the Sunlight Basin area, east of Yellowstone Park, the moose population of some 150 is reported to be barely maintaining itself. This is in spite of apparently abundant browse. Recent heavy snows and severe winters in this basin may be limiting the population.

There are approximately 35 moose on the Owl Creek hills in the northern section of the Wind River Shoshone-Arapahoe Indian Reservation. The southern limit of distribution of a constant population is probably Kemmerer.

Durrant (1952) states that the moose is

sporadic in Utah and that they are known to occur in the Uinta Mountains and in the Wasatch Mountains as far south as Utah County. Accidental individuals have been reported in Logan Canyon and near Lewiston. In 1947 a bull was killed in Ogden Canyon. In summary, moose occasionally are seen near the State line around the southwest corner of Wyoming.

The even rarer accidental moose in Colorado may come from this small Utah population or from nearby southwestern Wyoming. In 1941 a moose was shot near Steamboat Springs, Colorado. It is reported that Milton Estes shot a moose in what is now the Rocky Mountain National Park in the 1860s.

Dalquest (1948) refers to the casual occurrence of moose in Washington. He assumes that they came south from Canada, so that they may have been *A. a. andersoni* and not the Shiras subspecies. (See also Peterson (1950)).

In Idaho there is a little group of moose south of Gray's Lake and another group in Targhee National Forest southwest of Yellowstone National Park. A big game census made in 1950 by W. M. Shaw, Idaho State Conservation Officer, recorded 642 moose in Targhee forest of Idaho. Moose here and in the Beaverhead Forest northwest of the Park, where there are about 1,000 animals, have shown a gradual increase in recent years. North of the Park in the Gallatin Forest there are now approximately 790 head. This area was almost devoid of moose in 1910, so that they have shown a remarkable comeback, probably aided by migration from the protected breeding stock in the Park. These areas adjacent to the Park contain about half the moose in Montana, whose estimated total population is 3,587 animals. These southern areas are the only ones in Montana open to hunting of moose. Some 1,200 animals are reported for the Bitterroot Forest south of Missoula, although we feel that this may be somewhat of an over-estimate. Two groups of animals totaling 700 extend north and west along the Bitterroot range toward Coeur d'Alene. These groups and the 350 animals in and adjacent to Glacier Park do not seem to be reproducing adequately and are diminishing in numbers despite a lack of hunting pressure. In Glacier Park itself there are estimated to be 155 moose, with about 40 animals in Canada's adjacent Watertown Lakes Park. This population is barely maintaining itself.

In 1948 and 1950 the Wyoming Game and Fish Department transplanted two groups of about eight moose each to the east slope of the Big Horns about 100 miles east of the previous extremity of their range. These moose are said to be establishing themselves satisfactorily, and reproducing.

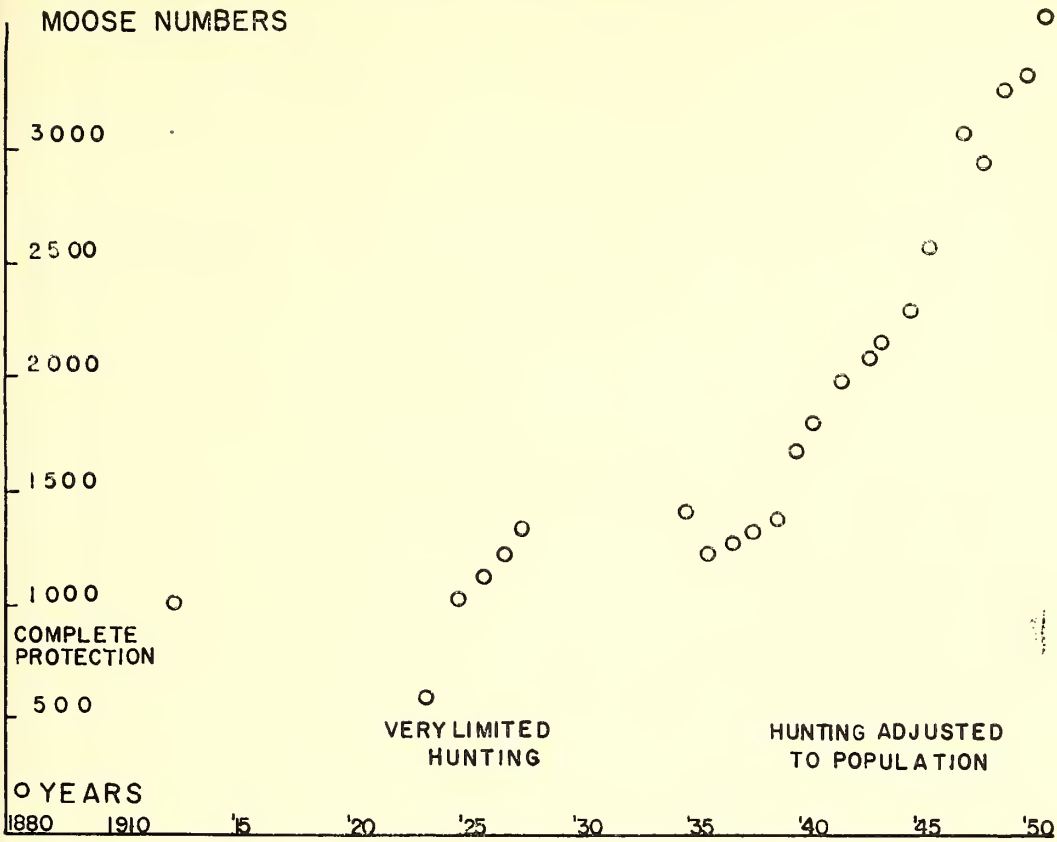
To summarize, it is estimated that there are today about 3,763 moose in Wyoming, including the Yellowstone National Park; 3,587 in Montana, and almost 1,000 in Idaho, Utah, Colorado and the Watertown Lakes area of Canada—in all, somewhat more than 8,000 individuals of *Alces alces shirasi.*

Relation between Counts and Estimated Totals

The proportion of the total population seen during a census check obviously depends on the methods employed. Even an experienced observer traveling casually will obtain only a very rough idea of the numbers of moose in the country through which he passes. More accurate is an airplane census in mid-winter. Similarly, an experienced observer living in a given small area for some length of time and systematically recording his observations may make a close estimate of the numbers of moose in the area. In this respect it is interesting to note the downward trend of estimates when the latter are based on objective data. In a series of reports to the Secretary of the Interior from the Superintendents of Yellowstone National Park we find the following: 1919 . . . "slightly more than 500 moose." 1920 . . . "I believe that there are about 800 moose in the Park." In 1924 and subsequent years the estimate of population was based on actual counts: 1924: count—121, estimate—385; 1925: count—170, estimate—525; 1926: count—103, estimate—575; 1927: count—73, estimate—600; 1928: count—111, estimate—650. Apparently when the population in the Park reached about its present level of 700 moose, the area had reached a saturation point and moose started to move out of the Park in increasing numbers, so that Wyoming was able to allow hunting. In 1912 the State Game Warden estimated the state population at 500 head and 50 hunting permits were issued. The number of permits remained constant for the next few years. We believe that the average legal kill in Wyoming represents about 8 per cent. of the total state population each year in terms of a sliding average. (See Text-figure 2).

The factors behind certain aspects of moose distribution seem fairly evident, while others are still puzzling. *Alces alces shirasi* must have established itself when it lost its connection with the much larger population of *A. a. andersoni* in Canada. This connection was later reestablished, giving a small area of overlap in Canada.

Shortly before the turn of the century the numbers of *A. a. shirasi* reached a dangerously low point. Thus Henry Avare, Montana State Game Warden, declared in 1910, "When the Game Warden's department was created ten years ago, these animals were practically ex-



TEXT-FIG. 2. Moose population changes in Wyoming and Yellowstone Park.

tinct in the state. Today, after ten years of careful protection, there are probably 300 moose in Montana. . ." In the forty-five years since 1910 there has been a ten-fold increase in moose in Montana, corresponding to the general trend in moose population elsewhere in recent years. As a practically monogynous deer, moose are peculiarly susceptible to hunting pressure, even if hunting is limited to bulls, since a bull usually mates with only one cow per season. Conversely, they have responded markedly to easing of hunting pressure through game law enforcement and the complete protection afforded by Yellowstone Park to a breeding nucleus. This response has taken the form of a great increase in numbers and a spreading to the south and east so that moose have been seen during the last dozen years where they have never been seen before.

SUMMARY

I

The behavior and ecology of the moose as made manifest in this study of the Wyoming or Rocky Mountain subspecies, *Alces alces shirasi*, are unique among deer to the extent that the moose is a solitary, somewhat amphibious form.

The daily cycle shows early and late feeding periods on littoral or aquatic flora. During the warmer part of the year these feeding periods are interrupted by a mid-day rest.

The annual cycle finds the moose in its greatest aggregations on winter food grounds, although Shiras moose do not form yards. Winter is the most difficult time for moose, bringing them into contact with man, stock and other game. Possibly because of the lack of effective social patterns occasioned by their solitary lives, moose are often inadequate in competition for food and space. Orphan calves may have a difficult time living through the winter, in the absence of the protection and leadership normally afforded by the mother. Most moose "attacks" on human beings come at this time of year. In the spring the winter aggregations split up with pregnant cows moving into secluded back country for calving. Bulls, and to a lesser extent dry cows, show a limited altitudinal migration in spring, following the snow line to graze on new

grass and browse on sage. From the time of their birth in May the calves are particularly dependent on their mothers. Since the moose has no nursery herd this dependence is accentuated, and "imprinting" in the Tinbergen sense may be to a *single* large individual, whereas in a herd form like the elk such "imprinting" may be to the herd itself. The reproductive behavior of the moose is unique among deer in that the male must attach himself to a cow-calf group and remains with a single cow for some weeks.

II

Alces alces shirasi apparently established itself as a subspecies when it lost its connection with the much larger population of *A. a. andersoni* to the north. After subspeciation had been completed the Shiras moose increased its range so that there is now a small area of overlap with the presumed parental stock in southern Alberta and British Columbia. At about the turn of the century the Shiras moose was nearly extinct, probably due to uncontrolled hunting. The protection afforded a breeding nucleus by the policies of Yellowstone National Park and the various State game and fish departments has enabled the subspecies to increase its numbers to more than 8,000 individuals, and its range to parts of six States and two Provinces.

Bibliography

Aldous, S. E. & L. W. Krefting
1946. The present status of moose on Isle Royale. N. Amer. Wildlife Conf. 11 pp. 296-308.

Asdell, S. A.
1946. Patterns of Mammalian Reproduction. Comstock Publishing Co. Ithaca, N. Y.

Beach, F. A.
1945. Concepts of play in animals. Amer. Nat. LXXIX.

Brown, R. C. & J. R. Simon
1947. Notes on wintering moose. Wyoming Wild Life. XI, pp. 4-8. June.

Dalquest, W. W.
1948. Mammals of Washington. Univ. of Kansas, Publ. Mus. of Nat. Hist. 2.

Daniels, T. W.
1953. Winter at Blackrock. Wyoming Wild Life. XVII, pp. 20-27. Feb.

Darling, F. Fraser
1937. A Herd of Red Deer. Oxford University Press, London.

Denniston, R. H., II
1954. A comparison of the natural responses and a recently learned response as an expression of the sex drive level. (abstract) Bull. Ecol. Soc. of Amer., vol. 35.

Durrant, S. D.
1952. Mammals of Utah. Univ. of Kansas Publ. Mus. of Nat. Hist. 6.

Hebb, D. O.
1949. The Organization of Behavior. John Wiley and Sons, N. Y.

Hess, D. E.
1951. Moose molesting. Yellowstone Nature Notes. XXV, March-April.

Hosley, N. W.
1949. The moose and its ecology. Wildlife Leaflet 312, U. S. Dept. of Int., Washington, D. C.

Merrill, S.
1916. The Moose Book. E. P. Dutton Co. New York, N. Y.

Murie, A.
1934. The moose of Isle Royale. U. of Mich. Museum of Zoology Misc. Pub. No. 25.

Murie, O.
1951. The Elk of North America. Stackpole, Harrisburg, Pa.

Peterson, R. L.
1950. A new subspecies of moose from North America. Occasional Papers of the Roy. Ont. Mus. No. 9.

Roberts, L. W.
1953. Technique for the extraction of chlorophyll pigments. Turtox News, vol. 31, p. 211.

Seton, E. T.
1928. Lives of the Game Animals. 3, p. 151.

15

Influence of Light and Darkness on the Pineal Body in *Astyanax mexicanus* (Filippi)

Marta Grunewald-Lowenstein

Department of Fishes and Aquatic Biology
The American Museum of Natural History, New York

(Plates I & II; Text-figures 1-4)

Introduction

THE problem of light sensitivity of the pineal complex in lower vertebrates has been discussed many times. Reactions to light have been demonstrated in both the pineal and the parapineal organ in cyclostomes (Young, 1935; Knowles, 1939) and in the parapineal organ in reptiles (Novikoff, 1910).

In teleosts von Frisch (1911.1, 1911.2) showed that illumination of the pineal region in blinded *Phoxinus laevis* resulted in melanophore reactions. When these reactions were still present after pinealectomy he concluded that light sensitivity is not confined to the pineal body alone but is characteristic of the entire pineal region. E. Scharrer (1928) came to the same conclusion when conditioned reflexes, which in blinded fish were elicited by light stimuli directed toward the region of the head immediately above the pineal, continued to exist after the pineal was destroyed.

Recently the problem was re-examined from new points of view by Breder & Rasquin (1950). These authors observed that phototropism was distinctly related to the degree of transparency of the tissues covering the pineal area. A predominantly positive phototaxis was seen in fishes with exposed pineal areas and a predominantly negative phototaxis in the groups of fishes in which this area is permanently and densely covered. Some phototactic instability was seen in an intermediate group in which the access of light to the pineal is controlled by melanophores. The influence of the pineal area on this behavior was also studied experimentally by covering the heads of blinded fish with India ink which caused the degree of positive phototaxis to be diminished or reversed.

From the results of these experiments and observations, the fact of light sensitivity of the pineal area in teleosts must be considered to be well established. The question remains to what degree the pineal body proper participates in bringing about the phenomena described above. The present study deals with this question by investigating morphologically demonstrable reactions of the pineal body in fish under the conditions of light and darkness.

Acknowledgements

The author wishes to express her indebtedness to Dr. C. M. Breder, Jr., and to Miss Priscilla Rasquin for their invaluable and much-appreciated advice and support of this work, especially for having made it possible to carry out these studies in the Laboratory of the Department of Fishes and Aquatic Biology of The American Museum of Natural History.

Miss Priscilla Rasquin kindly made available the slides from which the measurements given in Tables 1 & 2, and the photo-micrographs on Plate I, were taken.

Sincere thanks are extended to Dr. George Berg, Department of Biology at the Brookhaven National Laboratories, who most obligingly has given extensive and highly valuable information on the method of decalcification which meets with the requirements of glycogen preservation.

Material and Methods

All experiments and observations were done on the characin *Astyanax mexicanus* (Filippi).

Histological survey and comparative measurements were taken from 38 slides made in connection with studies by Rasquin & Rosenbloom (1954) on the effect of darkness on a number of organs in *Astyanax mexicanus* (Filippi). They contain the pineals of both eyed and blinded fish, part of which had been living

under the normal change of daylight and night, part under darkness. Some of the slides were stained with hematoxyline-eosine, some were unstained. The latter, for the purpose of the present study, were stained with Masson's trichrome stain as described by Rasquin & Rosenbloom (*l.c.*, p. 371). The areas of the pineals were measured by tracing the outlines of cross-sections of the pineal bodies in these slides with a camera lucida at table height; oc. Leitz, 6 × B, obj. 3, 10:1. Every second section was drawn. The areas of each drawing were determined with a planimeter, and the measurements are given in cm^2. For each pineal the average of the areas was taken. These are given in Tables 1 & 2, column 3. The maximum values for each case are given in column 4.

The formation of glycogen in the pineal of *Astyanax mexicanus* (Filippi) under the conditions of light and darkness was examined. Fifty fish, 7½ months old, were used; 25 of them were kept in darkness, 25 in constant light.

Darkness was obtained by keeping the fish in 15-gallon tanks which were surrounded by light-tight wooden boxes and provided with aerating equipment and a device which allowed feeding without exposing the fish to light; the tanks were placed in a darkroom, and red light was used each time it was necessary to lift the cover of the boxes in order to take out the specimens. Conditions of constant light were obtained by placing a fluorescent bulb (General Electric, warm-white, 20 Watt standard, 1860 Lumen) at a distance of 12 inches above the upper edge of the tanks; the brightness at the level of the water was 170 foot candles at the start of the experiment. To insure continual exposure of the fish to light, no plants were introduced into the tanks. Best's carmine stain for glycogen was used for the comparative study of the pineals of the fish that had lived under these conditions for various lengths of time. Controls were made by means of the saliva test. Decalcification was foregone in most of the individuals examined in order to avoid as much as possible any loss of glycogen. Therefore the pineals were taken out of the skulls either before or after fixation. Paraffine sections 5 μ thick were prepared. When, in the course of the investigation, it appeared desirable to examine the distribution of glycogen in the pineal *in situ*, the following method of decalcification proved to be applicable without any apparent detrimental effect on glycogen preservation: the fish were fixed in an ice-cold mixture of 8.5 parts dioxan saturated with picric acid, 1 part formol, 0.5 part glacial acetic acid. This mixture served as a fixer and simultaneously as a decalcifier; decalcification appeared complete after ten days; the specimens were kept cold during the time of decalcification.

Results

In cross-section, the pineal body in *Astyanax* is seen as an oval-shaped organ, the main part of which is located dorsally to the caudal part of the forebrain and immediately beneath the transparent foramen of the skull. It consists of a number of convoluted strands of epithelioid cells surrounding more or less narrow lumina (Plate I, Figure 1). The whole organ is sheathed by richly vascularized connective tissue which also fills the spaces between the epithelial folds. Plate I, Figures 2 & 3, show the pineal as maintained under conditions of light and darkness respectively. Plate I, Figure 1, represents the pineal of a fish which had been living under ordinary laboratory conditions, *i. e.*, the usual daily rhythm of light and darkness in one of the laboratory tanks. Plate I, Figure 2, shows the pineal of a specimen that had been kept for eight weeks in total darkness. Plate I, Figure 3, is taken from a blinded fish which had been living in the dark for 12 weeks. Comparison of these three pictures shows that they differ markedly with respect to the width of the lumen. This is seen to be very narrow in Plate I, Figure 1, *i. e.*, in the fish which had been living under normal conditions. The lumen is wider in the case of Plate I, Figure 2; the folds in the cases of Plate I, Figures 2 & 3, can be seen to be quite flat, surrounding a wide, hardly constricted lumen. Plate I, Figures 2 & 3, also show the pineal to have adopted a less slender, elongated shape than the one seen in Plate I, Figure 1. This is indicative of a tendency towards a change of shape of the pineal frequently seen to occur in the fish kept in constant darkness, where, in extreme cases, the pineal is nearly to the point of becoming a short, obtuse cone with its tip pointing towards the brain.

The picture of the pineal body in fish which had been exposed to constant light is similar to the one seen under the conditions of darkness insofar as the lumina were never seen to be as small and narrowed by convolutions as they are under the normal conditions of alternating day and night. They are, however, less wide than those seen in the more advanced cases in the fish exposed to constant darkness. The tendency to adopt a roundish, plump shape seen in the pineals of fish kept in darkness was not observed in the case of fish which had been exposed to constant light for up to seven months.

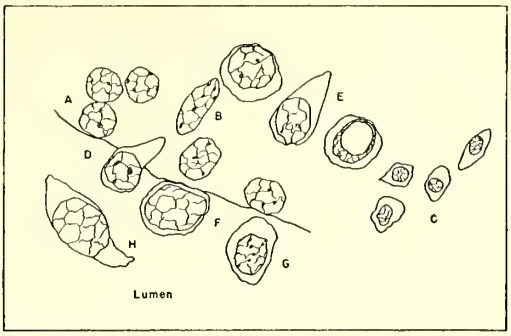
Text-figure 1 shows types of cells ocurring in the pineal body of *Astyanax*. The type most frequently seen is characterized by a rather large, round, vesicular nucleus which, in Mas-

son's trichrome stain, shows a ponceau red-stained chromatic network with one or several small red nucleoli (A, Text-figure 1). The plasma border surrounding these nuclei is very small and, if visible at all, shows a grayish-green color. Besides these cells, which are arranged in an epithelium of two or three layers surrounding the lumen, a number of oval, more or less elongated, nuclei can be seen; in B, Text-figure 1, they appear mostly in a deeper layer of the epithelium, less close to the lumen than the round nuclei (cf. also Plate I, Figures 2 & 3). Only occasionally they are seen protruding towards the lumen and lying between the large round nuclei. Also, irregularly shaped nuclei can be seen. Occasionally there appear nuclei which give the impression of being vacuolized—showing one single, large, completely colorless "vacuole" which is surrounded by deeply stained chromatic material (E, Text-figure 1).

A third group of cells, fewer in number, shows considerably smaller nuclei which are more deeply stained than those in the two groups described above. The narrow plasma border, as far as it is visible, is also more deeply stained. Small processes can be seen in many of them (C, Text-figure 1). Whether or not these processes are nervous in nature cannot be decided with the staining used in this study. The cells belonging to this group seem to be located mainly in the peripheral layers of the pineal body. Frequently they are situated at the dorsal edge. D, Text-figure 1, is typical of the pineal in *Astyanax, i.e.,* a cell of the type A which is protruding into the lumen. Its nucleus is located proximally to the lumen. Its cell body is seen to have formed a process which is extended away from the lumen. Cells of the same type are also frequently seen in the deeper layers of the epithelium, where their processes are extended towards the well-vascularized connective tissue which surrounds the pineal and fills the spaces between the epithelial folds (E, Text-figure 1; see also Plate I, Figures 2 & 3).

The cells, which protrude into the lumen, evidently represent the first stage of a process of migration out of the epithelium into the lumen. Some are partly and others completely detached from the epithelium and are lying freely within the lumen (D, F, G, H, Text-figure 1; see also Plate I, Figures 2 & 3).

Masson-stained slides show the lumen filled with cells, in various stages of disintegration, which have migrated out of the epithelium into the lumen: cells which still show the same structure and staining as the intact cells forming the epithelium, smaller cells with more deeply stained pycnotic chromatin (G, Text-figure 1), and, occasionally, also very large, quasi-



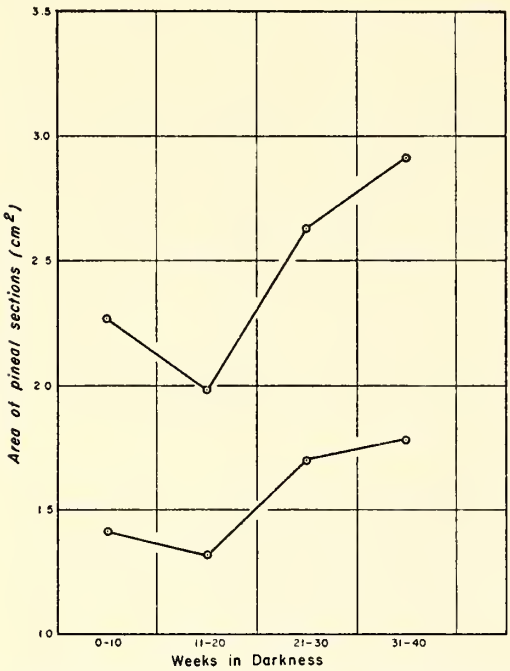
TEXT-FIG. 1. Types of cells occurring in the pineal body of *Astyanax mexicanus*. See text for full explanation.

"inflated" cells with clearly visible grayish-green stained cell body and a faintly ponceau red-stained, enlarged nucleus (H, Text-figure 1). The main part of the lumen is filled with strands of pale fast-green-stained material, which in all probability represents the end product of the disintegrated cells and nuclei. Their outlines are often still recognizable within the fibrous network in the lumen. These contents of the lumen show well in Plate I, Figures 2 & 3, but can also be recognized in the narrow lumen in Plate I, Figure 1. Normally, the cells of the epithelium are tightly packed, forming a well-defined border (Plate I, Figure 1). Under the conditions of prolonged darkness (Plate I, Figures 2 & 3) the nuclei surrounding the lumen are less densely arranged and sometimes the cells appear to be only loosely connected to each other. The oval shaped, elongated nuclei in these latter pineals are more conspicuous than those in the "normal" ones; they are seen to occur most numerously in the vascularized region of the ventral median connective tissue septum (cf. also Plate I, Figures 2 & 3).

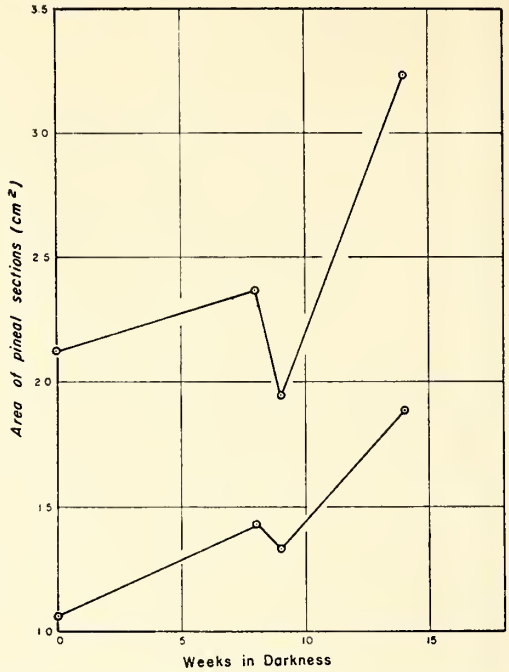
The histological picture of the pineal kept under the condition of constant light resembles the one seen in fish exposed to darkness. The epithelium surrounding the lumen consists of a comparatively small number of round nuclei. Elongated oval nuclei are mostly seen in the deeper layers of the epithelial folds. The small dark-stained cells appear to be numerous, especially at the dorsal edge of the pineal.

The blood supply of the pineal under the various conditions of illumination shows marked differences. Blood corpuscles are abundant in the pineals of the fish raised under normal conditions and under constant light. They are scarce under conditions of darkness.

Considerable amounts of fat have been seen to accumulate in the pineal region in the fish



TEXT-FIG. 2. Graphic representation of the data given in Table 1 (Effect of darkness on the size of the pineal body in eyed *Astyanax mexicanus*). Mean and maximum values.



TEXT-FIG. 3. Graphic representation of the data given in Table 2. These have been calculated by groups, comprising the data obtained in fish which had been living in darkness for less than 10, 20, 30, 40 weeks respectively. Mean and maximum values.

which had been kept in prolonged darkness. Droplets of fat are seen with the dissecting microscope within the loose network of connective tissue which fills the space between the skull and the brain and which surrounds the pineal body. Possibly due to these fat accumulations, the pineal is often seen, in slides, to be located less closely beneath the surface of the skull than in the fish kept under ordinary conditions. This looser connection to the skull could also be noticed when, under the dissecting microscope, the pineal had to be taken out for the purpose of glycogen staining; in general the pineals of the fish kept in darkness were much more easily loosened from their base than those of the fish kept under constant light conditions in which fat deposits at the site of the pineal were not noticed. Breder & Rasquin's (1947) observation of the development of "adipose tissue in great quantities between the bone and the epidermis and between the bone and the meninges governing the brain" but not "at the site of the pineal body" in blind cave fish should bear on this phenomenon. It should be also kept in mind, in this connection, that—according to Rasquin & Rosenbloom (1954, p. 419)—"the dark-reared fish were marked by large accumulations of adipose tissue and wasted musculature, indicating failing adrenal cortical function . . ." This shows that the development of fat in the pineal region of the dark-reared fish can be considered to be part of the phenomenon of the influence of darkness on the fat metabolism in fish.

Tables 1 & 2 and Text-figures 3 & 4 show the results of measurements of the pineals developed under normal conditions as compared with those under darkness. The measurements were made as previously described. Both tables as well as the graphs derived therefrom (Text-figures 3 & 4) show on the whole larger pineals in the fish kept in darkness than in those kept under normal conditions of alternating day and night illumination. The increase in size is not always in proportion to the number of weeks during which the fish were kept in darkness (cf. Nos. 131, 133, 135). A general tendency, however, towards an increase in size of the organ in accordance with the time spent in darkness is evident. Among the 16 cases of fish kept in darkness in Table 2 there are three in which the pineals appear oddly small. One of these cases (No. 150) which, after 15 weeks in darkness showed the extremely small surface average of 0.87 cm², is mentioned by Rasquin & Rosenbloom (1954, p. 388) as showing abnormalities

TABLE 1.[1] EFFECT OF DARKNESS ON THE SIZE OF THE PINEAL BODY IN NORMAL FISH

Fish No.	Time in darkness in weeks	Average area of cross-sections in cm.^{2}	Maximum area of cross-sections in cm.^{2}
126	0	1.03	1.94
127	0	1.07	2.52
128	0	1.08	1.94
129	8	1.23	1.94
130	8	1.31	2.59
131	8	1.76	2.59
132	9	1.34	1.94
133	9	1.32	1.94
135	14	1.88	3.23

[1]All fish recorded in Table 1 and Table 2 (column 1) belong to series 4 (eyed fish) and series 5 (blinded fish) in the experiments done by Rasquin & Rosenbloom (1954, pp. 367 & 369). The amount of time spent in darkness has been derived from their Table 2 (*l.c.*, p. 369). The values of the average and maximum areas of cross-sections of the pineal bodies (columns 3 & 4 in Tables 1 & 2) are obtained as indicated in "Material and Methods." The values indicating "Standard Length" and "Greatest Depth" referring to the body size of the fish (column 5 in Table 2) and the "Somatic Index" the quotient resulting from dividing the standard length by the greatest depth (column 6 in Table 2), are taken from Table 4 in Rasquin & Rosenbloom, 1954, p. 380.

in the cellular setup in the transitional lobe of the pituitary and as having "extremely small glands." In fish No. 141 it is mentioned (*l. c.*, p. 397) that — at the time when the fish was sacrificed — "the pseudo-branch appeared normal," indicating that at that time the impact of darkness was not yet seen in this gland. The fact, pointed out by Rasquin & Rosenbloom (*l.c.*, p. 374) "that darkness does not affect all the animals in the same way," may be emphasized in connection with the evaluation of the effects observed.

Columns 6 and 7 in Table 2 indicate the values of standard length and greatest depth of the whole fish and the somatic index calculated therefrom as given in Table 4 of the study by Rasquin & Rosenbloom (1954, p. 380). The values of the sizes of the pineal and the time spent in darkness can thus be compared to these indices of changes in body size under the condition of darkness. Although, again, a "direct correspondence" cannot be derived from this juxtaposition, it can be seen that, while the size of the pineal tends to increase with the length of time spent in darkness, the somatic index tends to decrease—the smallest values being found in those fish which have spent from 21 to 30 weeks in darkness. This means that the larger pineals tend to be associated with the plump-deep-bodied fish as they have been described to

TABLE 2. EFFECT OF DARKNESS ON THE SIZE OF THE PINEAL BODY IN BLINDED FISH

Fish No.	Time in darkness in weeks	Average area of cross-sections in cm.^{2}	Maximum area of cross-sections in cm.^{2}	Standard length by greatest depth, in mm. (body size of the fish)	Somatic index
136	0	1.27	2.59	55 × 18	3.06
137	0	1.62	2.26	58 × 19	3.05
138	0	1.68	2.65	42 × 12	3.50
139	0	1.08	1.94	45 × 14	3.21
141	8	1.19	1.94	35 × 11	3.18
147	9	1.47	2.00	34 × 11	3.09
148	9	1.55	2.52	29 × 9	3.22
161	12	1.16	1.94	42 × 13	3.23
162	13	1.33	1.94	—	—
150	15	0.87	1.62	—	—
153	19	1.74	2.48	—	—
163	19	1.52	1.94	46 × 13	3.07
164	20	1.36	2.13	39 × 13	3.00
154	21	1.36	2.00	37 × 13	2.85
156	22	1.55	2.59	35 × 14	2.50
157	23	1.85	3.23	45 × 13	3.46
158	25	2.09	3.23	39 × 15	2.60
151	28	2.01	2.59	42 × 13	3.23
160	30	1.68	2.59	40 × 14	2.86
152	38	1.88	3.23	—	—

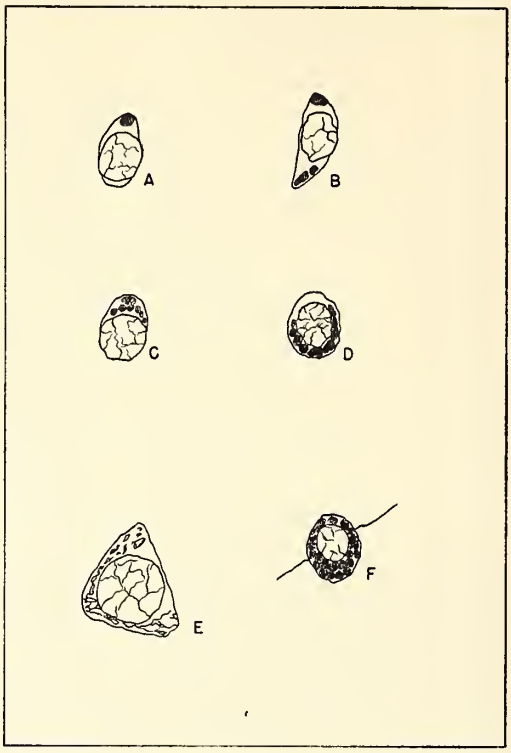
develop in darkness, partly as a result of "rapid accumulation of fat" (Rasquin & Rosenbloom, 1954, p. 372 f.)

The presence of glycogen in the pineal body was shown recently in mammals (Wislocki & Dempsey, 1948; Mikami, 1951). Wislocki & Dempsey (1948) have demonstrated "traces of glycogen" in the parenchymal cells of the pineal in the rhesus monkey; they emphasize that, of all parts of the brain examined by them (neurohypophysis, neurons and neuroglial cells of the white and gray matter in the hypothalamus or in the regions next to the pineal), only the pineal showed the occurrence of glycogen. Mikami (1951), in examining the pineal glands of goats and pigs, found glycogen deposits in all of them and he concludes that some metabolic activity takes place in the pineal which he denotes as being inconsistent with the still widely accepted concept of the pineal being a vestigial, rudimentary organ.

The occurrence of glycogen in the pineal in *Astyanax mexicanus* could be shown by means of the methods described earlier in this study. Plate II, Figures 1, 2 & 3, show pictures of glycogen in the pineal bodies of fish which had been kept under various conditions of illumination. They are taken from slides obtained after fixation and decalcification with the dioxan-mixture described earlier. Plate II, Figure 1, shows the pineal under the "normal" conditions of alternating day and night; Plate II, Figures 2 & 3, are taken from pineals of fish which had been kept for 15 weeks in darkness (Plate II, Figure 2) and in constant light (Plate II, Figure 3) respectively. After staining with Best's carmine, red-stained material, which by means of saliva tests is identified as glycogen, is seen accumulated in the ventral part of the pineals. Bigger and smaller brightly red-stained droplets are suspended within the ventrally located connective tissue septa. Single droplets are seen spreading from there into the more dorsally located parts of the pineal, where they appear within the parenchyma as well as within the lumina. Except for a few small droplets or granules seen in the plasma of some cells, most of the glycogen in these slides is extracellularly located, presenting the phenomenon which is known as "flight of glycogen."

Comparing the three pictures, it is seen that the amount of glycogen under "normal" conditions exceeds considerably the amounts observed under conditions of darkness or of constant light.

A different picture of glycogen occurrence is seen in those slides which were made from isolated pineals. The accumulations of glycogen in



TEXT-FIG. 4. Occurrence of glycogen in cells of the pineal body. See text for full explanation.

the ventral part of the pineal obtained when using the dioxan-method did not appear in these slides. Instead, Best's carmine-stained small granule-like droplets appear both extra- and intracellularly throughout the entire pineal, within the tissue as well as within the lumen. Text-figure 4 shows various pictures of glycogen within the cell. It is most frequently found in the cell with the round vesicular nucleus. There are cells containing just one small red droplet within the cytoplasm (A, Text-figure 4); in others the amount of droplets is increased (B, C, D, Text-figure 4). In other cases the whole cell body is filled with red droplets (F, Text-figure 4). Occasionally the glycogen is seen to fill the cell as a pale pink-stained homogeneous mass. The glycogen-filled cell pictured in F, Text-figure 4, is about to enter the lumen. Glycogen-filled cells within the lumen present the same stages of disintegration which were found in the histological picture of the pineal body after Masson staining (Text-figure 1), as described above. E, Text-figure 4, shows one of the big "inflated" cells within the lumen corresponding to those shown in F, G, H, Text-figure 1. Its glycogen content appears paler and less densely packed than in the intact cells. Red-stained

droplets and irregular fibrous strands are found lying free in the spaces between the disintegrating and disintegrated cells. Occasionally, droplets and strands of red-stained material are also seen in the parenchyma of the pineal body. However, it is difficult to decide whether these result from a process of disintegration of glycogen-bearing cells or from cases of "flight of glycogen." The features described are found in the pineals of fish kept in darkness as well as in those kept in constant light. In all probability, they can be interpreted as the morphological aspect of a process of apocrine secretion by which glycogen is being transported into the lumen where it is released. No marked quantitative differences such as were shown in Plate II, Figures 1, 2 & 3, can be demonstrated in these slides obtained from isolated pineals. It is thus a favorable circumstance that the two methods of demonstrating glycogen in the pineal, as applied in this study, are complementary to each other. The second has the advantage of showing the cytological details of glycogen occurrence within the cell. The advantage of the first method, apart from showing the pineal *in situ*, results from the otherwise undesirable phenomenon of "flight of glycogen" by which it was possible to compare the amounts of glycogen produced under the experimental conditions used. In combining the results of both methods it may be stated that at least part of the secretory processes, which in all probability take place in the pineal body, deals with the formation of glycogen. This is seen to be produced in greater quantities in fish which are kept under normal conditions of alternating day and night than in those which were kept in darkness or in constant light.

Discussion

Morphological and physiological changes in the pineals of *Astyanax mexicanus* have been shown to occur under the influence of light and darkness. The size of the organ increases when the fish are kept in darkness. The lumen is narrow under normal conditions of illumination and gets wider and less constricted by invaginations on exposure to either darkness or constant light. Red blood corpuscles are abundant in the fish kept in alternating light-darkness conditions; they are scarce in the fish kept in darkness. The number of cells surrounding the lumen decreases in both constant darkness and constant light; they are less closely connected to each other than in the fish raised under normal conditions and in extreme cases the epithelial unit seems to disintegrate. The amounts of glycogen produced under the conditions of constant darkness and constant light respectively are smaller than those produced under normal illumination.

The studies by Rasquin (1949) and by Rasquin & Rosenbloom (1954) have provided a great deal of information on the effect of darkness on the endocrine system, on growth, body shape, the kidneys and a number of other organs in *Astyanax mexicanus*. Most of the organs examined were distinctly affected when the fish was kept in darkness, *i. e.* in living conditions to this extent rescmbling those of its cave derivatives. "A condition of hormonal imbalance ordinarily inhibited by the presence of light and marked by somatic and pathological modifications" (Rasquin & Rosenbloom, 1954, p. 419) was seen by them to be produced in the fish raised in darkness—a condition which "can be considered a situation of long continued stress" (*l. c.*, p. 419).

The stimulus of constant light has been used in experiments on mammals, reptiles and amphibians. Prolonged application of constant illumination was seen to result in morphological and physiological changes, indicative of decreased function, in the pituitary (Stutinski, 1936; Florentin & Stutinski, 1936; Fiske, 1941; Woitkewitsch, 1944.1); in gonads (Fiske, 1941; Pomerat, 1942); and in the thyroid (Woitkewitsch, 1944.2, 1946; Puntriano & Meites, 1946).

According to Bissonnette (1938) it can be considered establishcd that "effects of light and darkness" are "mediated by the eyes, optic nerve and pituitary and accompanied by both cytological and physiological changcs in the gland and in pituitary activity" (*l. c.*, p. *372*). The pituitary, thus, is the mediating agent through which the effect of illumination is transferred to its target-organs. With reference to this fact it is interesting to note that Rasquin & Rosenbloom (1954, p. 306), among all the organs examined, found "no effects" (*sc.* "of darkness") in the pancreatic islets and corpuscles of Stannius, neither of which "has yet been proved to be directly under stimulation by the pituitary."

The pineal body also has never yet been found to be under direct stimulation by the pituitary. However, in contrast to the pancreatic islets and Stannius' corpuscles, the pineal in *Astyanax mexicanus* does show morphological and physiological changes under the influence of darkness and constant light. These changes, therefore, are strongly suggestive of being produced by direct photic stimulation of the pineal itself.

This conclusion may be considered to be supported by the fact that an independent reaction of the pineal body towards photic stimuli is

consistent with our knowledge about sensory cells in the pineal of some teleosts.

"Sensory cells" in the pineal in teleosts have been described by Studnicka (1905), Holmgren (1920) and Friedrich-Freksa (1932). Holmgren, in *Osmerus eperlanus* (1920), describes a very specific kind of cells which he considers to be photoreceptory, similar to those which he had described earlier in *Squalus acanthias* and in *Rana* (1917/18). In these he distinguishes two kinds of processes ("Aussen" and "Innenglied") which are seen to show special structures ("Spitzenstueck" and "Spiralfaden"). These processes, according to this author, detach themselves from the cells and migrate into the lumen where they are dissolved. Similar pictures are described in *Dermogenys pusillus* by Friedrich-Freksa (1932), who considers these cells to be sensitive to blood pressure fluctuations, and he describes a complicated system of capillaries, part of which he assumes to be filled with a blood pressure-regulating secretion produced by the pineal cells.

The elaborate structures described by these investigators could not be found in *Astyanax* with the methods used in this study. There are indications, however, that a sensory apparatus does exist in the pineal of *Astyanax mexicanus:* the small dark-stained cells (C, Text-figure 1) which were seen scattered in the pineal parenchyma are often seen to show processes of various shape; these appear more numerous at the dorsal edge of the pineal body than in other parts of the organ and they stain well with intravital methylene blue staining. Furthermore, by means of this staining, the existence of free nerve endings within the pineal parenchyma can be shown. These studies are still in progress and will be published at a later date. The possibility shoud be kept in mind that *Astyanax mexicanus* is not a particularly favorable object for the study of a sensory apparatus in the teleostean pineal. Other species seem to display better-defined specific structures. Further study in this direction therefore should be extended to other species.

Excluding the controversial question of direct effect of light on the skin, there are three ways in which light stimulates the fish: (a) directly through the eyes, (b) indirectly through the pituitary, (c) directly through the pineal body. With respect to the possible significance of this third way it may be emphasized that there is increasing evidence of an antagonistic relationship between the pineal body and the anterior lobe of the pituitary gland, as recently shown by Thiéblot (1954). The author of the present study has shown, in a previous paper (Grunewald-Lowenstein, 1952), that the activity of the pineal body affects the autonomous nervous system by increasing the activity of its sympathetic part and that, antagonistically, the activity of the anterior lobe of the pituitary increases the activity of the parasympathetic part of the autonomous nervous system. In view of these facts the concept might be advanced that, besides its main access to the body through the eyes, light, in fish, influences the vegetative neurohumoral system of the body by means of both the pituitary gland and the pineal body, the effects of which are antagonistic to each other. This concept should be considered in further research.

Summary

1. The influence of darkness and of constant light on the pineal body of *Astyanax mexicanus* (Filippi) was examined.
2. The influence of darkness shows in increased size of the organ, increased width of the lumen, diminished depth of the epithelial folds and gradual disorganization of the epithelium. Changes of the shape of the organ are observed. Accumulations of fat develop in its surroundings. The number of blood corpuscles is diminished.
3. Exposure of the fish to constant light results in similar pictures: the lumen is wide, the depth of the folds is diminished, the epithelium is less compact than under normal conditions. No effect on the shape of the organ, on the number of blood corpuscles or on the development of fat was observed.
4. Glycogen is seen to develop in the pineal under the conditions of darkness and of constant light as well as under normal conditions. Its amount in darkness and in constant light is less than in alternating light and darkness.
5. The pictures of the occurrence of glycogen in the pineal cells in *Astyanax mexicanus* are indicative of a process of apocrine secretion by means of which glycogen is emptied into the lumen.
6. The morphological and physiological changes in the pineal body in *Astyanax mexicanus* can be considered to be produced by direct photic stimulation of the organ.

References

Bissonnette, T. H.

1938. The influence of light upon pituitary activity. Assoc. Research in Nervous and Mental Diseases, 17: 361-376.

Breder, C. M., Jr., & P. Rasquin

1947. Comparative studies in the light sensitivity of blind characins from a series of Mexican caves. Bull. Amer. Mus. Nat. Hist., 89: 323-351.

1950. A preliminary report on the role of the pineal organ in the control of pigment cells and light reactions in recent teleost fishes. Science, 111, Jan. 6: 10-12.

Fiske, V. M.

1941. Effect of light on sex maturation, oestrous cycle and anterior pituitary of the rat. Endocrinology, 29: 187-196.

Florentin, P., & F. Stutinski

1936. Modification cytologique de la glande pituitaire des grenouilles maintenues à l'obscurité. Compt. Rendus Soc. Biol., Paris, 122: 674-676.

Friedrich-Freksa, H.

1932. Entwicklung, Bau und Bedeutung der Parietalgegend bei Teleostiern. Z. wiss. Zool., 141: 52-142.

Frisch, K. von

1911.1 Das Parietalorgan der Fische als funktionierendes Organ. Sitzgsber. Ges. Morph. und Physiol. München, 27: 16-18.

1911.2 Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Arch. Ges. Physiol., 138: 319-387.

Grunewald-Lowenstein, M.

1952. Studies on the function of the pineal body. Exper. Med. Surg., 10: 135-154.

Holmgren, N.

1917/18.1 Zum Bau der Epiphyse von Squalus acanthias. Arkiv för zoologi, 11(24).

1917/18.2 Zur Kenntnis der Parietalorgane von Rana temporaria. Arkiv för zoologi, 11(25).

1920. Zur Anatomie und Histologie des Vorder –und Zwischenhirns der Knochenfische. Acta Zool. Stockholm, 1: 137-315.

Knowles, F. G. W.

1939. Photomechanical changes in the pineal of lampreys. Jour. Exper. Biol., 16: 524-529.

Mikami, S.

1951. Cytological and histological studies on the pineal gland of domestic animals. Tohoku J., Agric. Res., 2: 41-48.

Novikoff, M.

1910. Untersuchungen uber den Bau, die Entwicklung und die Bedeutung des Parietalauges von Sauriern. Zschr. wiss. Zool., 96: 118-207.

Pomerat, G. R.

1942. Cell changes in the pituitary and ovary of the white rat following exposure to constant light or darkness. Anat. Rec., 82: 531-542.

Puntriano, G., & J. Meites

1951. The effects of continuous light or darkness on thyroid function in mice. Endocrinology, 48: 217-224.

Rasquin, P.

1949. The influence of light and darkness on thyroid and pituitary activity in the characin *Astyanax mexicanus* and its cave derivatives. Bull. Amer. Mus. Nat. Hist., 93: 501-531.

Rasquin, P., & L. Rosenbloom

1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Amer. Mus. Nat. Hist., 104: 365-425.

Scharrer, E.

1928. Die Lichtempfindlichkeit blinder Elritzen. Z. Vergleich. Physiol., 7: 1-38.

Studnicka, F. K.

1905. Die Parietalorgane. Lehrbuch der vergleichenden mikroskop. Anatomie der Wirbeltiere. Herausgeg. v. Dr. A. Oppel. 5. Teil. Jena.

Stutinski, F.

1936. Effets de l'éclairement continu sur la structure de la glande pituitaire de la grenouille. Compt. Rendus Soc. Biol., Paris, 123: 421-423.

Thiéblot, L.

1954. Le role fonctionnel de l'épiphyse. Revue Canadienne de Biologie: 189-207.

Wislocki, G. B., & E. W. Dempsey

1948. The chemical histology and cytology of the pineal body and neurohypophysis. Endocrinology, 42: 56-72.

Woitkewitsch, A. A.

1944.1 Influence of light upon thyrotropic activity of anterior lobe of pituitary body. Comp. Rendus Acad. Sci. U.R.S.S. 45: 357-360.

1944.2 Function of thyroid as dependent on pituitary body activized by light. Comp. Rendus Acad. Sci. U.R.S.S., 45: 396-400.

1946. Phenomenon of functional exhaustion of certain endocrine glands (thyroid gland and anterior lobe of pituitary body). Compt. Rendus Acad. Sci. U.R.S.S., 51: 729-732.

YOUNG, J. Z.

1935. The photo-receptors of lampreys. II. The function of the pineal complex. Jour. Exper. Biol., 12: 254-270.

EXPLANATION OF THE PLATES

PLATE I

Transverse sections of the pineal body of *Astyanax mexicanus*. Fix. Bouin. Masson's Trichrome Stain. Magnification 340×.

FIG. 1. Fish kept under the normal change of daylight and night. The lumen is extremely narrow. The epithelial folds are deep. The cells in the epithelium surrounding the lumen are closely arranged. Richly vascularized connective tissue fills the spaces between the folds.

FIG. 2. Fish kept in darkness (8 weeks). The lumen is wide. The epithelial folds are less deeply invaginated.

FIG. 3. Blinded fish kept in darkness (30 weeks). The lumen is wide. The cells in the epithelium are less numerous and less closely connected than in Text-figure 1 A and B.

PLATE II

Accumulation of glycogen in the ventral part of the pineal body in *Astyanax mexicanus*. Transverse sections. Fix: Dioxan—Picric Acid—Formal—Glacial Acetic Acid. Stain: Best's Carmine. Magnification: 1000×.

FIG. 1. Glycogen content in the pineal body of *Astyanax mexicanus* kept under the normal change of daylight and night.

FIG. 2. Glycogen content in the pineal body of *Astyanax mexicanus* kept in darkness (15 weeks).

FIG. 3. Glycogen content in the pineal body of *Astyanax mexicanus* after 15 weeks in constant light.

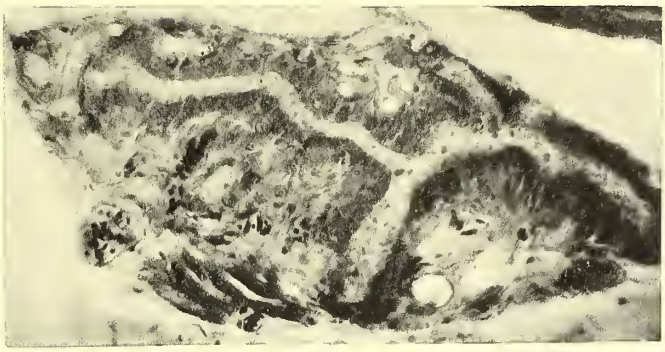


FIG. 1

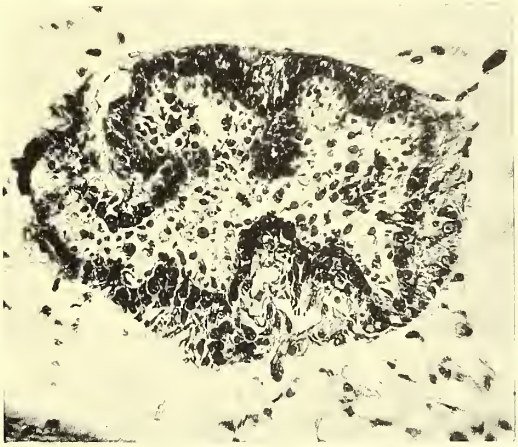


FIG. 2

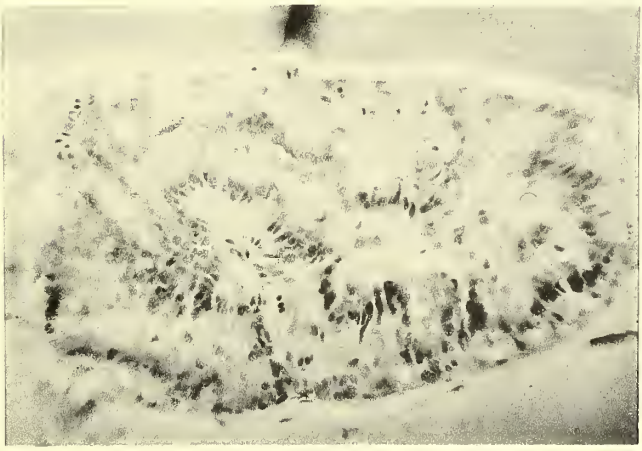


FIG. 3

INFLUENCE OF LIGHT AND DARKNESS ON THE PINEAL BODY
IN ASTYANAX MEXICANUS (FILIPPI)

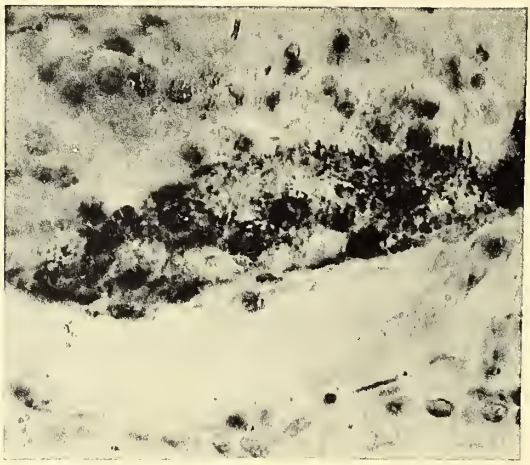


FIG. 1

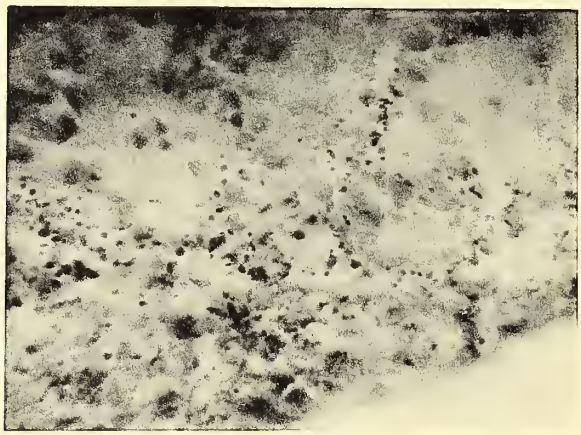


FIG. 2

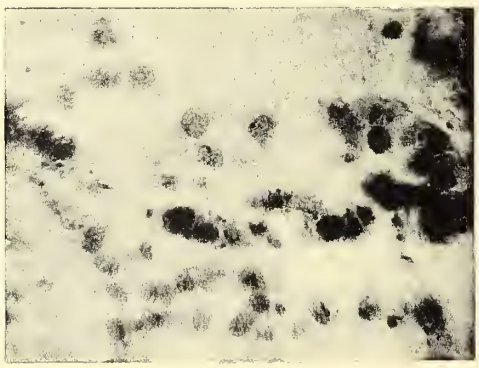


FIG. 3

INFLUENCE OF LIGHT AND DARKNESS ON THE PINEAL BODY
IN ASTYANAX MEXICANUS (FILIPPI)

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 41 • PART 4 • DECEMBER 31, 1956 • NUMBERS 16 TO 19

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

16

Aeration in Aquaria

A. L. Downing & G. A. Truesdale

Water Pollution Research Laboratory, Stevenage, Herts., England

(Text-figures 1-5)

Introduction

It is well known that to keep fish alive and healthy in aquaria it is necessary to ensure that the water contains an adequate concentration of dissolved oxygen. This can usually be arranged without a detailed knowledge of the physical and biochemical processes governing the level of dissolved oxygen attained under given conditions. In some cases when, for instance, there are few fish in a large volume of water with a large exposed surface and containing perhaps a few green plants, there is no apparent need to provide additional aeration. In other cases all that may be required is a stream of air bubbles passed through the water. In current research on the development of tests for the toxicity of effluents to be discharged into surface waters, however, it is sometimes desirable not merely to keep fish adequately supplied with oxygen, but also, since the toxicity of certain poisons is known to depend on the level of dissolved oxygen (Downing, 1954), to keep this concentration within prescribed limits without at the same time causing undue loss of volatile constituents. This demands a more detailed knowledge of the aeration process and of the other factors affecting the concentration of dissolved oxygen.

Factors Affecting the Concentration of Dissolved Oxygen

The concentration of dissolved oxygen is determined by the relative magnitudes of the rates of supply and demand. On the one hand oxygen is consumed by the respiration of fish and plants, if these are present, and possibly, to a much lesser extent, by the bio-oxidation of metabolic products; on the other it is re-supplied to the water by absorption from the atmosphere and by the photosynthetic activity of plants. The assumption of Breder (1931) that photosynthesis has little influence on the concentration of dissolved oxygen cannot be accepted as an accurate generalization, although it is true that in many cases its role may be unimportant. The difficulty is that its influence cannot often be calculated quantitatively because of an inadequate knowledge of the rates of consumption or evolution of oxygen by fish or plants under given conditions. Nevertheless approximate average values of these rates for certain fishes and plants are known, and they may be used when applicable to predict the variation of dissolved oxygen in an aquarium. Before this can be done, however, the rate of absorption of oxygen from the air must be measured or estimated.

Absorption of Atmospheric Oxygen by Water

In quiescent conditions oxygen penetrates into water by molecular diffusion in accordance with Fick's law. In moving water this process is augmented by turbulent mixing of the surface layers with the main body of the liquid and by the creation of fresh surfaces. It is proposed to consider the aeration of aquaria in which the general level of turbulence, created by the devices used to promote aeration, by the movement of fish, and by miscellaneous minor disturbances is sufficient to maintain a sensibly uniform concentration of dissolved oxygen at all points below the outermost surface layers, which are assumed to be saturated with oxygen. It seems very probable that this condition will be approximately satisfied in most small aquaria (not deeper than, say, about 1 metre) since experience shows that, unless special precautions are taken to control vibrations and the temperature and humidity of the air above the surface, gradients in the oxygen concentration

are not detectable even in apparently stagnant water. This is largely due to "streaming" resulting from density changes caused by cooling of the surface layers by evaporation. In very large aquaria in which aeration was promoted by a stream of bubbles liberated a long way from the center of the bottom of the tank, such conditions might not apply. In this case, however, the rate of change of oxygen concentration at any point would be governed by random movement of the fish and any other incidental disturbances; since this would be largely unpredictable it will not be considered further.

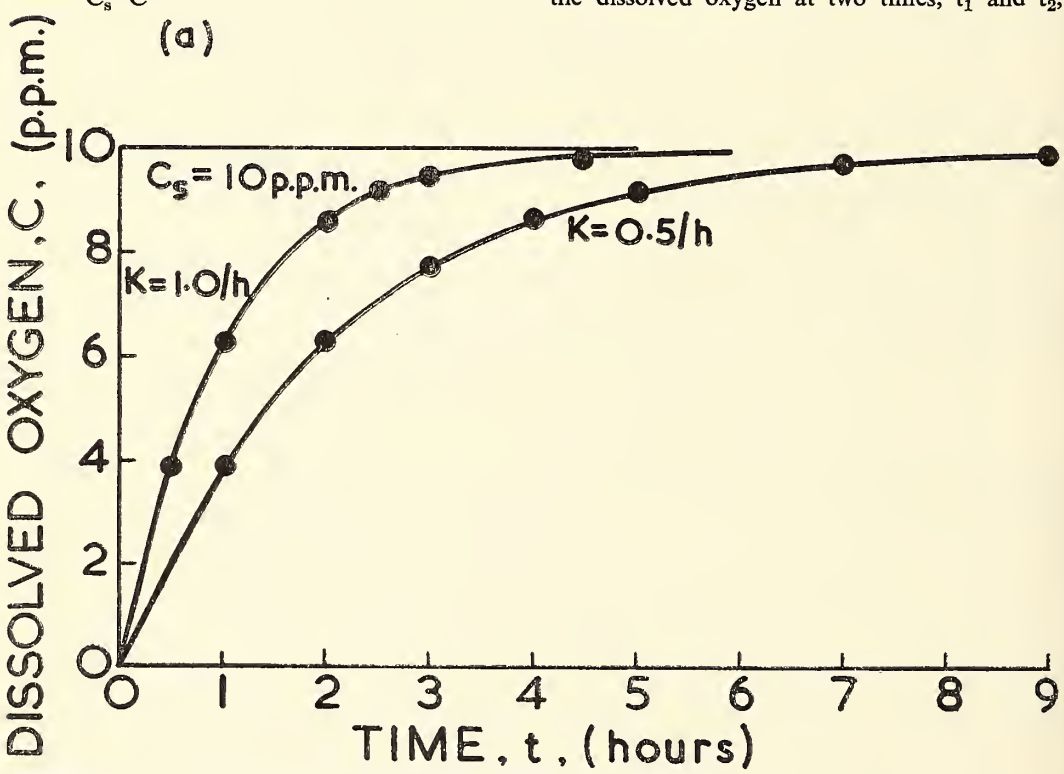
For aquaria in which there is reasonably uniform mixing it is found that the rate of change of dissolved oxygen at any time, t, is directly proportional to the difference between the saturation concentration, C_s, and the concentration, C, in the water (Lewis & Whitman, 1924). That is,

$$\frac{dC}{dt} = K\,(C_s - C) \quad \text{.....(1)}$$

and, integrating,

$$\log_e \frac{C_s}{C_s - C} = Kt \quad \text{.....(2)},$$

where K, the over-all absorption coefficient, measures the rate of change of concentration of dissolved oxygen when the saturation deficit, $C_s - C$, is unity. K has dimensions T^{-1} and is generally expressed in reciprocal hours. Thus the increase of dissolved oxygen with time, due to aeration, follows an exponential curve, the exact form of which depends on the magnitude of the absorption coefficient (Text-fig. 1a). There is however no simple method for predicting accurately the magnitude of K from a knowledge of the physical characteristics of the system; such theories as there are demand a knowledge of hypothetical or difficultly measurable quantities such as the thickness of the stagnant film at the water surface (Lewis & Whitman, 1924), the time of exposure of freshly created surfaces (Higbie, 1935; Pasveer, 1953), or the rate of mixing of the water (Phelps, 1947). Fortunately, however, if it is assumed that the effect on the rate of aeration due to the movement of fish is negligible, as will generally be the case except perhaps in stagnant water, then the rate of aeration can be measured directly in the aquarium in their absence. This is conveniently done by measuring the dissolved oxygen at two times, t_1 and t_2,



TEXT-FIG. 1(a). Rate of solution of atmospheric oxygen in water.
Temperature 13.90° C.
Solubility of oxygen, C_s, from moist air = 10 p.p.m.
Equation of absorption curves: $\log_e \frac{C_s}{C_s - C} = Kt$.

after partial deoxygenation of the water, followed by substitution of the values in an equation, derived from Equation (1), of the form

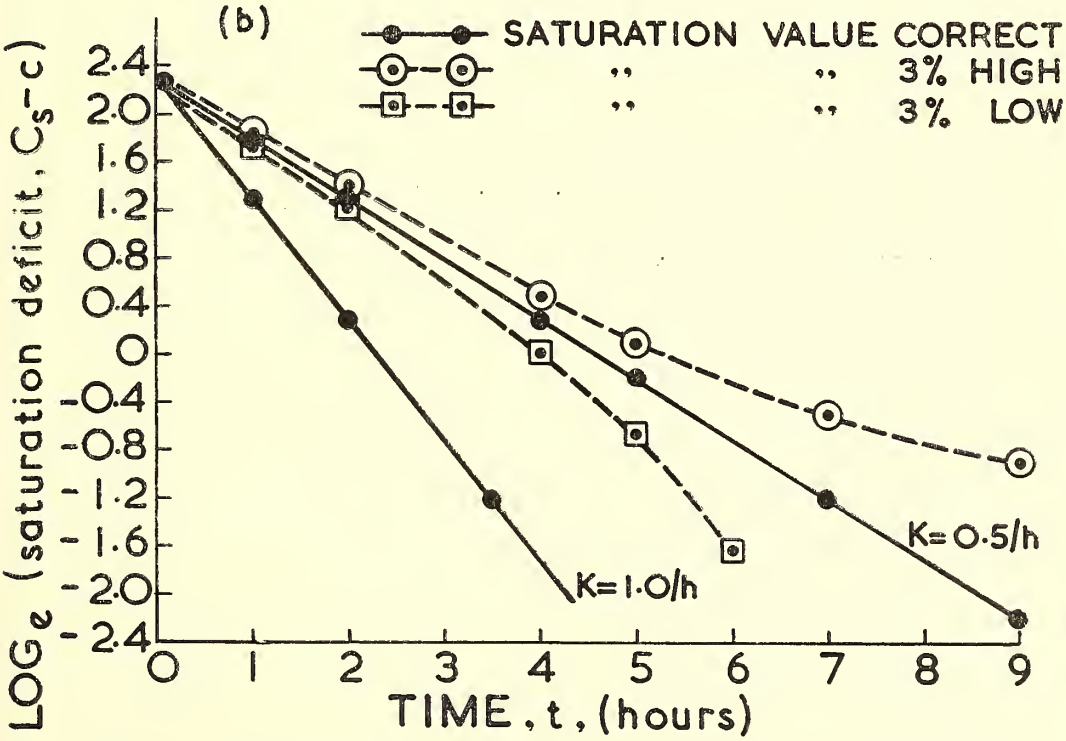
$$\log_e \frac{C_s - C_1}{C_s - C_2} = K(t_2 - t_1) \quad \text{.....(3)},$$

where C_1 and C_2 are the oxygen concentrations at t_1 and t_2. Alternatively K may be evaluated from the slope of the straight line obtained by plotting the logarithm of periodic observations of the saturation deficit against time. The importance of having an accurate knowledge of the solubility (Truesdale, Downing & Lowden, 1955) for these computations is demonstrated in Text-fig. 1b.

The value of K obtained in the manner indicated does however depend on the volume of water, V, and the area of the exposed surface, A, the ratio V/A being sometimes referred to as the "aeration depth." In considering the range of values in which the rate of aeration may vary under different conditions, it is therefore convenient to use a transfer coefficient, f, (sometimes called the "exit coefficient," (Gameson, Truesdale & Downing, 1955)) defined by the relation

$$K = \frac{f.A}{V}, \quad \text{.....(4)}$$

which measures the rate of change of dissolved oxygen in a unit depth of water when the saturation deficit is unity. It has dimensions LT^{-1} and is usually expressed in cm./h. It is also equivalent to the amount of oxygen passing through unit area of surface in unit time when the deficit is unity, 1cm./h. being equivalent to 1μg./cm.^{2}, p.p.m., h. For a system having f = 10cm./h. and an aeration depth 10 cm., the over-all absorption coefficient would be 1.0/h. so that the rate of solution when the deficit was 10 p.p.m. would be 10 p.p.m./h. In making predictions of this kind it is of course tacitly assumed that the mass transfer coefficient is unaffected by changes in the aeration depth provided there is no obvious difference in the conditions of agitation at the surface from those for which the coefficient was measured. This is generally only approximately true for limited changes in V/A, and if an accurate knowledge of the rate of aeration in a particular system is required it is much safer to determine this directly in the manner indicated. The published values of mass-transfer coefficients for different aeration systems show that they are extremely dependent on the degree and type of agitation in the water and may vary between values of less than 1.0 cm./h. for stagnant water to values



TEXT-FIG. 1(b). Linear variation of the logarithm of the saturation deficit with time; effect of incorrect saturation values.

of the order of 500 cm./h. for rapidly stirred water (Table 1).

The mass-transfer coefficient is also dependent on the temperature, the concentration of dissolved salts, and the condition of the surface. For instance, in the temperature range 0 to 35° C., f increases by between 2.2 and 2.3 per cent. of the value at 20° C. per degree Centigrade increase in temperature (Downing & Truesdale, 1955). On the other hand it appears to be reduced by dissolved salts since the rate of solution in pure sea water and in sodium chloride solution of the same salinity is lower than that in pure water under the same conditions (Department of Scientific and Industrial Research, 1956). Soluble or insoluble surface contaminants or surface-active agents also generally cause a decrease in the rate of solution although the magnitude depends very much on their concentration and the conditions of agitation (Downing & Truesdale, 1955).

Prediction of Dissolved Oxygen Concentrations in Aquaria

Given a knowledge of the rate of absorption of oxygen from the air and the rate of absorption or production of oxygen by respiration or photosynthesis, we are in a position to make approximate calculations of the variation of dissolved oxygen concentration with time. To simplify matters we may assume that fish and plants consume oxygen at a constant rate K_1 p.p.m./h. and K_2 p.p.m./h. respectively, and that during daylight oxygen is produced by photosynthesis at a constant rate K_3 p.p.m./h.

The rate of change of concentration of dissolved oxygen is given by

$$\frac{dC}{dt} = K(C_s - C) - K_1 - K_2 + K_3 \quad \text{....(5)}$$

and

$$\log_e \left\{ \frac{K(C_s - C_o) - K_1 - K_2 + K_3}{K(C_s - C) - K_1 - K_2 + K_3} \right\} = Kt \quad \text{.....(6),}$$

so that

$$C = e^{-Kt} C_o + \frac{(1 - e^{-Kt})\,(KC_s - K_1 - K_2 + K_3)}{K} \quad \text{.....(7),}$$

where K is the over-all absorption coefficient and C_o is the initial concentration of dissolved oxygen.

Table 1. Observed Values of the Mass-Transfer Coefficient, f, for Oxygen in Stagnant and Agitated Water

Description of aeration system	Approximate value or range of values taken by mass-transfer coefficient, f. (cm./h.)	Reference
Stagnant water	0.2 — 0.5	Holroyd & Parker (1952)
Slowly stirred water: no appreciable disturbance of the surface	1.0 — 5.0	Downing & Truesdale (1955)
Slowly stirred water: slight disturbance at the surface	1.0 — 10	do.
Rapidly stirred water: regular vortices at the surface	10 — 50	do.
Rapidly stirred water: irregular vortices and severely disturbed surface	*50 — 500	do.
Regular progressive waves (2.8-10.2 cm. high) in shallow water	*10 — 37	do.
"Choppy" waves (6.4-13.3 cm. high) in shallow water	*11 — 25	do.
Shallow and turbulent Lakeland beck	*30 — 200 (Depending on flow, depth, and roughness of bed)	Gameson, Truesdale & Downing (1955)
"Model Stream" flowing in channels, velocity 0.6-15 m./min.	1 — 30	Streeter, Wright & Kehr (1936)
Water surface ruffled by tangential air stream, velocity, measured near the surface, increasing to 13.5 m./sec.	1.5 — 63	Downing & Truesdale (1955)
Diffused air bubbles, 1-5 mm. in diameter	180	Holroyd & Parker (1952)
Diffused air bubbles, 2.4 mm. in diameter	Ca. 90	Scouller & Watson (1934)
Diffused oxygen bubbles, 2 mm. in diameter	96	Ippen & Carver (1954)

*Nominal values calculated using an aeration depth determined from the volume of water and the area of the undisturbed surface.

The use of this equation may be illustrated by inserting in it some typical values of the variable quantities.

Consider first the case in which no plants are present in the aquarium so that K_2 and K_3 are zero; suppose that the volume of fresh water is 100 litres with an exposed surface of 5,000 sq.cm., that this contains 10 brook trout each weighing 25 gm. and consuming oxygen at a rate of 100 ml. oxygen/Kg. fish, h., a reasonably approximate figure (Graham, 1949). Suppose further that the temperature is 13.9° C. and that the barometric pressure is 760 mm. Hg, so that the solubility of oxygen is 10 p.p.m. (Truesdale, Downing & Lowden, 1955). If no additional aeration is provided the mass transfer coefficient for the entry of oxygen through the surface will be approximately 1.0 cm./h. (Table 1). Thus

$$K = \frac{5 \times 10^3}{10^2 \times 10^3} = 0.05/\text{h}.$$

and

$$K_1 = \frac{10 \times 25}{1000} \times 100 \times 1.43 \times \frac{1}{100} = 0.36 \text{ p.p.m./h}.$$

The oxygen concentration will therefore tend to an equilibrium value given from Equation 5 when

$$\frac{dC}{dt} = 0,$$

such that

$$0.05(10-C) = 0.36$$
$$C = 2.8 \text{ p.p.m.},$$

which is approaching a dangerously low level for trout. In theory the equilibrium concentration will be reached only after an infinite time, but if the water is initially saturated with oxygen it can be shown, by substitution in Equation (5), that a concentration within about 0.1 p.p.m. of the equilibrium value will be reached after time, t, given from Equation (6) by

$$t = \frac{1}{0.05} \log_e \left(\frac{-0.36}{(0.05)\ (7.1) - 0.36} \right)$$
$$= 85.5 \text{ hours}$$

and that the variation of dissolved oxygen with time will follow curve AB (Text-fig. 2). Thus even in the short space of a day, the oxygen concentration would be lowered to half its initial value.

Validity of the Theoretical Relations

At present there is little or no evidence to support the validity of the hypothetical conclusions deduced from Equations (5), (6) and (7). Some preliminary experimental studies with rainbow trout do, however, indicate that approximately correct predictions of the average concentration of dissolved oxygen in an aquarium can be made in this way (Alabaster, 1956). It is found that the oxygen concentration at any instant fluctuates about the average value between quite wide limits. This is presumably due to variations in both the rate of solution of oxygen and the oxygen consumption of the fish. If the approximate variability in these two quantities is known we can determine the probable variation in the dissolved oxygen. Thus suppose that the random variations of K and K_1 about their mean values are represented by standard errors aK, and bK_1, then the variation in the equilibrium concentration of dissolved oxygen for an aquarium containing only fish may be expressed as the standard error, σ, in the quotient K_1/K in the equation $C = C_s - K_1/K$, derived from (5), i.e., by

$$\sigma = K_1/K \sqrt{(a^2 + b^2)}.$$

Thus the variation in the equilibrium dissolved oxygen concentration will increase as K decreases or as K_1 increases; that is, it will increase as the equilibrium concentration decreases.

For instance, in the case where $K = 0.05$, $K_1 = 0.36$, $a = b = 0.1$ and $C_s = 10$, we have

$$\sigma = 7.2 \sqrt{0.02}$$
$$= 1.02$$

Thus the mean equilibrium concentration and its standard error will be 2.8 and 1.02 p.p.m. For $K = 0.5$,

$$\sigma = 0.72 \sqrt{0.02}$$
$$= 0.1,$$

so that in this case the error in the equilibrium concentration, $C = 9.28$ p.p.m., will be only 0.1 p.p.m.

Adjustment of Conditions to Satisfy Oxygen Demands

The reduction of the oxygen concentration to a dangerously low level may be prevented by (i) reducing the population of fish, (ii) decreasing the aeration depth while keeping the volume constant, (iii) increasing the partial pressure of oxygen in the gas phase, (iv) changing the water at intervals or continuously, (v) increasing the rate of absorption of oxygen from the atmosphere, or (vi) making use of photosynthesis to produce oxygen. The first two possibilities (i) and (ii) are straightforward and will not be considered further.

Increasing the partial pressure of oxygen. (iii).

This will increase the saturation concentration of oxygen in the water, in accordance with Henry's law, so that for a given deficit, $C_s - C$, the concentration of dissolved oxygen will be greater.

Replacement of water. (iv).

If, as may sometimes be the case in toxicity tests, the volume of solution available for replacement is limited and we wish to maintain the oxygen concentration between prescribed limits, the minimum frequency of periodic replacement can be ascertained by substituting the appropriate values in Equation (6). Alternatively, the rate of change of oxygen concentration that would result from replacing the water in the aquarium continuously by water, saturated with oxygen, at a rate, v litres/h., assuming rapid and complete mixing, would be given by

$$dC/dt = v/V(C_s - C) \quad \text{.....(8)},$$

where V is the volume of the aquarium. Thus to maintain a given concentration, C, would require the water to be replaced at a rate given by

$$\frac{dC}{dt} = 0 = K(C_s - C) + \frac{v}{V}(C_s - C) - K_1 - K_2 + K_3 \quad \text{.....(9)}$$

so that

$$v = V\left[\frac{K_1 + K_2 - K_3}{(C_s - C)} - K\right] \quad \text{.....(10)}.$$

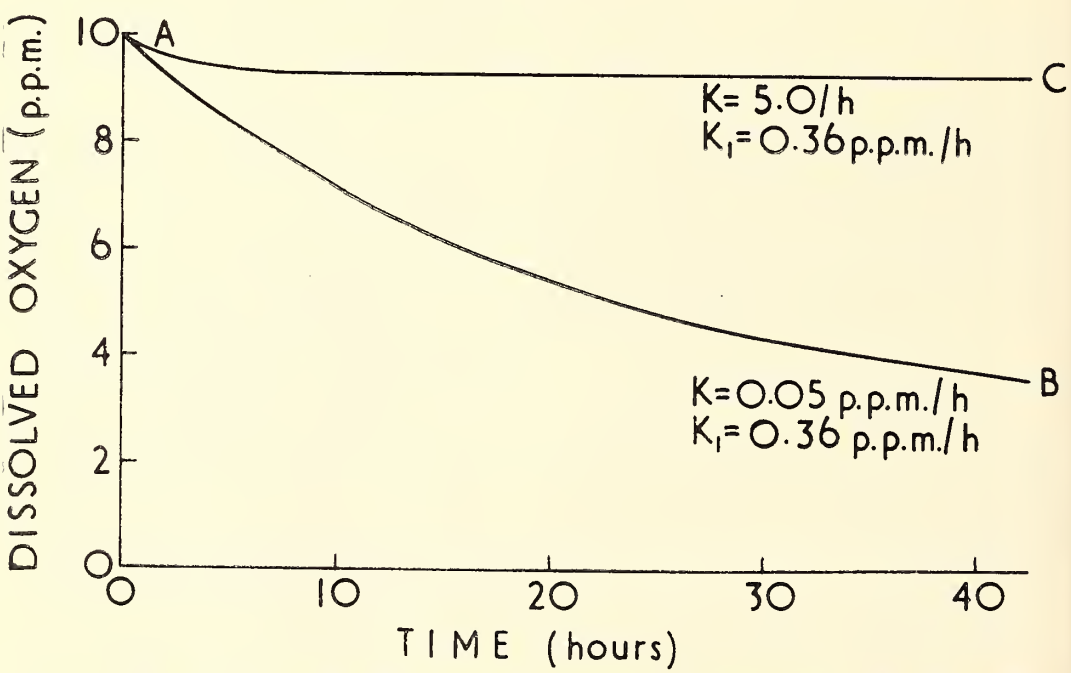
To maintain an equilibrium concentration C, of say 6 p.p.m., when $K_1 = 0.36$ p.p.m./h. and $K_2 = K_3 = 0$, would require a rate of replacement

$$v = 100\left(\frac{0.36}{4} - 0.05\right) = 4 \text{ l./h.}$$

These rates of replacement refer only to dissolved oxygen requirements, of course. In practice other factors such as the accumulation of noxious metabolic products might necessitate more frequent replacement.

Increasing the rate of aeration. (v).

If we prefer to increase the rate of aeration, then the minimum rate required to prevent the deficit, $C_s - C$, exceeding a given value D is given by $KD = 0.36$, ($K_2 = K_3 = 0$). For instance, if the previous equilibrium deficit is to be reduced from 7.2 p.p.m. to say 0.72 p.p.m., then K must be increased to 5.0/h. Once K had been increased to this value the variation of oxygen concentration with time would follow curve AC (Text-fig. 2). As already indicated, this rate of aeration could be obtained in several ways; in practice the most common and easiest method is to bubble air through the water. The approximate order of frequency of bubbles and rate of flow of air required may be determined using data published in the literature. For in-



TEXT-FIG. 2. Effect of the respiration of fish on the variation of concentration of dissolved oxygen in aquaria.

K: over-all absorption coefficient.

K_1: rate of consumption of dissolved oxygen by fish.

stance the average mass-transfer coefficient for streams of bubbles (from a porous diffuser) having an average radius of about 1 to 2 mm. has been found to be of the order of 200 cm./h. (Holroyd & Parker, 1949; Datta, Napier & Newitt, 1950), so that if we wish to increase K from 0.05 to 0.50, neglecting the effect of the bubbles on the aeration at the surface, the total surface area of the bubbles that must be present instantaneously is given from Equation (4) by

$$0.45 = \frac{200 \times A}{10^2 \times 10^3}.$$

$$\therefore \quad A = 225 \text{ sq. cm.}$$

Thus if we assume for this purpose that all the bubbles have a radius of 1 mm., the total number, N, that must be instantaneously present is $225/4\pi(0.1)^2$, that is, about 1,900. If the velocity of the bubbles is for simplicity assumed to be constant at the approximate terminal velocity for this size of bubble, 20 cm./sec. (Ippen & Carver, 1954), then the required frequency of production of bubbles, F, is given by

$$F = N \times \frac{\text{depth}}{\text{velocity}} = N \times 20/20, \text{ whence}$$

$$F = 1900 \text{ bubbles/sec.}$$

This corresponds to an airflow of about 475 ml./min.

In practice, it would be necessary to make preliminary trials with diffusers of different sizes and porosities to select one that gave bubbles of the size required, since the size is found to vary with porosity, loading, and the nature of the liquid.

It will of course be appreciated that this simple treatment neglects many factors such as the variation with depth of water of the bubble size and velocity as well as the partial pressure of oxygen within the bubble and hence of the saturation concentration of oxygen at the bubble interface. An interesting and more rigorous treatment of this subject is given by Pasveer (1954).

Production of oxygen by photosynthesis. (vi).

When living aquatic plants containing chlorophyll are illuminated, oxygen is produced by photosynthesis as a result of a complex series of reactions conventionally summarized by the equation

$$6\ CO_2 + 6\ H_2O + 675\ \text{K cals} \rightarrow C_6H_{12}O_6 + 6\ O_2 \quad \text{.....(11)}$$

The energy absorbed is supplied by the light.

The mechanism of this process is extremely complicated and is by no means fully understood. For a given plant the rate of production of oxygen depends upon the conditions of illumination, the concentration of carbon dioxide and on other physical variables such as temperature and turbulence. It is also a function of many physiological factors responsible for varied and as yet unpredictable phenomena such as resting, fatigue, aging, inhibition, adaptation and the like. The difficulties encountered in studies of the kinetics of such a complex process in living organisms can hardly be exaggerated, and indeed doubts have often been expressed as to the possibility of deriving significant kinetic relationships. Nevertheless, at least for short periods, the rate of photosynthesis appears to remain constant in a constant environment, and it is proposed to illustrate the influence of the process on the balance of oxygen in an aquarium under such conditions.

With both the two main variables, light and carbon dioxide, a saturation level is reached above which increasing the intensity or concentration causes no change in the rate of photosynthesis, which is at a maximum with respect to these two factors. Measurements of maximum photosynthetic activity recorded in the literature relate to conditions in which at least by intention neither light nor carbon dioxide was limiting. We shall consider the effect due to an aquatic plant of tropical origins, *Valisneria spiralis* (commonly found in indoor aquaria in the United Kingdom and elsewhere), leaves of which, 30 cm. in length, were observed to produce 0.63 mg. oxygen per hour per leaf during daylight (Manning, Juday & Wolf, 1938). Assuming the leaves to be about 1 cm. wide, this rate of production is equal to about 2 mg. O_2/h., 100 cm.^{2}, which is within the range of values 1.3 — 7 mg. O_2/h., 100 cm.^{2} observed for other aquatic plants (Rabinowitch, 1951, p. 961).

In addition to producing oxygen when illuminated, the living plant is, of course, continuously respiring and for any given conditions the ratio of respiration to photosynthesis governs the level of oxygen in an aquarium. On the average the *rate* of respiration appears to be about 5-15 per cent. of photosynthesis, according to Rabinowitch (1951, p. 7) and Verduin (1952). For detailed information on photosynthesis the reader is referred to the standard text on the subject by Rabinowitch (1951).

Let us suppose the aquarium contains 70 leaves of *Valisneria spiralis*, producing oxygen at a constant rate of 0.63 mg. O_2/h., leaf during daylight and consuming oxygen by respiration at about 10 per cent. of this rate. Then $K_2 =$ 0.04 p.p.m./h. and $K_3 =$ 0.44 p.p.m./h. (The total volume of water is taken to be 100 litres, as before.)

Case I. If the rate of aeration was small (K = 0.05/h.) and the water was initially

saturated with oxygen, then with the aquarium always in darkness (*i.e.*, $K_3 = 0$), the dissolved oxygen would fall toward an equilibrium value of 2 p.p.m. (Equation 5) in accordance with curve ABHI (Text-fig. 3) which has been deduced from Equation (7).

Case II. If on the other hand, the aquarium was continuously illuminated and initially saturated with oxygen, it would become supersaturated, the concentration varying in accordance with curve AJ (Text-fig. 3), reaching an equilibrium value, C = 10.8 p.p.m., given from Equation (5) by

$$\frac{dC}{dt} = 0 = 0.05\ (10-C) - 0.36 - 0.04 + 0.44.$$

It should be noted that in this case an increase in the rate of aeration, K, to, say, 0.5/h., would reduce the degree of supersaturation to the equilibrium concentration C = 10.08 p.p.m. If the concentration of dissolved oxygen was initially less than the saturation value, then upon continuous illumination it would approach the equilibrium value along a curve of the type BCFG (Text-fig. 3).

Case III. Considering a more typical day and night cycle, if the water was initially saturated, then during eight hours' darkness the dissolved oxygen would fall to about 7.4 p.p.m. in accordance with curve AB (Text-fig. 3), deduced from Equation (7), but would recover during daylight to 9.2 p.p.m., to fall again at night to 6.9 p.p.m. Finally an equilibrium would be established in which the maximum concentration, C_D, during daylight and the minimum concentration, C_N, during the night would be given by the simultaneous equations,

$$\log_e \left(\frac{K(C_s - C_D) - K_1 - K_2}{K(C_s - C_N) - K_1 - K_2} \right) = Kt_1 \quad \text{.....(12)}$$

$$\log_e \left(\frac{K(C_s - C_N) - K_1 - K_2 + K_3}{K(C_s - C_D) - K_1 - K_2 + K_3} \right) = Kt_2 \quad \text{.....(13)},$$

where $t_1 = 8$ hours (darkness), $t_2 = 16$ hours, and the other symbols have the same significance and values as before. Thus $C_D = 8.9$ p.p.m. and $C_N = 6.6$ p.p.m. Therefore the addition of plants to the water under these conditions prevents the concentration of dissolved oxygen from becoming dangerously depleted.

It is not the case, however, that adding plants to an aquarium will necessarily improve conditions for fish. In certain circumstances the concentration of oxygen may fall during the night below the equilibrium (minimum) concentration C_E, attained in the absence of plants. This would be undesirable if, as a result, the concentration fell below the minimum that could be tolerated by the fish. By combining the equation

$$C_E = C_s - K_1/K \quad \text{.....(14)}$$

with Equations (11) and (12), the relation between C_E and C_N becomes

$$C_N = C_E + K_3/K\ [(1 - e^{Kt_2})/(1 - e^{24K}) - K_2/K_3] \quad \text{.....(15)}$$

where $C_N, C_E \geqslant 0$.

Thus, $C_N \geqslant C_E$, provided that $(1 - e^{Kt_2})/(1 - e^{24K}) \geqslant K_2/K_3$. The variation of the function $(1 - e^{Kt_2})/(1 - e^{24K})$, which for convenience will be denoted as F, with the over-all absorption coefficient, K, for three different values of the period of illumination, t_2, is shown graphically in Text-fig. 4(a). It will be seen that when the over-all absorption coefficient, K, exceeds the values 0.28, 0.19, and 0.12 (according as $t_2 = 16$, 12 or 8 hours), the function F becomes less than 0.1 and therefore, if, as we have assumed $K_2/K_3 \cong 0.1$, the expression $(F - K_2/K_3)$ becomes negative, so that the minimum concentration during darkness, C_N, falls below C_E.

It will also be evident that for any given value of K, the tendency for C_N to exceed C_E increases as the period of illumination increases, which is perhaps obvious without mathematical derivation. On the other hand the conclusion that the tendency for C_N to fall below C_E increases with increase in the absorption coefficient for solution of oxygen from the air, may at first sight appear more surprising. This arises, however, because for given values of K_1, K_2, and K_3, as the absorption coefficient is increased, the level of the dissolved oxygen concentrations approach nearer to the saturation value, and the amount of oxygen dissolved from the air during daylight may be small by comparison with that dissolved when, although the absorption coefficients are lower, the oxygen deficits are higher and *in proportion* are not reduced so much by the oxygen liberated in photosynthesis. Thus there is not so much opportunity to build up a "reserve" of dissolved oxygen when the absorption coefficient is high, so that when illumination ceases, the dissolved oxygen concentration may quickly fall below the equilibrium value, C_E, that would have been attained in the absence of plants. However, by the same token, as the concentration of dissolved oxygen falls, the oxygen deficit, and hence the rate of solution of oxygen, increases much more rapidly, in proportion, when the absorption coefficient is high than when it is low and the deficits are much greater, so there is less tendency for C_N to fall so far below C_E, when K is high. Substitution of the values for

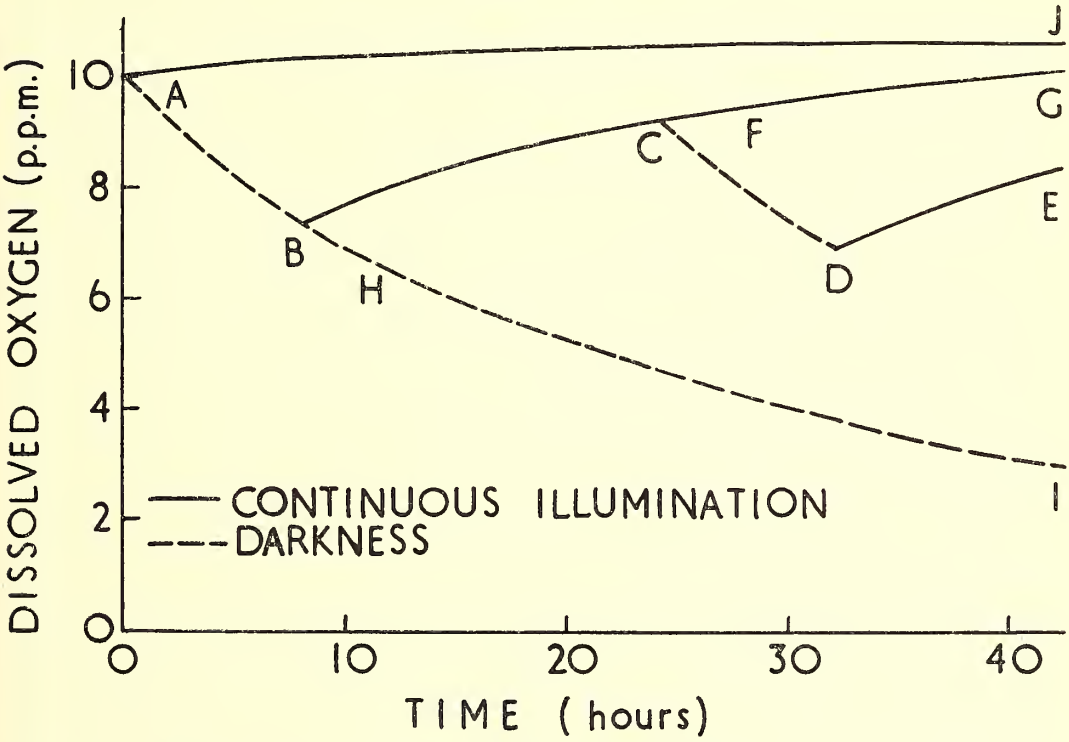
factor, F, from Text-fig. 4(a) in Equation (15) will show that, for given values of t_2, K_1, K_2, and K_3, as K is increased above the "critical values" at which $C_N = C_E$, then the difference $C_E - C_N$ at first increases (since the factor F decreases and $[F - K_2/K_3]$ increases) but then rapidly begins to decrease, since for values of $K > 0.5$, F becomes negligibly small and Equation 15 reduces to

$$C_E - C_N \cong 0.1\ K_3/K \quad \text{.....(15a).}$$

It will also be seen from Equations (15) and (15a) that the difference, $C_E - C_N$, which is positive or negative depending on the value of K, increases directly in proportion to K_3, the rate of photosynthesis. For instance for $t_1 = t_2 = 12$ hours, $K_2/K_3 = 0.1$, $K = 0.1$, C_N exceeds C_E by 1 p.p.m., for $K_3 = 0.75$ p.p.m./h. and K_3 must be increased by 0.75 p.p.m./h. for each increase of 1 p.p.m. in the difference $C_N - C_E$. On the other hand, for $t_1 = t_2 = 12$ hours, $K_2/K_3 = 0.1$, $K = 0.5$, C_N falls below C_E by 1 p.p.m. when $K_3 \cong 10K \cong 5.0$ p.p.m./h., and for each increase of 1 p.p.m. in the difference, $C_E - C_N$, K_3 must be increased by 5.0 p.p.m./h. For the conditions cited in the example in Case III above we had $C_N - C_E = 3.8$ p.p.m. To make $C_N - C_E = 5$ p.p.m., say, it would be necessary to increase K_3 to 0.61 p.p.m./h.; that is, to increase the number of leaves of *Valisneria spiralis* from 70 to about 97.

To summarize, the major conclusion that may be drawn from the preceding discussion is that it is inadvisable to attempt to improve conditions in an aquarium having a moderate rate of solution from the air ($K \cong 0.3$ to 0.5) in combination with a relatively high rate of absorption by fish, by introducing a high rate of photosynthesis, since under these conditions the dissolved oxygen will fluctuate quite widely about an already low level and may well fall below the tolerance level at night.

Of course, it may be that fish can withstand and thrive in conditions in which the dissolved oxygen falls below the tolerance level for short



TEXT-FIG. 3. Effect of the respiration of fish and plants and the production of oxygen by photosynthesis on the concentration of dissolved oxygen in an aquarium.

Over-all absorption coefficient, $K = 0.05$/h.

Rate of consumption of dissolved oxygen:

by fish, $K_1 = 0.36$ p.p.m./h.,

by plants, $K_2 = 0.04$ p.p.m./h.

Rate of production of dissolved oxygen by photosynthesis: $K_3 = 0.44$ p.p.m./h.

periods but in which the daily average concentration is relatively high. Under such circumstances, the effect of introducing plants could be expressed in terms of the difference between C_E and the average concentration, C_A, given by $C_A = (C_N + C_D)/2$. (The true "time-average" concentration is rather complicated to work out but will not be greatly different from C_A.) The relation between the two can be shown to be

$$C_A = C_E + K_3/K\,[1/2\,(F_1) - K_2/K_3] \qquad (16),$$

where $F_1 = (1 - e^{Kt_2})\,(1 + e^{Kt_1})/(1 - e^{24K})$. The variation of the numerical values of F_1 with variation in K, for three different values of t_2, is shown in Text-fig. 4(b). It will be seen that if $K_2/K_3 = 0.1$, $C_A > C_E$ under all conditions, since $F_1 > 0.1$, and $C_A - C_E$ may be increased by increasing t_2 or K_3. For $t_2 = t_1 = 12$ hours, $K_2/K_3 = 0.1$, then $C_A = C_E + 0.4\,K_3/K$ ..... (17) so that to make $C_A - C_N = 1$ p.p.m., we must make $K_3 = 2.5$ K.

Limitations to the Kinetic Equations for Photosynthesis

In addition to the assumption already referred to on page 132, the kinetic equations for aquaria demand that (i) the water remains in the aquarium long enough for equilibrium to be established, (ii) that photosynthesis is independent of the degree of agitation of the water, (iii) that all the oxygen evolved is absorbed by the water, and (iv) that photosynthesis may be varied without restriction by changing the number or size of plants. These assumptions will be briefly considered:

(i). In a crowded aquarium, accumulation of noxious metabolic products may make frequent replacement of water necessary. In a sparsely populated aquarium or pond biochemical self-purification may considerably reduce the frequency with which replacement becomes necessary.

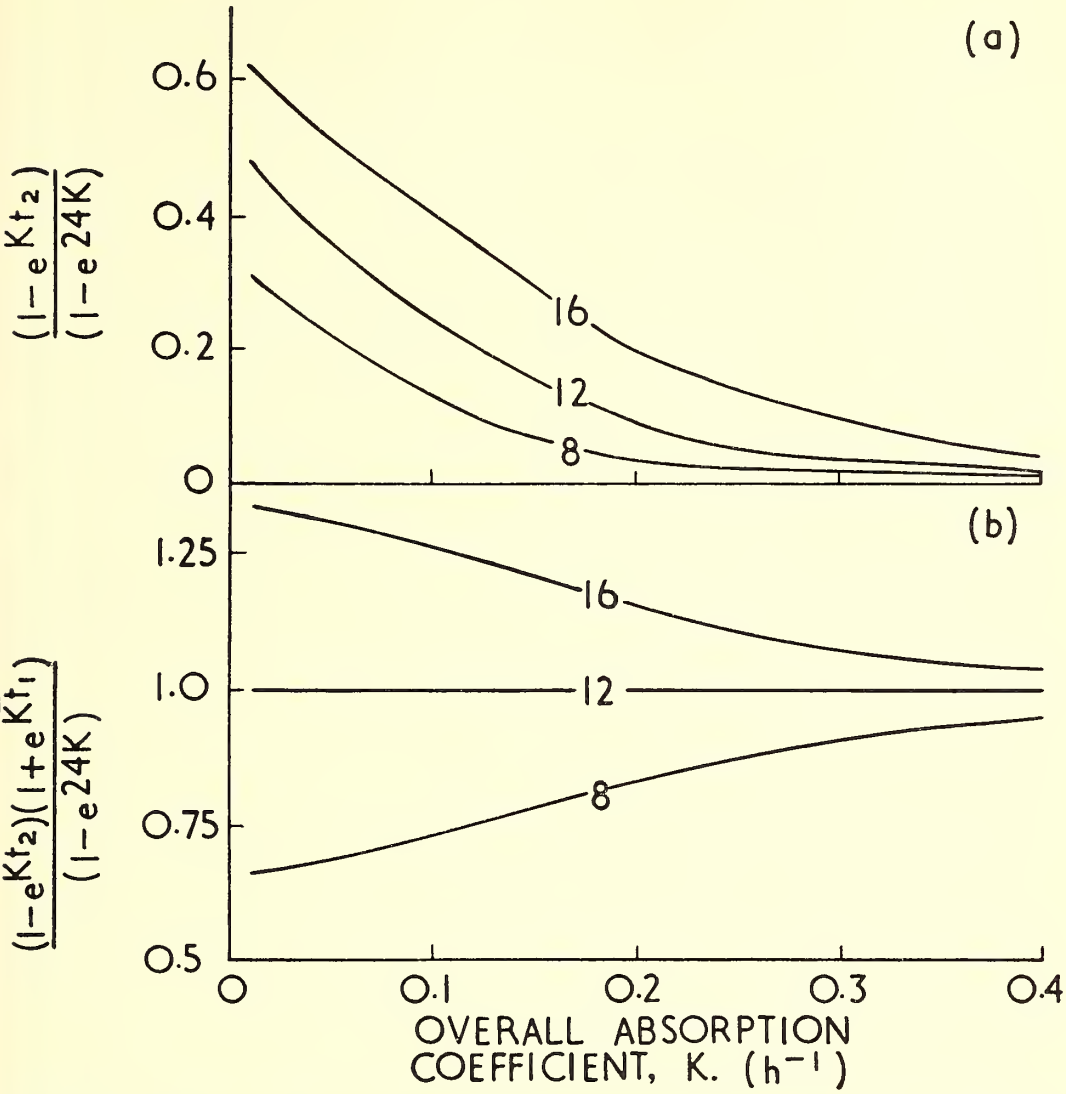
(ii). There appears to be no doubt that the rate of photosynthesis of aquatic plants depends upon the turbulence of the water, because of its effect on the rate of transport of carbon dioxide to the cells (Rabinowitch, 1951, Chapt. 27). Unfortunately, there appears to be little quantitative work on this subject, so for the present it must simply be noted as another variable to be considered.

(iii). When the rate of photosynthesis is high, oxygen may be liberated from the surface of aquatic plants in bubbles. The proportion of the liberated oxygen absorbed into the water will be a function of many factors, but it is doubtful if there is yet sufficient information to take this effect into account in aquaria.

(iv). In a given aquarium, the maximum rate of photosynthesis must inevitably depend on (a) the intensity of illumination and (b) the concentration and rate of supply of carbon dioxide.

(a). *Light intensity.*

It will be seen from Equation (11) on page 135 that 3.5 K cal. of energy are absorbed during the production of 1 gm. of oxygen. For a rate of production of oxygen of 1 p.p.m./h. (*i.e.*, $K_3 = 1.0$) in an aquarium, the equivalent rate of absorption of energy is about 84 gm. cal./litre, day. The daily amount of solar energy incident on a horizontal surface at sea level, of wave lengths which can promote photosynthesis and penetrate a smooth water surface, has been estimated by the U.S. Weather Bureau (Oswald & Gotaas, 1955). At latitude 52° the estimated monthly average amount of radiation in clear weather varies during the year from 21 gm. cal./cm.^{2}, day in December to 296 gm. cal./cm.^{2}, day in June; the corresponding average values for cloudy conditions are 4 and 176 gm. cal./cm.^{2}, day. If we consider a litre of water contained in a 10 cm. cube of glass the surface area will be 600 cm.^{2}, and assume that all this area receives radiant energy and neglect any absorption by the glass, the total amount of energy passing into the water will vary during the year from 21×600 to 196×600 gm. cal./day in bright weather, and from 4×600 to 176×600 gm. cal./day in cloudy weather. It has been calculated, however, that only some 2.0 per cent. of visible light incident on aquatic plants is utilized in photosynthesis (Rabinowitch, 1951, p. 1003), so that the net available energy for photosynthesis would vary during the year, taking the two extreme values, between $4 \times 600 \times 0.02$ and $296 \times 600 \times 0.02$ gm. cal./day, 10 cm. cube. If it is assumed that this energy is absorbed during 8 hours of daylight in December and 12 hours of daylight in June, the equivalent maximum rates of production of oxygen would be $3 \times 4 \times 600 \times 0.02/84 = 1.71$ p.p.m./h. and $2 \times 296 \times 600 \times 0.02/84 = 85$ p.p.m./h. However, the 10 cm. cube would be more similar in size to a gold-fish bowl rather than the average aquarium for which, as the size increased, the ratio of the surface area to volume would decrease, and also therefore the rate of increase of the concentration of dissolved oxygen. For instance, in an aquarium of the size of a 1 m. cube, the ratio of surface to volume would be only 1/10 that in the 10 cm. cube, and in consequence, other things being the same, the corresponding rates of production of oxygen would be 0.171 and 8.5



TEXT-FIG. 4. Values of functions in kinetic equations for photosynthetic production of oxygen. Period of daylight illumination, t_2, in hours shown against each curve.

(a) Variation of $\frac{1 - e^{Kt_2}}{1 - e^{24}}$ from the equation

$$C_N = C_B + \frac{K_3}{K}\left[\frac{1 - e^{Kt_2}}{1 - e^{24K}} - \frac{K_2}{K_3}\right]$$

with K and t_2.

(b) Variation of $\frac{(1 - e^{Kt_2})\ (1 + e^{Kt_1})}{(1 - e^{24K})}$ from the equation

$$C_A = C_B + \frac{K_3}{K}\left[\frac{1}{2}\left\{\frac{(1 - e^{Kt_2})\ (1 + e^{Kt_1})}{(1 - e^{24K})}\right\} - \frac{K_2}{K_3}\right]$$

with K and t_2.

p.p.m./h. In fact, however, the absorption of light by turbidity in the water and other "shading" effects would tend also to increase with increase in size (particularly with increase in depth), so that the actual practical values would be still further reduced. Despite this, it would appear that there is sufficient incident solar energy to support quite high rates of photo-

synthesis in summer, but that in winter, if one relies on daylight to provide energy, the maximum rate of photosynthesis is severely limited.

Carbon dioxide.

It is said that aquatic plants can assimilate carbon dioxide only in the form of neutral molecules, but there is also a possibility that in some cases bicarbonate ions may be utilized, although this contention has given rise to considerable controversy (Rabinowitch, 1951, Chapt. 27). According to Rabinowitch, the saturation level for carbon dioxide in photosynthesis occurs at concentrations in the range $1 - 10 \times 10^{-5}$ Molar, that is, 0.44 — 4.4 p.p.m.

Carbon dioxide is supplied to the aquarium by (i) absorption from the atmosphere, (ii) the dissociation and hydrolysis of bicarbonate and carbonate ions, and (iii) the respiration of fish and plants. The concentration and distribution of carbon dioxide between its various forms is governed by the constants of the following equilibria:

$$CO_2(g) \underset{k_s = 0.827}{\rightleftharpoons} CO_2(aq) \underset{k_{H_2O} = 2.2 \times 10^{-3}}{\rightleftharpoons} H_2CO_3 \underset{k_1 = 1.8 \times 10^{-4}}{\rightleftharpoons} H^+ + HCO_3^- \underset{k_2 = 5.61 \times 10^{-11}}{\rightleftharpoons} 2H^+ + CO_3^{--}.$$

N.B. Usually the apparent first dissociation constant, k_1', of carbonic acid is quoted as $k_1' = \frac{[H^+]\ [HCO_3^-]}{[CO_2] + [H_2CO_3]} = 4.54 \times 10^{-7}$.

If distilled water is allowed to come into equilibrium with normal air, it will be found to contain approximately 0.4 p.p.m. neutral carbon dioxide molecules (henceforth referred to as "free CO_2") and about 0.08 p.p.m. combined carbon dioxide present as bicarbonate ions. Owing to the small values of the constants k_{H_2O} and k_2, the quantities of carbonic acid and normal carbonate ion are negligible. Natural waters in equilibrium with normal air also contain about 0.4 p.p.m. free CO_2, but the amounts and proportion of combined carbon dioxide in the form of bicarbonate or carbonate ions depend on the pH value and alkalinity of the water. Since the concentration of free CO_2 is close to the lowest estimate of the saturation concentration for photosynthesis, it must be expected that removal of free CO_2 will tend to bring about a decrease in the rate of photosynthesis unless other factors are limiting. However, removal of free CO_2 will displace the CO_2-equilibrium and cause ionized carbon dioxide to recombine to form carbonic acid which dissociates to free CO_2, and additional carbon dioxide then dissolves from the air. This "resistance" to changes in carbon dioxide concentration, and hence to changes in the rate of photosynthesis, will increase with increase in the total combined carbon dioxide concentration, but will be governed by the rate at which the reactions influencing the equilibria take place; that is, by the rate at which carbon dioxide is made available by the processes (i), (ii) and (iii) above.

(i). If the exit coefficient for carbon dioxide is approximately the same as that for oxygen (a reasonable assumption for natural waters or tap waters but not necessarily for very alkaline solutions), then the maximum rate of supply of carbon dioxide from the air will be K times the equilibrium concentration of free CO_2, *i.e.*, 0.4K p.p.m./h. Therefore, unless the rate of aeration is relatively large, the supply of carbon dioxide from the air will not support a very high rate of photosynthesis.

(ii). When free CO_2 is removed from the system, it can be replaced by the dissociation of carbonic acid and, it is said, bicarbonate ion (Rabinowitch, 1951, Chapt. 27) in accordance with the reactions

$$H_2CO_3 \underset{k_A = 10/\text{sec.}}{\rightleftharpoons} H_2O + CO_2$$

$$HCO_3^- \underset{k_B = 0.47 \times 10^{-4}/\text{sec.}}{\rightleftharpoons} OH^- + CO_2.$$

For a water in equilibrium with the air, the concentration of carbonic acid is $0.4k_{H_2O} = 0.4 \times 2.2 \times 10^{-3} = 8.8 \times 10^{-4}$ p.p.m. Assuming that the reactions $H^+ + HCO_3^- \rightarrow H_2CO_3$ and $H^+ + CO_3^{--} \rightarrow HCO_3^-$ are virtually instantaneous, the maximum rate of supply of carbon dioxide from this source is therefore $8.8 \times 10^{-4}\ k_A$ p.p.m./sec. = 31.6 p.p.m./h.

The maximum rate of supply from the dehydration of bicarbonate ions will be $0.47 \times 10^{-4} \times 3.6 \times 10^3$ p.p.m./h., p.p.m. $HCO_3^- = 0.169$ p.p.m./h., p.p.m. HCO_3^-. Thus for a concentration of bicarbonate of 100 p.p.m. this corresponds to a rate of supply of 16.9 p.p.m./h.

These rates will tend to fall as carbon dioxide is removed from the system, since apart from the fall in the total concentration of carbon dioxide, the pH value will rise and in consequence the proportions of carbonic acid and bicarbonate will decrease. Ultimately if this trend continues and the water contains calcium ions, calcium carbonate will be precipitated so that some of the combined carbon dioxide hitherto available for photosynthesis will be effectively lost from the system. In a natural water containing an average concentration of combined carbon dioxide of the order of 100 p.p.m. or more, these changes will come about

slowly, and reasonably high rates of photosynthesis should be feasible for long periods. In distilled water, however, changes in the concentration of carbonic acid and bicarbonate ion will be directly proportional to the loss of carbon dioxide from the system, and since the total concentration initially present in water in equilibrium with the air is only about 0.5 p.p.m., even a comparatively low rate of photosynthesis may soon be restricted for lack of carbon dioxide.

Taking all these factors into account, it would seem reasonable to expect the supply of carbon dioxide from ionic dissociation to support continuously a rate of photosynthesis of at least $K_3 \cong X/24t$ p.p.m./h. where X p.p.m. is the total concentration of carbon dioxide in all forms and t is the period between replacement of the water, provided that other factors are not limiting and that K_3 does not exceed a value $K_3 \cong 31.6 + 0.169\ [HCO_3^-]$ p.p.m./h.

(iii). Fish and the plants themselves are the main respiring organisms to be considered in the aquarium. Since the respiratory quotient for fish is about 0.7 — 1.0 and one volume of oxygen is liberated per volume of carbon dioxide absorbed in photosynthesis, the maximum rate of photosynthesis from carbon dioxide produced from fish absorbing oxygen at K_1 p.p.m./h. will be $K_3 \cong 0.7 - 1.0K$. Plants will provide carbon dioxide at a rate K_2 equal to the rate of respiration.

If now we assume that the limiting rate of photosynthesis, K_3^L, in aquaria due to restriction in the supply of carbon dioxide is given by the sum of the individual maximum rates of supply due to (i), (ii) and (iii) above, we have

$$K_3^L = K_1 + K_2 + 0.4K + \frac{X}{24t}.$$

Thus if K, K_1, and K_2 take the values 0.05, 0.36, 0.04 as in previous examples, X = 240 p.p.m. (a reasonable value for tap water or natural fresh water) and t = 5 days we have

$$K_3^L = 0.36 + 0.04 + 0.4(0.05) + \frac{240}{24 \times 5} = 2.42 \text{ p.p.m./h.}$$

It will be noticed that although this rate is somewhat lower than the limiting rate for summer conditions, it is somewhat greater than the calculated limiting rate of photosynthesis caused by the low level of daylight illumination in winter. Thus the indication from these approximate calculations is that the maximum rate of supply of oxygen by photosynthesis may under some circumstances in the summer be limited by the supply of carbon dioxide rather than the intensity of illumination.

More Complex Systems

The only systems considered have been those in which the absorption or production of oxygen, apart from that due to atmospheric aeration, was a linear function of time and was independent of other variables. There is, however, no *a priori* reason for this other than shortage of detailed information about photosynthesis and respiration, and it should be possible to formulate equations representing the variation of dissolved oxygen with time, if, as information accumulates, these factors prove to be more complicated functions. As a simple example of a more complex system (in the mathematical sense, that is), we may consider the case in which the water in the aquarium has a biochemical oxygen demand (B.O.D.) exerted at a rate such that

$$L_t = L_0 e^{-kt} \quad \text{.....(18)},$$

where L_0 and L_t are the ultimate B.O.D.'s at times 0 and t hours and k is the velocity constant. This situation may well occur in work on the toxicity of effluents, or in aquaria or ponds in which dying plants are present. If absorption by fish is constant, K_1 as before, and $K_2 = K_3 = 0$, then

$$\frac{dC}{dt} = K(C_s - C) - K_1 - kL_0 e^{-kt} \quad \text{.....(19)}$$

and $$C = \frac{k}{K-k} L_0(e^{-Kt} - e^{-kt}) + C_0 e^{-Kt} + \frac{(KC_s - K_1)(1 - e^{-Kt})}{K} \quad \text{.....(20)}.$$

In this case, the oxygen concentration would pass through a minimum, C_M, given by

$$C_M = C_s - \frac{(kL_0 e^{-kt_M} + K_1)}{K} \quad \text{.....(21)}$$

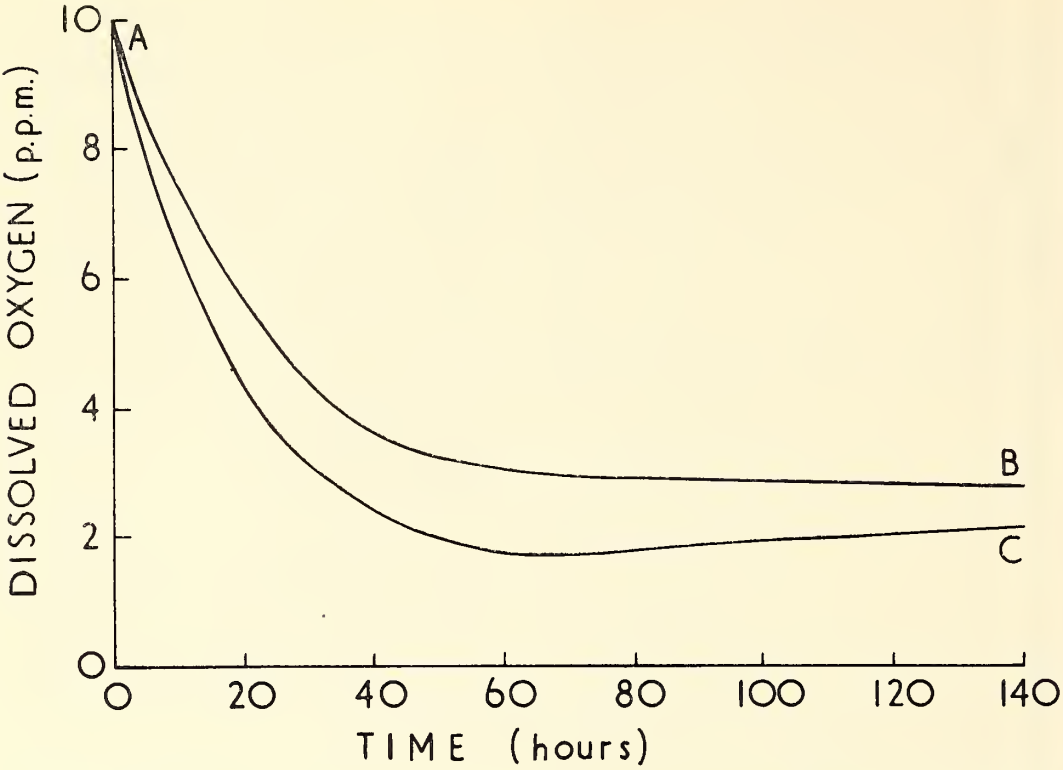
at time

$$t_M = \frac{1}{K-k} \log_e \left[\frac{K}{k} - \frac{K(C_s - C_0) - K_1(K-k)}{L_0 k^2} \right] \quad \text{.....(22)}$$

before finally reaching an equilibrium given by

$$K(C_s - C) = K_1.$$

Suppose, for instance, that in addition to the consumption of oxygen by fish at a rate $K_1 =$ 0.36 p.p.m./h., when the rate of reaeration is 0.05/h. (as in curve AB, Text-fig. 5), the water contains an initial ultimate biochemical oxygen demand, L_0, of 10 p.p.m. and that the velocity constant of the B.O.D., k, takes a value of 0.01/h. (Phelps, 1947). If these values are then substituted in Equations (20), (21) and (22), it will be found that the dissolved oxygen will fall to a minimum concentration of 1.77 p.p.m. in 67 hours before gradually recovering to the



TEXT-FIG. 5. Effect of the respiration of fish and of a biochemical oxygen demand on the variation of concentration of dissolved oxygen in aquaria.

Over-all absorption coefficient, K = 0.05/h.

Rate of consumption of dissolved oxygen by fish, K_1 = 0.36 p.p.m./h.

Initial ultimate biochemical oxygen demand, L_0 = 10 p.p.m.

Velocity constant of B.O.D., k, = 0.01/h.

Curve AB: L_0 = 0.

Curve AC: L_0 = 10 p.p.m.

equilibrium concentration of 2.8 p.p.m. in accordance with curve AC, Text-fig. 5. The chosen value for L_0 is, of course, quite low and could easily be exceeded—for instance in a pond in which there had been a profuse growth of plants during the summer, most of which died and decayed at the onset of colder weather. In such a case, the water might be completely depleted of oxygen and therefore quite unsuitable for fish.

Discussion

Methods for determining the rate of solution of atmospheric oxygen in water and the use of these data in the formulation of simple equations expressing the variation with time of the concentration of dissolved oxygen in aquaria have been described. These equations are based on a number of simplifying assumptions and approximations either because more detailed information is not available or because a more rigorous treatment has not been justified in view of the considerable variability of living organisms. It may well be a chimera to suppose that even with a great deal more information on some of the variable factors, it will be possible to make better than semi-quantitative predictions about variations in the dissolved oxygen content of aquaria, except perhaps in simple systems under rigidly controlled conditions. It is, however, difficult in the present state of knowledge to foresee to what extent more accurate information will be required. Meanwhile it is hoped that the present simple treatment will serve as an introduction to the subject to those who are unfamiliar with the general principles of the "dynamics of aeration," and will also prove a useful starting point

for anyone contemplating a detailed study of the part played by carbon dioxide in aquaria.

ACKNOWLEDGEMENT

This paper is published by permission of the Department of Scientific and Industrial Research of Great Britain.

BIBLIOGRAPHY

ALABASTER, J. S.
1956. Private communication.

BREDER, C. M., JR.
1931. On the organic equilibria in aquaria. Copeia, 1931: 66.

DATTA, R. L., D. H. NAPIER & D. M. NEWITT
1950. The properties and behaviour of gas bubbles formed at a circular orifice. Trans. Instn. chem. Engrs., Lond., 28: 14-26.

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH
1956. Water Pollution Research 1955. H. M. Stationery Office, London.

DOWNING, A. L. & G. A. TRUESDALE
1955. Some factors affecting the rate of solution of oxygen in water. J. appl. Chem., 5: 570-581.

DOWNING, K. M.
1954. The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout. J. exp. Biol., 31: 161-164.

GAMESON, A. L. H., G. A. TRUESDALE & A. L. DOWNING
1955. Reaeration studies in a lakeland beck. J. Instn. Wat. Engrs., 9: 571-594.

GRAHAM, J. M.
1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout Salvelinus fontinalis. Canad. J. Res., (D), 5: 270-288.

HIGBIE, R.
1935. The rate of absorption of a pure gas into a still liquid during short periods of exposure. Trans. Amer. Inst. chem. Engrs., 31: 365-389.

HOLROYD, A. & H. B. PARKER
1949. Investigations on the dynamics of aeration; the aeration of clean water. J. Inst. Sew. Purif., 1949 (3): 292-323.
1952. Investigations on the dynamics of aeration; the effects of some surface contaminants. J. Inst. Sew. Purif., 1952 (4): 280-305.

IPPEN, A. T. & C. E. CARVER
1954. Basic factors of oxygen transfer in aeration systems. Sewage industr. Wastes, 26: 813-829.

LEWIS, W. K. & W. G. WHITMAN
1924. Principles of gas absorption. Industr. Engng. Chem., 16: 1215-1220.

MANNING, W. M., C. JUDAY & M. WOLF
1938. Photosynthesis of aquatic plants at different depths in Trout Lake Wisconsin. Trans. Wis. Acad. Sci. Arts & Lett., 31: 377-410.

OSWALD, W. J., & H. B. GOTAAS
1955. Photosynthesis in sewage treatment. Proc. Amer. Soc. civ. Engrs., 81 (Separate No. 686): 27 pp.

PASVEER, A.
1953. Research on activated sludge. I. A study of the aeration of water. Sewage industr. Wastes, 25: 1253-1258.
1954. A study on the oxygenation of water with air bubbles. Afdeling Gezondheidstechniek T.N.O. Report No. 23. Nov. 1954.

PHELPS, E. B.
1947. Chapter 5, pp. 150-164 of "Stream Sanitation." John Wiley & Sons, Inc., New York.

RABINOWITCH, E. I.
1951. "Photosynthesis and Related Processes." Interscience Publishers, Inc., New York. p. 961.

SCOULLER, W. D. & W. WATSON
1934. The solution of oxygen from air bubbles. Surveyor, Lond., 86: 15-16.

STREETER, H. W., C. T. WRIGHT & R. W. KEHR
1936. Measures of natural oxidation in polluted streams. III. An experimental study of atmospheric reaeration under stream-flow conditions. Sewage Wks. J., 8: 282-316.

TRUESDALE, G. A., A. L. DOWNING & G. F. LOWDEN
1955. The solubility of oxygen in pure water and sea water. J. appl. Chem., 5: 53-62.

VERDUIN, J.
1952. The volume-based photosynthetic rates of aquatic plants. Amer. J. Bot., 39: 157-159.

17

Reproduction in the King Cobra, *Ophiophagus hannah* Cantor

JAMES A. OLIVER

Curator of Reptiles, New York Zoological Park

(Plates I-V)

INTRODUCTION

THE King Cobra (*Ophiophagus hannah* Cantor), the longest venomous snake in the world and one of the very few occasionally aggressive species, is one of a small number of snakes that guards the eggs during incubation, and is the only snake known to deposit its eggs in a nest made of vegetable matter. A good deal has been written about each of these attributes, but little is known of the details of the reproductive habits of the species.

That a snake constructs a sizeable nest of vegetable material is difficult to imagine. Colonel Frank Wall summarized the knowledge about this snake up to 1924 and concluded, "It seems most probable she selects a chance accumulation of debris which she can appropriate for her use." Wall also pointed out that little is known of the courtship, period of gestation or period of incubation in this species. He did report the killing of two pairs in the act of mating, one in March and the other in late April or early May, but gave no additional details. Little additional information on these topics has been published since the date of Wall's paper.

Wasey (1892) appears to be the first to report the deposition of eggs in a nest and the guarding of eggs. He killed a snake 9 feet 8 inches long on "a heap of dried leaves," at the bottom of which he found 33 eggs. Fenton (1917) reported finding an individual, also of unspecified sex, 9 feet 3 inches long, on a mound of leaves 2 feet high and 8 or 9 feet in circumference. He failed to note any eggs. H. C. Smith (1936) described a nest in detail and included a photograph of the nest, eggs and the dead snake, which was not sexed but measured 7 feet 5 inches. The nest contained 27 eggs and was a compact mass of dead leaves measuring 1 foot 7 inches in diameter and 9 inches deep. The eggs were covered with a layer of leaves that formed the bottom of the upper depression of the nest on which the snake was resting when discovered. Mustill (1936) described a nest observed by him in 1926. This contained 40 eggs and was guarded by a snake that measured "about 12 feet in length." The nest "was built in the base of a small shrub about 6 inches from the ground and at first sight it resembled the nest of an English magpie. It was composed of sticks and other debris and contained a hole in the top through which the snake could pass." Mustill estimated the nest to be 2 feet from top to bottom and about 2 feet 6 inches in diameter. He noted an upper and lower chamber, with the eggs in the latter and the snake resting in the former.

King Cobras in the collection of the New York Zoological Park have provided data on the reproductive habits of this interesting species. Three specimens were obtained from a dealer in Bangkok, Thailand, and reached the Park on October 10, 1953. At that time the larger male measured 14 feet 6 inches, the smaller male 11 feet 2 inches and the female 13 feet 4 inches. The snakes were kept in temporary quarters until April of 1954 when they were moved to their permanent home in the newly renovated Reptile House. Here the three snakes were placed in a cage 7½ feet long, 6½ feet wide and 6½ feet high. The cage is provided with a large water pool approximately three feet long and two feet wide. In one corner of the cage there is a raised planted area, containing small bamboo stalks with leaves. Several vertical sticks of bamboo are set in the floor of the cage for decorative purposes and are on the opposite side. A shift cage 6 feet long and 2 feet wide adjoins the main cage but is not visible to the public. The door to this cage is usually left open, permitting the snakes free access except when

it is desired to isolate one or more for feeding or some other reason. The shift cage provides the snakes with an area of seclusion from the public, although rarely do all three retire to the shift cage at the same time.

The floor is heated by hot water pipes located in the floor across the front of the cage. This provides a thermal gradient from the front to the back of the cage. The average temperature is between 27° C. and 30° C., with the front area being two or three degrees warmer than the rear. The floor is covered with white crystalline sand, from a half to an inch in depth. In the right rear corner there is a small pile of bamboo leaves and stalks. This is a favorite resting place of the three snakes and is the spot in which the nest has been built each year. The cage is lighted with incandescent lights, but also is provided with ultraviolet light that is turned on for daily intervals of 15 to 30 minutes.

The King Cobras are fed an average of once a week on dead snakes that have been frozen and thawed. Many species of snakes have been used as food in this manner, including boids, colubrids, viperids and crotalids. Once the cobras were accustomed to this type of food, they refused live snakes and would eat them only after they had been killed.

In 1955 and 1956 the smaller male and the female mated and the female laid eggs. Observations were made on these activities each year and both still photographs and motion pictures were obtained. I am grateful to Sam Dunton, Staff Photographer at the New York Zoological Park, for his cooperation and patience in getting an excellent photographic record. Head Keeper Stephen Spencook and Keepers Peter Brazaitis, Raymond Cummins and Robert Raabe all assisted greatly in recording the data reported herein and have my sincere thanks.

Courtship and Mating

Early in March of 1955 the larger male and the female were observed in intermittent courtship activity, but no record was made of the duration or exact nature of the activities and no mating was observed. On March 10, 1955, I observed the smaller male and the female in courtship. When first noted at 12:30 p.m. the female was crawling slowly around the cage. Her head and neck were raised obliquely about ten inches above the floor and her hood was spread (Fig. 1). The male was on top of the female with his head about three feet behind hers. He was moving forward along her back with his tongue flicking out frequently. When his head reached the hood of the female, he gently nudged her hood and head several times with his nose (Fig. 2). The male constantly flicked his tongue on either side of the female's head (Fig. 3). During the entire process both snakes crawled slowly around the cage,

The rear half of the male's body looped back and forth on either side of the female's body and slowly moved backward and forward as the two crawled along. The male occasionally slid off the female, at which time his head explored along her sides, nudging her. She paused several times and as soon as she started crawling again the male regained his position on top of her, usually about mid-body. His head quickly moved forward along her back, nudging her body with his snout as he moved along her back and continually flicking his tongue on her skin.

The female appeared to stimulate the male by merely crawling slowly away from him. When the female stopped crawling or if she attempted to coil, the male nudged her body sharply with his snout (Fig. 4). Either individual seemed able to initiate courtship. At the beginning, the male appeared to be stimulated visually and throughout courtship by chemical stimuli perceived through the tongue-Jacobson's organ system and possibly some tactile stimulation. In contrast the female appeared to respond primarily to tactile stimulation alone. Sex recognition probably involves visual and chemical cues, as well as behavior. Whenever the smaller male was actively prowling about the cage and touched the larger male, the latter reared, spread his hood and made threatening jabs that seemed to divert him. When the smaller male came upon the female, she would lie still until he pushed her vigorously with his head, or sometimes immediately start to crawl away, whereupon he would attempt to court her.

After the pair had courted for about thirteen minutes and shortly after a series of gentle nudges by the male, he lifted the rear of her body with his tail, brought his vent under hers (Fig. 5) and both hemipenes were everted about an inch but quickly withdrawn. Courtship was resumed and after another three minutes the male again raised the base of the female's tail. At this time his vent was approximately opposite and under hers but on the right side. His left hemipenis only was everted and copulation was effected. Immediately all movement stopped and the pair remained motionless for the next 15 minutes. Slowly the male moved off of the female so that the two were headed in opposite directions. The male tried to pull away, but the female began to crawl slowly, taking the male with her (Fig. 6). The two remained in contact for 57 minutes, after which time they separated, each going to opposite sides of the cage.

The temperature in the cage at the time of this mating was 27.5° C. (82° F.). The day was

clear and mild with an outdoor temperature of 21° C. (69° F.). A crowd of fifteen to twenty persons gathered in front of the cage from time to time but the snakes appeared completely undisturbed.

On two other occasions in 1955 this same pair was observed in a mating union when the keepers entered the Reptile House in the morning; once at 7:21 a.m. on March 14 and again at 7:23 a.m. on March 18. On neither of these days was the complete time of mating recorded, since the snakes were already in contact when noted. On the 14th the snakes separated at 8:07 a.m. and on the 18th at 7:31 a.m.

In 1956 the smaller King Cobra engaged in courtship with the female for several minutes on January 6, but no mating occurred. Courtship again occurred on January 12. On January 16 vigorous courtship took place for 22 minutes and culminated in mating.

On January 17 the same pair commenced courting at 9:40 a.m., during which time the male made three efforts to insert the hemipenes into the female. At 10:00 a.m. a successful union was made. As in the previous matings, all movement stopped as soon as one of the hemipenes was inserted. On this occasion copulation lasted 58 minutes, during the latter half of which time the female crawled around slowly, pulling the male with her. Fifty minutes after the union was effected and while the two snakes were still in copulation, the male was in the vicinity of the water pool and took a long drink. Throughout this courtship and mating the cage was brightly illuminated by photoflood lights, which appeared to have no effect on the snakes.

On January 26, the smaller male and female were observed in copulation at 8:31 a.m. They parted at 9:37 a.m. During the night of February 17 this pair shed their skins. About 3:00 p.m. on February 18, the two males participated in a brief and incomplete "combat dance." Each reared the anterior portion of its body and entwined it with that of the other while making short jabs with its head at its opponent's head. They separated after a minute or two and the larger male attempted to court the female, but was driven away by the smaller male. The latter courted the female and mating took place at 3:24 p.m. This union was broken at 4:23 p.m. After being driven away by the smaller male, the larger male remained quietly in a corner of the cage, apparently indifferent to the mating pair.

On March 8 and 14 the smaller male and female were observed mating, between 8:20 a.m. and 9:00 a.m. on the former occasion and between 8:05 and 8:55 a.m. on the latter. The start of neither of these matings was noted. Courtship and mating in all of the observed instances followed the pattern reported in detail for March 10, 1955.

Nest Building

The building of the nest was observed and photographed both years. In anticipation of this activity, freshly cut bamboo stalks were laid around the floor of the cage, and each year after the female began to build her nest, additional bamboo and some large dead magnolia leaves were introduced into the cage away from the side of the nest.

On April 21, 1955, at 7:40 a.m. the female was observed pushing the bamboo litter of the floor into a compact pile near the center of the cage. This was done by crawling part way around a pile of litter and then drawing the head and body back in an open loop. Between 8:00 a.m. and 9:00 a.m. she moved this pile into the rear corner of the cage behind several vertical sticks of bamboo. She soon had virtually all of the bamboo stalks back in the corner and began pushing sand on the pile. This was done by tilting the head about 45° to one side and pushing it through the sand for a short distance, then dragging the loosened sand back on the inside of an open loop. Moving the bamboo litter and the sand were both accomplished by movements that resulted in the anterior one-third to one-half of the body being employed as a large hook used on its side to drag in the loose material.

At 9:20 a.m. additional stalks of bamboo were spread around the floor of the cage and the two males were removed. (They were not returned to the cage until after the eggs had been removed on May 4, 1955). Neither had made any movement until just prior to this time, when the smaller male tried to crawl onto the growing heap of bamboo stalks. The female drove him away with two quick and forceful jabs of her head. This appears to be the customary method of avoiding intrustion or rejecting advances. I have seen these snakes use this movement many times when excited. The mouth is kept closed and the snake strikes out sharply with the head. It is a more forceful movement than the gentle jabs of courtship.

After driving away the male, the female rested on top of her pile of bamboo, lying in an elongated coil that covered the entire pile. She remained in this position with little movement until 3:30 p.m. when she began moving more bamboo toward the nest pile. At this time several clumps of dried magnolia leaves were put on the floor, but she ignored them. She coiled up on top of the pile again at 4:10 p.m. and remained quiescent the rest of the day.

Keeper Raabe came in early on the morning

of April 22. Before he turned on the lights at 7:16 a.m. the female was resting quietly on top of the pile of bamboo. As soon as the lights were turned on, she moved off the pile and started dragging in a clump of magnolia leaves. These were drawn to the top of the pile on the inside of a loop of her body held close against the floor of the cage. At 7:24 a.m. she crawled into the pile and began pushing out a chamber from the inside. She crawled round and round in a tight coil inside of the nest, sometimes pausing to push out a loop of her body to pack down the bamboo and leaves. Once she pushed the loop out with her head. After several minutes of this she emerged from the nest part way and, using her head to loosen the sand, drew some sand in on the pile of leaves.

Without resting, she crawled farther out into the cage, entwined her body through several bamboo stalks that were together and pulled them back in a loop of her body (Fig. 7). Then she went out and drew her body around a pile of magnolia leaves, which she dragged back with the forward part of her body (Fig. 8). She retired again to the center of the nest and, coiling tightly, revolved around in the middle of the structure, pausing occasionally to thrust the leaves and stalks back with a loop of her body. These movements became quite jerky and she appeared to be exerting considerable force to form the inner chamber of the nest. It became increasingly difficult to observe her movements since the nest had become quite large and most of her body was not visible when she retired into it. In entering and leaving the nest pile the female followed no regular path. One time she would enter from the side and go under or into the accumulated material. The next time she might go on top of it. She never entered from the front, but always from one side or the other. When the nest was nearly completed she appeared to go to the top of the pile rather than in the sides.

At 7:44 a.m. she again emerged from the nest, crawled out and around a pile of leaves she had previously moved into the corner of the cage. With her body looped tightly around this bunch of leaves she literally carried them to the pile, holding the coil about two inches off the ground. These were pulled inside the nest and she again revolved several times in a tight coil. Some leaves dropped out of her coil as she tried to pull them inside. She soon emerged and continued dragging in stalks and leaves. At 8:50 a.m. additional bamboo and magnolia leaves were placed around the floor of the cage. At this time the snake was spending longer and longer periods of time shaping the interior chamber and was almost entirely hidden from view. Occasionally she would come out and drag in small amounts of additional material.

At 10:00 a.m. the female emerged from the nest and crawled to the water pool where she took a long drink. She rested awhile beside the pool and then retired into the nest. Little movement of any sort was noted until shortly after 1:00 p.m. when she again began revolving inside the nest, making jerky pushes outward with a loop of her body. These movements lasted less than 15 minutes, after which time she remained quietly in the nest. No activity of any sort was observed on the 23rd of April, and the eggs were laid on the morning of the 24th. After the eggs were all laid in the central chamber, the female moved leaves over them and almost completely covered them with a layer of litter (Fig. 9). She then coiled on top of the leaf-covered egg heap. This is probably the basis for the statements of H. C. Smith and Mustill that the nest is divided into an upper and a lower chamber, with the eggs deposited in the latter and the snake resting in the former.

In 1956 the female started building her nest on the morning of April 20, almost exactly one year from the date she started her nest in 1955. The method of dragging in leaves and stalks was the same as that employed the previous year. The female worked on and off all day the first day and most of the second. During both days the cage was lighted by photoflood lights to permit the taking of motion pictures. The following morning the female tore the nest apart in some unobserved fashion, scattering many of the leaves and stalks all around the cage. She commenced to rebuild it shortly after 8:40 a.m. and completed the job on the afternoon of April 23. In the rebuilt nest the inner chamber was located about one foot away from where it had been situated in the two previous nests, and was shallower. The eggs were laid on the morning of April 24, the same date as in 1955. This time, possibly because of the shallower chamber, the female did not cover the eggs completely and coiled her body partly around the uppermost eggs.

Eggs

It is impossible to determine the period of development of the eggs since there is no way of knowing accurately when they were fertilized. In 1955 three matings were observed, all in March within a period of nine days. Whether or not there were other matings is unknown. In this year the eggs were laid approximately six weeks after the first recorded mating and about five weeks after the last. In 1956 the pair was observed mating on six occasions over a period of approximately sixty days. The last two mat-

ings, on March 8 and 14, fall within the period of the three observed the previous year and would give a developmental period of five to six weeks.

In their studies on reproduction in the Garter Snake (*Thamnophis sirtalis*), Frank N. and Freida Cobb Blanchard (1941) found that very early matings were not followed by correspondingly early births. Late April seemed to be the invariable start of the period of development. This, they inferred, was related to the arrival of more or less continuously warm weather. Such would not appear to be the case with snakes kept indoors at more or less constant temperatures all year. It is unwise to speculate on the basis of data from only two years, but it is interesting to contemplate whether the deposition of eggs on the same date each year is coincidental or the result of some regulatory factor.

In 1955 part or all of the egg clutch had been laid by 7:45 a.m. when Head Keeper Spencook checked the nest. Eggs were visible in the center of the nest at that time. The female continued to move around in the center of the nest, but we were unable to determine definitely whether she was continuing to lay eggs or simply covering them. Around 10:00 a.m. she became quiet, resting near the top of the central chamber with only part of her body visible. No eggs could be seen at this time. At 1:00 p.m. the leaves covering the chamber of the nest were gently lifted away so that photographs could be taken. As soon as we tried to uncover the eggs the female quickly covered them again with leaves or her body. She usually brought her head around quickly to wherever the leaves were removed and would push other leaves over the exposed eggs.

In order to photograph the eggs in the nest and to count and measure them, the female was removed from the nest and restrained behind a large plastic shield. After counting, 14 of the eggs were replaced and the shield was withdrawn. She immediately returned to the nest, covered the eggs and resumed her resting position in the upper part of the nest.

In 1956 all of the eggs had been laid by 8:00 a.m. when the nest was checked. Again most of the eggs were taken out of the nest, but five were returned. The female again covered these eggs and coiled on top of them.

The number of eggs previously reported in the literature varies from 21 to 40. In 1955, the female laid a total of 41 eggs, of which 11 were abortive. The abortive eggs were one-half to two-thirds the size of the normal ones and were covered with a more pliable shell. In 1956 a single small abortive egg was found on the floor of the cage each morning on April 17, 19 and 21. Sometime during the early morning of April 24 an additional 48 eggs were laid in the nest, making a total of 51. An exact count was not made of the abortive eggs in this clutch, but approximately forty appeared to be of that nature and soon spoiled. The remaining eggs became moldy and were destroyed.

Ten of the normal-appearing eggs of the 1955 clutch were weighed and measured the afternoon on which they were laid. The data obtained were as follows:

April 24, 1955

Egg No.	*Weight (in grams)*	*Length (in mm.)*	*Width (in mm.)*
1	40.0	60.0	34.0
2	40.0	57.7	34.3
3	40.5	58.1	35.0
4	40.0	57.6	34.6
5	42.5	58.5	32.8
6	41.0	58.8	35.0
7	40.5	59.2	34.8
8	42.8	64.3	34.6
9	40.9	63.2	32.3
10	40.3	58.1	36.0

The eggs removed from the nest were divided into three lots: one for incubation in leaf litter, one for incubation in damp sawdust and one for incubation on moist paper towels. The ten eggs that were weighed and measured were placed in damp sawdust. These were weighed and measured on three subsequent occasions, as follows:

May 23, 1955

Egg No.	*Weight (in grams)*	*Length (in mm.)*	*Width (in mm.)*
1	51.0	61.5	38.1
2	50.5	61.2	38.9
3	46.5	64.7	35.1
4	50.0	59.3	39.5
5	47.5	62.8	36.2
6	51.0	61.1	38.6
7	51.5	62.6	36.3
8	54.5	61.9	40.1
9	57.0	60.4	41.6
10	57.2	62.1	41.5

June 14, 1955

Egg No.	*Weight (in grams)*	*Length (in mm.)*	*Width (in mm.)*
1	56.5	61.2	41.2
2	55.3	60.8	40.0
3	50.5	64.9	36.1
4	53.0	58.7	41.0
5	50.7	61.8	38.8
6	56.2	61.2	41.0
7	54.5	63.3	38.1
8	59.5	61.5	41.7
9	73.3	63.7	46.8
10	74.0	65.4	46.5

July 5, 1955

Egg No.	Weight (in grams)	Length (in mm.)	Width (in mm.)
1	58.0	61.5	41.3
2	62.0	61.5	43.8
3	52.0	65.0	36.7
4	57.0	59.4	40.8
5	58.0	64.6	41.1
6	82.0	65.8	48.0
7	75.0	66.8	44.1
8	71.0	63.7	44.5
9	hatched	—	—
10	96.0	70.8	49.5

At 4:00 p.m. on July 4, egg No. 9 had a slit in the shell and the hatchling had its head and about 1 inch of the body protruding through the slit. The snakeling emerged sometime during the night. It measured approximately 460 mm. in total length and weighed 23 grams. During the following nine days thirteen other youngsters emerged, but four of these were crippled by deformities of the spinal column. Thus a total of nine healthy-looking youngsters were obtained from the clutch.

The period of incubation before the first egg hatched was ten weeks and one day, and the total period until the hatching of the last egg was eleven weeks and three days. During incubation the ten measured eggs increased an average of 66.1% in weight, 8.1% in length and 26.2% in width.

No data are available on the weights, measurements or incubation period of the eggs in the 1956 clutch.

Guarding the Nest

Early reports on the discovery of nests of King Cobras invariably include a guarding parent. Although the sex was not determined in any of the reports I have seen, Wall (1924) says, "All those who have met the hamadryad during the period of incubation have remarked upon the fact that the female has been coiled up on a nest of leaves, or vegetable rubbish." It appears to have been a common assumption that the guardian snake was the female, but whether this is invariably so or not I do not know.

In 1955 after the female had been moved off of her nest so that we could examine the eggs, she returned to it the afternoon of April 24 and remained coiled on the eggs until 4:40 p.m. on April 28 when she left and went to the water pool to take a long drink. The remaining eggs were removed from the nest on May 4 because they were becoming covered with mold. The female remained on the nest for a day or two longer, but the two males were returned to the cage and the nest was soon matted down into a common resting site for all three snakes. The female left the nest on May 4 to take her first meal since building it.

On April 25, 26 and 27, while the female remained on the nest, temperature readings were taken of the cage floor immediately beneath the nest, the interior of the egg cluster, between the coils of the snake, and the air one foot above the nest. These readings were taken with a Schultheis thermometer graduated in .2° C. There was not more than .4° C. difference in any of the readings, which were 27.4° to 27.8° C., and no constant pattern was noted in the differences. From these observations it is concluded that the presence of the female in the nest causes no rise in temperature that would aid the incubation process. Rather, her role in remaining with the eggs appears to be solely one of protection.

Many of the reports of unprovoked attacks by this snake attribute particular aggressiveness to the female when guarding her nest. Thus Wall (*op. cit.*) says, "The female when disturbed in the process of brooding her eggs, seems to be specially sensitive, and usually attacks the intruder at sight." This may be an exaggeration. There is no doubt that these snakes are sometimes aggressive, but whether this is the exception or the rule is open to question. G. H. Evans (1921) reports an encounter with a large King Cobra that was intent on escaping and made no effort to attack despite considerable provocation. He concluded with the remark, "I am more than ever convinced that hamadryads as a rule are as glad to escape as most other snakes." H. C. Smith (1936), referring specifically to a guardian snake on a nest close to a forest path, wrote, "It is interesting to note that fourteen people accompanied by seven dogs twice passed at different times within two yards of the nest and yet the hamadryad failed to show itself and the nest remained undiscovered until I prodded the heap of leaves with a small cane."

The three snakes whose activities are reported here were quite aggressive when they came to the Zoological Park, but, like most snakes under proper conditions of captivity, they became accustomed to the presence of visitors and keepers. By 1955, when the first matings occurred, none of the three seemed to pay attention to the keepers cleaning their cage except to investigate movements or cleaning implements. The snakes would not spread their hoods unless aroused vigorously. So far as we could tell, there was no change in disposition on the part of any of the snakes during the period of courtship and mating nor on the part of the female while guarding her nest, although the males were somewhat more alert to movements and were more active during this time. When the female was disturbed on her nest she appeared more concerned with

covering her eggs than with trying to drive off the intruder. Even when she was removed from the nest forceably to enable us to take out the eggs, she made no effort to strike or bite.

Young

The nine healthy young snakes, six males and three females, were marked very differently from the adults (Figs. 11 & 12), as had been noted by Wall (*op. cit.*).The ground color was black. This was interrupted by a series of narrow white to yellowish-green cross-bands approximately one scale wide and separated by spaces three to four scales wide. The scales bordering the light bands were mostly solid black, whereas those inside the dark interspaces were light in the center. The snout was white to yellow, with a narrow black band across the sutures of the internasals and prefrontals, extending ventrad onto the labials. There was a broader supraocular black band followed posteriorly by a narrow yellow area. The first of the black interspaces was on the rear of the head and neck.

The young varied from approximately 460 to 640 mm. in total length and in weight from 19 to 26 grams. They quickly assumed a defensive attitude when disturbed and struck vigorously if approached too closely (Fig. 12). They refused all food offered them and lived only four or five months. The food offered them included earthworms, mealworms (*Tenebrio*), Red-backed Salamanders (*Plethodon cinereus*), Red Efts (*Diemictylus v. viridescens*), American Toads (*Bufo terrestris americanus*), Spring Peepers (*Acris gryllus crepitans*), Carpenter Frogs (*Rana virgatipes*), Northern Fence Lizards (*Sceloporus undulatus hyacinthinus*), Green Anolis (*Anolis c. carolinensis*), Little Brown Skinks (*Lygosoma laterale*), Florida Green Water Snakes (*Natrix cyclopion floridana*), Northern Water Snakes (*Natrix s. sipedon*), Striped Keelbacks (*Natrix stolata*), DeKay's Snakes (*Storeria d. dekayi*), Red-bellied Snakes (*Storeria o. occipitomaculata*), Eastern Garter Snakes (*Thamnophis s. sirtalis*), Eastern Ground Snakes (*Haldea v. valeriae*), Eastern Ring-necked Snakes (*Diadophis punctatus edwardsi*), Eastern Worm Snakes (*Carphophis a. amoenus*), Black Racers (*Coluber c. constrictor*), Eastern Smooth Green Snakes (*Opheodrys v. vernalis*), Eastern Milk Snakes (*Lampropeltis doliata triangulum*), Florida Crowned Snakes (*Tantilla coronata wagneri*), Northern Copperheads (*Ancistrodon contortrix mokeson*), baby House Mice (*Mus musculus*), baby white mice and strips of horse meat.

All of these items were offered in an unaltered condition, and many of them were also rubbed with live and dead Asiatic snakes and lizards of several species not recorded. Several of the young were force-fed with some of the above-listed food animals and with a prepared liquid food mixture.

Summary

Two King Cobras (*Ophiophagus hannah* Cantor) from near Bangkok, Thailand, have mated during two successive years in the New York Zoological Park. Observations are reported on their courtship and mating. Three matings were recorded in 1955 and six in 1956. Five to six weeks after the last mating in each year the female constructed a large nest of bamboo stalks, bamboo leaves and magnolia leaves. On April 24 of each year she deposited her eggs, 41 the first year and 51 the second. A high percentage of each clutch consisted of abortive eggs. The female remained on the nest guarding the eggs until all had been removed. Young hatched ten to eleven weeks after the eggs were laid, but refused all food offered and lived only four to five months.

Literature Cited

Blanchard, Frank N. & Frieda Cobb Blanchard

1941. Factors determining time of birth in the Garter Snake (*Thamnophis sirtalis sirtalis* (Linnaeus). Papers Mich. Acad. Sci., Arts and Letters, 26: 161-176.

Evans, G. H.

1921. An encounter with a Hamadryad (*Naia bungarus*). Jour. Bombay Nat. Hist. Soc., 27, (4): 955.

Fenton, L. L.

1917. Note on the Hamadryad or King Cobra (*Naia bungarus*) in North Kanara. Jour. Bombay Nat. Hist. Soc.,25 (1): 151-2.

Mustill, F. J.

1936. A Hamadryad's nest and eggs. Jour. Bombay Nat. Hist. Soc., 39 (1): 186-7.

Smith, H. C.

1936. A Hamadryad's (*Naia bungarus*) nest and eggs. Jour. Bombay Nat. History. Soc., 39: 186, 1 photo.

Wall, F.

1924. The Hamadryad or King Cobra, *Naia hannah* (*Cantor*). Jour. Bombay Nat. Hist. Soc., 30: 189-195, 1 plate.

Wasey, George K.

1892. A nest of King Cobra Eggs. Jour. Bombay Nat. Hist. Soc., 7: 257.

EXPLANATION OF THE PLATES

(Note that in the photographs the neck of the female is sometimes inflated in an abnormal fashion. This is apparently a recurrent condition in this specimen). All photographs by Staff Photographer Sam Dunton.

Plate I

Fig. 1. The courting pair crawl slowly around the cage, with the male on top of the female. The water pool and planting area are at extreme left; the shift cage door is visible on the right.

Fig. 2. The heads and the anterior bodies of the courting pair. (Enlarged from a 16 mm. motion picture film).

Fig. 3 The male frequently flicks his tongue on the head of the female. (Enlarged from a 16 mm. motion picture film).

Plate II

Fig. 4. The male (upper) prods the female (lower) at the start of courtship. They are on a pile of bamboo leaves in a corner of the cage. Note vertical bamboo sticks. (Enlarged from a 16 mm. motion picture film).

Fig. 5. The posterior parts of the bodies showing how the male (right) places his tail under the female's tail at the moment the hemipenis is inserted. (Enlarged from a 16 mm. motion picture film).

Plate III

Fig. 6. The female (foreground) and the male (rear left) in copulation, approximately thirty minutes after the beginning of the mating. This picture shows almost the entire cage in which the snakes were kept. The water pool is in the left foreground and the main door to the cage in the left background.

Fig. 7. The female looping her body around some bamboo leaves and sticks preparatory to pulling them into the nest pile at the beginning of nest building. The site of the nest is behind the vertical sticks of bamboo in the right background.

Plate IV

Fig. 8. The female beginning to draw in a clump of magnolia leaves. Seen from above with nest site at right rear behind vertical bamboo sticks.

Fig. 9. The female coiled on her eggs at the conclusion of laying, April 24, 1955.

Fig. 10. The exposed egg cluster deposited on April 24, 1956. These were in second nest built in 1956 and forward from site of previous nest.

Plate V

Fig. 11. Five eggs and two hatchling King Cobras.

Fig. 12. A hatchling King Cobra spreading its hood in a defensive posture before fully emerged from the egg.

FIG. 1

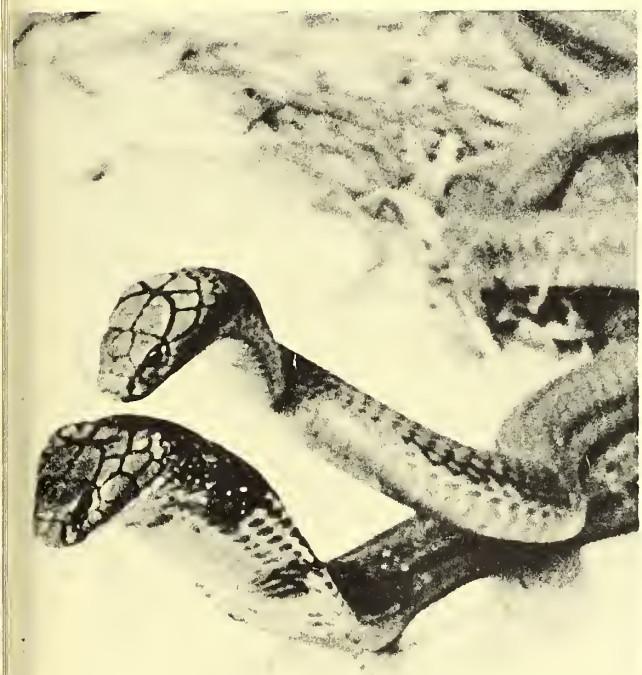


FIG. 2

FIG. 3

REPRODUCTION IN THE KING COBRA, OPHIOPHAGUS HANNAH CANTOR

FIG. 4

FIG. 5

REPRODUCTION IN THE KING COBRA, OPHIOPHAGUS HANNAH CANTOR

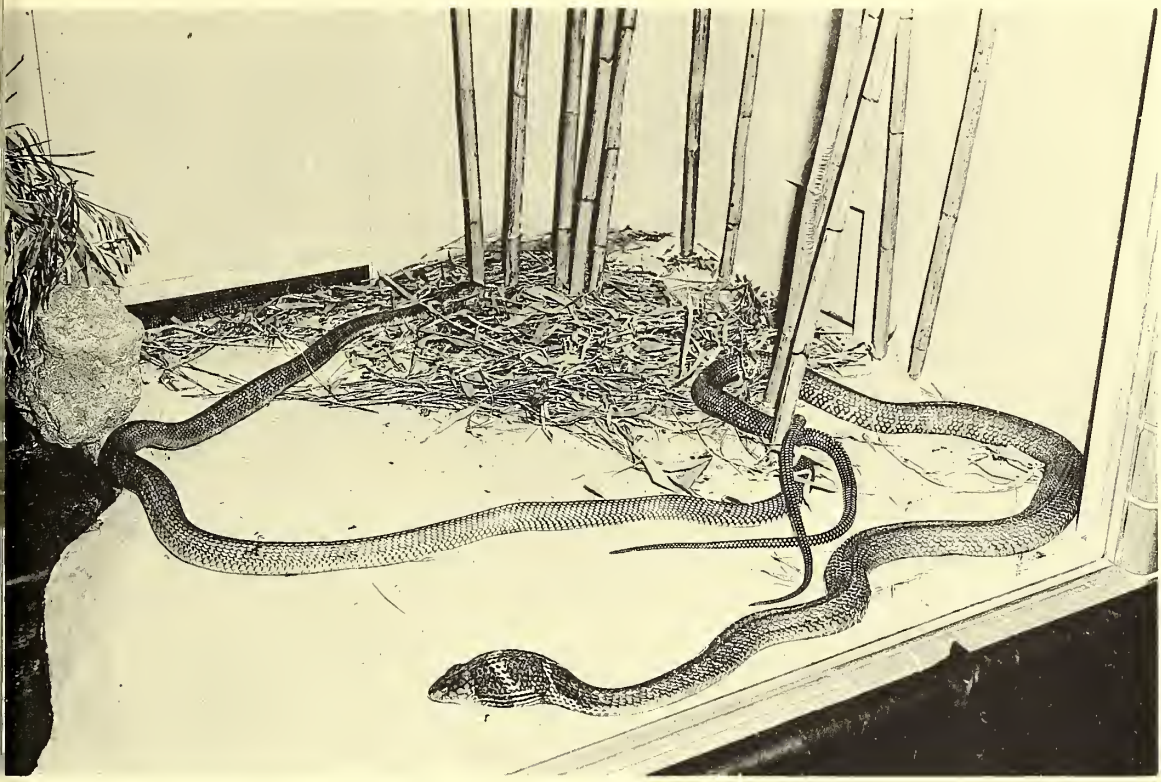


FIG. 6

FIG. 7

REPRODUCTION IN THE KING COBRA, OPHIOPHAGUS HANNAH CANTOR

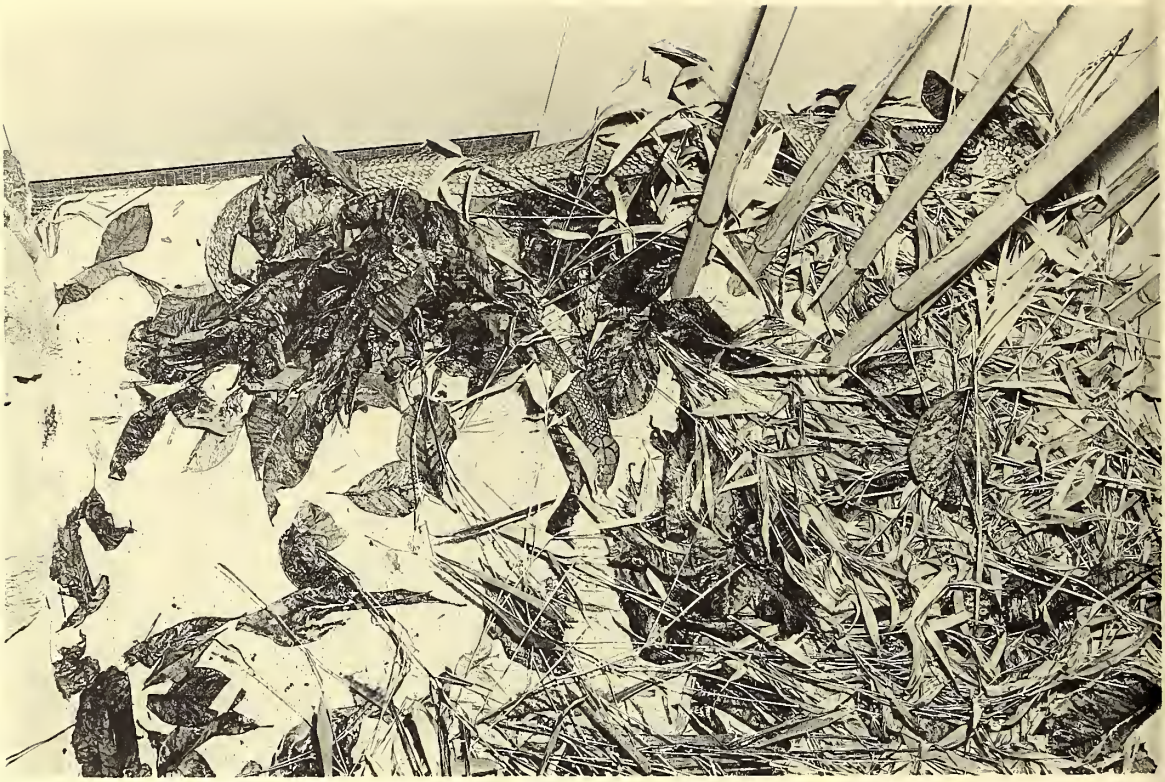


FIG. 8

FIG. 9

FIG. 10

REPRODUCTION IN THE KING COBRA, OPHIOPHAGUS HANNAH CANTOR

FIG. 11

FIG. 12

REPRODUCTION IN THE KING COBRA, OPHIOPHAGUS HANNAH CANTOR

18

An Intricate Genetic System that Controls Nine Pigment Cell Patterns in the Platyfish

MYRON GORDON

New York Aquarium, New York Zoological Society [1]

(Plate I; Text-figure 1)

THE genetic theory to account for the initiation and development of melanomas in platyfish-swordtail hybrids states that one principal gene, which controls the normal growth of large black pigment cells in the platyfish, interacts with a number of growth-modifying genes of the swordtail; as a consequence, the melanocytes, which normally would develop into macromelanophores, do not mature but retain their generalized juvenile characteristics and, as neotenous cells, the melanocytes accumulate, proliferate and eventually produce the melanoma in the hybrid organism (Gordon, in press).

One of the practical consequences of this theory is that it explains how it is possible for two normal parents (each representing a stock in which cancer has never been known) to have offspring in which cancer will develop in response to purely hereditary factors. The fortuitous combination of some primary gene for a specific cell and its growth-modifying genes may account for some puzzling manifestations of normal as well as atypical cell growth.

In view of the important role of gene modifiers (genes which have no apparent visible effect when present alone but which may have a profound effect when they are associated with an appropriate primary gene), it is valuable to have additional evidence of their specific genetic activities. The genetic reaction which will be described produces no atypical growth, but it establishes more firmly than before that some genes are capable of modifying the usual pattern of pigment cell growth. This paper is concerned with the transformation, through gene action, of one pigmentary pattern known as the *twin-spot* into another called the *Guatemala-crescent*.

In an examination of 5,019 sexually mature platyfish, *Xiphophorus maculatus*, that represented four natural populations from Mexico and Guatemala, Gordon (1947) detected only two specimens, both collected in 1931 from Lago de Petén, Guatemala, that had a unique color pattern which he called the *Guatemala-crescent*. He gave the rare pattern the genetic symbol of *Cg* to distinguish it from each of seven other common, hereditary patterns which some platyfish have at, or near, the caudal fin. He pointed out that the *Guatemala-crescent* closely resembled the *single crescent, C*, pattern but that *Cg* was "broader overall and particularly so at the ends of the crescent."

Additional collections of the platyfish from four other rivers in southern Mexico and British Honduras have now swelled the total number of adult platyfish examined to more than 9,000, and yet no other fish with the *Guatemala-crescent* pattern was found under natural conditions (Gordon & Gordon, in press). Several years ago, however, the author did find some live platyfish with the *Cg* pattern that were being maintained by a New York aquarist.

It is most unlikely that the aquarium-bred platyfish with the *Guatemala-crescent* pattern are traceable to the natural population of platyfish from the Lago de Petén, a location which is, and has been, inaccessible to the commercial aquarium fish collector. Gordon (1954) suggested that the earliest shipment of platyfish from "Central America" to Europe, in 1907, consisted of a few specimens that were most

[1] From the Genetics Laboratory at the American Museum of Natural History, New York 24, N. Y. Supported by a grant from the National Cancer Institute, National Institutes of Health, Public Health Service.

probably collected in the vicinity of Belize in British Honduras.

In a preliminary statement on the problem, Gordon (1951) had suggested that the *Guatemala-crescent* pattern may be referred to the complementary action of two dominant genes, one a primary allele, *T*, for *twin-spot*, which produces two discrete groups of small pigment cells near the tail fin, and a complementary gene, *Cg; Cg* produces no visible expression by itself, but in combination with *T* it acts to bring an additional number of small pigment cells to the area between the two discrete groups of micromelanophores. The result is that a crescent-like bridge of pigment cells is formed that unites the two outer dark clusters; the whole effect is then categorized as the *Guatemala-crescent,* Plate I, Fig. 1.

The Genetic Results

The general method of inheritance of the *Guatemala-crescent* pattern was demonstrated in the offspring of the mating of a purchased female platyfish having a *Guatemala-crescent* to a double recessive. From the results in the F_1, the P_1 were of the genetic constitution set forth in Table 1.

In another and apparently similar mating, slightly different results were obtained which indicated that the parents in the second mating were of the following genetic constitution:

Female		Male
T Cg/+ +	×	+ +/+ +

Phenotypes in F_1	Observed	Expected
1. *Guatemala-crescent, T Cg*	6	5.5
2. *Twin-spot, T*	6	5.5
3. No tail patterns, +	10	11.0

Autosomal or Sex-linked

The determination of whether the modifier *Cg* is sex-linked or autosomal was accomplished, in part, from a mating involving the dominant, sex-linked gene, *Sp*, for generalized black (macromelanophore) spotting on the body. A black-spotted female, (W) + (Y) *Sp*, was mated with a male that was recessive for the sex-linked gene, (Y) + (Y) +, but dominant for the *twin-spot* gene, *T*. The results in the F_1 were as shown in Table 2.

The criss-cross inheritance of the sex-linked *Sp* gene from the mother to her sons is characteristic of the domesticated strain of the platyfish in which the female is WY, the male YY, (Gordon, 1931); the *Sp* gene was presumably carried on the Y chromosome of the P_1 female. The *Cg* gene must have been carried by the *Sp* female parent because the male parent had the *twin-spot, T*, pattern only. The *Cg* gene, since it appeared in both sexes among the F_1, could not have been sex-linked. These results in addition to those obtained from the first mating, in which the female parent carried *Cg,* indicate that *Cg* is autosomal in its inheritance.

Test for Linkage to the Autosomal Gene *St* (+)

The inheritance of *Cg* was studied in association with a previously determined dominant, autosomal gene for *stippling, St* (Gordon, 1937). The gray color that the *St* gene produces, by a show of many micromelanophores, contrasts sharply with *golden*, which is its recessive effect. The P_1 were as shown in Table 3.

The results favor the conclusion that *Cg* is inherited independently of the *stipple* gene (*St*, +). Although the data are few, indeed, they indicate that if *Cg* were linked completely to *St* in the *Stippled* parent (*Cg St*) no *Stippled, Twin-spot* (*T* + *St*) F_1 should have appeared, yet nine did. If *Cg* were completely linked to the recessive + of the *Stippled* gene in the *Stippled* parent, *Cg* +, then no *Golden, Twin-spots* (*T* + +) F_1 should have appeared, yet four did.

While *Cg* and *T* appear in the expected ratios, the stippled fish outnumber the golden disproportionately. This has been observed and discussed by the author in previous papers but it is not pertinent to the study of the *T Cg* reaction.

Tests with Alleles, *Cc, C* and *O*

In the next series of experiments, the possible effects of *Cg* were tested on some of the other alleles of *T*, such as *Cc* for *complete-crescent, C* for *single crescent, O* for *one-spot* and *Co* for *comet* (Gordon, 1947).

1. Test with *Cc*, Complete-crescent

In order to determine whether *Cg* had any visible effect on the *complete-crescent* pattern, the genes *Cc* and *Cg* were combined in the following manner:

Guatemala-crescent		*Complete-crescent*
T Cg/+ +	×	*Cc* +/+ +

F_1 Phenotypes	Observed	Expected
1. *Complete-crescent, Guatemala-crescent, TCc Cg*	14	14.5
2. *Complete-crescent, Twin-spot, TCc*	7	14.5
3. *Complete-crescent, Cc*	35	29.0
4. *Guatemala-crescent, T Cg*	11	14.5
5. *Twin-spot, T*	16	14.5
6. No tail patterns, +	35	29.0
Totals	118	116.0

In counting the various F_1 phenotypes, the *complete-crescent, Cc,* pattern was identifiable in combination with the overlapping *T* and *T Cg* patterns, owing to the unobscured wedge-shaped mark of *Cc,* which is outside the range of *T* and *T Cg.*

In a follow-up experiment, the complete segregation of *T* from *Cc,* in the presence of *Cg,* was demonstrated by mating a platyfish with both a *complete-crescent* and a *Guatemala-crescent* back to one without a tail pattern as follows:

Complete-crescent, Guatemala-crescent		No Pattern
T Cg/Cc +	×	*+ Cg/+ Cg*

F_1 Phenotypes	Observed	Expected
Guatemala-crescent, T Cg	12	11
Complete-crescent, Cc	10	11

2. Test with Single Crescent

In order to determine whether *Cg* has any effect on the *single-crescent* gene, *C,* a platyfish with a *Guatemala-crescent* was mated to one with the *C* pattern. Results are seen in Table 4.

The expected frequencies given above are based upon two assumptions. First, that the genotypes of the parents were one or the other of the following:

Guatemala-crescent	×	*Single-crescent*
1. *T Cg/+ Cg*		*C +/+ +*
2. *T Cg/+ +*		*C Cg/+ Cg*

The second assumption is that when *C* is present in combination with *T Cg* the *single-crescent* pattern is completely obscured; *C* is not changed, and, certainly, *C* is not extended.

3. Test with One-spot

The ineffectiveness of the modifier *Cg* in changing the phenotypic expression of the *O*

Table 1.

Female *T Cg/T +* × Male *+ +/+ +*

Phenotypes in F_1	Female	Male	Observed	Expected
1. *Guatemala-crescent, T Cg*	11	7	18	21.5
2. *Twin-spot, T*	16	9	25	21.5

Table 2.

Phenotypes in F_1	Females		Males	
	Observed	Expected	Observed	Expected
1. *Spotted, Guatemala-crescent, Sp T Cg*	0	0	7	7
2. *Spotted, Twin-spot, Sp T*	0	0	7	7
3. *Guatemala-crescent, T Cg*	6	7	0	0
4. *Twin-spot, T*	8	7	0	0

Table 3.

Golden, Guatemala-crescent T Cg +/+ + + × *Stippled + Cg St/+ + +*

F_1 Phenotypes	Observed	No linkage	Expected if linked *Cg St*	*Cg +*
1. *Stippled, Guatemala-crescent, T Cg St*	23	18	24	12
2. *Stippled, Twin-spot, T + St*	9	6	0	12
3. *Stippled, + St*	29	24	24	24
4. *Golden, Guatemala-crescent, T Cg +*	14	18	12	24
5. *Golden, Twin-spot, T + +*	4	6	12	0
6. *Golden, + +*	16	24	12	24
	—	—	—	—
	95	96	96	96

Table 4.

Phenotypes in F_1	Presumed Genotypes	Observed	Expected
1. *Guatemala-crescent*	*T Cg/C +* or *T Cg/+ +*	10	12.5
2. *Single-crescent*	*+ Cg/C +*	7	6.25
3. No tail pattern	*+ Cg/+ +*	8	6.25

allele for *one-spot* was clearly demonstrated in three matings.

1. *O +/Cc +* × *T Cg/+ +*

F_1 Phenotypes	Observed	Expected
1. *One-spot, Guatemala-crescent, OT Cg*	6	7.5
2. *One-spot, twin-spot, OT*	9	7.5
3. *One-spot only, O*	19	15.0
4. *Crescent-complete, Guatemala-crescent, CcT Cg*	6	7.5
5. *Crescent-complete, twin-spot, CcT*	5	7.5
6. *Crescent-complete, Cc*	15	15.0

The 19 *one-spots* were not modified in appearance.

2. *O +/T +* × *Cc +/T Cg*

F_1 Phenotypes	Observed	Expected
1. *OT Cg*	7	6
2. *OT*	2	6
3. *OCc*	10	12
4. *CcT Cg*	8	6
5. *CcT*	8	6
6. *TT Cg*	6	6
7. *TT*	7	6

The *one-spot, O,* pattern was not modified in any of the three phenotypes in which it appeared.

3. The third mating involves, in addition to *O, T* and *Cg,* the independent autosomal gene *St* for the stippling effect of many micromelanophores which cover the entire body; the recessive (*stst*) effect is *golden* since most micromelanophores are lacking. One parent had *stipple, St, one-spot, O,* and *twin-spot, T;* the other was *golden* without tail markings, + + +. From the results of the mating it was demonstrated that the *golden* parent had been homozygous dominant for the *Cg* modifier.

O + St/T + + × *+ Cg +/+ Cg +*

F_1 Phenotypes	Observed	Expected
1. *Stippled, One-spot, O Cg St*	21	17.5
2. *Golden, One-spot, O Cg*	16	17.5
3. *Stippled, Guatemala-crescent, T Cg St*	16	17.5
4. *Golden, Guatemala-crescent, T Cg*	17	17.5

Separation of *Cg* from the Modifier *E*

The testing of the effect of *Cg* on the *comet, Co,* allele was complicated because the expression of *Co* is radically extended and changed by its own specific modifier *E.* Gordon (1946) showed that the combination *Co E* produces the *wagtail* pattern, in which all the fins and extremities are blackened by small black pigment cells. The question that needed clarification was whether the modifier *E* was synonymous with *Cg,* or whether there were, as originally suspected, two independent genetic modifiers, *E* that acts on *Co* and *Cg* that acts on *T.*

In two matings, a platyfish with *Co* and *T* was mated to a melanin-free, albino swordtail, *X. helleri,* because it was previously established by Gordon (1946) that many swordtails carry the modifier *E.* The following results were obtained in the F_1:

Phenotypes F_1	
1. *Wagtails, Co E*	26
2. *Guatemala-crescents, T Cg*	18

From the above results it was not clear whether there were one or two gene modifiers.

In some measure the distinctness of *Cg* was demonstrated by the results of mating a *comet* platyfish with one having a *twin-spot* pattern. The presumed genotypes of the P_1 were:

Comet *Twin-spot*

Co +/+ + × *T E/+ +*

F_1 Phenotypes	Genotypes	Observed	Expected
1. *Wagtail*	*Co E/T +*	3	3.50
Wagtail	*Co E/+ +*		
2. *Comet, twin*	*Co +/T +*	2	1.75
3. *Comet*	*Co +/+ +*	2	1.75
4. *Twin-spot*	*T E/+ +*	3	3.50
Twin-spot	*T +/+ +*		
5. No pattern	*+ E/+ +*	4	3.50
No pattern	*+ +/+ +*		
		14	14.0

The significant fact is that 3 of the 14 offspring had the *wagtail* pattern. This meant that the *E* modifier was carried by the *T* parent but that it had had no effect on the expression of *T.* Phenotypically, *Co E T* and *Co E* look alike and must be classified as *wagtails.*

In another mating a reverse effect was obtained, namely, the *comet* parent was harboring the *Cg* modifier. This was revealed when a *comet* was mated with a *twin-spot* platy as is indicated in Table 5.

While the matings involving *Co* and *T* yielded relatively few individuals, the very existence and recognition of some of the combinations support the conclusion that there are two specific modifiers, *E* and *Cg,* each of which reacts with its own specific primary gene, *Co* and *T,* respectively. For example, the combination of *Co Cg/T +* appeared four times in the last mating. If *Cg* modified *Co* as well as *T,* these four would have appeared as *wagtails,* but actually the patterns in the four were clearly recognized as being *comet* and *Guatemala-crescent;* there were no *wagtails* at all, Plate I, Fig. 2.

In order to get more evidence of this sort, another experiment was performed with similar phenotypes, that is, a *comet* platyfish was mated with a *twin-spot* as shown in Table 6.

In the F_1, 18 *comet, Guatemala-crescent* platyfish were observed, showing that the *Co* is not modified by the *Cg* gene. When one of the *Co Cg/T* + (*Comet, Guatemala-crescent*) F_1 platyfish was backcrossed to a platyfish that lacked a tail pattern, the results indicated that the parents must have had the genotypes set forth in Table 7.

No *twin-spot* patterns appeared among the F_1. From these results, it is clear that *Cg* has no visible effect on *Co* but rather that its action is restricted to the modification of *T*.

Presence of *Cg* in a Related Species

In a previous study of the modifier *E*, Gordon (1946) showed that this gene is found frequently in the swordtail, a close relative of the platyfish. In order to determine whether the swordtail, *Xiphophorus helleri*, carries the *Cg* gene, a *twin-spot* platyfish from a commercial stock was mated to a swordtail that belonged to a stock originally obtained from the Río Papaloapan in Mexico.

Twin-spot Platyfish		Plain Swordtail
T+/+ +	×	+ *Cg*/+ *Cg*

F_1 Phenotypes	
1. *Guatemala-crescent, T Cg*	35
2. No tail patterns, *Cg*	32

Since about 50% of the platyfish-swordtail hybrids showed the *Guatemala-crescent* and half showed no peduncular marking, the swordtail must have been homozygous for the modifier *Cg*. This indicates that some swordtails taken from natural habitats harbor a gene which apparently has no visible effect upon the members of its own species but which is capable of interacting with a specific gene of a different species, Plate I, Figs. 3 & 4.

Discussion

The data presented in this paper support the

Table 5.

Comet Co Cg/+ + × *Twin-spot T* +/+ +

F_1 Phenotypes	Genotypes	Observed	Expected
1. *Comet, Guatemala-crescent*	*Co Cg/T* +	4	1.5
2. *Comet, twin-spot*	*Co* +/*T* +	0	1.5
3. *Comet*	*Co Cg*/+ +	3	3.0
Comet	*Co* +/+ +		
4. *Guatemala-crescent*	*T Cg*/+ +	1	1.5
5. *Twin-spot*	*T* +/+ +	1	1.5
6. No pattern	+ *Cg*/+ +	3	3.0
No pattern	+ +/+ +		
		12	12.0

Table 6.

Comet		*Twin-spot*
Co Cg/+ +	×	*T* +/*T* +

F_1 Phenotypes	Genotypes	Observed	Expected
1. *Comet, Guatemala-crescent*	*Co Cg/T* +	18	23
2. *Comet, twin-spot*	*Co* +/*T* +	23	23
3. *Guatemala-crescent*	+ *Cg/T* +	32	23
4. *Twin-spot*	+ +/*T* +	19	23
		92	92

Table 7.

Comet, Guatemala-crescent		No Pattern
Co Cg/T +	×	+ *Cg*/+ *Cg*

Phenotypes in F_1	Genotypes	Observed	Expected
1. *Comet* only	*Co Cg*/+ *Cg*; *Co Cg*/+ +	14	15
2. *Guatemala-crescent*	*T Cg*/+ *Cg*; *T Cg*/+ +	16	15
		30	30

conclusion that in platyfish the *Cg* gene is a specific autosomal modifier of the *twin-spot* gene, *T; Cg* is not linked to *E*, which is a specific modifier of *comet, Co*. Nevertheless *T* and *Co* are alleles; both belong to a common series of seven dominant multiple alleles (Gordon, 1947). These facts reveal the existence of an intricate and interlocking genetic system for the production of nine distinctive patterns, all of which may appear only at the posterior region of the platyfish, and all of which are composed of a specific type of pigment cell, the micromelanophore. This interlocking and precise genetic system depends not only upon seven multiple alleles (plus one universal recessive), but also upon two independently inherited specific gene modifiers. In graphic form, the genetic system is represented by Text-fig. 1.

1. Frequency of the *Cg* Gene in Natural Populations

The frequency of the *Cg* gene modifier in the upper Río Usumacinta and the Lago de Petén area may be estimated on the basis of the frequency of the *T* allele; because only when the *T* allele is present can the presence of the *Cg* gene be detected. Gordon (1947) found that of 552 adult platyfish, which represented the entire Guatemala collection made in 1935, 31 specimens had the *T* allele either alone (13) or in combination with other members of its allelic series (18). In 2 of the 31, the *T* allele was modified by the *Cg* gene to produce the *Guatemala-crescent* pattern. The presence of the *Cg* gene on the basis of the 31 *twin-spot* platyfish is therefore 6.4%. In contrast, the presence of the *T* allele on the basis of 31 platyfish out of 552 is 5.6%. Thus, it would appear that the frequency of the *Cg* gene in the Río Usumacinta population as a whole is slightly greater than the frequency of the *T* allele it modifies. Actually, however, the frequency of the *Cg* gene might better be calculated from the limited number of platyfish from the Lago de Petén basin because the modifier *Cg* has been detected only in this area. In the Lago de Petén, which includes Petenxil and Ponteil but excludes Laguna de Zotz (Stations 12 to 20, Gordon, 1947), 79 platyfish were collected of which 14 had the *T* allele, the gene frequency of *T* in the platyfish from the Lago de Petén basin is 0.093, and of *Cg* 0.074; both calculated by the usual formula $f = 1 - \sqrt{1-p}$.

The distribution of the *Cg* gene is restricted not only in the platyfish of Guatemala, but it is probably non-existent among all the other known natural populations of the platyfish. For example, Gordon & Gordon (in press) evaluated the frequencies of the *twin-spot* and six other tail patterns among the platyfish of eight large river populations. To attain maximum accuracy, they based their evaluation on the frequencies of *single* tail patterns only; double patterns were deliberately excluded because many of them were difficult to identify with complete assurance. The frequencies of the single *twin-spot* patterns in each of eight natural populations of the platyfish were as follows:

	Number of Platyfish	Single *Twin-spots*	%
1. Jamapa	860	54	6.3
2. Papaloapan	3,492	138	4.0
3. Coatzacoalcos	1,334	252	18.9
4. Tonala	178	5	2.3
5. Grijalva	651	50	7.7
6. Usumacinta	552	13	2.4
7. Hondo	327	10	3.1
8. Belize	1,526	78	5.1

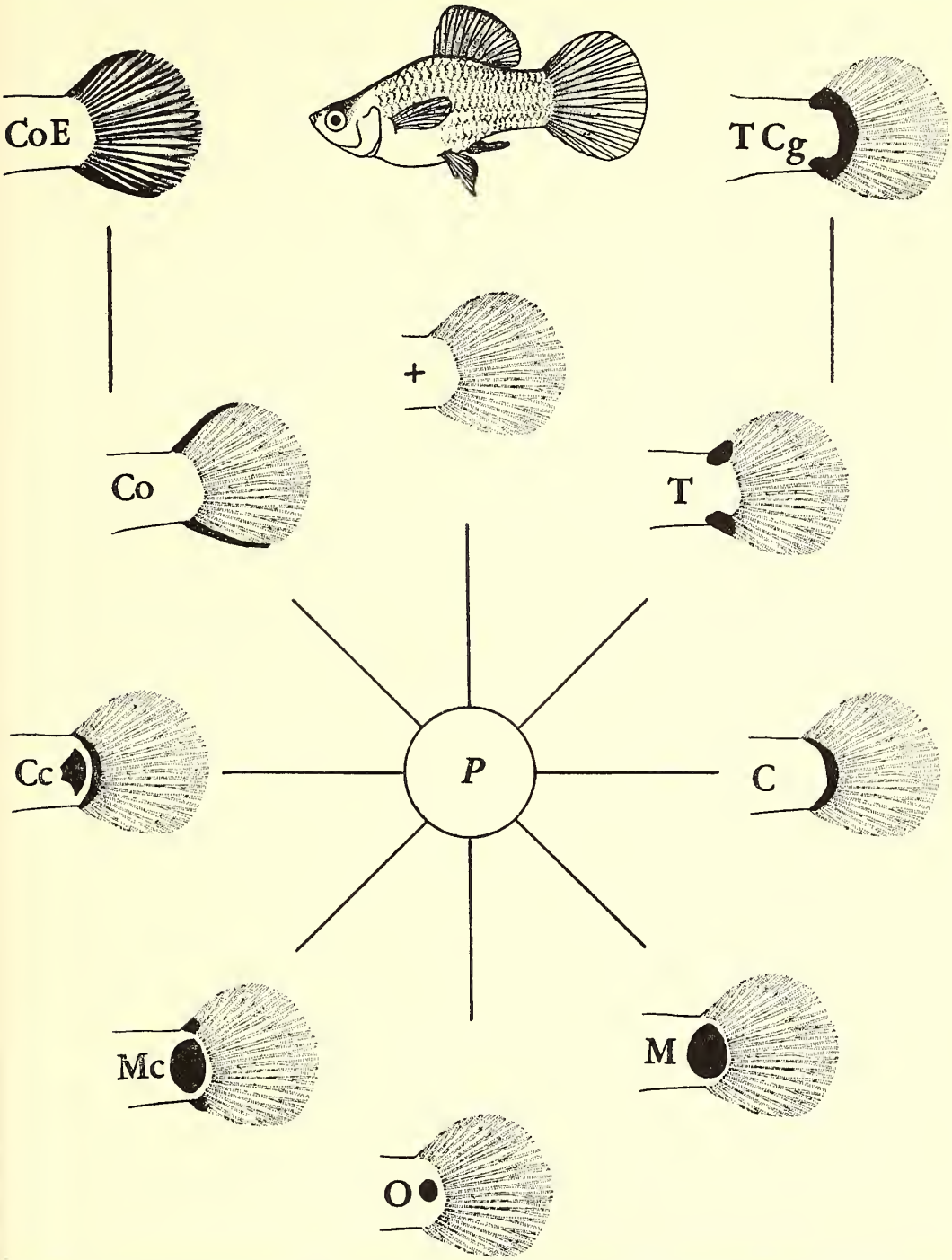
On the basis of this sample of Gordon & Gordon's (in press) data, it is evident that the *Cg* gene is either lacking in the eight river populations (except in Lago de Petén) or it is extremely rare. While the platyfish of the Lago de Petén have been assigned to the Río Usumacinta population, this analysis points up the fact that a local population within an assigned river system may differ genetically from its other local populations.

2. Introgression of the *Cg* Gene from One Species to Another

With regard to the mechanism of inheritance of the *Cg* gene in the platyfish, it is exactly similar to that of an equivalent color pattern of the domesticated swordtail which was previously analyzed by Kerrigan (1934) and confirmed by Gordon (1937). The genetic symbols these authors used varied, however:

	Xiphophorus maculatus	*Xiphophorus helleri*	
Phenotype	Gordon (in press)	Kerrigan (1934)	Gordon (1937)
Guatemala-crescent	*T Cg*	*C P*	*C P*
Twin-spot	*T* +	*C p*	*T P*
No pattern	+ *Cg*	*c P*	*C p*
No pattern	+ +	*c p*	*T p*

It seems strange that the inheritance of the *twin-spot* and *Guatemala-crescent* patterns should have been worked out first in the swordtail, because *X. helleri* in its many native habitats does not have any of these patterns. Aquarium-reared swordtails, however, obtained the *T* gene from the platyfish about 1911 by experimental introgressive hybridization made



TEXT-FIG. 1. Nine Pigment Cell Patterns. The intricate genetic system that controls nine specific pigment cell patterns in the platyfish, *Xiphophorus maculatus*, is built around the central gene *P* for seven melanic patterns located in the anterior part of the caudal fin and the posterior part of the caudal peduncle. The gene *P* has a series of seven dominant, autosomal, multiple alleles: *PT*, *PC*, *PM*, *PO*, *PMc*, *PCc*, *PCo* plus the multiple recessive *P+*. For convenience the symbol *P* is deleted from the formulae for the patterns which are written *T* for *PT*, etc.

Superimposed upon the *P* series of alleles are two independent, autosomal gene modifiers: *Cg* that interacts specifically with *T*, and *E* that interacts exclusively with *Co*. Neither *Cg* nor *E* have any visible expression by themselves, nor any visible effect upon the *P* multiple alleles other than *T* and *Co*.

T = *Twin-spot* *C* = *Crescent* *O* = *One-spot* *Co E* = *Wagtail complex* *Mc* = *Moon-complete*
Co = *Comet* *M* = *Moon* *T Cg* = *Guatemala-crescent* *Cc* = *Complete-crescent* + = No tail pattern

possible by the selective breeding and hybridization trials of German aquarists. When in 1911 the *twin-spotted* swordtails were sent by German aquarists to the British Museum (Natural History) for identification, Regan (1911) at first regarded the *Xiphophorus* with the *twin-spot* as a new species and named it *rachovii* on the assumption that it had been collected at Puerto Barrios, Guatemala. Later, when Regan (1913) obtained swordtails unquestionably from Puerto Barrios, he saw that the fish did not have any markings resembling the *twin-spot* or *crescent*. Consequently he suspected that the *"rachovii"* type was of hybrid origin, produced in the aquarium. He therefore recommended that the name *Xiphophorus rachovii* be regarded as invalid. As indicated in the present paper, a *twin-spot* platyfish mated with a swordtail produced a hybrid that had the *Guatemala-crescent* pattern. This confirms Regan's suggestion that *X. rachovii* was a fish of hybrid origin, Plate I, Figs. 5 & 6.

Once the *Guatemala-crescent* pattern was synthesized by fish fanciers in the platyfish-swordtail hybrids, some breeders, by backcrossing *T Cg* hybrids to platyfish, recreated the platyfish body type and distinguished it by the *T Cg* color pattern. Thus, while the aquarium-bred *"rachovii"* swordtails owe their color pattern to the *T* gene of the platyfish, the color pattern in the aquarium-bred *Guatemala-crescent* platyfish is traceable to the *Cg* gene of the swordtail. The development of *Guatemala-crescent* patterns in two related species of fishes illustrates an example of what may be called *reciprocal introgression.*

While precise data on the frequency of the *Cg* gene in the swordtail are not available, it would appear from the experiences of fish fanciers that the frequency of *Cg* is probably much higher in wild *X. helleri* than in wild *X. maculatus*. Just what function *Cg* serves in contemporary swordtails is unknown since, as is the case in the platyfish, *Cg* has no visible effect in the absence of the *T* gene. Perhaps in the early evolution of the xiphophorin fishes, *Cg* imparted some advantage to their members and was by this virtue retained in some of the present day species. It is odd, however, that the *Cg* gene, as far as can be determined, is restricted to a few local populations of *X. maculatus* within a small part of the Lago de Petén basin of Guatemala. This represents a tiny area considering the wide range of *X. maculatus,* which extends southeast from the Río Jamapa, near the city of Veracruz, Mexico, across southern Yucatan peninsula to the Belize River, near the city of Belize in British Honduras.

The discovery of the action of the *Cg* gene in the platyfish has cleared up the problem of the "rarity" of the *Guatemala-crescent* pattern, but it does not explain the significance of the gene's survival in only one of its many geographical races, nor its probable higher frequencies among races of swordtails.

Summary

A specific gene, *Cg,* was found to modify the *T* (*twin-spot*) allele which is one of seven multiple alleles of the *P* gene for seven pigmentary patterns in the platyfish. The combination of *T Cg* produces the *Guatemala-crescent* pattern which has been observed only twice in more than 9,000 adult specimens taken from eight natural populations of the platyfish. The distribution of *Cg,* under natural conditions, is restricted solely to the Lago de Petén area of Guatemala. There its frequency is not rare, for it approaches that of the *T* allele.

Previously, another gene modifier *E* was found to interact specifically with the *Co* (*comet*) allele of the *P* gene to produce the *wagtail* complex, *Co E*. Thus, on the basis of seven multiple alleles of the *P* gene and two independent gene modifiers, nine patterns are produced. Neither modifier *Cg* nor *E* alone has any visible effect in the platyfish or in the swordtail.

By introgressive hybridization, the genes *T* and *Co* have been transferred to swordtails under domestication to produce the *Guatemala-crescent* and *wagtail* swordtails. In a reverse direction aquarium-bred *Guatemala-crescent* and *wagtail* platyfish have obtained the gene modifiers *Cg* and *E* from the swordtail. Thus, the *Guatemala-crescent* and the *wagtail* platyfish and swordtails represent an example of reciprocal introgression.

These experiments show how it is possible for two parents, from stocks which never had a *Guatemala-crescent* or a *wagtail* pigmentary pattern, to have offspring in which one or the other of these patterns will appear. The results demonstrate the reality of specific gene modifiers.

Acknowledgements

I thank Pamela Alexander and James W. Atz for reading the manuscript and Sam C. Dunton for the first four photographs in Plate I.

References

Gordon, Hugh & Myron Gordon

[In Press] Maintenance of polymorphism by potentially injurious genes in eight natural populations of the platyfish, *Xiphophorus maculatus*. Jour. Genetics.

Gordon, Myron

1927. The genetics of a viviparous top minnow *Platypoecilus;* the inheritance of two kinds of melanophores. Genetics, 12: 253-283.

1937. Heritable color variations in the Mexican swordtail-fish. Jour. Heredity, 28: 222-230.

1946. Introgressive hybridization in domesticated fishes. I. The behavior of comet, a *Platypoecilus maculatus* gene in *Xiphophorus hellerii*. Zoologica, 31: 77-88.

1947. Speciation in fishes. Distribution in time and space of seven dominant multiple alleles in *Platypoecilus maculatus*. Advances in Genetics, 1: 95-132.

1951. A second specific gene-modifier of pigment cell growth in *Xiphophorus* (*Platypoecilus*) *maculatus*. Anat. Rec., 111 (3): 104 (Abstr. no. 168).

1954. Two opposing sex-determining mechanisms, one XX-XY, the other WY-YY, in different natural populations of the platyfish, *Xiphophorus maculatus*. Caryologia, 6: 960-964.

[In Press] Physiological genetics of fishes. *In:* "Physiology of Fishes," Vol. 2, chapter 10, Academic Press Inc., New York.

Kerrigan, A. M.

1934. The inheritance of the crescent and twin spot marking in *Xiphophorus helleri*. Genetics, 19: 581-599.

Regan, C. T.

1911. On some fishes of the family Poeciliidae. Ann. Mag. Nat. Hist., 8: 373.

1913. Ueber *Xiphophorus Rachovii*. Bl. f. Aquar.-u. Terrarienk., 24: 532-533.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. A platyfish, *Xiphophorus maculatus,* with a *twin-spot* (*T*) pattern is at the left. The one to the right has a *Guatemala-crescent* (*T Cg*) pattern; note that the *T Cg* complex also involves dot-like markings at the mandibular junction.

Fig. 2. The male platyfish, *Xiphophorus maculatus,* (to the left) has no tail pattern. The female (to the right) has *comet* (*Co*) and *Guatemala-crescent* (*T Cg*) patterns; note the small black dot at the mandibular junction in the female.

Fig. 3. The male platyfish, *Xiphophorus maculatus,* shown in the upper left, having a *comet, Co,* and *twin-spot, T,* pattern, was mated to a female albino swordtail, *X. helleri,* shown in the lower right. Their F_1 hybrids are shown in Fig. 4.

Fig. 4. The first-generation platyfish-swordtail hybrids; the one on the left shows the wagtail complex, *Co E*; the one on the right shows the *Guatemala-crescent* pattern, *T Cg.*

Figs. 5 & 6. The so-called *"rachovii"* swordtails are actually hybrids reconstructed to look like swordtails. These "swordtails" have been produced by fish culturists by a series of backcrosses of the hybrids to swordtails; that is, by introgressive hybridization. The female swordtail, Fig. 5, shows just the effects of the *T* gene. The *Guatemala-crescent* pattern which characterizes the male, Fig. 6, is traceable to the *T* gene of the platyfish and the *Cg* gene of the swordtail.

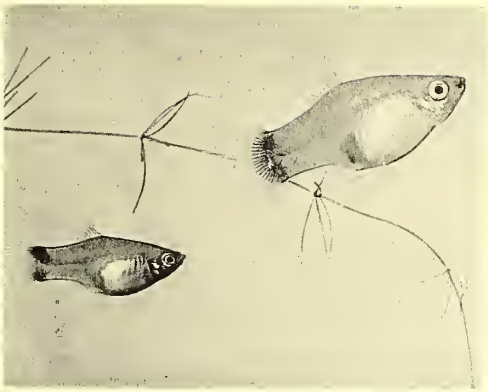


FIG. 1

FIG. 2

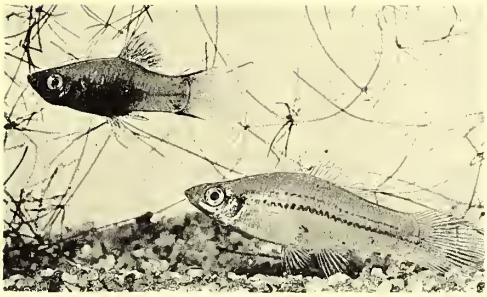


FIG. 3

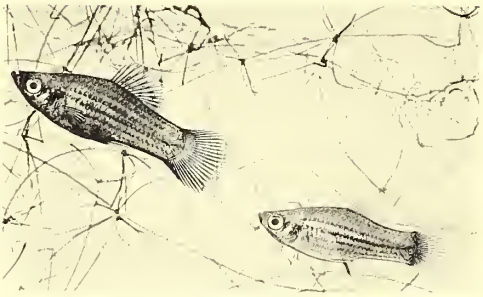


FIG. 4

FIG. 5

FIG. 6

AN INTRICATE GENETIC SYSTEM THAT CONTROLS NINE PIGMENT CELL PATTERNS IN THE PLATYFISH

19

Introduced Mammals and Their Influence on Native Biota[1]

ANTOON DE VOS, RICHARD H. MANVILLE
& RICHARD G. VAN GELDER

Ontario Agricultural College, Guelph, Ont., Canada; McGraw-Hill Book Company, New York; and American Museum of Natural History, New York

(Text-figure 1)

CONTENTS

INTRODUCTION

IT is characteristic of man to alter his environment, and he has attempted in various ways to improve upon the conditions of nature. Unfortunately some attempts, such as indiscriminate deforestation and draining of swamps, have had bad effects. The ruthless extermination of many forms of plants and animals, or their uncontrolled introduction into new environments, also fall into this category.

In recent years the faulty reasoning behind the introduction of animals has become increasingly apparent. Biologists now are generally opposing the liberation of exotics into the wild, because of the unpredictable nature and possibly unfavorable economic consequences of such action, and because of an aesthetic objection to mixing diverse faunas. In compiling the present data on introduced mammals our aim is to elucidate the results of this practice and to clarify certain principles involved.

Introductions may be classified as intentional or unintentional. Prior to and during the early stages of colonization of many parts of the world, most exotics were imported unintentionally, as "chance" introductions, for example as stowaways on ships. Intentional introductions, for the purpose of establishing foreign species in a new territory, reached a peak during the Nineteenth Century. This paper deals mainly with the intentional introduction of wild mammals. Some information is presented concerning feral mammals, but introduced domestic species are not discussed.

Although the majority of introductions of mammals have failed, most of those which succeeded have proved detrimental to man's interests; only a few have been advantageous. The most serious problem is that of predicting the consequences of an introduction. The exotic only rarely will occupy the niche which the introducer expects it to fill.

The expense involved in introducing a species and getting it established is usually very high and has proved to be a poor investment in most cases. From the economic viewpoint, it is often more practical to foster an increase of native mammals as a means of attaining the desired results.

By and large, it seems impossible to introduce animals under "scientifically controlled" conditions, because of our lack of knowledge of ecologic conditions. There is an obvious need for more detailed research here. Preferably, introductions should be undertaken only after they are carefully studied and approved by an international board of scientists, for too often exotics have not only become a nuisance in the country to which they were introduced, but also in ad-

[1] Received for publication December 1, 1955.

jacent countries where they spread as a "gift."

Overly successful introductions usually lead to a demand for control. This is a complex problem, and it is practically impossible or extremely costly to exterminate introduced species which have become well established.

We have attempted to assemble such data as are available on the introduction of alien species of mammals throughout the world, with a more detailed discussion of the consequences of such introductions in the case of certain conspicuous or economically important species. Our efforts have been more fruitful in certain groups and areas than in others. Orders best represented among introduced mammals are the Lagomorpha, Rodentia, Carnivora and Artiodactyla. Although it appears to be impossible to gather a complete record of all mammals which have been introduced or transplanted during historic time, a fairly good picture has been obtained of what has happened in the more recent past.

The subspecific identity of all forms could not be ascertained but this is presented when known. Space limitations do not permit the inclusion of all known cases of transplantations from one point to another within one continental area, as of deer, elk, rabbits and squirrels in North America.

The scientific and common names follow Ellerman & Morrison-Scott (1951), Miller & Kellogg (1955), Laurie & Hill (1954) and Troughton (1947). Where there is doubt as to the name of the animal in question, that given in the original publication is indicated.

The bibliography, although certainly not exhaustive, is intended as a helpful starting point for investigators interested in geographic areas or groups of mammals. The section on North America was prepared by Manville, that on South America by Van Gelder, and most of the balance by the senior author.

Acknowledgments

We are indebted to many colleagues for their cooperation and generosity in supplying data for this compilation. It is a pleasure to acknowledge our gratitude to all those who have helped, either by letters or by existing manuscripts, which are cited in the text, and we wish especially to mention the following in this connection: R. H. Baker, R. Bigalke, G. Bump, J. H. Calaby, J. A. Crespo, R. M. Gilmore, P. Hershkovitz, C. B. Koford, N. Kuroda, L. C. de Mesquita Maia, J. McNally, E. I. Sillman, J. H. Westermann and K. A. Wodzicki. We are especially grateful to V. H. Cahalane, H. W. Levi and T. I. Storer, who were kind enough to read the manuscript and make critical suggestions.

Intentional Introductions or Transplantations

Eurasia:

Introductions of mammals generally seem to have been less successful in Eurasia than on the other continents. This is the largest land mass and has more species of mammals than the New World. Competition there may be more severe, resulting in less chance of success for exotics. Because most emigrations of the white man have been *from* Eurasia, and because he is mainly responsible for recent animal introductions, the number of alien mammals released *in* Eurasia has been less than in other continents.

No data could be obtained for several countries in southeast Asia, and relatively few for the Soviet Union and China. It seems, however, that in the U.S.S.R. introductions and transplantations of fur-bearers have been accomplished on a large scale. From 1929 to 1948, in more than 500 areas inside the boundaries of the Soviet Union, no less than 21 species of fur-bearers, amounting to about 80,000 individuals, apparently were released (Schmidt, 1954). Another source (Naumoff, 1950) states that from 1925 to 1948 more than 115,000 mammals, of 32 species, were released in the Soviet Union.

Table 1 summarizes the introductions and transplantations in Eurasia.

The introduction of the Muskrat *(Ondatra zibethicus)* into Eurasia resulted in a serious threat to the local economy by damage to dikes and roadbeds and, conversely, in the addition of a valuable fur-bearer to Russia and Finland. Three females and two males from Alaska were introduced in the neighborhood of Prague by Prince Colleredo-Mannsfeld in 1905. More Muskrats, probably from Canada, were liberated later by the same person.

The spread of the species from this first site of release was rapid. In Bavaria, for example, in 1923 and 1924, the rate of emigration is said to have amounted to 30 to 45 miles yearly. In 1914, nine years after the first planting, the population in Bohemia alone was estimated at two million. In 1933, the colonized territory embraced about 64,000 square miles (Mohr, 1933). From Bohemia the Muskrat spread mainly in a northerly and easterly direction and now occupies most of Czechoslovakia, eastern Germany, Poland and parts of Yugoslavia, Romania and European Russia (Text-fig. 1). A good discussion of the early spread of the Muskrat in Europe is presented by Storer (1937).

In the 1920s several subspecies of Muskrat were introduced into fur farms in France. Muskrats escaped from several of these farms, and by 1933 were established in twelve areas. The first capture in the wild was made in 1930. Five

centers of infestation were in the basins of the Seine and the Somme, four were in eastern France and three others were in the center of southeastern France. An active campaign of destruction and the use of a virus resulted in the extermination of Muskrats in southeastern France (Bourdelle, 1939). Muskrats entered Switzerland from the area of infestation in Alsace, and by 1950 about 800 had been killed.

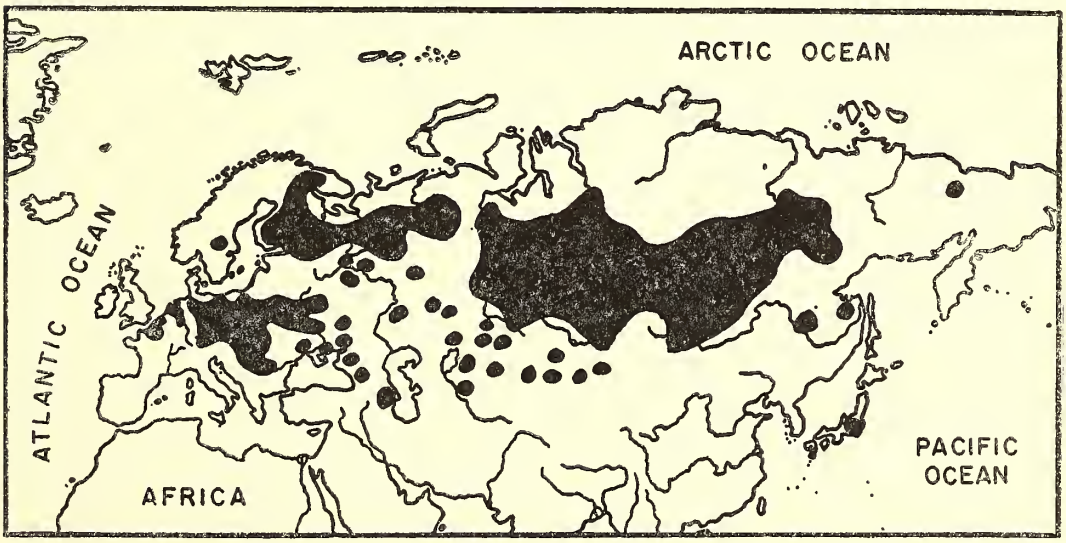
In the Low Countries, the Muskrat occupies nearly all of northern Belgium, as a result of introduction by 1930. In Holland the first Muskrats were caught in 1941, where they are now slowly penetrating along the southern boundary. Government trappers are still keeping the advance in check fairly well (van Koersveld, 1954).

Even before Poland was invaded by the progeny of the Muskrats from Czechoslovakia, some escapees from fur farms had already become established. Nearly all of Poland is now inhabited by the species. The Muskrat was introduced illegally into Sweden sometime before 1944 (Liljeström, 1954). The species was also deliberately introduced at three sites in the lakes region of Finland in 1922 and 1923, from Czechoslovakian stock; other specimens were introduced later from America. They spread from 18 to 25 miles yearly and are now present in most of the country except the extreme north. The Muskrat is now the most important furbearer in Finland, in point of value and of numbers, and 150,000 to 250,000 pelts are harvested annually (Schmidt, 1954; Hoffman, 1952).

The Soviet Union first introduced Muskrats in 1927; from then until 1945, 79,198 were released in the north European and Siberian taiga zone, even as far as Kamchatka, where a shipment arrived from Ontario in 1928 (Eyerdam, 1932). They are still spreading from centers of release (Berger, 1944). In 1941, 150,000 pelts were harvested, and in 1954 about 649,000 (Schmidt, 1954). Nothing specific seems to be known about a supposed introduction into China (Bachrach, 1953). The Muskrat was also introduced in Japan from America, perhaps before 1945; it is confined to Tokyo and environs (Kuroda, 1955).

The only area where the Muskrat has been successfully eradicated is the British Isles. Since 1929 there have existed 87 farms from which animals escaped to establish colonies in England, Scotland and Ireland. Around 1930 it was forbidden to keep Muskrats on fur farms, and a successful system was devised to exterminate them (Warwick, 1934). By 1935, young Muskrats were no longer captured, and by 1939 the campaign was considered terminated; 4,299 animals had been caught.

Only in Finland and Russia is this rodent of value as a fur-bearer, and here no problem of damage seems to exist. In western Europe, however, its burrowing is extremely harmful to dikes, roads and fish-ponds. The freshwater pondfishing industry accuses it of eating fish and damaging nets; also it raids garden crops. Certainly the sums expended on destruction of Muskrats and repairing their damages are much higher than the profits obtained from fur and flesh. It is still doubtful whether further spread of this introduced mammal can be curtailed.



TEXT-FIG. 1. The present distribution of the Muskrat in Eurasia, derived from stock originally introduced from North America (modified after M. Hoffman, 1952).

TABLE 1: MAMMALS INTRODUCED OR TRANSPLANTED IN EURASIA

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of Origin	Country or Place of Introduction	Reason for Introduction
A: *Established; increased in numbers and/or range:*					
1. *From another continent*					
Gray Squirrel	*Sciurus carolinensis*	1890	N. America	British Isles	Pet
Muskrat	*Ondatra zibethicus*	1905	Canada?	Czechoslovakia	Fur
Nutria	*Myocastor coypus*	1930s	S. America	Several countries	Fur farming
American Beaver	*Castor canadensis*	...	N. America	Russia	Fur
American Mink	*Mustela vison*	Recent	N. America	Scandinavia, Iceland	Fur farming
Alaskan Fox	*Vulpes fulva alascensis*	Recent	Alaska	Eur. Russia	Fur
American Red Fox	*Vulpes fulva*	Recent	N. America	Eur. Russia	Fur
White-tailed Deer	*Odocoileus virginianus*	1934, 1947	U.S.A.	Finland	Hunting
2. *From the continent to islands or vice versa, or between islands*					
Pig-tailed Macaque	*Macaca nemestrina leonina*	Recent	Burma	Andaman Is.	?
Formosan Macaque	*Macaca cyclopis*	Since 1942	Formosa	Oshima, Japan	Escaped
Crab-eating Macaque	*Macaca irus*	?	Western Indonesia	Celebes, Lesser Sunda Islands	?
Crested Celebes Macaque	*Cynopithecus niger*	?	Western Indonesia	Batchian Is.	?
Black-naped Hare	*Lepus nigricollis*	?	India or Ceylon	Java	Hunting
Golden-backed Squirrel	*Callosciurus caniceps*	1940s	Formosa	Japan	Zool. Gardens
Northern Red-backed Vole	*Clethrionomys rutilus*	1870	Kamchatka	Bering Is.	?
Fat Dormouse	*Glis glis*	1886	Europe	British Isles	?
Javan Mongoose	*Herpestes javanicus*	?	Indonesia	Ambon	?
European Red Fox	*Vulpes vulpes*	Recent	British Isles	Sweden	Fur
Siberian Weasel	*Mustela sibirica coreana*	1930s	Korea	Japan	Fur farming
Siberian Weasel	*Mustela sibirica itatsi*	Before 1901	Japan	Hokkaido	Fur farming, rat control
Ermine	*Mustela erminea*	1931	Holland	Terschelling Is.	Rat control
Weasel	*Mustela nivalis*	1931	Holland	Terschelling Is.	Rat control
Masked Palm Civet	*Paguma* sp?	?	Formosa or China	Japan	Cage animal
Malay Civet	*Viverra tangalunga*	?	Indonesia	Celebes	?
Common Palm Civet	*Paradoxurus hermaphroditus*	?	Indonesia	Moluccas	?
Sika Deer	*Cervus nippon hortulorum (mantchuricus)*	1880s	Manchuria	Europe	...
Sika Deer	*Cervus nippon nippon*	1880s	Japan	Europe	...
Sika Deer	*Cervus nippon taiouanus*	1942	?	Japan	...
Fallow Deer	*Dama dama*	Middle Ages	Asia Minor	Central Europe	Parks
Rusa Deer	*Cervus timorensis moluccensis*	1855 1913, 1920	Ceram Moluccas	Aru Islands New Guinea	Hunting Hunting
Rusa Deer	*Cervus timorensis russa*	1680	Java	Borneo	Hunting
Hog Deer	*Axis porcinus*	18th Century	India	Ceylon, Denmark	Hunting
Mouflon	*Ovis musimon*	1869	Sardinia or Corsica	Slovakia	Hunting

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of		Reason for Introduction
			Origin	Introduction	
Indian Buffalo	*Bubalus bubalis*	Recent	India	Andaman Islands	Hunting
3. *Transplanted on the continent*					
Golden Hamster	*Mesocricetus auratus*	?	Syria	Germany	?
European Rabbit	*Oryctolagus cuniculus*	Middle Ages	Europe	Central Europe	?
European Hare	*Lepus europaeus*	?	C. Europe	Sweden, Far East	Hunting
Raccoon-Dog	*Nyctereutes procyonoides*	Recent	E. Asia	Soviet Union	Fur
Sable	*Martes zibellina*	Recent	Trans. to several places		Fur
Stone Marten	*Martes foina*	Recent	Trans. to several places		Fur
Ferret	*Mustela putorius eversmanni*	Recent	Trans. to several places		Fur
Sea Otter	*Enhydra lutris*	Recent	Trans. to several places		Fur
European Mink	*Mustela lutreola*	Recent	Siberia	Far East	Fur
Arctic Fox	*Alopex lagopus*	Recent	Siberia	Kola Peninsula	Fur
B: *Failed to survive:*					
Hog Deer	*Axis porcinus*	1700s	India	Ceylon	Hunting
		1880	India	Denmark	Hunting
Nubian Ibex	*Capra ibex nubiana*	1910s	Asia Minor	Czechoslovakia	Hunting
Goat	*Capra hircus*	1910s	India	Czechoslovakia	Hunting
Chinese Water-Deer	*Hydropotes inermis*	1850	China	British Isles	Parks
Black-tailed Deer	*Odocoileus hemionus columbianus*	1850	N. America	British Isles	Parks
Axis Deer	*Axis axis*	1846	India	Java, Nicobar Is.	Hunting
Indian Muntjac	*Muntiacus muntjak*	1850	India	British Isles	Parks
Reeves's Muntjac	*Muntiacus reevesi*	1850	China	British Isles	Parks
C: *Transplantations of subspecies to range of other subspecies:*					
Roe Deer	*Capreolus capreolus pygargus*	Before 1914	Siberia	Czechoslovakia	Hunting
Maral Deer	*Cervus elaphus asiaticus*	19th Century	Siberia	Eur. Russia	Hunting
American Wapiti	*Cervus canadensis*	19th Century	N. America	Austria	Hunting
Red Squirrel	*Sciurus vulgaris exalbidus*	Recent	Siberia	C. Russia	Fur
European Beaver	*Castor fiber*	1927	Norway	Latvia	Fur
		1935	Norway	Letland, Finland	Fur
Red Deer	*Cervus elaphus*	?	Germany	Norway	Fur
English Red Fox	*Vulpes vulpes*	Recent	British Isles	Sweden	Fur
Siberian Sable	*Martes zibellina*	Recent	Several parts of Soviet Union		Fur
D: *Status unknown:*					
Alpine Marmot	*Marmota baibacina*	Recent	Soviet Union		Fur
Himalayan Marmot	*Marmota bobak*	Recent	Soviet Union		Fur
Large-toothed Souslik	*Citellus fulvus*	Recent	Soviet Union		Fur
Raccoon	*Procyon lotor*	Recent	N. America	Soviet Union	Fur
Corsac Fox	*Vulpes corsac*	Recent	?	Soviet Union	Fur
Arctic Fox	*Alopex lagopus groenlandicus*	Recent	Greenland	Soviet Union	Fur
Russian Desman	*Desmana moschata*	?	?	?	?
Striped Skunk	*Mephitis mephitis*	Recent	N. America	Soviet Union	Fur

The American Gray Squirrel *(Sciurus carolinensis)* has become a serious liability since its introduction into the British Isles. Although several early introductions failed, others resulted in restricted local establishments. The present population probably dates from introductions since 1890, although there are several records of the species before that date. Specimens were liberated in southern and central England, in Scotland and in one locality in Ireland. By 1930, Gray Squirrels occupied approximately 13,350 square miles in the greater part of southeastern England, the Midlands and Yorkshire (Middleton, 1930).

Little is known of the present distribution of the Gray Squirrel in Ireland, where it was introduced before 1938. It is, however, extending its range, and is also spreading in Scotland (Shorten, 1953).

The spread of the Gray Squirrel in England is well documented by Shorten (1946, 1953) who devised a system of grids 10 km. square and related the distribution and spread of the squirrel to them. She also tried to correlate the disappearance of the native Red Squirrel *(Sciurus vulgaris leucourus)* with the spread of the Gray Squirrel. While the American intruder was present in 274 grids in 1937, it occurred in 708 in 1944 and 1945, and had spread into an additional 199 by 1952. Apparently in some areas the spread may be halted by such natural barriers as large rivers with few bridges, or by mountainous or treeless country. In no place does the Gray Squirrel inhabit dense coniferous woods, which are the preferred habitats of the Red Squirrel (Middleton, 1930).

After a period of years, the presence of Gray Squirrels may cause the disappearance of Red Squirrels. In 1952, Red Squirrels seemed to have disappeared from areas in eight counties where Gray Squirrels had become established since 1945 (Shorten, 1953). There is little evidence available regarding competition or antagonism between the two species. Possibly the Gray Squirrel carries a disease which is fatal to the Red Squirrel but not to itself. The occurrence of many epidemics after the first appearance of Gray Squirrels supports this contention (Middleton, 1930).

This American immigrant is a serious pest in Great Britain, where only the Norway Rat is considered worse. It does much damage to many kinds of trees, fruits and plants.

The Nutria *(Myocastor coypus)*, a native of South America, has been introduced on many fur farms in Europe since 1926. In many cases the animals were released when they were considered a liability, and sometimes they escaped. Wild populations are now present in Russia, England, Holland, Denmark, Germany, France and Japan.

In Russia, Nutrias have been imported into Transcaucasia, the Kuban delta, the downstream parts of the Kura and Terek Rivers and the southern part of the Amu-Darja Basin, where they have multiplied. Since 1949, increasing numbers of Nutria pelts have been offered on the market (Schmidt, 1954). Efforts to introduce this fur-bearer into central Russia have failed because of unsuitable climatic conditions. However, the Nutria is successfully established in Armenia, Georgia and the steppes of Shirwan (Lindemann, 1956). Because of low fur prices in 1949, Nutrias were released from several fur farms in Holland. Although the animals are well established, they are readily controlled because of their large size, diurnal habits and trusting ways. According to P. V. Jensen *(in litt.)* several escaped animals are now living in the wild in Denmark, where they are reproducing. In France, Nutrias have escaped from parks in several parts of the country, mostly in the central section. They appear to be maintaining their numbers in the wild state. These rodents were imported from Europe to fur farms in Japan in 1931. A few escaped and established a small breeding population; in 1949 about 500 were known to be present south of Okayama City. Nutrias trample down and eat the rice in the paddy fields (Kuroda, *in litt.*).

Apart from the more spectacular introductions already mentioned, several other rodents have been imported or transplanted. The Red Squirrel *(Sciurus vulgaris exalbidus)* was introduced into central Russia from several parts of Siberia with the result that thousands are now being harvested (Schmidt, 1954).

European Beavers *(Castor fiber)* were reintroduced from Norway into their former range in Sweden in the 1920s. Specimens also were imported from Norway into Latvia in 1927, and again into Latvia as well as Finland in 1935 (Harper, 1945). Beavers also were transplanted to several parts of the Soviet Union, such as the Kola Peninsula, western Siberia and the Volga delta. The American Beaver *(Castor canadensis)* has also been imported into Russia (Naumoff, 1950).

The Syrian Golden Hamster *(Mesocricetus auratus)* has become established locally in the wild in parts of Germany. Here there exists the possibility that it may become a pest in crop lands and food storage places.

The Fat Dormouse *(Glis glis)* was introduced into the British Isles from continental Europe. Rothschild released a few pairs near Tring in 1886, and there were several subsequent importations. After a slow establishment, the spe-

cies gradually spread into several adjoining counties. It seems to be doing little serious damage, and keeps largely to the neighborhood of houses (Cansdale, 1953).

The Formosan Golden-backed Squirrel *(Callosciurus caniceps thaiwanensis)* was introduced in zoological gardens on the Island of Oshima, south of Tokyo, some time after 1940. It escaped, and in 1950 an estimated population of 20,000 inhabited many parts of the island. These squirrels chase White-eyes *(Zosterops palpebrosa)* from the flowers of the camellia, resulting in lack of fertilization of this plant. Other trees and shrubs are stripped of their bark, and nuts also are eaten. These activities result in a considerable decrease in the production of camellia oil, the island's principal commodity.

The northern Red-backed Vole *(Clethrionomys rutilus)* was introduced in 1870 from Kamchatka to Bering Island, one of the Commander group. Within ten years it spread over all the island from the beaches to the interior mountains. It occurs both in the swamps and on the sand dunes, and has become a pest in the huts of the natives (Palmer, 1899).

Marmota baibacina, Marmota bobak and *Citellus fulvus* have also been transplanted within the Soviet Union (Naumoff, 1950).

The European Rabbit *(Oryctolagus c. cuniculus)* undoubtedly has a much wider distribution in central and western Europe now than in Roman times, and it still seems to be extending its range, assisted by man. It was introduced in the Middle Ages into Germany and Holland for hunting purposes and reached the British Isles from central Europe in the Twelfth Century (Cansdale, 1953). In Denmark it has been released in several places in the past fifty years, but has not increased greatly. About 1920 it crossed the Danish border from an isolated German population released about 1900 (Jensen, *in litt.*). The rabbit has also been released in the Soviet Union (Naumoff, 1950) and in China (Allen, 1938). Rabbits were liberated on one of the islands of the Madeira group in the Fifteenth Century; they increased so rapidly as to seriously deplete the vegetation.

Although rabbits are popular game animals and provide much meat and many skins, they are nevertheless destructive pests in that they raid gardens, kill many tree seedlings and reduce the carrying capacity of pastures for livestock. They also compete seriously for food with the European Hare. The recent myxomatosis outbreak in western Europe has changed the picture completely; rabbits have become very scarce.

Other introduced lagomorphs include the European Hare (*Lepus e. europaeus*) in the Far East (Lindemann, 1956) and also in Sweden, where it is gradually replacing the Alpine Hare *(Lepus timidus)*, possibly because of a higher reproductive potential. A pair of Alpine Hares was introduced from Norway to the Faroe Islands in 1854-55; their descendants have given rise to a new subspecies, *seclusus*. Immediately following their introduction, all the hares turned white in winter, but today they retain their dark coats throughout the year (Bourlière, 1954). This species has also been transplanted from the Scottish Highlands to the southern uplands and to the island areas of the Highlands (Darling, 1947). The Black-naped Hare *(Lepus nigricollis)*, a native of India and Ceylon, has been introduced around Djakarta, Indonesia, and now occurs also near Bogor and Bandoeng (van Bemmel, *in litt.*).

The few transplantations of monkeys in Asia include the Celebes Crested Macaque *(Cynopithecus niger)* to the island of Ambon and the Crab-eating Macaque *(Macaca irus)* to Celebes and the Lesser Sunda Islands from the more westerly islands in the Indonesian Archipelago. The Formosan Rhesus Monkey *(Macaca cyclopis)* escaped from captivity on Oshima Island, south of Tokyo, and multiplied rapidly in the absence of natural enemies; there is some doubt as to whether it occurs in the wild at Kivozumi (Prefecture Chiba), Hindo. The Pig-tailed Macaque *(Macaca nemestrina leonina)* was introduced from India to the Andaman Islands (S. L. Hora, *in litt.*).

The Javan Mongoose *(Herpestes javanicus)* has been introduced to Ambon from other islands in the Indonesian Archipelago. One Palm Civet *(Paradoxurus hermaphroditus)* was introduced throughout the Moluccas and the Lesser Sunda Islands, and the Malay Civet *(Viverra tangalunga)* was imported into Celebes. The Masked Palm Civet (*Paguma* sp.) was imported into Japan in ancient times and again more recently as a cage animal; it probably came from Formosa *(P. taivana)* or from South China *(P. larvata)*. Some evidently escaped, according to sporadic records from Central Hondo and Shikoku. The animal is said to have been caught in the Prefecture of Yamanashi in early times, and again more recently.

The Mouflon *(Ovis musimon)*, although reduced in numbers in its native Sardinia and Corsica, appears to have thrived in various continental areas. It has become established in Germany, Czechoslovakia, Austria, Rumania, European Russia, Holland and Denmark. In 1869, ten were transferred to the Tribec Mountains of Slovakia, where they became established. Several herds are now dispersed over Germany and

Austria. The herd in Holland, numbering about one hundred head, was started in 1918-19. In 1951-52 Mouflons were released on private lands in Southfyn, Denmark (Jensen, *in litt.*). The species was introduced into Italy during the mid-1800s, but has long since disappeared (Harper, 1945). According to Turcek (*in litt.*) Mouflons were introduced to the Crimea and South Ukraine before World War I. It is of interest that a new race developed in the Slovakia range; about 1910 a distinctly different type of sheep was common, in which the rams are darker in color, lack the saddle patch and possess short, thick horns with converging tips (Allen, 1954).

In 1910 two goats, the Bezoar Goat *(Capra hircus)* and the Nubian Ibex *(Capra ibex nubiana)* were introduced from Asia Minor into the High Tatra Mountains of Czechoslovakia. They interbred with the native Ibex *(Capra i. ibex)*, reintroduced since 1901. The hybrids did not do well, possibly because they were born during the dead of winter rather than in spring, as are the native Ibex (Turcek, 1951).

The Indian Buffalo *(Bubalus bubalis)* has been recently introduced into the Andaman Islands from India. The availability of considerable food and the absence of predators have aided its establishment, and it is slowly extending its range (Hora, *in litt.*).

In 1929, seventeen Muskoxen *(Ovibos moschatus)* were introduced from East Greenland to Spitzbergen. This herd appeared to be thriving in 1950 (Anon., 1952a).

Several species of deer have been introduced or transplanted in Eurasia. The Fallow Deer *(Dama d. dama)*, originally from Asia Minor, has been widely imported in captivity, and also liberated in the wild. In Denmark it was mentioned in the literature as early as 1231, having been introduced by the Danish Kings for hunting; it now occurs in deer parks as well as in the wild, and its numbers were estimated at 3,300 head in 1950 (Jensen, *in litt.*). Fallow Deer have been in Germany since the Middle Ages, but their population is small. They were introduced into the Bialowies Forest of western Russia about 1890, but required special care; none have been observed since 1930 (Lindemann, 1956).

Sika Deer have been introduced at several places in western Europe as well as in eastern Asia. Two subspecies reached the British Isles, *Cervus n. nippon* from Japan and *C. n. mantchuricus (= hortulorum)* from Manchuria, near Loch Rosque from 1880 to 1890. Their range still centers about the Achnasheen area. Another introduction in 1893 still survives at Carradale, Kintyre (Darling, 1947). Sika Deer are numerous in parts of the Midlands and in the southern counties from Kent to Dorset (Matthews, 1952). H. G. Lumsden (*in litt.*) reports them present near Inniskillen, Ireland. The Japanese race first reached Denmark around 1900, and was later liberated from captivity; about 500 of these animals are now living in the wild (Jensen, *in litt.*). Sika Deer are still reported in the wild in France. In Japan, *Cervus nippon taiouanus* was released on Oshima Island, south of Tokyo, about 1942-43; fifty were observed here in 1950 (Kuroda, 1955). The Manchurian Sika *(C. n. hortulorum)* has been released in the Soviet Union (Naumoff, 1950).

The Hog Deer *(Axis porcinus)* was introduced to the western parts of Ceylon during the Dutch occupation in the Eighteenth Century. Here it multiplied and persisted until about 1920, but since then, with increased human population and heavier hunting pressure, it has been practically extirpated (C. W. Nicholas, *in litt.*). This species, from India, was liberated on Samsö Island, Denmark, in 1880, but no longer exists there (Jensen, *in litt.*).

The Axis Deer (*Axis axis*), introduced from India to the Nicobar Islands in 1846, seems to have disappeared there, for reasons unknown (Hora, *in litt.*).

Reeves's Muntjac *(Muntiacus reevesi)* and the Indian Muntjac *(Muntiacus muntjak)*, as well as the Chinese Water Deer *(Hydropotes inermis)* and the Black-tailed Deer *(Odocoileus hemionus columbianus)*, have apparently escaped or been released from parks in the British Isles since 1850 (Matthews, 1952). They seem to survive in the wild in small numbers. Barking Deer have been reported introduced from Bali to Lombok (Everett, *in litt.*). Sambar Deer *(Cervus unicolor)* have been transplanted from the Philippine Islands to Guam and Rota (Baker, 1946).

White-tailed Deer *(Odocoileus virginianus)* from Minnesota have twice been released in Finland. One buck and four does, liberated 100 miles north of Helsinki in 1934, had multiplied to more than 200 by 1947. A further introduction of three bucks and three does was made in 1948 (Connelly, 1948). The next year some damage to forests and isolated farms was noted (Salmi, 1949).

Deer have been widely transported throughout the eastern part of the Indonesian Archipelago; in some instances the circumstances are known. Rusa Deer *(Cervus timorensis russa)* were introduced into Ambon from Java and later from Celebes (where they were not native) during the Seventeenth Century. In the Aru Islands, deer *(Cervus timorensis moluccensis)* were imported from Ceram in 1855, and are now numbered in the thousands. In South Borneo Rusa Deer were

introduced near Mataram about 1680; these increased to enormous herds in the Nineteenth Century, but have since declined (van Bemmel, 1952). In Netherlands New Guinea, *C. t. moluccensis* now occurs on the Onin Peninsula (introduced from Ceram in 1913) as well as on the eastern coast of the "Birdshead" (around Manokwari) and near Hollandia, the latter stock from Halmaheira in 1920 (Westermann, 1947).

Three species of deer have been transplanted in Europe with poor results. The Siberian race of the Roe Deer *(Capreolus capreolus pygargus)*, released in Czechoslovakia prior to World War I, hybridized in some localities with the smaller native race; crosses involving a native female produced a fetus too large for parturition. Effects of crosses with native males are still perceptible in bucks with abnormally high and thick antlers (Turcek, 1951). In the British Isles, Siberian Roebuck have escaped from parks since 1850 (Matthews, 1952); they have also been transplanted within the Soviet Union (Naumoff, 1950).

The transplantation of German Red Deer *(Cervus elaphus)* into Norway has apparently resulted in the virtual extermination of the species there, probably because the German strain was less hardy. A few hundred American Elk *(Cervus canadensis)* were introduced to Austria by Francis Joseph I (Lorenz, 1953). Asiatic elk have also been transplanted in Russia (Naumoff, 1950).

Central Maral Deer *(Cervus elaphus asiaticus)* from Asia and American elk *(Cervus canadensis)* were introduced into European Russia in the days of the Czars. Hybridization between the two species resulted in the development of animals with antlers having less spread, fewer points and poorly developed burrs (Lindemann, 1956).

Other ungulates transplanted within the Russian orbit include the European Bison *(Bison bonasus)*, the Siberian Ibex *(Capra ibex sibirica)* and the Wild Boar *(Sus scrofa)* (Naumoff, 1950).

Many carnivorous fur-bearers have recently been transplanted in the wild, especially in the Soviet Union. The Raccoon-dog *(Nyctereutes procyonoides)* from extreme eastern Asia was introduced into the Baltic Republics and into White and Middle Russia from Smolensk to the Urals. In central Russia this animal is now one of the principal fur producers, but in Siberia its fur proved to be less valuable and the animals soon competed seriously with the more highly regarded native mustelids (Lindemann, 1956). It is reported that in the Caucasus the Raccoon-dog changed its food habits from fish and crabs to game birds, hares and poultry.

Several forms of Mink have been transplanted for their furs. *Mustela vison* from North America has escaped from fur farms in Norway, Sweden, Denmark, the Soviet Union and Iceland. Their status in Norway is described by Wildhagen (1956). In the Scandinavian countries they are a serious problem because of their depredations; whether they are yet fully established in Denmark is not certain (Jensen, *in litt.)*. The Iceland escape is rather recent, but damage to native wildlife already has been reported (Anon., 1953b).

The Siberian Weasel *(Mustela sibirica coreana)* was imported from South Korea to Japan after 1930, where it escaped from fur farms and bred with the native *M. s. itatsi* which it is gradually replacing; it has already extended into southwestern Hondo and the eastern parts of Shikoku. The Japanese race, introduced into Hokkaido before 1901, has increased and spread; it was further transplanted to two small islands off Hokkaido in 1933, and in 1948 was imported into Okujirijima in the hope of exterminating *Rattus norvegicus* and *Apodemus*.

Lindemann (1956) refers to the introduction of the European Mink *(Mustela lutreola)* into the Far East.

An interesting case of differential survival is recounted by van Koersveld *(in litt.)*. In 1931 nine Ermines *(Mustela erminea)* and 102 weasels *(Mustela n. nivalis)* were introduced on the island of Terschelling, Holland, to diminish an abundance of rabbits and rats. By 1953, the Ermines had increased to a high population, but the weasels had entirely disappeared. A bounty is now set on the Ermines, which kill many wild birds and poultry.

Sables have been transplanted from the Trans-Baikal to several parts of the Ural and Altai Mountains. The Kamchatkan Sable *(Martes zibellina kamtshadalica)* was introduced into western Siberia, where it has interbred with the native form. The resultant hybrids have heavier and more valuable furs than the native animal (Lindemann, 1956).

Other mustelids introduced or transplanted in the Soviet Union include the Stone Marten *(Martes foina)*, the Skunk (probably *Mephitis mephitis)*, the Siberian Weasel *(Mustela sibirica)*, the Sea Otter *(Enhydra lutris)* and the Ferret *(Mustela putorius eversmanni)* (Naumoff, 1950).

The North Siberian Polar Fox *(Alopex lagopus)* has been released on the Kola Peninsula (Lindemann, 1956). The introduction of Red Foxes *(Vulpes vulpes)* from England to Sweden may be responsible for the appearance of numerous "Samson foxes"—individuals lacking guard hairs and therefore of little value for their fur. Alaskan Silver Foxes *(Vulpes fulva alascensis)* were released in Finland in 1938 with the idea of producing a good cross-fox hybrid between it

and the native Red Fox, and interbreeding seems to be occurring. In European Russia large numbers of North American Silver Foxes were released (Schmidt, 1954). Naumoff (1950) lists also *Vulpes fulva, Vulpes corsac* and *Alopex lagopus groenlandicus* as introductions in the Soviet sphere.

The Raccoon *(Procyon lotor)* is the only other carnivorous fur-bearer introduced into the Soviet area which is mentioned by Naumoff (1950). It is present in the Far East (Ussouri) and in European Russia.

North America:

North America, and the United States in particular, seems to have received an unduly large number of introductions. Why this continent, with a rich and varied native mammalian fauna, should have been subjected to the importation of numerous exotics is not clear, but a possible explanation is the dependence of the colonists upon hunting and trapping for food and clothing. The spread of settlements from Atlantic to Pacific led to the virtual annihilation of some important and valuable animals, such as the Beaver, the Bison and other ungulates. The depletion of several major game species did not necessarily result in the loss of hunting as a privilege, but led to the use of other, perhaps less desirable, species as game. North Americans have come to consider hunting and trapping as a portion of their heritage, and many of the introductions and transplantations of mammals may be related directly to attempts to provide added materials to bolster the reduced numbers of native mammals.

In no case have foreign implants been an unqualified success; generally they have been failures. Several colonies of exotic monkeys, of various species, are established in the Caribbean region (Miller & Kellogg, 1955). The last few years have seen the successful introduction of the Barbary Sheep *(Ammotragus lervia)* into New Mexico (O'Conner, 1953), the release of the European Rabbit *(Oryctolagus cuniculus)* in Pennsylvania and the liberation of the Chinchilla *(Chinchilla* sp.) in California (Voris *et al.*, 1955).

Accidental introductions or escapes take place from time to time, but seldom are these followed by the permanent establishment of the species. Examples of failures are the Blue Fox *(Alopex lagopus)* in Minnesota (Bailey, 1929) and the Coatimundi *(Nasua narica)* in Oklahoma (Glass & Hanson, 1952) and in Indiana (Lyon, 1923).

Worthy of special studies in themselves are the details of the many transplantations, from state to state, of Pronghorns *(Antilocapra americana)* (Fisher, 1942; Nichol, 1942), American Elk *(Cervus canadensis)* (Atwood, 1938; Kirk, 1923; Scheffer, 1941), Muskrat *(Ondatra zibethicus)* (Dickey, 1923; Eyerdam, 1932; Storer, 1937) and other favored game and fur-bearing mammals (Dice, 1927; Dixon, 1929; Bailey, 1936). These species have been transplanted widely and, as a result, several local races, particularly of the Elk and Muskrat, have been mixed.

Countless experimental plantings have been tried and a few examples may be cited. On Anticosti Island, in the Gulf of St. Lawrence, have been made introductions of Moose *(Alces alces)*, American Elk, Whitetail Deer *(Odocoileus virginianus)*, Bison *(Bison bison)*, Mink *(Mustela vison)*, Fisher *(Martes pennanti)*, Red Fox *(Vulpes fulva)*, Beaver *(Castor canadensis)*, Muskrat and Varying Hare *(Lepus americanus)* (Newsom, 1937). On Lanz Island, British Columbia, Mink were imported from Vancouver Island about 1938; by 1950 they were so plentiful as to force out the pelagic birds which formerly nested there. From here, these Mink populated Cox Island, on which Raccoons *(Procyon lotor)* from Vancouver had also been planted in 1938. The Raccoons became established, but had not by 1950 become as injurious as the Mink (Clifford *et al.*, 1951).

Massachusetts has introduced, on the islands of Nantucket and Martha's Vinyard, Varying Hares, Black-tailed Jackrabbits *(Lepus californicus)*, European Hares *(Lepus europaeus)*, Florida Cottontails *(Sylvilagus floridanus)*, Red Fox, Prairie Dogs *(Cynomys ludovicianus)* and Fallow Deer *(Dama dama)*. The foxes and Prairie Dogs became such pests that they were exterminated; the Fallow Deer seemingly compete for food with the White-tailed Deer on Martha's Vinyard; the Florida Cottontail probably competes with the native *Sylvilagus transitionalis*. It is believed that western rabbits introduced tularemia to these islands (Starrett, *in litt.*).

In New York State, since 1886, released game and fur-bearing mammals have included Raccoon, Red Fox, Coyote *(Canis latrans)*, Timber Wolf *(Canis lupus)* Beaver, Muskrat, Fox Squirrel *(Sciurus niger)*, Varying Hare, Cottontail, European Hare, Pronghorn, Black-tailed Deer *(Odocoileus hemionus)*, White-tailed Deer, Moose, Elk, Red Deer *(Cervus elaphus)*, Japanese and Siberian Deer and Wild Boar *(Sus scrofa)*. Only the Beaver and White-tailed Deer plantings were successful (Bump, 1940). The State of Washington has witnessed the establishment of the Virginia Opossum *(Didelphis marsupialis)*, eastern Fox Squirrel, Gray Squirrel *(Sciurus carolinensis)*, eastern Cottontail, Nutria *(Myocastor coypus)* and the Pronghorn (Buechner, 1953; Dalquest, 1948). In Michigan, Jack-

rabbits *(Lepus townsendii)*, European Hares, Nutria, Reindeer, Moose and Elk have been introduced (Blouch, 1954; Ruhl, 1940). Alaska has seen the introduction of ground squirrels *(Citellus undulatus)*, Raccoons, Blue Foxes, Black-tailed Deer, Reindeer, Roosevelt Elk, Bison and Muskoxen (Murie, 1940; Scheffer, 1947; Palmer, 1954). Muskrats and ground squirrels failed to survive on the Pribilof Islands (Preble, 1923).

In Texas, several ranchers have experimented with exotic game species. At least some of this stock is on open range and hence might possibly become established in the wild. The King Ranch has introduced White-tailed Deer, Elk, Japanese Fallow Deer, Indian Blackbuck *(Antilope cervicapra)* and Nilgai Antelope *(Boselaphus tragocamelus)*; only the last two survived (Lehmann, 1948). The Bar-O Ranch has stocked the Sardinian Mouflon *(Ovis musimon)* which later crossed with domestic sheep, the Blackbuck, Asiatic Serow *(Capricornis sumatraensis)*, and Aoudads. The Rickenbacker ranch has kept European Fallow Deer, Roe Deer *(Capreolus capreolus)*, Asiatic Sambar *(Cervus unicolor)* and Blackbuck on a large scale (O'Conner, 1953). Some zoological gardens in Texas are selling Axis Deer and antelopes for the stocking of ranches. It is estimated that at least 1,000 wild Blackbuck exist in Texas alone today, and they are on the increase (Stilwell, 1955). Similar instances of large scale introductions or transplantations might be related for California (Storer, 1931, 1933), North Carolina (Anon., 1953a), Ohio (Hicks, 1940), Ontario (Soper, 1923), Pennsylvania (Anon., 1944), Saskatchewan (Forsyth, 1942), Utah (Popov & Low, 1953) and elsewhere.

Kangaroo Rats *(Dipodomys ordii)* have been introduced on the sand dunes on the shores of Lake Erie, near Fairport, Ohio, and are established (Bole & Moulthrop, 1942).

Happily, the short-sighted policy of wholesale introduction on a hit or miss basis is no longer as common as it once was. Missouri has recently stopped its long practice of supplying Cottontails to many other states in the northeastern United States. Still, there are reports of plantings of Great Plains Jackrabbits *(Lepus californicus)* in Kentucky (Myers, 1952) and of European Rabbits from the San Juan Islands off the coast of Washington to Pennsylvania and elsewhere (Anon., 1954b).

Table 2 summarizes the status of introduced mammals in North America. The following introductions or transplants are discussed in detail.

The Nutria *(Myocastor coypus)* has been imported for its fur in many places in the United States and Canada. Escapees or releases from fur farms have survived in the wild and several local populations have increased. The animal seems best established in the marshes of the southeastern states (Dozier, 1951) and of Oregon, where it is the subject of much debate. Its pelt commands a low price and is in little demand. In Louisiana it is increasing rapidly and competing seriously for food with the more valuable muskrats, which are reported on the decline (Ashbrook, 1953). It was reported on the Pacific coast by 1942 (Larrison, 1943) and is now in Washington (Dalquest, 1948). In California most of the escaped Nutrias were killed (Storer, *in litt.*). Colonies are reported living in a feral state in Montana (Jellison, 1945), Texas (Petrides, 1950; Swank & Petrides, 1954), Ohio, Kansas and Michigan.

The Muskrat was transplanted to Vancouver Island, to other islands off the coast of British Columbia, and to several localities in California (Storer, 1937). The population has spread since the original releases were made.

From 1888 to 1911 European Hares were liberated at various places, and some of these releases survived (Osborn, 1933). The earliest well-authenticated releases were in Brant County, Ontario, in 1912 (Dymond, 1922). From here they spread rapidly, and by 1923 were reported in several localities in Wellington and Waterloo Counties (Soper, 1923). They were further introduced near Thunder Bay, Ontario (Allin, 1950). They are now reported in all of southern Ontario (Reynolds, 1952). These hares, although good game animals, are often pests, consuming crops and damaging orchards. By 1950, they were considered to be established beyond hope of eradication in southern Ontario and in Michigan (Cahalane, 1950). In addition, they have also invaded northern Wisconsin and Minnesota, and range from the St. Lawrence across northeastern New York (Hamilton, 1952) and extreme western New England into New Jersey and eastern Pennsylvania.

The introduction of the European Rabbit on the mainland of North America is recent and may still be susceptible of control. On other continents this burrowing rabbit seriously competes for forage even with sheep, undermines buildings, kills vegetation and causes erosion. A population has existed on the San Juan Islands, off the coast of Washington, perhaps since the days of the Hudson's Bay Company occupation (Thompson, 1955). In 1900 and thereafter more "Belgian Hares" or "tame rabbits," a domestic form raised for fur and meat, were released by the lighthouse keeper on Smith Island to supplement the earlier population. Introductions followed on several other islands of the San Juan group. Numbers increased and reached plague

TABLE 2: MAMMALS INTRODUCED OR TRANSPLANTED IN NORTH AMERICA

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of Origin	Country or Place of Introduction	Reason for Introduction
A: *Established; increased in numbers and/or range:*					
Raccoon	*Procyon lotor*	1932	Florida	Bahamas	Curiosity
Mink	*Mustela vison*	1938	Vancouver	Scott Islands, B.C.	Fur
European Red Fox	*Vulpes vulpes*	18th Century	W. Europe	N.E. U.S.A.	Hunting
Nutria	*Myocastor coypus*	1940?	S. America	Louisiana	Fur
European Hare	*Lepus europaeus*	1912	Europe	Ontario	Sport, food, fur
European Rabbit	*Oryctolagus cuniculus*	1900 1953	Europe	Washington Pennsylvania	Fur, food Hunting
Wild Boar	*Sus scrofa*	1912	Germany	N. Carolina	Sport
Fallow Deer	*Dama dama*	1938	?	Nebraska	Sport
Peccary	*Pecari angulatus*	?	Yucatan	Cozumel Island, Mexico	Food?
Kangaroo Rat	*Dipodomys ordii*	?	S.W. U.S.A.	Ohio	Curiosity
Jaguarundi	*Felis yagouaroundi*	Before 1942	Central or S. America	Florida	?
B: *Survived but did not spread:*					
Three-toed Sloth	*Bradypus griseus*	1925	Panama	Barro Colorado Island	Curiosity
Sambar Deer	*Cervus unicolor*	1900	Asia	Florida	Sport
Fallow Deer	*Dama dama*	?	Europe	Texas	Sport
Roe Deer	*Capreolus capreolus*	?	?	Texas	Sport
Blackbuck	*Antilope cervicapra*	?	India	Texas	Sport
Nilghai	*Boselaphus tragocamelus*	?	India	Texas	Sport
Mouflon	*Ovis musimon*	?	Sardinia	Texas	Sport
Serow	*Capricornis sumatrensis*	?	Asia	Texas	Sport
Aoudad	*Ammotragus lervia*	1950	Africa	New Mexico	Sport
Axis Deer	*Axis axis*	1930s	India	Florida	Escapees
C: *Failed to survive:*					
Coatimundi	*Nasua narica*	1950	?	Oklahoma	Escaped
Blue Fox	*Alopex lagopus*	1927	Arctic	Minnesota	Escaped
Ground Squirrel	*Citellus undulatus*	1899	Alaska	Pribilof Is.	Food for foxes
Muskrat	*Ondatra zibethicus*	1913	Nushagak	Pribilof Is.	Fur
Red Deer	*Cervus elaphus*	?	?	?	Sport
Sika Deer	*Cervus nippon*	?	Japan	?	Sport
German Deer	*Cervus elaphus*	?	Germany	?	Sport
Fallow Deer	*Dama dama*	?	Japan	Texas	Sport
Roe Deer	*Capreolus capreolus*	?	Siberia	?	Sport
D: *Successful transplantations* (only a partial list, covering little-known cases):					
Marten	*Martes americana*	1953 1954	Ontario Montana	New Hampshire Wisconsin	Aesthetic Aesthetic
Muskox	*Ovibos moschatus*	1930	Greenland	Alaska	Food, clothing
Armadillo	*Dasypus novemcinctus*	1918	Mexico	Florida	Curiosity
Mountain Goat	*Oreamnos americanus*	1948	Rocky Mts.	S. Dak., Colo.	Hunting

proportions by 1924, when the rabbits were estimated at over thirty to the acre on Smith Island. Wholesale poisoning was undertaken, and nearly 2,000 were probably killed (Couch, 1929). But the rabbits have persisted, and of late are being introduced elsewhere. Feral stocks also occur on South Farallon Island, 30 miles west of San Francisco (Storer, *in litt.*). Shipments have recently reached Ohio, Pennsylvania, Indiana and Wisconsin (Barnes, 1955). The implicit dangers have been publicized (Anon., 1954b; Thompson, 1955). Perhaps, as was the case in Australia and New Zealand, these initial releases may prove unsuccessful, and this new exotic may not further extend its range into North America.

The Nine-banded Armadillo (*Dasypus novemcinctus*), which gradually has been extending its range northward into Texas since the 1880s, was probably introduced into Florida during World War I (Bailey, 1924). It thrived, and by 1952 occurred over all the state except the swampy southwestern portion and possibly the western panhandle (Neill, 1952). It is regarded as highly undesirable because its burrowing activities undermine buildings, damage gardens and penetrate dikes and levees; on the other side of the picture, the armadillo's burrows provide homes for other animals, its meat is edible, and its horny armor is made into novelties for the tourist trade. It now also occurs in Arkansas, Louisiana, New Mexico and Oklahoma, and has been reported from Alabama, Georgia, Kansas and Missouri (Fitch *et al.*, 1952). In part, this spread appears to be by natural means; its course has been well summarized by Buchanan (1955).

Introduction of Wild Boars (*Sus scrofa*) from the Harz Mountains of northern Germany, primarily for sport, began in 1912 with the arrival of fifteen males and fifteen females. They were kept in a 600-acre enclosure near Hooper Bald, North Carolina, until 1920, when about 100 escaped and persisted in the wild (Stegeman, 1938). They were decimated by hog cholera in 1932, but by 1937 it was estimated that there were about 230 in the region. Some of these were released near Carmel, California, in 1924 and persisted at least until 1938 (Shaw, 1940). Wild Boars readily breed with domestic swine. They are regarded by some as desirable game animals, but also they are harmful to vegetation and to ground-dwelling animals. Their greatest population is currently in the mountain forests of eastern Tennessee, but they exist also in Georgia, North Carolina, Oregon, Texas and on the Corbin Preserve in New Hampshire (Baynes, 1923; Cahalane, 1950; Scheffer, 1941).

The introduction of the Moose into Newfoundland (Pimlott, 1953) may be cited as an instance of the establishment of a mammal which greatly augmented the big game resources of the province and which apparently filled a niche at least partly vacant. Two introductions were made, in 1878 and in 1904. The second attempt resulted in complete establishment. In twenty years the radius of distribution had extended to at least 80 miles from the point of release. At present the species is well established on the entire island, with a bull kill of approximately 14,000 over a period of eight years. Moose have also been introduced into Labrador.

As recently as about 1938, some sixty white Fallow Deer (*Dama dama*), original source unknown, were released on the Hall Ranch ten miles northwest of Petersburg, Boone County, Nebraska. They have reproduced and spread, being reported in 1955 from five counties in central Nebraska (Packard, 1955), where they do some damage to orchards and to crops.

Sambar Deer (*Cervus unicolor*) from a zoological park were released on St. Vincent's Island, near Appalachicola, Florida, in 1900. They have since reproduced and thrived.

The Greenland Muskox (*Ovibos moschatus*), indigenous to the northern mainland and to many parts of the Arctic islands, has been introduced on Nunivak Island, Alaska, where it competes directly with the introduced Reindeer.

Aoudads or Barbary Sheep (*Ammotragus lervia*) have recently been introduced into New Mexico (O'Connor, 1953) and have been promoted for release as game animals in Texas. A large herd is established in the wild on the St. Simeon Ranch near San Luis Obispo, California (Lindemann, *in verbis*).

Dwindling herds of Bighorn Sheep (*Ovis canadensis*) in our western mountains have been supplemented by stock from British Columbia. Twenty individuals were released in the Hart Mountains of eastern Oregon in 1954, and others in several localities in Colorado, Montana and New Mexico (Buechner, 1956).

There is some evidence that the present Red Fox of the eastern United States is a direct descendant of the European Red Fox (*Vulpes vulpes*) which was introduced between 1650 and 1750 from England for fox hunting (Gilmore, 1946).

On Barro Colorado Island, in the Panama Canal Zone, six Three-toed Sloths (*Bradypus griseus*) from nearby Frijoles were released in 1925 (Enders, 1930). Their present status is not known.

In 1942, Jaguarundis (*Felis yagouaroundi*) were reported in Florida. These animals are believed to have been released deliberately at Chiefland and Hillsborough River State Park. Repeated observations of animals believed to be Jaguar-

undis suggest that they are present in the following areas: Dunellon through Chiefland to Jena, eastern Marion County; the south end of the central Florida ridge in Osceola, Polk and Highlands Counties; Everglades National Park; and possibly Hillsborough County. Specimens identified as Jaguarundis have been obtained from Jena and near Lake Placid. The animals seem to prefer areas of thick brush near water. Most of the sight-records were made while they were raiding chicken coops and poultry yards (Neill, *in litt.*).

Axis Deer (*Axis axis*) escaped in Volusia County, Florida, in the 1930s, and they are now known from four counties east of the St. John's River. Since 1951, they have been protected by state law (Allen & Neill, 1954).

The Mountain Goat (*Oreamnos americanus*) was transplanted from its original range in the Rocky Mountain area to the Black Hills in South Dakota, where the species is thriving. It was also introduced into Colorado, at least three of the eleven animals released in 1948 still surviving in 1954 (Yeager, *in litt.*), and into several areas in central Montana (Cahalane, *in litt.*).

Pine Martens (*Martes americana*) have been transplanted during recent years to states where they had been exterminated. Reintroduction from Ontario into New Hampshire appears to be successful (Monahan, 1953). Martens also were transplanted from Montana to Wisconsin.

Bison have been introduced (reintroduced?) and established in northern Sonora, Mexico. Whitetail Deer, reintroduced in the mountains of central Mexico, have failed to survive because of heavy hunting pressure to which they are subjected (Villa-R., *in verbis*). Elk have also been introduced or reintroduced in Waluula.

Peccaries (*Pecari angulatus*) on Cozumel Island, Quintana Roo, Mexico, have been described as a subspecies (*nana*), but it is believed that these animals were introduced from the mainland and that the subspecific characters (small size) are the result of heavy hunting pressure which allows few animals to attain more than two years of age (Hershkovitz, 1953).

South America:

South America, with a continental area larger only than Australia, has been isolated for long periods of geological history. This separation from the other continents, together with major barriers within South America such as the western Cordillera, the tropical forests of the Amazon basin, and the Pampas, has resulted in differentiation of a unique mammalian fauna. Because many native mammals, especially carnivores and ungulates, although specialized, seem to be more primitive than other allied forms living elsewhere, the South American fauna may be highly susceptible to deleterious effects by more highly developed competitors from other continents as well as by intracontinental introductions.

Table 3 summarizes the status of introduced and transplanted mammals in South America.

The European Rabbit (*Oryctolagus cuniculus*) was introduced on islands in the Beagle Channel, Tierra del Fuego, about 1880, by Thomas Bridges, a missionary. These rabbits, brought from the Falkland Islands, were introduced to provide food both for castaways and for the natives, and care was taken to avoid introduction on the mainland. On some islands the rabbits reproduced rapidly, while on others they failed to survive because of predation by birds, hunting pressure by the Indians (who used dogs), or because the land was too rocky or wet for burrowing (Bridges, 1949). Eventually the rabbits reached the mainland and have spread northward, west of the Cordillera, at least as far as Vallenar, Chile. They caused considerable damage to the flora, destroying young pines (*Pinus insignis*) and the native grass cover (Mann, *in litt.*). The European Rabbit is also reported established in Argentina (Sanborn, *in litt.*).

The European Hare (*Lepus europaeus*) was introduced into Argentina in 1880, and has spread to Chile during the present century. It causes much damage to pastures and is found in large numbers in central and southern Chile, north at least to Illapel, and at elevations as high as 2,600 meters (L. E. Peña, *in litt.*). The pelts of the European Hare and European Rabbit are used in various industries, and 50,000 skins were sold in Punta Arenas in 1939. According to C. C. Sanborn (*in litt.*) this hare was introduced into Uruguay and also Tierra del Fuego. He reported range damage by these animals in Patagonia.

The Mongoose (*Herpestes auropunctatus*) was introduced into British Guiana on the mainland of South America from Caribbean stock after 1872. It has multiplied and spread to settled areas, but does not seem to have penetrated farther. The dense tropical forests, the many wide rivers and competition with native predators have been suggested as factors which have limited its spread (Westermann, 1953).

Like other continents, South America has received its share of exotic ungulates. About thirty years ago Red Deer and Fallow Deer were imported for aesthetic and game purposes from a German zoo and liberated on various estates between Temuco and Puerto Montt, Chile; they have reproduced and exist in semi-

confinement (Mann, *in litt.*). On the island of Lago Ranco, Province of Valdivia, wild herds of Red Deer have been reported (Peña, *in litt.*). Red Deer were also introduced between 1916 and 1918 by Pedro Luro in the province of La Pampa, Argentina, and they compete for food with domestic stock. They thrive in the region of the Cordillerana de Neuquen and north of Chubut, and compete strongly with the native Huemul (*Hippocamelus bisulcus*) and Pudu, and are a serious pest to agricultural interests (Crespo, *in litt.*). In Argentina, Fallow Deer have been introduced on various estates in the vicinity of Buenos Aires, but they have not become serious pests (Cabrera & Yepes, 1940; Crespo, *in litt.*). Axis Deer (*Axis axis*) have been introduced in the province of Santa Fé in Argentina (Crespo, *in litt.*) and on farms near Belo Horizonte in Brazil (Maia, *in litt.*); they are in semi-confinement and exist in small numbers. American Elk (*Cervus canadensis*) have been imported to Argentina, according to Murie's (1951) map, but whether these animals were liberated or confined is not known. The report of the introduction of the Bush-Pig (*Potamochoerus*) to South America by the early slave-traders (Simoons, 1953) is viewed with doubt, for the observations are probably confused with descriptions of the native peccaries.

Several mammals have been transferred from one part of the continent to another, occasionally with unfortunate results. The Kinkajou (*Potos flavus*) was introduced (from northern South America?) to the Isle of Juan Fernandez, Chile, "for the purpose of eating the rats which exist there (!), but today is a serious danger for birds . . . destroying them." (Peña, *in litt.*).

Two kinds of Armadillo (*Euphractus sexcinctus* and *Zaedyus pichiy*) have been recorded as introduced as pets from Argentina (as early as 1847) and established in central Chile (Osgood, 1943). Correspondents (Mann, Peña) have failed to confirm the existence of these two animals in the wild state in Chile, and doubt the veracity of the records. Seemingly, if these armadillos are established in Chile, they are neither numerous nor widespread.

The introduction of several pairs of coati (*Nasua* sp.) and of a mustelid (*Vison* sp. [*sic* —the mink?]) about 1940 in the vicinity of Lago Todos Santos, Chile, seems to have been unsuccessful, for these animals have not been seen again (Mann, *in litt.*).

It is gratifying to learn that some South Amer-

TABLE 3: MAMMALS INTRODUCED OR TRANSPLANTED IN SOUTH AMERICA

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of Origin	Country or Place of Introduction	Reason for Introduction
A: *Successful introductions:*					
European Rabbit	*Oryctolagus cuniculus*	1880	Europe via Falkland Is.	Tierra del Fuego	Food
European Hare	*Lepus europaeus*	1880 ?	Europe Europe	Argentina Brazil	Food
Red Deer	*Cervus elaphus*	1920s 1916	Germany	Chile Argentina	Sport and Aesthetic
Fallow Deer	*Dama dama*	1920s	Germany Europe	Chile Argentina	Sport and Aesthetic
Axis Deer	*Axis axis*	?	? India	Brazil Argentina	Sport and Aesthetic
Mongoose	*Herpestes auropunctatus*	After 1872	India via West Indies	British Guiana	Rat control
B: *Successful transplantations:*					
Six-lined Armadillo	*Euphractus sexcinctus*	?	Argentina	Central Chile	?
Pichiy	*Zaedyus pichiy*	1847	Argentina	Central Chile	As pets
Kinkajou	*Potos flavus*	?	Northern S. America	Isle of Juan Fernandez, Chile	Rat control
C: *Unsuccessful transplantations:*					
Coati	*Nasua* sp.	1940	Northern S. Amerca?	Lake Todos Santos, . . . Chile	
Mink?	*Vison* sp. (*sic*)	1940	?	Lake Todos Santos, . . . Chile	

duction of foreign animals: "In the past few years strict government control has been exercised over the introduction of new animals, paying attention to the evident danger which these animals signify for a country of so labile a biotic equilibrium as Chile, whose flora and fauna are, for the most part, of ancient stock and therefore very susceptible to destruction by modern competitors." (Mann, *in litt.*).

Australia:

Australia furnishes one of the best examples of the destructive effect of exotics. Introductions, both of the rabbit and other mammals, have had results disastrous both to the native fauna and to man himself.

Free from the controlling factors of their original habitat, European Rabbits attained incredible numbers. They converted millions of acres of good agricultural country into semi-desert by denuding the plant cover and by girdling trees. This resulted not only in a decreased carrying capacity of the range for domestic stock, but also in the rapid disappearance of many specialized marsupials which could not stand the competition. Some marsupials have thus become extinct; others, fortunately, can live in areas too dry for rabbits, and some are arboreal.

Introduced foxes, ferrets, weasels, and feral dogs and cats have wrought havoc among the indigenous fauna. Generally the small marsupials with poorly developed defense mechanisms suffer severely from predation by these placental mammals which are more effective than the native marsupial predators.

Introduced deer have done less harm in Australia than in New Zealand, probably because the Australian habitat is less favorable for them. However, an appraisal of deer damage to forest reproduction and agricultural crops may prove them to be more destructive in Australia than has been supposed.

Table 4 lists the mammals which have been introduced into Australia.

Although further introductions are now rigidly prohibited, legislation regarding exotics already present does not always appear to be reasonable. The contradictory nature of the game laws is shown by the complete protection of deer in Queensland and their partial protection in Tasmania, while there are open seasons on kangaroos in both these states.

Australia's greatest curse, the European Rabbit (*Oryctolagus cuniculus*), was brought to Port Jackson with the first settlers in 1788, and presumably to the other early settlements in Australia and Tasmania. The progenitors of the present hordes are generally believed to be the 24 released for sporting reasons at Barwon Park (near Geelong, Victoria) by Thomas Austin in 1859 (Stead, 1928). It is possible that domestic escapees may have become feral before 1859. Old records indicate many rabbits in Tasmania by 1825. They were also released on various islands at early dates as food for castaways. On many of these islands they did not prosper (Calaby, *in litt.*). There are none today on Kangaroo, King, Flinders or Cape Barren Islands.

Six years after the initial release, Austin had killed 20,000 rabbits on his estate and estimated that at least 10,000 remained. From Victoria, rabbits spread rapidly in all directions, unhampered by any natural controls. By the late 1870s they had crossed the Murray River into New South Wales; they soon populated thousands of square miles here and crossed into Queensland. In vain, thousands of miles of rabbit-proof fences were thrown up; poisoning campaigns killed not only large numbers of rabbits, but also livestock and native species (Stead, 1928). The rabbit has now apparently reached the limits of its range; it seems unable to penetrate the tropical zone, and is very scarce in the arid interior.

The rabbits' damage to vegetation is unbelievable. Vast areas of tree scrub have been cleared by debarking mature trees and eating young seedlings, and in turn unpalatable plants have developed to the detriment of the pastures.

The virus disease myxomatosis has recently proved fatal to millions of rabbits. Pathologists expect the virus and the rabbits to reach a dynamic balance, with the population of the latter at a less destructive level. It is hoped the virus will not attack other mammalian hosts.

Of the carnivores, the Dingo (*Canis familiaris dingo*) was almost certainly introduced by early aborigines or Malayans. The native Tasmanian Wolf (*Thylacinus cynocephalus*) and the Tasmanian Devil (*Sarcophilus harrisii*) apparently were exterminated from the Australian mainland by competition with Dingos. Interbreeding of the latter with domestic dogs is increasingly common and has already eliminated "pure" Dingos from the more settled areas (Troughton, 1947).

The Red Fox (*Vulpes v. crucigera*) was introduced to Victoria from England in the 1870s for sporting purposes (Jones, 1925). It spread rapidly across the continent, but slowed to a stand-still in the last twenty years. It appears to be absent in the most northern part of the Northern Territory and on the Cape York Peninsula, and is still sparse in the Kimberley Division of Western Australia. Calaby (*in litt.*)

states that the fox's northern limit is beyond that of the rabbit. Foxes prey on most native mammals except the larger kangaroos, and they chase even these until the young ones fall out of the pouch (Stead, 1928).

Ferrets, stoats and weasels (*Mustela* spp.) were brought to Australia in large numbers to prey on rabbits, but proved to be extremely destructive to native birds and smaller mammals. No information was obtained regarding their present status.

The American Gray Squirrel (*Sciurus carolinensis*) occurs in only a few parks within the city of Ballarat, Victoria. These specimens are said to be descended from a single pair liberated about fifteen years ago. This species is also present in the city of Melbourne (Barrett, 1934).

The Indian Palm Squirrel (*Funambulus palmarum*) has become established near the Taronga Park Zoo in Sydney as well as near a local zoo at South Perth (W.A.). These populations represent escapees from the zoos, not deliberate releases.

The European Hare (*Lepus europaeus occidentalis*) was introduced in the 1870s from England to various sites in Victoria for sporting purposes. It is now well distributed over Victoria, New South Wales, parts of southern Queensland and South Australia. It seems not to present any great problem. Apparently it is not affected by myxomatosis.

Fallow Deer were first introduced into the Australian region by Captains Dumaresque and Kemp in 1829. Other species have since been introduced into Victoria (Bentley, 1954). Deer also occur in New South Wales, Queensland and Western Australia.

No less than seventeen different forms of deer were imported by the Victorian Acclimatization Society to their grounds at Royal Park from the 1840s to 1890. Those known to have been liberated in the wild are listed in Table 4. The Roe Deer failed to survive. Others which were introduced, but of which there is no record of their liberation, include the Luzon Sambar, the Moluccan Rusa, the Mule Deer, Chinese Water Deer and Musk Deer. According to offi-

TABLE 4: MAMMALS INTRODUCED INTO AUSTRALIA

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of Origin	Country or Place of Introduction	Reason for Introduction
A: *Successful introductions:*					
European Rabbit	*Oryctolagus cuniculus*	1788	England?	Several states	Hunting
European Hare	*Lepus europaeus*	1870s	England	Victoria	Hunting
Dingo	*Canis dingo*	?	?	?	
European Red Fox	*Vulpes vulpes crucigera*	1870s	England	Victoria	Hunting
European Red Deer	*Cervus elaphus*	1870s	. . .	Several states	Hunting
Sambar Deer	*Cervus unicolor*	1860s	?	Victoria	Hunting
Hog Deer	*Axis porcinus*	1866	?	Victoria	Hunting
Fallow Deer	*Dama dama*	1840s and later	England	Several states	Hunting
B: *Partly successful, success unknown, or unsuccessful introductions:*					
Indian Palm Squirrel	*Funambulus palmarum*	?	?	N.S.W., W.A.	Escaped from zoos
Ferret	*Mustela putorius furo*	?	?	?	Rabbit control
Stoat	*Mustela erminea*	?	?	?	Rabbit control
Weasel	*Mustela* sp.	?	?	?	Rabbit control
Axis Deer	*Axis axis*	1860-1870	?	Victoria	Hunting
Barasingha Deer	*Cervus duvauceli*	1871-1885	?	Victoria	Hunting
Rusa Deer	*Cervus timorensis*	1890	Indonesia	Victoria	Hunting
Sika Deer	*Cervus nippon nippon* and *C. n. taiouanus*	1890 1850s	? ?	Tasmania	Hunting Hunting
Roe Deer	*Capreolus capreolus*	?	?	Victoria	Hunting

cial records from about 1884, several species of deer were introduced into the National Park, N.S.W. (Troughton, *in litt.*).

Most deer have failed to become established in the wild. In Victoria, the most common deer at present are the Sambar and the Hog Deer. In areas where Red and Fallow Deer do occur, they are not common. Little information is available on the browsing pressure exerted by deer; in localized areas, destruction of farmers' crops has been reported.

Sambar Deer (*Cervus unicolor*), first liberated in 1860, inhabit the hills northeast of Melbourne, and also West Gippsland. The northern and eastern limits of their range are uncertain, but they seem to persist despite considerable hunting pressure. On French Island, a population of Sambar traces back to a number that swam from the mainland in the early 1900s. The species is present in the Grampians and about Mount Cole in western Victoria. The National Park Guide of New South Wales, for 1893, states that Sambar were received via New Caledonia, but there is no indication of their ultimate survival.

Hog Deer (*Axis porcinus*) occur in the coastal areas of Gippsland in eastern Victoria, as far as the region between Nowa and Orbost. A herd is rumored to be present on the Murray River.

Red Deer (*Cervus elaphus*) occur in Victoria, New South Wales, Queensland and Western Australia. The Werribee herd in Victoria developed from six animals of Windsor Park blood imported in 1860. Stock from this herd was distributed throughout Australia and New Zealand. Another herd, introduced about the same time and owned by the Melbourne Hunt, was bred from Windsor Park and Knowsley Park stock; it contributed to the present Victoria population. In Western Victoria, Red Deer are reported from the timbered ranges about twenty miles south of Ballarat, west to Ararat, in the Wartook and Victoria valleys of the Grampians, and possibly in the Otway Ranges. In eastern Victoria, these deer are occasionally taken near Warburton and Gembrook.

Red Deer were liberated near Aston in New South Wales about 1918 and appear to have extended down the Bemm and Brodribb Rivers in Victoria (Bentley, 1954). They presumably also occur in the timbered hills along the Brisbane and Mary Rivers of Queensland. The New South Wales official National Park Guide, for 1893, refers to a donation of five Red Deer in November, 1886 (Troughton, *in litt.*). Their progeny appear to survive. Red Deer have been in the North Dandalup-Pinjarry area, south of Perth, for about fifty years, and seem to be barely holding their own (Calaby, *in litt.*).

Fallow Deer (*Dama dama*) were introduced into Tasmania in 1829 and now represent the bulk of the deer population, although covering less than one-twentieth of the island (H. A. Cox, *in litt.*). Some of these were later shipped to Melbourne and elsewhere. Wild herds were mentioned in the ranges northwest of Bacchus Marsh, Victoria, after 1810. They are now found near Casterton, on the Murray River flats near Wodonga, in the Healesville-Norbethong area and in west Gippsland and the Dandenong Ranges. Hunting has greatly reduced the populaton in several places. In the Glen Innes district (N.S.W.), seven individuals gave rise to a herd of 1,000 by 1939; they proved very destructive, and shooting parties reduced the population to about 200. Much the same history took place in the National Park after 1884 (Troughton, *in litt.*).

Axis Deer (*Axis axis*) were liberated at Bunyip and Wilson's Promontory between 1860 and 1870, but now occur in Victoria only in the Grampians. Some were also liberated in Tasmania about 1834 (Cox, *in litt.*).

Barasingha (*Cervus duvaucelli*) were liberated in Victoria late in the Nineteenth Century and before World War I. A few seem to persist in the Mirboo North area, and possibly elsewhere in remote sections.

Rusa Deer (*Cervus timoriensis*) were released in 1890 from the Victorian Acclimatization Society's grounds at Gembrook, and were flourishing in several places in 1900. They are now occasionally shot in Gippsland.

Japanese Sika Deer (*Cervus n. nippon*) were imported from 1868 to 1887 by the Victorian Acclimatization Society, as were a number of Formosan Sika Deer. A number were liberated at Gembrook between 1887 and 1900. They have been reported on Wilson's Promontory.

Africa:

It is regrettable that no definite information could be collected regarding introduced mammals in most of northern and central Africa. Ellerman & Morrison-Scott (1951) speak of Fallow Deer in North Africa. Other possible introductions have gone unrecorded; various individuals questioned about this matter were unable to provide the requested data.

In Tanganyika, the Pemba and Zanzibar Islands are inhabited by two introduced forms: the Thick-tailed Shrew (*Suncus caerulaeus*) and the Rasse (*Viverricula indica rasse*). The Indian Mongoose (*Herpestes auropunctatus*) has been introduced on Mafia Island (G. A. Swynnerton, *in litt.*).

Only three mammals are known as exotics in South Africa: the European Rabbit, American Gray Squirrel and Fallow Deer. None of these has spread very widely.

The European Rabbit (*Oryctolagus cuniculus*) appears to be the only introduced mammal in South Africa which potentially might become a widespread, serious pest. Fortunately, it is now restricted to Robben Island, an islet in Table Bay near Capetown. As early as 1656, rabbits were sent there by Van Riebeeck to provide food for vessels on their way to the East. These lagomorphs are stunted, and have become a pest by nearly eliminating all plants except inedible species. Consequently, the vegetation of the island is now characterized by its great uniformity (Adamson, 1934). Some years ago an attempt was made to exterminate the rabbits by digging them out, but this failed.

The American Gray Squirrel (*Sciurus carolinensis*) was imported into Capetown by Cecil John Rhodes, probably early in the present century. It was intended to fill a "vacant habitat," namely oak forests (also exotics), but seems to prefer the neighboring orchards (Bigalke, 1937a). A few pairs liberated on an estate on the eastern slopes of Table Mountain increased to occupy an area of land within forty miles of Capetown; this spread of range is well documented by Davis (1950). The species is still expanding its range, and seems likely to continue as afforestation proceeds and the pine plantations mature. This squirrel has become a nuisance to fruit growers, and was placed on the vermin list from 1918 to 1922, when rewards were paid for no less than 11,188 specimens. Destruction of seed has become a serious problem in some plantations. The chief factor limiting the spread of Gray Squirrels is the absence of tall seed-bearing trees; it therefore seems unlikely that they will extend much farther in Cape Province. The groves of oak and stone pine (*Pinus pinea*) in the western Cape satisfy their requirements. Although Gray Squirrels prey on small birds, they do not appear to affect the mammalian fauna (Davis, 1950).

The Fallow Deer (*Dama dama*) was introduced on the lands of the Vereeniging Estates Ltd. until about 1914. A nucleus herd was set free on a well-wooded area of some 3,500 acres, and the population has now increased to approximately 50 individuals. Fallow Deer also occur on two other estates (Bigalke, 1937b.)

Oceanic Islands:

Particular islands and island groups are discussed separately because they are inhabited by specialized faunas which, as a result of virtual freedom from predation, have developed few defense mechanisms and in general are easy prey to aggressive introduced mammals. The environmental resistance encountered by exotics on islands is usually much less than in their countries of origin because of the availability of considerable forage as well as limited competition with native species.

New Zealand, the Caribbean Islands and Hawaii will be discussed separately. On the Galapagos Islands the introduced mammals which have a harmful effect on the native animals and plants are feral species (cattle, horses, donkeys, pigs, goats) and Black Rats (Lack, 1947). Rabbits (sp.?) have been responsible for a reduction of the native finch on Laysan Island.

New Zealand.—No other island group in the world provides such an interesting example of the havoc which introductions cause among native animals and plants as New Zealand. In no other area of comparable size have such a variety of mammals been introduced. Because of the long separation from other large land areas, a flora and fauna have evolved here which are very different from anything found elsewhere.

A major reason for the introduction of so many exotics was the virtual absence of native mammals, limited to only two species of bats. Maoris introduced a rat (*Rattus exulans*) and a dog before the arrival of white settlers. Since a meatless diet is unattractive to white settlers, Captain Cook and other early explorers took particular pains to import goats and other mammals. Still more exotics were introduced to provide hunting, furs for garments and controls for species overabundant due to lack of predation.

Wodzicki (1950) divides these exotics into two major groups: those which are widely distributed, *e.g.* Red Deer and rabbits, and those whose distribution has remained localized.

Many of the mammals in the first group may be considered serious pests. They have spread rapidly and become widely established by taking advantage of the large supply of available food and by considerable protection during the early stages of acclimatization. In some cases, a species first reached an excessively high population, and then declined to a more stable level after the exploitation of the reserve food. The continued spread of exotics has seriously affected the native flora as well as man's agricultural activities. In an attempt at control, as many as 100,000 deer have been shot in a single year.

With the possible exception of the Weasel, the mustelids and the hedgehog are well established throughout the country. Most of these were liberated for the purpose of controlling other animal pests, but they have had detrimental effects on the native and introduced

birds which considerably outweigh their beneficial activities.

Many mammals in the group of localized distribution have also proved troublesome. Their economic importance varies considerably. Chamois (*Rupicapra rupicapra*) and Tahr (*Hemitragus jemlahicus*) are dispersed over areas of many hundreds of square miles, while wallabies and several kinds of deer are restricted to relatively small areas.

When first introduced, each species increased in inverse ratio to the resistance by the environment. In some cases, this full resistance may not have been encountered until the species reached a very high population.

According to Wodzicki (1950), four periods of liberation of exotics may be recognized as follows:

1. From the settlement by the Maoris to the visits of Captain Cook,
2. From the time of Captain Cook's visit until 1840,
3. From the period of regular settlement until about 1900, and
4. The present century.

During the first period, the Polynesian cat and dog and the Maori rat were introduced; these species did no appreciable damage to the native flora and fauna. The second period brings the first intentional introductions of exotics. During the third period, white settlers profoundly changed the nature of the vegetation and also deliberately attempted to acclimatize many new animals.

Wodzicki tabulated exotic mammals according to the country of origin and the number of species that became established. Table 5 is modified from his table. Thirty-six per cent. have failed completely. The native rat and dog succumbed to the competition of their European counterparts. Two species of deer, after a slight initial success, are, so far as is known, either on the decrease (Moose) or completely absent (Mule Deer).

TABLE 5: COUNTRY OF ORIGIN AND TOTAL NUMBER OF MAMMAL SPECIES IN NEW ZEALAND (Modified from Wodzicki, 1950)

Country of Origin	Mammal Species	
	Liberated	Established
Europe and England	20	19
Australia	13	4
Polynesia	2	2
North America	7	4
South America	4	—
Asia	5	5
Africa	2	—
	53	34

Table 6 (modified from Wodzicki) tabulates the successful, partially successful, and unsuccessful introductions into New Zealand, with pertinent details.

Caribbean Islands, West Indies.—Considerable damage has been done to the autochthonous fauna and flora of the Caribbean Islands by introduced mammals. Unfortunately, this damage still continues. The Mongoose may be singled out as exceedingly detrimental; it has probably been instrumental in the complete or nearly complete extirpation of several species of mammals and birds. Many members of the indigenous fauna are continually falling prey to such introduced mammals as cats, dogs, monkeys and opossums. Table 7 lists the known introduced mammals of the Caribbean area. Many exotics introduced during the early days of settlement are unrecorded.

The Mongoose (*Herpestes auropunctatus*), the most destructive exotic animal in the Caribbean area, was brought from India to Trinidad in 1870 and to Jamaica in 1872, for the purpose of controlling introduced rats that were destructive in the sugar cane fields (Westermann, 1953). In later years the Mongoose was introduced in the West Indies. Its introduction on Martinique and St. Lucia was intended to reduce the numbers of the poisonous Fer-de-lance (*Bothrops atrox*), but this met with only partial success (Lewis, 1953). The Mongoose soon became well established and spread extensively; it now occurs on most large and moderately large islands of the Caribbean, but is absent on most of the smaller islets, which therefore serve as useful refuges for species decimated elsewhere.

The Mongoose multiplied rapidly in most places. The four males and five females released on Jamaica increased so quickly, and attacked the rats with such ardor, that in 1882 it was estimated they had saved the planters nearly 45,000 pounds annually. But as soon as the rats were appreciably reduced in numbers, the Mongoose began preying upon native mammals and birds that feed or nest on or near the ground, as well as on such small, harmless creatures as terrestrial snakes, lizards, toads and other amphibians, and land crabs. Eggs of birds and reptiles are also eaten. This havoc has greatly reduced the indigenous fauna and has all but exterminated several species of mammals, birds and reptiles. The killing of small domestic animals has caused serious economic repercussions. Thus, within twenty years after its introduction, the Mongoose had come to be regarded on several islands as the worst of all pests (Westermann, 1953). Furthermore, it has recently been

TABLE 6: INTRODUCTIONS INTO NEW ZEALAND
(Modified from Wodzicki, 1950)

Common Name	Scientific Name	Year(s) of Liberation	Country of Origin	Reason for Introduction
A: *Successful introductions:*				
Brush-tail Opossum	*Trichosurus vulpecula*	1858	Australia	Utility
Scrub Wallaby	*Thylogale* sp.	1870	Australia	Sport and fur
Swamp Wallaby	*Wallabia ualabatus*	1870	Australia	Sport and fur
Black-striped Wallaby	*Wallabia dorsalis*	1870	Australia	Sport and fur
Dama Pademelon	*Thylogale eugenii*	1870	Australia	Sport and fur
Brush-tailed Rock Wallaby	*Petrogale penicillata*	1870	Australia	Sport and fur
Wallaby	Species unknown	1903	Australia	Sport and fur
Hedgehog	*Erinaceus europaeus*	1885	England	"Escapees"
Stoat, Ermine	*Mustela erminea*	1885	England	"Natural enemies" of rabbit
Ferret	*Mustela putorius furo*	1886	England	"Natural enemies" of rabbit
Weasel	*Mustela nivalis*	1885	England	"Natural enemies" of rabbit
European Rabbit	*Oryctolagus cuniculus*	Before 1838	England	Utility
Hare	*Lepus europaeus*	1867	England	Sport
Himalayan Tahr	*Hemitragus jemlahicus*	1904	Asia	Sport
Chamois	*Rupicapra rupicapra*	1889	Austria	Sport
Red Deer	*Cervus elaphus*	1851	England	Sport
Axis Deer	*Axis axis*	1867	India	Sport
Sambar Deer	*Cervus unicolor*	1875	India	Sport
Wapiti	*Cervus canadensis*	1870	Canada	Sport
Japanese Deer	*Cervus nippon*	1885	Manchuria	Sport
Fallow Deer	*Dama dama*	1864	England	Sport
White-tailed Deer	*Odocoileus virginianus*	1901	America	Sport
Moose	*Alces americanus*	1900	Canada	Sport

Common Name	Scientific Name	Year(s) of Liberation	Country of Origin	Reason for Introduction	Reason for Failure
B: *Unsuccessful introductions:*					
Marsupial Cat	*Dasyurus* sp.	1868	Australia	?	Only 2 introduced
Short-faced Bandicoot	*Isoodon* sp.	1873	Australia	?	...
Ring-tailed Opossum	*Pseudocheirus peregrinus*	1867	Australia	Utility	Only 2 introduced
Kangaroo	*Macropus* sp.	1868	Australia	Sport	Only 6 introduced
Roan Wallaroo	*Osphranter erubescens*	1860-1870	Australia	Sport	...
Long-nosed Kangaroo	*Potorous tridactylus*	1867	Australia	?	...
Raccoon	*Procyon lotor*	about 1905	America	Escapees	Only 2 introduced
Chipmunk	*Tamias striatus*	1906	America	...	Only 2 introduced
California Ground Squirrel	*Citellus* ?	1906	America	...	Only 2 introduced
Guinea Pig	*Cavia porcellus*	1869	America	Stowaways	...
Bharal or Blue Sheep	*Pseudois nayaur*	1909	Asia	Sport	Only 3 introduced
Gnu	*Connochaetes gnou*	1870	S. Africa	?	Only 1 introduced
South American Deer	*Cariacus chilensis* (=*Hippocamelus bisulcus?*)	1870	S. America	Sport	Only 1 introduced
Alpaca	*Lama glama huanacus*	1878	S. America	Sport	Only 3 introduced
Zebra	*Equus zebra*	1870	S. Africa	?	Only 2 introduced
Mule Deer	*Odocoileus hemionus*	1905	America	Sport	...

implicated as a reservoir of the virus of canine rabies (Seaman, 1952).

Two species of monkeys have been introduced into the Caribbean area. The Green Monkey (*Cercopithecus aethiops sabaeus*) from West Africa was introduced on Barbados between 1673 and 1750; in 1903 it was found at only a few points where woodland persisted; it is still present today, in small numbers. The species was also introduced on St. Kitts and Nevis where it is still rather common. These monkeys prey on the eggs and young of birds, and are believed to have exterminated the St. Kitts Bullfinch (*Loxigilla portoricensis grandis*). The Mona Monkey (*Cercopithecus mona*), now present on Grenada, is native to the Cameroons and adjacent parts of West Africa and was possibly introduced by slave traders (Westermann, 1953).

The Opossum (*Didelphis marsupialis insularis*) was introduced from Trinidad into Grenada, the larger Grenadines, St. Vincent, St. Lucia and Dominica, where it has apparently played a role in the nearly complete disappearance of the Ground Dove (*Scothrygon mystacea*) (Barbour, 1930). It is uncertain whether another species of opossum (*Marmosa mitis chapmani*), found on Grenada and some of the Grenadines, is native to these islands.

On Great Bahama Island one pair of Raccoons (*Procyon lotor*) from the Florida mainland were released about 1932; they are now considered a nuisance over the entire island (Sherman, 1954). It is conjectural whether or not another species of Raccoon (*Procyon maynardi*) was imported by man to New Providence Island in the Bahamas. *Procyon minor* of Guadeloupe, and *Procyon* sp. of Barbados, may also be exotics (Allen, 1911).

Several species of deer have been introduced on various Caribbean Islands. Whitetail Deer (*Odocoileus virginianus*) were brought to Cuba from Mexico and/or the southern United States, presumably about 1850. They formerly were

TABLE 7: SUCCESSFUL INTRODUCTIONS ON SELECTED ISLAND GROUPS*

Common Name	Scientific Name	Year(s) of Liberation	Country or Place of		Reason for Introduction
			Origin	Introduction	
Opossum	*Didelphis marsupialis insularis*	?	Trinidad	Caribbean Is.	?
Mona Monkey	*Cercopithecus mona*	?	West Africa	Grenada	Pets
Green Monkey	*Cercopithecus aethiops sabaeus*	1673-1750	West Africa	Caribbean Is.	Pets
European Hare	*Lepus europaeus occidentalis*	?	Europe	Caribbean Is.	Hunting
European Hare	*Lepus europaeus*	?	Russia	Islets off Oahu	Hunting
European Rabbit	*Oryctolagus cuniculus*	?	Europe	Caribbean Is.	Hunting
Black-naped Rabbit	*Sylvilagus nigronuchalis*	?	Venezuela?	Curaçao, Aruba	?
Agouti	*Dasyprocta aguti*	Before 1852	Brazil?	St. Thomas Is.	?
Red Agouti	*Dasyprocta punctata*?	1890s	C. America	Cayman Is.	?
Mongoose	*Herpestes a. auropunctatus*	1870 and later	India	Caribbean Is.	Rat control
Mongoose	*Herpestes a. auropunctatus*	1883	Jamaica	Hawaii	Rat control
Axis Deer	*Axis axis*	?	?	Hawaii	Hunting
Mouflon	*Ovis musimon*	1954	Europe	Hawaii	Hunting
White-tailed Deer	*Odocoileus virginianus*	1850s	Mexico, U.S.A.	Cuba and other islands	Hunting
White-tailed Deer	*Odocoileus virginianus curassavicus*	?	Colombia	Curaçao	Hunting
Sambar Deer†	*Cervus unicolor*	During Spanish occupation	Philippine Is.	Guam, Rota	Hunting

* Except New Zealand.
† R. H. Baker (1946).

more abundant, but have disappeared from many areas because of deforestation. A South American race (*O. v. curassavicus*) is thought to have been imported from Colombia to Curaçao. Deer of this or other genera have reached the Dominican Republic, Jamaica, U. S. Virgin Islands, Barbuda, Dominica, Grenada and possibly other islands (Westermann, 1953).

The European Hare (*Lepus europaeus*) and the European Rabbit (*Oryctolagus cuniculus*) have been reported introduced into Barbados, one of the Grenadines, and Guadeloupe (Allen, 1911): Another rabbit (*Sylvilagus nigronuchalis*) is supposed to have been introduced into Curaçao and Aruba.

Agoutis have been introduced into St. Thomas and the Cayman Islands. The species of St. Thomas is *Dasyprocta aguti*, probably obtained from Brazil prior to 1852 (Miller, 1918). The Cayman animal is a Red Agouti derived from Central America in the 1890s or earlier. It has rendered certain areas virtually useless for cultivation, and at present the Government pays a bounty of nine pence per head for its destruction (Swabey & Lewis, 1946).

Hedgehogs may have been introduced from Africa to Porto Rico before 1877, for the type specimen of *Erinaceus krugi* Peters (=*E. algirus caniculus*) was killed in Mayaguez, Porto Rico. Seemingly the species did not survive, for there are no recent records of them on this island.

Hawaii.—The Hawaiian Islands have only one native mammal, the Hawaiian Hoary Bat (*Lasiurus*). Introduced mammals have therefore exerted pressure mainly on the native birds, and there is little doubt that they have contributed to the decline of several species of land and sea birds. Depredations by certain mammals have been more serious in regard to eggs and young than to adult birds. It is likely that certain species of plants which served either as cover or as food, or as a link in the food chain of the nectar-feeding and insect-feeding birds, were destroyed or sufficiently reduced by introduced forms to contribute to the decline of certain native bird species (Schwartz, *in litt.*).

Table 7 includes the known introduced mammals of Hawaii.

Axis Deer (*Axis axis*) occur in limited numbers, but in dense concentrations, on Molokai, Oahu and Lanai. They cause damage by browsing where native flora still exists. Some legal hunting is permitted, but since the species is found on private land, no regulation of herds is attempted (Schwartz, *in litt.*).

A hare, believed to be of Russian origin (species unknown), is found on two small uninhabited islets consisting of only a few acres each, off Oahu. The animal is not abundant (Schwartz, *in litt.*).

A herd of Mouflon (*Ovis musimon*) was liberated on Lanai during the summer of 1954, according to a report of the Committee on Fauna Conservation of the Conservation Council for Hawaii (1955).

The Mongoose (*Herpestes auropunctatus*) was introduced from Jamaica in 1883. It now occurs on all major islands except Kanai, Lanai and Niihau. It is abundant and the heaviest populations are found near sea level. The merits of the Mongoose in the control of rodents are doubtful; the sugar producers have their own regular program of rat control. In the past, futile attempts have been made to control the animals. Territorial laws prohibit transporting them between any of the islands of the group. The Mongoose has probably contributed to the destruction of some ground-nesting sea birds, as well as of the Nene Goose, Hawaiian Owl, Hawaiian Duck, Hawaiian Coot and Hawaiian Gallinule (Schwartz, *in litt.*).

Introduced Rats, Mice and Shrews

The introduced Old World murids are among the most notorious of mammalian pests. The history of their spread (Silver, 1927) has been recounted so frequently that it need not be detailed here. They affect man's economy by eating and contaminating food, and they also carry epidemic diseases and parasites. As stowaways in ships, two species of rats and the House Mouse have been involuntarily spread by man throughout most of the world, including many scattered islands. These exotics are usually restricted to human habitations and their environs.

The rats concerned are the Norway or Brown Rat (*Rattus norvegicus*) and the Black or Roof Rat (*Rattus rattus*), several subspecies of which have been involved. The Norway Rat has by far the wider distribution and the greater abundance. It occurs in almost all coastal cities of the world, but also has spread widely inland. The Black Rat is generally considered to be less aggressive than the Norway Rat, and where the two compete it is often forced into the less desirable habitats. The Black Rat came to Europe in early times from Asia, probably on ships of the Crusaders sometime after 1191, and by the Middle Ages it had spread over most of the Old World. The Norway Rat arrived in Europe early in the Eighteenth Century, crossing the Volga in great numbers in 1727, reaching East Prussia in 1750 and Spain in 1800. The Black Rat and the House Mouse arrived in North America very soon after the first European visitors. The Alexandrine Rat (*Rattus r. alexandrinus*) is restricted to rather limited

areas on all continents except Australia. *Rattus r. frugivorous* has been recognized in recent years as part of the population in the United States.

Introduced rats are blamed for the destruction of small insectivores (*Nesophontes*) and native Rice Rats and Spiny Rats on the Caribbean Islands (Westermann, 1953). In Australia, the native rats are normally slower breeders than the two introduced species and have consequently been displaced in many places by these exotics (Troughton, 1947).

The House Mouse (*Mus musculus*), originating in Asia, has established itself not only around human habitations but also in the field, especially in the more moderate climates. In Africa and Australia it is now present over large areas, even very far from settlements. On the latter continent, as well as in North America, it is subject to irregular irruptions and during peaks of population it does tremendous damage to the vegetation. Introduced House Mice have developed new subspecies in several parts of the world. On the Faroe Islands, for example, four distinct subspecies exist today (Bourlière, 1954).

The House Shrew (*Suncus murinus*) has been spread by man throughout most of the southeastern Asian islands.

Feral Mammals

Feral mammals have been transplanted by man as domestic stock or as pets, and have since escaped or been purposely released and then "reverted to nature" to various degrees. Although feral mammals may be of considerable biological and economic importance, they have received little attention from zoologists.

Domestic horses, cattle, sheep, goats, pigs, dogs and cats are now almost cosmopolitan in distribution, and also occur widely in the wild state. Many less known feral mammals such as buffaloes and camels populate certain parts of the world.

Feral ungulates provide meat in considerable quantities, and sport for the hunter. Some are considered harmful because of damage to the native fauna and flora and because they may harbor diseases affecting domestic stock.

The domestic goat (*Capra prisca*) undoubtedly has been the most destructive of feral mammals to native vegetation, particularly as it travels over all types of terrain and consumes all kinds of browse and herbaceous material. Feral goats occur on all continents, even in such places as the Scottish Highlands. In Australia they are well established in the dry interior (Calaby, *in litt.*). They are a serious problem on many islands, such as New Zealand (Thomson, 1922), Hawaii and several of the Caribbean islands. A typical picture is that presented by Wallace (1880) of the island of St. Helena: "At the time of its discovery, about the beginning of the sixteenth century, it is said to have been covered by dense forest; to-day it is described as a comparatively barren, rocky desert. This change has been brought about by goats first introduced by the Portuguese in 1513, and which multiplied so fast that in seventy-five years they existed by thousands." Heavy browsing destroyed the vegetation, followed by erosion of the soil by tropical rains. The story is similar on Guadalupe Island, in the Pacific, where goats were released by Russians in the Eighteenth Century. Many species of plants and animals, particularly birds, apparently disappeared forever.

Feral sheep (*Ovis aries*) cause considerable damage to the forests on the Hawaiian Islands. Elsewhere they are not considered a menace by agriculturalists.

Feral pigs (*Sus scrofa*) are widespread throughout the world. In Australia, where they occur in almost every state, they are a problem for graziers (Pullar, 1953). In Hawaii they are sometimes a serious threat to wildlife, including the nearly extinct Hawaiian Goose (Schwartz, *in litt.*; Bryan, 1937). Man imported them to most of the South Sea islands and to the Galapagos Islands. In parts of New Zealand they are very destructive to vegetation. The pigs in the eastern part of the Indonesian Archipelago are presumably descendants of domestic stock, but in some places they may have hybridized with wild species. It is probable that the pigs on New Guinea are direct descendants of domestic stock.

Feral horses (*Equus caballus*) still occur in Australia, New Zealand, North and South America and Asia, and where they become too plentiful they compete critically for food with domestic stock and native wildlife. Feral donkeys (*Equus asinus*) are present in northwestern Australia and the Northern Territory (Calaby, *in litt.*). Some "burros" also are present in herds in places on the southwestern deserts of the United States (Anon., 1952b; Davis, 1953).

Feral cattle (*Bos taurus*) survive in fair numbers chiefly in Australia, where they are restricted to parts of Western Australia, the Northern Territory and western Queensland. They also are found in some numbers in New Zealand and in parts of the western United States (Linger, 1943). Indian Water Buffaloes (*Bubalus bubalis*) occur in large herds in certain valleys of northern Australia (Calaby, *in litt.*). Feral Water Buffaloes also occupy many

countries in southeastern Asia and the island of Marajo in Brazil.

Feral Camels (*Camelus dromedarius*) are now found in large numbers in the more arid parts of Australia (Calaby, *in litt.*) and of central Asia, and in small numbers in the Kalahari Desert of southern Africa (Cahalane, *in litt.*). Several attempts, prior to 1860, to establish them in Texas, Cuba, Jamaica and South America were unsuccessful (Goodwin, 1925).

Llamas (*Lama glama*) may have been introduced in Mexico as beasts of burden in the Sixteenth Century. None are present there today.

Reindeer (*Rangifer tarandus*), introduced into Alaska and Canada, have hybridized to some extent with the native Caribou (*R. arcticus*), with resultant genetic changes in each. These Reindeer have done considerable localized damage to Caribou range by overgrazing lichens (Cahalane, 1950; Hanson, 1952). A recent introduction of Reindeer into Scotland has failed, but the species has been successfully introduced to Iceland. The establishment of Reindeer in Tierra de los Estados is reported by Cabrera & Yepes (1940), but details of the results are lacking.

Among the feral carnivores, cats and dogs are the most destructive species. Both revert to the wild readily and, being efficient predators, they may do much damage to wildlife. Dogs and cats are responsible for the disappearance of several forms, particularly in the Caribbean area, in Australia and on small islands.

Introduction of Diseases and Parasites

A consequence of animal introductions generally not fully appreciated is the importation of diseases and parasites of which the exotics are hosts. Most diseases are more dangerous to a previously unexposed population, since no immunities have been developed. Introduced diseases may easily become established in native species, and may prove impossible to eradicate.

Two epizootic diseases, rinderpest and foot-and-mouth disease, are particularly destructive after introduction. Rinderpest, or cattle plague, introduced from Asia to Africa, killed many ungulates. The Cape Buffalo (*Syncerus caffer*), common before the disease was introduced, was almost extirpated in the first outbreak. Although methods of artificially immunizing domestic stock are available, these cannot be applied to wild game.

Foot-and-mouth disease, endemic to Eurasia, has been introduced twice with domestic stock to North America, but fortunately was extinguished in the United States and Canada. It is, however, present in Africa, Mexico and South America, where thousands of wild ungulates have been killed in a futile attempt to combat it.

There are few data on the introduced parasites of exotic mammals and their possible influences on native hosts. Two pertinent papers dealing with the endoparasites of the European Rabbit in New Zealand (Bull, 1953) and of the Muskrat in Great Britain (Warwick, 1936) have come to our attention. These papers raise several interesting points, on which further information is desirable.

First, they indicate that parasites do get introduced, and sometimes several species at once. For example, of five nematodes and two cestode species found in the rabbits of New Zealand, three of the nematodes and both cestodes also are found among rabbits in Wales. The other two nematodes perhaps were acquired in New Zealand. The rabbit is one of the intermediate hosts for the two cestodes, which are carried in vesicles within the body, in the larval stage only. Another cestode, *Cittotaenia* sp., for which the rabbit is the definitive host of the adult stage, apparently is absent from rabbits in New Zealand, as well as in Australia. Possibly it failed to survive the long voyage from England (and the periods of confinement before and after the voyage) because of the absence of its only known intermediate host, a free-living oribatid mite.

At least two parasites, a cestode and a trematode, were introduced with the Muskrat hosts from North America to Great Britain. Two others might also have been introduced, had they not already been present in Great Britain. One other parasite was apparently acquired from the native fauna.

Second, it is possible for introduced parasites to be transferred to closely related or associated species of hosts. In North America, parasites of the Muskrat often are shared with the Meadow Vole (*Microtus pennsylvanicus*). Such an exchange might take place between introduced Muskrats in Europe and the Water Vole (*Arvicola terrestris*) or other species of voles. Table 8

Table 8: Parasites Shared by the Muskrat and the Meadow Vole

	Species of Parasites in the Muskrat*	Species also Present in the Meadow Vole
Trematodes	30	4
Cestodes	10	4
Nematodes	9	2

* Data summarized from various reports.

lists the number of species of helminth parasites in North American Muskrats, and the number of those species which have also been found in the Meadow Vole.

Third, the more complicated the life cycle of the introduced parasite, the less likely is its chance for survival. Trematodes with a life history involving two or more intermediate hosts may not find suitable hosts in a new environment. Nematodes may be introduced with relative ease because many of them are transmitted directly from one final host to another, the intervening stages being free-living. Introduction of cestodes in the larval stages may be fairly easy, but survival depends upon the presence of a suitable final host (a carnivorous bird or mammal) in the new area.

Rausch & Schiller (1954) mention the introduction from Siberia to Bering Island of the larval stage of a possible Siberian cestode, *Echinococcus* sp., with its host the Red-backed Vole; the island foxes became infected with this cestode.

Little information could be secured about the transfer of ectoparasites with mammals. It seems likely that the tick, *Trombicula akamushi,* which harbors the rickettsial disease, scrub typhus, has been spread widely with introduced rats or other rodents. The Indian rat flea, *Xenopsylla cheopis,* which transmits bubonic plague, has also been spread widely with rats on ships into many parts of the world.

Summary

For a variety of reasons, man has seen fit to transport mammals from one part of the world to another. These introduced forms have frequently failed to become established; when establishment did result, the exotics have usually failed to achieve the purpose of the introduction. Sometimes they have become serious economic pests, as well as exerting an unbalancing force on the local biotic equilibrium.

This paper, largely restricted to the problem of deliberately introduced mammals, discusses more than 200 cases of exotic species on different continents and islands.

Certain principles may be deduced from the study of introductions and transplantations of mammals. Some of these principles are described by Dice (1952); some principles and implications are discussed by Pierce (1956).

1. The result of an introduction is unpredictable; both the relative success with which it establishes itself and the amount of disturbance produced in a community through its presence depend upon the newcomer as well as upon the composition of the invaded community. The more specialized a species is, the more difficulty it usually encounters in becoming established, and the less likely it is to become a pest.

2. No exotic mammal is identical in its various requirements with any native member of a community; this results in conflicts and causes numerous readjustments, often detrimental, in the ecologic organization of a community. One result is the reduction or elimination of certain native species. Established exotics may supplant closely related native species, often because they have a higher reproductive potential.

3. When an introduced species differs greatly in habits from existing members of a community, it may produce serious changes in the entire community. Thus goats introduced on certain oceanic islands have caused the virtual annihilation of vegetation and the consequent disappearance of many native forms. Mammals which are apparently harmless in one area may become serious pests in another. For example, the Phalanger is innocuous in Australia, but very harmful in New Zealand.

4. Genetic changes may occur in species after introduction to a new habitat. This is possibly due to the limited quantity of genetic material available when only a few individuals are introduced. For example, Mouflons introduced into Slovakia developed a new race expressed by different color of hair and shape of horns.

5. The transplantation of one form into the existing range of another form of the same species often results in the production of a mixed race, with sometimes serious complications, especially when the introduced form is in some respect less desirable than the native. In Czechoslovakia, for example, two introduced species of goat have interbred with the native Ibex, resulting in poor survival of the hybrids.

6. Some species change their habits in a new environment, often with serious consequences for existing communities and for man. The Raccoon-dog has apparently altered its food habits after introduction into the Caucasus; this is also the case with the American Gray Squirrel in South Africa and with the Formosan Gray-headed Squirrel in Japan.

7. An introduced species which establishes itself successfully seems to pass through two definite phases of population behavior: at first it multiplies rapidly and builds up to a peak population; thereafter it levels off to a point of moderate abundance, or may even become rare. The initial increase may be due to the lack of natural parasites or other controlling factors, which in time develop to again produce a stabilized community, but one with a changed composition.

8. Exotic species which become established as thriving populations usually do so at the expense of one or more native species. There is little evidence to indicate that the total supply of game will be increased by adding a new game species to a habitat already occupied by one with similar requirements; the habitat can support permanently only a certain population. Occasionally "vacant" niches may be occupied by exotics; this may be the case with the European Hare on farm lands in eastern North America, or with the Moose in Newfoundland.

9. The problems of chance introductions and of feral mammals are similar to those of deliberate introductions of wild species.

10. Control of a well-established introduction is extremely difficult. The only known case of the eradication of an introduced mammal which was distributed over a fairly wide range is that of the Muskrat in the British Isles.

11. There is evidence that parasites and diseases of introduced mammals are at least partly transferred with their hosts to new biota.

12. The evidence accumulated in this paper indicates that the introduction of mammals is a hazardous undertaking and that further introductions should be more carefully considered in advance. The introduction of a mammal for the sole purpose of controlling pests is almost certainly doomed to failure. Food habits are rarely so specialized that a mammal will feed entirely on the pest to be controlled. Few mammals introduced for sport have proved eminently successful.

Bibliography

Adamson, R. S.

1934. The vegetation and flora of Robben Island. Trans. Roy. Soc. South Africa, 22: 4.

Allen, D. L.

1954. Our Wildlife Legacy. New York: Funk & Wagnalls Co., 422 pp.

Allen, G. M.

1911. Mammals of the West Indies. Bull. Mus. Comp. Zool., 54: 175-263.

1938, 1940. The Mammals of China and Mongolia. New York: American Museum of Natural History, 2 vols.

Allen, R., & W. T. Neill

1954. The Florida deer. Florida Wildlife, 7 (9): 21, 37.

Allin, A. E.

1950. European hare introduced into the district of Thunder Bay, Ontario. Canad. Field Nat., 64: 122-124.

Anonymous

1944. Hits, misses, and home runs. Penna. Game News, 15 (4): 4-5, 28, 30.

1952a The musk ox is a-coming. Life Mag., April 14.

1952b Wild burros in California. Outdoor California, 13: 4-5.

1953a Tar Heel Wildlife. Raleigh, N. Car., 98 pp.

1953b Iceland's mink are a menace to wildlife. Modern Game Breed. Hunt. Club News, 23 (8): 13.

1954a Die Vermehrung wertvoller Pelztiere in der Sowjetunion. Umschau, 54: 456.

1954b A new threat: European rabbits in the United States. Nat. Conserv. News, 4 (5): 1.

Ashbrook, F. G.

1953. Muskrat—nutria. Louisiana Conserv., 5 (6): 16-17.

Atwood, E. L., Jr.

1938. Some observations on adaptability of Michigan beavers released in Missouri. Jour. Wildlife Mgt., 2: 165-166.

Bachrach, M.

1953. Fur. New York: Prentice-Hall, Inc., 660 pp.

Bailey, B.

1929. Mammals of Sherburne County, Minnesota. Jour. Mamm., 10: 153-164.

Bailey, H. H.

1924. The armadillo in Florida and how it reached there. Jour. Mamm., 5: 264-265.

Bailey, V.

1936. The mammals and life zones of Oregon. N. Amer. Fauna, 55: 1-416.

Baker, R. H.

1946. Some effects of the war on the wildlife of Micronesia. Trans. N. Amer. Wildlife Conf., 11: 205-213.

Barbour, T.

1930. Some faunistic changes in the Lesser Antilles. Proc. New England Zool. Club, 11: 73-75.

Barnes, W. B.

1955. Status of the San Juan rabbit in the Midwest. Trans. 17th Midwest Wildlife Conf., Lafayette, Indiana: 1-7, mimeo.

Barrett, C.

1934. The grey squirrel in Melbourne. Victoria Nat., 51: 108-110.

Baynes, E. H.

1923. Tusker—a wild boar. Nature Mag., 2: 91-92.

BEMMEL, A. C. V. VAN
1952. Revision of the Rusine deer in the Indo-Australian Archipelago. Treubia, 20: 191-262.

BENTLEY, A.
1954. The introduced deer of Victoria. Unpublished MS., Dept. of Fisheries and Game, Melbourne.

BERGER, N. M.
1944. "Establishment of new fur-bearing animals in Western Siberia." Zoologicheskii Zhurnal, 23: 267-274. (In Russian).

BIGALKE, R.
1937a The naturalisation of animals, with special reference to South Africa. S. Afr. Jour. Sci., 33: 46-63.
1937b The adulteration of the fauna and flora of our national parks. S. Afr. Jour. Sci., 33: 221-225.

BLOUCH, R. I.
1954. Introductions all around. Mich. Conserv., 23 (3): 6-9.

BOLE, B. P., JR. & P. N. MOULTHROP
1942. The Ohio Recent mammal collection in the Cleveland Museum of Natural History. Sci. Publ. Cleveland Mus. Nat. Hist., 5: 83-151.

BOURDELLE, E.
1939. American mammals introduced into France in the contemporary period, especially *Myocastor* and *Ondatra*. Jour. Mamm., 20: 287-291.

BOURLIÈRE, F.
1954. The Natural History of Mammals. New York: Alfred A. Knopf, 363 pp.

BRIDGES, E. L.
1949. Uttermost Part of the Earth. New York: E. P. Dutton, 558 pp.

BRYAN, L. W.
1937. Wild pigs in Hawaii. Paradise of the Pacific, 49 (12): 31-32.

BUCHANAN, G. D.
1955. Nine-banded armadillo—invader from the south. Animal Kingdom, 58 (3): 82-88.

BUECHNER, H. K.
1953. Some biotic changes in the State of Washington, particularly during the century 1853-1953. Research Studies, State Coll. Washington, 21: 154-192.
1956. The future of the bighorn sheep. Animal Kingdom, 59 (1): 2-10.

BULL, P. C.
1953. Parasites of the wild rabbit, *Oryctolagus cuniculus* (L). in New Zealand. New Zeal. Jour. Sci. & Tech., Sec. B, 34: 341-372.

BUMP, G.
1940. The introduction and transplantation of game birds and mammals into the State of New York. Trans. N. Amer. Wildlife Conf., 5: 409-420.

CABRERA, A. & J. YEPES
1940. Mamiféros Sud-Americanos. Comp. Argentina de Editores, Buenos Aires.

CAHALANE, V. H.
1950. Some effects of exotics on nature. Proc. Internat. Tech. Conf. Prot. Nat., UNESCO, Paris, 396-405. Also in Atlantic Nat., 10 (4): 176-185.

CANSDALE, G. S.
1953. Animals and Man. New York: F. A. Praeger, 1-200.

CLIFFORD, C., C. J. GUIGUET & G. A. HARDY
1951. Biology of the Scott Island Group, British Columbia. Prov. Mus. Nat. Hist. and Anthrop., Report for 1950: B21-B63.

CONNELLY, J.
1948. Minnesota whitetails flown to Finland. The Conservation Volunteer. Minn. Dept. Cons., XI: 29-30.

COUCH, L. K.
1929. Introduced European rabbits in the San Juan Islands, Washington. Jour. Mamm., 10: 334-336.

DALQUEST, W. W.
1948. The Mammals of Washington. Publ. Univ. Kans. Mus. Nat. Hist., 2: 1-444.

DARLING, F. F.
1947. Natural History in the Highlands and Islands. Collins, London.

DAVIS, D. H. S.
1950. Notes on the status of the American grey squirrel (*Sciurus carolinensis* Gmelin) in the southwestern Cape (South Africa). Proc. Zool. Soc. London, 120: 265-268.

DAVIS, G. S.
1953. Respite for the burro. Nature Mag., 46 (7): 370-374.

DICE, L. R.
1927. The transfer of game and fur-bearing mammals from state to state, with special reference to the cotton-tail rabbit. Jour. Mamm., 8: 90-96.
1952. Natural Communities. Ann Arbor: Univ. Michigan Press.

DICKEY, D. R.
1923. An extension of the range of the muskrat in California. Jour. Mamm., 4: 55-56

DIXON, J.
1929. Artificial distribution of fur-bearing mammals. Jour. Mamm., 10: 358-359.

DOZIER, H. L.
1951. The present status and future of nutria in the southeastern states. SE Assn. Game Fish Comm., 5th Ann. Meeting, Gulfport, Miss., 10 pp.

DYMOND, J. R.
1922. The European hare in Ontario. Canad. Field Nat., 36: 143.

ELLERMAN, J. R. & T. C. S. MORRISON-SCOTT
1951. Checklist of Palaearctic and Indian Mammals 1758 to 1946. British Museum, London.

ENDERS, R. K.
1930. Notes on some mammals from Barro Colorado Island, Canal Zone. Jour. Mamm., 11: 280-292.

EYERDAM, W. J.
1932. A shipment of muskrats to Kamchatka. Jour. Mamm., 13: 281-282.

FISHER, L. W.
1942. Live-trapping Texas antelopes. Jour. Wildlife Mgt., 6: 231-236.

FITCH, H. S., P. GOODRUM & C. NEWMAN
1952. The armadillo in the southeastern United States. Jour. Mamm., 33: 21-37.

FORSYTH, E. S.
1942. Our stocking experience in the Province of Saskatchewan. Trans. N. Amer. Wildlife Conf., 7: 152-161.

GILMORE, R. M.
1946. Mammals in archaeological collections from southwestern Pennsylvania. Jour. Mamm., 27: 227-235.

GLASS, B. P. & W. R. HANSON
1952. A feral coatimundi in northwestern Oklahoma. Jour. Mamm., 33: 108-109.

GOODWIN, G. G.
1925. The first living elephant in America. Jour. Mamm., 6: 256-263.

GRAF, W.
1955. Cottontail rabbit introductions and distribution in western Oregon. Jour. Wildlife Mgt., 19: 184-188.

HAMILTON, W. J., JR.
1952. The European hare in New York. Bull. to Schools, Univ. State of New York, 38: 189-190.

HANSON, H. C.
1952. Importance and development of the reindeer industry in Alaska. Jour. Range Mgt., 5: 243-251.

HARPER, F.
1945. Extinct and Vanishing Mammals of the Old World. New York: Amer. Comm. Int. Wildl. Prot. Spec. Publ., 12.

HERSHKOVITZ, P.
1953. Mammals from British Honduras, Mexico, Jamaica and Haiti. Field-Zool. 31: 547-569.

HICKS, L. E.
1940. The role of exotics in the Ohio Valley. Trans. N. Amer. Wildlife Conf., 5: 420-424. Also in Ohio Wildlife Res. Sta. Release, 133: 1-4.

HOFFMAN, M.
1952. Die Bisamratte. Die Neue Brehm-Bücherei. Akademische Verlags Gesellschaft, Geest und Portig, Leipzig, 1-43.

HOWARD, W. E.
1953. Nutria (*Myocastor coypus*) in California. Jour. Mamm., 34: 512-513.

JELLISON, W. L.
1945. Spotted skunk and feral nutria in Montana. Jour. Mamm., 26: 432.

JONES, F. W.
1925. The Mammals of South Australia. Govt. Printer, Adelaide, 3 parts.

KIRK, G. L.
1923. Beaver introduced in Vermont. Jour. Mamm., 4: 59-60.

KOERSVELD, E. VAN
1954. De muskusrat, *Ondatra zibethica* L., in Nederland en zijn bestrijding. No publisher listed.

KURODA, N.
1955. The present status of the introduced mammals in Japan. Jour. Mamm. Soc. Japan, 1: 13-18.

LACK, D.
1947. Darwin's Finches. Cambridge Univ. Press, Cambridge.

LARRISON, E. J.
1943. Feral coypus in Pacific Northwest. Murrelet, 24: 3-9.

LAURIE, E. M. O. & J. E. HILL
1954. List of land mammals of New Guinea, Celebes and adjacent islands 1758-1952. British Museum, London.

LEHMANN, V. W.
1948. Restocking on King Ranch. Trans. N. Amer. Wildlife Conf., 13: 236-242.

LEWIS, C. B.
1953. Rats and the mongoose in Jamaica. Oryx, 2: 170-172.

LILJESTRÖM, C.
1954. Bisam i Norrbotten, Svensk Jakt, 10: 341.

LINDEMANN, W.
1956. Transplantation of game in Europe and Asia. Jour. Wildlife Mgt., 20: 68-70.

LINGER, A. L.
1943. Wildlife and livestock on western ranges. Trans. N. Amer. Wildlife Conf., 8: 62-66.

LORENZ, K. Z.
1953. King Solomon's Ring. The Reprint Soc., London, 1-202.

LYON, M. W., JR.
1923. A stray coati in Indiana. Jour. Mamm., 4: 184-185.

MATTHEWS, L. H.
1952. British Mammals. London: Collins.

MIDDLETON, A. D.
1930. The ecology of the American grey squirrel (*Sciurus carolinensis* Gmelin) in the British Isles. Proc. Zool. Soc. London, paper 38: 809-849.

MILLER, G. S., JR.
1918. Mammals and reptiles collected by Theodoor de Booy in the Virgin Islands. Proc. U. S. Nat. Mus., 54: 507-511.

MILLER, G. S., JR., & R. KELLOGG
1955. List of North American Recent mammals. U. S. Nat. Mus. Bull., 205: viii+ 954.

MOHR, E.
1933. The muskrat, *Ondatra zibethica* (Linnaeus), in Europe. Jour. Mamm., 14: 58-63.

MONAHAN, R. S.
1953. Bringing back the marten. Dartmouth Alum. Mag., 46: 88.

MURIE, O. J.
1940. Wildlife introductions in Alaska. Trans. N. Amer. Wildlife Conf., 5: 432-436.
1951. The Elk of North America. Harrisburg: The Stackpole Co.

MYERS, R. H.
1952. New game animal for Kentucky has been planted. Ky. Happy Hunt. Ground, 8 (4): 16, 30.

NAUMOFF, ——
1950. "Vertebrate Zoology" (in Russian). Moscow.

NEILL, W. T.
1952. The spread of the armadillo in Florida. Ecology, 33: 282-284.

NEWSOM, W. M.
1937. Mammals on Anticosti Island. Jour. Mamm., 18: 435-442.

NICHOL, A. A.
1942. Gathering, transplanting, and care of young antelopes. Jour. Wildlife Mgt., 6: 281-286.

O'CONNOR, J.
1953. The bearded Texan. Outdoor Life, 112 (2): 44-45, 91-92.

OSBORN, T. A. B.
1933. Effect of introductions of exotic plants and animals into Australia. Proc. 5th Pacific Sci. Cong., 1: 809-810.

OSGOOD, W. H.
1943. The mammals of Chile. Publ. Field Mus. Nat. Hist., 542, Zool. Ser., 30: 1-268.

PACKARD, R. L.
1955. Release, dispersal, and reproduction of fallow deer in Nebraska. Jour. Mamm., 36: 471-473.

PALMER, R. S.
1954. The Mammal Guide: Mammals of North America North of Mexico. Garden City: Doubleday & Co.

PALMER, T. S.
1899. The danger of introducing noxious animals and birds. U. S. Dept. Agric. Yearbook for 1898: 87-110.

PETRIDES, G. A.
1950. The nutria comes to Texas. Texas Game & Fish, 8 (5): 4-5, 27.

PHILLIPS, J. C.
1928. Wild birds introduced or transplanted in North America. U. S. Dept. Agri. Tech. Bull., 61: 1-63.

PIERCE, R. A.
1956. Some thoughts concerning the introduction of exotic game birds. Wilson Bull., 68: 80-82.

PIMLOTT, D. H.
1953. Newfoundland Moose. Trans. N. Amer. Wildlife Conf., 18: 563-581.

POPOV, B. H. & J. B. LOW
1953. Game, fur animal and fish introductions into Utah. Utah Dept. Fish & Game Misc. Publ., 4: 1-85.

PREBLE, E. A.
1923. Mammals of the Pribilof Islands. N. Amer. Fauna, 46: 102-120.

PULLAR, E. M.
1953. The wild (feral) pigs of Australia: their origin, distribution and economic importance. Mem. Nat. Mus. Melbourne, 18: 7-23.

RAUSH, R. & E. L. SCHILLER
1954. Studies on the helminth fauna of Alaska. XXIV. *Echinococcus sibiricensis* n. sp. from St. Lawrence Island. Jour. Paras., 40: 659-662.

REYNOLDS, J. K.
1952. The biology of the European Hare (*Lepus europaeus* Pallas) in southwestern Ontario. Ph. D. Thesis, Univ. of W. Ontario, London.

RUHL, H. D.
1940. Game introductions into Michigan. Trans. N. Amer. Wildlife Conf., 5: 424-427.

SALMI, A. M.
1949. The white-tailed deer in Finland. Suomen Riista, 4: 124.

SCHEFFER, V. B.
1941. Management studies on transplanted beavers in the Pacific Northwest. Trans. N. Amer. Wildlife Conf., 6: 320-326.
1947. Raccoons transplanted in Alaska. Jour. Wildlife Mgt., 11: 350-351.

SCHMIDT, F.
1954. Pelztierzucht in freier Wildbahn. Kosmos, 50: 73-77.

SCHMIDT, V.
1935. Das Muffelwild. Neudamm.

SEAMAN, G. A.
1952. The mongoose and Caribbean wildlife. Trans. N. Amer. Wildlife Conf., 17: 188-197.

SHAW, A. C.
1940. The European wild hog in America. Trans. N. Amer. Wildlife Conf., 5: 436-441.

SHERMAN, H. D.
1954. Raccoons of the Bahama Islands. Jour. Mamm., 35: 126.

SHORTEN, M.
1946. A survey of the distribution of the American grey squirrel (*Sciurus carolinensis*) and the British red squirrel (*S. vulgaris leucourus*) in England and Wales in 1944-5. Jour. Anim. Ecol., 15: 82-92.
1953. Notes on the distribution of the grey squirrel (*Sciurus carolinensis*) and the red squirrel (*S. vulgaris leucourus*) in England and Wales from 1945 to 1952. Jour. Anim. Ecol., 22: 134-140.

SILVER, J.
1927. The introduction and spread of house rats in the United States. Jour. Mamm., 8: 58-60.

SIMOONS, F. J.
1953. Notes on the bush-pig (*Potamochoerus*). Jour. Uganda Soc., 17: 80-81.

SOPER, J. D.
1923. The mammals of Wellington and Waterloo counties, Ontario. Jour. Mamm., 4: 244-252.

STEAD, D. G.
1928. The rabbit menace in New South Wales. Sydney, privately printed: 1-108.

STEGEMAN, L. C.
1938. The European wild boar in the Cherokee National Forest, Tennessee. Jour. Mamm., 19: 279-290.

STILWELL, H.
1955. Old-world hunt in Texas. Field & Stream, 60 (1): 68-69, 143-146.

STORER, T. I.
1931. Known and potential results of bird and animal introductions with special reference to California. Calif. Dept. Agric. Monthly Bull., 20: 267-273.
1933. Economic effects of introducing alien animals into California. Proc. 5th Pacific Sci. Cong., 1: 779-784.
1937. The muskrat as native and alien. Jour. Mamm., 18: 443-460.

SWABEY, C. & C. B. LEWIS
1946. Forestry in the Cayman Islands. Development and welfare in the West Indies. Bull. 23, 11.

SWANK, W. G. & G. A. PETRIDES
1954. Establishment and food habits of the nutria in Texas. Ecology, 35: 172-175.

SWIFT, L. W.
1945. A partial history of the elk herds of Colorado. Jour. Mamm., 26: 114-119.

TESDORPF, O. L.
1910. Einbürgerung des Muffelwildes auf dem europäischen Festlande. Selbstverlag, J. Neumann, Neudamm.

THOMPSON, H. V.
1955. The wild European rabbit and possible dangers of its introduction into the U.S.A. Jour. Wildlife Mgt., 19: 8-13.

THOMSON, G. M.
1922. The Naturalization of Animals and Plants in New Zealand. Cambridge Univ. Press, 1-607.

TROUGHTON, E.
1947. Furred Animals of Australia. Angus & Robertson Ltd., Sydney.

TURCEK, F. J.
1951. Effect of introductions on two game populations in Czechoslovakia. Jour. Wildlife Mgt., 15: 113-114.

VIDRON, F.
1939. Le cerf sika. P. Lechevallier, Paris.

VORIS, J. C., J. D. YOAKUM & C. F. YOCUM
1955. Notes on chinchilla liberations in California. Jour. Mamm., 36: 302.

WALLACE, A. R.
1880. Island Life. Harper & Bros., 1-522.

WARWICK, T.
1934. The distribution of the muskrat (*Fiber zibethicus*) in the British Isles. Jour. Anim. Ecol., 3: 250-267.
1936. The parasites of the muskrat (*Ondatra zibethica* L.) in the British Isles. Parasitology, 28: 395-402.

WESTERMANN, J. H.
1947. Fauna en natuurbescherming in Nederlandsch Nieuw Guinea. Nederlandsche Commissie voor Internationale Natuurbescherming. Mededeelingen, 13: 1-107.
1953. Nature preservation in the Caribbean. Publ. Found. Sci. Res. in Surinam and Netherlands Antilles, 9: 1-106.

WILDHAGEN, A.
1956. Present distribution of North American mink in Norway. Jour. Mamm., 37: 116-118.

WODZICKI, K. A.
1950. Introduced Mammals of New Zealand. Wellington: Dept. Sci. & Ind. Res., Bull.

INDEX

Names in **bold face** indicate new genera, species or subspecies; numbers in **bold face** indicate illustrations; numbers in parentheses are the series numbers of papers containing the plates listed immediately following.