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WILLIAM B. JEFFRIES
Department of Biology
Dickinson College
Carlisle, Pennsylvania 17013

HAROLD K. VORIS
Department of Zoology
Field Museum of Natural History

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CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABSTRACT

Blue crabs (*Callinectes sapidus* [Rathbun, 1896]) bearing barnacles (*Octolasmis mülleri* [Coker, 1902]) in the branchial chambers were collected at Beaufort, North Carolina, to study the abundance, distribution, and reproduction of the symbiotic barnacles. Branchial chambers within, as well as between, crabs were inhabited with barnacle populations having different densities and size frequencies. Barnacle densities did not correlate with crab size; most barnacles were attached to the inner sides of the gills. Barnacle densities did not correlate with gill size; barnacles were significantly more abundant on the large gills and more abundant proximally than distally on each gill. The minimum size of reproductively active barnacles varied among crabs, with the smallest being 1.14 mm in capitular length. There was no significant correlation between the size of the smallest gravid barnacle and the density of barnacles in the chamber. Brood size ranged from 21 to 4,459 and correlated well with barnacle size. Members of a brood were at the same stage of development.

INTRODUCTION

Octolasmis mülleri (Coker, 1902), a pedunculate barnacle, is an epizoite frequently found within the branchial chambers of the blue crab *Callinectes sapidus* (Rathbun, 1896). Two inhalent apertures, about 4–6 cm apart in mature crabs, service separate branchial chambers and provide access for the barnacle larvae. Barnacles attach to the gills, most often on the concave side (Humes, 1941), where as much as 83% of the barnacle population may reside (Walker, 1974). The largest number of barnacles attaches to gill 3 (= gill 6 [Pyle & Cronin, 1950], since the gills were numbered in reverse order), the numbers decreasing progressively on gills 5–1 and 7–8 (Walker, 1974). More female than male crabs are infested, and the number of barnacles per crab is greater among females (Coker, 1902; Humes, 1941).

This study was undertaken to investigate factors affecting the distribution of barnacles between crabs, gill chambers, gills, and portions of gills; to determine the effect, if any, of population density on the size of gravid barnacles; and to compare barnacle size with brood size.

MATERIALS AND METHODS

Blue crabs were collected from Beaufort Inlet, North Carolina, from late May until mid-July 1978. Those crabs which had not recently molted, based on the presence of balanoid barnacles, were collected. The carapace of each crab was measured from spine tip to spine tip. The carapace and the limbs were removed before the body of the crab was fixed in formalin. Subsequently, the gills in each

branchial chamber were removed, measured, and examined for barnacles. The sites of barnacle attachment, whether along the inner (efferent) or outer (afferent) margin and whether on the proximal, medial, or distal third of the gill, were recorded. The barnacles were removed with watchmaker's forceps under a dissecting microscope, the length of each capitulum was measured with an ocular micrometer, and the presence or absence of ovigerous lamellae within each capitulum was noted.

Ten crabs each bore less than 30 gravid barnacles. All of these broods were counted. Two crabs had large numbers of gravid barnacles, and random samples (broods) were counted (70 of 194 from crab 22, and 32 of 83 from crab 26) (table 1).

To determine brood size, barnacles were placed individually in distilled water in three-spot depression slides. Using watchmaker's forceps under low magnification of a dissecting microscope, the capitulum was partially torn away and the lamellae removed. The embryo masses were transferred to another depression containing a digestive mixture of 8 mg of pepsin/ml in 0.01 N HCl. Each slide was placed in a 125-mm covered Petri dish containing some water and incubated on a slide warmer at 38.5° C. The lamellae were periodically agitated by sucking them into and out of a widemouthed pipette. The embryos were washed in several changes of distilled water, stained in a mixture of fast green and acetocarmine, rinsed in distilled water, dehydrated, spread on a slide, affixed with parlodion, and covered with mounting medium and a cover glass.

A background grid for counting was improvised by attaching a piece of graph paper to a plate of clear plastic. A frame superimposed on the paper permitted each slide to be situated in the same way. The graph paper was sufficiently translucent so that the stained organisms could be seen clearly with transmitted light when viewed with low magnification under a dissecting microscope. All of the counts were made by the same individual, following a prescribed pattern.

TABLE 1. Abundance and size of *Octolasmis mülleri* (N = 1,832) on 17 blue crabs. In this table, gravid barnacles comprise a subset of the total barnacles.

	Numbers			Capitular length (mm)				
				Barnacles		Gravid barnacles		
Crab	Barnacles	Gravid barnacles	Percent gravid	$\bar{x}$	s	$\bar{x}$	s	Range
22	344	194	56.4	1.90	.44	2.07	.48	1.14–3.58
26	94	83	88.3	3.19	.79	3.17	.50	1.72–4.72
30	66	0	0	.81	.39	. . .	. . .	. . .
31	10	3	30.0	1.70	1.49	3.53	.54	3.15–4.15
38	29	2	6.9	1.06	.90	4.00	.60	3.58–4.43
39	9	1	11.1	1.37	.96	3.58	. . .	. . .
40	160	29	18.1	1.26	1.04	3.25	.42	2.29–4.00
41	44	26	59.0	2.75	1.20	3.53	.58	2.57–4.72
42	13	1	7.7	1.12	1.21	2.86	. . .	. . .
43	35	0	0	1.30	.66	. . .	. . .	. . .
45	42	0	0	.87	.21	. . .	. . .	. . .
48	329	2	.6	.79	.37	4.00	0	0
49	91	3	3.3	1.16	.64	3.10	.36	2.72–3.43
50	89	2	2.2	.86	.44	2.65	.30	2.43–2.86
51	434	0	0	.69	.20	. . .	. . .	. . .
52	174	8	4.6	.98	.77	2.99	.32	2.57–3.58
55	6	0	0	1.93	1.62	. . .	. . .	. . .

RESULTS

Distribution of Octolasmis mülleri

Between crabs.—A total of 59 crabs having a mean carapace width of 143 mm was studied. Fifteen were males with an average width of 131 mm and 44 were females with an average width of 147 mm. Twenty-nine crabs, including eight males ($\bar{x}$ carapace width = 114 mm) and 21 females ($\bar{x}$ carapace width = 148 mm), were found to bear *O. mülleri*. Seventeen crabs, each bearing at least six (nonlarval) barnacles (range = 6–434) were arbitrarily selected for detailed studies of barnacle distribution and reproduction. Table 1 presents the number, size, and reproductive condition of the barnacles inhabiting these 17 crabs.

Within branchial chambers.—Pedunculate barnacles were never observed externally on the crabs. They were rarely attached to the maxillipeds, e.g., on the epipodites (flabella) in the branchial chambers. Occasionally they were attached to the walls of the branchial chambers, especially beneath the gills, but most frequently they were cemented to the gills.

The branchial chambers on the right side did not consistently have more barnacles than the branchial chambers of the left side. Of the 17 crabs, eight had more barnacles in the right chambers, eight had more in the left, and one crab had equal numbers in the two chambers.

The branchial chambers are, however, physically separate, and nine crabs had significantly (chi-square tests, $P < .05$) more barnacles in one chamber than in the other. Some of these comparisons were striking; e.g., crab 40—left = 31, right = 129; crab 48—left = 50, right = 279; crab 51—left = 267, right = 168.

To investigate the relationship between the size of the gill chamber and the number of barnacles observed, the 17 crabs were ranked according to carapace width and the number of barnacles found in their branchial chambers. Barnacle densities did not vary significantly with crab size, using a Spearman rank correlation test adjusted for ties (Siegel, 1956).

On the gills.—Does the density of barnacles affect their distribution over the eight gills? The number of barnacles on each of the gills of five crabs with few barnacles (<50) was compared with five crabs having substantially more barnacles (>100). In neither group did the distribution of barnacles deviate significantly from the overall distribution observed in the 17 crabs taken as a group (chi-square test, $P > .05$).

Gill length.—The right and left branchial chambers in *C. sapidus* each contain eight gills numbered 1–8 from anterior to posterior (Pyle & Cronin, 1950). For the above 17 crabs, the average gill lengths (in mm) were: gill 1 = 10.6; 2 = 17.7; 3 = 26.7; 4 = 33.3; 5 = 36.5; 6 = 37.0; 7 = 35.5; and 8 = 31.4. More barnacles were found on the larger gills than on the smaller gills; these results are consistent with Walker (1974) (fig. 1).

Gill length did not correlate significantly with barnacle abundance. For example, gill 1 represented 4.6% of the total gill length in a chamber and, over the 17 crabs, would be expected to support about 85 barnacles (4.6% of 1,832 total barnacles). However, only seven barnacles (0.4% of 1,832) were observed on gill 1. A comparison of the observed distribution over all gills with that expected based on gill length resulted in a highly significant deviation (chi square = 613.4, $P < .001$).

Whole gill area.—The areas of the gills were calculated using the formula for the area of a right cone ($a = \pi rh$). Area was not significantly correlated with

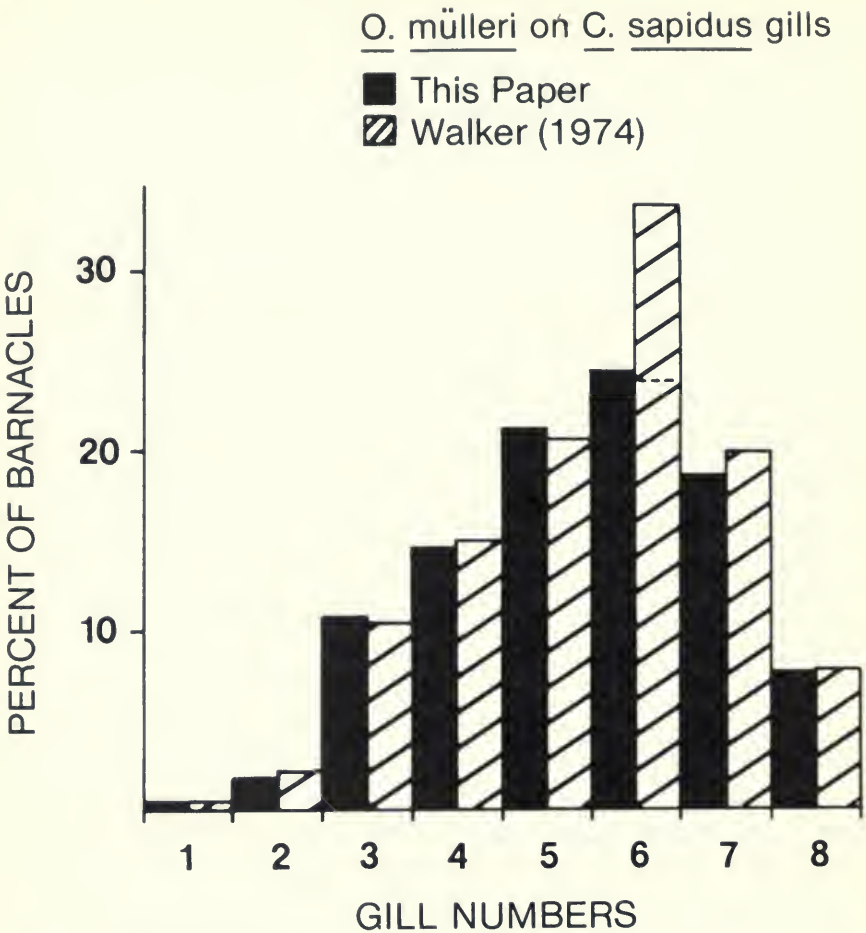

FIG. 1. The relative frequency of *Octolasmis mülleri* on the eight pairs of gills in the blue crab, *Callinectes sapidus*. These data are compared with those of Walker (1974). The histograms are indistinguishable if Walker's "extra" 10% (the mean ratio values plotted in his Figure 2 add up to 110% instead of 100%) is removed from the gill pair 6 category. The dashed line on the barred gill 6 indicates his data with the 10% removed.

barnacle abundance. The expected numbers of barnacles on the basis of area and the observed counts (in parentheses) for gills 1–8 were: 29 (7), 72 (35), 128 (197), 308 (268), 418 (391), 317 (449), 290 (341), and 273 (144). These expected and observed distributions were significantly different from each other (chi square = 205.4, $P < .001$).

Inner versus outer gill surface.—Barnacles were more abundant on the inner side of the gills than the outer. This was illustrated by an examination of gill pairs 5 and 6, which bore the largest numbers of barnacles over the 17 crabs:

Gill pair	Barnacles (N)	Inner	Outer
5	391	365 (93.4%)	26 (6.6%)
6	449	431 (95.9%)	18 (4.0%)

This pattern was characteristic of all gills. Walker (1974) found 83% of the barnacles on the inner side versus 17% on the outer side over all the gills of 25 crabs.

Linear distribution on the gills.—Barnacles were observed to be differentially distributed along proximal, medial, and distal gill segments of equal lengths. The numbers of barnacles on the gills of the 17 crabs were:

Gill segment	Barnacles (N)	Percent of total
Proximal	920	50.2
Medial	774	42.3
Distal	138	7.5

Clearly, disproportionate numbers of barnacles were found on the proximal and medial portions of the gills.

Areas of gill segments.—Barnacle abundance was not proportionate to the surface area taken up by each portion of the gill. For example, for gill 5, the proximal portion comprised 58% of the total area of the gill; the medial portion, 27%; and the distal portion, 15%. Based on these percentages, 1,062.6 barnacles would be expected on the proximal portions of the gills, whereas only 920 were observed. On the medial portions only 494.6 would be expected, but 774 were observed; and on the distal portions 274.8 would be expected, but only 138 were observed. In summary, for the 17 crabs, the distribution of barnacles over these three gill regions did not correspond to that predicted on the basis of surface area (chi square = 42.11, $P < .001$).

Size of Octolasmis mülleri

Barnacles ranged in capitular length (height) from 0.14 to 5.58 mm. Of the nearly 2,000 specimens measured (1,832 from the gills, 137 found elsewhere within the branchial chambers), most were between 0.5 and 1.0 mm.

The size-class distribution of barnacles varied a great deal between crabs (table 1). The mean capitular length varied from 0.69 mm (N = 434, range 0.43–1.43 mm, s = 0.20) for crab No. 51, to 3.19 mm (N = 94, range 0.43–5.58 mm, s = 0.79) for crab 26. A plot of the size distribution of barnacles found in the gill chambers of crab 22 followed a normal curve (fig. 2), but for the populations found in other crabs, size distributions varied from asymmetrical (crabs 30 and 41) to bimodal (crab 40). The cause of this variability is not known.

The size distributions of barnacles between chambers of the same crab also varied. Only barnacles attached to gills were included in these comparisons. Examination of the mean sizes of the capitulum of the two barnacle populations (in the left and right branchial chambers) of each crab showed five pairs of chambers that had populations significantly different from each other (table 2). In 12 other crabs, the mean sizes of the barnacles in the two chambers were not significantly different ($P > .05$).

Reproduction of Octolasmis mülleri

Gravid barnacles were present in 12 of the 17 crabs sampled from late May to mid-July 1978 (table 1). Data from crab 22 demonstrate that *O. mülleri* with a capitular length of 1.14 mm can become reproductively active, but the gravid condition was not common when the capitular length was less than 1.75 mm.

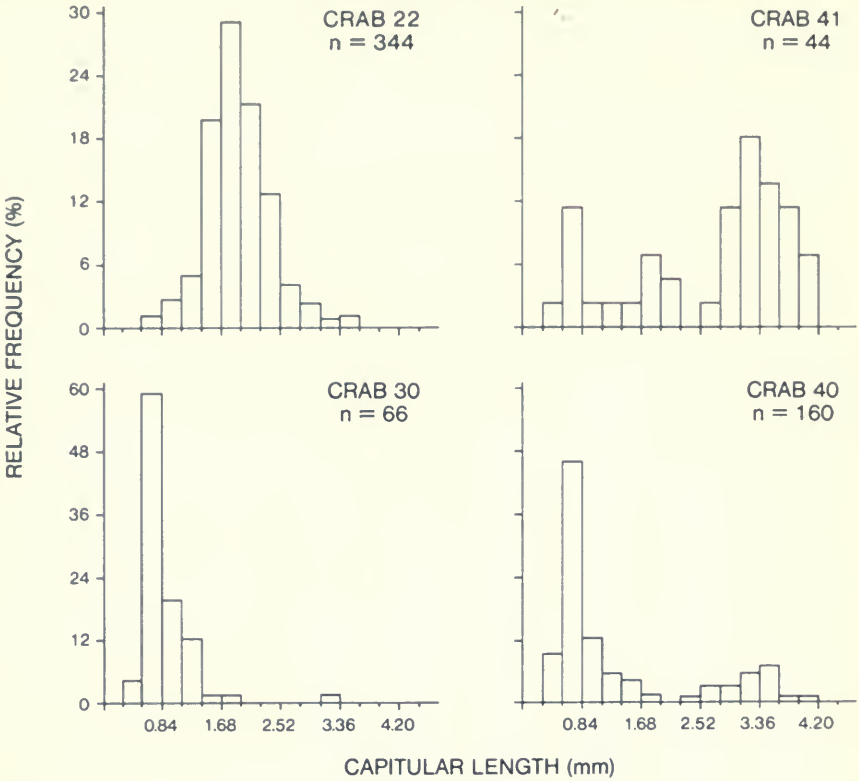

FIG. 2. The relative frequency distribution of barnacles according to their size (capitular length) for populations from crabs No. 22, 30, 40, and 41.

TABLE 2. Size of barnacles (capitular length) in the branchial chambers of five crabs in which the mean size of barnacles in the two chambers was significantly different.

	Left chamber			Right chamber			
Crab	N	$\bar{x}$	s^2	N	$\bar{x}$	s^2	*P*
22	153	1.81	.15	106	1.93	.19	<.05
40	25	1.50	1.82	124	1.06	.64	<.05
41	17	1.73	1.27	23	3.34	.41	<.001
45	35	.92	.04	7	.63	.01	<.001
52	62	1.04	.73	95	.79	.30	<.05

The minimum size of reproductively active barnacles varied from crab to crab. For example, both crabs 40 and 41 had barnacles with capitular lengths of 1.0 to 2.0 mm, but none were gravid. On the other hand, about half of the barnacles in this size range from crab 22 were gravid. Figure 3 illustrates these data for the four crabs in which there were 10 or more gravid and 10 or more nongravid barnacles. These illustrate the major patterns observed.

Does the density of barnacles in a given chamber determine the size of the smallest gravid individual? Barnacle populations in 19 of 24 branchial chambers

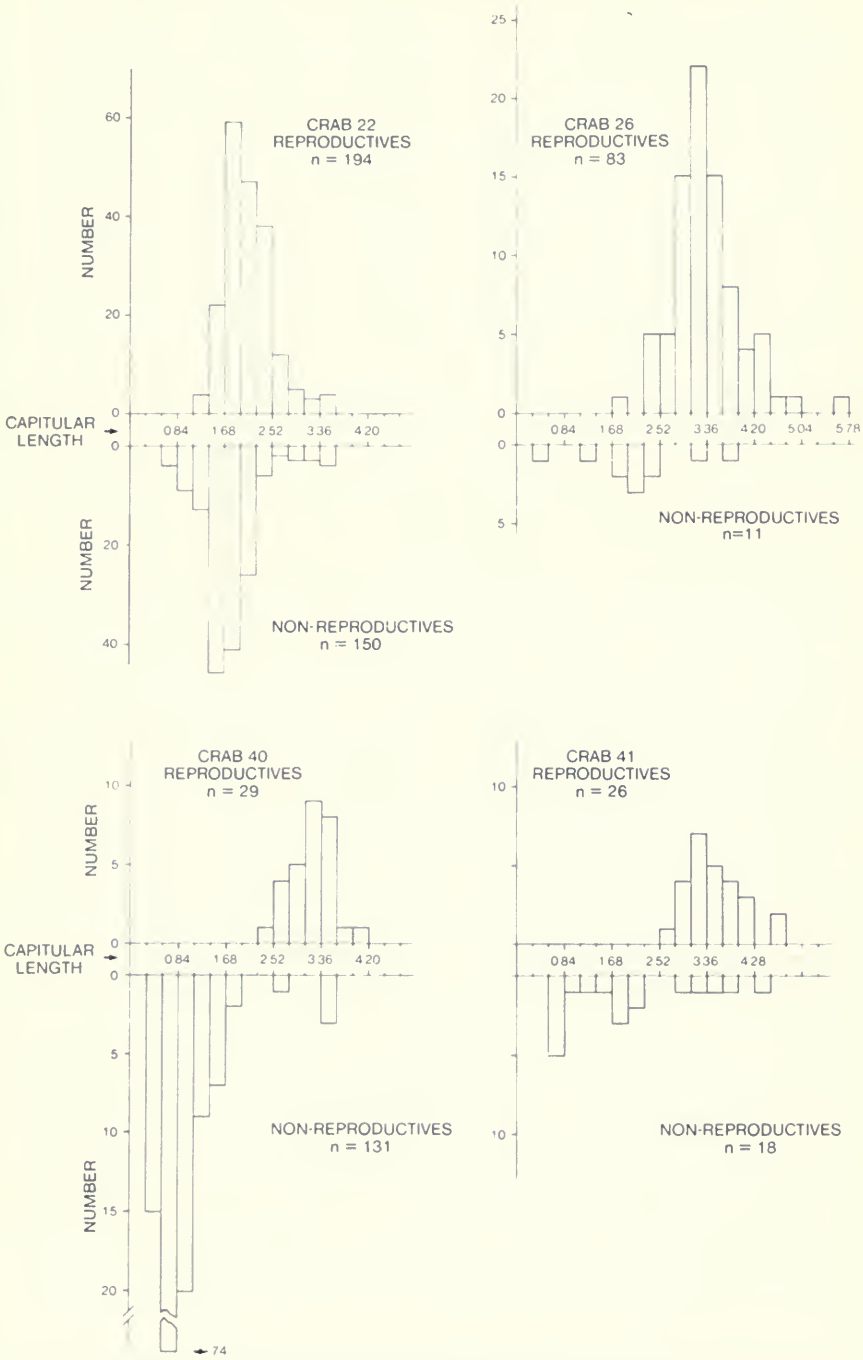

FIG. 3. Size frequency distribution of reproductive and nonreproductive barnacles for populations from crabs No. 22, 26, 40, and 41.

in 12 crabs where there was at least one gravid barnacle were examined. A least-squares regression analysis of the barnacle-chamber density versus the size of the smallest gravid barnacle showed no significant correlation between these two factors ($r = 0.21$, $P > .05$).

Is brood size related to barnacle size? Brood size was highly variable but was related to barnacle size. In Figure 4 capitular length is plotted versus brood size for gravid barnacles from crabs 22 (N = 70), 26 (N = 32), 40 (N = 29), 41 (N = 26), and 52 (N = 8). The upper boundary in this scattergram is rather sharp and seems to indicate a straight line relationship up to capitular lengths of about 2.5 mm and then a curvilinear relationship for the larger sizes. The many values substantially below the upper boundary may be a function of partial release of larvae or may reflect variations in fecundity. Thus, a regression of all the data is of questionable value. The upper boundary of points is the best indicator of the overall relationship between brood size and barnacle size.

The average sizes of the gravid barnacles from crabs 22, 26, 40, and 41 differ, $P < .02$ (table 1). For example, the points below 1.7 mm (fig. 4) are data on barnacles from crab 22 only. These data further suggest that the dynamics of growth and reproduction of barnacles can differ between crabs.

DISCUSSION

Given the proximity of the inhalent apertures to each other and the fact that the densities of barnacles in the two branchial chambers are not correlated with each other suggests that factors affecting colonization and survivorship may be complex, and mere exposure to larvae may not ensure colonization.

The results demonstrated that barnacles are concentrated in the middle sections of the gills and on gills more centrally located in the chambers. The flow of water through the crab respiratory system may be responsible for this distributional pattern. The usual route of water flow through the crab is inhalent aperture, hypobranchial chamber, by the gill lamellae, hyperbranchial chamber, and exhalent aperture (Pyle & Cronin, 1950) (fig. 5). Occasional reversal of flow results in flushing of the hypobranchial chamber. The gills are in close apposition and they arch dorsomedially over the hypobranchial chamber. The hypobranchial chamber narrows dorsally and posteriorly, being most spacious beneath gills 4, 5, 6, and 7. *Octolasmis* cyprids, carried with other plankters, must impinge against the underside of the gills and then maneuver to the final attachment sites. Although cyprid movement was observed in a dish of sea water, the extent they can move in the branchial chamber is not known. A greater volume of water probably passes through the central part of the hypobranchial chamber. The interlamellar spaces are larger in the bigger gills and are larger proximally than distally, thereby providing less resistance to flow. This flow may account for the deposition of the greatest number of cyprids and subsequently allow the greatest number to settle.

Examination of the age and size distribution of barnacles in the branchial chambers may provide information about the process of colonization. In addition to metamorphosed barnacles, cyprid larvae were observed in all but one of the 17 crabs, and in some cases comprised up to 45% of the total *Octolasmis* population. The attached cyprids, the size range of the metamorphosed barnacles (capitular length 0.14–5.58 mm), and the gravid barnacles suggest many sequential invasions. The number, nature, and duration of the pre-cyprid larval

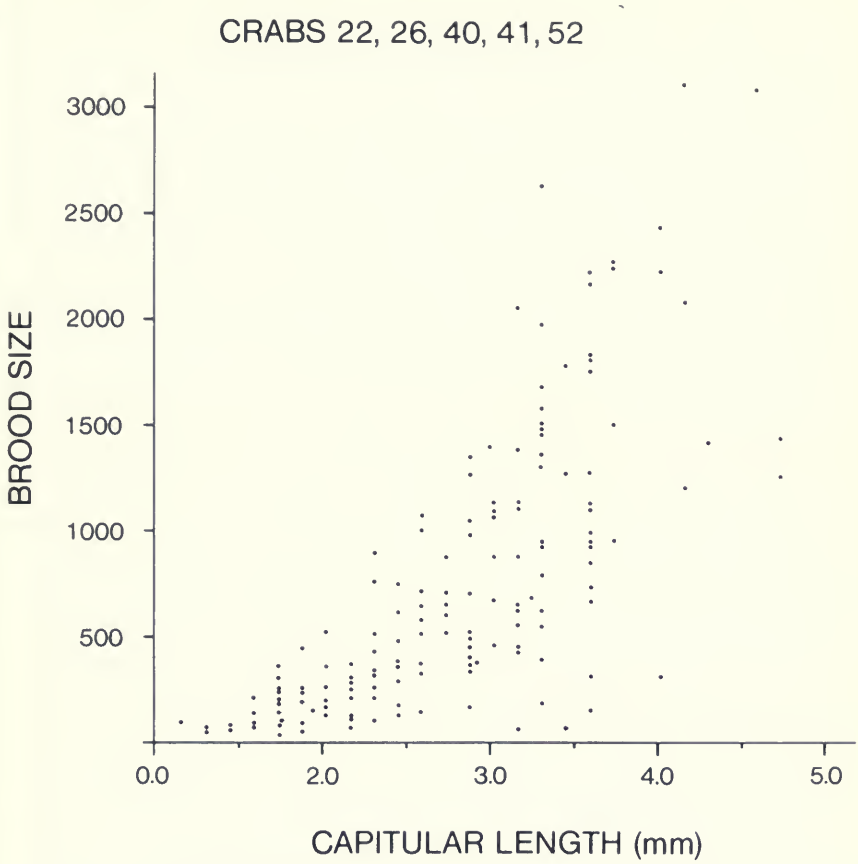

FIG. 4. Barnacle capitular length versus brood size for barnacles from crabs No. 22, 26, 40, 41, and 52.

FIG. 5. Diagram of respiratory water flow through the branchial chambers of *Callinectes sapidus*.

stages (Lang, 1976) virtually preclude the chance that a life cycle could be completed within the branchial chamber. The size frequency patterns shown in Figure 2 differed from each other and from computer-generated patterns for a random "rain" of larvae. Thus, cyprids either do not attach in a random sequence, or some other mitigating factors such as temporal changes in susceptibility or differing survival rates produce the nonrandom distributions observed.

The data suggest that the pattern of brood production in *O. mülleri* resembles the third category of Hines (1978), i.e., that of warm temperature and subtropical species which produce many broods during the summer; however, collections in the present study were limited to summer months.

Brood sizes ranged from 21 to 4,459 and, in general, correlated well with barnacle size. The young, which were stained for contrast rather than cytological detail, were difficult to categorize according to age. Microscopic examination indicated, however, that the members of a brood were at essentially the same stage of development.

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Field Museum of Natural History
Roosevelt Road at Lake Shore Drive
Chicago, Illinois 60605-2496
Telephone: (312) 922-9410