A STUDY OF REPRODUCTION IN THE INTERTIDAL BARNACLE, MITELLA POLYMERUS, IN MONTEREY BAY, CALIFORNIA[1,2]

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The goose barnacle, *Mitella polymerus* (Sowerby, 1833), sometimes referred to as *Pollicipes polymerus* or the leaf barnacle, is distributed along the exposed rocky coast of Western North America from British Columbia to the middle of Baja California (Cornwall, 1925). It is generally abundant here in the upper two-thirds of the intertidal belt (Ricketts and Calvin, 1952), though it is occasionally found below this level where there is considerable surging wave action. Along the central California coast, clusters of *Mitella* patch the exposed rocky regions, the barnacles usually attaching themselves to rock, to *Mytilus californianus,* or to other individuals of *Mitella.* Individuals are seldom isolated and one sees among the mussel beds or on the rocks rosette-shaped clusters in which large barnacles are at the center and smaller barnacles grade toward the periphery. In other aggregations, individuals of nearly the same size are packed closely together, frequently with such uniformity in size and orientation that their valves form a geometric pattern, and their closely packed bodies make a strong but resilient mat against the pounding surf. Often where the animals are attached to rock beneath the mussel beds, their stalks extend up eight inches or more to the surface. Occasionally, solitary animals occur on tables of rock exposed to strong wave action; in these the stalks remain short and stubby while the shells and bodies grow.

While *Mitella polymerus* is abundant, conspicuous, and well-known taxonomically, almost nothing is known of its reproductive biology. Nussbaum (1890) has described the anatomy of *M. polymerus* in some detail. The only published study of reproduction and development in this genus is that of Batham (1944–45), on the New Zealand species, *Mitella spinosus.* Since Batham's study of the reproductive cycle was carried out at latitude 44°52′ South, a comparison of her results with the situation occurring in *M. polymerus* at Monterey Bay (36°40′ North) seemed particularly interesting. The following study of reproduction in *M. polymerus* includes: the anatomy of the reproductive system, the relationship between size and sexual reproduction, the seasonal reproductive cycle, the rate of egg and embryo development, an estimate of fecundity, and evidence concerning self-fertilization.

[1] This investigation was carried out as partial fulfillment for the degree, Master of Arts.

[2] The author wishes to express her gratitude to Dr. Donald P. Abbott, under whose guidance this work was carried out; and to acknowledge with appreciation his generous help with arrangement and discussion of the data.

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The Reproductive System

The gross reproductive anatomy of *Mitella polymerus* is shown in Figure 1. Ovarian tissue is found in the upper portion of the peduncle. From the ovaries, a pair of oviducts lead up into the body proper, emptying into the glandular oviducal atria in the bases of the first thoracic cirri. Eggs pass down the oviducts to the

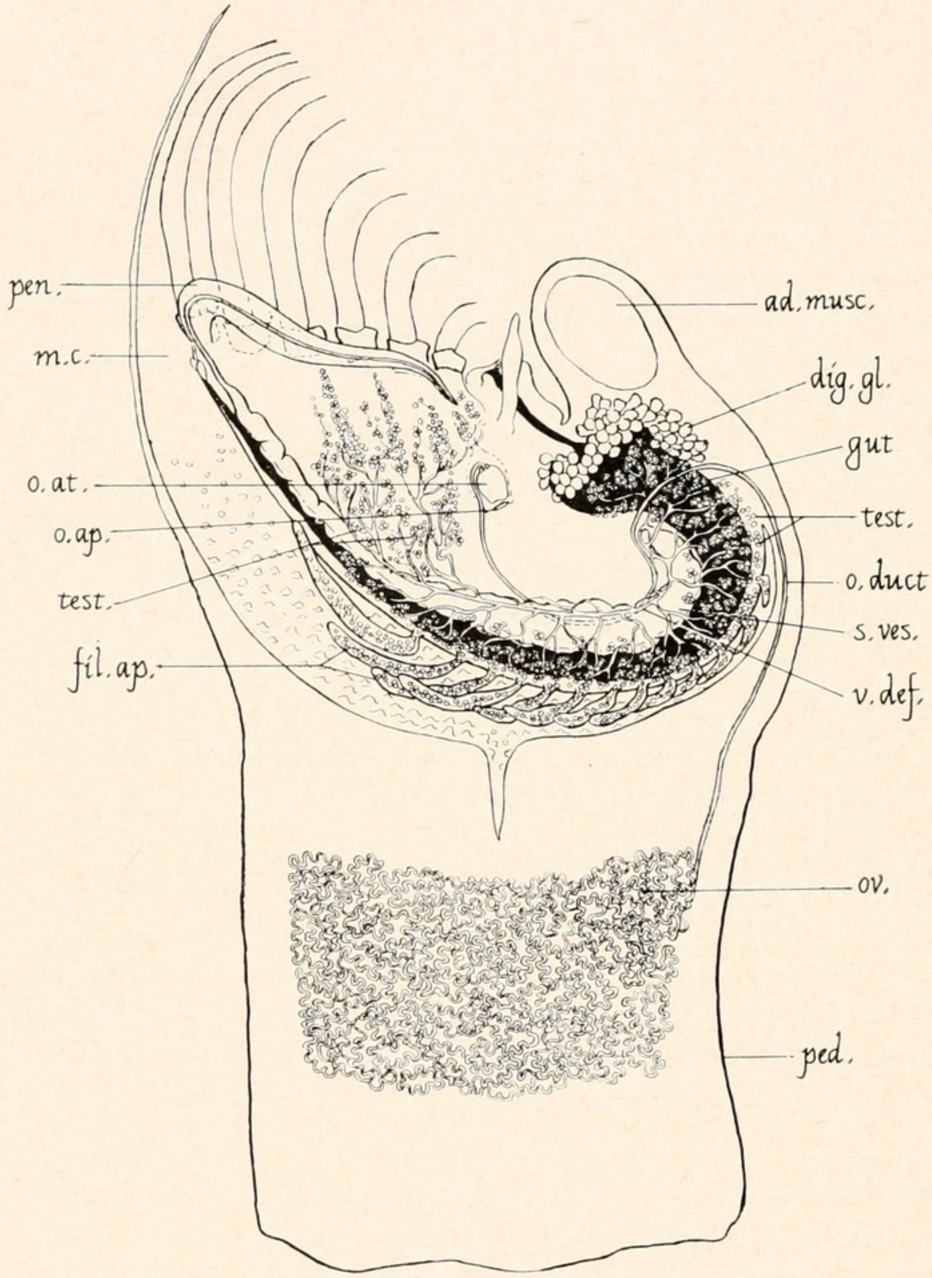

Figure 1. Gross anatomy of the reproductive system of *M. polymerus*, exposed by dissection from the left side. ad. musc. = adductor muscle; dig. gl. = digestive gland; fil. ap. = filamentary appendages; gut = gut; m. c. = mantle cavity; o. ap. = oviducal aperture; o. at. = oviducal atrium; o. duct = oviduct; ov. = ovaries; ped. = peduncle; pen. = penis; test. = testes; s. ves. = seminal vesicle; v. def. = vas deferens.

atria, where they receive a protective coating which glues them together into masses. The egg masses are then extruded through the oviducal apertures and come to lie in the mantle cavity on either side, where they are pressed flat to form the two ovigerous lamellae.

The numerous small testes are found on either side of the gut, extending ventrally into the coxopodites of the first four pairs of thoracic appendages and dorsally into the numerous paired filamentary appendages. Fine efferent ducts connect the testes to the paired vasa deferentia, which lead to the paired storage organs or seminal vesicles. These in turn join posteriorly at the base of the penis, and form a single duct extending to its tip.

Copulation was not observed, but sperm are deposited in the mantle cavity. The embryos are brooded in the ovigerous lamellae in the mantle cavity until they are hatched out as nauplius larvae.

The gross reproductive anatomy of *M. polymerus* is similar to that of *M. spinosus;* but *M. spinosus* has no filamentary appendages and the testis is described as (p. 370) "a median structure lying closely in the U-bend of the gut," with a pair of ducts leading from either side of it which expand to form the seminal vesicles (Batham, 1944–45).

Materials and Methods for the Study of Reproduction

Nearly all living material used in the present study was collected in or very near Monterey Bay, California, and studies were carried out at the Hopkins Marine Station of Stanford University, Pacific Grove.

For the study of the reproductive cycle, a population of *Mitella polymerus* was sampled at approximately monthly intervals for a period of fifteen months. All individuals were collected within an area approximately fifteen feet square on granite rocks and adjacent beds of *Mytilus californianus* on the northern shores of Mussel Point, Pacific Grove, California, at an intertidal level corresponding to the upper middle horizon of Zone Three of Ricketts and Calvin (1952). Care was taken to insure that all barnacles large enough to be reproductively mature were collected in close proximity to other individuals of a reproductive size, and to avoid taking isolated individuals which had lacked the opportunity for cross-fertilization.

Animals were anaesthetized for four hours in a solution of magnesium chloride isotonic with sea water. This was sufficient to relax the cirri and peduncle. The animals were then preserved in 10% formalin buffered with borax.

Individuals of all sizes were collected and examined. For most of the reproductive data, the largest common animals available were used. All of these were of a reproductive size, and for purposes of consistency, large animals which ranged in breadth (distance from rostrum to carina) from 27.5 mm. to 32.5 mm. were used (occasionally larger animals are found).

When maturing, the ovarian tissue in the peduncle (Fig. 1) undergoes marked and visible changes. Tiny eggs appear and grow, accumulating yolk until they are passed up through the oviducts and are extruded into the mantle cavity. Preliminary observations showed that throughout the ovarian tissue, eggs are generally of about the same size and stage of development at any one time; an exception to this is provided where a new batch of tiny eggs appears in the ovary before the previous batch of very much larger eggs is extruded. With a compound microscope and calibrated ocular micrometer, the greatest diameters of five to ten eggs were measured (in each animal), and an average ovarian egg size was recorded for the individual. Where two batches of eggs were present at once in an ovary (*i.e.*, large and small), this situation was quite evident, and the two were treated separately. The average egg sizes were then grouped into three useful classes for purposes of comparison: small eggs (diameter 0.016 mm. to 0.065 mm.), medium eggs (diameter 0.066 mm. to diameter 0.099 mm.), and large eggs (diameter 0.100 mm. to diameter 0.127 mm.). The few individuals in which no eggs could be seen were treated separately.

As the male gonads of *Mitella* mature, they also show easily visible changes: testes in the filamentary appendages and throughout the body lose their translucency and become opaque white with sperm. Sperm then travel through efferent tubules and vasa deferentia to the paired seminal vesicles, where they are stored. As more and more sperm accumulate in the seminal vesicles, these, too, change from translucent to opaque white and increase in diameter. Diameter of the seminal vesicles affords a fairly good index for the maturation of the male reproductive organs. Width was measured with a pair of dividers at a point just back of the anterior sharp bend in the seminal vesicle (Fig. 1). Conditions of the male organs were finally grouped into three categories: (1) seminal vesicles translucent and apparently empty of sperm; (2) seminal vesicles ranging in width from one to two mm.; and (3) seminal vesicles more than two mm. in width (ranging up to a maximum observed width of 3.8 mm.).

Fertilized eggs and developmental stages present in ovigerous lamellae were also studied. Embryos were examined in the ovigerous lamellae from animals taken in the monthly samples. In a pair of ovigerous lamellae taken from any one parent, embryos are all at about the same stage of development. As the fertilized egg develops, it increases slightly in size, but not enough for size to yield a good criterion for stage of development. Major morphological changes can be studied fairly readily, and it proved practicable for the purpose of the present problem to divide embryonic development into three stages: (1) early stages, with neither limb buds nor nauplius eye; (2) middle stages, with limb buds developing but no nauplius eye; and (3) late stages, with well-formed limbs and median eye present. Studies were also made of lamellae removed from the parent and raised *in vitro*. Ovigerous lamellae were isolated from their parent barnacles, placed in clear glass dishes of filtered sea water, and kept at a constant 13° C. in a water bath or a refrigerated room. Sea water was changed approximately daily, at which times the embryos were examined in small sections of the ovigerous lamellae plucked off with glass needles. Many embryos raised *in vitro* were observed through development to hatching, and many larvae were raised beyond this through several naupliar molts.

Size and Sexual Reproduction

Figure 2 shows the occurrence of ovigerous lamellae in animals of different sizes at selected times during the breeding season (May through December). Animals with a breadth of more than 27.5 mm. were always found to be sexually mature during the reproductive season; all individuals examined bore ovigerous lamellae, or enlarging ova in the ovaries, or both. In the individuals below 27.5 mm. breadth, the percentage of animals with ovigerous lamellae can be seen to decrease more or less directly with decreasing size. For individual months, the tendency is not always clear, and this may be due to sampling deficiencies (*e.g.*, class III in July and September). However, the summary graph, which represents values for the total number of animals of each size class, points out the trend well, and the differences shown here are statistically significant. The variation in reproductive activity with size may be explained in either of two ways: possibly a lower percentage of the smaller animals are sexually mature; or (less likely) the smaller class animals produce ovigerous lamellae less frequently than the larger animals. No ovigerous lamellae were ever found in any barnacle with a breadth of less than 17.2 mm.

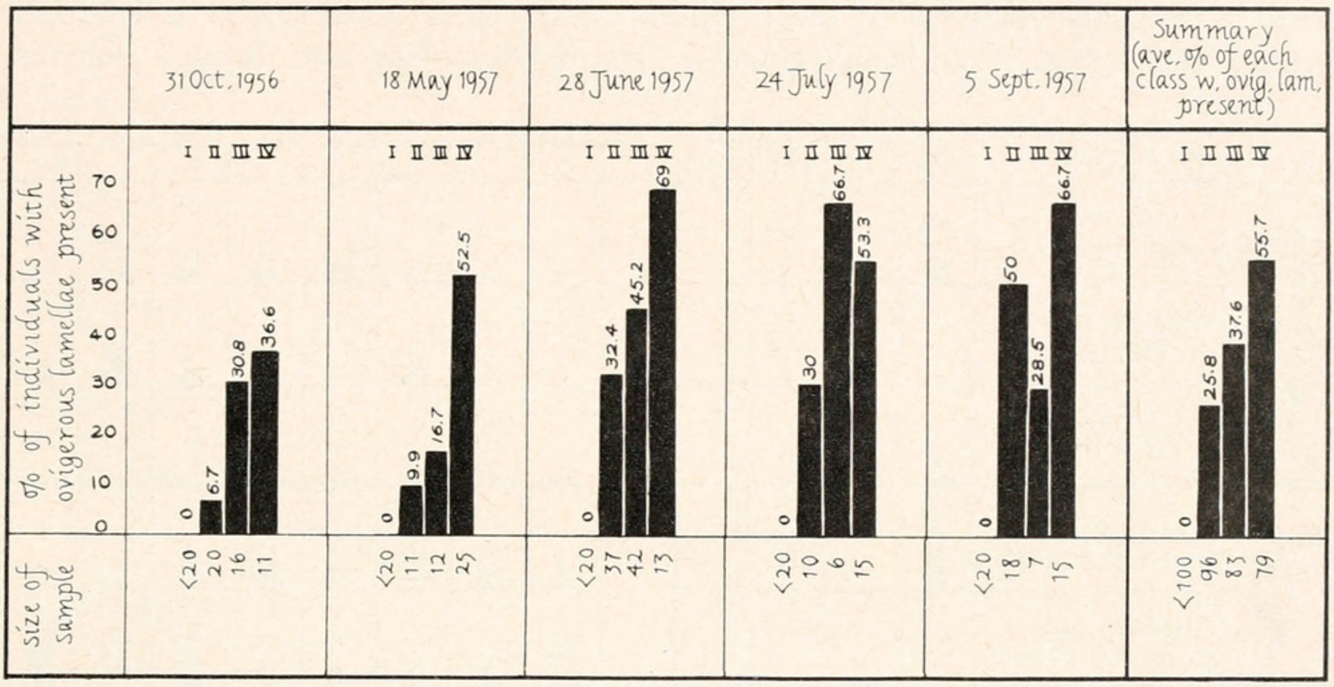

FIGURE 2. Variation in reproductive activity with size of animals. Vertical bars indicate percentage of individuals of each size class which contained ovigerous lamellae. Size classes are designated as follows: I, breadth less than 17.2 mm.; II, breadth 17.2 to 22.5 mm.; III, breadth 22.6 to 27.5 mm.; and IV, breadth 27.5 to 32.5 mm.

THE REPRODUCTIVE CYCLE IN THE MITELLA POLYMERUS POPULATION

Each month, 10 to 25 large animals were collected, measured, and examined for reproductive condition. The number of individuals carrying ovigerous lamellae was noted; conditions of the female and male gonads, and stage of development of the embryos (where present) were studied.

The distribution of egg sizes and larval stages is shown in Table I and Figure 3. Table I shows for each sample the number and per cent of *individuals* which contained given egg size classes and which brooded particular embryonic stages. However, in Figure 3, each separate mass of eggs or embryos is treated as a separate unit. For example, in cases where a given individual contained simultaneously small eggs, large eggs, and late embryos, these appear in Figure 3 in three different horizons in the same date column.

It is apparent in Figure 3 that between November, 1956, and January, 1957, breeding waned and was discontinued until the spring of 1957. Then, during March, eggs began to enlarge, but did not exceed the upper limits of the class of small eggs. By April, the first medium and large ovarian eggs were present, and the first fertilized egg mass appeared. Egg production continued through the year to January, 1958, when the number of ovigerous lamellae present in the population dropped significantly. The season of reproductive activity for the population covers three-quarters of the year, and it is of interest that all stages of egg and embryo development were found throughout the season. On the average, during the height of the breeding season, between 50% and 60% of the population of large animals are carrying ovigerous lamellae at any one time.

Data for the male gonads are plotted in Table I and in Figure 4. Sperm is present in at least some members of the population throughout the year. While there is seasonal variation in the condition of the male gonads, this is less well defined than is that of the female gonads. In the fall of 1956 and through January,

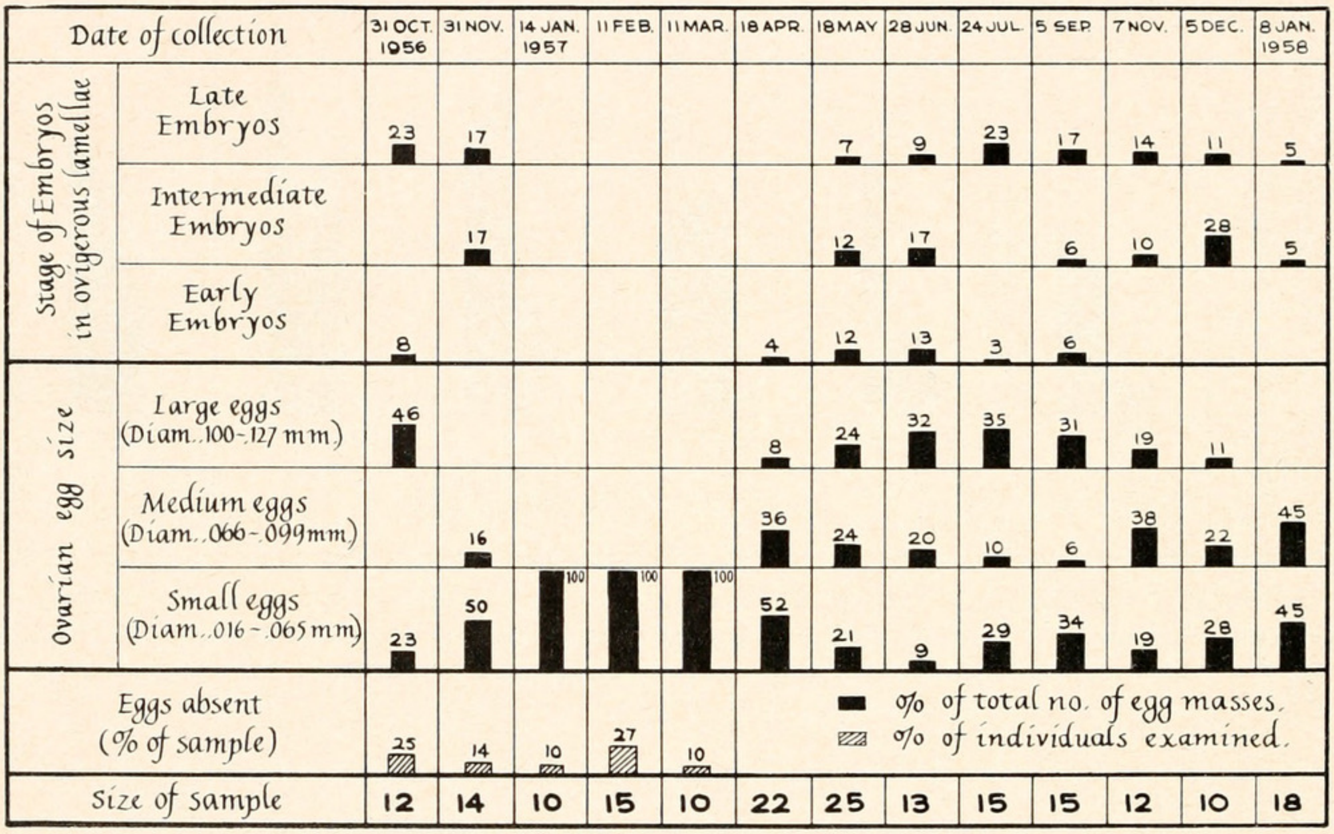

FIGURE 3. Distribution of egg and embryo masses of various stages of development in large *M. polymerus*.

1957, some seminal vesicles were very thick with sperm, while others appeared spent. In February and March, 1957, most of the vesicles had a meager amount of sperm, presumably building up, and some were empty. In April, most vesicles were quite full with sperm, others had a meager amount, and none were empty, and from this point on through the summer and fall of 1957, the mean vesicle width was high (well over two mm.). Empty vesicles were not observed again until January, 1958. Thus during the winter months, the quantity of sperm present in the seminal vesicles of most of the population was considerably less than the amount present during the rest of the year.

There is a suggestion of waves of reproductive activity during the breeding season, in the data presented in Figure 3 and Table I. This appears most clearly in Table I in the column showing the percentage of animals which are carrying

TABLE I

Seasonal variation in egg size, seminal vesicle width, and stage of development of brooded embryos in M. polymerus

Dates	1956 Oct. 31	Nov. 30	1957 Jan. 19	Feb. 11	Mar. 11	Apr. 18	May 18	Jun. 28	Jul. 24	Sept. 5	Nov. 7	Dec. 5	1958 Jan. 8
No. of animals examined:	12	14	10	15	10	22	25	13	15	15	12	10	18
No. and per cent of animals with:													
Eggs absent													
No.	3	2	1	4	1	—	—	—	—	—	—	—	—
%	25	14	10	27	10	—	—	—	—	—	—	—	—
Small eggs													
No.	3	9	9	11	9	11	5	1	1	2	—	4	9
%	25	65	90	73	90	50	20	8	7	13	—	40	50
Medium eggs													
No.	—	3	—	—	—	9	10	6	3	2	8	4	9
%	—	21	—	—	—	41	40	46	20	13	67	40	50
Large eggs													
No.	6	—	—	—	—	—	6	5	3	1	—	1	—
%	50	—	—	—	—	—	24	38	20	7	—	10	—
Both small and Large eggs													
No.	—	—	—	—	—	2	4	1	8	10	4	1	—
%	—	—	—	—	—	9	16	8	53	67	33	10	—
No. and per cent of animals with ovigerous lamellae:													
No.	4	6	—	—	—	1	13	9	8	10	5	7	2
%	33	43	—	—	—	5	52	69	53	67	42	70	11
No. and per cent of animals with ovigerous lamellae bearing:													
Early embryos													
No.	1	—	—	—	—	1	5	3	1	2	—	—	—
%	8	—	—	—	—	5	20	23	7	13.5	—	—	—
Middle embryos													
No.	—	3	—	—	—	—	5	4	—	2	2	5	1
%	—	21.5	—	—	—	—	20	31	—	13.5	17	50	5.5
Late embryos													
No.	3	3	—	—	—	—	3	2	7	6	3	2	1
%	25	21.5	—	—	—	—	12	15	46	40	25	20	5.5
Conditions of seminal vesicles present in the population													
No. of animals examined:	12	13	18	15	10	21	23	13	14	14	12	10	18
No. of animals with seminal vesicles in following conditions:													
Apparently empty	1	1	1	4	3	—	—	—	—	—	—	—	2
Width 1–2 mm.	5	6	5	9	7	4	1	2	2	5	4	9	7
Width 2–3.8 mm.	6	6	12	2	—	17	22	11	12	9	8	1	9

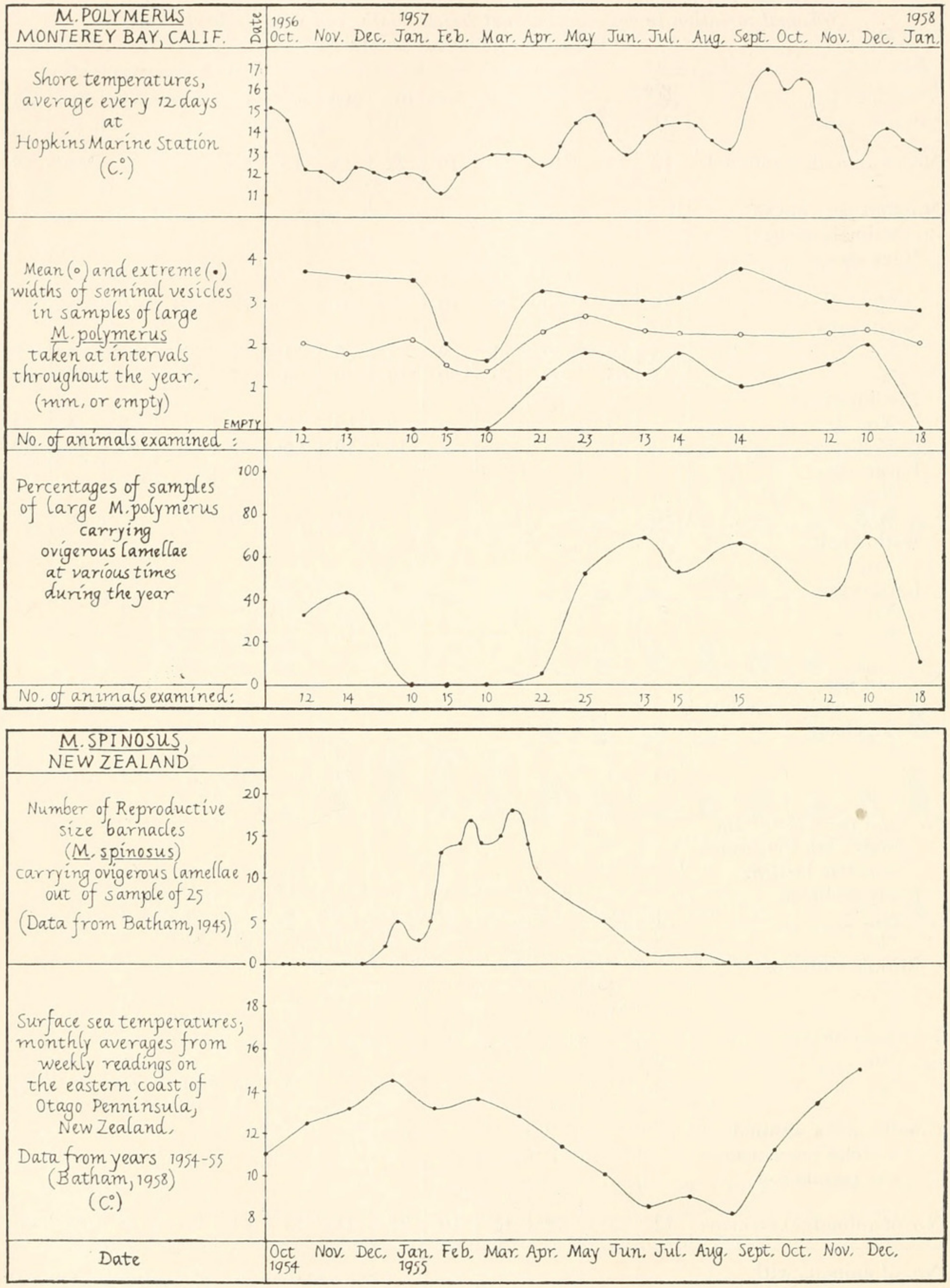

FIGURE 4. Temperature and reproductive activity in *M. polymerus* in Monterey Bay and in *M. spinosus* in New Zealand.

ovigerous lamellae at different times during the year. The highest values, *i.e.*, those for June, September, and December (69%, 67%, and 70%, respectively), alternate with the lower values shown for May, July, and November (53%, 53%, and 42%, respectively), and therefore suggest that the population as a whole may be reproducing in waves, more or less simultaneously. As will be indicated later, this phenomenon is very likely related to the successive waves of reproductive activity shown by *individual* barnacles during the breeding season. However, not all individuals start to reproduce at precisely the same time, and waves of activity do not proceed in all individuals at exactly the same rate. The differences between alternate highs and lows and their deviations from the mean for the population are not statistically significant; and a larger sample might be expected to yield a smoother plateau for reproductive activity of the population.

The Reproductive Cycle in Individuals

The reproductive cycle in the population of *M. polymerus* represents a summation of the reproductive processes in individual barnacles. The animals are hermaphroditic and ovoviviparous, brooding their young for a period of time in the mantle cavity. It is accordingly of interest to examine the relative degrees of development of the two gonads in individual animals, and to relate the ovarian egg size with the stage of development of brooded embryos present simultaneously in individual barnacles.

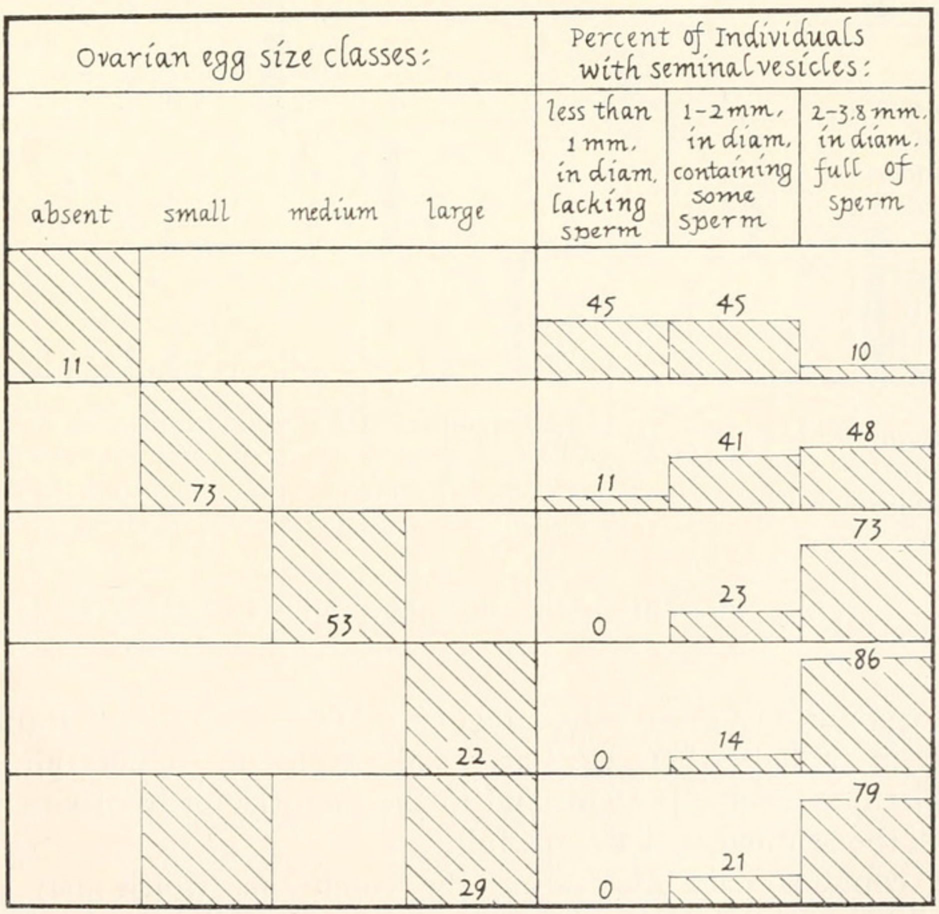

Figure 5. Egg sizes and seminal vesicle widths found simultaneously in large individuals. Left-hand side: numbers within the blocks represent numbers of individuals examined; right-hand side: numbers above blocks represent percentages of individuals with seminal vesicles of given sizes.

For studies of the two gonads, the large animals taken at intervals throughout the study period were examined. Animals were grouped into four categories according to egg size: animals with small eggs, those with medium eggs, those with large eggs, and those with both small and large eggs. The seminal vesicle width of each animal of a given egg category was noted. The data for all animals examined are shown in Figure 5.

In Figure 5, the bars on the right hand side represent the frequency of occurrence of the various conditions of the seminal vesicle which may be found in individuals with eggs of any given size category. We see from the graph that sperm is absent only in animals where eggs are small or absent. Under all other ovarian conditions, sperm is always present, and in the majority of barnacles with medium or large eggs, the seminal vesicles are full.

TABLE II

Correlation of size of ovarian egg with developmental stage of brooded embryos present in the same individual. Numbers of individuals showing each combination of conditions are indicated by numbers within the squares. Heavy vertical lines enclose the "peak" months of the breeding season.

Egg size	Embryos	31 Oct.	30 Nov.	14 Jan.	11 Feb.	11 Mar.	18 Apr.	18 May	28 June	24 July	5 Sept.	7 Nov.	5 Dec.	8 Jan.
Small & Large	Absent						2	4	1	4	6	3	1	
	Early													
	Middle													
	Late									5	4	1		
Small	Absent	2	5	9	11	9	11			1				8
	Early							4	1		1			
	Middle		2					1			1		3	
	Late	1	2										1	1
Medium	Absent		1				9	3				4	1	8
	Early							1	1	1				
	Middle		1					4	4		1	2	2	1
	Late		1					2	1	1	1	2	1	
Large	Absent	4						5	4	2			1	
	Early													
	Middle													
	Late	2						1	1	1	1			
Eggs Absent	Absent	3	2	1	3	1								
	Early													
	Middle													
	Late													
		12	14	10	14	10	22	25	13	15	15	12	10	18

Table II contains data illustrating the conditions which may occur simultaneously in the ovary and in the brooded ovigerous lamellae, in the same individual at different times of the year. From these data, the information on large animals collected during the breeding season (October to November, 1956; and May, 1957 to January, 1958) and containing both ovarian eggs and ovigerous lamellae, has been selected and is shown graphically in Figure 6.

It is evident from Figure 6 that there is a general relationship between ovarian egg size and brooded embryo stage in the same individual, particularly during the height of the breeding season. In general, where eggs are small in the ovaries, embryos are in an early stage of development in the mantle cavity; where eggs are medium-sized in the ovaries, embryos are in the middle stage of development in the mantle cavity; and where eggs in the ovaries are large (nearly ready to be extruded), embryos in the mantle cavity are advanced (nearly ready to be liberated). The parallel diagonal lines drawn through successive peak conditions show clearly the synchronized pattern of development of ovaries and embryos during the peak months of the reproductive season. Certainly such a condition, in which eggs and embryos show more or less parallel development, appears to

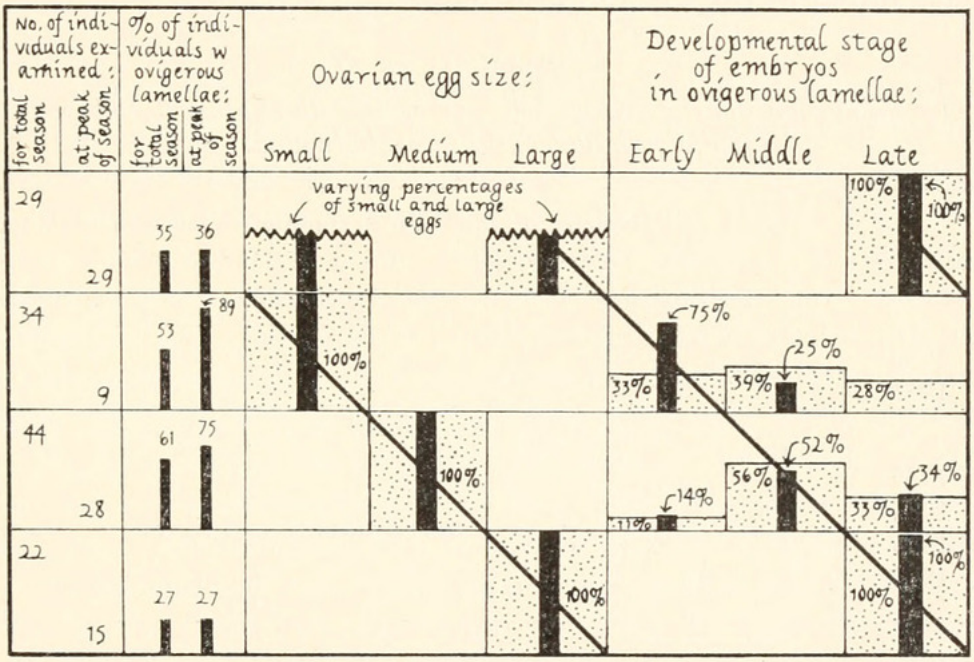

FIGURE 6. Developmental stages, within individuals, of ovarian eggs and brooded embryos, where both are present simultaneously. Numbers pointing to black bars show percentages of given stages present during the "peak" months of the reproductive season; numbers within the larger bars show percentages of given stages present during the total reproductive season.

represent the most efficient timing pattern for an ovoviviparous organism which produces successive broods of young in a single breeding season. However, it is clear from the graph and the table that precise coordination between rates of egg enlargement and larval development does not exist for all animals examined during the reproductive season. For example, some animals with small eggs in the ovaries were found brooding middle or late stage embryos. In such cases, it appears that the interval between successive broods of larvae is greater than in cases where eggs and embryos develop in phase, and that ovarian egg development is retarded with respect to embryonic development. Table II shows clearly that these animals with relatively retarded ovarian development tend to be localized in time, predominating during the later months and not the peak months of the reproductive season. Thus, the five cases in which late embryos accompany small ovarian eggs occurred in October and November of 1956, December of 1957, and January of 1958, suggesting that at this time development of the ovaries had slowed down or stopped for the season, and that the final batch of nauplii would soon have been shed. Also, the combination of small eggs with middle embryonic stages occurred, with one exception, either at the beginning (May, 1957) or near the end (November, 1956 and December, 1957) of the breeding season. Animals with medium eggs and *no* ovigerous lamellae occurred in April and May, 1957 (when they were presumably giving rise to their first batch of eggs of the season) and in November, 1956 and 1957 (when they may represent individuals in which the ovarian growth has slowed down toward the close of the season). In contrast, a few animals contained medium-sized eggs along with early embryos in the ovigerous lamellae, suggesting a relative acceleration of egg development. However, in all three cases, the eggs measured fell close to the lower size limits of the medium egg size class, and the apparent acceleration is exaggerated by the positioning of size-class limits in the grouping of data.

Table III

Apparent conditions of ovaries (ovarian eggs) with relation to embryos (found simultaneously in an individual)

Dates	Number of times ovaries appeared in following conditions:		
	Accelerated	Synchronized	Retarded
31 Oct. 1956	—	2	1
30 Nov. 1956	—	1	5
14 Jan. 1957	no reproductive activity		
11 Feb. 1957			
11 Mar. 1957			
18 Apr. 1957			
18 May 1957	1	9	3
28 Jun. 1957	1	6	1
24 Jul. 1957	1	6	1
5 Sept. 1957	—	7	2
7 Nov. 1957	—	3	2
5 Dec. 1957	—	2	5
8 Jan. 1958	—	1	1

Table III summarizes the condition of the ovaries with relation to embryonic stages. We see that during the early and middle months of the reproductive season, most of the animals examined appeared to show synchrony in rates of ovarian and embryonic development. Toward the end of the reproductive season, a relative lag in ovarian egg development became noticeable. By combining data for the months of May through September (the main reproductive period) and for the period of October through December (the end of the breeding season), we can show that the prevalence of animals with synchronized ovaries during the former months, and of animals with retarded ovaries during the latter months, is indeed statistically significant.

While animals with apparently well synchronized brood development are probably producing batches of eggs at a relatively efficient rate, there is evidence that even in such animals some time does elapse between broods. We might expect, in a perfectly synchronized animal, that immediately following the liberation of a batch of larvae, a second batch of eggs would be extruded, and that such an animal would be carrying ovigerous lamellae virtually all of the time. However, at no time during the reproductive period were all reproductively mature members of the population carrying ovigerous lamellae; even during the height of the breeding season, only 50% to 60% of the population were brooding embryos. The occurrence of a very few slow developers during this time could hardly account for such a discrepancy; and it must be assumed that even during the height of reproduction, some time does elapse between the hatching of a batch of larvae and the extrusion of the next batch of eggs from the same individual.

Number of Broods and Larvae Liberated by Mitella polymerus, and its Relative Fecundity

No direct observations are available on the number of broods produced by an adult barnacle per year. However, by combining all the lines of evidence at hand, we can arrive at a hypothetical figure for the number of batches of larvae liberated by a large individual during one reproductive season. Three lines of evidence are considered here: the duration of the reproductive season, the rate of development of embryos in the ovigerous lamellae, and the developmental stages of eggs and embryos present at any one time in an individual during the reproductive season.

We have already seen that the reproductive period for most barnacles extends through about eight months or about 240 days (Fig. 3). The approximate duration of the lamellar brood period was determined in the laboratory. Several batches of larvae were raised *in vitro* at 13° C., starting with what appeared to be the two-cell stage, and continuing to hatching. Periodic examination of these ovigerous lamellae showed that embryos remain in the "early" stage for the first nine days, are in the "middle" stage from the tenth through the fifteenth day, and remain in the "late" stage from the sixteenth day to hatching, which occurs on the twenty-ninth to the thirty-first day. Development from fertilization to hatching of the nauplius thus averages about thirty days. This rate is substantiated for field conditions in Figure 3, where we find the first large eggs being extruded in April and the first late embryo just one month later. It is interesting to note that the brood period for "normal" *M. spinosus,* raised under conditions where temperature was not controlled but averaged 14° C. to 15° C., was thirty to thirty-two days (Batham, 1946).

The developmental stages present simultaneously in individuals have been shown in Figure 6 and Table II. Here, the simultaneous presence in single animals of three stages (small eggs, large eggs, and late embryos, especially in animals taken in July and September) strongly suggests that an animal may give rise to *at least* three batches of embryos during one reproductive season. Tables II and III show that during the first six months of the reproductive season, egg and embryo stages tended to be more or less synchronized in individuals; and during the last two months of the breeding season, the majority of animals carried broods which tended to be out of phase by two stages. In a perfectly synchronized animal, in which ovarian eggs and lamellar embryos develop in phase, we might assume that the interval between the average small egg and the average early embryo is equal to the lamellar brood period, or thirty days. In animals one step out of phase, that is, in animals in which *medium* embryos accompany *small* eggs, or *late* embryos accompany *medium* eggs, we might assume that the interval between broods is thirty-eight days (or thirty days plus the difference between the average age of an early embryo and the average age of a middle stage embryo—eight days). In animals two steps out of phase, in which *late* embryos accompany *small* eggs, or (specifically in November, 1957) *no* embryos accompany *medium* eggs, we might assume that the interval between successive broods is at least 48.5 days (or thirty days plus the difference between the average age of an early embryo and the average age of a late embryo—18.5 days).

Through the breeding season (eight months), these various intervals were found to be more or less localized in time. That is, for approximately the first six months, most of the population seemed to be producing broods either in phase or one stage out of phase. Hence, at the assumed rate of one brood for every thirty to thirty-eight days, a minimum of 4.7 broods and a maximum of six broods could have been extruded by an individual during this time. For the last two reproductive months, a majority of the population carried broods two steps out of phase. At the assumed rate, then, of one brood for every 48.5 days, one to 1.2 broods may have been extruded by an individual during these two months. During the TOTAL reproductive period, then, it appears that a single large barnacle *could* have liberated from five to 7.2 broods of larvae.

If an average animal gives rise to six broods in a season, each brood developing in the mantle cavity for thirty days, we would expect such an animal to be carrying embryos for approximately 180 days out of the total 240 days, or approximately 75% of the total time. Since no single individual was followed during the breeding season, no data are available to provide a direct means of checking this figure. However, 59% of the large individuals collected during the eight-month reproductive season bore ovigerous lamellae. This suggests that perhaps large individuals contain ovigerous lamellae for only about 59% of the total reproductive period, or 142 out of 240 days. On this basis, then, the average animal probably produced only four or five broods during the season.

There seem to be two good reasons for the discrepancy between the two estimates for average number of broods per season. The first, already mentioned, is that even in well-synchronized animals, some time did elapse between successive lamellar broods. There was no direct measurement of the duration of this minimum interval, and thirty days was used as the minimum time for the enlargement and extrusion of eggs; but actually, the period during which eggs remain in the ovary is probably a few days longer. A suggestion providing some independent support for this is seen in the data in Figure 3, where on March 11, 1957, all ovarian eggs were in the small size class (though some approached the upper limits of this class), and on April 18, 1957, 36 days later, one individual was just extruding eggs from the oviduct. The other possible reason for the discrepancy between the two estimates of number of broods produced per year is shown by the fact that many stages of development were found in the population at any one time, and that the whole population did not produce broods synchronously. It is probable that the average animal did not reproduce at the given rates for a total of 240 days; that animals may have either started reproducing later than the earliest assumed limit or stopped reproducing earlier than the latest assumed limit. Such animals may have produced only five or four, or possibly only three broods during a season. In contrast, the results of Batham (1944–45) show that *M. spinosus* in New Zealand probably liberates only two per season.

Counts have been made of the number of larvae contained in the ovigerous lamellae of both species of *Mitella*. It was found that a large *M. polymerus* may liberate from 104,000 to 240,000 larvae from a single brood contained in one pair of ovigerous lamellae; the slightly smaller *M. spinosus* liberates approximately 3000 larvae per brood (Batham, 1944–45). It appears that a single adult *M. polymerus* may produce roughly 52 to 280 times as many larvae per year as a single adult *M. spinosus*.

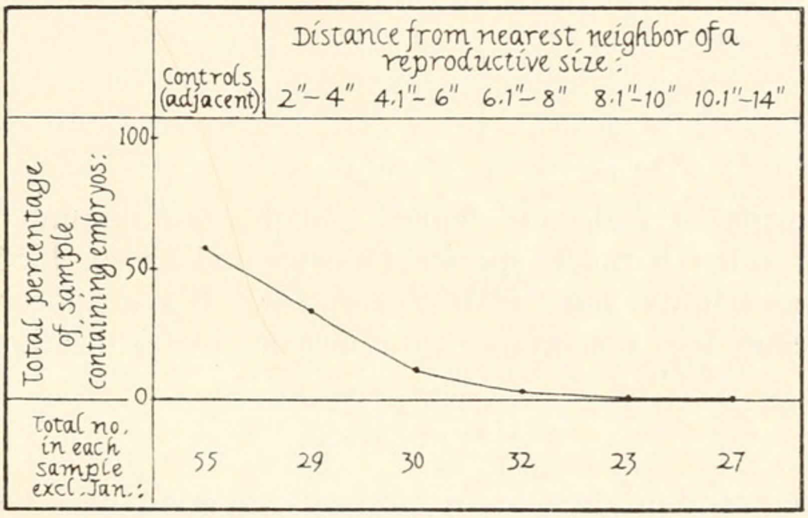

FIGURE 7. Summary graph showing percentages of large *M. polymerus* containing ovigerous lamellae when separated from other large specimens by given distances.

SELF-FERTILIZATION

For a study of self-fertilization, a series of large and relatively isolated barnacles, situated at various measured distances from their nearest neighbors of reproductive size, were collected and inspected for the presence of ovigerous lamellae. These samples were collected at different times throughout the reproductive season. The data, grouped for the months of collection, and for the distances by which the sexually mature barnacles were separated, are presented in Table IV and Figure 7. It can be seen that over 50% of the control animals for each month (except January, 1960) carried ovigerous lamellae; animals separated by two to four inches appeared to carry ovigerous lamellae less frequently, but the differences are not statistically significant. There is a statistically significant drop in the presence of embryos in animals separated from their nearest neighbor of a reproductive size by over four inches, and an absence of embryos in animals separated by more than eight inches. Thus it appears that eight inches was the maximum distance over which copulation of large animals collected could take place, and that animals separated by greater distances failed to receive sperm. Despite the

Table IV

Numbers of barnacles of reproductive size isolated from other such barnacles by given distances, showing presence (+) and absence (−) of ovigerous lamellae

Date	Distance from nearest neighbor of reproductive size:											
	Controls (adjacent)		2″–4″		4″–6″		6″–8″		8″–10″		10″–14″	
	+	−	+	−	+	−	+	−	+	−	+	−
1958	12	8	3	2	1	3	0	5	0	2	0	8
1959												
20 July	10	5	3	3	1	6	1	6	0	8	0	3
7 Aug.	6	4	2	8	0	10	0	12	0	7	0	6
2 Nov.	4	6	2	6	1	8	0	8	0	6	0	10
1960												
2 Jan.	0	30	0	8	0	6	0	5	0	8	—	—
Summary, excluding Jan. 1960	32	23	10	19	3	27	1	31	0	23	0	27

simultaneous maturity of male and female gonads, and despite the evidence for self-fertilization in other barnacle species (Barnes and Crisp, 1956), self-fertilization apparently does not take place in *M. polymerus*. It also appears that formation of ovigerous lamellae does not occur in the absence of fertilization.

Discussion

On the subject of reproduction in cirripeds, a good deal has been written. Some accounts, such as those of Darwin (1851), Broch (1922), and others are concerned with the questions of hermaphroditism, the existence of complemental males, etc., rather than reproductive anatomy, reproductive cycles, and fecundity.

Comparison of the seasonal reproductive cycle of *M. polymerus* in Monterey Bay with that of *M. spinosus* on the New Zealand coast (Batham, 1944–45) shows some interesting features. Figure 4 summarizes reproductive and temperature data for the two species. In Monterey Bay, from November, 1956, through the greater part of April, 1957, shore temperatures remained between 11.2° C. and 12.4° C., but showed a definite rise toward 13° C. during February and March. From May, 1957, through about one-half of November, shore temperatures remained above 13° C., rising to over 14° C. in May, July, and August, and to above 16° C. during September and October. Thus, the year may be roughly divided into the colder winter months and the warmer spring, summer, and fall months.

In *M. polymerus,* the increase in mean seminal vesicle width during the early spring roughly parallels the rise in shore temperature; the decrease in mean seminal vesicle width in the winter follows roughly the winter decrease in temperature. Likewise, the earliest occurrence of embryos follows closely behind the seasonal rise in temperature, and embryos continue to be present in the population until just after temperatures begin to drop during the winter. There is thus a clear correspondence between reproduction and shore temperature. The three peaks seen in the occurrence of embryos in the latter half of 1957, while not statistically significant, show a relationship with the temperature peaks of May, July, and September which is perhaps suggestive.

The data given by Batham (1944–45) for *M. spinosus,* from the open coast of Otago Peninsula, New Zealand, and the corresponding temperature curve (from two later years and a neighboring vicinity; Batham, 1958) show a similar relationship between temperature and reproductive activity. As might be expected for a related species occurring at a slightly higher latitude but in the southern hemisphere, the reproductive cycle is a rough mirror image of that of *M. polymerus* in Monterey Bay, California, though the breeding season is somewhat shorter.

Other barnacle species have been observed to be reproductively active primarily during the months of warmer water temperatures: *Balanus crenatus,* studied on the Atlantic coast of Canada (Bousfield, 1952–53) and in San Francisco Bay, California (Herz, 1933); *Balanus improvisus,* studied on the Atlantic coast of Canada (Bousfield, 1952–53); and *Chthamalus stellatus,* studied in Great Britain (Crisp, 1950).

While such a correlation between temperature and reproductive activity might seem obvious and only reasonable, a number of barnacles reproduce primarily when water temperatures are low or at a minimum. This group includes the following: *Balanus balanoides,* studied on the Atlantic coast of Canada (Bousfield, 1952–53), at Woods Hole, Massachusetts (Fish, 1925), and in Great Britain (Moore, 1935; Crisp and Patel, 1960); *Balanus hameri,* studied on the Atlantic coast of Canada (Bousfield, 1952–53), and in Great Britain (Crisp, 1954); *Balanus porcatus,* studied in Great Britain (Crisp, 1954); and *Balanus glandula,* studied at Vancouver, B. C., and La Jolla, California (Barnes and Barnes, 1956) and in San Francisco Bay, California (Herz, 1933).

There is evidence that still another group of barnacles reproduce (perhaps with some variation in rate) throughout the entire year. These include *Elminius modestus,* studied in Great Britain (Crisp and Davies, 1955), *Verruca stroemia,* studied in Great Britain (Pyefinch, 1948), and *Balanus tintinabulum,* observed at La Jolla, California (Coe, 1932).

Bousfield (1952–53) has studied the distribution and spawning seasons of the barnacles of the Atlantic coast of Canada, and reviewed the evidence supporting temperature as a principal factor governing reproduction in cirripeds. He clearly indicated that there is variability in reproductive period within a given species at different latitudes within its geographic range, and showed that reproductive activity tended to occur at times when temperatures were similar, regardless of latitude.

The relationship of temperature and food supply to rate of reproduction has been studied by Crisp and Davies (1955) in the barnacle *Elminius modestus.* By growing these barnacles on glass slides and observing development through the translucent bases, these workers were able to follow, *in vivo,* the development of both ovarian eggs and lamellar embryos. They found that (p. 379) "the time interval occupied by successive broods varies among individuals, and with the season of the year. Rate of development of embryos seems to be a function of temperature alone, but regeneration of the ovary depends on nutrition and food supply." Crisp (1959), working with *Balanus balanoides,* showed that the rates of development of the early embryonic stages (through the limb bud stage) are temperature-dependent up to 12°–14° C., but that the later stages vary little in rate of development between 3° C. and 12° C.

Further points of comparison may be brought out between *M. polymerus, M. spinosus,* and other barnacles. The present study indicated that sexual maturity is not necessarily a function of size of the animals alone. Results of studies on *M. spinosus* and *Elminius modestus* showed similarly that in populations of smaller barnacles, sexually mature individuals are found, but less frequently than in populations of larger barnacles.

Self-fertilization apparently does not occur in large isolated individuals of *M. polymerus,* and cross-fertilization appears to be the rule. Crisp (1954) and Crisp and Patel (1960) pointed out that cross-fertilization also appears obligatory in *B. crenatus, Elminius modestus,* and in *B. balanoides.* However, self-fertilization very probably can occur in at least three species of acorn barnacles. Barnes and Crisp (1956) experimentally isolated individuals of *Chthamalus stellatus, Verruca stroemia,* and *Balanus perforatus* and found that they frequently produced ovigerous lamellae. They also observed that fertilized eggs found in such isolated individuals are frequently less viable than cross-fertilized eggs. The genetic advantage of cross-fertilization is well known, and it appears that the *Mitella polymerus* population, composed usually of closely-packed individuals, is well adapted for cross-fertilization.

Summary

1. The gross structure of the reproductive system of *M. polymerus* is described and compared with that of the southern hemisphere species, *M. spinosus* (studied by Batham, 1944–45).

2. Size and reproductive activity in *M. polymerus* are related. All animals over 27.5 mm. in breadth (distance from rostrum to carina) are found to reproduce; smaller animals are found to contain developing embryos less frequently. No sexually mature animals less than 17.2 mm. in breadth were found.

3. A fifteen-month study of the reproductive cycle in the population is described. Reproductive activity is evident during the greater part of the year. For the year of 1957, developing embryos were present in the population for a period of eight months during which time the shore temperature ranged from 12.3° C. to 17° C. The reproductive season for the southern hemisphere species likewise occurs during the warmest months; thus the yearly cycle of *M. polymerus* shows a perhaps expected mirror image of the situation occurring in the southern hemisphere.

4. Within individuals, male and female gonads mature at approximately the same time during the year, and during the greater part of the year, an individual contains both developing eggs and seminal vesicles full of sperm.

5. Stages of development of ovarian eggs and brooded embryos found simultaneously in individuals are compared. During the early and middle months of the reproductive season, ovarian eggs and brooded embryos tend to be in similar stages of development (that is, small eggs are found with early stage embryos, large eggs with late stage embryos, etc.). During the later reproductive months, a relative lag in ovarian egg development is evident.

6. Embryos were raised *in vitro* under controlled temperatures. The embryos took an average time of thirty days for development from fertilized egg to free-swimming larva.

7. Estimates are given of the number of broods of young and the number of larvae liberated by a large individual during a year, and these are compared with the results of Batham (1944–45) for *M. spinosus*. Studies of the larval brood period, the stages of eggs and embryos found simultaneously in individuals, and the length of the reproductive season allow an hypothesis that five to seven broods may be liberated by a large individual during a year. Three to four broods appears more probable for an average large animal. A pair of ovigerous lamellae may contain from 104,000 to 240,000 larvae. Batham's data (1944–45) showed that probably two broods, each containing approximately 3000 larvae, are liberated by *M. spinosus* during a year. Thus *M. polymerus* may liberate from 52 to 280 times as many larvae per year as a single large *M. spinosus*.

8. The possibility of self-fertilization in *M. polymerus* is studied. Relatively isolated large animals are found to carry ovigerous lamellae less frequently than those adjacent to each other; and large animals isolated from each other by over eight inches were never found carrying embryos. From this evidence, it appears that self-fertilization does not occur in this species, and that cross-fertilization is necessary for the formation of ovigerous lamellae.

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