

OBSERVATIONS *IN VIVO* ON THE BREEDING OF *ELMINIUS MODESTUS* GROWN ON GLASS SLIDES

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(Text-figs. 1-7)

Periodic sampling and examination of the gonads or retained embryos from a given species may fail to give reliable information on the number and frequency of its broods. When several broods follow one another in rapid succession and are not synchronous, as occurs at the height of the breeding season in some animals, the gonads are to be found in all stages between the immature and spent condition. If embryos are retained they also occur in all stages of development. The relative time occupied by any one of these stages, expressed as a fraction of the time taken by a single brood, is equal to the proportion of the population which occurs in that stage of development. This can readily be determined by appropriate sampling.

On the contrary the actual time occupied by a single brood (namely the time between the liberation of one brood and the next) cannot be obtained from such records. Only when continuous observations can be made on the breeding of separate individuals is it possible to determine directly the duration of each brood. Where the proportion of breeding individuals in a population increases only slowly, but the brood cycles are short, this may indeed afford the only satisfactory method.

It is well known that the state of the female gonad in operculate barnacles can be determined visually if the individual is removed from its substratum and viewed from below. Moore (1935) illustrates the difference between unfertilized and fertilized individuals of *Balanus balanoides*. Individuals carrying on each side of the mantle cavity recently extruded egg masses have a characteristic appearance both in the Balanidae and in the Veruccidae. Moreover, so far as is known, oviposition in these groups occurs only immediately after copulation. Unfortunately removal from the substratum is normally necessary for the purpose of such an examination and this precludes further observations on that individual. If, however, the species has a trans-

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parent basis, and can be grown on a transparent substratum, visual observations may be continued indefinitely. Moreover, since during development the egg masses become increasingly pigmented the darkening of colour provides a useful guide to the rate of development.

Elminius modestus, having a transparent membranous basis and breeding most prolifically during the warmer months (Crisp & Chipperfield, 1948), was considered to be a very suitable organism for this work. Unlike *Chthamalus stellatus*, which also breeds prolifically over the greater part of the summer (Crisp, 1950), *Elminius* will settle readily on, and remain attached to, smooth glass surfaces.

METHODS OF INVESTIGATION

The observations described below were carried out in Brixham Harbour, South Devon, on individuals of *E. modestus* attached to glass plates of dimensions $4 \times 3 \times \frac{1}{4}$ in. Thinner glass plates improve visibility, but are liable to accidental damage during immersion in the sea. These plates fitted into bakelite frames, which were bolted to panel holders (Fig. 1*a*), enabling them to be suspended from a raft at a depth of 6 ft. below the surface of the sea. It was found advisable to use the inner wells of the raft (Fig. 1*b*) in order to reduce incident illumination, since strong light in clear water promoted dense algal growth, which smothers *Elminius*. As a further precaution against interference by other organisms, the barnacles were occasionally cleaned by gently scrubbing the surface of the plate with a nail-brush and washing with fresh water, to which *Elminius* is known to be extremely tolerant.

Although a small regular settlement of *E. modestus*, of the order of 0.01 per sq.cm during the season, is now (1952) maintained at Brixham, this was insufficient for our work. The initial settlements were therefore obtained by exposing plates in the River Crouch, Essex, where *Elminius* is exceedingly abundant, settlements of 50–100 spat per sq.cm having sometimes been obtained during only a week's exposure in June and July. The plates, each bearing an identification number, were taken to Brixham harbour (where all the observations were carried out), and immersed as soon as possible. When the spat had attained a diameter of 2–3 mm about fifty to sixty of the more healthy and larger individuals were selected, and the remainder prised off the panel, but no individual was allowed to be separated by a distance of more than 2 cm from its fellows. The panel was then photographed at intervals from the underside, the individuals in the photographs being numbered for reference.

When the spat reached maturity daily visits were paid to the raft whenever possible, in order to observe the condition of the female gonad of each individual. The newly fertilized egg masses can easily be picked out; by this means it became a simple matter to determine, to the nearest 1 or 2 days, the time at which eggs were extruded and hence when fertilization had occurred. Moreover, it is possible with practice to judge both the condition of the unfertilized

ovary, from its colour and texture, and the approximate state of development of embryos, from the degree of darkening of the egg masses. These are at first a pale cream, then ochre, fawn and finally brown. A table was compiled in which was entered against the date and number of each barnacle, either a letter indicating the colour of the fertilized egg mass (e.g. W white, C cream,

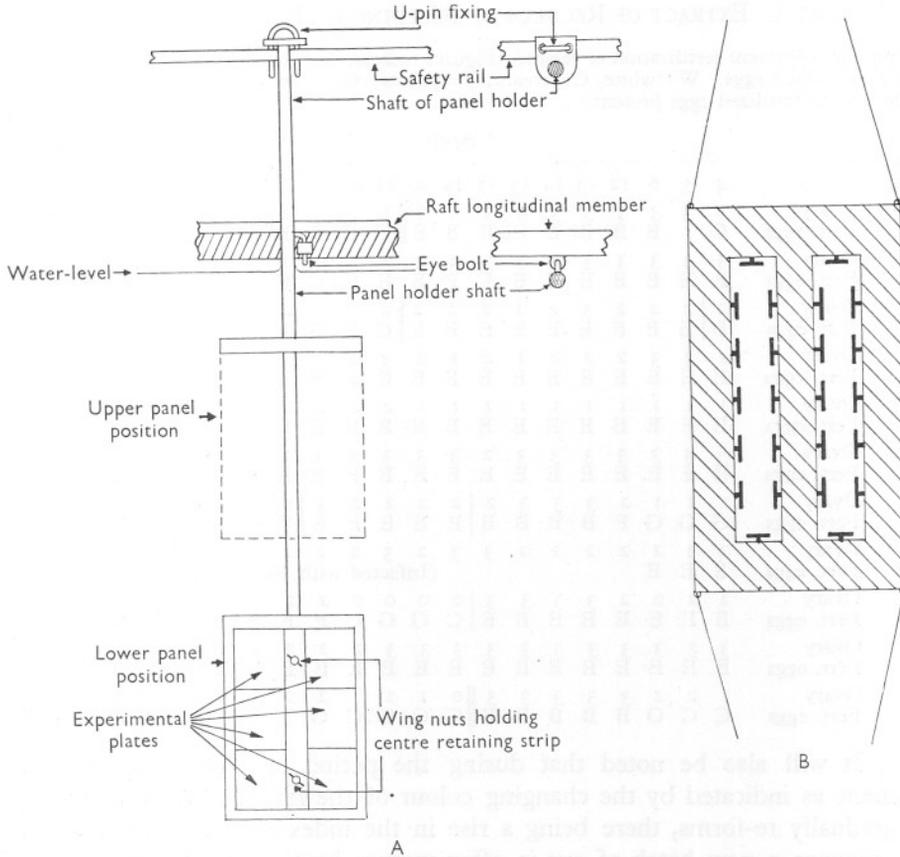


Fig. 1. Diagram of method of exposure of glass plates. A, Panel holder and fixing arrangements. B, Plan of raft showing wells for panel holders. The wells are darkened by wooden covers when the panel holders are in position.

G grey, O ochre, F fawn, B brown), or E (empty), when no egg masses were present. The state of the ovaries was recorded by numbering 0, 1, 2 or 3, according to whether the ovaries were not discernible or were of small, moderate, or large size. A typical extract of such records is shown in Table I.

In this table heavy lines are inserted to indicate either the liberation of nauplii or the fertilization and discharge of egg masses into the mantle cavity. It will be seen from the table that whenever a fertilization occurs, as shown by

new ovarian tissue next to the basis sometimes obscured the darker colour and characteristic shape of the egg masses. This difficulty occurred mainly towards the end of development when the reconstituted ovary made it increasingly difficult to decide with certainty whether release had occurred (e.g. individual 1, 5-15 April). Sometimes, too, release appeared to be partial, and some doubt was entertained as to the exact time. The most reliable method of observation late in development was found to be as follows: the plate was held against a strong light, when the dark kidney-shaped egg mass, if present, showed up against the translucent shell. Under these conditions the ovary, not being pigmented, only slightly masked the egg masses.

A second difficulty in assessing the state of the gonads arose from infections by *Hemioniscus balani*, a parasite which lives in the mantle space where the fertilized eggs are normally found. This parasite was rife in south Devon during the period of these experiments (Southward & Crisp, 1952, 1954). When an individual first became infected no obvious change could be observed, but such individuals, though retaining ovaries, became suspect as they ceased to be fertilized for an abnormally long period. Later the condition of the ovary gradually changed, the healthy yellow or cream lobular tissue becoming amorphous and milky in appearance. When microscopically examined it was found to lack developing eggs and to be composed of diffuse fatty tissue like that of the immature ovary. At this stage the parasite could often be seen through the thin sheet of ovarian tissue as a yellow or reddish brown mass, easily to be confused with developing egg masses, but distinguishable in the records because of the sterile history of the individual carrying it. These parasitized individuals were subjected to prolonged observation, but were excluded from analyses of breeding behaviour. On no occasion was an individual bearing an obvious parasite found to be fertile, yet in spite of the relatively large size of the parasite the host did not appear to suffer otherwise. Many individuals tolerated parasites for periods up to a year. In larger species, such as *Balanus balanoides*, *B. porcatus* and *B. hameri*, this parasite usually, but not invariably, produces similar castration of the female gonad.

DEFINITION OF TERMS

The reproductive cycle of a single brood occupies the time from one fertilization (oviposition) to the next; this will be called the brood period T . It may be subdivided into the fertilized period T_F which covers the time from oviposition to liberation of the nauplii, and the empty period T_E from liberation to the next fertilization and oviposition. There will also be a period of early embryonic development, recognizable from the colour change of the egg masses from cream to the brown and apparently fully developed state, which is normally shorter than the fertilized period (see p. 360); this will be called T_D .

During the breeding season there will be a certain fraction θ of the population bearing eggs, and a complementary fraction $(1 - \theta)$ empty.

On the assumption that individual values of T and T_F are narrowly dispersed about their means \bar{T} and \bar{T}_F ,

$$\theta \simeq \frac{\bar{T}_F}{\bar{T}}. \quad (1)$$

This relation holds approximately only when T and T_F do not vary widely about their means, since strictly θ is equal to the mean value of (T_F/T) taken over the population, i.e.

$$\theta = \frac{1}{n} \left[\left(\frac{T_F}{T} \right)_1 + \left(\frac{T_F}{T} \right)_2 + \dots + \left(\frac{T_F}{T} \right)_n \right]. \quad (1a)$$

The average frequency of fertilization is most conveniently defined as the proportion of individuals fertilized per day; this will be called ϕ_F . The corresponding quantity for the rate of liberation will be the proportion of individuals liberating nauplii per day, and will be termed ϕ_L . Clearly in the steady state with θ constant,

$$\phi_F = \phi_L.$$

If the brood periods are narrowly dispersed about a mean \bar{T} , then clearly

$$\phi \simeq \frac{1}{\bar{T}}. \quad (2)$$

If the variations in T are wide this relation does not hold very approximately since

$$\phi = \frac{1}{n} \left(\frac{1}{T_1} + \frac{1}{T_2} + \frac{1}{T_3} + \dots + \frac{1}{T_n} \right). \quad (2a)$$

Now since the average fertilization rate ϕ_F will be dependent on the proportion of individuals, $(1 - \theta)$, at that time in the unfertilized condition—since these alone are capable of being fertilized—it would be more natural to consider the fertilization rate only in relation to these individuals. Accordingly, we may introduce a fertilization constant, α , theoretically independent of θ , defined as

$$\alpha(1 - \theta) = \phi_F. \quad (3)$$

Similarly, a liberation constant, independent of θ , will be given by

$$\beta\theta = \phi_L. \quad (4)$$

Thus the constant α gives the absolute rate of fertilization were the population composed entirely of unfertilized individuals; the constant β gives the absolute liberation rate on the basis of fertilized individuals only. This overcomes the difficulty which would arise in comparing average rates taken over populations containing different proportions of fertilized individuals.

Since under actual conditions and with small samples θ is not constant, the variation of θ with time will be

$$\frac{d\theta}{dt} = \phi_F - \phi_L. \quad (5)$$

Hence
$$\frac{d\theta}{dt} = \alpha(I - \theta) - \beta\theta. \quad (6)$$

Combining equations (1), (2) and (4),

$$\beta \approx I/\bar{T}_F, \quad (7)$$

i.e. the liberation constant approximates to the reciprocal of the fertilized period.

Combining (1), (2) and (3),

$$\alpha \approx I/(\bar{T} - \bar{T}_F).$$

Hence clearly

$$\alpha \approx I/\bar{T}_E, \quad (8)$$

i.e. the fertilization constant approximates to the reciprocal of the empty period.

SEASONAL CHANGES IN REPRODUCTIVE ACTIVITY

Table I shows that the behaviour of individuals can differ very considerably; for example, during the period covered by the table, the specimen numbered 3 gave evidence of four broods and continued to breed throughout the following summer, while number 10 showed no breeding activity till considerably later in the season. It was therefore necessary to combine the results from many individuals; in this survey the number examined varied from about 170 at the outset to 50 at the end of the experiment. This was considered sufficient to give a reliable picture of the reproductive activity of the population as a whole. The number of individuals which underwent fertilization (or liberation) between successive observations was counted, and the results presented in the form of a histogram showing the percentage of the population fertilized (liberated) per day. The recorded heights of the histogram therefore give the sample values of the average fertilization (or liberation) rate ϕ_F (ϕ_L). The number of individuals bearing fertilized eggs was also counted and expressed as a fraction of the total population examined. The values of θ so obtained were also plotted against time (in days). The full results covering a period from August 1949 until May 1951 are shown in Fig. 2. The results were broken on 26 September 1949, when the original plates (A) were replaced by a new set (B) containing many immature individuals; this changeover is marked by a dotted line. The set of plates (B) were left out for the remainder

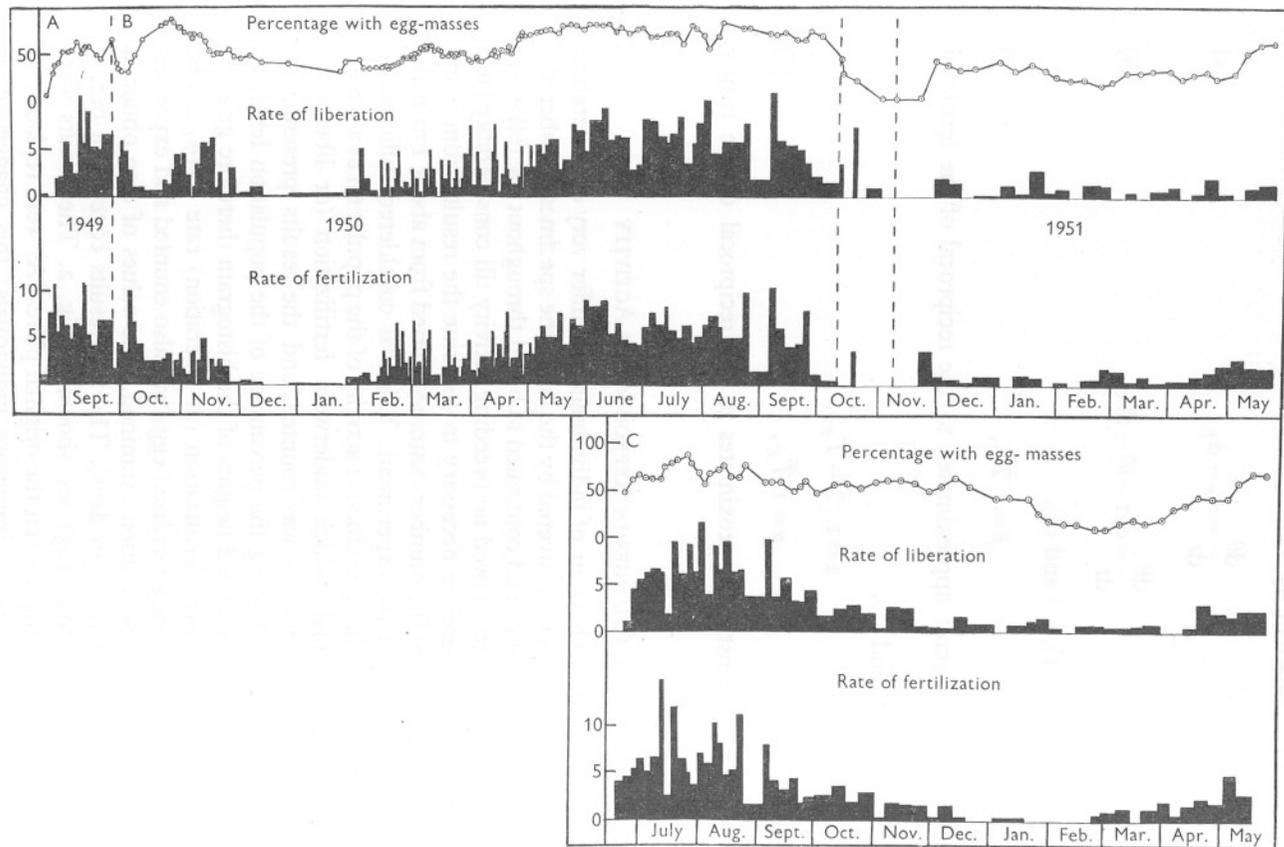


Fig. 2. Histograms showing frequency of liberation (upper) and fertilization (lower) for three series of barnacles, as percentage of population per day. Series A covers the period from August to September 1949, series B from September 1949 to May 1951, and series C, shown in the lower part of the figure, from June 1950 to May 1951. Series B were starved from October to November 1950, as shown by dotted lines. The points graphed above each pair of histograms give the percentage of individuals bearing fertilized egg masses.

of the experiment. In mid-June 1950 a further set of plates (C) were put out containing younger barnacles most of which had settled very late in the preceding year. These gave results closely similar to series B which had settled early in 1949, and are shown separately for comparison. The only noticeable difference between the B group and the younger C barnacles occurred in October and November 1950, when the B series were subjected to experimental treatment (see p. 372).

The seasonal character of the breeding activity is very clear from these results, both fertilization and liberation rates rising steadily to a maximum in mid-summer and falling to a minimum in mid-winter. In the equable climate of south-west England the breeding of continuously submerged specimens does not quite cease even in the coldest months, though it appears to do so in the normally more severe winters in south-east England. Furthermore, the rates of fertilization and of liberation (ϕ_F and ϕ_L) remain closely similar to each other at all times of year, hence apart from statistical fluctuations the proportion of individuals carrying egg masses (θ) varies only very slowly in accordance with equation (5) above.

The seasonal change in θ from a value of about 80% in summer to about 15–20% in winter is due to a slight excess in the number of fertilizations over the number of liberations throughout the spring and to the reverse occurring during autumn.

The initial changes that occur when a group of individuals come to maturity is shown best by series A which consisted of individuals of closely similar age. Maturity was reached at a remarkably early age. A few individuals were found to possess fertilized eggs within 8 weeks of settlement, just at the commencement of the records shown in Fig. 2. These individuals were about 4–6 mm. in diameter measured across the basis. From the middle until the end of August the fertilization rate was high, and since few egg masses were sufficiently far advanced in development, liberations were only occasional and θ consequently rose steeply, reaching 50% by 31 August. Thus half the population had become mature within 10 weeks of settling. Thereafter the remainder of the population matured, but since by now many egg masses had ripened the rate of liberation approached the rate of fertilization causing θ to increase more slowly. By mid-September θ was about 60–70%, a value close to that normal for fully mature populations at that season. An examination of the eggs showed that all stages of development were present and their distribution did not differ significantly from that of samples taken at random on the shore. Thus within 12 weeks of settlement this young population had not only become fully mature, but had reached a breeding equilibrium, or steady state, indistinguishable from that found in colonies of older individuals.

Fig. 3 gives the values of the fertilization constant α and the liberation constant β obtained by the application of equations (3) and (4) above and the

elimination thereby of θ , the fraction bearing fertilized eggs. The sea-water temperatures and the phases of the moon are shown in the same figure. The results of series A, B and C (omitting all records where the barnacles were subjected to experimental treatments) have been combined, in order to obtain the most representative figures covering the whole period of the experiment from August 1949 until May 1951.

The constant β is inversely related to the average length of time that each individual carries its eggs (\bar{T}_F , eqn. 7 above), which clearly cannot normally be less than the time of embryonic development T_D (p. 361). Hence, over an extended period, β cannot be greater than the rate of development of the egg masses, though it may well be less if fully developed embryos are retained beyond full term. If release occurs at the end of embryonic development and is not determined by external stimuli, the variation in \bar{T}_F and β broadly represents the temperature dependence of the physiological changes occurring during development.

The constant α depends inversely upon the average empty period \bar{T}_E (eqn. 8 above) and would become very large if \bar{T}_E were reduced to a very short interval of time. For this to occur fertilization must regularly take place just after release, and the ovaries must therefore have reached maturity during the fertilized period T_F . If some ovaries remain immature the empty period will be correspondingly increased and α reduced. Variations in α therefore indicate how far the re-development of the ovary keeps pace with embryonic development, high values implying that the ovaries are maturing very rapidly. This in turn indicates rapid assimilation, either through food being abundant, or because a high level of feeding activity results in much sea water being filtered.

The seasonal variation of β is seen from Fig. 3 to be clearly less than that of α , for whereas in winter β is sometimes greater than α it is always considerably less in summer, never exceeding 20 liberations per 100 individuals per day.

It follows therefore that the rate of development of embryos (nauplii), though considerably increased in summer, is not affected to the same degree as is the rate of maturation and re-fertilization of the eggs. In other words, during spring and summer the supply of food is sufficient to allow a steady output of broods of nauplii, the interval between broods being limited mainly by the time taken by the egg masses to develop. During late autumn and winter, on the other hand, the rate of assimilation appears to be the limiting factor, for α is so much reduced that in spite of the considerable retardation in embryonic development the greater part of the population remains in the empty condition. The ovaries tend to be small and poorly developed at this season, whereas the male organs, though somewhat reduced, are still present. Breeding therefore appears to be limited more by lack of food for the eggs than by any deficiency in the male organs.

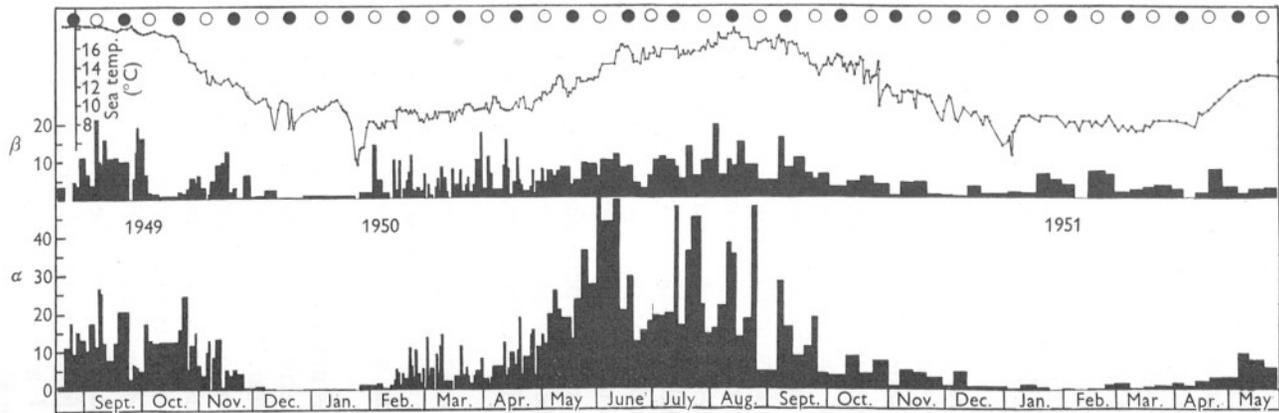


Fig. 3. Histograms showing the fluctuations in the values of the fertilization constant α and the liberation constant β over the period August 1949 to May 1951 for all series of barnacles. The sea temperatures are graphed above the histograms, and the times of maximum tides corresponding to full and new moons are shown.

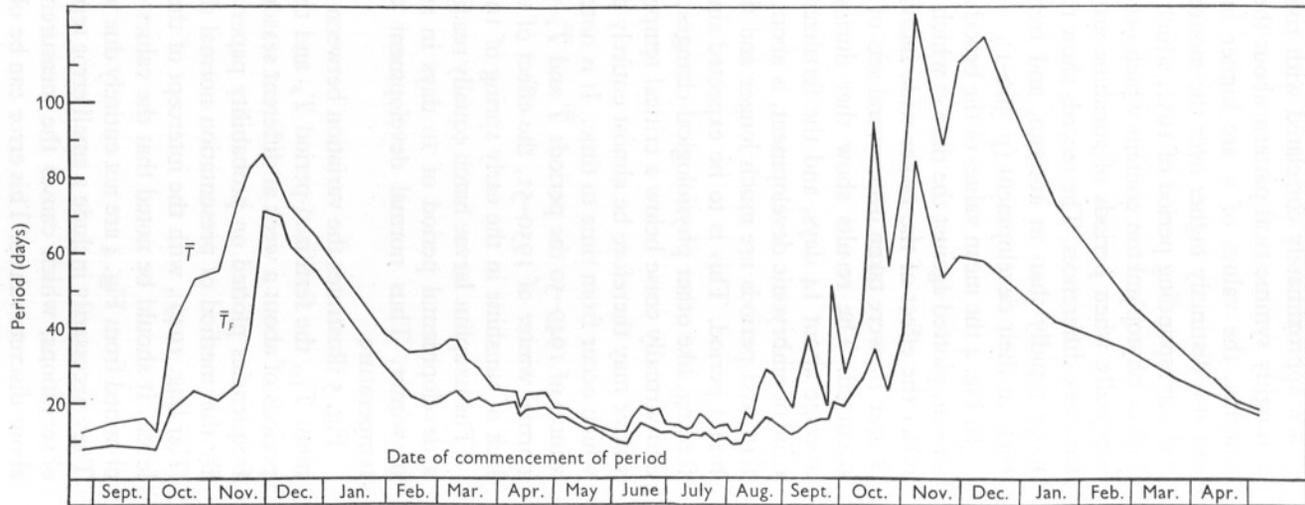


Fig. 4. Mean values of brood period \bar{T} (upper), and fertilized period \bar{T}_p (lower), from August 1949 to May 1951. The values of \bar{T} and \bar{T}_p are plotted against the date of commencement of the brood and fertilized periods respectively.

It will be noted from the histograms of α and β that whereas variations of β are approximately correlated with rise and fall in temperature, and form a roughly symmetrical pattern about the seasonal temperature maxima and minima, the values of α are higher in early than in late summer. They are also distinctly higher over the months of February–May in 1950 than in the corresponding period of 1951, which was relatively wet and sunless. The higher phytoplankton content which occurs during the first half of the year, especially when periods of sunshine are prolonged, is probably responsible for these differences. The records show that during spring the ovaries develop more rapidly than in autumn, and frequently mask the egg masses fairly early in their development (p. 360–1).

In Fig. 4 the mean values of the brood and fertilized periods (\bar{T} and \bar{T}_F) are shown, plotted against the time at which the period commenced. In order to offset the effect of the rather wide individual variations, the mean values of T and T_F were taken over several sets of observations when few fertilizations occurred. The results show that during summer the brood period is on average about 14 days, and the fertilized period, probably representing the time of embryonic development, is about 10 days. In winter both brood and fertilized periods are much longer and fluctuate considerably, especially the brood period. This is to be expected since the rates of development and of feeding, like other physiological changes, have a high temperature coefficient and virtually cease below a critical temperature level; these processes during winter may therefore be almost entirely dependent upon short warm periods which occur from time to time. It is noteworthy that in the particularly mild winter of 1949–50 the periods \bar{T} and \bar{T}_F were not so extended as in the more normal winter of 1950–51, the effect of which was further prolonged by the lack of sunshine in the early spring of 1951.

The nauplius larvae hatch equally readily from the egg masses at the end of a developmental period of 10 days in summer as after one of 60–80 days in winter. Thus normal development can tolerate a very wide range of temperature.

Fig. 5 illustrates the variation between individuals in the times of development T_D , the fertilized period T_F and the brood period T , taken over short periods of about a week at different seasons of the year. The data are given as frequencies plotted on probability paper against time on the horizontal axis. By this method of presentation normal distributions appear as straight lines (Harding, 1949), with the intercept of the mean value at the 50% frequency level. It should be noted that the values of standard deviation for T and T_F obtained from Fig. 4 are not entirely due to individual variations in behaviour. They necessarily include a small error term arising from the interval between observations, which causes the measurements of T and T_F to be grouped about discrete values. This error can be obtained by Sheppard's correction in particular cases, but except where the interval between observations was a long one it was small and generally insignificant.

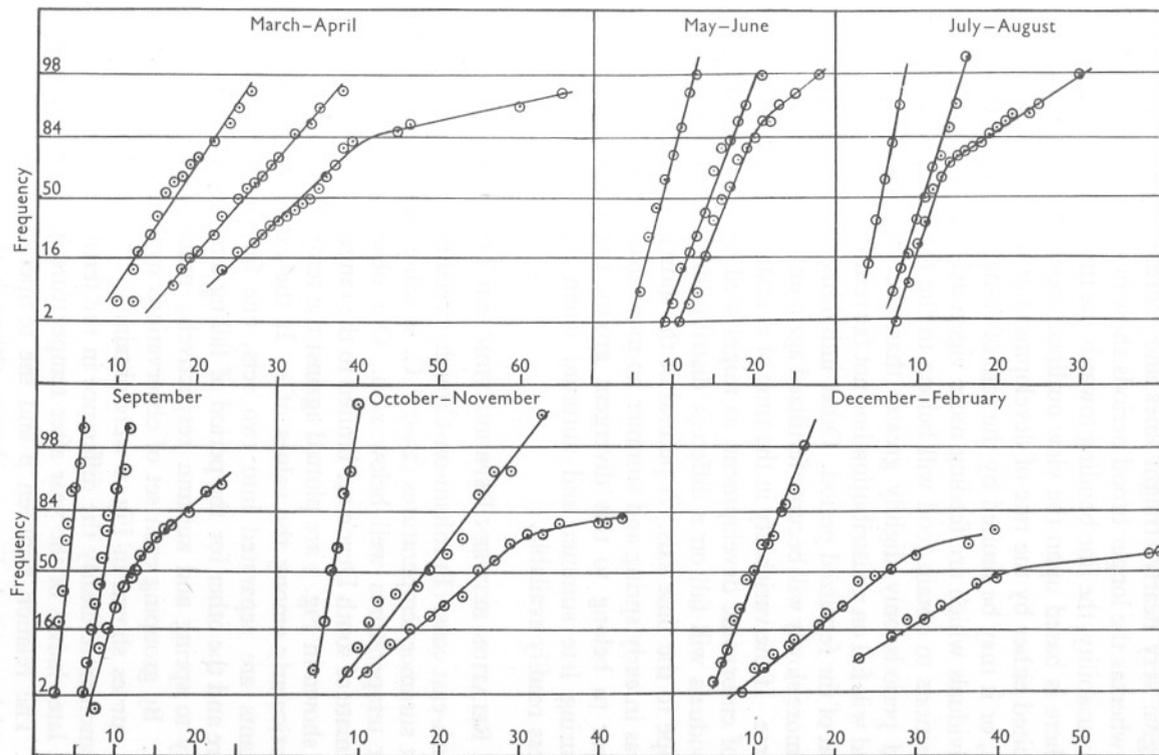


Fig. 5. Cumulative frequency curves for time of development T_D (left), the fertilized period T_p (centre) and the brood period T (right) at various seasons. The frequency axis is drawn on a probability scale, so that normal distributions are presented as straight lines. Horizontal axis gives the time in days.

The graphs reveal an interesting difference in distribution between T_D and T_F on the one hand and T , the brood period, on the other. The former distributions give very nearly straight lines and therefore are approximate to normality, whereas the longer brood periods show in every instance a marked increase in variability, the line bending towards the time axis. The explanation suggested here is based upon the view outlined above that the brood period may be limited either by the rate of development of the previously fertilized egg masses, or it may be limited by the rate of re-development of the ovary. Those individuals which are feeding more vigorously or are in more advantageous positions to obtain food will belong to the former group. They will have brood periods only slightly greater than the corresponding fertilized periods, and will fall on a distribution line not far removed from and of similar slope to that of the fertilized period. Other individuals less fortunately placed or feeding more slowly will become fertilized again only as their ovaries mature in due course. If the variability in the time of ovarian development is greater than that of embryonic development, as might well be expected, then these latter individuals will fall on a different distribution line which diverges at a lesser slope to the time axis. A perusal of the individual graphs will show that whereas in early spring and summer no more than 10 or 20% of individuals appear to belong to this divergent group, the proportion increases steadily during late summer and autumn, when food material probably becomes less readily available.

RELATION BETWEEN REPRODUCTION AND TEMPERATURE

Off the south-east coast (Burnham-on-Crouch) breeding occurs in *Elminius* at the highest summer temperatures (23–25° C. in some years), and survival is possible at temperatures well below zero. Our observations in the more equable climate of south Devon are limited to the range 5–18° C. If all values of α and β shown in Fig. 3 are plotted against the temperature a wide scatter results, particularly among the values of α . If the values of the reproduction constants are separated into two sets, one for the period of rising temperature and the other for the period of falling temperature, corresponding broadly to spring and autumn respectively, much of the variation is eliminated. By grouping each set of observations over limited temperature ranges the curves shown in Fig. 6 were obtained.

They demonstrate clearly the difference in the fertilization rates α for the earlier and later halves of the year after temperature has been eliminated as a variable. The relation between β and the temperature, however, can be approximated by a single line, as shown, although the points on the lower section show a tendency towards asymmetry, with β somewhat greater on the rising than on the falling temperature gradient. This would imply a tendency towards longer retention of the embryos in late autumn than at corresponding temperatures in early spring.

Fig. 7 gives the average values for the period of embryonic development, for the fertilized period, and for the total brood period, again divided into two series according to whether the seasonal temperature is rising or falling. These results are based on the individual breeding times, whereas those shown in Fig. 6 are derived from population values of the rates of fertilization and release; the values of α , however, are of the same order as $1/(\bar{T} - \bar{T}_F)$ (eqn. 8) and β fairly closely approximates to $1/\bar{T}$ (eqn. 7) at a given temperature and season.

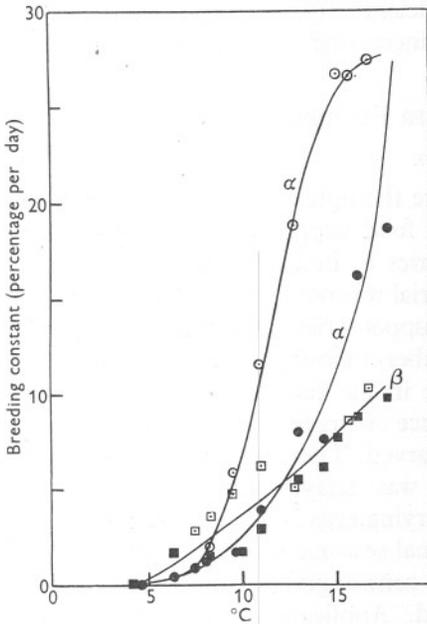


Fig. 6

Fig. 6. Temperature dependence of the fertilization constant α and the liberation constant β . \circ , α , rising temperatures; \bullet , α , falling temperatures; \square , β , rising temperatures; \blacksquare , β , falling temperatures.

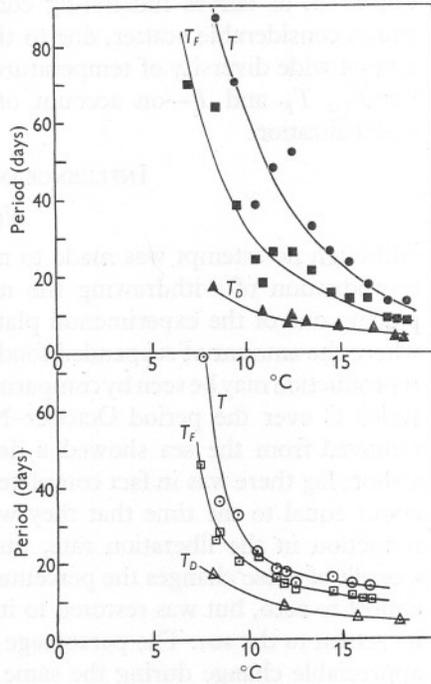


Fig. 7

Fig. 7. Temperature dependence of development, fertilized, and brood periods. Upper figure on falling, lower figure on rising temperature gradient. \circ , brood period T ; \square , fertilized period T_F ; \triangle , time of development T_D .

The results given in Fig. 7 show rather more clearly the temperature dependence of the various processes. The period of early development T_D , given by the lowest curve in each graph, is practically identical in spring and autumn, and appears to depend only upon the temperature, as might be expected. The curves for the period of retention of embryos T_F , and for the brood period T , taken on a rising temperature gradient in the spring (lower

part of figure) exhibit a relation to temperature closely similar to that of the rate of embryonic development. The values for Q_{10} based on the more reliable sections of the curves, namely those between 9 and 16° C., are 3.6, 3.3, and 3.6 for T_D , T_F and T respectively. These values are of a reasonable order for temperature coefficients of developmental processes (see Barnes, 1937). Their identity points to a common rate-governing process—that of the development of the embryos, which when completed leads to their release from the mantle cavity and then allows another fertilization to take place. The upper set of curves illustrating conditions in autumn, on the other hand, shows considerable scatter, due to the less regular individual behaviour, and gives a wide diversity of temperature coefficients— Q_{10} being 3.6, 5.4, and 5.8 for T_D , T_F and T —on account of the increasing delay in liberation and re-fertilization.

INFLUENCE OF OTHER FACTORS

Nutrition

Although no attempt was made to measure the uptake of food, the effect on reproduction of withdrawing the normal food supply was investigated by placing one of the experimental plates (series B) in a well-aerated aquarium where the amount of suspended food material was not replaced. The effect on reproduction may be seen by comparing the upper series B with the lower control series C over the period October–November 1950 (Fig. 2). The barnacles removed from the sea showed a decrease in the rate of fertilization; after a short lag there was in fact complete absence of any fertilizations for a period about equal to the time that they were starved. There was a corresponding reduction in the liberation rate, but this was delayed about a month. As a result of these changes the percentage carrying eggs in the starved group fell almost to zero, but was restored to its normal seasonal level a few weeks after its return to the sea. The percentage in the control group C did not show any appreciable change during the same period. Application of the t test to the difference between starved and control barnacles over the period extending from the beginning of the experiment until mid-December showed that the fertilization constant α of the starved individuals was lowered (significance level = 7%) but that the liberation constant β was not significantly altered in relation to controls.

Water Movement

The effect of water movement on reproduction was studied in a separate series of experiments. Since *Elminius* feeds on suspended material in the water, the effects of water current and population density are linked. Either may, under appropriate circumstances, limit the rate of feeding and thereby possibly the reproductive behaviour. In a population growing on a flat plate, those in the centre will be receiving water which passes across the surface of the plate, and which has probably been filtered to some extent by neighbouring

individuals, whereas those growing at the sides will receive water flowing not only in the plane of the plate but also at right angles to it; moreover, they will have immediate access to unfiltered water. We may therefore regard the latter as having better access to water than the former.

A series of panels well covered with young barnacles which had settled over a short period at Burnham-on-Crouch was put out at Brixham in January 1949 and examined at different dates during the following spring. Those which approached mature size (4-7 mm rostro-carinal diameter) were sampled from positions near the centre and at the edges of the plate. It was noted at the time that those at the edges invariably showed greater growth than those at the centre.

TABLE II. ANALYSIS OF VARIANCE OF FACTORS INFLUENCING THE BREEDING OF *ELMINIUS MODESTUS*

Source of variation	Degrees of freedom	Mean squares		
		A Fertilized and fully developed ovary	B Fertilized	C Fully developed embryos
Dates and position	4	1695.6	532.1	314.8
Sizes	2	1407.9	850.7	367.5
Access to water	1	3333.1	6579.3	1146.7
First-order interaction	14	130.6	98.5	120.5
Replicates and second-order interaction (residual)	38	67.2	60.2	55.3

Two samples each of ten to twenty individuals belonging to each sub-group (i.e. of a given size range, position, and relation to water access) were examined on each date and the number of individuals falling into classes A, B, or C recorded as a percentage. Percentages were transformed to angular values, and the variation about the mean (mean square) was calculated in respect of each of the sources of variation. The combined mean square due to replication and second-order interaction was used to determine the significance of each source of variation in turn, $P=0.05$ as the limit of significance. All factors except first-order interactions had a significant effect.

The samples taken at different times in different positions were therefore grouped according to size, in order to remove this effect from the comparison. The percentage (A) having well-developed or fertilized eggs, (B) having fertilized eggs and (C) having fully developed embryos was recorded for each sample and size group. In order to make the variances homogeneous percentages (p) were converted to angles (θ), using the transformation $p = \sin^2 \theta$ (Fisher & Yates, 1943, p. 50). The success of this transformation is well illustrated in the analysis of variance (Table 2) from which it will be seen that the residual variances for A, B and C are very similar. The analysis of variance shows that the fecundity, judged on criteria A, B or C, is influenced by all the main variables, date of sampling, size and access to water. Comparison of the mean values for main effects (Table 3) shows that there was the expected rise in the percentage bearing well-developed ovaries and egg masses as the season advanced. Also, individuals exceeding 6 mm in diameter

were more fecund than those between 5 and 6 mm, and these in turn bred more freely than those of less than 5 mm. There was a slight but significant difference between the fronts (illuminated side) and backs of the panels, those on the darker side breeding slightly better, although the amount of algal growth was not very different on the two sides. The difference between those settled at the centre and those at the edges, however, was very pronounced, and ranked in importance with the difference between the two extreme size groups. All the results given in the table are based on samples balanced in respect of all effects and interactions other than those which are being compared. The difference in breeding attributable to variations in access to water is therefore quite independent of the greater growth rate which also takes place at the edges.

TABLE III. EFFECT OF VARIOUS FACTORS ON REPRODUCTION
IN *ELMINIUS MODESTUS*

Source of variation	Details	A	B	C
Dates of settlement and examination	Settled Sept. 1948			
	Ex. 19. i. 49	32.7*	18.4	14.4*
	Ex. 10. iii. 49	46.9*	18.3*	9.05
	Ex. 16. iii. 49	60.9*	24.8*	5.14*
	Ex. 30. iii. 49†	83.1	72.7	17.3
Size ranges	3-5 mm	48.2**	14.5*	4.4**
	5-6 mm	53.4*	20.7*	6.9*
	> 6 mm	62.7	27.5	12.8
Position†	Fronts of panels	64.3	34.2	11.8*
	Backs of panels	62.9	43.0	18.8
Access to water	Centre of panel	47.8*	10.4*	3.6*
	Outside	61.8	31.4	12.3

Mean values given as angular transformations from percentages (p). $p = \sin^2 \phi$. A, proportion with fertilized or well-developed ovaries; B, proportion fertilized; C, proportion with well-developed embryos.

† Contains information additional to that included in analysis of variance.

* Indicates significant difference from entry one line below ($P < 0.05$).

** Indicates significant difference from entry two lines below ($P < 0.05$).

The significance was determined by means of a t test applied to the differences in the mean values. The residual variance of Table 2 was used to determine in each case the standard error of the mean, taking into account the number of observations contributing to the mean.

Age of individuals

In his study of breeding in *Balanus balanoides* Moore (1935) suggested that older individuals frequently showed sterility. No evidence of sterility in relation to age was found among individuals of *B. porcatus* (Crisp, 1954). Examination of glass panels bearing identified individuals of *Elminius modestus* in their third summer (1947-50) showed that at least some bore fertilized eggs and were, therefore, capable of reproducing. These individuals must have given rise to successions of broods for 3 years; at a conservative estimate they must have produced 30-40 broods in all. It seemed possible, however, that there might be a gradual slowing down in the rate of reproduction in older

individuals. To test this possibility a series of panels bearing younger individuals (series C, p. 365) was observed over the same period as the older individuals of series B. Both sets were of mature age and therefore not markedly different in average size. A comparison of the reproduction constants α and β was then made, excluding the short period when the two sets of panels were receiving different treatments. The difference between the constants α for the older and younger series (B-C) based on forty-three pairs of parallel observations was $+0.407\%$ per day, and the standard error of the difference was 1.30% per day. The observed difference of means for β was -0.667% per day and the corresponding standard error 0.481 . In neither therefore was the difference significant.

Tidal periodicity

Tidal periodicity in breeding has been established in a number of marine animals, notably in certain lamellibranchs and annelids, and in some marine algae. A test for tidal periodicity in the breeding of *Elminius* was carried out as follows. Each tidal cycle was divided about the dates midway between the largest and smallest tides into two equal parts, a spring tide period and a neap tide period. The fertilization rate ϕ_F was then averaged over each successive spring and neap tide period and the difference $\Delta\phi_F$ noted. The same treatment was applied in regard to rates of liberation ϕ_L . A *t* test was applied to these differences over forty-three pairs of tidal periods, with the following results:

$\Delta\phi_F$ mean difference = -0.0028 , standard error of difference = 0.211 ;

$\Delta\phi_L$ mean difference = -0.167 , standard error of difference = 0.246 .

The result is therefore clearly negative, breeding occurring at all times and with equal intensity during the lunar cycle. Since these results were obtained from raft exposures where no hydrostatic changes accompanied the tide, it does not follow that tidal periodicity may not be found in barnacles which are growing on the shore. If observations are carried out on the developmental stages of the fertilized eggs found in the mantle cavity of such individuals, however, both early and late stages may be identified at any time during the greater part of the year, whether spring or neap tides prevail. There seems therefore little indication of any tidal influence on breeding in this species.

BREEDING IN *ELMINIUS* IN RELATION TO SEASON

In *Elminius* the individual cycles or broods are non-seasonal, although the rate of breeding varies with the season. It therefore differs fundamentally from those species, such as *Balanus balanoides* (Moore, 1935) and *B. porcatus* (Crisp, 1954), in which the cycle occurs only once a year. In these species the gonads show a regular seasonal change and are at any one time in a uniform state throughout the population. These species are therefore adapted to an

annual rhythm, and since sea temperatures do not vary widely from year to year each part of the breeding cycle takes place within a fairly narrow temperature range. *Elminius*, on the other hand, is able to breed over very wide temperature limits, the whole cycle being capable of completion at any temperature from 6 to 20° C, and probably higher. Even at temperatures below 6° C embryonic development may continue, but it is extremely slow.

There is in *Elminius* no evidence of any pace-making mechanism to the breeding cycle, the population at all times having a random selection of embryonic and ovarian developmental stages. Indeed, the observed variations in rates of fertilization and liberation were never greater than would have been expected from chance. The breeding rates are consistent with the view that they are entirely dependent upon temperature and food supply. The possibility that there is a seasonal rhythm superimposed is unlikely, for when young individuals were transferred from Burnham-on-Crouch to Brixham in autumn or winter they bred earlier in the south-west. Moreover, there is no definite cessation of breeding, but rather a slowing down or pausing when the temperature falls.

The concept of a fertilization constant (α) and a liberation constant (β) as defined above can be employed only in animals which reproduce by a continuous succession of broods, as for example *Elminius* and *Chthamalus stellatus*. These constants are useful not only in determining the influence of temperature and other variables, but they also give information on the mechanism of breeding. In *Elminius* it has been shown that α depends mainly upon the food supply, while β is determined by the rate of development of the eggs in the mantle cavity. This condition must result from the physiological mechanisms which control breeding. The ovary appears to develop as rapidly as assimilation can provide for it, and is frequently ahead of the development of the eggs; when this occurs liberation is immediately followed by copulation and oviposition, provided there is another individual in the vicinity to function as a male. It is noteworthy that oviposition never occurs until the previous brood is liberated. If, as seems probable, oviposition is dependent upon copulation, either there is a refusal on the part of the gravid barnacles to allow copulation, or else individuals acting as males require some stimulus which is provided only by individuals which have liberated the previous brood. Some such mechanism seems to be general, for only one brood of eggs is found in the mantle cavity of *Balanus improvisus*, *B. amphitrite*, *B. crenatus* and *Chthamalus stellatus*, all of which exhibit continuous breeding. The only exception noted by one of us (D. J. C.) was the presence of two pairs of egg masses in one or two out of several thousand specimens of *C. stellatus*, where the earlier egg masses were already fully developed, and the later egg masses only just deposited. Since *Chthamalus* lives high up the intertidal zone it may only infrequently be submerged, and it is possible that one brood might be retained beyond the normal physiological time limit. Alternatively, the removal of a specimen

with very ripe ovaries might induce a false spawning of unfertilized eggs which would not be readily distinguishable from a true fertilization. Both exceptional specimens were taken at the peak of the breeding season in a situation where the ovaries were developing very rapidly.

It appears from our observations that liberation occurs soon after the eggs are fully developed. It involves activity on the part of the parent, for the eggs are discharged forcibly through the aperture between the opercular plates. Liberation of eggs does not appear to depend primarily upon the condition of the ovary of the parent, for it continues normally in specimens which have been starved and which have no new ovary developing. There was, however, some evidence that such individuals may retain the eggs a little longer, particularly at low temperatures, in that the value of β was lower and the fertilized period T_F distinctly longer on a falling temperature gradient than on a rising one.

We have assumed that the effect of the falling temperature gradient is ultimately attributable to diminished food supply in autumn and winter, leading to poorer ovarian growth and a decrease in the percentage of gravid individuals. It is equally possible that the food supply is there, but that on a falling temperature gradient the rate of feeding or perhaps the whole metabolic process is depressed by temperature adaptation.

Evidently *Elminius* is capable of being fertilized at any time of year as soon as the ovaries have regenerated. Other barnacles, particularly northern forms, show a delay after the regeneration of the ovary, fertilization only being possible at certain seasons (Crisp, 1954).

FECUNDITY OF *ELMINIUS* IN RELATION TO ITS ECOLOGY

The breeding behaviour of *Elminius* is well suited to its environment, namely sheltered coasts and estuaries. The remarkable eurythermy of its reproduction is clearly adapted to the wide fluctuations of temperature which occur in such habitats. Moreover, land-washed nutrients normally allow a high level of production to be maintained in enclosed waters throughout all but the coldest months. *Elminius* therefore has available abundant suspended food material for purposes of reproduction throughout most of the year. The continuous succession of broods necessitates intensive feeding, and it is noteworthy that *Elminius* has a faster rate of beat of the cirri than is found in most of the other British species, and far more rapid than that of *Balanus balanoides*. The advantage of feeding with access to unfiltered water, not only to growth but also to reproductive rate, has been shown. This effect is very pronounced in individuals exposed to a fast-flowing stream, as for example those attached to piles standing in a narrow part of a tidal estuary. Such individuals grow rapidly to a large size (1.0–1.5 cm) and a high percentage are fertile in the warmer months. The influence of water current on growth rate renders size

a poor criterion of age, and large individuals cannot necessarily be regarded as having been settled for a long period. When intense settlements occur in situations exposed to strong currents, growth may be restricted laterally, the result being columnar individuals with narrow parietes tapering towards the basis. Columnar growth is less evident, however, than in *B. balanoides* under comparable conditions.

The remarkable fecundity of *Elminius* brought out in this paper is largely responsible for its rapid colonization of new shores in Britain and Europe (Crisp & Chipperfield, 1948). Assuming a span of life of three breeding seasons, and an average number of 500 nauplii in each brood, and twelve broods per season, the total output of young approaches 20,000 per individual. This is equivalent to the output over the same period of three broods of a large specimen of *Balanus balanoides* which would occupy a considerably greater area. Moreover the generation time of *Elminius* is very short compared with that of *Balanus balanoides*, which leads to a potentially much greater rate of multiplication. Taking a value of 10 weeks for the period of growth from settlement to maturity in summer (p. 365) and an assumed value of 4 weeks of planktonic life, the total generation time is only 14 weeks, compared with 1-2 years in *B. balanoides* (Moore, 1935). The generation time of *Elminius* maturing over the cooler months will of course be greater.

Another advantage held by *Elminius* over *Balanus balanoides* consists not only in the greater number of embryos produced per unit area of substratum, but also in the greatly extended period over which they settle. Under the very crowded conditions which frequently obtain in estuaries and shores where *Elminius* abounds, new individuals can only survive if they settle in the spaces from which old ones have broken away. Hence the long settling period of *Elminius* offers ample opportunities for the colonization of bare areas, whereas *Balanus balanoides* can only occupy such spaces as are available during the four weeks or so in which it settles. On the other hand, since *B. balanoides* settles about a month before *Elminius* it can take advantage of sites left available by losses during the preceding winter months. The disparity is, therefore, not as great as might be anticipated from the difference in the lengths of the settling periods. To some extent, too, the very intense settlements of *Elminius* which occur in mid-summer in shallow estuaries, such as the Crouch and Blackwater, fail to benefit the species since they produce many small individuals which retard each other's growth and mature only slowly except where conditions such as exposure to strong water currents are especially favourable.

We are indebted to Mr A. H. Molesworth who carried out some of the observations for us on occasions when we were unable to visit the raft, and to Mr P. Bowles for the drawing of Fig. 1.

SUMMARY

A more complete study of reproductive behaviour is possible where observations can be made on individuals. This is especially so in a population where each individual has a succession of breeding cycles which are not synchronous with those of other individuals. Continuous records of the breeding of individual barnacles is possible in species which have a membranous base. Individuals may be grown on glass slides and the reproductive condition determined by observations through the base.

Reproduction in *Elminius modestus* takes the form of a succession of breeding cycles or broods, each cycle being initiated by copulation, oviposition and fertilization of the eggs in the mantle cavity. Here the eggs develop, and embryos are eventually liberated through the opercular valves.

The time interval occupied by a brood varies both among individuals and with the season of the year. The time of development of the embryos appears to be a function of temperature alone, but the regeneration of the ovary depends upon the nutrition and food supply. When the ovary regenerates rapidly, as in the period of rising temperature, viz. in spring and early summer, another fertilization follows closely upon liberation, but in autumn and winter the ovary may not mature for some time after liberation, with the result that a large proportion of the population do not contain egg masses. In spring and summer the fecundity is probably limited by the rate of development of the embryos; in autumn and winter by nutrition.

There is a slight tendency for eggs to remain in the mantle cavity for a longer period during the falling temperatures of autumn and winter than at corresponding rising temperatures in spring and summer. It is possible that a rapidly maturing ovary exerts some stimulus accelerating liberation; nevertheless, liberation can take place without ovarian regeneration.

Access to previously unfiltered water, and to rapidly moving water increases both the rate of growth and the fecundity.

Older individuals do not differ significantly from young mature individuals in their breeding behaviour, unless infected by *Hemioniscus balani*, which prevents maturation of the ovary and so renders *Elminius modestus* sterile. Parasitized individuals may live for at least a year without showing any other symptom.

There is no lunar periodicity in the breeding cycle.

The breeding of *Elminius* is characterized by extreme eurythermy. It can breed at any season, provided that the temperature does not fall below 6° C and the food supply is adequate. This type of breeding is well suited to life in shallow estuaries and sheltered coasts of temperate latitudes and accounts for its success in Britain in competition with the indigenous *Balanus balanoides*.

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