J. mar. biol. Ass. U.K. (1959) 38, 589–597
Printed in Great Britain

THE INFLUENCE OF TEMPERATURE ON THE REPRODUCTION AND MOULTING OF *LEPAS ANATIFERA* L. UNDER LABORATORY CONDITIONS

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(Text-figs. 1–2)

Lepas anatifera L. is generally found on floating objects in tropical and sub-tropical oceanic waters, where sea temperatures exceed 18–20° C.

A drifted buoy covered with recently settled and adult *Lepas anatifera* L., washed ashore near Cable Bay on the south-west of Anglesey, however, provided an opportunity to study in detail the processes of moulting and reproduction in this species under laboratory conditions.

Animals were carefully detached from the buoy and were kept in glass crystallizing dishes. Sea water was circulated through a long thin-walled coiled glass tube immersed in a tank controlled by a thermostat. A constant flow of warm water was thus maintained over the animals throughout the experiment. This was found necessary to keep them healthy and in normal condition; they rapidly became lethargic and moribund if the water was allowed to become stagnant.

The barnacles were fed on *Artemia* larvae and on a paste prepared from powdered mammalian liver. Small pieces of *Mytilus* tissue (1–2 mm) were also offered; these were rapidly grasped by the cirri and engulfed. Very large pieces (about 5 mm) were accepted, but the animals had difficulty in swallowing them (cf. Howard & Scott, 1959). However, uneaten *Mytilus* tissue and liver caused fouling of the water, so *Artemia* larvae were generally used and the animals seemed more healthy when fed on live food.

We found that in isolation the barnacles lived healthily with the stalks unattached. The stalks which had been carefully freed from their original attachment showed no evidence of refixing themselves, for example to the glass dish. However, when several such individuals were kept in the same dish, they were often found grasping and attempting to feed on each other's stalk, thus occasionally causing injury. The injured stalks decayed, followed often by the death of the animal. In one instance the stalk started to decay and a few days later cirral activity ceased and the animal appeared dead. When the stalk of the animal was lifted the thick outer integument separated, leaving the muscular part of the stalk attached to the animal. On the following

day the fleshy stalk disappeared, the other barnacles in the dish having devoured it. The stalkless animal was then isolated; it resumed its normal activity and lived for a further 2 weeks. It is unlikely that this type of damage would arise if the animals were growing together attached to a common object, because then only the leathery outer surface of the stalk would be exposed to attack and the individuals would also be able to move away from each other by bending the stalk.

Colour of ovary

The normal colour of the ovary in *Lepas* is deep blue (Darwin, 1851; von Willemöes Suhm, 1876; Groom, 1894) as are the recently fertilized egg masses, though later the embryos turn to purple, red and pink as development proceeds. The original colour of the ovaries of the specimens washed ashore was blue, as also were the egg masses. On the Munsell Colour Chart they matched purple blue 5·0, value 4/chroma 6. However, after the animals had been kept in the laboratory and fed on *Artemia* larvae, the newly developed ovaries were seen to be distinctly pink (Munsell red 5·0, value 6/chroma 10) and the colour changed only slightly to a salmon peach tint (Munsell red 5·0, value 8/chroma 4) by the time the embryos were ready to hatch. A similar though less dramatic difference in colour of the ovary has been noticed also in operculate barnacles, such as *Balanus crenatus* Bruguière, *B. amphitrite* var. *denticulata* (Broch), *B. perforatus* Bruguière and *Chthamalus stellatus* Poli, when fed on *Artemia* in the laboratory, the ovaries being slightly more peach coloured in comparison with the yellow or orange ovaries of naturally fed specimens. The type of food thus plays an important role in determining the ovary colour.

In *Lepas anatifera* at least, this difference appears not to be due simply to an additional pigment derived from the *Artemia*, for there is no sign of the presence of any deep blue colour. The blue colour in *Lepas* eggs is considered to be an astaxanthin-protein complex (Ball, 1944), as also is the blue pigment of the oceanic Siphonophore, *Velella lata*, which Fox and Haxo (1958) believe to be derived from crustacean food. It seems probable therefore that an element in the oceanic plankton on which these animals feed contains an essential precursor of the blue astaxanthin-protein complex.

Reproduction

Like operculate Cirripedes and unlike a few pedunculate forms, *Lepas* is an hermaphrodite animal; it bears embryos four to five weeks after settlement (Skerman, 1958; Evans, 1958). Groom (1894) observed in detail the process of fertilization in *Lepas anatifera* L. and our observations confirm his. Occasionally some undeveloped and cytolysed eggs were observed in the egg mass; it was thought that these eggs may not have been fertilized. Failure to effect complete fertilization of the egg mass was considered not unlikely,

because the animals, not being attached to their natural substratum, were unable to copulate and insert the penis normally in the mantle space of the adjacent specimens. Laboratory conditions might also have reduced the potency of spermatozoa. At every successful copulation the individual acting as female contracted its stalk as though to squeeze the ova through the oviducts and up into the mantle cavity where they became attached to the fraenae, the semi-circular folds of skin at the base of the mantle space (Darwin, 1851).

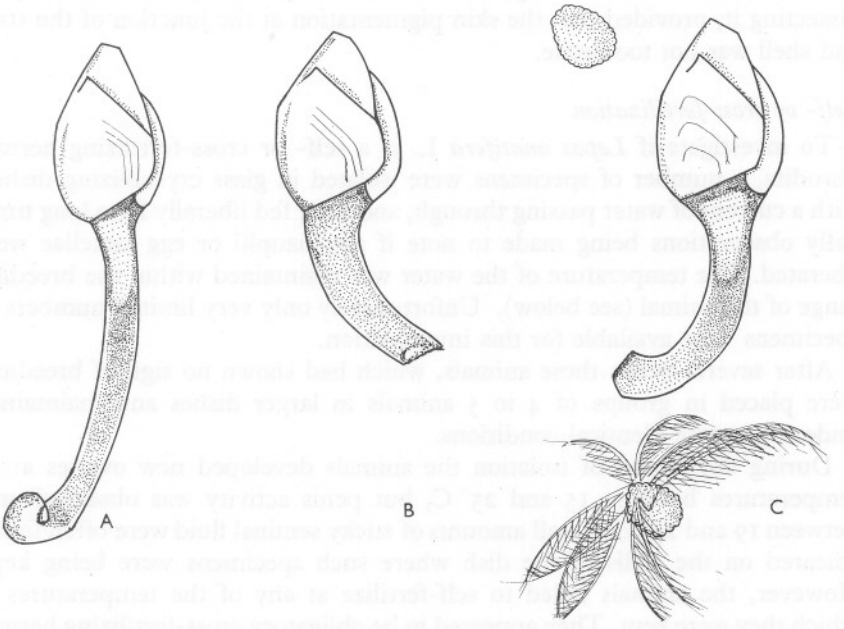

Fig. 1. A single individual of *Lepas anatifera* L. at different phases of breeding cycle. A, Before copulation. The large ovary is visible through the stalk. B, Just after copulation. The ovary has disappeared and the stalk is contracted. C, After liberation and moulting. The two egg lamillae are visible, one still attached to the cast skin. The ovary has redeveloped.

The ovary, originally visible through the stalk, was observed to have disappeared after copulation was completed. This fact could be used to ascertain when an animal had been fertilized. The clear stalk generally remained contracted for two to three days till a new ovary was formed and began to show through the stalk.

The sequence of changes visible outside the barnacle is presented in Fig. 1. One *Lepas*, which had a clearly defined pink ovary showing through the stalk, was marked and photographed (Fig. 1A). It was then put into contact with other animals. Some time later, the marked individual was found in copula; after copulation the ovary disappeared and the stalk remained contracted (Fig. 1B). The *Lepas* was then kept isolated and fed liberally. Three to four

days later a new ovary appeared. On completion of the embryonic development (after seven days at 25° C) it liberated egg masses which were seen to be hatching as stage I nauplii and were accompanied by the cast skin. In Fig. 1 C one of the egg masses is still attached to the cast skin. This sequence of events could be observed in most specimens. The changes in the appearance of the ovary as seen through the upper part of the stalk might usefully be employed in the field to determine whether an animal had recently been fertilized or whether it still carried a large unfertilized ovary, without the necessity of dissecting it, provided that the skin pigmentation at the junction of the stalk and shell was not too dense.

Self- or cross-fertilization

To investigate if *Lepas anatifera* L. is a self- or cross-fertilizing hermaphrodite, a number of specimens were isolated in glass crystallizing dishes, with a current of water passing through, and were fed liberally for a long time, daily observations being made to note if any nauplii or egg lamellae were liberated. The temperature of the water was maintained within the breeding range of the animal (see below). Unfortunately only very limited numbers of specimens were available for this investigation.

After several weeks these animals, which had shown no sign of breeding, were placed in groups of 4 to 5 animals in larger dishes and maintained under otherwise identical conditions.

During the period of isolation the animals developed new ovaries at all temperatures between 15 and 25° C, but penis activity was observed only between 19 and 25° C. Small amounts of sticky seminal fluid were often found smeared on the walls of the dish where such specimens were being kept. However, the animals failed to self-fertilize at any of the temperatures at which they were kept. They appeared to be obligatory cross-fertilizing hermaphrodites like *B. balanoides* L., *B. balanus* (= *porcatus*, da Costa) (Crisp, 1954; Barnes & Crisp, 1956) and *Elminius modestus* (Crisp, 1958). No sooner were they grouped than copulations were observed at temperatures between 19 and 25° C. Clearly under the conditions of these experiments self-fertilization does not occur, but cross-fertilization takes place readily (Table 1).

Breeding temperature

The results of a number of experiments in which groups of animals were maintained for several weeks at approximately constant temperatures are shown in Table 2. It will be noted that breeding was possible between 19 and 25° C but not at or below 15° C, nor at temperatures higher than 30° C. These results were confirmed by the disappearance of ovaries only from the barnacles in dishes kept at 19 and 25° C, and later by the presence of liberated nauplii only in the same dishes. Probably 16° C is close to the lower limit of the breeding range because Skerman (1958) found *L. anatifera* var. *testudinata*

in New Zealand waters bearing embryos at temperatures varying from 17 to 20° C, while Darwin's records (1851), with one exception, are from areas where the temperatures generally exceed this range. Darwin includes a record from the Bass Straits, where the range lies between 13 and 17° C. Evans' (1958) observations during a voyage from Dakar to Barbados indicate that this species breeds readily between 24 and 26° C, and also agree with the behaviour of specimens in the laboratory.

TABLE 1. ABSENCE OF SELF-FERTILIZATION IN *LEPAS ANATIFERA* L. IN THE LABORATORY

(The temperature range of the experiment was 19–25° C)

Number of *Lepas* available during experiment	Condition of specimens	Total no. of barnacle days*	No. of liberations of nauplii seen
25	Isolated	667	0
31	Grouped	619	26

TABLE 2. INFLUENCE OF TEMPERATURE ON BREEDING ACTIVITY IN *L. ANATIFERA* L.

Temperature (°C)	No. of barnacles used	Total no. of barnacle days	No. of liberations observed	Embryonic period (days)	Size of ripe embryos
8–10	9	53	0	—	—
15–16	4	96	0	—	—
19–20	19	431	12	11–12	290 × 136 μ
24–25	12	188	14	6–7	266 × 122 μ
30–31	7	160	0	—	—
34–36	12	Died	—	—	—

* 'Barnacle days' = no. of specimens × no. of days under observation.

It is interesting to note that the breeding optimum, 19–25° C, is close to the optimum range of cirral activity as shown by Southward (1957), (Fig. 2). It is surprising that Boëtius (1952–3) found recently settled *Lepas* in Danish waters where, except in very shallow and locally warm pools, such temperatures would not be expected, though it is also possible that, if its normal planktonic food were available, breeding might occur over a wider temperature range than when fed on *Artemia*. However, the specimens kept in the laboratory at 15–16° C appeared healthy, developed large ovaries, and became fertilized within a few days of their temperature being raised to 24–25° C.

Rate of embryonic development

Groom (1894), following the work of von Willemöes Suhm (1876), studied all the stages of embryonic development, but did not mention the time required for complete development. The exact time required by a fertilized egg to reach its final stage capable of hatching as a stage I nauplius is important in an animal which breeds continuously, as this determines its fecundity

(Crisp & Davies, 1955). The time of the embryonic development was investigated as follows:

The fertilized animals were removed and kept isolated as soon as they were observed in copula or when their ovaries had disappeared from the stalk. They were kept under observation and examined every 6–12 h until liberation of egg masses or nauplii occurred. This was always coincident with moulting.

The time of the embryonic development varied with temperature as shown in Table 2.

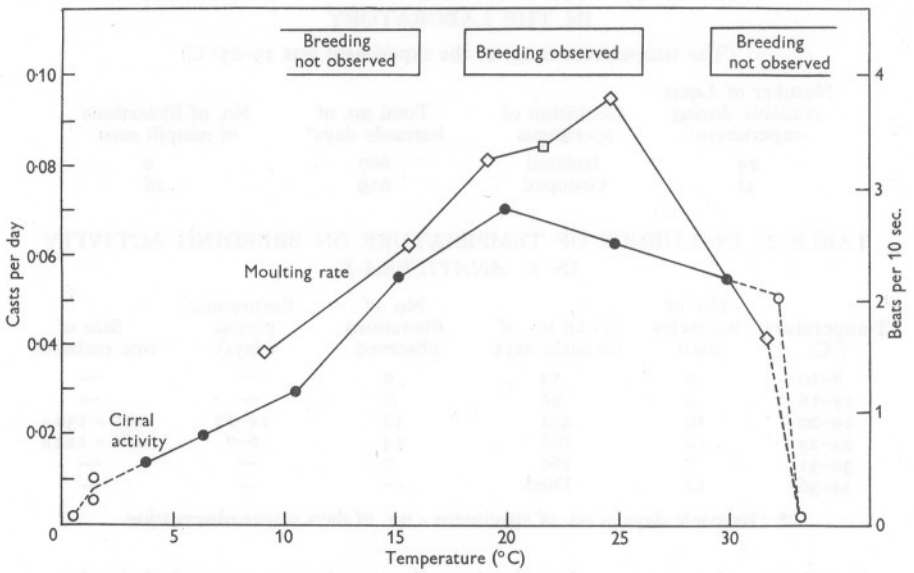

Fig. 2. The effect of temperature on cirral activity (after Southward, 1957), on moulting rhythm and on breeding activity in *Lepas anatifera* L.

The ripe embryos were measured and were found larger both in length and in breadth when the parents had been maintained at the lower temperature and smaller in size when the parent was kept at the higher temperature (Table 2). This phenomenon has been found also in other operculate barnacles. Groom (1894) measured the sizes of the eggs containing ripe embryos from *Lepas anatifera* L. grown under natural conditions, and found their length to be 250 μ. His measurement of the breadth of ripe embryos as stated seems absurdly small and is probably so due to a misprint; it should surely read 145 μ not 45 μ. His figures are then of the same order as ours. Evidently the eggs produced in the laboratory with abnormal food were of the normal size found in nature, as also were the stage I nauplii.

Ecdysis

Within the sub-class Cirripedia the mode of ecdysis differs between sessile and pedunculate forms, as Darwin (1851) noticed. In the sessile forms the chitinous layers of the animal body and also the inner lining of the operculum and the shell are moulted regularly. We confirmed that in *Lepas* the chitinous layers of the prosoma, cirri and penis, together with the lining of the oesophagus and rectum, were regularly cast, but on no occasion did the cast skin include the membrane lining the walls, nor was there any ecdysis of the integument of the stalk. On average an animal took about 15–30 min in completely relieving itself of the cast.

TABLE 3. EFFECT OF TEMPERATURE ON THE FREQUENCY OF MOULTING IN *LEPAS ANATIFERA* L.

	Isolated specimens				Grouped specimens				All specimens		
Temperature (°C)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	Barnacle days	No. of casts	Moulting rate (cast/day/barnacle)
8–10	—	—	—	—	9	53	2	26·5	53	2	0·0380
15–16	29	1214	76	16·0	4	96	6	16·0	1310	82	0·0625
18–20	3	84	6	14·0	19	431	33	13·0	515	39	0·0760
21–22	11	363	30	12·0	—	—	—	—	363	30	0·0840
24–25	11	220	26	8·5	12	188	22	8·5	508	48	0·0950
30–31	3	9	—	—*	7	160	7	23·0	169	7	0·0415
	Starved specimens										
15–16	11	495	16	31·0	—	—	—	—	495	16	0·0320

* Only three *Lepas* were kept for about 3 days. None moulted during this period, hence no definite figure for the inter-moult period.

All except the last row were fed liberally on *Artemia* and dried liver.

During the experiments described in the section on reproduction observations were made on moulting. Table 3 shows the effect of temperature on the rate of moulting (Fig. 2). It will be seen that with the increase of temperature from 10 to 25° C, the rate of moulting increased in an approximately linear fashion, but at 30–31° C the rate of moulting decreased and the animals subsequently died at 34–35° C. It can be seen from Fig. 1 that the three activities, namely cirral activity (Southward, 1957), moulting rate and breeding increased regularly to an optimum at about 20–25° C, and thereafter decreased sharply.

A few animals were kept without food at 16° C and compared with animals fed on *Artemia* the moulting rate fell considerably (Table 3), cirral activity ceased in the starved individuals, and eventually many died. The fact that even at the lower part of its temperature range *Lepas* cannot survive long without food suggests that it is less able to withstand starvation than most sessile barnacles, since forms such as *Balanus balanoides* L. and *Chthamalus stellatus* Poli can be kept for many months in the laboratory without food.

Relation between moulting and reproduction

Crisp and Patel (1958) showed for the first time that there was generally a relationship between moulting and reproduction in sessile Cirripedes.

In *Lepas*, fertilized specimens in which the ovaries had disappeared from the stalk were placed each in a separate dish and examined daily. None of these individuals moulted whilst carrying embryos. After embryonic development was completed, they usually liberated nauplii and simultaneously underwent ecdysis. In a few instances the animals liberated first and moulted on the following day, but in most cases the salmon-peach-coloured egg masses containing unfertilized eggs with free stage I nauplii (and sometimes a few stage II nauplii) were given off at the same time as, or within an hour or so of, the act of moulting. In one instance only, a *Lepas* kept at 24–25° C moulted although bearing egg masses. This occurred eight days after the ovary had disappeared, by which time the eggs should have been hatching. At the end of another three days it gave off salmon-peach-coloured egg masses. On microscopical examination it was found that none of the eggs, though normally oviposited and cemented together, had developed, and they appeared to be unfertilized.

From Tables 2 and 3 it may be seen that the duration of embryonic development is on average slightly less at all temperatures than the inter-moult period of unfertilized specimens. Thus if the recently moulted individual is able to be fertilized immediately, there would be no interference between the moulting cycle and breeding cycle, the moult normally taking place simultaneously with, or soon after, the completion of embryonic development. On one occasion two animals were put together at 19–20° C, one of them moulted (it was marked) and after a few hours it was found receiving sperms. After the ovary had disappeared from the stalk this specimen was separated and it liberated nauplii as expected after 12 days and moulted on the 13th day.

I am greatly indebted to Dr D. J. Crisp for his generous help and guidance in the experimental work and in the preparation of the manuscript.

SUMMARY

The moulting and breeding activities of *Lepas anatifera* L. were studied under laboratory conditions.

When removed from the substratum and fed on *Artemia* and powdered mammalian liver the animals remained healthy and resumed their normal activities if kept in constantly changing sea water. If the animals were not fed or water ceased to circulate they became lethargic and slowly died.

The ova, normally of a blue colour, develop instead to a pink when the animals are fed on *Artemia* larvae.

Isolated specimens showed penis activity but did not self-fertilize; when

grouped together they became fertilized and produced viable nauplii at temperatures between 19 and 25° C. After successful copulation the acting female shed the ova by contraction of the stalk into the mantle space, and a new ovary developed after 4–5 days.

The rate of moulting increased with rise in temperature from 10 to 25° C but fell after further rise in temperature, 34–36° C being rapidly lethal.

Gravid *Lepas* did not moult while they were carrying embryos, and the liberation of nauplii was always accompanied or followed shortly by ecdysis.

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