The general biology of *Verruca stroemia* (O.F. Müller)

with

some additional observation on penis development and moulting frequency in

Balanus balanoides

Thesis for the Doctor of Philosophy in the University of Stirling

by

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Dedication

To Leon L. and Ruth H. Stone

Contents

Preface

This thesis is largely concerned with the biology of *Verruca stroemia* (O. F. Müller), a common species of the sublittoral and near-littoral of the eastern Atlantic. A small animal even when fully grown, it is not very conspicuous, and there is very little information on its general biology. *V. stroemia* is the only easily accessible species of the genus, all others being found only in relatively deep water. Two features of its biology are of particular interest: first, although boreo-arctic in its distribution it is not, like some other species of similar geographical distribution, restricted to a single annual brood; secondly, the genus *Verruca* is a primitive one, in many respects more closely allied to the Lepadomorpha than to the Balanomorpha with which it is classified. Both these aspects of the species have been considered, the latter particularly with reference to feeding and cirral activity. The relation of the major nauplii release to the spring diatom outburst and the effects of desiccation have been investigated since these are important in the ecology of the species and there is much comparative data on both for other cirripedes.

In addition, two aspects of the biology of *Balanus balanoides*, a common littoral species, have been given some attention, namely the effect of light and temperature on the development of the penis, and the effect of the same two factors on moulting frequency.

Verruca stroemia (O.F.Müller)

Introduction

Systematics

With the single exception of that of Gruvel (1905), all classifications of the sub-class Cirripedia are based on that put forward by Darwin (1854) who divided the group into three orders, namely, Thoracica, Abdominalia, and Apoda. We are concerned here with Verruca stroemia (O. F. Müller) belonging to the family Verrucidae with only a single genus, Verruca, in the order Thoracica. The genus is readily distinguished by the asymetry of its shell. The possession of moveable opercular plates which effectively occlude the aperture of the shell distinguishes it from the Lepadidae, although this character is shared with the Balanidae and Chthamalidae. This lends the common name operculates to the latter families while the Lepadidae are often referred to as pedunculates; this does not, however, imply any agreement with the sub-order ranks proposed by Gruvel (1905). Pilsbry's (1916) modification of Darwin's classification has now been widely accepted. He raised the Verrucidae, Balanidae and Lepadidae of Darwin to sub-order rank as the Verrucomorpha, Balanomorpha and Lepadomorpha. Most of Darwin's taxa at or below the level of family were retained within the modified scheme.

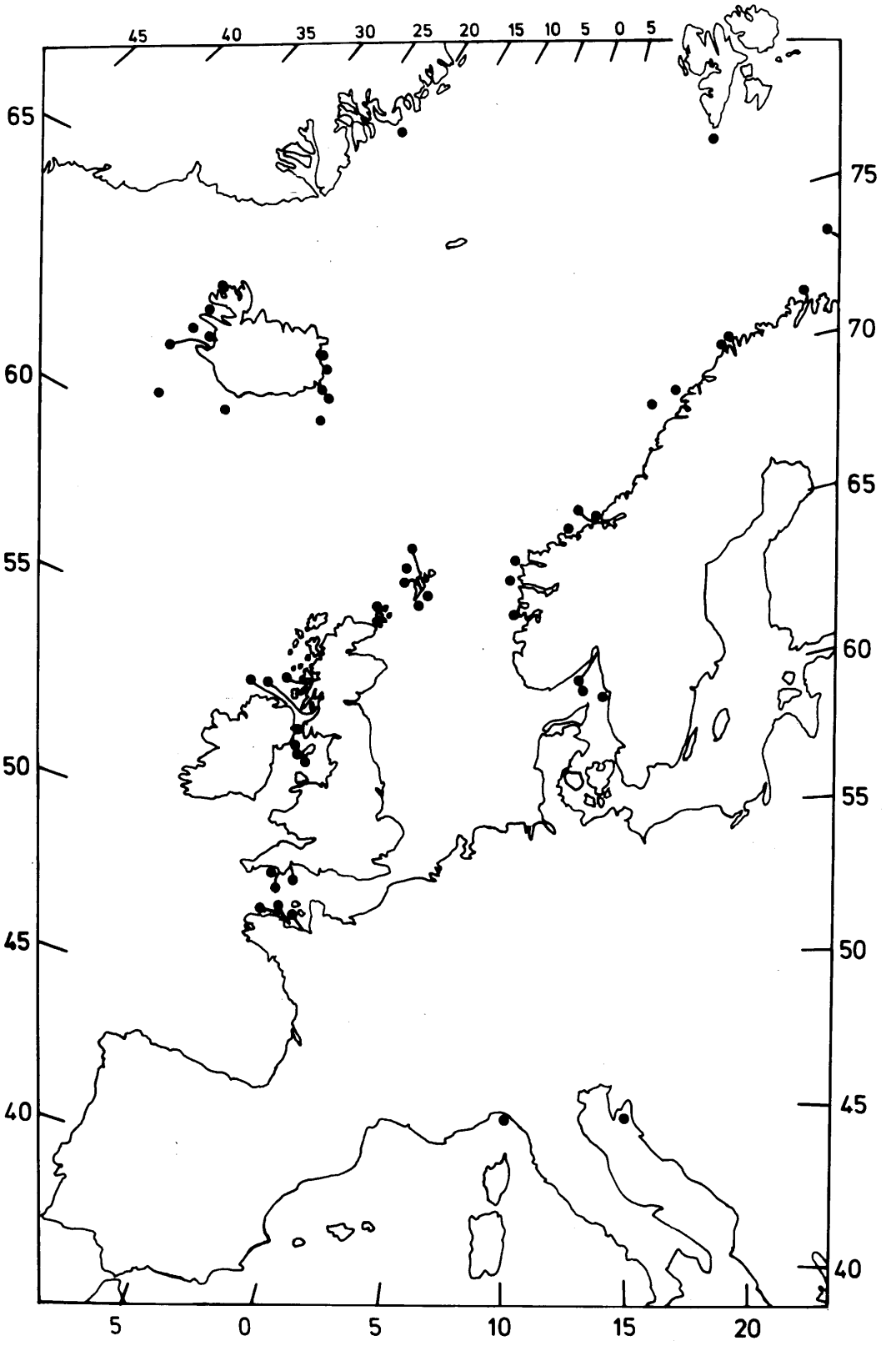

Fig. 1. Verruca stroemia: geographical distribution.

Distribution

Geographical

V. stroemia is essentially an eastern North Atlantic species (Fig. 1); according to Broch (1924) it ranges from the Mediterranean to Finmark all along the eastern Atlantic coast of Europe. It occurs northwest of the British Isles from the Faroes (Weltner, 1922; Sars, 1886) to Iceland (Weltner, 1900; Schaper, 1922; Stephensen, 1938; Pelsbry, 1916) and Greenland (Pelsbry, 1916). It is common on the coasts of Britain being recorded from the south coast at Plymouth (Plymouth Marine Fauna, 1957), and the west coast at Millport, (pers. obs.), the Isle of Mull and the Shetlands (Darwin, 1854). It is recorded from the southern tip of the Isle of Man (Marine Fauna of the Isle of Man, 1963), the Irish Channel (Pilsbry, 1916) and Ireland (MacDonald, 1941). Darwin (1854) and Sars (1886) recorded it from the North Sea, which Pilsbry (1916) regarded as the type locality. It is common off the Northumberland coast. The species has also been found in Dannish waters (Darwin, 1854); Nilsson-Cantell (1921) recorded it in both the Skagerak and Kattegat. Broch (1924) has given considerable details of its distribution in Norwegian waters from Drøbak and Bergen northwards through the

Lofotens to the Porsanger Fjord in the North. Sars (1911) extended its northerly range to Spitzbergen. It has not been found on the Atlantic coast of North America.

In contrast to the area of distribution delineated above, there are two records, both of Darwin (1854), one from the Mediterranean; (also quoted by Nilsson-Cantell, 1921 and others), and another from the Red Sea (Darwin, 1854), which have been questioned (Broch, 1924); Pilsbry, 1916). Although it is always difficult to deny the veracity of published records, single occurrences well beyond the normal area in which ~~it~~ an animal is found must always be critically examined in attempting to define ~~the~~ its distribution. ~~of any animals.~~ Darwin's record of V. stroemia from the Red Sea may be valid, since larval transport into that sea is possible, but it may perhaps be discounted. This record was based upon his finding a specimen in a box labelled for that locality; although realising this was far from the normal area of distribution, he considered the record valid because of the presence in the same container of a Pyrgoma sp. well known from the Red Sea. In view of the extremely high temperatures in the Red Sea and the otherwise north-boreal distribution of the species this record must remain doubtful. It seems more likely that if the label on the container in the British Museum be correct, the specimen was transported into the area, perhaps attached to a

rock dredged up and used for ballast in northern waters and subsequently dropped when the ship reached a loading dock in the Red Sea. This argument may well be equally valid for the specimen recorded from the Mediterranean Sea by Darwin (1854), a record repeatedly quoted by others. Greater credence must, however, be placed in these records in the light of recent observations of Dr. G. Relini of Genoa who graciously sent samples for confirmation. He has found Verruca stroemia on Posidonia oceanica and on 'coralligenous' substrata down to 40m in the Ligurian Sea: his samples only contain very small individuals ($\simeq$ 2mm basal diameter). At this size they are not sexually mature but whether they are carried into the area as larvae from the Atlantic never to grow much larger than $\simeq$ 2mm and never to become mature, or whether there is a parent population - perhaps at ~~lower~~ greater depths where temperatures will be more moderate - which gives rise to these small adults can only be a matter of speculation.

The depth and salinity distribution

According to Darwin (1854) in the waters surrounding Great Britain V. stroemia may be found from 5 to 50 fathoms (10 to 100m). Later reports for this area give 35 fathoms (70m) (Plymouth Marine Fauna, 1957) and 30 fathoms (60m) (Marine Fauna of the Isle of Man,

1963). In British waters it reaches the low littoral. Weltner (1922) reported the species at 496 m "north of Scotland" while Stephensen (1929) stated that the range of depth in Faroese waters was from 20-200m, although further offshore to the north west it was found at 249m. Stephensen (1938) reported V. stroemia in the waters around Iceland at depths of from 4 to 6m to 170m, with some dead fragments-quite possibly washed down-being recovered even at 320m. In Greenland waters it was found down to 90 fathoms (180m) (Pilsbry, 1916). It is apparent from these reports that V. stroemia is commonly found in inshore or nearshore waters; the greatest number of reports, however, put the species well within 5 miles of the coasts around which it is found (Broch 1924). It does, however, occur at considerable depths offshore. It was found 40 miles west of Stromo and about 10 miles N.W. of Sando in the Faroes by Stephenson (1929) who (1938) also reported it as far as 230 miles off the shores of Iceland. It would be reasonable to assume that the greatest depths recorded are exceptional and some of these records may even refer to material that has been carried down or dropped from ships.

V. stroemia was considered by Broch (1924) to have moderately euryhaline tendencies which allow it to penetrate far into Oslo fjord. He also stated that this tendency enabled the species to extend its distribution into the Arctic although he did not elaborate on this point. Bertrand (1943) has reported the barnacle from the

Rance at Saint Servan and of fairly common occurrence in the river Trieux as well as on the southern shore of the Fosse de Lezardrieux. Sars (1911) said that it was found at the mouth of the Stordal. The species has been found in Loch Etive near the S.M.B.A. laboratory at Oban, Scotland where at times surface salinities are low. Schaper (1922) did not report it from the Baltic. Although there is, therefore, some field evidence for a tolerance of low salinities no experimental evidence has thus far been reported.

There is a single report of a certain tolerance to desiccation by Bertrand (1943), based apparently on observations at Plouha in the bay of Saint-Brieuc, but his evidence is not clearly given. There is no other information concerning the resistance of V. stroemia to desiccation.

Substrata

V. stroemia is commonly found attached to the shells of bivalves (Darwin, 1854; Sars, 1911; Pilsbry, 1916; pers. obs.) In the Clyde it is found on Pecten sp. and Chlamys and commonly with Balanus balanus which is also at the same time epizoic on these shells. The species is at times found on the stalks and holdfasts of Laminaria plants at low tide (MacDonald, 1941). It is common on loose rocks when they are not grossly disturbed as in Loch Etive, and as well as on the underside of stones (Marine Fauna of the Isle of Man, 1963; Plymouth Marine Fauna, 1957). It has been reported on such things as floating pieces of wood

and the carapaces of crabs (MacDonald, 1941).

General morphology

The shell of Verruca stroemia is very depressed and asymmetrical. It is subcircular and varies in coloration from one individual to another from white to a dark yellow-brown. The species is quite small with rare individuals reaching a basal diameter of 9 to 9.5mm. None of the six plates making up the shell resembles any other. The two moveable valves, the scutum and tergum, close upon the fixed scutum and tergum thus closing the opercular opening. When compared with the scuta of other balanids the moveable scutum of V. stroemia is unusual in being smaller than the moveable tergum. It may be recognized most easily by its attachment to the scutal adductor muscle in a dissected animal. The operculum is surrounded by four plates, the fixed scutum, fixed tergum, carina and rostrum, and these enclose the body of the animal. The moveable scutum and tergum together form a lid which closes down on to the fixed scutum and tergum. The fixed scutum bears on its inner wall a much enlarged adductor ridge which serves for the attachment for the adductor scutorum muscle. The four plates which surround the body of the animal are joined by more or less distinct sutures. The most obvious of these is that between the rostrum and carina. It is formed by the junction of several interdigitating folds from each plate: the folds at the top of the

plates are larger than those at the basis. There are comparable sutures between the rostrum and fixed scutum and the carina and fixed tergum, but these are smaller and their folds shorter and fewer in number. Darwin (1854) has discussed the growth of the shell; lateral growth takes place at these sutures. Such growth would add to the diameter of the shell; upward growth occurs at the basis. There is no growth at the suture between the fixed scutum and tergum ~~and it~~ which is difficult to see, and, instead of being formed by folds as are the other sutures, it is straight.

Between the two scuta runs the adductor scutorum muscle and attached to it is the body of the animal which is held parallel to the substratum when the shell is closed. The position of the body, parallel to the substratum, is brought about by the arrangement of muscle attachments for the adductor scutorum on the fixed scutum (with its peculiarly developed adductor ridge) and moveable scutum.

The body of V. stroemia is not short and rounded within the shell as is that of, a typical balanid; instead it is relatively longer and laterally compressed. The body and curled cirri fill much of the mantle cavity under the rostrum and carina, but space still remains above the membranous basis which, in the mature animal, becomes filled with ovary and later with egg masses.

The breeding cycle and population size structure

It is evident from the results of Pyefinch (1948) that the early nauplius stages of Verruca are present in the plankton throughout the year at Millport, Scotland: he gave no data on the adult animals, but suggested that some settlement probably takes place at least over the period from April to September, and notes that Fischer-Piette (1932) had recorded settlement in northern France during early December. Pyefinch (loc. cit.) also found that the early nauplius stages were abundant in the plankton by mid-February, that there was a second large peak in early March, and that this was followed by a number of smaller peaks - mid-May, June-July, and early October - later in the year. All these data indicate the production of a number of broods during the year but do not give any information as to whether they arise as a result of the same animal giving more than one brood. This can most easily be determined with certainty if a single animal is followed throughout the year; this cannot, however, be done if animals have to be sacrificed in order to determine the state of their gonads. Crisp & Davies, (1955) overcame this difficulty in the case of Elminius modestus, which has a relatively thin membranous basis, by growing the animals on glass slides and examining 'known' animals from the underside without removing them from the substratum. Only natural populations of Verruca have been examined here.

The reproductive organs

Verruca is a hermaphroditic species: although, since isolated individuals have been found with egg masses in the mantle cavity, self-fertilization appears possible, cross-fertilization is believed to be normal. (Barnes & Crisp, 1956).

The ovaries, as in other Balanomorpha, are paired structures lying between the mantle cavity epithelium and that lying over the basis. They are tubular structures with many branches which distally unite to give common paired oviducts. The ova are produced in the blind tubules which resemble those of the testes (Darwin, 1854). At the time of copulation ova pass up the oviducts (which open to the exterior at the base of the first cirri) and it is there that they are fertilized. The whole mass of eggs is surrounded by a thin membrane which is secreted by the oviducal gland, itself situated at the distal end of the oviduct. Following fertilization, the egg masses, while still very 'fluid', are manoeuvered downwards on either side of the body into the mantle cavity. Because of the marked asymmetry of Verruca neither the ovaries nor the egg masses are equal in size as is the case in all Balanus sp: nor do they lie symmetrically on either side of the cavity (see Fig. 20).

The male organs consist of paired testes, paired seminal vesicles, and a penis capable of considerable extension. The

testes are found in the prosoma. In the mature animals they surround the 'U'-bend of the alimentary ~~tract~~ tract of the animal and also extend into the thorax. They are not visible through the body wall of an immature animal. When the animal begins to produce spermatozoa the testes are easily seen as grey-white to dense white areas within the body. They are composed of a mass of minute tubules (Darwin, 1854) which are of similar diameter throughout their length when development first starts: later they expand at their blind ends and so become more club-shaped. The spermatozoa pass along the tubules and enter the vesiculae seminales which are present on either side of the body. The vesiculae seminales are hardly visible through the body wall until they contain spermatozoa, when they first appear as narrow white cords on either side of the gut. As gamete production proceeds the vesiculae seminales become more and more distended with semen and they are eventually very conspicuous on dissection of the animal.

The material and methods

Samples were taken at intervals over a fifteen-month period (June, 1970 to August, 1971) from a natural population growing largely on Pecten (but some on stones) in the Fairlie Channel, Millport, Scotland, at a depth of $\approx$ 20 m. A sample of this

population*, from which all small animals had been removed so that it consisted only of the 5.0-7.0 mm basal diameter size-class, was transferred to Oban, Scotland, maintained on a raft, and sampled from ~~July~~ February, 1971 - ~~August~~ March, 1972. A limited number of samples were obtained over the spring months of 1971 and 1972 from material

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* The top valves of about 120 Pecten bearing small to medium-sized Verruca stroemia were selected. All adhering material other than the barnacles required for the experiments was scrubbed from the shells with a brush. The remaining V. stroemia were then 'thinned out' so that those left on the shells would have enough room in which to grow, yet would be close enough to each other for copulation. The clam shells were then bored with a $\frac{3}{8}$in. drill, bolted to plastic panels mounted on metal frames, and suspended from a raft in the bay near the laboratory. The frames were brought in to the laboratory at roughly monthly intervals, when the shells were cleaned and the barnacles thinned out when necessary.

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dredged at 30 m in the upper part of Loch Etive, at Bonawe; these animals were growing on stones.

On each sampling occasion the basal diameter of 100 animals, randomly chosen, was measured under a dissecting microscope with an ocular micrometer. A random sample of 30 animals was removed from the substratum after measuring their basal diameters: the state of the gonads was recorded and the presence or absence of egg masses determined: a further 70 animals were then examined for the presence of egg masses. After taking note of the appearance of the ovary of each animal in the sample (30 individuals) the ova were lightly teased out and those readily separating, pooled, allowed to 'round off' and the diameter of 20, chosen at random, measured under the microscope using an ocular micrometer.

The size at maturity

During the whole of this investigation one animal with a basal diameter of 1.5 mm had egg masses: otherwise, below a size of 2.3 mm no fertile animals were found.

The breeding cycle

Since the animals exposed on the raft were all large adults the observations on the reproductive cycle are not complicated by

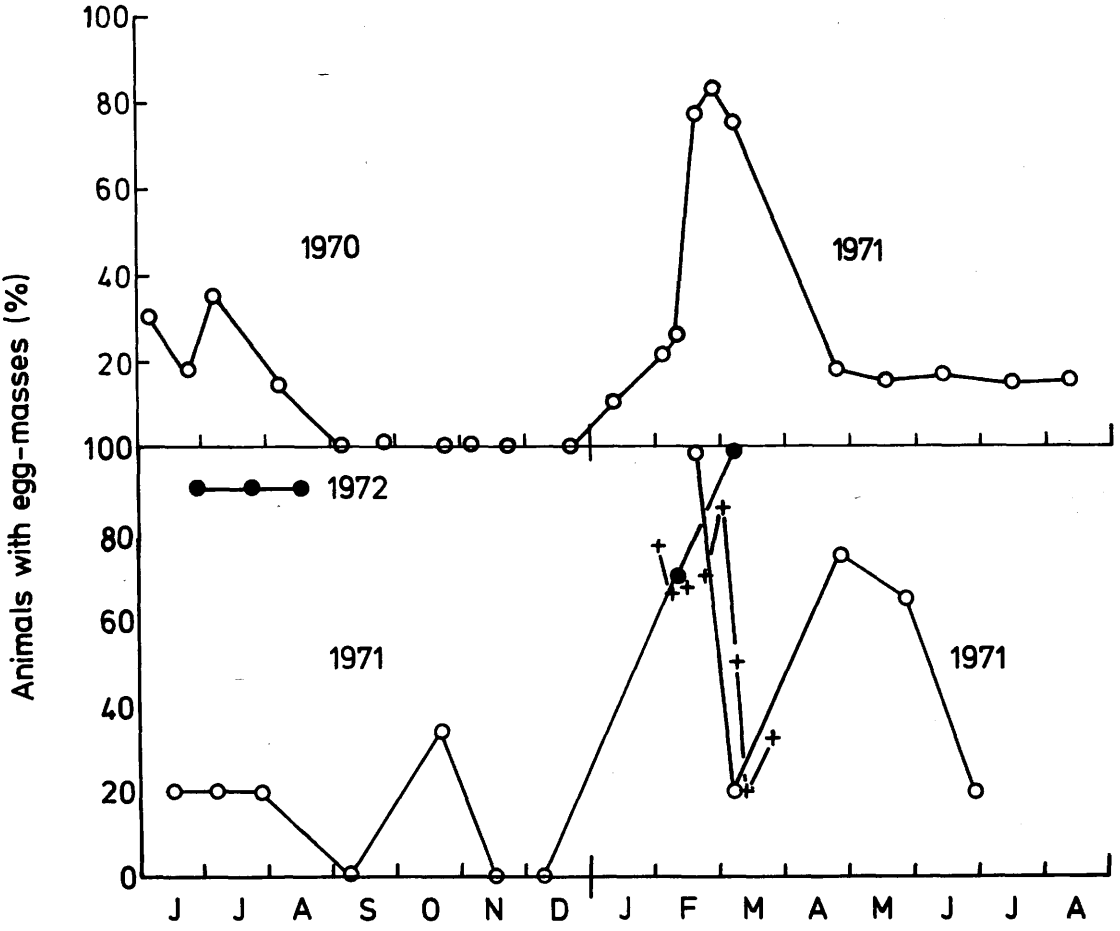

Fig. 2. Verruca stroemia: seasonal changes in % animals with egg masses in mantle cavity: upper, natural populatiom (Millport); lower, large animals only from the same population transferred to and maintained on, raft (Oban); +—+—+, additional spring samples, Bonawe.

Table I

Verruca stroemia: raft exposure: %animals with egg masses on various sampling occasions.

Date	% animals with egg-masses.	Date	% animals with egg-masses.
18/2/71	100	7/9/71	0*
16/3/71	25	19/10/71	33
26/4/71	75	16/11/71	0
26/5/71	65	8/12/71	0
28/6/71	20	9/2/72	70
26/7/71	20	6/3/72	100

* The Sept. sample was smaller than the others (5 animals) and this may be reflected in this result.

the entry, growth, and possibly breeding of new recruits into the population sampled; they will be considered first (Fig.2; Table I). In both years (1971 and 1972), 100% of the population contained egg masses in early to mid-February and the embryos in these were well advanced and in a very uniform state of development. Release of these embryos apparently took place in late February and the early part of March as is indicated by the fall in numbers of the animals with egg masses: this would correspond to the appearance of a large population of stage I nauplii observed by Pyefinch (loc. cit.) in his plankton hauls at about this time: there is no indication in these results - or indeed in any of those discussed below - of two maxima in the numbers of animals with egg masses in early spring which would correspond to his two peaks (mid-February and early March) of nauplii abundance. These two nauplii maxima may have arisen from the consecutive two sets of the appropriate kind of conditions stimulating the release of nauplii from a developmentally synchronous population, and perhaps related to a diatom outburst and not from distinct broods: more likely, his results were influenced by the problems associated with single-station plankton sampling and, indeed, his sampling methods themselves were only semi-quantitative.

It is evident from Table II that gonad development leading to the production of this spring brood took place during the winter months. There was a second maximum in the numbers with egg masses

in late April (26th) but at the maximum only 75% of the population were involved in contrast to 100% for the first maximum of the year. This second maximum was much less sharply defined and it would seem that the population was less synchronous. The age structure of the population of embryos with the animals bearing egg masses was not, however, determined. ~~There can be littl~~ ~~Evidently~~ most - and ~~there is little reason not to~~ probably all - the animals gave two broods in the early months of the year. The interval between these two maxima is some 60 days which, although adequate for brood development in a warm or even warm - temperate species under optimal conditions, in other characteristically boreo-arctic species that have been investigated has not been found adequate for the re-development ab initio of the ovary, fertilization, and embryonic development (Crisp, 1959; Barnes & Barnes, 1954 ; Crisp, 1954): in Balanus balanus, and B. balanoides for example, embryonic development alone takes about 30-40 days. It is evident, however, from Table II , that while the egg masses of the first spring brood were developing in the mantle cavity, in a large proportion of the population well developed ovaries were already present underneath them and the female gonads continued their development during the spring. It has been shown for Elminius modestus that even when breeding is at its height, with broods succeeding one another in rapid

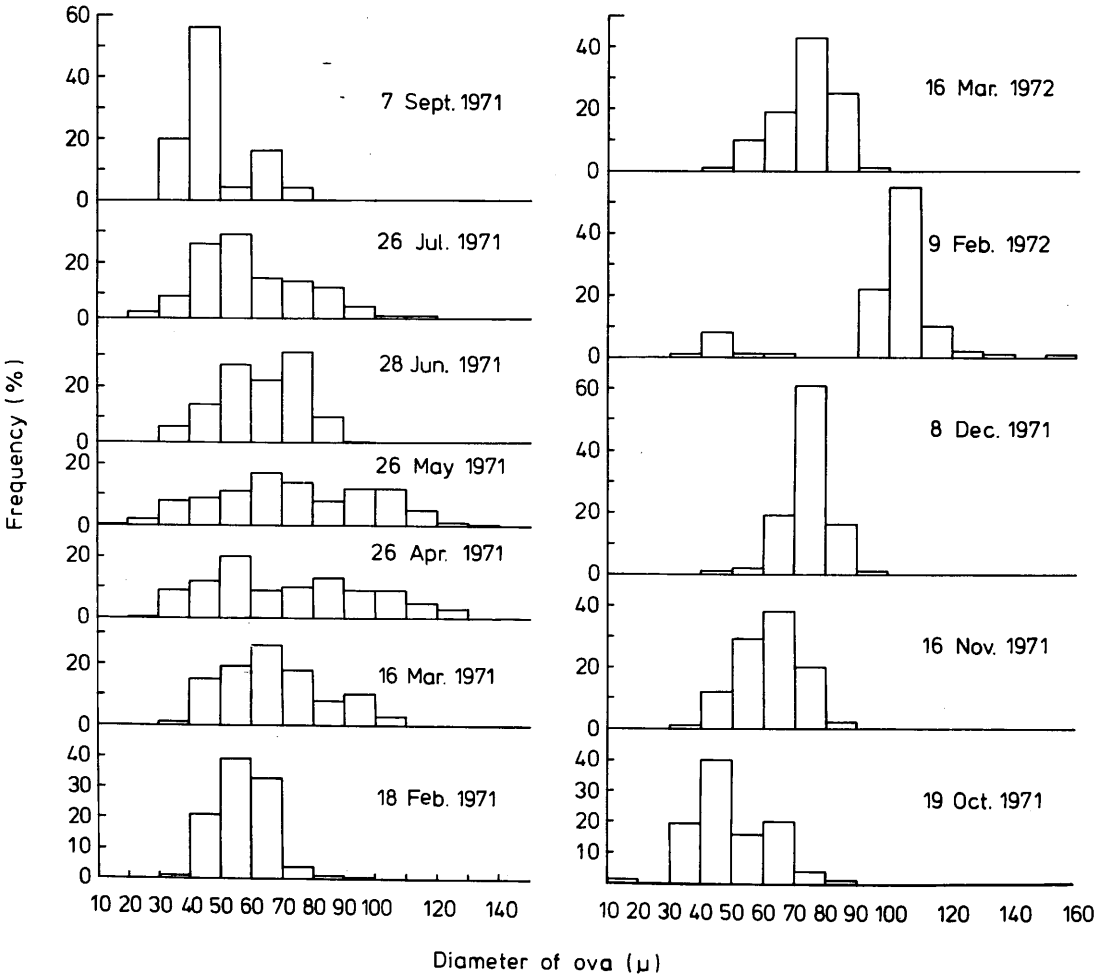

Fig. 3. Verruca stroemia: frequency histograms for mean diameter of ova; population transferred and exposed on a raft (Oban).

Table II

Verruca stroemia: numbers of ova in size classes (μ); large animals from Millport population maintained on raft at Oban.

	SIZE CLASS														
	10 - 19	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	70 - 79	80 - 89	90 - 99	100 - 109	110 - 119	120 - 129	130 - 139	140 - 149	150 - 159
18th Feb. 1971	0	0	3	46	85	71	8	2	1	0	0	0	0	0	0
16th Mar. 1971	0	0	3	31	40	55	38	16	22	6	1	0	0	0	0
26th Apr. 1971	0	1	27	34	58	28	30	37	28	26	15	10	1	0	0
26th May 1971	1	8	35	36	42	67	54	30	48	47	19	5	1	0	0
28th Jun. 1971	0	0	14	34	72	58	83	24	1	0	0	0	0	0	0
26th Jul. 1971	0	4	11	39	44	21	20	17	6	1	1	0	0	0	0
7th Sep. 1971	0	0	5	14	1	4	1	0	0	0	0	0	0	0	0
19th Oct. 1971	1	4	53	109	43	58	12	3	0	0	0	0	0	0	0
16th Nov. 1971	0	0	4	38	95	124	64	5	0	0	0	0	0	0	0
8th Dec. 1971	0	0	0	2	6	50	157	41	2	0	0	0	0	0	0
9th Feb. 1972	0	0	1	14	2	1	0	0	36	90	16	3	1	0	1
16th Mar. 1972	0	0	0	3	32	59	134	76	4	0	0	0	0	0	0

succession, a second brood is never brought into the mantle cavity of a given adult until the previous one has been released (Crisp & Davies, 1955). In this raft-maintained population of Verruca, then, it would appear that as soon as the spring brood is released in February, the well-developed ovaries, already present throughout much of the population, complete their development, become fertilized, and the egg masses are transferred to the mantle cavity: the maximum is less sharply defined and the synchrony is less marked (maximum numbers with egg masses, 75%) than is the case with the very first brood of the year. Breeding continues at a low intensity throughout the summer months with little synchrony throughout the population; between June and August the number of animals with egg masses is ≈ 20%. Fig. 2 indicates a minor maximum in October but it must be emphasized that this is dependent upon the reliability of the September results - and this was from only a small sample (5 animals instead of the usual 20): in spite of this reservation it does, nevertheless, correspond with the autumn maximum of nauplii observed by Pyefinch at Millport. Only in November and December are egg masses absent. The results of the measurements of the ova are given in Fig. 3 and Table II, and they correspond with the above observations on brood production. On 8th December, 1970

there is a single maximum at 70–80 μm indicating a considerable degree of homogeneity in the population: these are the ova that give rise to the major spring brood: by 9th February 1972 the maximum frequency was at 100–110 μm. Fertilization began in early January and was not completed until early February. In these late winter and early spring ~~(except January)~~ months the size-distribution of the ova is indicative of a single population being sharply defined and this is responsible for the considerable degree of synchrony which becomes evident in March when the whole population has egg masses. On the 9th February a second population of eggs with a smaller mean size (maximum, 40–50 μm) is also present. This population of ova, developing under the egg masses of animals which become fertilized, increases in size in February and March (peak 70–80 μm). In 1971, by February 18th when 100% of the animals had egg masses it appears that no large eggs (100–110 μm) remained to be fertilized: they had all contributed to the 100% of egg masses. A single population (maximum, 50–60 μm) was present underneath these egg masses. It is this brood, somewhat less synchronous, than the first, which gives rise to the peak in the population of egg masses in April. Between April and September there is a wide spread in the ova sizes and clearly some are continuing to contribute to the succession of broods produced

Table III

Verruca stroemia: Condition of the testes and seminal vesicles on various sampling occasions: raft exposure: -, empty; (-), residual semen in seminal vesicles; +, slight development of testes or slight filling of seminal vesicles; ++ increased development of content; +++, maximum development or moderate distention; ++++, maximum distension of seminal vesicles.

Date	% of sample	Testes	Seminal vesicles	Date	% of sample	Testes	Seminal vesicles
1971							
Feb.18	100	-	(-)	Sept. 7	60	-	+++
					40	++	+
Mar.16	20	-	(-)	Oct.19	30	+++	+
	40	-	-		70	-	+++
	40	+	-				
Apr.26	95	++	+	Nov.16	95	++	+++
	5	-	(-)		5	+++	+
May 26	55	-	+++	Dec.8	100	-	++++
	20	++	+				
	15	+	-				
	10	-	-				
Jun.28	50	+++	+	1972	65	+	+
	25	+	-	Feb.9	10	-	-
	20	-	+++		25	++	+++
	5	-	-				
Jul.26	30	+++	+	Mar.6	70	-	(-)
	10	-	++		20	+	++
	60	+ or ++	+		10	-	+++

throughout the summer as is clearly evident from the examination for egg masses. The rather more clearly defined population of ova present on the 7th September may have given rise to the small peak in egg mass numbers observed on 19th October (see, however, the reservations above).

Observations were also made on the male gonads (Table III). As would be expected in view of the considerable synchrony in the population, the testes were poorly developed and there was very little semen in the vesiculae seminales in February 1971 or March 1972 when all the animals contained egg masses; this is clearly the result of the loss of semen at the copulation which produced the first spring brood. By March 1971 when many of the animals had released their nauplii and ova were rapidly maturing, 40% of the animals had poorly developed testes, and no semen in the vesiculae seminales, in 40% the testes were clearly re-generating, and in the remainder some semen was present in the vesiculae seminales. In April testis development had advanced and a majority of the animals had semen in the vesiculae seminales. In May, 55% of the animals had their seminal vesicles destended with semen. Re-development of the testes and production of ripe spermatozoa had proceeded along with that of the ova so that semen was present in a large proportion of the animals and copulation to give the second spring brood could

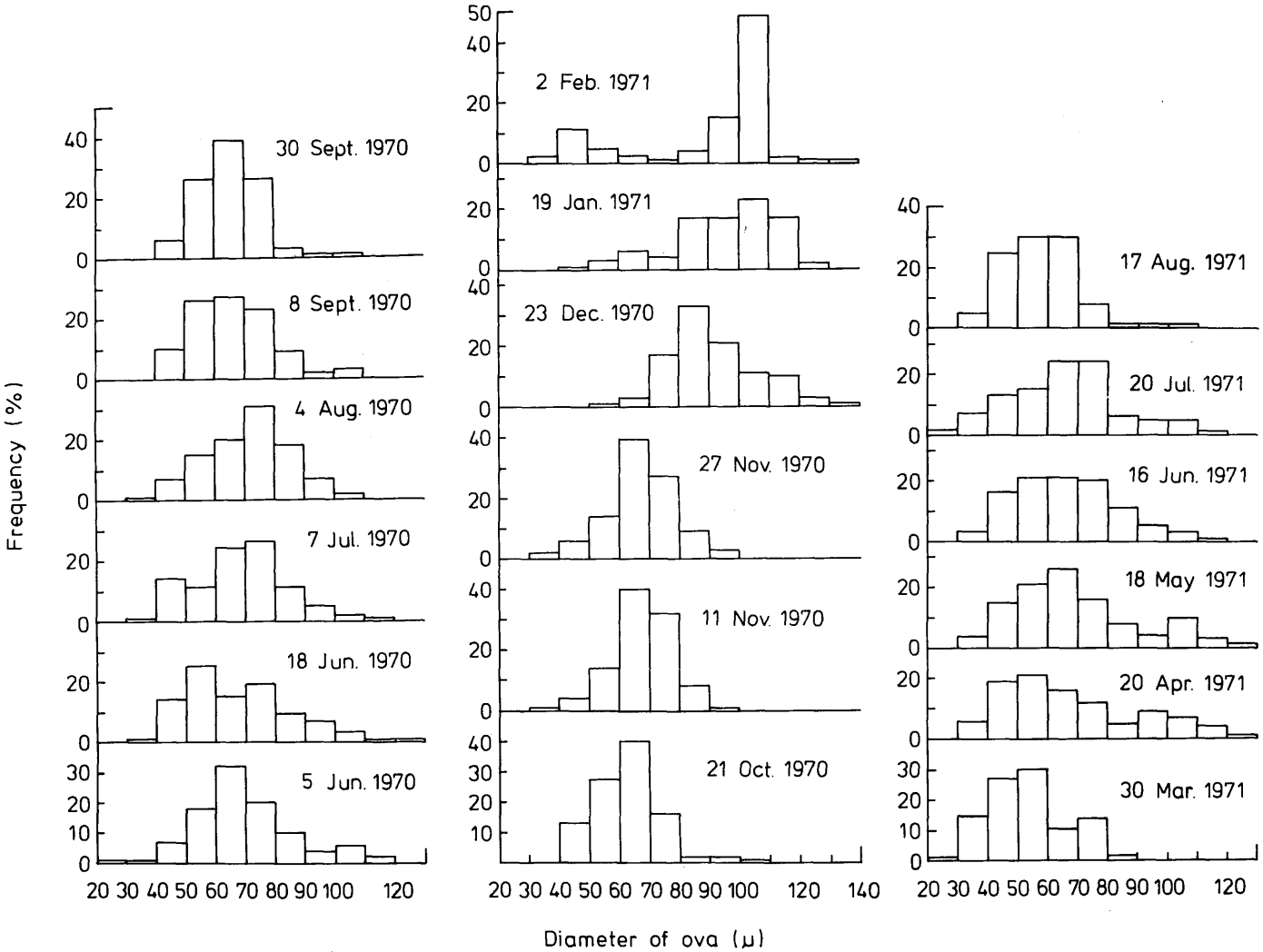

Fig. 4. Verruca stroemia: frequency histograms; ova from natural population (Millport).

take place. During the summer 30-50% of the animals had well developed testes, the numbers with their vesiculae seminales full of semen being variable. By September, although 60% of the animals had distended vesiculae- the contents of which no doubt fertilized the ova responsible for the October maximum in the numbers with egg masses - the testes themselves were relatively poorly developed. Between October and December there was a continuous development of the testes and, by late winter, the vesiculae seminales had become vastly distended with semen; they were the most conspicuous feature seen on dissecting an animal. It seems that the conditions found in the female gonads were paralleled in the state of the male gonads at the same time. From a comparison of the February and March samples in the two years 1971 and 1972 it seems possible that the male gonads were further advanced in their development in 1972: the spring diatom increase began earlier in 1972 and the earlier provision of nutrients may have been responsible for this difference.

The results of the observations on the natural population (Fig. 2,4; Table IV) will now be considered and may be discussed relative to the above. Once again the annual cycle is clearly dominated by a peak in the 'breeding' activity culminating in 83% of the animals having egg masses in the mantle cavity by mid-February.

Table IV

Verruca stroemia: % animals from Millport with egg-masses on various sampling occasions.

Date	% with egg-masses	Date	% with egg-masses
1970		1971	
Jun. 5	30	Jan. 19	10
Jun. 16	18	Feb. 2	22
Jul. 7	35	Feb. 9	26
Aug. 4	14	Feb. 16	77
Sept. 8	0	Feb. 23	83
Sept. 30	1	Mar. 2	75
Oct. 21	0	Apr. 20	18
Nov. 11	0	May 18	15
Dec. 23	0	Jun. 16	17
		Jul. 20	15
		Aug. 17	16

The maximum is less sharply defined and it would seem that the synchronization is less than that in the raft-maintained animals which, it should be stressed, originated from the same sublittoral Millport population. Furthermore, there is no evidence for a second spring brood in this natural population. Conclusions about breeding periods as determined from data such as these must always take into account the intervals between sampling and it must be emphasized that no samples were taken from this natural population between 2nd March and the 20th April. A maximum in this interval would not have been detected. It is interesting to observe that the few spring samples taken from a natural population in Loch Etive at Bonawe in both 1971 and 1972 indicate that in this sublittoral population the maximum was, like the raft population more sharply defined, (although the numbers with egg masses never reached 100%) and there is an indication of a second spring maximum. The breeding activity is at ≈ the 20% level during the whole summer i.e., similar to that of the raft-maintained population, perhaps with a small increase in June and July, In marked contrast with the raft-exposed animals none contained egg masses between September and December. In contrast to the raft-exposed animals, it is also evident (Table V) that those animals from the natural population with egg masses had less well developed ovaries underneath them. The size of the ova may now be considered. In the natural population there

Table V

Verruca stroemia: numbers of ova in size classes (μ); natural population (Millport).

	SIZE CLASS												
	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	70 - 79	80 - 89	90 - 99	100 - 109	110 - 119	120 - 129	130 - 139	140 - 149
5th Jun. 1970	1	1	17	43	77	48	24	10	14	4		0	0
18th Jun. 1970	0	1	20	53	33	40	21	15	7	1	2	0	0
7th Jul. 1970	0	2	57	42	87	95	40	20	7	2	0	0	0
4th Aug. 1970	0	1	11	25	32	51	29	11	4	0	0	0	0
8th Sep. 1970	0	0	20	55	57	49	19	5	6	0	0	0	0
30th Sep. 1970	0	0	11	46	66	45	6	2	1	0	0	0	0
21st Oct. 1970	0	0	34	71	103	42	4	4	1	0	0	0	0
11th Nov. 1970	0	3	13	47	134	108	28	2	0	0	0	0	0
27th Nov. 1970	0	6	19	49	133	93	31	12	1	0	0	0	0
23rd Dec. 1970	0	0	0	3	14	68	134	83	44	41	14	3	0
19th Jan. 1971	0	0	1	9	16	38	50	50	68	50	5	0	0
~~9th~~ 2nd Feb. 1971	0	3	15	6	3	1	5	22	69	12	3	2	1
30th Mar. 1971	1	24	42	46	17	22	3	0	0	0	0	0	0
20th Apr. 1971	0	16	53	58	46	34	13	26	20	10	4	0	0
18th May 1971	0	6	32	53	46	33	15	9	20	7	2	0	0
16th Jun. 1971	0	12	57	77	63	71	39	18	12	2	1	0	0
20th Jul. 1971	2	15	28	40	46	52	14	11	10	2	0	0	0
17th Aug. 1971	0	8	41	54	46	14	1	1	1	0	0	0	0

seems only a gradual change towards a larger size between October and November, the maximum values (60–70 μm) which are less sharply defined, remaining the same. By February, however the majority of the ova are in the 100–110 μm size-class and will soon be fertilized. As in the raft-maintained animals a second population of ova also with its maximum frequency in the 40–50 μm size-class is present in February and this suggests that a second spring brood may have been missed. Throughout the late spring and summer the spread in the sizes of the ova is extremely wide, which corresponds to the continuous production of broods, so that at any one time only a small number of animals have egg masses: generally, in all the samples from the natural population the spread at a given date is always greater than in the raft-maintained population.

Population size-structure

The basal diameter of a random sample of animals was measured at about monthly intervals (Fig.5; Table VI). The smallest size-class (0–1 mm) was always present (June 5th, 1970, excepted) and this suggests continuous input into the population. The small numbers in the 0–1 mm size-class during the winter corresponds to the absence of any input since in this natural population egg masses were absent from the adult mantle cavities at this time. During the spring and summer months when growth is maximal, in the absence of any input, the

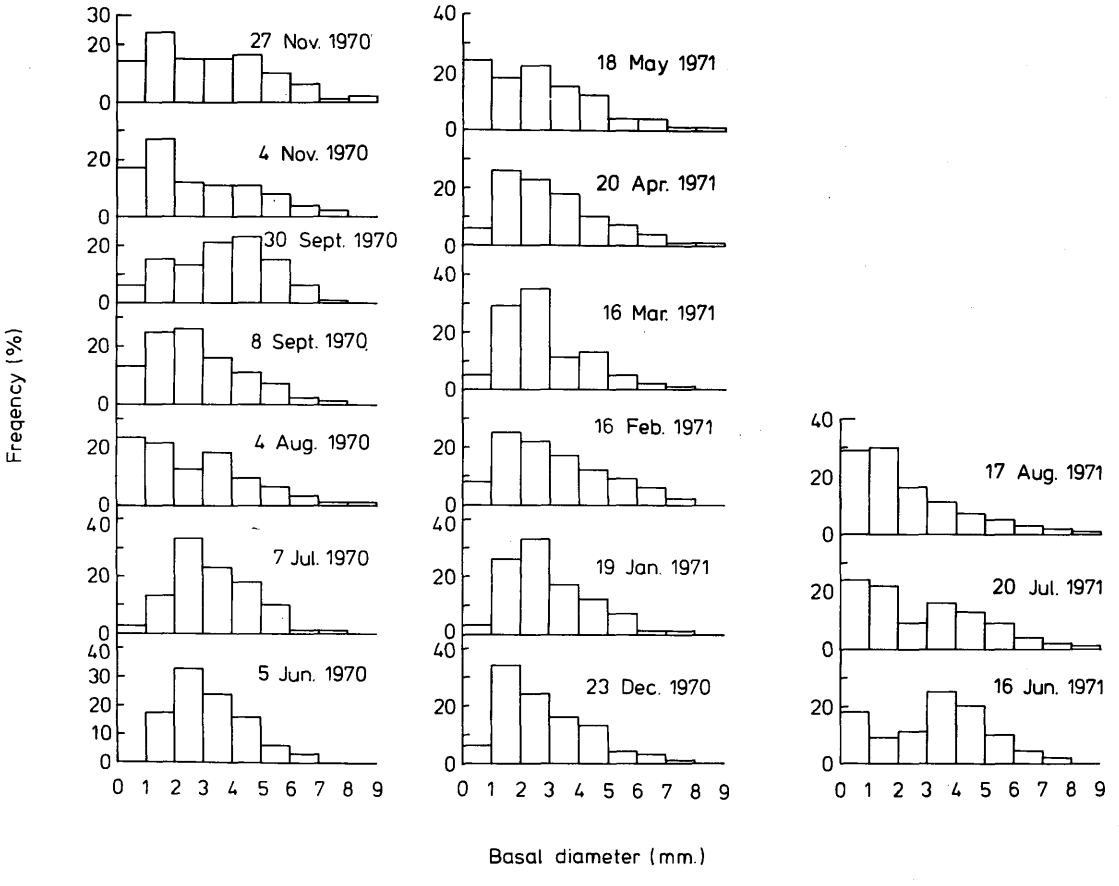

Fig. 5. **Verruca stroemia: frequency histograms for basal diameter; natural population (Millport).**

Table VI

Verruca stroemia: numbers of animals in size classes (mm); natural population (Millport).

	SIZE CLASS									
	0.0 - 0.99	1.0 - 1.99	2.0 - 2.99	3.0 - 3.99	4.0 - 4.99	5.0 - 5.99	6.0 - 6.99	7.0 - 7.99	8.0 - 8.99	9.0 - 9.99
5th Jun. 1970	0	28	53	38	26	10	5	0	0	0
7th Jul. 1970	4	24	59	41	33	18	1	1	0	0
4th Aug. 1970	112	106	61	89	47	34	17	4	3	0
8th Sep. 1970	45	85	93	54	37	25	6	1	0	0
30th Sep. 1970	11	27	23	37	40	27	11	1	0	0
4th Nov. 1970	66	105	66	63	60	29	13	6	0	0
27th Nov. 1970	30	50	31	32	33	21	12	1	2	0
23rd Dec. 1970	20	109	76	50	42	13	11	1	0	0
19th Jan. 1971	9	80	100	51	35	21	4	3	0	0
16th Feb. 1971	19	58	50	39	29	20	14	4	0	0
16th Mar. 1971	12	71	87	26	33	13	5	1	0	0
20th Apr. 1971	19	83	71	57	48	22	13	1	0	0
18th May 1971	72	54	64	43	34	11	13	2	1	0
16th Jun. 1971	59	30	37	81	67	33	13	7	0	0
28th Jul. 1971	102	93	39	66	56	37	18	9	2	0
13th Aug. 1971	96	100	54	39	26	16	8	2	1	1

0-1 mm size-class would not have been represented, since these small animals would have passed into the next size-class: in winter, however, the specific growth rate is low even at a small size, so that even in the absence of any input to the population from a further brood, small numbers of this 0-1 mm size-class persist throughout the winter and early spring. Juveniles probably take 3-4 weeks to reach 1.0 mm basal diameter (Barnes, 1958): the nauplii stage I were being released throughout March and probably some three weeks are needed for larval development so that the increase in the 0-1 mm size-class appears in April (Fig. 5), but is only marked in May. The size-frequency histograms for the summer months again indicate a continuous overlap of size-classes.

Discussion

It is evident from the foregoing that Verruca stroemia although largely a boreo-arctic species with a geographical distribution in the eastern Atlantic similar to that of both Balanus balanoides and B. balanus, and B. hammeri has a more complex breeding cycle (Fig. 6) than all the three latter species, with their single annual brood which is released in the spring at a time when, in northern waters, there is abundant planktonic food

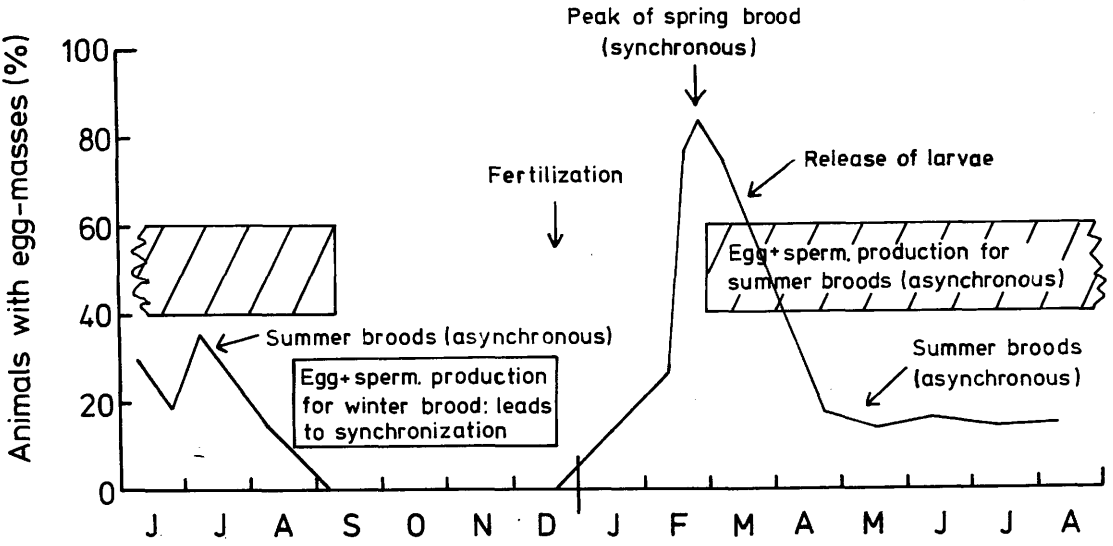

Fig. 6. Verruca stroemia: diagram of breeding cycle of the natural population.

for the larvae. The major, virtually synchronous, brood in Verruca is, nevertheless, still produced in the spring. The completeness of the spring synchrony may depend largely on nutrient conditions: when the animals from a sublittoral population are exposed on a raft and kept clean from all other organisms and non-living debris and mud the synchrony, as demonstrated in two successive years, is complete so that in early spring all the animals have egg masses; Barnes (1955) has shown that under these conditions growth rate is far in excess of that of natural shore populations. The 'same' population (Millport) in its natural habitat was almost, but not completely, synchronous, as was the sublittoral population from another locality (Bonawe). The greater availability of food in the photic zone, the better conditions of water-flow, and the absence of mud and competing organisms on the raft all contribute to the better environment. It seems evident that in the natural population some of the animals settling in late summer and early autumn do not reach the size for maturity of ≈ 2.0 mm (see presence of 0-1.0 mm size-class in the autumn and winter months, Fig. 5) in time to contribute to the first spring brood. Since water temperatures fall throughout the winter and do not reach their minimum until March it does not seem likely that low temperatures are responsible for this reduction in breeding potential during the winter. This fall in the reproductive potential - most marked in the natural population in early winter-may therefore also be ascribed to the poor nutrient

conditions at this time in northern waters. Nevertheless, Verruca would seem to be able to utilize organic debris too (see p. 79) and ovarian development proceeds and, over this long period, all the ova are brought into the same condition by spring.

As already pointed out (p.10) direct proof that a given animal can produce more than one brood in succession requires observations on individual animals without sacrificing them, but the production of a maximum with 83% of the animals with egg masses following one with 100% with egg masses (both estimated on random samples) in restricted raft-maintained population (with no input of breeding animals from the previous brood) indicates that a large proportion and, when the greater spread of the second maximum is taken into account, probably all, the animals can produce two major broods. The succession of overlapping broods during the summer indicates further non-synchronous breeding activity - at a much lower level - by the same adult population. In the natural population the situation is more complex: members of the spring brood and some of the latter broods may grow to sexual maturity and contribute to the summer breeding population of the same year.

One further point may be mentioned regarding the low level of breeding activity and absence of synchrony during the summer. If the reproductive development of a population is not synchronous

then there is no reason to expect the neighbours of a given animal, spatially near enough to allow copulation to take place, to be in the appropriate reproductive condition to lead to successful insemination which may, therefore, in an obligately cross-fertilizing species, be delayed (or even prevented if the waiting time is so long as to allow gonad regression) ~~be delayed~~ until a neighbour is in the appropriate state. In Verruca such a delay might also lead to self-fertilization probably with the production of fewer and less viable eggs (Barnes & Crisp, 1956). Any small synchronous peaks that might be expected from the mean state of the gonads would be damped down under these conditions.

The fact that Verruca can develop the female gonads during the winter months so that by the time the population has 100% egg masses many of the animals have well-developed ovaries, suggests that this species is far less directly dependent on diatoms for its nutrition (see p. 79). Furthermore, when a second brood follows, as in the raft population, reproductive anecdysis cannot be prolonged. This is, perhaps, not surprising, since unlike Balanus balanoides, it is a sublittoral species. On the other hand, B. balanus is also sublittoral yet its life history seems, like that of B. balanoides, to be closely 'geared' to the spring diatom increase of northern waters. Perhaps the synchrony in the

spring is brought about by the poor nutrient conditions in winter which only allow the ova to develop slowly over a relatively long period of time so that they are brought into a similar state at about the time of maturation: beyond this time conditions are more favourable. It has been shown that the breeding cycle of Balanus balanoides is under control both from the environment and by endogenous factors. There is an upper temperature above which gonad maturation is not completed and a requirement regarding light period; about 4–6 weeks at a temperature lower than $\approx 10^{\circ}C$ and with less than 12 h light per day is essential for maturation (Barnes, 1963). Even when optimum conditions for development are available it has, so far, not been possible to shorten the breeding period more than about 4 weeks: by starving the animals at the appropriate time the onset of breeding can, however, be delayed for several months. Nothing is known concerning the effect of light or temperature on the breeding of Verruca, not is anything known of the breeding cycle in other localities or of any changes with depth: it would not be surprising if, in deeper waters with their more constant environment, breeding were continuous throughout the year.

It should be pointed out that the breeding cycle cannot be directly correlated with the littoral or sublittoral habitat, at

least as regards the few species on which information is available. Balanus balanoides, B. balanus and B. hammeri all have a single, annual spring brood: the first is littoral, the second and third largely, or completely, sublittoral species. On the other hand, B. glandula and B. crenatus, the one littoral and the other largely sublittoral, produce more than one brood (Pyefinch, 1948; Barnes & Barnes, 1956). In the case of B. balanoides the reason for a single brood may be sought in its adaptation to the extreme conditions of the Arctic where it is present under the shore ice foot for many months. B. glandula does not extend into such a severe environment. The other anomalies, however, remain.

Nauplii release, the spring diatom outburst, and hatching substance.

It has been known for a long time that many boreo-arctic animals 'release' their young at about the time of the spring diatom outburst and, in the absence of any evidence to the contrary, common environmental factors - probably related to the marked seasonal changes in northern waters - have usually been assumed to be responsible for this synchronization. The terms nauplii or larval release are preferred to the commonly used 'spawning' since in many invertebrates 'spawning' refers, as it should, to the release of gametes and this may be - and usually is - initiated by entirely different factors. The use of 'spawning' to describe the release of larvae has lead to confusion and some misunderstanding. The survival value of such a synchrony is not far to seek; when larvae are liberated at the time of the spring diatom outburst abundant food will be available for their development; this becomes more and more important on proceeding further north until, in Arctic waters where the season is short and the waters particularly barren except at this time, it is an over-riding consideration. How a common environment factor, or factors, that would initiate the diatom outburst, which depends upon the establishment of a stable water column and adequate light energy, and at the same time cause the release of invertebrate larvae is difficult to imagine even though the ambient light regime is well

known to affect many animal processes and activities. A more simple causal mechanism would postulate the larval release to be directly dependent upon - and, indeed, triggered by, the diatom outburst. Such a causal relation has been demonstrated for Balanus balanoides. Nauplii release occurs at the time of the spring diatom increase whenever that takes place over the whole area of distribution of this species and extracts of adult animals will stimulate the release of nauplii from free egg masses (Crisp, 1956; Barnes, 1957; Crisp & Spencer, 1958). The effect of extracts is immediate and direct stimulation of the embryo rather than changes in any gross metabolic process seems to be involved. The substance responsible for hatching appears to be restricted to cirripedes but, as far as has been tested, is non-specific within the group. Although for one species, B. nubilus, the effect can be mimicked by 5-hydroxy-tryptamine (Barnes & Barnes, 1959) this does not appear to be the factor responsible in B. balanoides (Crisp, 1969). It is a product of the animal's own metabolism and, according to Crisp (1969), is produced in vitro only on maceration of the tissues. Crisp and his co-workers are of the opinion that in B. balanoides the presence of a hatching factor, derived from the adult either naturally or applied as an extract, is essential to ensure liberation of nauplii. Embryos, however, developed outside the mantle cavity will certainly hatch without the addition of any extracts (Barnes & Barnes, 1963) and certainly the shore population will release nauplii,

although over an extended period, in the absence of a typical 'sharp' diatom outburst; since the embryos themselves contain the hatching substance it may well be that when conditions severely limit the intensity of the spring increase, nauplii release is initiated by a feed-back mechanism from the embryos themselves, which perhaps tend to accumulate hatching substance when the release is delayed.

It is evident from the foregoing that the major brood in Verruca, developed during the winter, is released at about the time of the spring diatom increase; the closeness of this synchrony has been investigated and some experiments carried out on stimulated hatching. In the spring of 1971 samples of Verruca were collected at short intervals offshore at Millport and in Loch Etive, and in 1972 in Loch Etive only; a hundred animals were dissected and the percentage with egg masses was determined. For comparison, samples of Balanus balanoides on the adjacent shores in both localities were also collected and the percentage with egg masses also determined. In order to determine the onset as well as to follow the course of the spring diatom outburst in a relatively simple, but for the present purpose, adequate way, the chlorophyll content of regular surface water samples (10 litres) in each locality was measured. The water samples were filtered through glass fibre filters (Whatman, GF/C) and dried in a desiccator in the dark. After cutting up into small pieces the pigment

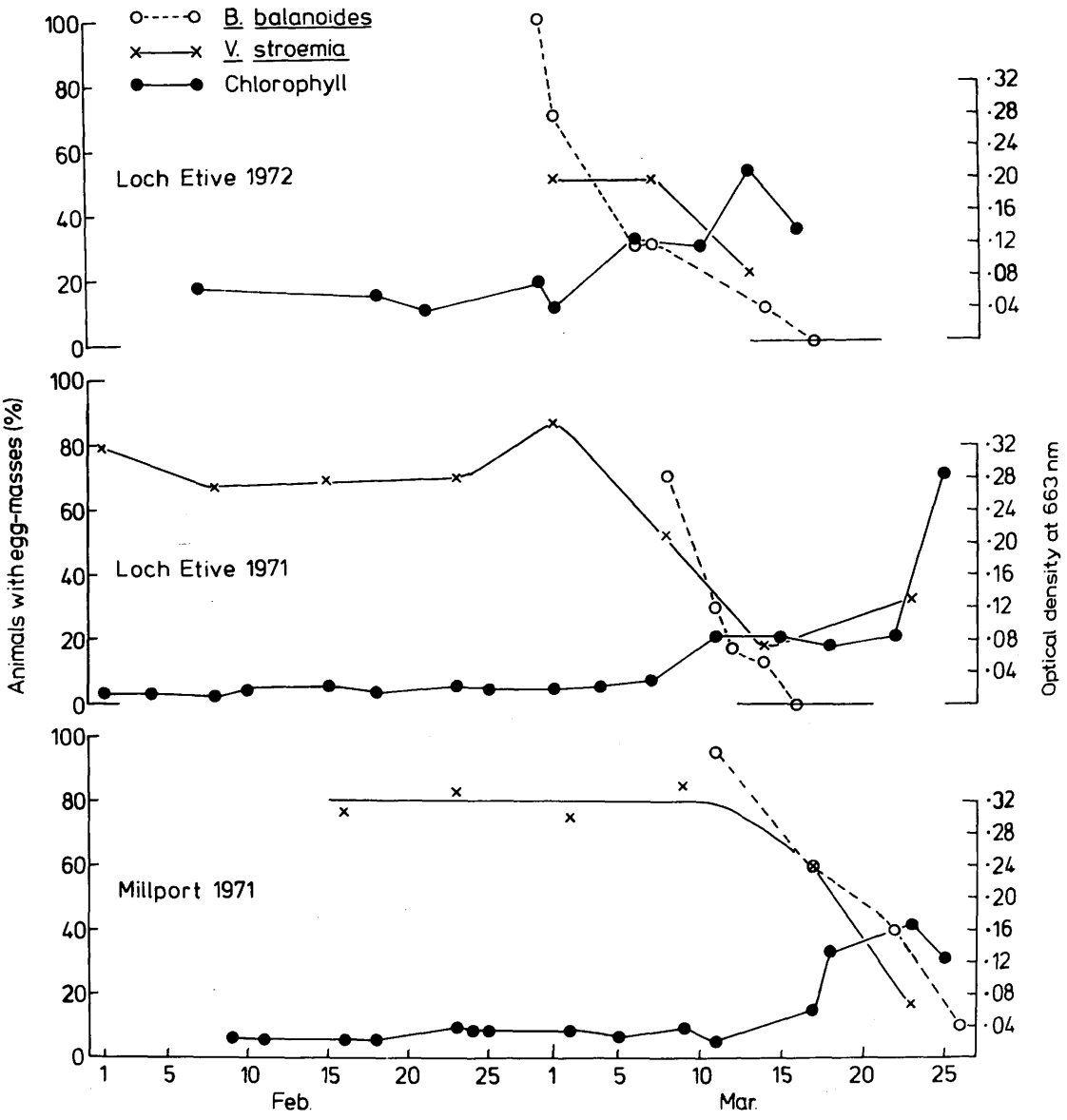

Fig. 7. Relation between spring release of nauplii (expressed in terms of % of animals with egg masses) for Verruca stroemia and Balanus balanoides and chlorophyll content (in terms of optical density of a standard sample of 10 l) of surface water samples: Loch Etive, two consecutive years, Millport, one year only.

Table VII

Chlorophyll estimation and animals with egg-masses, 1971. 10 liters of sea water filtered through G F C filters using a Millipore apparatus: chlorophyll extracted with 70% acetone (10 ml): after standing overnight and centrifuging read spectrophotometer at 663 nm. % V. stroemia and B. balanoides with egg masses at various sampling occasions.

Loch Etive				Millport			
Date	O.D. at 663 nm	Animals with egg-masses (%) V.stroemia	B. balanoides	Date	O.D.at 663 nm	Animals with egg-masses (%) V.stroemia.	B.balanoides
1971				1971			
Feb.2	0.014	79					
4	0.013						
8	0.012	67		Feb.9	0.026	23	
10	0.018			11	0.021		
15	0.022	69		16	0.023	77	
18	0.014			18	0.022		
23	0.021	70		23	0.040	83	
25	0.018			25	0.035		
Mar.1	0.019	86		Mar.2	0.035	75	
4	0.021			5	0.027		
8	0.029	52	70	9	0.036	85	
11	0.083		30	11	0.020		96
12			17	17	0.016	60	60
14			13	18	0.133		
15	0.083	18		22			40
16			0	23	0.166	17	
18	0.071			25	0.126		
22	0.085			26			10
23		33					
25	0.285						

Table VII

Chlorophyll Estimation and

animals with egg-masses continued : 1972.

Loch Etive

Date	O.D. at 663 nm	Animals with egg-masses (%) V. stroemia	B. balanoides
Feb.7	0.070		
18	0.062		
28	0.075		100
29		50	70
Mar.1	0.040		
6	0.125		30
7		50	30
9			
10	0.118		
13	0.210	21	
14			10
16	0.138		
17			0

was then extracted by macerating each filter with 10ml of 70% acetone in a boiling tube and allowing to stand overnight in a refrigerator (no magnesium carbonate was added since only comparative values were required). After centrifuging, a sample of supernatant was transferred to a 1cm cell and the optical density determined at 663nm using a Pye S.P. 600 spectrophotometer (Barnes, 1972). Since only comparative values were needed the results are plotted in terms of the optical density so determined.

The results are given in Fig. 7 and Table VII. It is evident that in 1971 the major release of nauplii in Loch Etive was at the time of a diatom outburst. The percentage of animals with egg masses in the mantle cavity remained relatively constant during February (fluctuations reflect sampling errors) but during the period 8 - 16th March fell markedly at the same time as chlorophyll values indicated the development of a diatom bloom: a large proportion of the animals had, however, released their nauplii before the bloom reached its maximum intensity later in March: during this same period, Balanus balanoides also released its nauplii. At Millport the release of the nauplii of both species did not take place until somewhat later (18 - 25th March) but this was again synchronous with a marked rise in pigment values. The fact that in both species there was synchrony between nauplii release and the rise in chlorophyll values in two quite different

localities even though synchrony was at different times lends considerable support to the contention that in Verruca, as in Balanus balanoides where the phenomenon has been adequately substantiated, the two are causally related. In view of the fact that the Verruca were collected from the sublittoral one might have expected the initiation by feeding to have been delayed relative to that of Balanus balanoides: there is no evidence of this on the time scale of the present data. In 1972 the evidence for causality in B. balanoides is stronger, the major relase of nauplii taking place early in March (29th February - 8th March) at the same time as a marked increase in chlorophyll values. At this time 50% of the Verruca had released their nauplii: it may be noted that in this year chlorophyll values throughout February had been higher than in 1972. There was a major release during the second half of March at the time of a marked rise in chlorophyll but by this time Balanus balanoides had released over 70% of its egg masses. In 1972, then, the relatively sudden release of nauplii in Verruca was somewhat delayed relative to that of Balanus balanoides.

In view of the foregoing the stimulatory effects of extracts of Balanus balanoides, B. balanus, Chthamalus stellatus, and Verruca stroemia itself on ripe egg masses of Verruca were investigated. Although for the most part the stimulating effect has been found to be common to all the

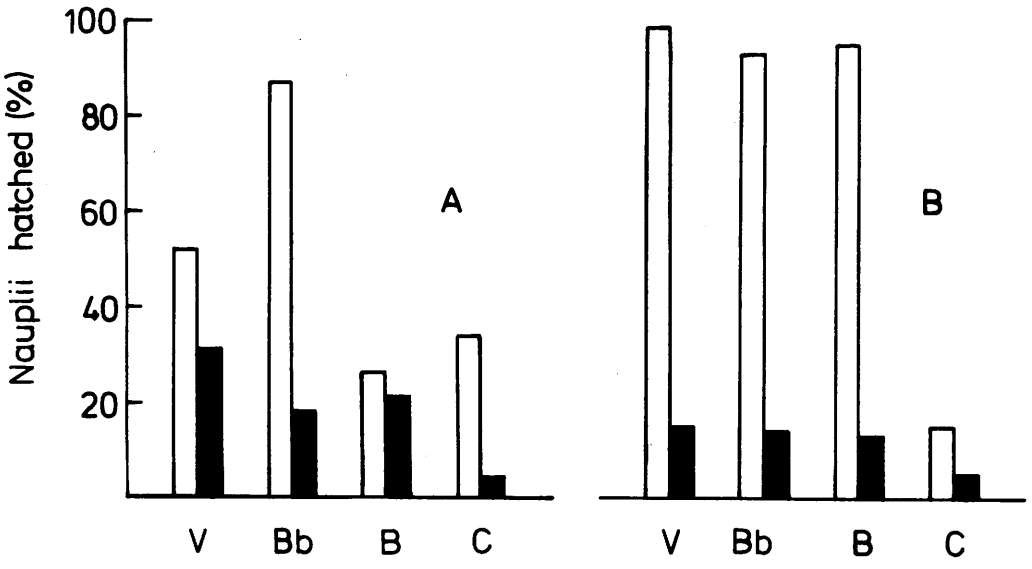

Fig. 8. Verruca stroemia: the effect of extracts of V. stroemia (V), Balanus balanoides (Bb), B. balanus (B) and Chthamalus stellatus (C) on the hatching of nauplii from the egg masses of V. stroemia: open blocks experimentsl animals, closed blocks controls: A, 1.5ml extract: B, 0.5ml extract (for preparation see text).

species tested, Crisp & Spencer (1958) found that extracts of Verruca stroemia did not stimulate the liberation of Balanus balanoides nauplii.

After removing dirt and epifauna by scraping and scrubbing whole, freshly collected (8th March) animals they (including shells) were ground with a pestle and mortar for 5 min, under sea water (25g/30ml) and allowed to stand for 30 min in the refrigerator. The material was then centrifuged. The supernatant was removed and heated in a boiling water bath for 5 min to precipitate proteins and other materials. After cooling, the precipitate was removed by centrifuging and the supernatant, after dividing into several replicate portions, deep frozen. For the tests, a replicate was thawed and used immediately. Lamellae of Verruca were removed from the mantle cavity of the animal and washed several times with fresh sea water; this allowed a selection of lamellae which were sufficiently, but not too, ripe to be used for the tests. From each animal one of the paired lamellae was used for the test and the other for the control in sea water. For the test 1.5 or 0.5 ml of extract was made up to 2 ml with sea water in a test tube and the selected lamellae added: after standing 30 min at room temperature, inverting the tubes gently at 10 min-intervals, formalin was added and the hatched and unhatched nauplii counted.

The results are shown in Fig. 8 which gives the percentage of Verruca hatched (mean of 3 replicate) in both the test solution and control on lamellae from the same animal. It is evident that at both

concentrations extracts of all the species tested gave an increase in the hatching of Verruca nauplii. Except for Chthamalus stellatus the relative effect (ratio of extract/control) was greater at the lower concentration: Crisp & Spencer (1958) found a similar effect at high concentrations of their extracts and they considered that this was possibly due to contaminating substances exerting a toxic effect in such crude extracts. It is evident that if, as the results of Crisp & Spencer (1958) indicate, extracts of Verruca stroemia are without stimulatory activity on the embryos of Balanus balanoides the converse is not true. At the lower concentration the effectiveness of Verruca stroemia, Balanus balanoides and B. balanus is very similar (7.0 relative to the control) while that of Chthamalus stellatus (3.4) is less: however, at the higher concentration (partial inhibition in the other species) the value for Chthamalus is 8.8, suggesting a lower titre of activator for this species together, apparently, with a lower level of extracted toxic material. It may be doubted, whether this indicates a specific difference: it may represent inadequate conditions for extraction in this species, or relative differences in shell and body weights, but more probably does indicate a truly lower level of active material in Chthamalus stellatus at this time of the year since, unlike the other species tested, this is a warm temperate form reaching greater activity later in the season when temperatures have risen. Barnes (1972) has shown that in other respects the response of C. stellatus to the spring diatom increase is far less than that of the two boreo-arctic species

Seasonal changes in body weight and biochemical composition

In order to compare only the seasonal changes in the body weight and its biochemical composition it is essential to obtain results which are independent of the growth of the animal and for which some measure of size is used that is independent of storage or other seasonal changes. The simplest method is to use animals of the same 'size' on each sampling occasion (Heath & Barnes, 1970). Since this is often impractical, one or more 'size' ranges are often used (see, e.g., Ansell, Loosmore & Lander, 1964). An alternative is to take animals of all sizes for analysis, but express the results in terms of a 'standard animal' of selected size either by setting up the appropriate calibration curve on each sampling occasion or directly through a parameter such as the 'component index' of Giese (1967). Various measures of 'size' have been used; they include valve weight in barnacles (Barnes, Barnes & Finlayson, 1963a,b), shell length in molluscs (Ansell & Trevallion, 1967) body volume in echinoids (Moore, 1934) and body weight in numerous other groups (Giese, 1966a).

It should be emphasized that when the whole of the population sampled is synchronous as regards any seasonal changes then the

'standard animal' will be representative of the whole of that population: such is the case with Balanus balanoides and B. balanus in which, at any one time, (except for immature animals) the reproductive state is the same throughout the whole population, both species having an annual breeding cycle synchronous throughout the population at a given locality throughout the year (perhaps one should specify not only the locality but also the littoral level and depth). When this is not the case, the standard animal determined from the calibration will, if all the samples are taken at random, represent only the mean state of the population: such is the case when, for example, at any given time, the reproductive state is not the same throughout a given population; this will be the case when there is more than one brood and when the broods are not synchronised throughout the population. (In this case the spread of the values about the regression line of weight on, say, basal diameter will be greater than in a synchronous population). It is open to the investigator to subdivide such a heterogeneous population into subsets of parts homogeneous with respect to any feature and then on each sampling occassion to take separate samples of these homogeneous subsets and to define a 'standard animal' - as determined from random samples within these subsets - for each homogeneous subset at various times throughout the year:

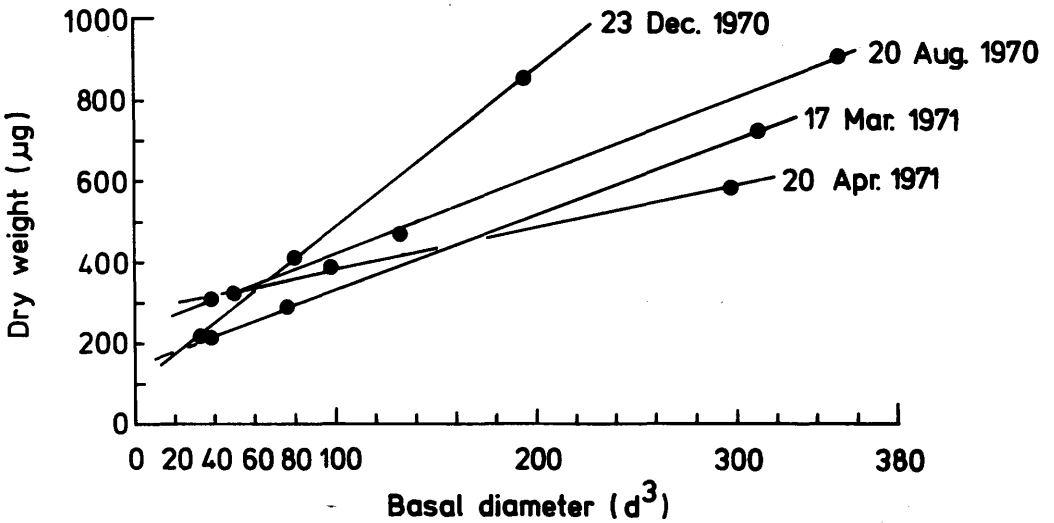

Fig. 9. Verruca stroemia: representative calibration curves of basal diameter (d^3) against desiccator dry body weight (µg) for several sampling occasions; used to determine weight of a standard animal.

such samples could be analysed separately and their time course followed. In some cases, for example, in separating juveniles from later stages the criterion chosen to define homogeneity could be some measure of size. In the present instance a random sample of the population was taken so the 'standard animal' is representative of the mean state of the population.

Size-body weight calibration

In the present instance the animals were little distorted and so rather than use the weight of the opercular valves as a measure of size as has been done with Balanus balanoides it was adequate, and much simpler and quicker, to use the basal diameter. Each time a collection of animals was made for analysis a calibration curve was set up relating basal diameter to freeze dried body weight (Fig.9): for convenience the cube of the former was plotted against the latter since the relation was then linear. Since the animals were small, the calibration had to be based on the mean value of a small number of limited size-groups rather than on individual animals. On each sampling occasion, the basal diameter of each animal in a number of selected size groups was measured and then the bodies carefully dissected from the valves: each size-group was arbitrarily selected to cover the range of sizes available. After dissection from the shell the bodies of each group were pooled, lightly blotted with filter paper, and transferred to tared foil: after freeze drying

the samples were reweighed and the weight of tissue calculated. The 'standard' animal was taken as one of 5.8mm basal diameter (i.e. 200 mm^3 on the calibration curves), and its body weight for each sampling occasion read off from the graph relating mean basal diameter (of each size group) to body weight.

A bulk collection of adult animals was made for biochemical analysis: the bodies, after dissection from the shells and blotting with filter paper, were all transferred to small planchettes and after being lypholized were stored in the deep-freeze until analysed. The 'body' of the animal includes the soft parts attached to the opercular valves. The ovary lies at the base of the mantle cavity: its consistency is variable according to the stage of development and a good separation for analytical purposes is difficult and was not attempted.

The methods of analysis

Total carbohydrate

Total carbohydrate was determined by the method of Kemp & Van Heijningen (1954), which depends upon the formation of a

bluish-pink furfural derivative when glucose is treated with hot concentrated sulphuric acid: glycogen is hydrolysed to glucose. A known amount ($\approx$ 5mg) of the homogenous dry powder was weighed out into a graduated centrifuge tube using a micro-balance. 1.0 ml of 10% (w/v) trichloroacetic acid (TCA) and about 10 mg of silver sulphate (to remove halides which would interfere with the subsequent reaction) were added, and the mixture heated in a boiling water bath for 30 min. The tubes were cooled under running water, centrifuged at 14,000 rpm (15,000 g) for 15 min and the supernatant decanted into a clean, dry graduated centrifuge tube. The precipitate was washed with 1.0 ml of 10% TCA, again centrifuged and the washings added to the supernatant. The combined washings and supernatant were made up to 2.5 ml by the further addition of TCA. Aliquots of 1.0 ml were carefully layered over 3.0 ml of concentrated H_2SO_4 in test tubes. Once the sample had been added the contents were thoroughly and rapidly mixed, heated at

100°C for exactly 6.5 min and cooled quickly in running tap water. The optical density was measured on a spectrophotometer (Unicam, SP 600) at 520 nm. The results are expressed as µg/100mg freeze dried material.

Total lipids

Barnes & Blackstock (in prep.) have discussed the relative merits of methods for the estimation of total lipids in marine biological material. They recommend the phosphosulphovanillin method of Zollner & Kirsch (1962) which, unlike some other methods, may be applied directly with relative little error to crude chloroform-methanol extracts of dried tissues without purification by shaking with salt solutions: this is a great advantage when only small quantities of material are available for analysis.

About 5 mg of the freeze dried material, accurately weighed, was extracted with 5 ml of 2:1 (v:v) chloroform-methanol: because of the small quantities of material available the extraction was made by allowing the solvent to stand in contact with the material for a day, warming, and grinding thoroughly with a glass rod at frequent intervals. After centrifuging and washing the residue with chloroform-methanol, the supernatants were combined and made up to 10 ml in a standard flask. Replicate 2 ml aliquots were taken for further analysis. 2 ml of the extract were evaporated to dryness

under vacuum (or on a rotary evaporator), 0.5 ml concentrated sulphuric acid added and, after shaking, the mixture was heated for 10 min in a boiling-water bath: after cooling, 0.2 ml of the digest was transferred to a clean test tube, 5.0 ml of the phosphosulphovanillin reagent (Boehringer Co. Total Lipid Estimation) added, and, after standing 15-30 min, the optical density was measured at 520 mμ in a Unicam spectrophotometer. A calibration curve (10-100 ug total lipid/0.1ml) was set up by carrying appropriate dilutions of the Boehringer Standard Solution (which is in ethanol) in concentrated sulphuric acid through the above procedure. The appropriate blanks were run. All estimations were done in duplicate or triplicate and the mean values calculated as μg lipid/100 mg freeze dried material.

Trichloroacetic acid soluble and insoluble ('protein') nitrogen

Nitrogen was estimated using a micro-Kjeldahl technique: after digestion, the ammonia was distilled off and the ammonia produced estimated using a phenol-hypochlorite reagent which forms a blue product. Within the range used the optical density at 630 nm of the coloured solution is proportional to the amount of ammonia.

About 5 mg of dry material were weighed into a Pyrex centrifuge tube and 5 ml of 5% trichloroacetic acid (TCA) were added. Extraction with the acid was assisted by gently heating at 50°C with intermittent vigorous grinding and stirring with a glass rod. The tubes were then

allowed to stand at room temperature (20-25°C) overnight and centrifuged for 15 min at 14,000 rpm (15,000 g). The supernatant and washings ('soluble' nitrogen)were transferred to a Kjeldahl flask. The volume of the liquid in the flask was reduced under vacuum. The residue remaining in the centrifuge tube ('insoluble' nitrogen, protein) was transferred with small quantities of water to a Kjeldahl flask. Both fractions were then treated in the same manner. Approximately 0.5 g of Kjeldahl catalyst (B.D.H.) and 2.5 ml nitrogen-free sulphuric acid (BDH) were added and the flasks were then heated, gently at first, and then more vigorously; digestion was continued for a further 2 h after the solutions had cleared. Steam distillation was carried out in a Markham apparatus which was steamed out for at least an hour before use. The cooled acid digest was diluted with a few ml of water and then transferred to the distillation flask together with the washings. A receiving flask containing 10 ml of 0.1 N HC1 (B.D.H. Volumetric Ampoules) was placed under the condenser so that its tip was below the level of the acid. 15 ml of 60% NaOH solution were carefully run into the distillation flask and the distillation was begun. Distillation was allowed to proceed for 2 min and then for another min with the tip of the condenser removed from the acid to wash down the latter. The distillate was transferred to a 10 ml volumetric flask, the washings added, and made up to volume. Aliquots of 0.2 ml were taken and made up to 2 ml with 0.1 N HC1 in a clean dry test tube.

1 ml of phenol reagent was added, the contents immediately mixed thoroughly and then 1 ml of alkaline hypochlorite was added and the solutions again mixed. The tubes were then heated in a water bath at 40°C for 20 min. While the tubes were cooling, 2 ml of water were added and the contents again mixed. The optical density was measured at 630 nm. A calibration curve was set up using standard solutions of 1-5 μg NH_3/ml prepared from a stock standard solution of ammonium chloride. The appropriate blanks were run.

DNA

To conserve material, DNA was estimated on the residue from the lipid analyses: insufficient material was available for pentose estimation so, after separation from RNA, the DNA was estimated in terms of its phosphorus content for which sensitive methods are available.

The residue remaining after the extraction of lipids was incubated with 1 ml of 1N KOH at 37°C for 16 h with intermittent shaking. The digest was neutralized with 1 ml of 1N HCl and 2 ml of trichloroacetic acid was added to precipitate the DNA: the precipitate was washed with a little TCA. The tubes were centrifuged again and the supernatant containing the RNA discarded. The RNA-free residue was then wet-ashed: it was transferred to a boiling tube and digested with 0.5 ml concentrated H_2SO_4 for 15 min (until clear). After cooling, 1 drop of 100 volume hydrogen peroxide was added

followed by vigorous heating: if the digest still showed any yellow colour the hydrogen peroxide treatment was repeated. About 5 ml of water were added and the volume reduced to about 1 ml by boiling to destroy any traces of hydrogen peroxide. The cooled acid solution was then neutralized by means of drop-wise addition of dilute 0.1N NaOH using one drop of phenolphthalein (0.1%) as indicator (phenolphthalein has been shown not to interfere with subsequent estimation procedure).

The neutral solution and washings were transferred to a 10 ml volumetric flask and made up to about 6 ml with water, and the inorganic phosphorus estimated by the Fiske-Subbarov method. The optical density was measured at 625 nm; a calibration curve was set up from standards containing 10 - 40 ugP/ml and prepared from potassium hydrogen phosphate. Blanks were taken through the colorimetric procedure.

Results

The seasonal changes in the body weight of a standard animal - representing, as already stressed, the mean population value of the natural sublittoral population at Millport - are shown in Fig.10 & Table VIII Since only the 'body', excluding female gonads but including the male gonads, is included in the weight of such a standard animal, and since by expressing the results in these terms the effect of growth has been eliminated, changes in weight represent either changes in

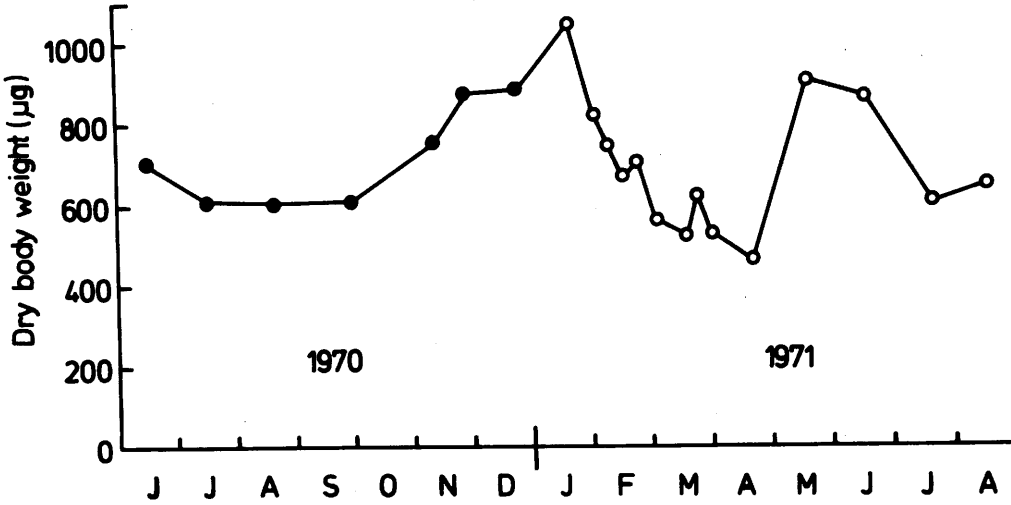

Fig. 10. Verruca stroemia: seasonal changes in total dry body weight for a standard animal.

Table VIII

V. stroemia: dessiccator dry wt. of a "standard animal" with 5.8 mm basal diameter. Data from calibration graphs produced on each sampling occasion.

Date 1970	Dry wt. (ug)	Date 1971	Dry wt. (ug)	Date 1971 (cont.)	Dry wt. (ug)
Jun. 16	705	Jan. 19	1040	Mar. 23	620
Jul. 16	610	Feb. 2	820	Mar. 31	520
Aug. 20	605	Feb. 9	740	Apr. 20	460
Sept. 29	610	Feb. 16	670	May 18	900
Nov. 11	750	Feb. 23	700	Jun. 16	860
Nov. 27	870	Mar. 2	560	Jul. 20	600
Dec. 23	880	Mar. 17	520	Aug. 17	640

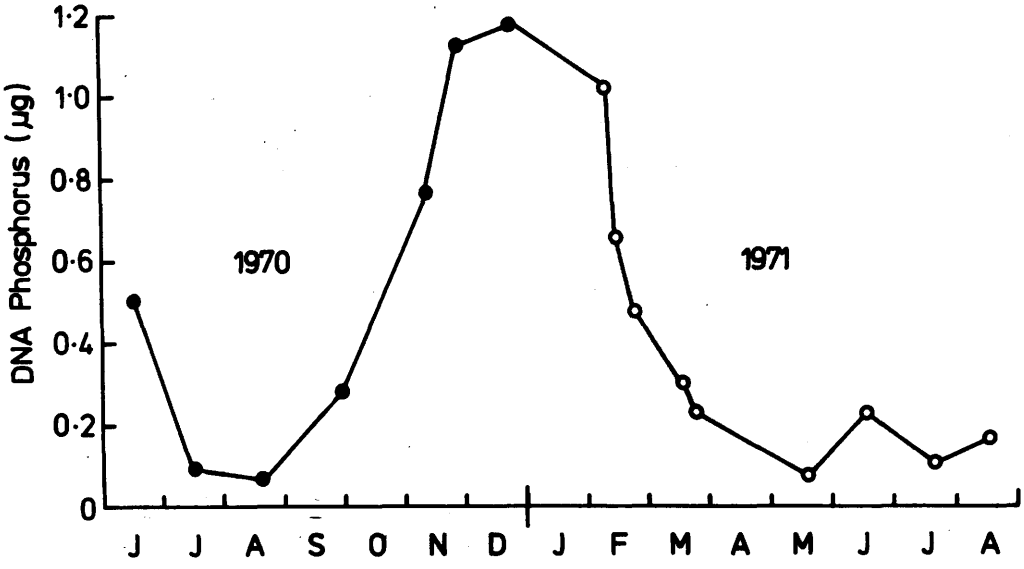

Fig.11. Verruca stroemia: seasonal changes in DNA for a standard animal.

stored materials or in the state of the male gonads. The rise in autumn is clearly associated with the development of the male gonads throughout the whole population at this time, when no further broods are being developed in the mantle cavity. Similarly, the fall in weight during the early months of the year is the result of a loss of semen as the greater part of the population is fertilized (maximum numbers with egg masses, 83% in late February, see p. 20). The body weight continues to fall until April during a period when nauplii are being released: this suggests that during this period there is little re-development of the male gonads or that any such development is accompanied by other changes which result in a net loss; thus, it may well be that some stored material is being utilized for ovarian development since at this time nutrient conditions following upon the spring diatom increase are near optimal. By May there is further reproductive activity (see p.21) but only in part (20%) of the population, and the rise in body weight, which approaches values near to the winter maximum seems too great to be accounted for by an increase in male gonads alone: there is almost certainly an accumulation of stored material at this time. That this is the case is further indicated by the changes in the DNA values (Fig. 11; Tables IX, X) which, since growth has been 'eliminated', represent cellular increase in the form of

Table IX. - Verruca stroemia: biochemical composition; mg/100 mg dry wt.; total carbohydrate as glucose; total lipid as cholesterol standard; trichloracetic acid (TCA) - insoluble nitrogen (protein) and TCA soluble nitrogen; DNA as phosphorus.

Date	Dry wt. Standard animal (ug)	Total carbohydrate	Total lipid	TCA - insoluble nitrogen	TCA - soluble nitrogen	DNA phosphorus	Ratio: insoluble to soluble nitrogen
1970							
June 16	705	4.22	7.32	5.603	1.800	0.072	3.113
July 16	610	5.56	6.13	6.446	3.881	0.015	1.661
Aug. 20	605	4.91	9.98	6.005	2.544	0.011	2.360
Sept.29	610	6.54	6.65	6.296	2.154	0.046	2.923
Nov. 11	750	5.79	7.38	6.825	3.054	0.102	2.235
27	870	5.92	8.74	6.213	2.771	0.130	2.242
Dec. 23	880	3.42	8.70	5.037	2.968	0.133	1.697
1971							
Jan. 19	1040						
Feb. 2	820	5.83	8.50	7.207	5.000		1.441
9	740	3.40	7.27	6.576	4.271	0.138	1.533
16	670	4.23	7.12		1.936	0.097	
23	700	3.51	7.01	6.955	3.426	0.070	2.030
March 2	560	3.65	6.30	5.646	2.860		1.974
17	520	4.83	7.84	6.596	2.659	0.057	2.481
23	620	2.98	4.76	6.563	2.915	0.037	2.251
31	520	2.72	5.40	7.220	3.074		2.349
Apr. 20	460	4.29	6.29	6.469	2.839		2.279
May 18	900	7.93	6.64	6.017	2.426	0.009	2.480
June 16	860	8.26	5.92	6.600	2.891	0.027	2.283
July 20	600	5.82	6.57	6.593	4.519	0.017	1.459
Aug. 17	640	5.59	5.82	6.173	2.581	0.026	2.392

Table X - Verruca stroemia: biochemical composition of standard animal (ug): total carbohydrate as glucose; total lipid: trichloracetic acid (TCA) - insoluble nitrogen (protein) and TCA soluble nitrogen; DNA as phosphorus.

Date	Desiccator dry wt. standard animal (ug)	Total carbohydrate (ug)	Total lipid (ug)	TCA - insoluble nitrogen (ug)	TCA - soluble nitrogen (ug)	DNA phosphorus (ug)
1970						
Jun. 16	705	29.75	51.61	39.50	12.69	0.5074
Jul. 16	610	33.92	37.39	39.32	23.67	0.0932
Aug. 20	605	29.70	60.38	36.33	15.39	0.0678
Sept.29	610	39.94	40.57	38.41	13.14	0.2818
Nov. 11	750	43.43	55.35	51.19	22.91	0.7655
27	870	51.50	76.04	54.05	24.33	1.126
Dec. 23	880	30.10	76.56	44.32	26.12	1.117
1971						
Jan. 19	1040					
Feb. 2	820	47.81	69.70	59.10	41.00	
9	740	25.16	53.80	48.44	31.61	1.017
16	670	28.34	47.70		12.97	0.652
23	700	24.57	49.07	48.69	23.98	0.487
Mar. 2	560	30.44	35.28	31.62	15.96	
17	520	25.12	40.77	34.30	13.82	0.299
23	620	18.48	29.51	40.69	17.07	0.2300
31	520	14.14	28.09	37.54	15.98	
Apr. 20	460	19.73	28.93	29.76	11.06	
May 18	900	71.37	59.76	54.15	21.83	0.078
Jun. 16	860	71.04	50.90	56.76	24.86	0.228
Jul. 20	600	34.92	39.42	39.56	27.11	0.101
Aug. 17	640	35.78	37.25	39.51	16.52	0.167

spermatozoa. The DNA increases sharply during the autumn and winter and falls during the early months of the year indicating that the changes in body weight, are as suggested above, largely dependent upon the accumulation of semen: there is, however, no rise in DNA which corresponds to the rise in body weight in May - that male gonadal tissue contributes something to the high value of the body weight in June is indicated by the increase in DNA in that month - more marked in 1970 than in 1971; this is in accordance with the fact that the level of reproductive activity (Fig. 2) during the summer of 1970 appears to be somewhat higher than that of 1971.

The changes in both nitrogen fractions follow closely those in the body (Fig.12; Tables IX,X). The increase during the autumn may again be ascribed to the accumulation of semen as the male gonads ripen, and the subsequent fall to its loss. The high values in May and June, when reproductive activity is low, confirm the suggestion that at this time material is being stored. It is notable that these high values in May-June are most marked in the total carbohydrate (a $>$3-fold increase) (Fig.13;Tables IX,X). Now semen is relatively low in carbohydrate but, as glycogen, carbohydrate is a common storage product. Lipid also appears to be accumulated at this time but to a relatively smaller extent (Fig.14; Tables IX,X). Although there was a rise in carbohydrate, nitrogen fractions, and lipids during the

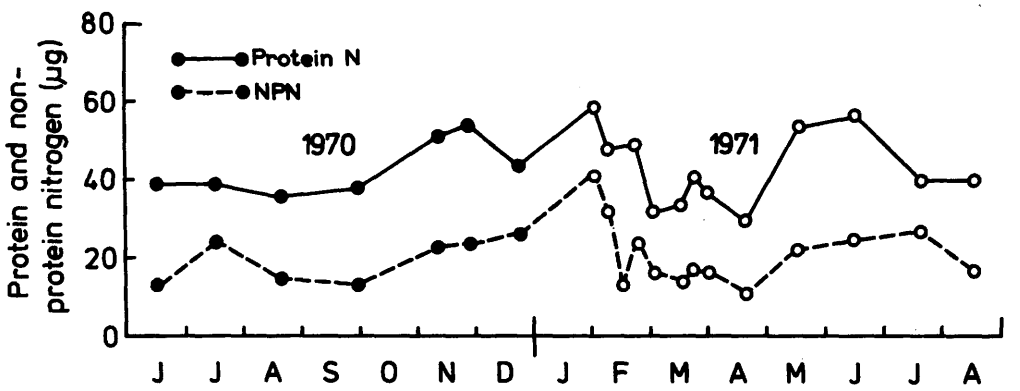

Fig.12. Verruca stroemia: seasonal changes in 'protein' and 'non-protein' nitrogen.

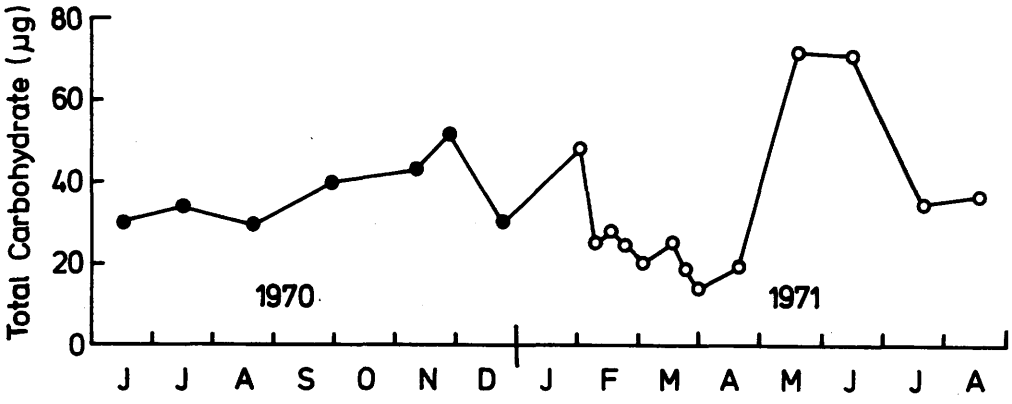

Fig. 13. Verruca stroemia: seasonal changes in total carbohydrate for a standard animal.

Fig. 14. Verruca stroemia: seasonal changes in total lipid for a standard animal.

autumn and a fall in the early part of the year which has been ascribed to the accumulation of semen and its subsequent loss, it must be emphasized that the maximum in the number of animals with egg masses was in late February: fertilization, with the consequent loss of semen must, therefore, have taken place probably at least one month prior to that date. Yet DNA, and some of the other biochemical constituents continued to fall - to a varying degree - during March and even in the case of the nitrogen fractions during April. It seems possible that at least part of this fall is due to limited reproductive anecdysis (Barnes, 1962) with consequently little feeding activity and also to the poor nutrient conditions during the winter. It is unfortunate that sufficient material was not available for analyses to be made on the population that was transferred to the raft, and which, because of the better nutrient conditions to which they were exposed, gave a second brood in the spring; they might have helped to confirm the above interpretation of the changes found in the natural population: there would, presumably have been a large rise in both body weight and DNA values to correspond with the second and still relatively large spring brood.

It is possible to calculate the changes in the body weight

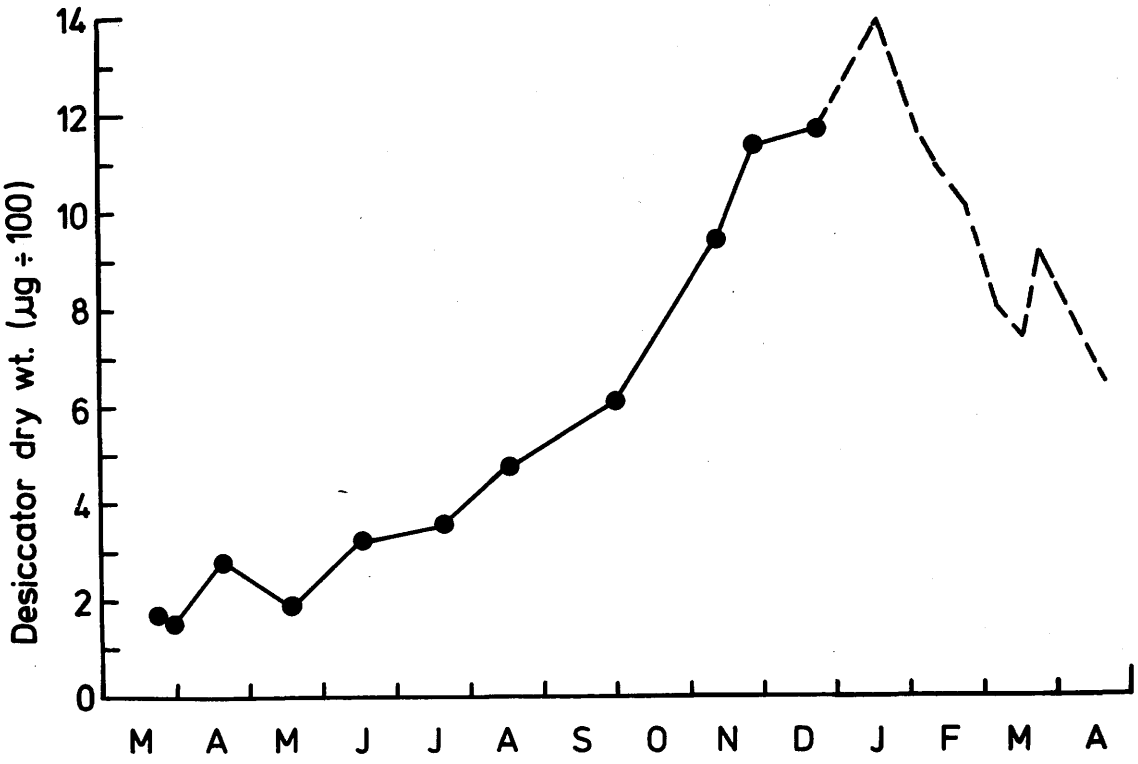

Fig. 15. Verruca stroemia: increase in dry body weight with growth over 14 months: weights estimated from seasonal calibration curves of body weight against basal diameter and growth curves of Barnes (1958); dotted line extrapolated values less reliable.

Table XI

Verruca stroemia: seasonal growth rate (dry body wt. (µg)); interpolation of values beyond 23rd Dec. only approximate; dry wt. data taken over 1970-1971; growth data from Barnes (1958).

Date	Longest basal diameter (mm)	Basal diameter $(mm)^3$	Desiccator dry wt. of animals (µg)
23rd Mar.	0.35	0.04	170
31st Mar.	0.65	0.27	155
20th Apr.	1.45	3.04	279
18th May	2.50	15.63	178
16th Jun.	3.65	48.63	320
20th Jul.	4.60	97.34	352
17th Aug.	5.10	132.65	475
30th Sept.	5.85	200.20	610
11th Nov.	6.35	256.05	940
27th Nov.	6.45	268.34	1135
23rd Dec.	6.50	274.63	1160
19th Jan.	6.55	281.01	1390
3rd Feb.	6.60	287.50	1138
16th Feb.	6.65	294.08	1050
23rd Feb.	6.70	300.76	1005
2nd Mar.	6.75	307.55	805
17th Mar.	6.85	321.42	745
23rd Mar.	6.90	328.51	915
31st Mar.	6.95	335.70	835
20th Apr.	7.10	357.91	645

of an animal as it grows from the data on growth rate (raft-maintained animals) (Barnes,1958) and on the relation between body weight and basal diameter determined in this investigation: for each occasion on which a body weight - basal diameter calibration is available the body weight which corresponds to the interpolated basal diameter at that time on the growth curve may be found. The results are given in Fig. 15 and Table XI. Because of the restriction in Verruca that the calibration curve is only representative of the population the growth curve so determined is similarly restricted. Body weight increases during the spring and early summer when growth is maximal. By late summer the animal is reaching its maximum size and the rate of increase in body weight falls: the fall in body weight noted in the standard animal also contributes to this decrease in rate. Later, however, although growth as measured by the increase in basal diameter is very small, the accumulation of semen leads to a rise in body weight at this time. When semen is lost, body weight falls dramatically (January-February) while the basal diameter changes but little.

Discussion

The fact that there is a spring brood which is virtually

synchronous throughout the whole population in Verruca tends to 'dominate' the changes in both the body weight of a standard animal and the biochemical composition; these changes come, therefore, to resemble closely those found in Balanus balanoides and B. balanus both of which have only a single annual brood (Barnes, Barnes & Finlayson, 1963). In B. balanoides, semen is largely accumulated during the late summer and early autumn from the time when the penis begins markedly to lengthen in August and is followed by copulation in October-November, but in B. balanus semen is accumulated over a longer period and copulation does not take place until February. The phasing of the similar changes in body weight and biochemical composition is, therefore, different. It is now believed that the earlier copulation in B. balanoides is related to its adaptation to truly arctic conditions of ice-cover during the winter, so that it is not surprising that the changes in the largely sublittoral Verruca more closely resemble those seen in B. balanus. It may be noted that the percentage change in the body weight over this period of copulation is very similar in all three species indicating that a similar, and large (60-70%) proportion of the body weight at these times is made up of semen. The fall in body weight is less rapid in Verruca

and this is to be ascribed to the smaller degree of synchrony in this major spring brood; and this finds expression in the fact that at no time in Verruca does the body weight remain at a low and virtually constant value as is the case in the other two species. Furthermore, copulation in some animals of the Verruca population may even overlap with the beginning of the accumulation of reserves so that the net population change (loss) is reduced. The possession of a major spring brood, whatever the survival value, clearly brings Verruca into close similarity to Balanus balanoides and B. balanus and is in accord with its largely boreo-arctic distribution. (It would be extremely interesting to know something of the breeding cycle in those Verruca populations far removed from its centre of distribution). There seems no reason to believe other than that the survival value of this major spring brood is the same as that postulated for the two balanids, namely, the availability of phytoplanktonic organisms at this time for the planktonic larvae which are released at this time and which migrate to the upper waters. It should, perhaps, be stressed, however, that there is evidence that the release of this brood in Verruca takes place - or at least begins - somewhat earlier than in the balanids. Although there is great variation from one year to another in the course of the body weight-time curves in Chthamalus stellatus (Barnes, 1972)

a comparison of these, which represent the behaviour of a warm water species near its northern limit, with those of the two balanids and Verruca, clearly indicate that Verruca, in spite of its continued breeding during the summer is a boreo-arctic species which has 'escaped' from the complete restriction of a single annual brood. Since the changes in weight during the winter and early spring are closely associated with the production and loss of semen it is not surprising that in all three boreo-arctic species they are closely paralleled by the changes in the DNA content of a standard animal.

The rise in body weight and other materials observed in late spring in Balanus balanoides and B. balanus is also found in Verruca: in the two former, which are not at this time developing male gonadal tissue, it was ascribed to the storage of reserve materials. Although there is some re-development of the male gonads in Verruca at this time - preparatory to the production of summer broods - storage, doubtless, also plays a major rôle. Similarly, the fall in July probably represents the transfer of material to the female gonads, but in Verruca this leads to maturation at a low level during the summer months, whereas maturation is much delayed in the other two species.

In Balanus balanoides the late spring maximum of body weight, lipid, and soluble and insoluble nitrogen is reached in late April and that of total carbohydrate possibly a little later: in B. balanus these maxima are a month later. The evidence from Figs 10-14 indicates that in Verruca too these maxima are not reached until May-June. Like Balanus balanus, the population of Verruca sampled was sublittoral and the delay, relative to the littoral species, in reaching a maximum value for stored material may, as with the balanid, be due to ~~this~~ habitat conditions. The spring diatom population is immediately available to a littoral species as soon as it is covered by the tide; the 'true', as distinct from the predicted, immersion by water of the high level Chthamalus stellatus has been shown to be correlated with the year to year anomalies of changes in body weight (Barnes, 1972) and the time of immersion of Balanus balanoides is directly correlated with the growth rate (Barnes and Powell, 1953). On the other hand, there is a delay in this food - either primary as living diatoms or secondary as zooplankton remains and faecal material - reaching a sublittoral species. It is, perhaps, worth noting that the relative increase in carbohydrate (260%) during this time (20th April-18th May) is greater than that for the other fractions ($\approx$ 100%).

The effect of desiccation

Verruca stroemia is largely sublittoral: however, it does extend into the lower levels of the littoral, where it occurs on any suitable substrata. Under these conditions the animals will be regularly exposed to air and consequently to desiccation stress. The fact that Verruca does not extend higher up the shore suggests that its ability to withstand desiccation is not great, although factors such as settlement behavior and competition might be involved.

The materials and methods

Experimental investigations on desiccation in littoral marine animals in which an attempt has been made to simulate conditions on the shore are few, since little attempt has been made to control the velocity of the air over the animals. The velocity of the air is important since not only does it determine the thickness of the boundary layer (in which diffusion is molecular) but it has an influence on evaporative cooling (see Ramsey, 1935). Most usually, animals have been exposed to static air of known humidities (sometimes at a series of temperatures) and the median lethal time determined for each set of conditions: such experiments may be useful in comparing different species. In the present work an apparatus very similar to that of Kensler (1967)

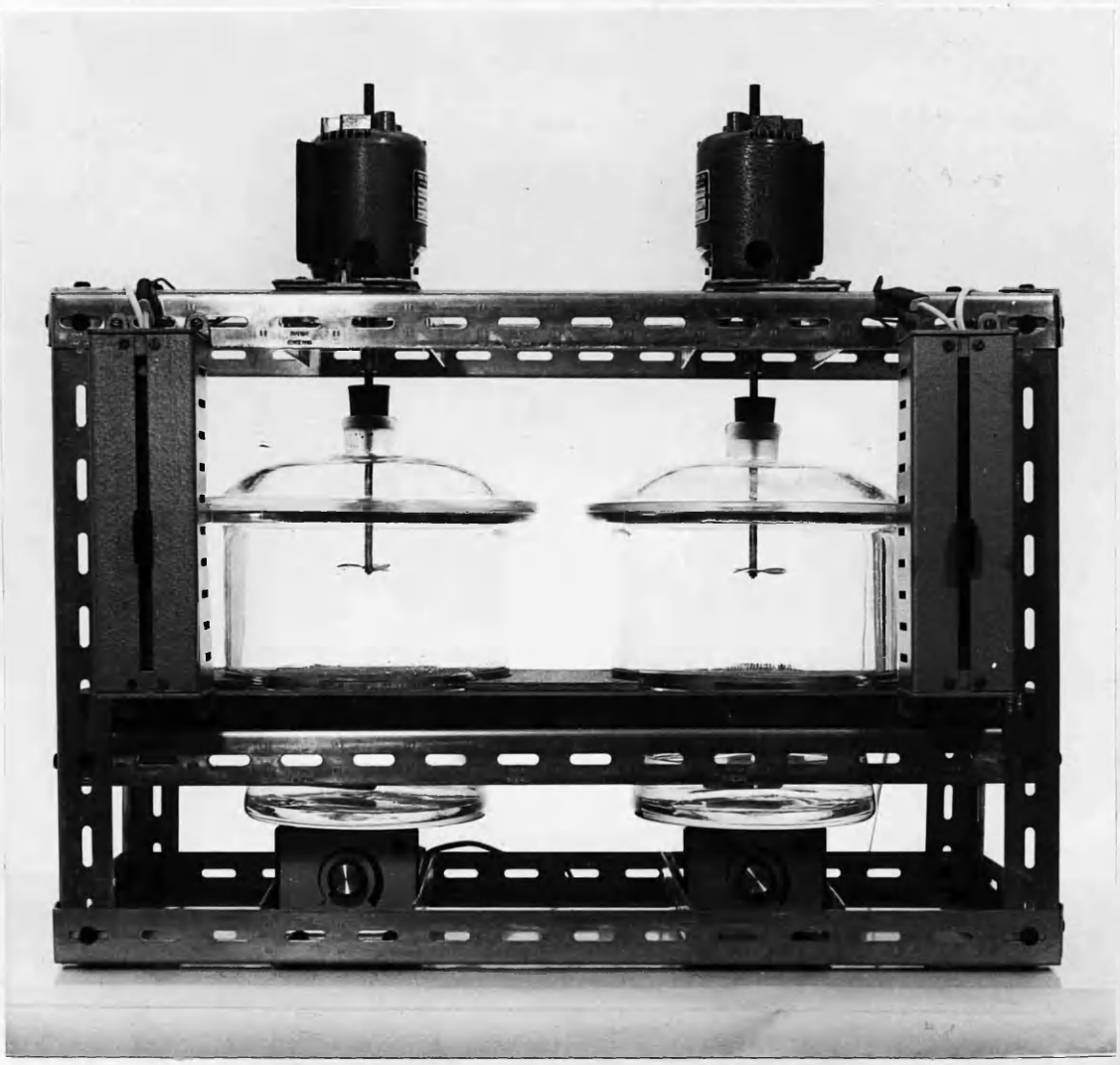

Fig. 16. Apparatus used in desiccation experiments.

has been used, in which the air within the closed chamber is kept moving (Fig. 16). To avoid independent thermostating of the whole apparatus, it was maintained in a constant temperature room throughout all the experiments. A pair of matched desiccators contains the animals held on racks above a solution giving a known, constant humidity. In the present instance, since under natural conditions the animals are rarely exposed to low humidities, only one R.H. was used, namely ≈ 50% (43% w/w sulphuric acid, Solomon, 1951). This condition pertained to one desiccator, the other contained filtered sea water and served for control animals. The shafts of the stirrers passed into the desiccators through a close-fitting brass tube sealed with vaseline. The stirrers were under separate control and the speeds were adjusted to be equal at the beginning of a run and then checked at intervals. In order to reduce any laminar effects at the adsorbing acid - air interface the former was stirred from below by a conventional magnetic stirrer; the precaution was not taken by Kensler (loc. cit.).

Animals living on live Pecten were used: the upper valves of the latter having about 50 Verruca on them were separated, scrubbed clean on the underside, and lightly cleaned on the upper surfaces: they were then drilled, fastened to Tufnol panels and exposed on a raft for several weeks. In this way all remaining

molluscan tissue is removed since otherwise this would 'foul' the desiccation chambers. The shells were removed from the panels and held overnight in running sea water before each experiment. After drying the animals and shell, first by draining and then with filter paper, the shells were placed in the desiccators and left for a known time under the conditions described above. At the selected time they were taken out, left in running sea water overnight, and the mortality assessed. Some workers (e.g., Kensler, loc. cit.) have used a ranking system to express the viability of the animals after subjecting them to stress. In the present instance, and after much experience, it was felt that by careful examination and probing under a dissecting microscope a reliable decision could be made as to whether the animal would survive (Re-exposure and subsequent examination is the only completely reliable test). Only two categories were recognized, namely, dead and alive. In one case (4-h experiment) there was abnormal mortality in the controls. This may have been due to physical damage during handling; while there is no a priori reason to expect that those animals under desiccation in the same run should have been similarly damaged it is notable that they too showed a greater than expected mortality relative to all the others. Before expressing the results in terms of percentage mortality at various times the control value was

subtracted from that at 50% R.H. Since so much of the work on desiccation in cirripedes has been carried out on Balanus balanoides a check on the present methodology and for comparative purpose separate experiments were carried out on this species under the same conditions as used for Verruca: the balanids were on stones and two size groups were used since it has been shown that the median lethal time under desiccation stress is size-dependent.

In addition, the behaviour of the animal on taking out of water and exposing to air, as well as on re-immersion, was observed repeated by under a dissecting microscope.

Results and discussion

On exposure to air the cirri, usually in an unexpanded 'mass' are protruded from the mantle cavity at irregular intervals: on withdrawal the valves close. Sometimes the curled cirri only reach the edge of the mantle cavity where they may remain for 30-40 sec, before being again withdrawn: on other occasions the cirri are brought, in successive movements, further and further outside the mantle cavity while the valves remain open. The valves may remain open after the cirri have been withdrawn, until the latter are again protruded. During all these movements water is 'expelled' from the mantle cavity: when most of the water has been expelled the cirri may expand somewhat as they

Table XII

Verruca stroemia: mortality under 50% relative humidity at various times in moving air: animal sizes 1.0 - 7.0 mm: control animals over sea water.

Time	No. animals	No. alive	No. dead	% alive	% dead	Corrected mortality
2 h	44	43	1	97.73	2.27	0
control	40	39	1	97.50	2.56	
3 h	61	55	6	90.17	9.83	9.83
control	57	57	0	100.00	0	
4 h	34	13	21	38.24	61.76	51.12
control	47	42	5	89.36	10.64	
5 h	58	39	19	67.24	32.76	32.76
control	63	63	0	100.00	0	
6 h	63	23	40	36.51	63.49	59.49
control	50	48	2	96.00	4.00	
7 h	57	27	30	47.37	52.63	52.63
control	46	46	0	100.00	0	
8 h	59	3	56	5.08	94.92	94.92
control	52	52	0	100.00	0	
12 h	42	1	41	2.38	97.62	95.12
control	40	39	1	97.50	2.50	
18 h	65	0	65	0	100.00	97.27
control	37	36	1	97.30	2.70	
24 h	39	0	39	0	100.00	75.00
control	60	45	15	75.00	25.00	

Table XIII

Balanus balanoides: mortality under 50% relative humidity at various times in moving air: small animals (1.0 - 3.9 mm): control animals over sea water.

Time	No. animals	No. alive	No. dead	% alive	% dead	Corrected mortality
12 h				100.00	0	0
control				100.00	0	
24 h	75	42	33	56.00	44.00	44.00
control				100.00	0	
48 h	51	31	20	60.78	39.22	39.22
control				100.00	0	
84 h	67	4	63	5.97	94.03	94.03
control				100.00	0	
96 h	70	5	65	7.14	92.86	92.86
control				100.00	0	
144 h	24	1	23	4.17	95.83	90.20
control	71	67	4	94.37	5.63	
192 h	26	0	26	0	100.00	100.00
control				100.00		

Table XIV

Balanus balanoides: mortality under 50% relative humidity at various times in moving air: large animals (> 4.0 mm): control animals over sea water.

Time	No. animals	No. alive	No. dead	% alive	% dead	Corrected mortality
12 h				100	0	0
control				100	0	
24 h	62	61	1	98.40	1.60	1.60
control				100.00	0	
48 h	66	64	2	96.97	3.03	3.03
control				100.00	0	
84 h	82	55	27	67.07	32.93	32.93
control				100.00	0	
96 h	153	123	30	80.39	19.61	19.61
control				100.00	0	
144 h	127	36	91	28.35	71.65	70.16
control	67	66	1	98.51	1.49	
192 h	87	5	82	5.75	94.25	94.25
control				100.00	0	

are protruded: presumably in the earlier stages 'surface tension effects' prevent the wet cirri from being separated when they are protruded in air. As far as could be ascertained all these movements were part of the normal sequence seen under water (see p. 69). After 30 min, these movements become very intermittent and relatively infrequent: the valves may open and slight movements of the cirri within the mantle cavity may be seen. This may be accompanied, from time to time, by more vigorous cirral movements giving the appearance of 'struggling' whilst they are protruded. After 1 h many animals have their valves open and on dissection the animals, which were clearly drying out, failed to respond to touch by a probe. When the animals are returned to sea water after only short periods of exposure to air, then air bubbles are expelled as normal cirral activity is resumed.

It is evident from the above that Verruca, on exposure to air, behaves quite unlike a typical littoral species; the latter, e.g., Balanus balanoides or Chthamalus stellatus, after repeatedly extruding the cirri and so expelling the water from the mantle cavity remain with their valves still somewhat raised in the mantle cavity and with the membrane of the operculum forming a micropylar opening (Barnes, Finlayson & Piatigorsky, 1963; Grainger & Newell, 1965: the latter authors termed the micropylar opening a pneumostome).

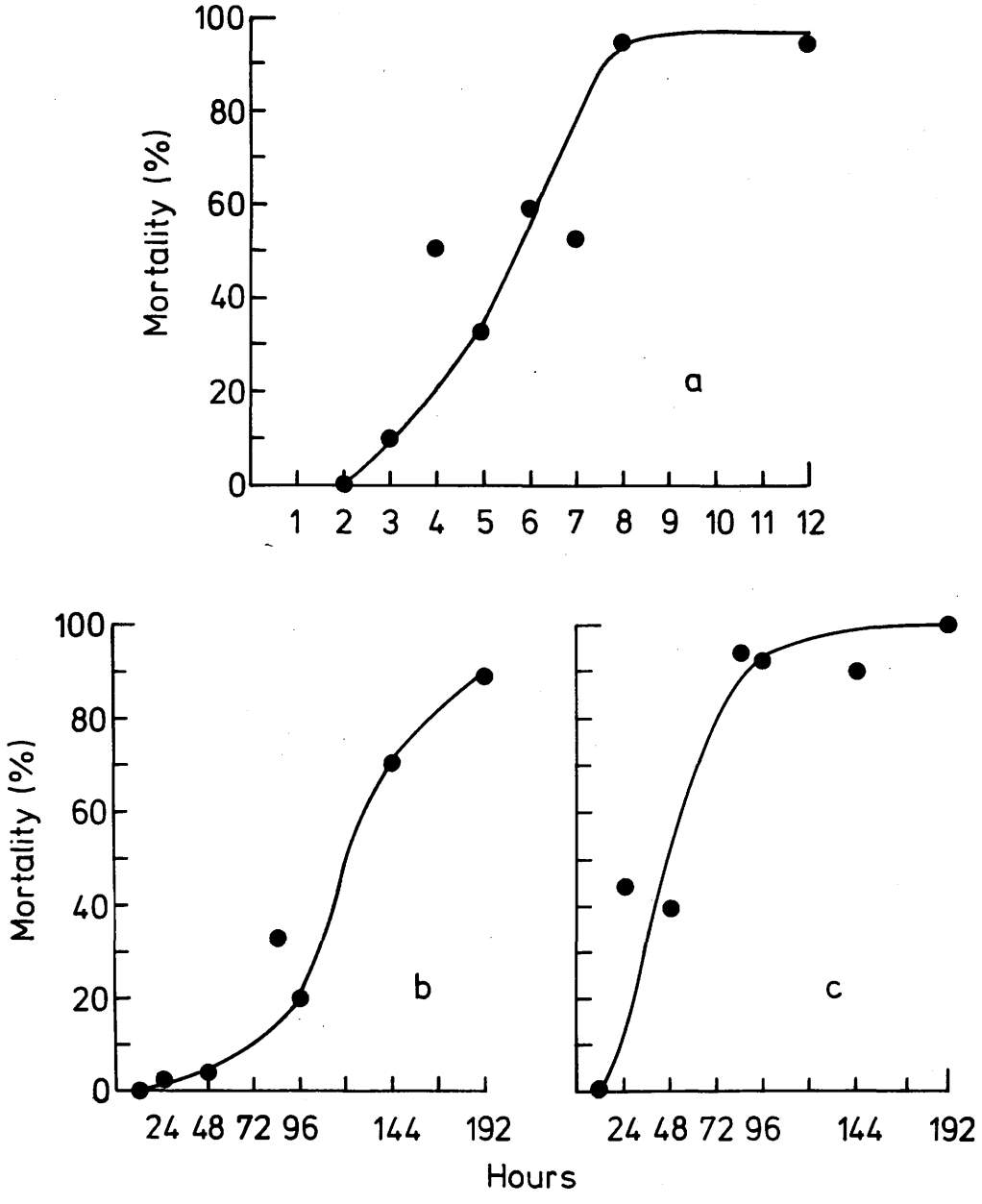

Fig. 17. The mortality with time under ≈50% R.H.; a, Verruca stroemia 1.0–7.0 mm basal diameter; b, Balanus balanoides >4.0 mm basal diameter; c, B. balanoides <4.0 mm basal diameter.

Subsequently, the micropylar opening controls water loss. The behavior of Verruca is very similar to that of Balanus crenatus and B. balanus - both also largely sublittoral species, but both found in the low littoral at a similar level to Verruca (locally the two balanids appear to be able to occupy somewhat higher levels than Verruca, being present on mussels and pier pilings).

The results of the desiccation experiments are shown in Fig. 17 and Tables XII,XIII,XIV. The median lethal time for Verruca is $\approx$6h which stands in marked contrast to that of small ($<$ 3.9mm basal diameter) Balanus balanoides, namely, $\approx$ 2 days, or large ($>$ 4.0mm basal diameter) B.balanoides - a value of $\approx$5 days)*. The resistance of Verruca to desiccation is clearly far less than that of a typical littoral species, and indeed less than that of Balanus crenatus for similar-sized animals; the median lethal time of the latter estimated from published data may be 10-20h.

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* Although conditions were not identical these results are in good agreement with those of Barnes, Finlayson & Piatigorsky, (1963) and of Foster (1971): the former found a M.L.T. for 'adult' animals ($<$ 5.0mm basal diameter) of $\approx$ 3.8 days in still air at zero R.H., and the latter using Kensler's apparatus a M.L.T. of $\approx$ 60h for small and 140h for large animals.

Whatever other factors are involved in determining the distribution of Verruca the inability to resist desiccation on exposure to air will limit its ability to occupy the littoral. Under 50% R.H. the median lethal time is some 6h: conditions at low levels on rocky shores, where there is often a dense cover of large algae such as Laminaria are such that in the immediate vicinity of the Verruca with its extremely low growth form, the humidity in northern water is rarely likely to fall below 50%: indeed for much of the year it is probably far higher. Under these conditions Verruca is quite clearly capable of surviving the relatively short periods of exposure to air at such tidal levels. Its relative sporadic occurrence in the low littoral must be ascribed to other factors than air exposure: doubtless the nature of the substrata available, degree of wave exposure, and competition are some of the important factors.

Cirral activity and feeding

Introduction

In all operculate cirripedes feeding is very closely associated with movements, particularly cirral beat, which also subserve respiratory activity. Crisp & Southward (1961) have investigated cirral activity in some detail, describing various forms which they term "fast beat","normal beat", "pumping" and "extension"; the distinction between the first two is somewhat arbitrary and it may be argued that all the first three are expressions of the same pattern of movements carried out to different degrees of completion. The part played by body, opercular, and cirral movements in driving a stream of water through the mantle cavity was also considered: the importance of this in the release of nauplii together with an estimate of the pressures involved had already been established (Barnes, 1955). Feeding and cirral activity has been investigated by Barnes & Reese (1959), in the common littoral pedunculate of the American Pacific coast, Pollicipes polymerus and to a lesser extent in Pollicipes spinosus (Batham, 1945). In these species there is little or no regular cirral beat; the cirri, either individually or collectively, close down

on the prey as a result of contact stimulus and respiration does not depend upon a distinct mantle cavity current. Crisp & Southward (loc. cit.) focussed their attention on Balanus balanoides and, although some comments are made on other species particularly relative to cirral activity and habitat, they make only a passing reference to Verruca stroemia in which they refer in a Table to its testing, pumping, normal beat, and in particular to extension.

The morphology of the mouthparts, cirri and 'operculum'

The following details supplement those of Darwin (1854), Pilsbry (1916) and Nilsson-Cantell (1921).

Mouthparts

The labrum is thin, neither notched nor bullate; it bears 6-9 teeth on its inner margin. The upper edges of the palps, roughly oblong in outline, are closely applied to the outer rim of the labrum; setae are present on the outer edge and tip while the lower surfaces are partially covered with small setules. The mandibles are strong with three main teeth, the first and second double at their tips, and the lower third bears numerous small teeth and the upper, lower and medial

(oral) surfaces bear many short setae. The maxillae are strongly toothed on two projections separated by a narrow notch. The upper projection has two teeth - with smaller teeth between them: the lower projection has four strong teeth almost as long as the first pair on the first projection. There are smaller teeth within the notch and on the strong teeth of the projections. The surfaces of the maxillae are beset with many setae on both upper and lower surfaces. The bilobed, red-coloured outer maxillae are prominent when the mouth is viewed from above. Their oral, upper and lower surfaces are generously covered with strong setae and a pair of strong teeth are present on each lobe.

The cirri

Except for the first pair which are attached at the sides of the mouth, and closely associated with it, and so stand some way apart from the more posterior ones the cirri are somewhat different from those of the Balanidae. The second and third pairs differ from the remainder. The first pair, with broad sub-equal rami are short and densely covered with bristles; the second has a

the posterior ramus
short and broad anterior ramus, much longer than the anterior; the segments of the former are broad and protuberant in front; terminally there are groups of strong pectinated spines. The third pair resemble the second. The remaining three pairs are similar to one another and very much longer: the number of setae increase distally. The thickness of the spinous covering on the first pair of cirri recalls a similar situation in Pollicipes.

The caudal appendages are long and consist of numerous thin cylindrical segments of unequal length with a ring of setae at the junction of the segments: the final segment bears a brush-like tip of fine setae. Within the shell the caudal appendages are closely applied to the cirri.

The 'operculum'

In the Lepadidae the membranes connecting the valves which make up the two halves of the capitulum, lie, where they do not touch, around the symmetrical opening leading into the mantle cavity and resemble the outer layers of the peduncle: the two halves are joined internally by the adductor scutorum muscle. In the Balanidae, however, which have lost the peduncle, the entrance to the mantle cavity is closed by an operculum containing the scuta and

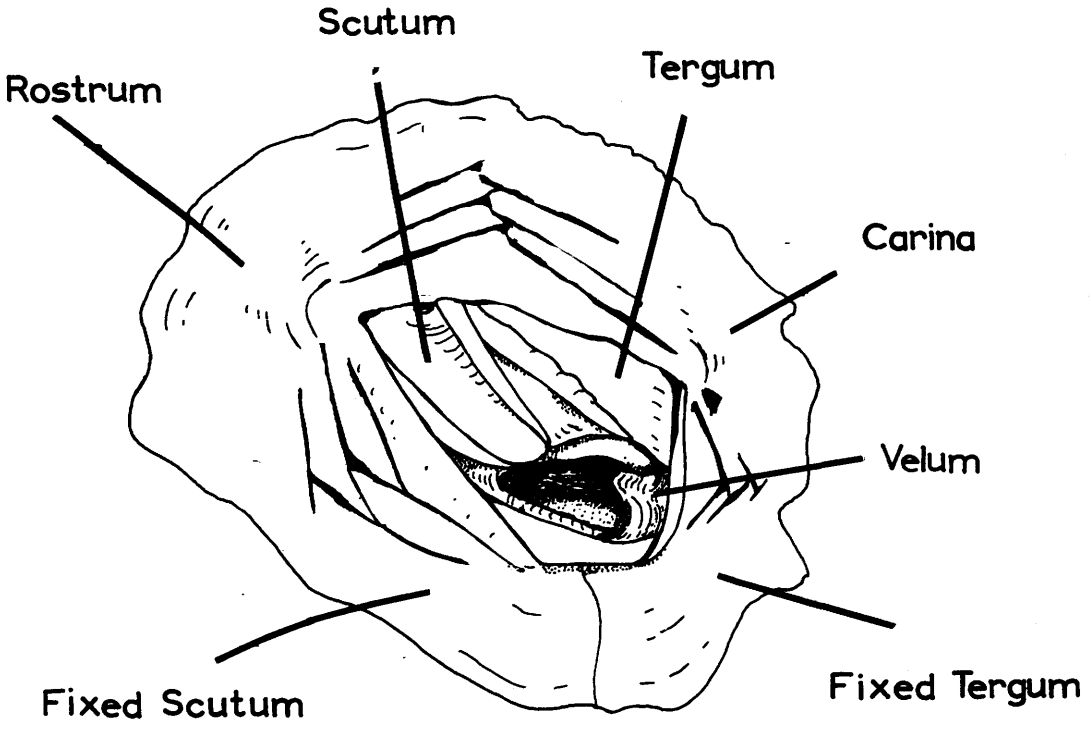


Fig. 18. Verruca stroemia: viewed from above to show position of moveable valves in relation to velum and opercular membrane.

terga joined in pairs to form two valves which open on the rostro-carinal axis and allow access to the mantle cavity. Along the edges of the opening the operculum is lined by tissue, more or less prominent according to the species. In Verruca, where only one tergum and scutum are moveable, the 'opercular' membrane forms a continuous cushion round the fixed and moveable valves (Fig. 18). At its rostral and carinal ends it is attached somewhat lower down and the apices, particularly carinally, are folded as may readily be seen when the valve is open. (It can be most easily seen by examining an animal in situ after it has been decalcified in dilute acid). This carinal fold recalls the velum of Lithotrya (Cannon, 1947); as the valves open this velum flattens and Cannon considered that this resulted in a suction pressure which caused water together with any contained food to enter the mantle cavity. When the moveable valves are closed against the fixed scutum and tergum the entrance to the mantle cavity is effectively sealed.

Cirral movements

Cirral movements in still water

The cirri may be extended in still water. As the

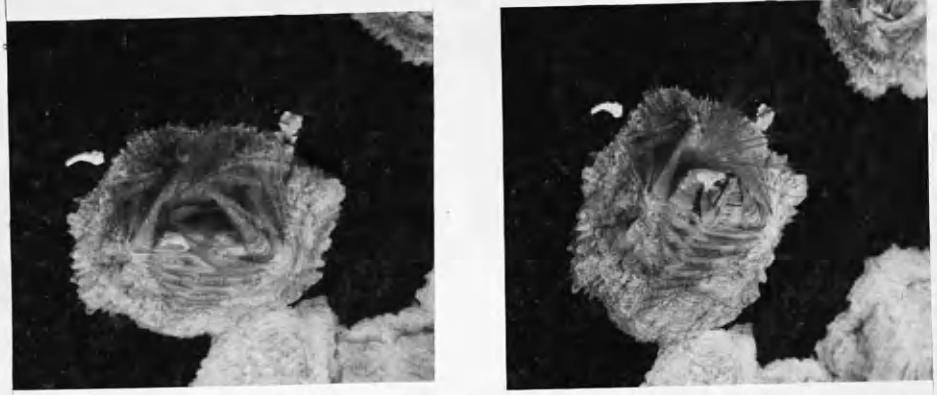
operculum opens the body rises in the mantle cavity- largely under the action of the attrahens muscle and the cirri uncurl relatively slowly: this takes ≈ 0.5 sec. After pausing at the top of this phase the cirri begin to curl towards the body: there is little appearance of any marked forward sweeping movement so characteristic of the balanid cirral beat. The cirri - partially curled up - are brought down as the body is retracted into the mantle cavity: as seen from outside the animal, apart from the curling up of the cirri, the movement is only up and down with no movement of the valves. The frequency of a complete cycle of these movements is ≈ 32/min. This is very similar to the cirral beat of Balanus balanoides (30-36/min according to conditions) but larger than that (maximum, 18/min) given by Southward (1957) for Lepas anatifera (see also Patel, 1959).

If Artemia nauplii are present they may be trapped in the downward-curling cirri and carried towards the mouth parts. These relatively slow movements would hardly be classified as beating as the term is usually understood, even though this term was used by Southward (loc.cit.) for cirral movements in Lepas anatifera. Apart from the absence of any pressure changes because there are no changes

Plate I

a b c

d e



f g

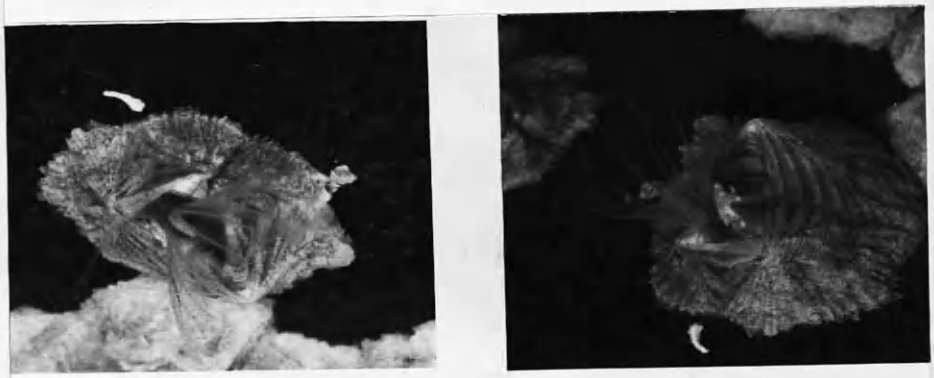


Plate I

A series of photographs to illustrate the movements involved in the protrusion and retraction of the cirri.

a. Moveable valves beginning to open: opercular membrane visible; cirri not yet extruded.

b. Moveable valves further open: cirri slightly protruded; body not yet visible.

c. Similar point in extrusion sequence as Fig.b looking at the side of the cirri; entrance to mantle cavity more evident; body not yet visible.

d. Cirri further out of mantle cavity and more widely extended; body somewhat raised; penis visible.

e. Cirri still further out of mantle cavity and rami more widely spread.

f. Cirri virtually fully extended and rami widely spread; mantle cavity and penis (held in a U-shape) evident; body is now lifted up; caudal appendages fully extended backwards; viewed from above the animal.

g. Similarly to (f) more from the side.

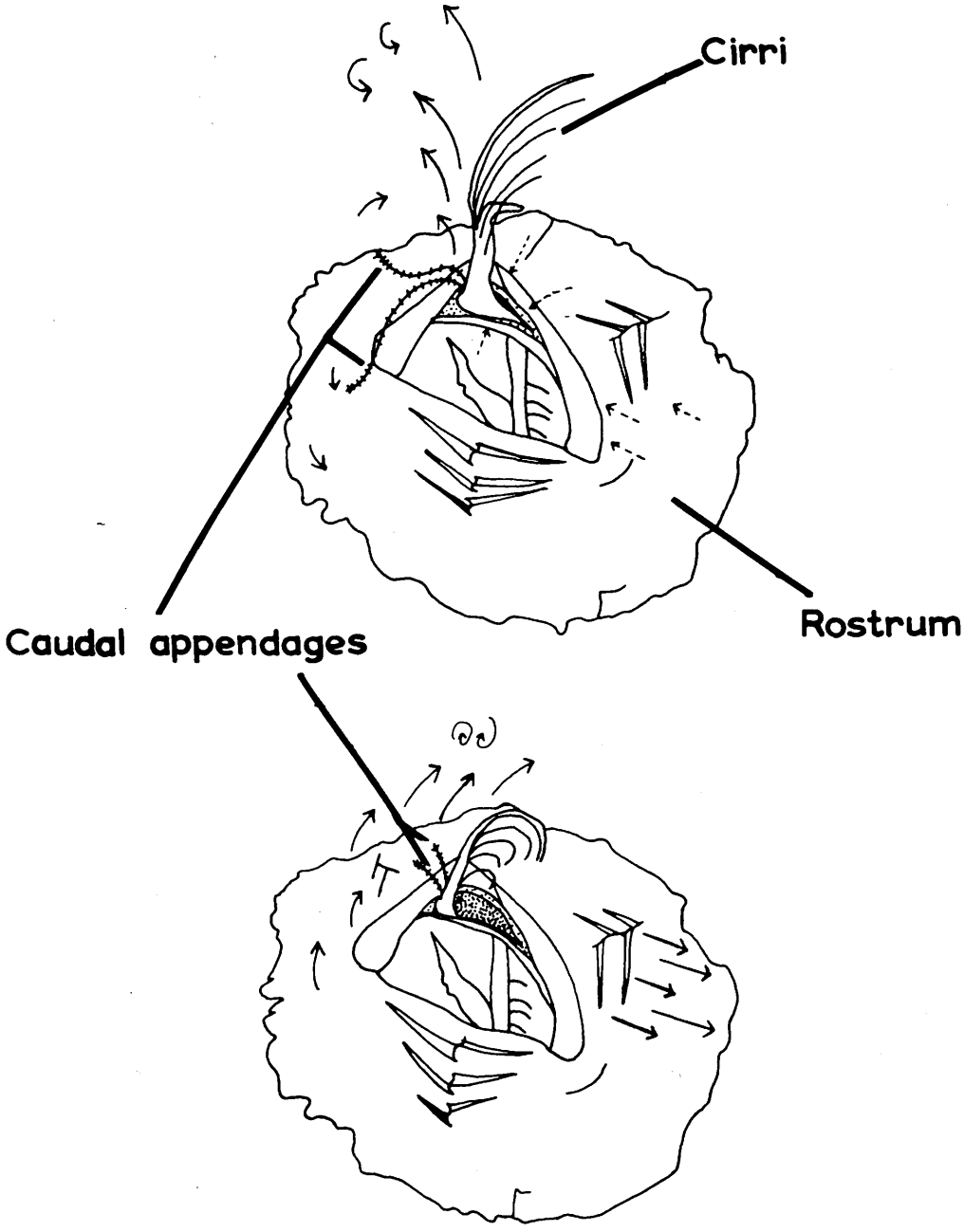


Fig. 19. Diagram to show water currents during cirral activity in Verruca stroemia.

in the volume of the mantle cavity, the fact that during retraction the cirri are held close to the velum gives no space behind them through which water could easily be expelled.

During the course of these movements no through mantle cavity current could be detected. The movement of the cirri causes external water currents to be generated (Fig. 19) much as in a balanid and the fact that the long posterior cirri are much curled during both extension and retraction and will, therefore, present a continuous surface to the water, may be more effective in setting up a water current than if the cirri were widely extended during their movement. Curling of the cirri takes place very early in the retraction sequence at which time there is relatively little forward movement of the cirri. Animals were allowed to remain in sea water to which methylene blue had been added so that after opening and closing a few times the water in the mantle cavity was blue: they were then taken out and, whilst still closed, washed thoroughly with clean sea water and then replaced in fresh sea water. As the cirri retracted a 'puff' of blue water was expelled from the mantle cavity forming a crescent-shaped band in front

Plate II

a

b

c

d

Plate II

A sereis of photographs to illustrate the various movements of cirri.

a. Top right, animal with cirri fully extended: penis somewhat extended from U-shape; valves in extreme position; caudal appendages extended over carina; note close opposition of basal parts of cirri to operculum.

b. Cirri in same position as Fig.a; fully extended but terminal part of some rami curled, probably as a result of stimulus by contact with a particle.

c. Beginning of retraction sequence: body is lower in the mantle cavity, cirri beginning to curl, and penis beginning to disappear from view; caudal cirri brought together.

d. Two animals (top right) fully extended: one animal (bottom left) with cirri only partially extended; penis not visible.

of the cirri: at the next extension of the cirri this crescent moved some way back towards the opening of the mantle cavity, indicating a partial ingress of water, and particles lying at the sides of this current were drawn inwards: no inward movement of water behind the cirri was detected. Further pulses of water formed successive drawn inwards: crescentic bands with successive retraction and extension of the cirri. It is possible that when the valves open and close, small pressure changes resulting from the opening and closing of the folds at the two ends of the operculum also contribute to this exchange of water (Cannon, 1947).

Cirral movements in a water current

To observe the activity in the presence of currents, animals attached to small stones were held in a glass vessel, the water in which could be stirred at variable speeds by a magnetic stirrer. The magnet was so placed that it did not interfere with the animals. Current velocities were estimated by timing the movement of small particles in the water over known distances marked on a card placed under the vessel.

At very low water velocities the cirri were expanded

Plate III

a

b

Plate III

a. Beginning of retraction phase: tips of cirri curling; caudal appendages still widespread but lifted up away from the carina; penis held U-shaped.

b. Later in retraction phase: cirri curled; caudal appendages closer to one another and nearer cirri being retracted; penis held in U-shape.

and contracted much as in still water but with increasing velocity fewer animals of a group showed this beating, but those that did uncurled and curled somewhat more rapidly, and more and more animals showed a typical extension reaction; the cirri were rigidly extended in a fan-like manner - immediately recalling the extension position in balanids or the normal activity in Pollicipes. From time to time if no food is captured, the cirri may be partially curled and retracted after which they return to the extended position without total withdrawal into the mantle cavity of either cirri or body. Unlike the cirri of balanids they do not appear to be able to respond to the direction of the current by twisting movements of the body: they always face the rostrum. For extension, a current velocity of ≈ 7-8cm/sec is required - a value similar to that found by Crisp & Southward (1961) for Balanus balanoides: in contrast to B. balanoides, however, if no food is captured the cirri will remain extended for long periods (in B. balanoides extension lasts only 1.3-1.8 sec according to Crisp & Southward). Current velocities of ≈ 16cm/sec caused the animals to close and this value is smaller than that

for Balanus balanoides ($\subset$ 20cm/sec): As the waterspeed is increased the cirri in B. balanoides may however, first reduce the pressure on them by turning sideways to the current: this course is not open to Verruca.

When potential food particles are present in the water a single cirrus may react when hit by a particle by immediately curling and so bringing it down towards the mouth and then, after the food has been cleaned off by the anterior cirri, the cirrus returns to its fully extended position during which time the other cirri have remained fully extended. In other cases after a food particle - particularly a large one such as an Artemia nauplius - has struck a cirrus others may react to ensure more certain capture. It was not found possible to stimulate a single cirrus alone to react to touch by even the lightest probe, as may be done with Pollicipes; on touching a single cirrus all the other cirri reacted together immediately and the whole cirral net was contracted.

Restricted ventilation of the mantle cavity

On occasions, and particularly in still water, the animals may open the opercular valve a very short distance without extruding the cirri. This seems to resemble pumping

or testing activity but for the reasons already given there can be no enlargement of the mantle cavity itself: however, because of the negative pressure developed towards the mantle cavity as the operculum moves against the surrounding water there will be some exchange of water - possibly enhanced by body movements within the mantle cavity: water will flow into the mantle cavity around the edges of the operculum as the latter opens.

Activity during pre-copulatory periods

That the penis of an operculate barnacle performs characteristic searching movements to locate a functional female is well-known (Barnes & Barnes, 1956), and during this activity the cirri are fully extended. In the normal extension position in Verruca the whole penis is always brought outside the mantle cavity with the cirri: it forms an inverted U, the free end pointing downwards towards the mantle cavity. Only a limited number of animals have been observed showing searching activity but in all cases the movements were as described below. As a preliminary to searching behaviour the cirri are further extended to lie almost parallel to the substratum and, with the body well out of the mantle cavity, the penis is unrolled and passes

backwards behind the cirri. Only the area behind the cirri was explored and then only in an arc extending some 10-15° on either side of the mid-line: in Balanus balanoides the whole region both in front and behind the cirri can be explored. In Verruca much of the penis was straight during this activity with only the distal region bent in exploring for a functional female. Copulation and emission of semen has not been observed, but there is little reason to believe it would be anything but similar to that in B. balanoides. Although it was not found possible to stimulate a single cirrus to react alone during normal extension by touching with a probe this was possible when the cirri were in the extreme extended position during active searching. The tip of the cirrus may only curl in response to light touch but on stronger stimulation the whole ramus will curl up, sometimes to be followed by curling of the other cirri, in which case the penis too rolls up.

The emission of nauplii

In a typical balanid such as Balanus balanoides the two similar egg masses lie free and symmetrically placed at the base of the mantle cavity. The eggs are fertilized as they

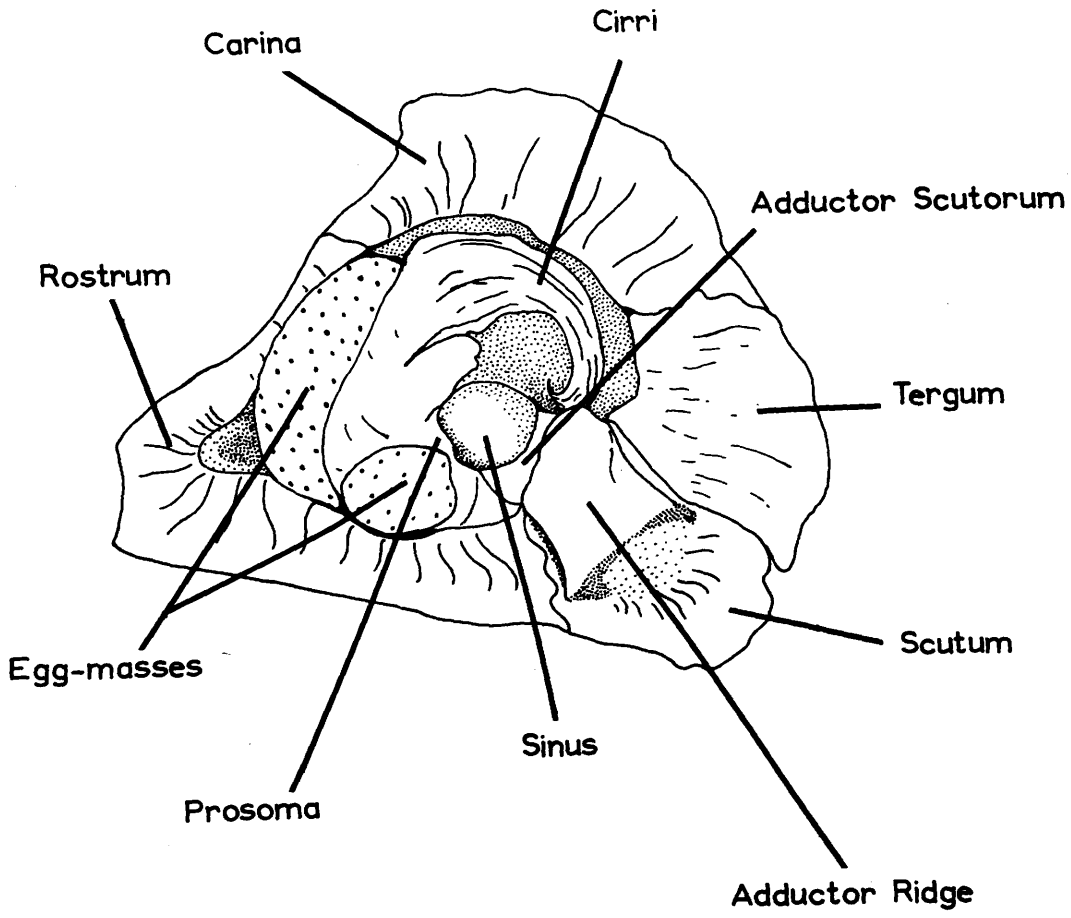


Fig. 20. Verruca stroemia: general view of animal after removal from substratum and viewed from the under side showing disposition of 'body' and egg masses.

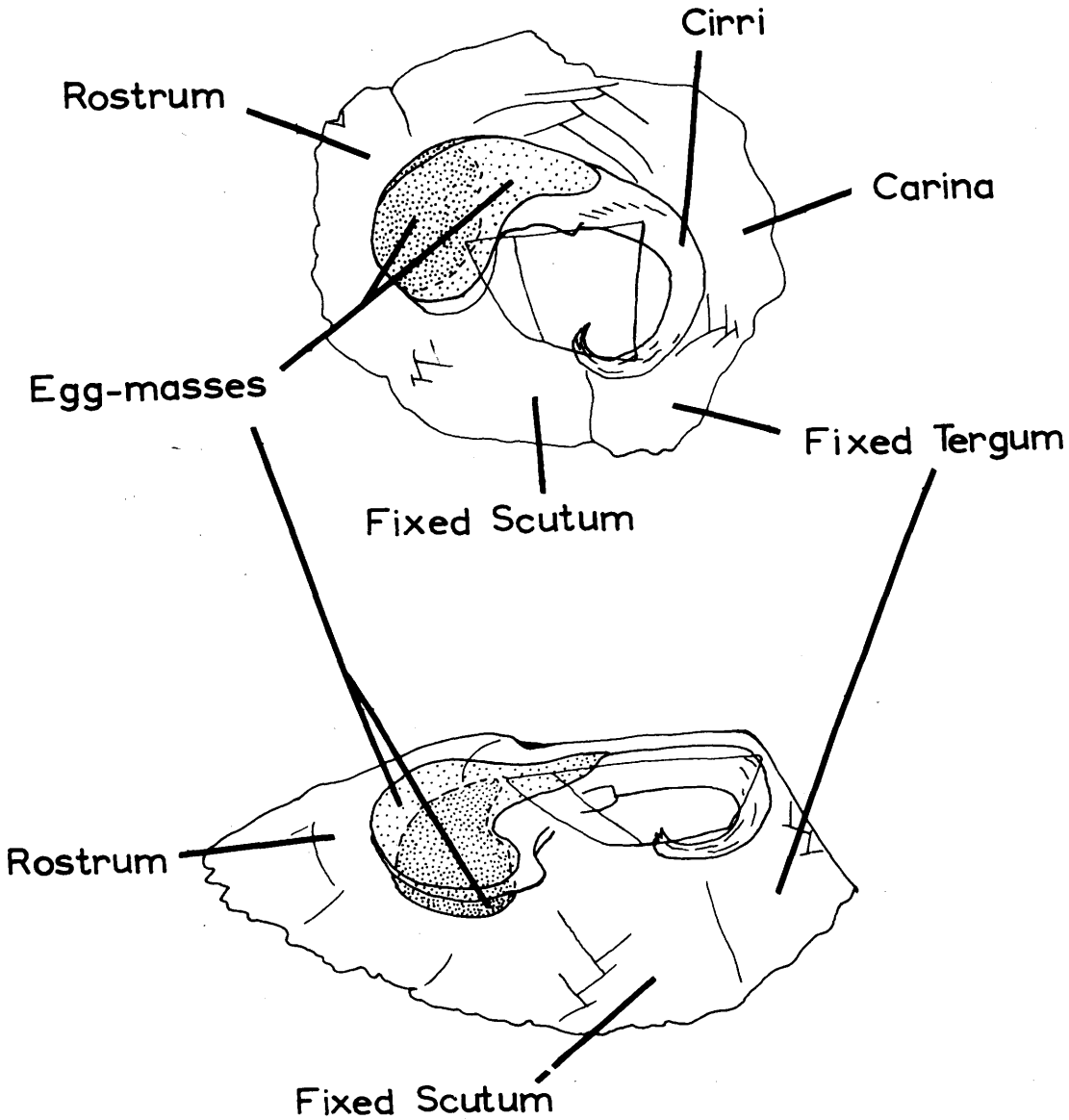


Fig. 21. Verruca stroemia. Upper: position of egg masses seen from above i.e., through the opercular opening after displacing the moveable valves. Lower: same viewed laterally.

issue from the oviduct which opens near the base of the first cirrus and within a 'bag' secreted by the terminal oviducal gland; they are subsequently manoeuvered round the sides of the body to the position described above; this is possible because of the extreme fluidity of the egg masses at this stage. The asymmetry of Verruca and its much reduced mantle cavity consequent upon its greatly flattened form, leads to differences in the size and position of the egg masses (Fig. 20+21): both are closely appressed to the prosoma. The larger has on the average 1.5 times as many eggs. The larger egg mass lies above the prosoma (as the latter lies parallel to the substratum) virtually covering it: it is curved and elongated. The smaller lies below the body in its resting position and is almost a flat curved circular plate of eggs (in small animals).

Nauplii have been seen to be expelled on three occasions. They were freed from their egg cases before emission; in this respect they resemble Balanus balanoides (Barnes, 1955). They issue from behind the cirri (in still water, in which the details can be more easily seen) as the body is raised and the cirri expanded. Each time the cirri unrolled free-swimming larvae were released in small groups but were not expelled away from the parent with any considerable force

as is the case when expulsion takes place in the exhalent current of a balanid. The caudal appendages seem to play a part in this activity. In the resting animal they are curled over the posterior cirri and as the cirri expand they spread widely over the carina and behind the cirri. Each time the nauplii are expelled the spreading and retraction of the caudal appendages helps to disperse them away from the parent. The free larvae are further separated on the downward movement of the cirri, presumably by the small external current then generated.

Gut contents

The gut contents of *Verruca* have been examined: samples were collected on three occasions - in January, March, and August, representative of the different nutrient conditions in this locality. The animals were fixed in formalin immediately after collection to stop enzymatic breakdown of the gut contents which were subsequently dissected out: after separation, the material was examined for any recognizable remains such as broken diatom frustules or zooplankton skeletons. The total gut contents of a large number of animals were then pooled and shaken for 2 h in sea water

using a mechanical shaker to aid dispersion.

It is realized that in the absence of any added dispersing agents such as are used, for example, in soil and sediment analysis the particle-size distribution subsequently determined may not represent that of the fully dispersed material: however, it seems likely that it will more closely correspond to the particle-size distribution of the food as collected by the animal. After dispersal in this way a sub-sample of the suspension was spread on a microscope slide and 64 fields, selected at random, photographed under phase contrast. The particles on each frame were measured with a mm ruler under an enlarger, taking the largest and smallest dimension: the means of these two measurements (for brevity termed the diameter) were used to set up size-frequency distributions shown in Fig. 22 and Table XV . It is evident from this figure that on all three occasions small particles up to 4 µm in diameter dominated the gut contents (January, 71%; March, 64% and August, 93%): most of this was unrecognizable organic material with some small grains of sand. In January many (30%) of the guts were virtually empty; two setae, and a small copepod (?) - present in one large animal (5.3 mm

Table XV

Verruca stroemia: size (mean of largest and smallest dimension) - frequency distribution (%) of gut contents; animals preserved immediately after collection: dispersed by shaking in water.

Size-frequency class µm	January	March	August
0 - 1.9	25.3	19.8	70.6
2.0 - 3.9	45.6	44.3	23.2
4.0 - 5.9	16.3	15.9	3.5
6.0 - 7.9	5.9	7.8	1.0
8.0 - 9.9	3.1	5.1	0.8
10.0 -11.9	1.9	3.6	0.2
> 12.0	1.9	3.6	0.8

basal diameter) - were the only recognizable remains found. In March, when none of the guts were empty, there was a slight shift towards more of the larger particles; damaged frustules of Skeletonema costatum Grev. were found in many of the guts and the size-frequency distribution (length of

Plate IV

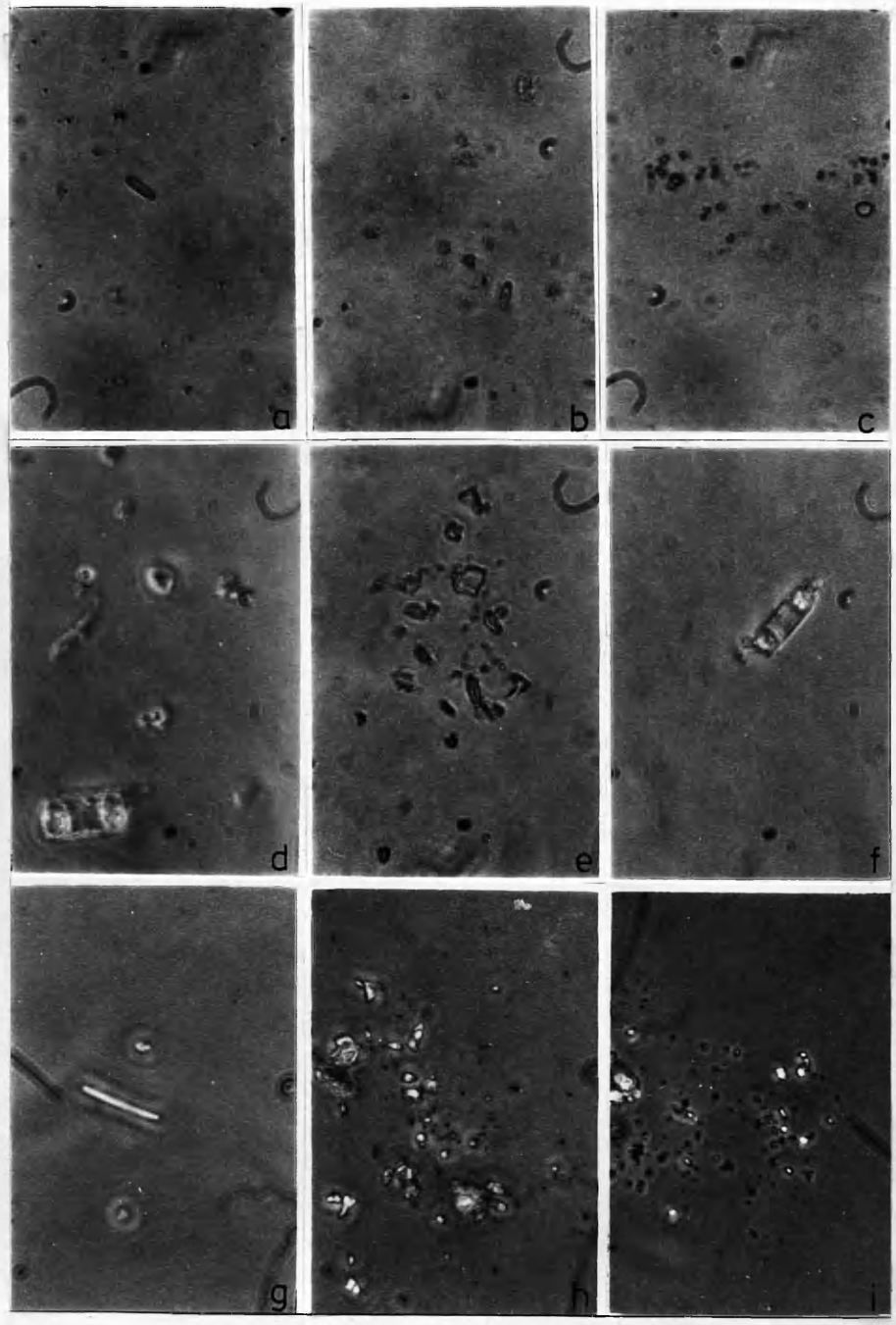


Plate IV

Verruca stroemia. random samples of gut contents examined at three different seasons representative of different nutrient conditions : a-c, January; d-f, March, note broken Skeletonema frustule in d and f; g-i, August : all at x 600.

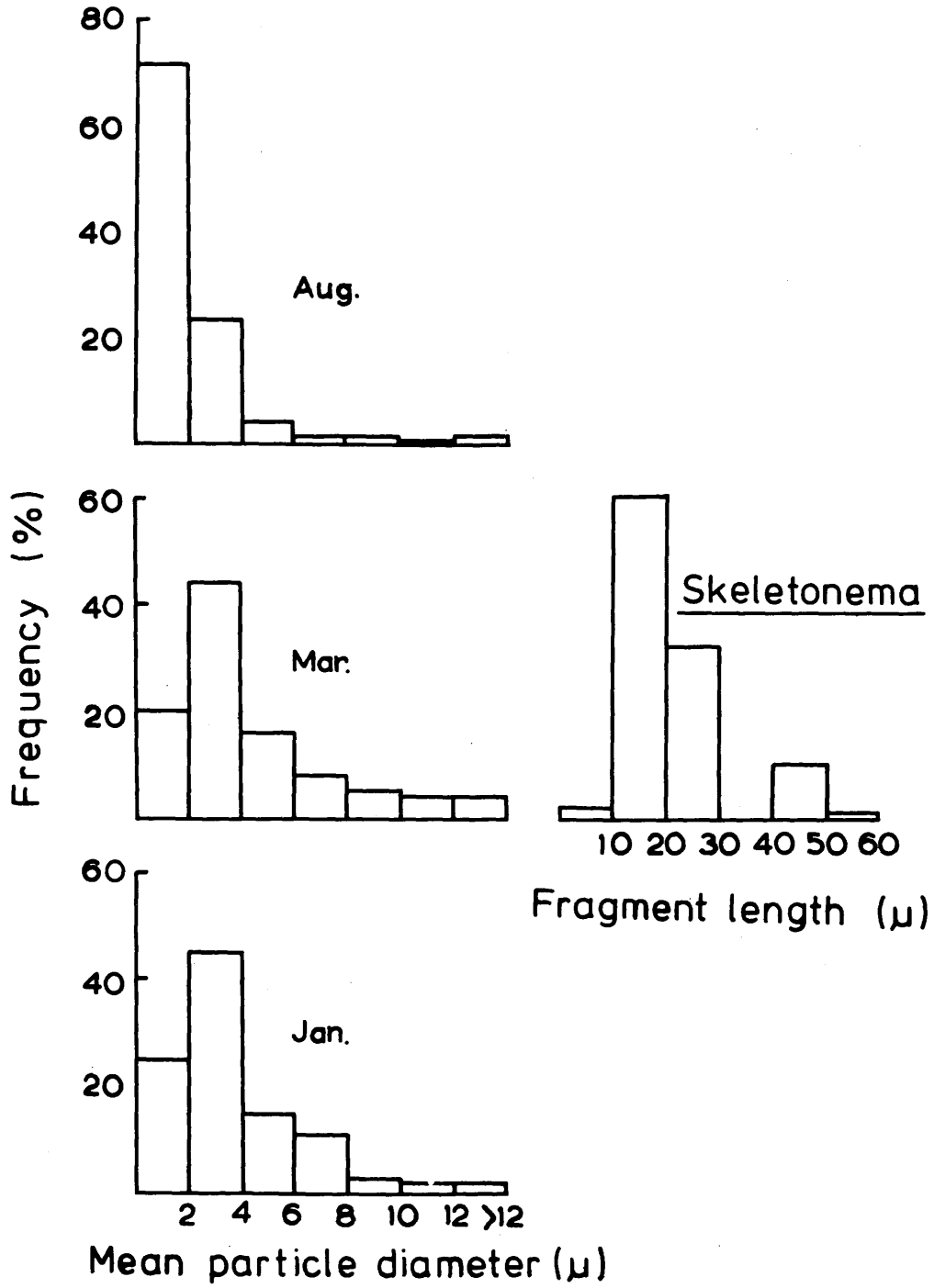


Fig. 22. Verruca stroemia: Gut contents; particle-size distribution on three occasions representative of varying nutrient conditions; particle-size distribution for Skeletonema costatum frustules shown separately on right.

frustules) of these is shown separately in Fig 22 . The 10-20 µm size class dominates this distribution but since particles > 12 µm contribute only 3.6% to the overall size-frequency distribution their effect on this latter is small. Lebour (1930) states that the diameter of S. costatum lies between 8-17 µm and its length from 10-16 µm. Skeletonema normally occurs in chains and while many single damaged cells were present it is evident that larger material in the form of chains of the diatom are taken. One large animal (7.3 mm in basal diameter) had several stage I cirripede nauplii (one 178 µm) and the other 240 µm long (probably Verruca) as well as the remains of a copepod (?), 130 µ x 240 µm. The predominance of small particles in the August sample - even though an occasional frustule of Skeletonema (8-17 x 5-15 µm), a naviculoid (18-25 x 4-6 µm) and a centric diatom (19 x 14 µm), were present - is marked: fragments of macroscopic algae with cells 57 x 4 µm and an occasional crustacean appendage (168 x 124 µm) was also found in some individuals.

Discussion

It has already been stressed and was so cogently pointed

out by Darwin (1854) that in many respects Verruca closely approaches the Lepadidae and in particular Pollicipes: this is true with regard to its cirral activity. The membranous basis of a Verruca is homologous with the pedicel but the latter having been lost, the whole animal cannot respond to a current: the wall plates are firmly fixed to the substratum. In many balanids this loss of reaction to water currents in the absence of a peduncle has been 'compensated' for by the capacity of the cirri to respond and to initiate movements of the body so that they face into the current - a character of obvious survival value in captorial activity. In Verruca no such 'compensating' mechanism has evolved, and the cirri do not respond to a water current by turning to face it.

More important in the overall cirral activity is the fact that Verruca differs from all other Balanidae in that the whole shell, including the moveable valves, has no muscles other than the adductor scutorum. Quite clearly the whole sequence of events of the cirral beat and the production of a distinct mantle cavity current in the balanids in which the raising and lowering of the opercular valves, by means of retractor muscles attached to the basis

of the animal is involved, cannot be carried out by a Verruca. The only movements other than those under the control of the body muscles (which are probably similar to those described by Cannon (1947) for Lithotrya) and the cirral retractors are those associated with hydrostatic (turgor) pressure and the adductor scutorum; in fact, the operculum acts as a simple hinged 'lid' under the opposing effects of turgor pressure and the adductor scutorum muscle. The possibility of movement of the 'lid' is, as in other species, dependent upon the flexibility of the opercular membrane at its junction with the wall plates. It is as if a Pollicipes were shorn of its peduncle and one half of the capitulum were solidly attached to the substratum. This analogy also stresses the fact that in the extremely asymmetrical Verruca, the body, attached between the moveable and the fixed scuta is, in its resting position, virtually parallel to the basis; indeed the whole animal is much flattened. The adductor scutorum is attached basally to a prominent projection of the fixed scutum so that the whole muscle still virtually makes a right angle with the body. The body is only brought into the near vertical position when the valve is fully opened.

The cirral movements in still water do not seem very effective in the capture of food, largely because the cirri curl so soon during the retraction phase. It would seem that these movements largely subserve a respiratory function. Captorial feeding by extension is efficient: the whole cirral net is extremely sensitive to even quite small food particles. The fact that although individual cirri were seen to respond to particles and yet even the lightest touch by a probe caused the whole net to contract, indicates a greater sensitivity to tactile stimuli than is the case with Pollicipes: this latter species is largely a zooplankton feeder and the greater sensitivity in Verruca will allow it to respond to much smaller organisms such as diatoms. The fact that after individual cirri have responded to particles striking them the other cirri may subsequently react indicates some central control, whilst the reduction in sensitivity during searching movements, unless it be due entirely to extreme extension, implies some central inhibition.

Some ventilation of the mantle cavity that can subserve a respiratory function and may also contribute to micro-feeding clearly takes place in the movements that lead to extension and its reversal. In addition, whilst

the posterior cirri remain in the fully extended condition the anterior cirri make virtually continuous vigorous 'reciprocating' movements which may indicate micro-feeding at this time.

The searching activity of the penis resembles that of other operculates. It is, however, of interest to note that unlike Balanus balanoides the searching is restricted to the region behind the cirri. It has already been pointed out (Barnes & Klepal, 1971) that the penis of Verruca lacks a distinct pedicel (that in Pollicipes sp. and other Lepadidae is vastly modified from that seen in the Balanidae) and so the complex musculature associated with Balanus species (Klepal, Barnes & Munn, 1972) which is related to the presence of the appropriate girdle structures for their attachment, may well be absent in Verruca: it is probably for this reason that the searching area of the penis is so restricted.

In the absence of an internal mantle cavity current nauplii cannot be ejected with any great force. The part played by the caudal appendages is of interest and more observations are clearly desirable. Caudal appendages are characteristic of the Lepadidae and Verrucidae: in the Balanidae they only occur in the two species of Pachylasma

and in one of Catophragmus. In the former genus, however, the caudal appendages are small and in one, Pachylasma aurantiacum, very small; and in Catophragmus imbricatus minute: even in the Lepadidae they are absent in some genera and rarely do they consist of more than a very small single segment. Only in Verruca are they extremely long and only in this species would it seem that they are capable of assisting in the emission of nauplii. One of the functions of the considerable mantle cavity current in balanids would seem to be to expel the nauplii to a sufficient distance from the parent to avoid them being immediately eaten by the latter. In this respect expulsion by Verruca is much less effective and it is perhaps, therefore, not surprising that the stomach contents at certain times of the year contained nauplii of the same species. In general, however, the strong photopositive reaction of the larvae will quickly take them away from the parent into the upper water layers.

Little further can be said about the feeding mechanism once the food, captured by the cirri, has been transferred to the proximity of the anterior cirri. As far as can be seen in this small species, the anterior cirri appear to

make similar movements to those described for Balanus balanoides and Pollicipes polymerus. The particularly dense covering of spines on the first two cirri which show much activity recalls the situation in Pollicipes and may reflect the capacity to micro-feed while the cirri are extended. It should, however, be remembered that much diatom.material is rejected by the latter species.

In view of the resemblance of the cirral activity to that of Pollicipes polymerus which feeds largely on zooplankton and the fact that some operculates such as Tetraclita sp. which feed largely by extension contain abundant zooplankton remains in their guts, (Mori, 1958; Barnes, 1959) the virtual absence of zooplankton remains in Verruca is perhaps surprising. The guts of most species, however, contain much fine particulate material and while the cirri of Verruca react to very small particles it does seem that some form of microphagy is used - either in the form of particles entering the mantle cavity in the weak currents or by the vigorous activity of the brush-like anterior cirri. Even at the height of the spring diatom increase, diatoms did not, apparently form the main constituent of the food. Verruca is essentially a deep-

water genus; *V. stroemia* is the only species occurring in shallow water. *V. stroemia* is quite sensitive to water currents and, even near the bottom, currents of 15 cm/sec (0.03 kt) which are adequate to stimulate cirral extension are likely to be common: the species seems to be most common, at least in shallow water, where there are considerable water currents. It would seem probable that the species largely relies on debris swept from the bottom for its major nutrient requirements.

To summarize; in its feeding and respiratory activity *Verruca* is very similar to the littoral lepadid *Pollicipes* common on the North American west coast. This results from the characters of *Verruca* which, in spite of the absence of a peduncle, require it to be considered as a primitive genus more closely related in many respects to the Lepadidae than the Balanidae.

Suppression of penis development in Balanus balanoides (L.).

Introduction

The reproductive organs of Balanus balanoides are developed annually. The new ovarian tissue is visible at the base of the mantle cavity sometimes below the egg masses, in older animals even before the embryos are released: the ova increase in size to reach their maximum just before copulation in early autumn. The testis begins to re-develop simultaneously but is not conspicuous on dissection until the summer: by autumn the vesiculae seminales are gorged with spermatozoa. The penis degenerates and is lost leaving only a stump at a moult soon after the egg masses have been laid down: the vesiculae seminales also shrink (Crisp & Patel, 1960); according to them a new penis "gradually developed during the period of summer growth reaching its maximum length before the onset of the next breeding season" but, an inspection of their figure relating penis length to season indicates that the growth was by no means gradual but that there was a marked increase in the growth rate in mid-August, as is also evident from Fig.1. The ova are eventually shed into a sac

formed by an oviducal gland situated at the end of the oviduct and lying in the basal segment of the first cirrus. After copulation the epithelial cells which secrete the sac are small. This oviducal gland increases in size during the next year and about six weeks before copulation there is a burst of mitotic activity associated with a large increase in size of the gland before it enters the secretory phase about three weeks before copulation (Walley, 1965). The lengthening of the penis and the changes in the oviducal gland may be considered as secondary sexual characteristics - even though occurring in a hermaphroditic species.

Barnes (1963), and Barnes & Barnes (1967) have shown that there is an upper critical temperature above which breeding will not take place and that continuous illumination is also inhibitory (see also Crisp & Patel, 1969). Tighe-Ford (1967) has shown that constant illumination not only inhibits the production of egg masses but also inhibits the formation of the oviducal gland sac: when transferred, however, from constant illumination to a dark environment at a low temperature the inhibition was removed and the oviducal gland enlarged, formed a sac, and the animals produced egg masses. Clearly the same environmental factors inhibiting the production

of egg masses, however this is mediated, also inhibit the female secondary sexual characteristic. It is, therefore, of interest to see whether these same environmental factors will also inhibit the secondary male sexual character, namely, the lengthening of the penis.

Material and Methods

Crisp & Patel (1960) measured the length of the penis directly on the exuviae of individuals of the same approximate "age" group, "mostly exceeding two years": Crisp (1954) working with Balanus balanus measured the length of the contracted penis and to investigate the seasonal changes applied a correction factor for the size of the animal. The penis is a highly muscular and extensible organ so that comparable measurements on different individuals are difficult to make with accuracy. In the present work, therefore, the number of annulations, which is a measure of penis length, has been determined for animals of a very narrow size range (basal diameter) by counting them under a binocular microscope.

In the first series of observations, animals from the shore on stones were held at 10° and $15 \pm 1^{\circ}C$ in each case under constant illumination (fluorescent light) and constant darkness (known not to inhibit the laying down of egg masses below the critical temperature of $\approx 10^{\circ}C$).

The sea water was aerated and a liberal supply of food in the form of Artemia larvae supplied. Every few days the animals were removed and cleaned and the sea water renewed. The experiment was begun on the 18th July, 1970 and the first samples removed for examination on the 29th September, 1970, by which time elongation of the penis was marked, in the shore population: a second sample was removed on the 4th November 1970, when the shore population had already laid down egg masses: since the experimental animals had been virtually continually immersed it would be expected that copulation would be retarded relative to the shore population.

Results and discussion

General observations

29th September 1970

10°C-continuous illumination. The ovary was in a poor condition thin, white and 'watery' with few ova, not enough in some animals to make satisfactory measurements of their size: it was difficult to remove without disintegration. The ova themselves were abnormal, with little development of yolk. The mean diameter of the few ova measured was 117 μ. The testis was hardly developed and contained much oil and the seminal vesicles were different to follow on dissection.

10°C-continuous darkness. The ovary looked in good

condition though not large. In some animals no ova could be shaken out: the mean diameter of the ova measured was 141 μ. The testis was well developed with spermatozoa having rounded testicular or accessory droplets although some had a thickening characteristic of vesicula spermatozoa (Barnes, Klepal & Munn, 1971). The vesiculae seminales were moderately well developed and contained many spermatozoa with thickened droplets; the proportion of fully filiform spermatozoa was variable (20-95%) in the few sampled.

15°C-continuous illumination. The ovary was poorly developed: in some animals only very small ova were present with little yolk. The mean diameter of separable ova was 99 μ. The testis was small or undeveloped full of globules and without spermatozoa while the vesiculae seminales were hardly discernible.

15°C- continuous darkness. The ovary, firm yellow, and easily removed without disintegration was in much better condition than in either the 15°C-continuous light or even the 10°C-continuous light suggesting that even at this relatively high temperature its development can take place in the absence of light. Ova (mean diameter 125 μ) could be shaken out of all the animals examined but seemed to have less yolk than normal. The testis was moderately-well developed and contained spermatozoa, largely with testicular droplets, although some had thickened vesicula-type droplets.

The vesiculae seminales were clearly visible and in some animals distended with spermatozoa, many of which still had, however, testicular droplets but a few with thickened accessory droplets were present. Clearly, as with the ovary, the male gonads had developed far better than at 15°C-continuous light or even at 10°C-continuous light.

It is clear that, after just over two months under the experimental conditions, continuous light had depressed the development of both male and female reproductive organs, thus confirming earlier work. The results on the animals at 15°C in continuous darkness are a further indication that a previous suggestion for apparently 'anomalous' results regarding the critical temperature for breeding- namely that the critical level is higher in the absence of light- is correct; under these conditions both male and female reproductive products as regards size, appearance and state of development were not too different (except perhaps for the amount of ovary) from those pertaining in the 10°C-dark cultures. It was noted that under continuous light, at either 10 or 15°C, the mortality during the two months was considerable whereas few animals died in both the dark cultures, particularly that at 10°C.

4th November, 1970

10°C-continuous illumination. The white-creamy, rather

watery ovary was in poor condition; it readily broke up. Free fat globules were abundant. The individual ova were somewhat more dense than previously but were often misshapen and their membranes had a typical 'frothy' appearance at the edges: the few ova shaken out had a mean diameter of only 98 μ, indicating no increase in size since the 29th December. In one animal the testis was moderately developed with some spermatozoa possessing testicular droplets and some with thickened droplets; in this animal the thin seminal vesicles also contained spermatozoa in these same stages. In all the other animals of this sample the testis had not developed, no spermatozoa were found and the vesiculae seminales were difficult to distinguish.

10°C-continuous darkness. The ovary, of a moderately thick consistency, was quite well developed and ova were readily shaken out (mean diameter, 109 μ but ranging from 70-169 μ). The testis was well developed and contained spermatozoa largely with testicular droplets but also some with thickened droplets: the vesiculae seminales were fairly well developed and contained spermatozoa of both types seen in the testis.

15°C-continuous illumination. No compact ovary was present: it was very 'watery'. The few small irregular shaped ova present, easily broken on teasing,and none were available for measurement. The testis was degenerate

with no spermatozoa and the vesiculae seminales could not be seen through the body: on dissection they were found to be thin, irregular, and empty.

15°C-continuous darkness. The light-yellow ovary was quite well developed and on teasing out in sea water readily shed ova (mean diameter, 144 μ) with normal membranes and contents. The testes were largely empty but the well-developed vesiculae seminales, distended and easily seen through the body wall, had spermatozoa with both testicular and thickened droplets; and filiform spermatozoa were also present (up to 20%).

The events set in motion between July and September had clearly continued until the November sampling, by which time the shore population had laid down egg-masses. Under continuous illumination at both 10° and 15° the gonads had not developed and at 15°C degeneration was marked. At 10°C in the dark gonad development had proceeded normally with abundant yolky ova, spermatozoa, and well-developed vesiculae seminales. The 15°C-dark culture had to some extent at least developed normal gonads so that the raising of the critical temperature by darkness was not limited to the early period: indeed the presence of egg masses in some animals of this culture indicated the viability of the reproductive products since copulation must have taken place. The development of the gonads in the 10°-dark culture seemed somewhat retarded when compared with the 15°C-dark culture although in both

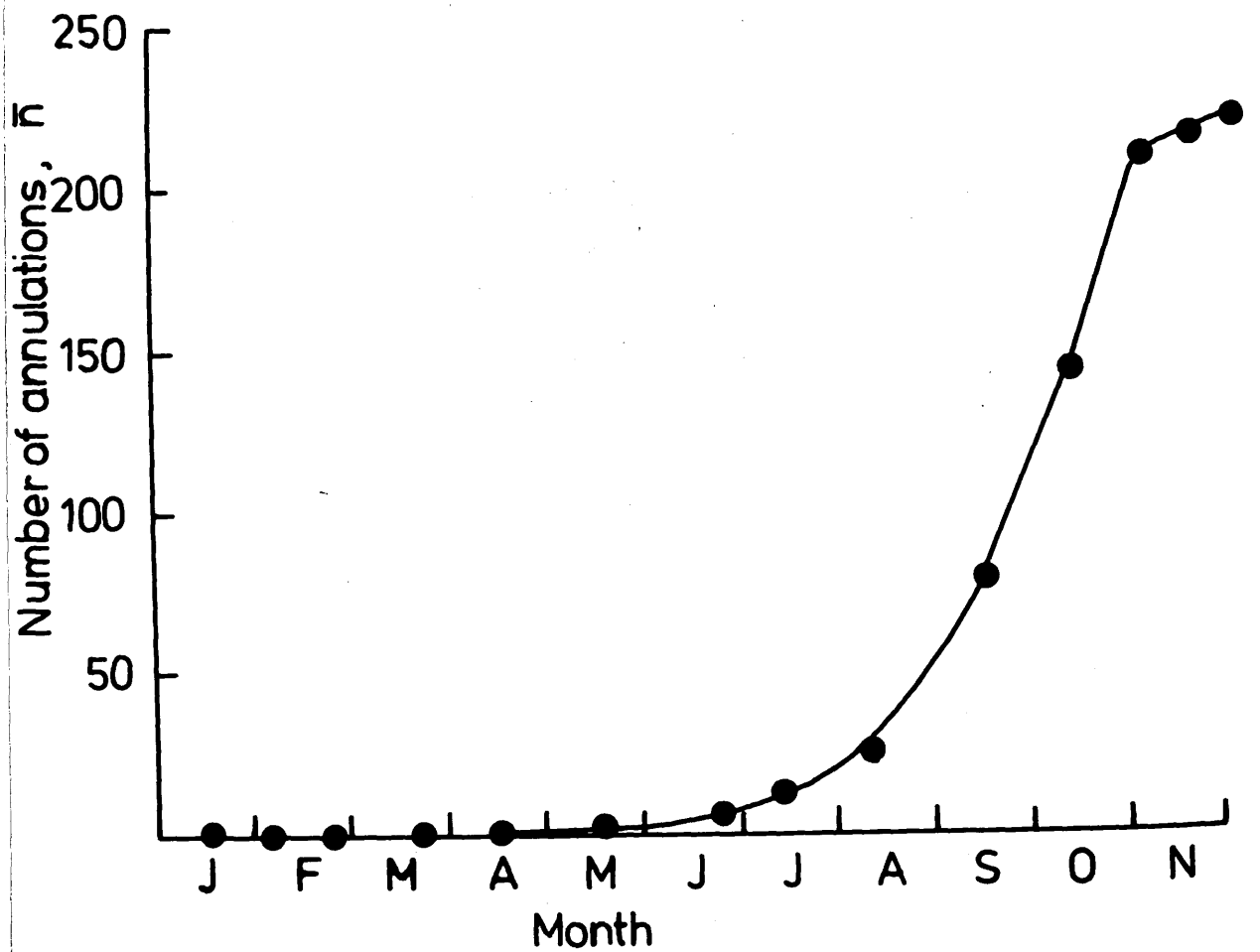


Fig. 23. Balanus balanoides: change in the number of annulations, used as a measure of penis length, during a season after loss in the previous autumn.

cases viable reproductive products were found. Observations in the field have indicated that higher summer temperature and an adequate food supply can lead to a more rapid development of the ova (at least as indicated by their size). Thus at Point Lookhout in an embayment of Great South Bay, Long Island, New York, where summer temperatures are high (20°C) the maximum size (154 μ) is reached by the end of September, whereas at Millport, Scotland (10-15°C) it (170 μ) is not reached until the end of October. Mortality was high in this second period in both cultures under constant illumination and particularly severe at the higher temperature.

The growth of the penis

The changes in the number of annulations of the penis for an upper shore population of B. balanoides at Millport, Scotland are shown in Fig. 23. The penis grows only slowly during the spring and early summer but as the length increases in August-September the number of annulations increases rapidly to reach its maximum at the time of copulation in November. The changes on the lower shore are similar but the time scale is somewhat different.

Table XVI

Number of annulations of the penis of B. balanoides maintained under a variety of experimental conditions from 17th July, 1970: values are the mean of 20 animals, basal diameter, 0.9-1.3 cm: animals collected at Oban, Scotland.

Sample		No. of annulations
17th July	Shore	18.9
29th September	Shore	145.2
" "	10°C light	41.6
" "	10°C dark	129.7
" "	15°C light	42.7
" "	15°C dark	198.0
7th November	Shore	172.9
" "	10°C light	33.0
" "	10°C dark	90.8
" "	15°C light	69.0
" "	15°C dark	189.5

Table XVI gives the number of annulations for the experimental animals; the actual values are not comparable with those given in Fig.1 since a different size of animal had to be selected for comparison, but the changes with time are directly comparable. At the time when the animals were placed under the experimental conditions the sharp increase in the number of annulations was

about to begin in the shore population (see Fig. 23). On the first sampling occasion (29th September) the number of annulations had increased in the shore animals and was approaching its maximum value. At either 10 or 15°C under constant illumination gonad development was markedly depressed; there was little development of the penis. At both temperatures growth and development of the penis had taken place in animals maintained in the dark - that at 15°C exceeding the natural population. By 7th November the development of the penis of the shore animals was maximal. There was little change from the very low values of the previous sampling date in the number of annulations at either temperature when the animals were maintained under constant illumination. At 10°C in the dark growth was similar to that of the shore animals: the lower value for the number of annulations may be due to sampling errors. At 15°C under dark regime the number of annulations was very similar to that of the natural population.

It is clear that under constant illumination gonad development - both male and female - is suppressed: this is in agreement with previous work in which it was shown that no egg masses are produced under these conditions. Both secondary sex characters, namely, growth of the penis and development of the oviducal gland, are therefore, suppressed by conditions which suppress gonad development itself. Whether these two secondary sexual characteristics are directly influenced by the light and temperature regime or whether they are secondarily determined by one or both gonads, is unknown.

Light period and moulting frequency in Balanus balanoides

It is evident from the foregoing that temperature and light period are important determinants in the breeding cycle of Balanus balanoides. These two factors may act, together or separately, either through their influence on general metabolic activities or as a 'trigger' to some specific endogenous behaviour. If they affect the general activity then this might well be reflected in the moulting frequency of the animals: there might well be a seasonal change in any such phenomena. To cover completely, say, six light periods, with the animals starved or fed, at more than one temperature at a number of times during the year, demands an enormous amount of control equipment. So far, a selection of the combination of variables has had to be made: three series have been carried through: in each case at two temperatures, 10 and 15°C (which have been found to be important in the life cycle), namely:

Series 1. June - October, all animals fed (1971);

Series 2. April - May, all animals starved (1972);

Series 3. August - September, all animals fed (1972);

Series 4. September - onwards (still in progress and not considered here) (1972).

The materials and methods

The animals were maintained throughout (in plastic containers) under sea water which was continuously agitated by bubbling air

through it. The containers were held in a specially made set of cabinets the compartments of which were illuminated by fluorescent lighting, the light periods (0, 2, 8, 12, 18 and 24 h) being set by standard time switches. All the cabinets were kept in constant temperature rooms (10, 15°C) and some additional control of temperature was effected by air-blowers to the individual compartments of the cabinets. The temperature of the sea water in each container was checked daily: maximum variability was $\pm$ 1°C.

The animals were collected on moderate-sized stones and cleaned, leaving ~~about~~ a known number ($\approx$ 100) animals on each: they were kept clean throughout the experiments. The exuviae were collected and counted three times each week, and at the same time the sea water was changed: The rare animals which died were noted. When food was supplied it was in the form of Artemia nauplii (San Fransisco product) added in excess at the time the water was changed.

The moulting frequency (number of exuviae/100 animals/day) was calculated for each 2 or 3 day period (in the few cases where animals died the mean number alive for that particular interval was used) and the mean weekly frequency taken as the mean value of these three sets of results: since there are two 2-day periods

and one 3-day period in each week the mean so calculated will be somewhat biased, but in view of the overall variability it was considered that any such bias could be neglected. In all cases the results for at least the first week were neglected since during this period the results tend to be abnormal due to transfer to the experimental conditions. The detailed results are given in the Appendix.

Results and discussion

The analyses of variance for the three series are given in Table XVII. With the exception of the mean square for 'Weeks' in Series 1 at both 10° and 15°C none of the values are significant at the $P = 0.05$ level: this significant value only reflects a change in the moulting frequency with time. Light period had no significant effect on moulting frequency. In view of this, the mean moulting frequency at the two temperatures over all the light periods was calculated. The results are given in Table XVIII from which it is evident that in these experiments the moulting frequency was unaffected by a rise in temperature from 10 to 15°C. Although the 'Light period' mean square was not significant, inspection of the individual means for the two extremes, 0h and 24h, i.e., constant darkness and constant light, suggests that these may have been different: however, the appropriate analysis (Table XIX) shows this not to be the case.

Table XVII

Analyses of variance

	10°C				15°C			
	Sum of squares	D.f.	Mean square	F	Sum of squares	D.f.	Mean square	F
Series 1.								
Weeks	27.28	5	5.455	4.45*	20.23	5	4.046	4.46*
Light periods	6.79	7	0.970	-	11.39	7	1.627	1.79
Residual	42.93	35	1.227	-	31.75	35	0.907	-
Total	76.99	47	-	-	63.37	47	-	-
Series 2.								
Weeks	13.86	5	2.772	5.13	30.20	5	0.604	2.35
Light periods	10.23	3	3.409	6.31	1.67	3	0.557	2.17
Residual	8.18	15	0.541	-	3.86	15	0.257	-
Total	32.21	23	-	-	8.55	-	-	-
Series 3.								
Weeks	43.85	5	8.770	3.07	42.06	5	8.412	2.69
Light periods	13.00	4	3.250	1.14	23.68	4	5.920	1.89
Residual	57.14	20	2.860	-	62.61	20	-	-
Total	113.99	29	-	-	128.35	29	-	-

Table XVIII

A comparison of the mean moulting frequency (exuviae/100 animals/day) at 10 and 15°C over all light periods.

	10°C	15°C	D.f.	t.
Series 1.	6.84	5.55	46	0.53
Series 2.	3.90	3.47	22	0.23
Series 3.	8.86	9.13	58	0.07

Table XIX

A comparison of the mean moulting frequency (exuviae/100 animals/day) in total darkness (0 h) and total light (24 h) neglecting temperature.

	0 h light	24 h light	D.f.	t.
Series 1.	6.09	6.95	14	0.13
Series 2.	3.90	3.47	22	0.23
Series 3.	9.62	8.84	18	0.24

The ability of B. balanoides to continue to moult over long periods of starvation, even though at a decreasing frequency, has been pointed out by Barnes, Barnes & Finlayson (1963) who showed that carbohydrate, lipids, and protein could be utilized as metabolic substrates under inanition: it was suggested that moulting frequency might be coupled to the general metabolic processes rather than under direct hormonal control. Nevertheless, the two latter may be interrelated. In agreement with the above, the moulting frequency observed in the present work is much lower for the starved animals (Tables I A, XIV A & XXVII A). The suppresseion of breeding by starvation at the appropriate time and its initiation when feeding is resumed may then, like the effect of feeding on moulting frequency, be ascribed to this general lowering of metabolism (Barnes & Barnes, 1967). The fact that under starvation ecdysis continues whereas ovarian development does not, indicates the more specialized nature of gonadal metabolism.

Within the limits of variability of the results there is complete homeostasis between 10 and 15°C. A marked homeostasis between April and October in the oxygen uptake of 'isolated bodies' over this temperature range has been observed by Barnes & Barnes (1969) who pointed out that this homeostasis extended over periods which represented very different phases of the life cycle of the animal so that it could not be related to a particular internal state. When the general

metabolism as measured by the moulting frequency is considered then this homeostasis is even more pronounced. There are no data on the effect of temperature on specific tissues since, except for the female gonad, they are difficult to separate, but it is of interest to note that normal Q_{10} values have been found for the effect of temperature on both the rate of development and the oxygen uptake of embryos (Crisp & Davies, 1955; Barnes & Barnes, 1959). Barnes, Barnes & Finlayson (1963) found that although in the early stages (neglected in the present calculations) the Q_{10} of the moulting frequency of starved animals was near to 2.0, after 25 days it was virtually independent of temperature. Patel & Crisp (1960), however, found that in both Elminius modestus and Balanus perforatus starved animals showed a similar rise in moulting frequency to fed barnacles but that of starved Chthamalus stellatus and Balanus amphitrite showed little change with temperature: they considered that the condition of the latter two species in their experiments was such that their reserves were unable to maintain a moulting frequency in step with the rise in temperature. In the present instance, however, the reserves are high during the period when the starvation experiments were carried out and the homeostasis was also shown by liberally fed animals.

Crisp & Patel (1960) found that over a wide range of temperature (3-20°C) the moulting frequency of fed B. balanoides increased linearly

with temperature: the present results are at variance with these observations. It does, however, seem that while their temperature control was adequate to establish a general relation between moulting frequency and temperature over a wide range of the latter it was perhaps inadequate to establish homeostasis over a small range. Furthermore, in all their experiments the animals were out of water for 8 hours each day and the sea water was not agitated in any way. The effect of water velocity on both cirral activity and activity rhythms is well known (Southward, 1955; Southward & Crisp, 1965) so their comparison between the moulting frequency of their animals, in still water and exposed to air each day, and the cirral activity in the presence of a current as determined by Southward (loc. cit.) may be misleading. To some extent the results of Newell & Northcroft (1965), in spite of some criticisms of their statistical methods, may help to resolve the anomaly. They found that their so-called 'active rate' - the higher rate of oxygen consumption of intact animals-showed a marked homeostasis between 10 and 15°C while the 'standard' or 'basal rate' did not: the 'active' rate corresponded to normal cirral activity. If their moulting frequency is related to 'active' and 'standard' rates the homeostasis in the present experiments, in which the animals were actively feeding and beating, and the lack of it in Crisp & Patel's experiments with the animals in still water would be

explained. That moulting frequency is related to the cirral beat, in turn representing metabolic activity, is in accord with the suggestion made previously.

Although there is some evidence that light can affect the moulting activity in some crustaceans, Costlow & Bookhout (1956) found that it did not affect the moulting frequency of juvenile B. amphitrite: Crisp & Patel (1960) found that at 10°C the moulting frequency of adult B. balanoides was the same in constant light and constant darkness: Southward & Crisp (1965) have shown that activity rhythms in a variety of species are, except for an initial 'shading' response, unaffected by light. The present results are in agreement: light period had no effect at either temperature on either fed or starved animals. The critical period of a 12 h light-dark regime essential to ensure the final stages of gonad development is not mediated through any effect upon the moulting frequency.

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Appendix

Details of biochemical methods

Table XX — Verruca stroemia: mean basal diameter of three size classes chosen for measurement at each sampling occasion. Mean dry weight of bodies from each of the size classes is given. The data were used to construct calibration curves yielding a "standard animal" for each sample.

Date	Mean dry wt. (ug)	Mean basal diameter (mm)	Date	Mean dry wt. (ug)	Mean basal diameter (mm)	Date	Mean dry wt. (ug)	Mean basal diameter (mm)
1970			1971					
June 16	335	2.98	Jan. 19	348	3.43	Mar. 23	225	3.17
	675	4.96		535	4.44		445	4,35
	941	7.34		993	5.73		536	5.66
Jul. 16	202	3.35	Feb. 3	212	3.17	Mar. 31	313	4.18
	397	5.24		380	4.44		448	5.18
	944	6.78		857	5.91		500	6.14
Aug. 20	310	3.40	Feb. 9	263	3.24	Apr. 20	323	3.66
	470	5.10		498	4.34		385	4.62
	800	7.06		650	6.39		578	6.67
Sept. 30	232	3.35	Feb. 16	150	3.15	May 18	250	3.30
	445	4.84		368	4.28		450	4.37
	714	6.36		807	6.12		907	5.87
Nov. 11	199	3.35	Feb. 23	202	3.32	Jun. 16	258	3.51
	456	4.70		365	4.39		568	4.53
	861	6.20		921	6.52		843	6.10
Nov. 27	203	3.17	Mar. 2	153	3.25	Jul. 20	208	3.45
	679	4.86		323	4.42		318	4.36
	1,129	6.44		336	5.82		607	5.87
Dec. 23	217	3.17	Mar. 17	210	3.35	Aug. 17	189	3.36
	413	4.28		293	4.21		420	4.33
	850	5.79		714	6.76		543	5.67

Preparation of the material

On each sampling occasion 10 to 20 'bodies' i.e., soft parts excluding ovary, were dissected from animals in three restricted size ranges based on basal diameter and dried for 5 days over silica gel in a dessicator under reduced pressure: as far as possible the same three size ranges were used on each sampling occassion to ensure similarity in the calibration curves of size against body weight (Table xx). The bodies were then taken from the desiccator, allowed to come to equilibrium with the ambient air of the laboratory for 2 h and weighed on a precision microbalance. The vacuum dried body weight for each size range was plotted against the mean basal diameter and from this calibration the dry weight of an arbitrarily chosen 'standard animal' was determined. For the biochemical estimations about 200 bodies were dissected as quickly as possible from medium sized animals selected at random from the collection. They were quickly dissected and transferred in small batches (with individual animals well spaced) on aluminium foil planchettes and cooled to -10°C. When the collection was complete, all of the planchettes were placed for

16 h in a freeze-dryer (Vertis, Model, 10-148 MR-BH). The freeze dried material was pooled, transferred to a small vial, stoppered and stored at -20°C over silica gel. Just prior to analysis the bodies from each collection were ground to a fine powder using a mortar and pestle; this powder was used for biochemical analysis. Tests indicated that mean body weights derived from freeze-dried and vacuum dessicator dried material were the same.

Chemical Analysis (Table XXI; Fig.24)

Analytical grade reagents were used unless otherwise stated and distilled or deionized water was used throughout for preparation of solutions and sample dilutions.

Estimation of total carbohydrate

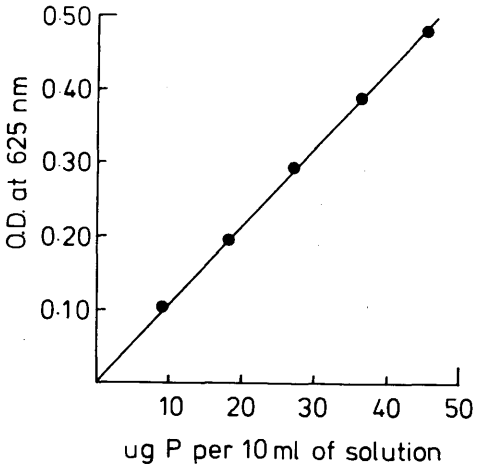
The estimation of carbohydrate was based on the method of Kemp & Van Heijningen (1954) and depends on the formation of a bluish-pink coloured furfural derivative when glucose reacts with hot concentrated sulphuric acid. Carbohydrates other than glucose will be extracted with trichloroacetic acid and glycogen is hydrolyzed by the hot acid and is consequently estimated as glucose. The optical

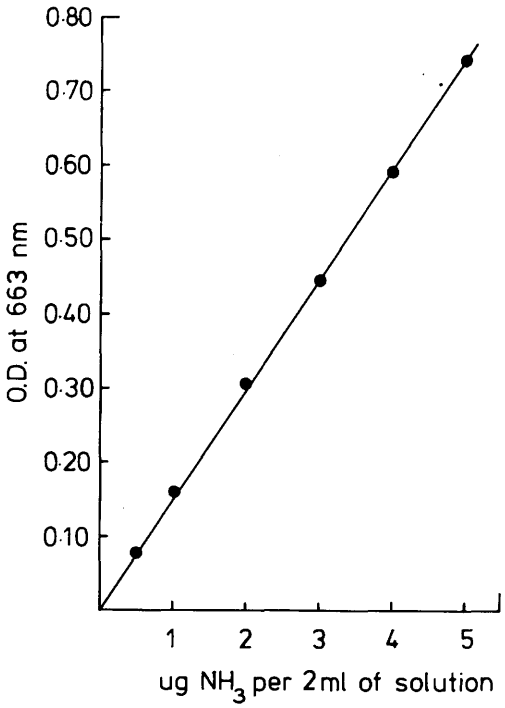
Table XXI

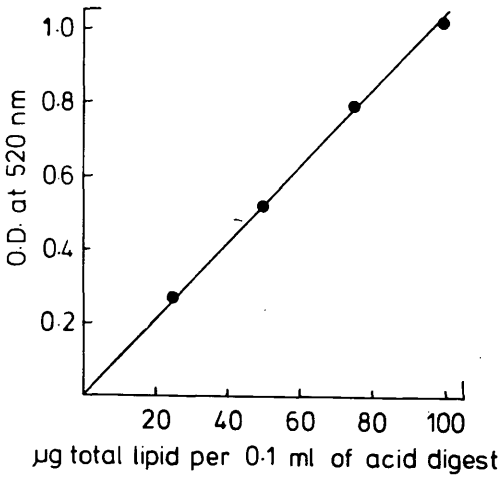
Optical densities and concentrations of standard solutions used in the estimation of total carbohydrate, total lipid, trichloro-acetic acid (TCA) - soluble and - insoluble nitrogen and DNA phosphorus

Total Carbohydrate		Total Lipid	
O.D. at 520 nm	ug glucose in ml of solution	O.D. at 520 nm	ug per 0.1 ml of acid digest.
0.022	20	0.268	25
0.072	50	0.511	50
0.142	100	0.786	75
0.208	150	1.02	100
0.263	200		

TCA - soluble and - insoluble nitrogen		DNA phosphorus	
O.D. at 663 nm	ug NH_3 in 2 ml kf solution	O.D. at 625 nm	ug P in 10 ml of solution
0.081	0.5	0.103	9.10
0.159	1.0	0.196	18.21
0.303	2.0	0.293	27.31
0.444	3.0	0.389	36.41
0.594	4.0	0.475	45.51
0.743	5.0		







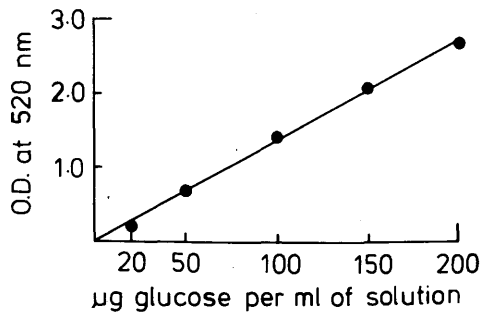


Fig. 24. Calibration curves for the estimation of various biochemical components.

density at 520 nm of the coloured product is directly proportional to the concentration of glucose in the test solution up to 200 ug/ml.

About 5 mg of material were weighed into a Pyrex centrifuge tube. 1.0 ml of 10% (w/v) trichloroacetic acid (TCA) and about 10 mg of silver sulphate (to remove halides which would interfere with the subsequent reaction) were added, and the mixture heated in a boiling water bath for 30 min. The tubes were cooled under running water, centrifuged at 14,000 rpm (15,000 g) for 15 min. and the supernatant decanted into a clean, dry graduated centrifuge tube. The precipitate was washed with 1.0 ml of 10% TCA, centrifuged again and the washings added to the supernatant. The combined washings and supernatant were made up to 2.5 ml in the graduated centrifuge tube by the further addition of TCA. Aliquots of 1.0 ml were carefully layered over 3.0 ml of concentrated H_2SO_4 in test tubes. Once the sample had been added the contents were thoroughly and rapidly mixed, heated at 100°C for exactly 6.5 min. and cooled quickly in running tap water. The optical density was measured on a spectrophotometer (Unicam, SP 600) at 520 nm using the blue sensitive photocell

and 1 cm light path (4.0 ml cells). A calibration curve was set up from solutions containing 20 - 200 µg glucose/ml similarly treated. The calibration values were corrected by deducting the optical density of a 'blank' consisting of 1 ml of water mixed with acid and heated with the standard solutions; the reagent blank for the unknowns was obtained by taking 1.0 ml TCA through the whole procedure. The results were expressed as µg glucose/ml of solution by reference to the calibration curve, and then converted to µg/100mg freeze dried material.

Estimation of total lipid

The method is based on the procedure of Zoller & Kirsch (1962) in which lipids after treatment with hot concentrated sulphuric acid and a phosphoric acid - vanillin reagent give a red coloured complex. Over the range used, the optical density at 520 nm of the coloured product is directly proportional to the amount of lipid present. For convenience with the relatively small number of estimations the Boerhinger Test Kit was used in which the lipid is estimated against a cholesterol standard which may be related to a gravimetric value.

About 5 mg of freeze dried material was weighed into

Pyrex centrifuge tubes and extracted with 5 ml of a 2:1(v/v) chloroform-methanol solution. Extraction was facilitated by gentle heating in a water bath (50°C) with occasional vigorous grinding and stirring (with a glass rod) followed by standing for 16 h at room temperature. The tubes were then centrifuged at 14,000 rpm (15,000 g) and the supernatant decanted into a clean dry 10 ml volumetric flask. The residue was washed twice with 2 ml of the chloroform-methanol solution, centrifuged followed each washing, and the washings added to the supernatant. The contents of the flask were made up to 10 ml with ~~the~~ chloroform -methanol. 2.0 ml of the solution were then transferred to a clean, dry test tube and evaporated to dryness under vacuum in a desiccator. Following the addition of 0.5 ml of conc. H_2SO_4 tube was heated at 100°C in a boiling water bath for 10 min., and then cooled quickly under running tap water. Duplicate 0.2 ml aliquots of the acid solution were removed to clean, dry test tubes, 5.0 ml of the phosphoric acid-vanillin reagent were added to each and the contents of the tubes thoroughly mixed. After allowing to stand at room temperature for 15-30 min/ to allow the pink colour (which is stable for up to

60 min.) to develop. The optical densities were then measured at 520 nm in a spectrophotometer (Unicam SP 600) using a 1 cm light path (4 ml) and blue sensitive photocell. A calibration curve was set up using standard solutions (0.5 ml) containing the equivalent of 25 - 100 µg of toal lipid/0.1 ml and following the same procedure. Reagent blanks consisting of 0.5 ml of chloroform-methanol were run with each analysis. Results were recorded as µg total lipid/0.1 ml of solution. All estimations were done in duplicate or triplicate and the mean values calculated as µg lipid/ 100 mg freeze dried material.

Estimation of TCA-soluble and insoluble (protein) nitrogen

Nitrogen was estimated using a micro-Kjeldahl technique: after digestion, the ammonia was distilled off and the ammonia produced ~~is reacted with~~ estimated using a phenol-~~and~~ hypochlorite reagent which forms a blue product. Within the range used the optical density at 630 nm of the coloured solution is proportional to the amount of ammonia.

Reagents

Phenol reagent: 50 g of phenol and 0.25 g of sodium nitroprusside were dissolved in 400 ml of water and made up to 1 ℓ in a volumetric flask.

Alkaline hypochlorite solution: 40 g of NaOH pellets were dissolved in 400 ml of water, cooled, and 40 ml of a sodium hypochlorite solution added (BDH) containing 10 - 14% w/v available chlorine).

Both reagent solutions are stable for at least 2 months when stored at 0 - 4°C in amber, glass stoppered bottles.

Procedure

About 5 mg of dry material were weighed into a Pyrex centrifuge tube and 5 ml of 5% trichloroacetic acid (TCA) were added. Extraction with the acid was assisted by gentle heating at 50°C with intermittent vigorous grinding and stirring with a glass rod. The tubes were allowed to stand at room temperature (20 - 25°C) overnight and centrifuged for 15 min, at 14,000 rpm (15,000 g). The supernatant fluid (soluble nitrogen) which contained the non-protein nitrogenous material, was transferred to a Kjeldahl flask. The precipitate was washed with about 1 ml of TCA, again centrifuged and the washings added to the supernatant. The volume of the liquid in the flask was reduced under vacuum. The residue remaining in the centrifuge tube(insoluble nigrogen, protein) was transferred with

small quantities of water to a Kjeldahl flask. Both fractions were then treated in the same manner. Approximately 0.5 g of Kjeldahl catalyst was added (a mixture of equal parts of BDH Kjeldahl catalyst tablets, (a) 1g Na_2SO_4 and 0.1 g of $CuSO_4 : 5H_2O$; and (b) 0.05g selenium, and 1 g Na_2SO_4 per tablet and 2.5 ml nitrogen-free sulphuric acid (BDH). The flasks were heated gently at first and then more vigorously; digestion was continued for a further 2 h after the solutions cleared.

Steam distillation was carried out using the Markham apparatus which was steamed out for at least an hour before use. The cooled acid digest was diluted with a few ml of water and ~~it was~~ then transferred to the distillation flask together with the washings. A receiving flask containing 10 ml of 0.1 N HCl (B.D.H. Volumetric Ampoules) was placed under the condenser so that its tip was below the level of the acid. 15 ml of 60% NaOH solution were carefully run into the distillation flask and the distillation was begun. Care was taken to maintain a liquid seal on the still. It was allowed to proceed for 2 min, and then for another min, with the tip of the condenser removed from the acid to wash down

the latter. The distillate was transferred to a 10 ml volumetric flask, the washings added, and made up to volume. Aliquots of 0.2 ml were taken and made up to 2 ml with 0.1 $\frac{N}{7}$ HCl in a clean dry test tube. 1 ml of phenol reagent was added and the contents immediately mixed thoroughly: 1 ml of alkaline hypochlorite was then added and the solutions again mixed. The tubes were then heated in a water bath at 40°C for 20 min. While the tubes were cooling, 2 ml of water were added and the contents again mixed. The optical density was measured at 630 nm on a spectrophotometer (Unicam, S.P. 600) with 1 cm light path (4 ml cells) using the red sensitive photocell.

A calibration curve was set up using standard solutions of 1-5 µg NH_3/ml prepared from a stock standard solution of ammonium chloride. Blanks were run containing 0.5 g Kjeldahl catalyst and 2.5 ml of nitrogen-free H_2SO_4 being taken through the whole procedure.

Estimation of DNA

To conserve material, DNA was estimated on the residue from the lipid analyses: insufficient material was available for pentose estimation so, after separation

from RNA, the DNA was estimated in terms of its phosphorus content for which sensitive methods are available.

Reagents for phosphorus estimation

Reducing agent: 0.5 g of 1-amino-2-naphthol-4-sulphonic acid was gradually added with stirring to 200 ml of a 15% solution of sodium metabisulphite in a 250 ml beaker and warmed to hasten solution. 5 ml of a 20% solution of sodium sulphite were then added. The solution was kept 3-4 days before use: between batches of estimations it was stored in an amber bottle in the refrigerator.

Ammonium molybdate solution: A 2.5% aqueous solution of ammonium molybdate, also stored in a glass stoppered bottle in the refrigerator.

Method (Volkin & Cohn, 1954)

The residue remaining after the extraction of lipids was incubated with 1 ml of 1N KOH at 37°C for 16 h with intermittent shaking. The digest was neutralized with 1 ml of 1N HCl and 2 ml of trichloroacetic acid was added to precipitate the DNA: the precipitate was washed with a little TCA. The tubes were centrifuged again and the supernatant

containing the RNA discarded. The RNA-free residue was then wet-washed: it was transferred to a boiling tube and digested with 0.5 ml concentrated H_2SO_4 for 15 min (until clear). After cooling, 1 drop of 100 volume hydrogen peroxide was added followed by vigorous heating: if the digest still showed any yellow colour the hydrogen peroxide treatment was repeated. About 5 ml of water was added and the volume reduced to about 1 ml by boiling to destroy any traces of hydrogen peroxide. The cooled acid solution was then neutralized by means of drop-wise addition of dilute 0.1N NaOH using one drop of phenolphthalein (0.1%) as indicator (Phenolphthalein has been shown not to interfere with subsequent estimation procedure.

The neutral solution and washings were transferred to a 10 ml volumetric flask and made up to about 6 ml with water. 0.4 ml of 1N H_2SO_4 were added followed by 0.2 ml of reducing agent, 0.8 ml of ammonium molybdate solution and a further 0.2 ml of reducing agent. The splitting of the addition of the reducing solution in this way ensures that any traces of hydrogen peroxide still remaining are reduced before the addition of the ammonium molybdate reagent. The contents of the flasks were shaken after each addition of reagents. The flasks

were allowed to stand for 20 min, at room temperature (20 - 25°C) to allow colour development. The optical density was measured at 625 nm using a red sensitive photocell and 1 cm light path (4 ml cell). A calibration curve was set up from standards containing 10 - 40 µgP/ml and prepared from potassium hydrogen phosphate. Reagent blanks were taken through the colorimetric procedure.

Appendix

Balanus balanoides: data on moulting frequency

Series 1

Table IA

Balanus balanoides: 'mean weekly' moulting frequency (number of exuviae per 100 animals per day) at 10 and 15°C with variable light regime: excess Artemia supplied as food.

	Light period (h)					
	0	2	8	12	18	24
15°C	4.72	7.22	6.20	5.50	6.51	6.69
	4.52	2.92	6.24	4.69	6.52	4.61
	6.38	6.64	6.43	3.95	5.38	7.56
	7.53	8.25	5.34	5.78	5.55	8.12
	5.76	4.76	4.63	3.80	5.45	6.23
	4.57	4.47	5.05	5.84	6.36	6.80
	4.10	4.57	5.38	4.86	4.70	6.06
	3.74	5.27	4.47	5.09	5.55	5.72
Mean	5.165	5.513	5.468	4.939	5.753	6.474
10°C	8.66	8.02	6.37	8.10	6.88	8.39
	6.57	6.75	7.75	6.23	5.55	5.15
	6.87	5.72	6.76	5.21	6.77	8.57
	8.78	8.04	7.90	6.12	7.30	9.14
	6.03	5.36	5.99	5.27	6.08	5.09
	6.75	5.80	5.07	7.76	8.89	7.27
	7.25	6.15	7.10	7.76	9.33	7.00
	5.19	5.45	4.26	6.88	5.93	8.83
Mean	7.013	6.411	6.400	6.667	7.091	7.430

Table IIA

Balanus balanoides; animals fed; 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	95				
21st June	94	95	18	6.32	
23rd June	93	94	16	8.51	7.45
25th June	93	93	14	7.53	
28th June	93	93	20	7.17	
30th June	93	93	19	10.22	8.66
2nd July	93	93	16	8.60	
5th July	93	93	16	5.73	
7th July	93	93	11	5.91	6.57
9th July	93	93	15	8.06	
12th July	93	93	17	6.09	
14th July	93	93	10	5.38	6.87
16th July	93	93	17	9.14	
19th July	93	93	21	7.35	
21st July	93	93	19	10.22	8.78
23rd July	93	93	16	8.60	

Table IIA cont.

Balanus balanoides: animals fed; 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	93	93	19	6.81	
28th July	93	93	12	6.45	6.03
30th July	93	93	9	4.84	
2nd Aug.	93	93	10	3.58	
4th Aug.	93	93	13	6.98	6.75
6th Aug.	93	93	18	9.68	
9th Aug.	93	93	26	9.32	
11th Aug.	93	93	10	5.38	7.25
13th Aug.	93	93	13	6.98	
16th Aug.	93	93	9	3.22	
18th Aug.	93	93	11	5.91	5.19
20th Aug.	93	93	12	6.45	
23rd Aug.	93	93	17	6.09	
25th Aug.	93	93	14	7.52	7.76
27th Aug.	93	93	18	9.68	

Table IIA cont.

Balanus balanoides: animals fed: 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	93	93	9	3.22	
1st Sept.	93	93	21	11.29	7.34
3rd Sept.	93	93	14	7.52	
6th Sept.	93	93	10	3.58	
8th Sept.	93	93	13	6.99	6.39
10th Sept.	93	93	16	8.60	
13th Sept.	93	93	15	5.38	
15th Sept.	93	93	13	6.98	6.45
17th Sept.	93	93	13	6.98	
20th Sept.	93	93	14	5.18	
22nd Sept.	88	88	5	2.84	4.19
24th Sept.	88	88	8	4.55	
27th Sept.	88	88	5	1.89	
29th Sept.	88	88	4	2.27	2.71
1st Oct.	88	88	7	3.97	

Table IIA cont.

Balanus balanoides: animals fed: 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	88	88	5	1.89	
6th Oct.	87	88	6	3.40	2.15
8th Oct.	87	87	2	1.15	
11th Oct.	84	86	7	2.71	
13th Oct.	84	84	3	1.79	2.49
15th Oct.	84	84	5	2.98	
18th Oct.	84	84	3	1.19	
20th Oct.	84	84	9	5.36	2.58
22nd Oct.	84	84	2	1.19	
25th Oct.	84	84	9	3.57	
27th Oct.	84	84	3	1.79	2.38
29th Oct.	79	82	3	1.79	

Table IIIA

Balanus balanoides: animals fed: 10°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	118				
21st June	118	118	26	7.34	
23rd June	117	118	10	4.24	5.71
25th June	117	117	13	5.56	
28th June	116	117	33	9.40	
30th June	116	116	15	6.47	8.02
2nd July	116	116	19	8.19	
5th July	116	116	15	4.31	
7th July	116	116	14	6.03	6.75
9th July	116	116	23	9.91	
12th July	116	116	22	6.32	
14th July	116	116	11	4.74	5.72
16th July	114	115	14	6.09	
19th July	114	114	21	6.14	
21st July	114	114	19	8.33	8.04
23rd July	114	114	22	9.65	

Table IIIA cont.

Balanus balanoides: animals fed: 10°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	114	114	25	7.30	
28th July	114	114	12	5.26	5.36
30th July	114	114	8	3.51	
2nd Aug.	114	114	7	2.05	
4th Aug.	114	114	21	9.21	5.80
6th Aug.	114	114	14	6.14	
9th Aug.	114	114	13	3.80	
11th Aug.	112	113	19	8.41	6.15
13th Aug.	112	112	14	6.25	
16th Aug.	112	112	13	3.86	
18th Aug.	112	112	17	7.59	5.45
20th Aug.	112	112	11	4.91	
23rd Aug.	112	112	14	4.17	
25th Aug.	112	112	13	5.80	6.89
27th Aug.	112	112	24	10.71	
30th Aug.	112	112	10	2.98	
1st Sept.	110	111	21	9.46	6.11
3rd Sept.	110	110	13	5.91	

Table IIIA cont.

Balanus balanoides: animals fed; 10°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
6th Sept.	110	110	15	4.55	
8th Sept.	110	110	7	3.18	5.00
10th Sept.	110	110	16	7.27	
13th Sept.	110	110	17	5.15	
15th Sept.	106	108	9	4.17	4.05
17th Sept.	106	106	6	2.83	
20th Sept.	106	106	6	1.89	
22nd Sept.	101	101	4	1.98	1.95
24th Sept.	101	101	4	1.98	
27th Sept.	101	101	2	0.66	
29th Sept.	101	101	0	0	0.22
1st Oct.	101	101	0	0	
4th Oct.	101	101	1	0.33	
6th Oct.	101	101	0	0	0.11
8th Oct.	101	101	0	0	

Table IIIA cont.

Balanus balanoides; animals fed : 10°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
11th Oct.	101	101	1	0.33	
13th Oct.	101	101	1	0.55	0.44
15th Oct.	101	101	1	0.55	
18th Oct.	101	101	1	0.33	
20th Oct.	101	101	0	0	0.11
22nd Oct.	101	101	0	0	
25th Oct.	101	101	7	2.31	
27th Oct.	101	101	1	0.50	1.43
29th Oct.	101	101	3	1.48	

Table IVA

Balanus balanoides; animals fed; 10°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	131				
21st June	131	131	27	6.87	
23rd June	130	131	11	4.20	6.25
25th June	129	130	20	7.69	
28th June	129	129	29	7.49	
30th June	129	129	17	6.58	6.37
2nd July	129	129	13	5.04	
5th July	129	129	30	7.75	
7th July	129	129	15	5.81	7.75
9th July	129	129	25	9.69	
12th July	129	129	29	7.49	
14th July	129	129	12	4.65	6.76
16th July	128	129	21	8.14	
19th July	128	128	37	9.64	
21st July	128	128	20	7.81	7.90
23rd July	128	128	16	6.25	

Table IVA cont.

Balanus balanoides; animals fed; 10°C

8 hrs Light

Date	No. animals	Mean no, animals	No. exuviae	No. 100 exuviae animals/ day	Weekly mean values
26th July	128	128	24	6.25	
28th July	128	128	16	6.25	5.99
30th July	127	128	14	5.47	
2nd Aug.	127	127	10	2.62	
4th Aug.	127	127	14	5.51	5.07
6th Aug.	127	127	18	7.09	
9th Aug.	124	126	15	3.97	
11th Aug.	124	124	29	11.69	7.10
13th Aug.	124	124	14	5.64	
16th Aug.	124	124	13	3.49	
18th Aug.	124	124	11	4.44	4.26
20th Aug.	124	124	12	4.84	
23rd Aug.	124	124	11	2.96	
25th Aug.	123	124	7	2.84	3.83
27th Aug.	123	123	14	5.69	

Table IVA cont.

Balanus balanoides: animals fed; 10°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	123	123	14	3.79	
1st Sept.	123	123	10	4.07	3.84
3rd Sept.	122	123	9	3.66	
6th Sept.	122	122	9	2.45	
8th Sept.	122	122	7	2.87	2.73
10th Sept.	122	122	7	2.87	
13th Sept.	122	122	4	1.09	
15th Sept.	122	122	3	1.23	0.91
17th Sept.	122	122	1	0.41	
20th Sept.	121	122	1	0.27	
22nd Sept.	116	116	1	0.41	0.34
24th Sept.	116	116	0	0	
27th Sept.	116	116	0	0	
29th Sept.	116	116	0	0	0
1st Oct.	115	116	0	0	

Table IVA cont.

Balanus balanoides: animals fed; 10°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No, exuviae 100 animals/ day	Weekly mean values
4th Oct.	113	114	1	0.27	
6th Oct.	113	113	1	0.41	0.36
8th Oct.	113	113	1	0.41	
11th Oct.	110	112	2	0.61	
13th Oct.	110	110	4	1.82	1.11
15th Oct.	110	110	2	0.91	
18th Oct.	110	110	1	0.27	
20th Oct.	110	110	4	1.82	1.76
22nd Oct.	110	110	7	3.18	
25th Oct.	110	110	16	4.85	
27th Oct.	110	110	8	3.64	4.65
29th Oct.	110	110	12	5.45	

Table VA

Balanus balanoides; animals fed ; 10°C

12 hrs Light

Date	No. animals	Mean No. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	103				
21st June	101	102	21	6.86	
23rd June	98	100	15	7.50	7.34
25th June	98	98	15	7.65	
28th June	98	98	31	10.54	
30th June	98	98	9	4.59	8.10
2nd July	98	98	18	9.18	
5th July	98	98	13	4.42	
7th July	98	98	20	10.20	6.23
9th July	98	98	8	4.08	
12th July	98	98	7	2.38	
14th July	98	98	12	6.12	5.21
16th July	98	98	14	7.14	
19th July	98	98	15	5.10	
21st July	98	98	12	6.12	6.12
23rd July	98	98	14	7.14	

Table VA cont.

Balanus balanoides: animals fed: 10°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	98	98	9	3.06	
28th July	98	98	12	6.12	5.27
30th July	98	98	13	6.63	
2nd Aug.	98	98	19	6.46	
4th Aug.	98	98	16	8.16	7.76
6th Aug.	98	98	17	8.67	
9th Aug.	98	98	19	6.46	
11th Aug.	98	98	9	4.59	7.76
13th Aug.	97	98	24	12,24	
16th Aug.	97	97	18	6,19	
18th Aug.	97	97	16	8.25	6,88
20th Aug.	97	97	12	6,19	
23rd Aug.	97	97	25	8.59	
25th Aug.	96	97	11	5,67	6,84
27th Aug.	96	96	12	6,25	

Table VA cont.

Balanus balanoides: animals fed: 10°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	95	96	24	8.33	
1st Sept.	95	95	21	11.05	8.74
3rd Sept.	95	95	13	6.84	
6th Sept.	95	95	20	7.02	
8th Sept.	95	95	16	8.42	8.83
10th Sept.	95	95	21	11.05	
13th Sept.	95	95	14	4.91	
15th Sept.	95	95	19	10.00	8.65
17th Sept.	95	95	21	11.05	
20th Sept.	95	95	22	7.72	
22nd Sept.	90	90	15	8.33	7.76
24th Sept.	90	90	13	7.22	
27th Sept.	90	90	9	3.33	
29th Sept.	90	90	5	2.78	4.44
1st Oct.	90	90	13	7.22	

Table VA cont.

Balanus balanoides: animals fed: 10°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	90	90	13	4.81	
6th Oct.	90	90	12	6.67	5.31
8th Oct.	90	90	8	4.44	
11th Oct.	90	90	12	4.44	
13th Oct.	90	90	11	6.11	4.44
15th Oct.	90	90	5	2.78	
18th Oct.	90	90	9	3.33	
20th Oct.	90	90	9	5.00	3.70
22nd Oct.	90	90	5	2.78	
25th Oct.	90	90	3	1.11	
27th Oct.	90	90	4	2.22	1.85
29th Oct.	90	90	4	2.22	

Table VIA

Balanus balanoides; animals fed; 10°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	106				
21st June	106	106	31	9.75	
23rd June	105	106	14	6.60	7.98
25th June	105	106	16	7.62	
28th June	105	105	20	6.35	
30th June	105	105	18	8.57	6.88
2nd July	105	105	12	5.71	
5th July	105	105	18	5.71	
7th July	105	105	8	3.81	5.55
9th July	105	105	15	7.14	
12th July	105	105	19	6.03	
14th July	105	105	13	6.19	6.77
16th July	105	105	17	8.10	
19th July	105	105	21	6.67	
21st July	105	105	20	9.52	7.30
23rd July	105	105	12	5.71	

Table VIA cont.

Balanus balanoides; animals fed; 10°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	105	105	20	6.35	
28th July	105	105	12	5.71	6.08
30th July	105	105	13	6.19	
2nd Aug.	105	105	15	4.76	
4th Aug.	105	105	25	11.90	8.89
6th Aug.	105	105	21	10.00	
9th Aug.	103	104	22	7.12	
11th Aug.	103	103	21	10.19	9.33
13th Aug.	103	103	22	10.68	
16th Aug.	103	103	13	4.21	
18th Aug.	103	103	16	7.77	5.93
20th Aug.	103	103	12	5.82	
23rd Aug.	103	103	17	5.50	
25th Aug.	103	103	15	7.28	7.33
27th Aug.	103	103	19	9.22	

Table VIA cont.

Balanus balanoides; animals fed: 10°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	103	103	8	2.59	
1st Sept.	103	103	13	6.31	4.58
3rd Sept.	101	102	10	4.85	
6th Sept.	101	101	26	8.58	
8th Sept.	101	101	9	4.46	6.33
10th Sept.	101	101	12	5.94	
13th Sept.	101	101	20	6.60	
15th Sept.	101	101	18	8.91	6.82
17th Sept.	101	101	10	4.95	
20th Sept.	101	101	12	3.96	
22nd Sept.	96	96	8	4.17	5.31
24th Sept.	96	96	15	7.81	
27th Sept.	96	96	12	4.17	
29th Sept.	96	96	4	2.08	3.82
1st Oct.	96	96	10	5.20	

Table VIA cont.

Balanus balanoides: animals fed: 10°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	96	96	11	3.81	
6th Oct.	96	96	9	4.69	5.26
8th Oct.	96	96	14	7.29	
11th Oct.	96	96	4	1.39	
13th Oct.	96	96	5	2.60	2.20
15th Oct.	96	96	5	2.60	
18th Oct.	96	96	9	3.12	
20th Oct.	96	96	1	0.52	1.73
22nd Oct.	96	96	3	1.56	
25th Oct.	96	96	7	2.43	
27th Oct.	96	96	8	4.17	4.80
29th Oct.	96	96	15	7.81	

Table VIIA

Balanus balanoides: animals fed: 10°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	96	96			
21st June	96	96	34	11.81	
23rd June	96	96	10	5.21	8.10
25th June	96	96	14	7.29	
28th June	96	96	20	6.94	
30th June	96	96	17	8.85	8.39
2nd July	96	96	18	9.38	
5th July	96	96	10	3.47	
7th July	96	96	7	3.64	5.15
9th July	96	96	16	8.33	
12th July	96	96	15	5.21	
14th July	96	96	19	9.90	8.57
16th July	96	96	22	11.46	
19th July	96	96	19	6.59	
21st July	96	96	23	11.98	9.14
23rd July	96	96	17	8.85	

Table VIIA cont.

Balanus balanoides; animals fed; 10°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	96	96	11	3.82	
28th July	96	96	11	5.73	5.09
30th July	95	96	11	5.73	
2nd Aug.	95	95	13	4.51	
4th Aug.	95	95	16	8.33	7.23
6th Aug.	95	95	17	8.85	
9th Aug.	95	95	20	6.94	
11th Aug.	95	95	17	8.85	7.00
13th Aug.	95	95	10	5.21	
16th Aug.	95	95	23	8.07	
18th Aug.	95	95	21	11.05	8.83
20th Aug.	95	95	14	7.37	
23rd Aug.	95	95	21	7.37	
25th Aug.	95	95	21	11.05	8.94
27th Aug.	95	95	16	8.42	

Table VIIA cont.

Balanus balanoides; animals fed; 10°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	95	95	10	3.51	
1st Sept.	95	95	16	8.42	6.96
3rd Sept.	95	95	17	8.94	
6th Sept.	95	95	16	5.61	
8th Sept.	95	95	23	12.11	8.36
10th Sept.	95	95	14	7.37	
13th Sept.	95	95	16	5.61	
15th Sept.	95	95	16	8.42	6.60
17th Sept.	95	95	11	5.78	
20th Sept.	95	95	15	5.26	
22nd Sept.	90	90	12	6.67	5.83
24th Sept.	90	90	10	5.56	
27th Sept.	90	90	6	2.22	
29th Sept.	90	90	7	3.89	2.96
1st Oct.	90	90	5	2.78	

Table VIIA cont.

Balanus balanoides; animals fed; 10°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	90	90	14	5.19	
6th Oct.	90	90	15	8.33	7.10
8th Oct.	90	90	14	7.78	
11th Oct.	90	90	7	2.59	
13th Oct.	90	90	15	8.33	7.16
15th Oct.	90	90	19	10,56	
18th Oct.	90	90	11	4.07	
20th Oct.	90	90	15	8.33	7.28
22nd Oct.	90	90	17	9.44	
25th Oct.	90	90	11	4.07	
27th Oct.	90	90	6	3.33	7.10
29th Oct.	90	90	25	13.89	

Table VIIIA cont.

Balanus balanoides: animals fed ; 15°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	108				
21st June	107	108	28	8.64	
23rd June	107	107	15	7.01	7.09
25th June	107	107	12	5.61	
28th June	107	107	8	2.49	
30th June	107	107	11	5.14	4.72
2nd July	107	107	14	6.54	
5th July	107	107	6	1.87	
7th July	107	107	13	6.07	4.52
9th July	107	107	12	5.61	
12th July	107	107	18	5.60	
14th July	107	107	11	5.14	6.38
16th July	107	107	18	8.41	
19th July	107	107	17	5.30	
21st July	107	107	23	10.75	7.53
23rd July	107	107	14	6.54	

Table VIIIA cont.

Balanus balanoides: animals fed: 15°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	107	107	24	7.47	
28th July	107	107	13	6.07	5.76
30th July	107	107	8	3.74	
2nd Aug.	107	107	14	4.36	
4th Aug.	107	107	7	3.27	4.57
6th Aug.	107	107	13	6.07	
9th Aug.	107	107	11	3.43	
11th Aug.	107	107	11	5.14	4.10
13th Aug.	107	107	8	3.74	
16th Aug.	107	107	12	3.74	
18th Aug.	107	107	9	4.21	3.74
20th Aug.	107	107	7	3.27	
23rd Aug.	107	107	11	3.43	
25th Aug.	107	107	9	4.21	3.48
27th Aug.	105	106	6	2.80	

Table VIIIA cont.

Balanus Balanoides; animals fed; 15°C

Dark

Date	No. animals	Mean No. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean value
30th Aug.	105	105	11	3.49	
1st Sept.	105	105	12	5.71	4.02
3rd Sept.	105	105	6	2.86	
6th Sept.	105	105	6	1.90	
8th Sept.	105	105	9	4.29	3.02
10th Sept.	105	105	6	2.86	
13th Sept.	105	105	5	1.59	
15th Sept.	105	105	10	4.76	3.55
17th Sept.	105	105	9	4.29	
20th Sept.	105	105	12	3.81	
22nd Sept.	100	100	13	6.50	3.94
24th Sept.	100	100	3	1.50	
27th Sept.	100	100	16	5.33	
29th Sept.	100	100	10	5.00	3.94
1st Oct.	100	100	3	1.50	

Table VIIIA cont.

Balanus balanoides: animals fed; 15°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	100	100	15	5.00	
6th Oct.	100	100	10	5.00	4.16
8th Oct.	100	100	5	2.50	
11th Oct.	100	100	16	5.33	
13th Oct.	100	100	9	4.50	4.61
15th Oct.	100	100	8	4.00	
18th Oct.	100	100	14	4.67	
20th Oct.	100	100	18	9.00	5.22
22nd Oct.	100	100	4	2.00	
25th Oct.	100	100	14	4.67	
27th Oct.	100	100	19	9.50	5.22
29th Oct.	100	100	3	1.50	

Table IXA

Balanus balanoides: animals fed: 15°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	99				
21st June	97	98	12	4.12	
23rd June	97	97	16	8.25	6.01
25th June	97	97	11	5.67	
28th June	97	97	18	6.19	
30th June	97	97	16	8.25	7.22
2nd July	97	97	14	7.22	
5th July	97	97	9	3.09	
7th July	97	97	6	3.09	2.92
9th July	97	97	5	2.58	
12th July	97	97	28	9.62	
14th July	97	97	9	4.64	6.64
16th July	97	97	11	5.67	
19th July	97	97	21	7.22	
21st July	97	97	23	11.86	8.25
23rd July	97	97	11	5.67	

Table IXA cont.

Balanus balanoides: animals fed; 15°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	97	97	13	4.47	
28th July	97	97	14	7.22	4.76
30th July	97	97	5	2.58	
2nd Aug.	97	97	9	3.09	
4th Aug.	97	97	13	6.70	4.47
6th Aug.	97	97	7	3.61	
9th Aug.	97	97	10	3.40	
11th Aug.	97	97	6	3.09	4.57
13th Aug.	97	97	14	7.22	
16th Aug,	96	96	11	3.82	
18th Aug.	96	96	13	6.77	5.27
20th Aug.	96	96	10	5.21	
23rd Aug.	96	96	11	3.82	
25th Aug.	96	96	11	5.73	4.92
27th Aug.	96	96	10	5.21	

Table IXA cont.

Balanus balanoides: animals fed: 15°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	96	96	10	3.44	
1st Sept.	96	96	13	6.77	5.14
3rd Sept.	96	96	10	5.21	
6th Sept.	96	96	7	2.43	
8th Sept.	96	96	7	3.65	3.42
10th Sept	96	96	8	4.17	
13th Sept.	96	96	17	5.90	
15th Sept.	96	96	7	3.65	4.23
17th Sept.	96	96	6	3.13	
20th Sept.	96	96	11	3.82	
22nd Sept.	91	91	15	8.24	6.03
24th Sept.	91	91	11	6.04	
27th Sept.	91	91	15	5.49	
29th Sept.	91	91	8	4.40	4.76
1st Oct.	91	91	8	4.40	

Table IXA cont.

Balanus balanoides: animals fed; 15°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	91	91	9	3.30	
6th Oct.	91	91	8	4.40	3.67
8th Oct.	91	91	6	3.30	
11th Oct.	91	91	11	4.03	
13th Oct.	91	91	12	6.59	4.64
15th Oct.	91	91	6	3.30	
18th Oct.	91	91	12	4.40	
20th Oct.	91	91	13	7.14	5.13
22nd Oct.	91	91	7	3.85	
25th Oct.	91	91	10	3.63	
27th Oct.	91	91	20	10.00	5.46
29th Oct.	91	91	5	2.75	

Table XA

Balanus balanoides: animals fed: 15°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	104				
21st June	103	104	26	8.33	
23rd June	103	103	19	9.22	7.95
25th June	103	103	13	6.31	
28th June	103	103	20	6.47	
30th June	103	103	11	5.34	6.20
2nd July	103	103	14	6.80	
5th July	102	103	17	5.50	
7th July	102	102	18	8.82	6.24
9th July	102	102	9	4.41	
12th July	102	102	14	4.58	
14th July	102	102	14	6.86	6.43
16th July	102	102	16	7.84	
19th July	102	102	13	4.25	
21st July	102	102	13	6.37	5.34
23rd July	102	102	11	5.39	

Table XA cont.

Balanus balanoides: animals fed: 15°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	102	102	17	5.56	
28th July	102	102	9	4.41	4.63
30th July	101	102	8	3.92	
2nd Aug.	101	101	10	3.27	
4th Aug.	101	101	17	8.42	5.05
6th Aug.	101	101	7	3.47	
9th Aug.	101	101	10	3.27	
11th Aug.	101	101	13	6.44	5.38
13th Aug.	101	101	13	6,44	
16th Aug.	101	101	10	3.27	
18th Aug.	100	101	6	3.00	4.47
20th Aug.	77	77	11	7.14	
23rd Aug.	77	77	12	5.19	
25th Aug.	77	77	9	5.84	5.84
27th Aug.	77	77	10	6.49	

Table XA cont.

Balanus balanoides: animals fed: 15°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	77	77	12	5.19	
1st Sept.	77	77	13	8.44	6.06
3rd Sept.	77	77	7	4.55	
6th Sept.	77	77	4	1.73	
8th Sept.	77	77	17	11.04	4.91
10th Sept.	77	77	3	1.95	
13th Sept.	77	77	4	1.73	
15th Sept.	77	77	11	7.14	3.39
17th Sept.	77	77	2	1.30	
20th Sept.	77	77	9	3.90	
22nd Sept.	72	72	12	8.33	5.23
24th Sept.	72	72	5	3.47	
27th Sept.	72	72	11	5.09	
29th Sept.	72	72	9	6.25	4.01
1st Oct.	72	72	1	0.69	

Table XA cont.

Balanus balanoides: animals fed; 15°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/day	Weekly mean values
4th Oct.	72	72	5	2.31	
6th Oct.	72	72	9	6.25	3.55
8th Oct.	72	72	3	2.08	
11th Oct.	72	72	5	2.31	
13th Oct.	72	72	6	4.17	3.55
15th Oct.	72	72	6	4.17	
18th Oct.	72	72	10	4.58	
20th Oct.	72	72	10	6.94	5.23
22nd Oct.	72	72	6	4.17	
25th Oct.	72	72	13	6.02	
27th Oct.	72	72	12	8.33	6.64
29th Oct.	72	72	8	5.56	

Table XIA

Balanus balanoides: animals fed : 15°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	99				
21st June	98	99	34	11.56	
23rd June	98	98	13	6.63	9.63
25th June	97	98	21	10.71	
28th June	97	97	15	5.15	
30th June	97	97	5	2.58	5.50
2nd July	97	97	17	8.76	
5th July	97	97	11	3.78	
7th July	97	97	9	4.63	4.69
9th July	97	97	11	5.67	
12th July	97	97	15	5.15	
14th July	97	97	7	3.61	3.95
16th July	96	97	6	3.09	
19th July	96	96	17	5.90	
21st July	96	96	17	8.85	5.78
23rd July	96	96	5	2.60	

Table XIA cont.

Balanus balanoides: animals fed: 15°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	96	96	6	2.08	
28th July	96	96	16	8.33	3.80
30th July	96	96	2	1.00	
2nd Aug.	96	96	13	4.51	
4th Aug.	96	96	17	8.85	5.84
6th Aug.	96	96	8	4.17	
9th Aug.	96	96	12	4.17	
11th Aug.	96	96	10	5.20	4.86
13th Aug.	96	96	10	5.20	
16th Aug.	96	96	14	4.86	
18th Aug.	96	96	11	5.72	5.09
20th Aug.	96	96	9	4.69	
23rd Aug.	96	96	14	4.86	
25th Aug.	96	96	0	0	4.01
27th Aug.	96	96	14	7.29	

Table XIA cont.

Balanus balanoides: animals fed: 15°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	96	96	10	3.43	
1st Sept.	96	96	15	7.81	4.79
3rd Sept.	96	96	6	3.13	
6th Sept.	96	96	16	5.56	
8th Sept.	96	96	9	4.69	4.98
10th Sept.	96	96	9	4.69	
13th Sept.	96	96	10	3.47	
15th Sept.	96	96	2	1.00	2.35
17th Sept.	96	96	5	2.60	
20th Sept.	96	96	10	3.47	
22nd Sept.	91	91	12	6.59	4.09
24th Sept.	91	91	4	2.20	
27th Sept.	91	91	13	4.76	
29th Sept.	91	91	5	2.75	3.05
1st Oct.	91	91	3	1.65	

Table XIA cont.

Balanus balanoides: animals fed: 15°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	91	91	12	4.40	
6th Oct.	91	91	6	3.30	2.57
8th Oct.	91	91	0	0	
11th Oct.	91	91	11	6.23	
13th Oct.	91	91	15	8.24	5.90
15th Oct.	91	91	6	3.30	
18th Oct.	91	91	28	10.26	
20th Oct.	91	91	13	7.14	7.45
22nd Oct.	91	91	9	4.95	
25th Oct.	91	91	12	4.40	
27th Oct.	91	91	16	8.79	5.31
29th Oct.	91	91	5	2.75	

Table XIIA

Balanus balanoides: animals fed: 15°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	104				
21st June	104	104	27	8.65	
23rd June	104	104	22	10.58	6.89
25th June	104	104	3	1.44	
28th June	104	104	28	8.97	
30th June	104	104	13	6.25	6.51
2nd July	104	104	9	4.32	
5th July	104	104	16	5.13	
7th July	104	104	19	9.13	6.52
9th July	104	104	11	5.28	
12th July	104	104	13	4.17	
14th July	104	104	12	5.77	5.38
16th July	104	104	13	6.25	
19th July	104	104	16	5.13	
21st July	104	104	16	7.69	5.55
23rd July	104	104	8	3.84	

Light XIIA cont.

Balanus balanoides: animals fed: 15°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	104	104	15	4.81	
28th July	104	104	15	7.21	5.45
30th July	104	104	9	4.33	
2nd Aug.	104	104	13	4.17	
4th Aug.	104	104	16	7.69	6.36
6th Aug.	104	104	15	7.21	
9th Aug.	104	104	14	4.49	
11th Aug.	104	104	11	5.29	4.70
13th Aug.	104	104	9	4.32	
16th Aug.	104	104	10	3.20	
18th Aug.	104	104	18	8.65	5.55
20th Aug.	104	104	10	4.81	
23rd Aug.	104	104	14	4.49	
25th Aug.	104	104	12	5.77	4.86
27th Aug.	104	104	9	4.32	

Table XIIA cont.

Balanus balanoides: animals fed: 15°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	104	104	14	4.49	
1st Sept.	104	104	13	6.25	5.34
3rd Sept.	104	104	11	5.29	
6th Sept.	104	104	13	4.17	
8th Sept.	104	104	13	6.25	4.75
10th Sept.	104	104	8	3.84	
13th Sept.	103	104	11	3.53	
15th Sept.	103	103	12	5.82	5.40
17th Sept.	102	103	14	6.86	
20th Sept.	101	102	13	4.25	
22nd Sept.	101	101	9	4.41	4.97
24th Sept.	96	96	12	6.25	
27th Sept.	96	96	12	4.17	
29th Sept.	96	96	9	4.69	3.99
1st Oct.	96	96	6	3.13	

Table XIIA cont.

Balanus balanoides: animals fed: 15°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	96	96	15	5.20	
6th Oct.	96	96	20	10.42	6.25
8th Oct.	96	96	6	3.13	
11th Oct.	96	96	9	3.13	
13th Oct.	96	96	18	9.38	5.04
15th Oct.	96	96	5	2.60	
18th Oct.	96	96	6	2.08	
20th Oct.	96	96	23	11.98	5.73
22nd Oct.	96	96	6	3.13	
25th Oct.	96	96	25	8.68	
27th Oct.	96	96	7	3.65	5.67
29th Oct.	96	96	9	4.69	

Table XIIIA

Balanus balanoides: animals fed : 15°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
18th June	101				
21st June	101	101	31	10.23	
23rd June	101	101	19	9.41	9.52
25th June	100	101	18	8.91	
28th June	100	100	20	6.67	
30th June	100	100	16	7.92	6.69
2nd July	100	100	11	5.50	
5th July	100	100	16	5.33	
7th July	100	100	12	6.00	4.61
9th July	100	100	5	2.50	
12th July	100	100	23	7.67	
14th July	100	100	14	7.56	7.74
16th July	100	100	16	8.00	
19th July	98	98	18	6.00	
21st July	98	98	27	13.78	8.12
23rd July	98	98	9	4.59	

Table XIIIA cont.

Balanus balanoides: animals fed: 15°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
26th July	98	98	7	2.38	
28th July	98	98	19	9.69	6.23
30th July	98	98	13	6.63	
2nd Aug.	98	98	27	9.18	
4th Aug.	98	98	15	7.65	6.80
6th Aug.	98	98	7	3.57	
9th Aug.	98	98	16	5.44	
11th Aug.	98	98	9	4.59	6.06
13th Aug.	98	98	16	8,16	
16th Aug.	98	98	16	5.44	
18th Aug.	98	98	14	7.14	5.72
20th Aug.	98	98	9	4.59	
23rd Aug.	98	98	14	4.76	
25th Aug.	98	98	9	4.59	5.67
27th Aug.	98	98	15	7.65	

Table XIIIA cont.

Balanus balanoides: animals fed: 15°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
30th Aug.	98	98	11	3.74	
1st Sept.	98	98	15	7.65	6.01
3rd Sept.	98	98	13	6.63	
6th Sept.	98	98	15	5.10	
8th Sept.	98	98	15	7.65	6.12
10th Sept.	98	98	11	5.61	
13th Sept.	98	98	11	3.74	
15th Sept.	98	98	10	5.10	4.65
17th Sept.	98	98	10	5.10	
20th Sept.	98	98	7	2.38	
22nd Sept.	93	93	21	11.29	6.53
24th Sept.	93	93	11	5.91	
27th Sept.	93	93	14	5.02	
29th Sept.	93	93	17	9.13	6.33
1st Oct.	93	93	9	4.84	

Table XIIIA cont.

Balanus balanoides; animals fed; 15°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
4th Oct.	93	93	18	6.45	
6th Oct.	93	93	7	3.76	4.84
8th Oct.	93	93	8	4.30	
11th Oct.	93	93	19	6.81	
13th Oct.	93	93	26	13.98	7.83
15th Oct.	93	93	5	2.69	
18th Oct.	93	93	14	5.02	
20th Oct.	93	93	26	13.98	8.12
22nd Oct.	93	93	10	5.38	
25th Oct.	93	93	22	7.89	
27th Oct.	93	93	23	12.37	8.01
29th Oct.	93	93	7	3.76	

Series 2

Table XIVA

Balanus balanoides : 'mean weekly' moulting frequency (number of exuviae per 100 animals per day) at 10 and 15°C with variable light regime: all animals starved.

	Light period (h)					
	0	2	8	12	18	24
	3.56	3.14	3.34	4.16	4.32	3.70
	2.76	3.63	3.43	3.99	3.95	3.43
15°C	2.67	2.86	3.43	3.38	1.55	3.38
	3.77	3.49	3.26	4.47	3.91	3.77
Mean	3.19	3.28	3.37	4.00	3.43	3.57
	4.55	4.52	4.80	5.27	5.50	6.36
	3.58	3.01	3.01	3.69	4.52	4.30
10°C	2.36	1.16	3.05	3.69	5.31	3.31
	2.11	4.43	3.31	3.09	4.34	4.30
Mean	3.15	3.28	3.54	3.94	4.92	4.57

Table XVA

Balanus balanoides: animals starved: 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	Mean no. 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	28	14.00	
21st April	99	99	24	12.12	12.46
24th April	99	99	34	11.45	
26th April	99	99	21	10.61	
28th April	99	99	25	12.63	10.33
1st May	99	99	23	7.74	
3rd May	99	99	9	4.55	
5th May	99	99	10	5.05	4.55
8th May	99	99	12	4.04	
12th May	99	99	11	2.78	3.58
15th May	99	99	13	4.38	
19th May	99	99	8	2.02	2.36
22nd May	99	99	8	2.69	
26th May	99	99	10	2.53	2.11
29th May	99	99	5	1.68	

Table XVIA

Balanus balanoides; animals starved; 10°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	90	99	28	14.14	
21st April	98	98	17	8.67	10.32
24th April	98	98	24	8.16	
26th April	98	98	9	4.59	
28th April	97	98	29	14.80	8.64
1st May	97	97	19	6,53	
3rd May	97	97	10	5.15	
5th May	97	97	9	4.64	4.52
8th May	97	97	11	3.78	
12th May	97	97	14	3.61	3.01
15th May	97	97	7	2.41	
19th May	97	97	5	1.29	1.16
22nd May	97	97	3	1.03	
26th May	97	97	17	4.38	4.43
29th May	97	97	13	4.47	

Table XVIIA

Balanus balanoides: animals starved: 10°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	98	99	21	10.61	
21st April	98	98	23	11.73	10.28
24th April	98	98	25	8.50	
26th April	98	98	23	11.73	
28th April	98	98	10	5.10	6.97
1st May	98	98	12	4.08	
3rd May	97	98	7	3.57	
5th May	97	97	11	5.67	4.80
8th May	97	97	15	5.15	
12th May	97	97	10	2.58	3.01
15th May	97	97	10	3.44	
19th May	97	97	9	2.32	3.05
22nd May	97	97	11	3.78	
26th May	97	97	15	3.87	3.31
29th May	97	97	8	2.75	

Table XVIIIA

Balanus balanoides: animals starved: 10°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	27	13.50	
21st April	99	99	17	8.59	10.17
24th April	98	99	25	8.42	
26th April	96	97	17	8.76	
28th April	96	96	21	10.94	8.88
1st May	96	96	20	6.94	
3rd May	96	96	9	4.69	
5th May	96	96	8	4.17	5.27
8th May	96	96	20	6.94	
12th May	96	96	15	3.91	3.69
15th May	96	96	10	3.47	
19th May	96	96	15	3.91	3.69
22nd May	96	96	10	3.47	
26th May	96	96	13	3.39	3.09
29th May	96	96	8	2.78	

Table XIXA

Balanus balanoides: animals starved: 10°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	100	100	25	12.50	
21st April	99	100	18	9.00	10.68
24th April	97	98	31	10.54	
26th April	96	97	12	6.19	
28th April	96	96	13	6.77	7.79
1st May	96	96	30	10.42	
3rd May	96	96	16	8.33	
5th May	96	96	9	4.69	5.50
8th May	95	96	10	3.47	
12th May	95	95	17	4.47	
15th May	95	95	13	4.56	4.52
19th May	95	95	23	6.05	
22nd May	95	95	13	4.56	5.31
26th May	95	95	13	3.42	
29th May	95	95	15	5.26	4.34

Table XXA

Balanus balanoides: animals starved: 10°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100	100			
19th April	99	100	44	22.00	
21st April	97	98	27	13.78	14.33
24th April	97	97	21	7.22	
26th April	97	97	18	9.28	
28th April	97	97	14	7.22	7.79
1st May	97	97	20	6.87	
3rd May	97	97	19	9.79	
5th May	97	97	6	3.09	6.36
8th May	97	97	18	6.19	
12th May	97	97	20	5.15	4.30
15th May	97	97	10	3.44	
19th May	97	97	15	3.87	3.31
22nd May	97	97	8	2.75	
26th May	97	97	12	3.09	4.30
29th May	97	97	16	5.50	

Table XXIA

Balanus balanoides: animals starved; 15°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	30	15.0	
21st April	97	98	22	11.22	11.37
24th April	97	97	23	7.90	
26th April	96	97	19	9.79	
28th April	96	96	17	8.85	7.83
1st May	95	96	14	4.86	
3rd May	95	95	12	6.31	
5th May	95	95	3	1.57	3.56
8th May	95	95	8	2.80	
12th May	95	95	9	2.36	2.76
15th May	95	95	9	3.15	
19th May	95	95	11	2.89	2.67
22nd May	95	95	7	2.45	
26th May	95	95	10	2.63	3.77
29th May	95	95	14	4.91	

Table XXIIA

Balanus balanoides: animals starved; 15°C

2 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	96	98	41	20.92	
21st April	93	95	17	8.95	12.35
24th April	93	93	20	7.17	
26th April	93	93	15	8.06	
28th April	92	93	19	10.22	7.54
1st May	92	92	12	4.35	
3rd May	92	92	7	3.80	
5th May	92	92	5	2.72	3.14
8th May	92	92	8	2,90	
12th May	92	92	16	4.35	3.63
15th May	92	92	8	2,90	
19th May	92	92	5	1.36	2.86
22nd May	92	92	12	4,35	
26th May	92	92	11	2.99	3.49
29th May	92	92	11	3.99	

Table XXIIIA

Balanus balanoides: animals starved; 15°C

8 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	26	13.0	
21st April	98	99	17	8.59	10.60
24th April	98	98	30	10.2	
26th April	97	98	14	7.14	
28th April	97	97	17	8.76	7.59
1st May	97	97	20	6.87	
3rd May	96	97	9	4.64	
5th May	96	96	7	3.65	3.34
8th May	96	96	5	1.74	
12th May	96	96	13	3.39	3.43
15th May	96	96	10	3.47	
19th May	96	96	9	2.34	3.43
22nd May	96	96	13	4.51	
26th May	96	96	9	2.34	3.26
29th May	96	96	12	4.17	

Table XXIVA

Balanus balanoides: animals starved: 15°C

12 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	25	12.5	
21st April	97	98	18	9.18	9.75
24th April	97	97	22	7.56	
26th April	97	97	21	10.82	
28th April	96	97	13	6.70	8.27
1st May	96	96	21	7.29	
3rd May	96	96	12	6.25	
5th May	96	96	4	2.08	4.16
8th May	96	96	12	4.16	
12th May	96	96	16	4.16	
15th May	96	96	11	3.81	3.99
19th May	96	96	10	2.60	
22nd May	96	96	12	4.16	3.38
26th May	96	96	17	4.42	
29th May	96	96	13	4.51	4.47

Table XXVA

Balanus balanoides; animals starved; 15°C

18 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	99	100	31	15.50	
21st April	99	99	30	15.15	12.27
24th April	99	99	18	6.06	
26th April	99	99	24	12.12	
28th April	99	99	12	6.06	6.96
1st May	99	99	8	2.69	
3rd May	99	99	13	6.56	
5th May	99	99	8	4.04	4.32
8th May	99	99	7	2.35	
12th May	99	99	18	4.54	3.95
15th May	99	99	10	3.36	
19th May	99	99	7	1.76	1.55
22nd May	99	99	4	1.34	
26th May	99	99	11	2.77	3.91
29th May	99	99	15	5.05	

Table XXVIA

Balanus balanoides; animals starved; 15°C

24 hrs Light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
17th April	100				
19th April	98	99	20	10.10	
21st April	96	97	17	8.76	8.95
24th April	96	96	23	7.98	
26th April	96	96	7	3.64	
28th April	96	96	13	6.77	5.09
1st May	96	96	14	4,86	
3rd May	96	96	9	4.68	
5th May	96	96	9	4.68	3.70
8th May	96	96	5	1,73	
12th May	96	96	13	3.38	3.43
15th May	96	96	10	3.47	
19th May	96	96	14	3,64	3.38
22nd May	96	96	9	3.12	
26th May	96	96	13	3.38	3.77
29th May	96	96	12	4,16	

[illegible] [illegible] [illegible]. mean weekly [illegible] frequency [illegible]
[illegible] per 100 animals per day) at 10 [illegible] with variable
[illegible] Artemia supplied as food.

Series 3

			Light period (h)			
			[illegible]	[illegible]	18	
		[illegible]	7.44	8.76	10.37	[illegible]
		[illegible]	[illegible]	[illegible]	8.01	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]	6.78	
	[illegible]	10.10	9.74	8.10	10.76	[illegible]
	[illegible]	6.91	11.36	9.44	7.37	[illegible]
[illegible]	[illegible]	9.06	[illegible]	7.56	8.21	
	[illegible]	7.73	8.07	[illegible]	8.97	
		8.20	9.97	10.12	11.76	
	[illegible]	7.01	5.44	8.10	7.48	
	[illegible]	8.11	9.75	9.08	5.33	
	[illegible]	12.36	9.83	12.34	10.98	

Table XXVIIA

Balanus balanoides: 'mean weekly' moulting frequency (number of exuviae per 100 animals per day) at 10 and 15°C with variable light regime: excess Artemia supplied as food.

	Light period (h)					
	0	2	8	12	18	24
15°C	10.11	10.47	7.44	8.78	10.37	10.89
	12.00	12.47	10.18	9.33	8.91	10.00
	8.00	10.03	8.57	4.44	3.78	6.78
	9.56	10.30	9.14	6.33	10.76	10.00
	8.67	6.51	11.34	9.44	7.21	12.22
Mean	9.67	9.96	9.33	7.66	8.21	9.98
10°C	7.58	7.78	9.07	7.41	8.37	7.78
	10.99	8.00	9.97	10.12	13.16	9.44
	9.66	5.67	5.44	9.13	7.48	6.67
	10.39	8.11	9.75	9.09	5.33	7.63
	9.19	12.56	9.83	12.24	10.88	7.00
Mean	9.56	8.42	8.81	9.60	9.04	7.70

Table XXVIIIA

Balanus balanoides : animals fed : 10°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	14	9.33	
26th July	50	50	6	6.00	8.44
28th July	50	50	10	10.00	
31st July	50	50	6	4.00	
2nd Aug.	48	49	9	9.18	7.58
4th Aug.	46	47	9	9.57	
7th Aug.	46	46	14	10.14	
9th Aug.	46	46	12	13.04	10.99
11th Aug.	46	46	9	9.78	
14th Aug.	46	46	10	7.25	
16th Aug.	46	46	8	8.70	9.66
18th Aug.	46	46	12	13.04	
21st Aug.	46	46	10	7.25	
23rd Aug.	46	46	10	10.89	10.39
25th Aug.	46	46	12	13.04	
28th Aug.	46	46	8	5.80	
30th Aug.	46	46	10	10.89	9.19
1st Sept.	46	46	10	10.89	
4th Sept.	46	46	5		

Table XXIXA

Balanus balanoides : animals fed : 10°C

2 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	13	8.67	
26th July	50	50	4	4.00	5.89
28th July	50	50	5	5.00	
31st July	50	50	11	7.33	
2nd Aug.	50	50	9	9.00	7.78
4th Aug.	50	50	7	7.00	
7th Aug.	50	50	21	14.00	
9th Aug.	50	50	8	8.00	8.00
11th Aug.	50	50	2	2.00	
14th Aug.	50	50	12	8.00	
16th Aug.	50	50	4	4.00	5.67
18th Aug.	50	50	5	5.00	
21st Aug.	50	50	14	9.33	
23rd Aug.	50	50	11	11.00	8.11
25th Aug.	50	50	4	4.00	
28th Aug.	50	50	16	10.67	
30th Aug.	50	50	14	14.00	12.56
1st Sept.	50	50	13	13.00	
4th Sept.	50	50	13	13.00	

Table XXXA

Balanus balanoides : animals fed : 10°C

8 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	12	8.00	
26th July	50	50	7	7.00	8.00
28th July	49	50	9	9.00	
31st July	49	49	7	4.76	
2nd Aug.	49	49	7	7.14	9.07
4th Aug.	49	49	15	15.30	
7th Aug.	49	49	14	9.52	
9th Aug.	49	49	7	7.14	9.97
11th Aug.	49	49	13	13.26	
14th Aug.	49	49	9	6.12	
16th Aug.	49	49	5	5.10	5.44
18th Aug.	49	49	5	5.10	
21st Aug.	49	49	16	10.88	
23rd Aug.	49	49	11	11.22	9.75
25th Aug.	49	49	7	7.14	
28th Aug.	49	49	10	6.80	
30th Aug.	48	49	10	10.20	9.83
1st Sept.	47	48	12	12.50	
4th Sept.	47	47	11	7.80	

Table XXXIA

Balanus balanoides : animals fed : 10°C

12 hrs. light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	14	9.33	
26th July	49	50	9	9.00	10.44
28th July	48	49	13	13.26	
31st July	45	48	5	3.47	
2nd Aug.	45	45	8	8.89	7.41
4th Aug.	45	45	6	6.67	
7th Aug.	45	45	11	8.15	
9th Aug.	45	45	12	13.33	10.12
11th Aug.	45	45	8	8.89	
14th Aug.	45	45	16	11.85	
16th Aug.	45	45	8	8.89	9.13
18th Aug.	44	45	6	6.67	
21st Aug.	44	44	9	6.82	
23rd Aug.	44	44	8	9.09	9.09
25th Aug.	44	44	10	11.36	
28th Aug.	44	44	14	10.61	
30th Aug.	44	44	12	13.64	12.24
1st Sept.	44	44	11	12.50	
4th Sept.	44	44	10	7.58	

Table XXXIIA

Balanus balanoides : animals fed : 10°C

18 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	11	7.33	
26th July	50	50	6	6.00	7.78
28th July	50	50	10	10.00	
31st July	50	50	12	8.00	
2nd Aug.	49	50	12	12.00	8.37
4th Aug.	49	49	5	5.10	
7th Aug.	49	49	19	12.93	
9th Aug.	49	49	13	13.27	13.16
11th Aug.	49	49	13	13.27	
14th Aug.	49	49	3	2.04	
16th Aug.	49	49	10	10.20	7.48
18th Aug.	49	49	10	10.20	
21st Aug.	49	49	7	4.76	
23rd Aug.	49	49	4	4.08	5.33
25th Aug.	49	49	7	7.14	
28th Aug.	49	49	6	4.08	
30th Aug.	49	49	15	15.31	10.88
1st Sept.	49	49	13	13.26	
4th Sept.	49	49	6	4.08	

Table XXXIIIA

Balanus balanoides : animals fed : 10°C

24 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	10	13.33	
26th July	50	50	6	6.00	7.77
28th July	50	50	4	4.00	
31st July	50	50	5	3.33	
2nd Aug.	50	50	10	10.00	7.78
4th Aug.	50	50	11	11.00	
7th Aug.	50	50	14	9.33	
9th Aug.	50	50	7	7.00	9.44
11th Aug.	50	50	12	12.00	
14th Aug.	50	50	6	4.00	
16th Aug.	50	50	16	16.00	6.67
18th Aug.	50	50	0	0.00	
21st Aug.	50	50	15	10.00	
23rd Aug.	50	50	10	10.00	7.67
25th Aug.	50	50	3	3.00	
28th Aug.	50	50	12	8.00	
30th Aug.	50	50	9	9.00	7.00
1st Sept.	50	50	4	4.00	
4th Sept.	50	50	7	4.67	

Table XXXIVA

Balanus balanoides : animals fed : 15°C

Dark

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	23	15.33	
26th July	50	50	8	8.00	9.44
28th July	50	50	5	5.00	
31st July	50	50	14	9.33	
2nd Aug.	50	50	14	14.00	10.11
4th Aug.	50	50	7	7.00	
7th Aug.	50	50	21	14.00	
9th Aug.	50	50	11	11.00	12.00
11th Aug.	50	50	11	11.00	
14th Aug.	50	50	6	4.00	
16th Aug.	50	50	12	12.00	8.00
18th Aug.	50	50	8	8.00	
21st Aug.	50	50	10	6.67	
23rd Aug.	50	50	10	10.00	9.56
25th Aug.	50	50	12	12.00	
28th Aug.	50	50	6	4.00	
30th Aug.	50	50	14	14.00	8.67
1st Sept.	50	50	8	8.00	
4th Sept.	50	50	9	9.00	

Table XXXVA

Balanus balanoides : animals fed : 15°C

2 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	8	5.33	
26th July	50	50	7	7.00	6.67
28th July	49	50	8	8.00	
31st July	42	46	5	3.62	
2nd Aug.	41	42	9	10.72	10.47
4th Aug.	41	41	14	17.07	
7th Aug.	41	41	16	13.01	
9th Aug.	41	41	10	12.20	12.47
11th Aug.	41	41	10	12.20	
14th Aug.	41	41	10	8.13	
16th Aug.	41	41	8	9.76	10.03
18th Aug.	41	41	10	12.20	
21st Aug.	41	41	8	6.50	
23rd Aug.	41	41	13	15.85	10.30
25th Aug.	41	41	7	8.54	
28th Aug.	41	41	6	4.88	
30th Aug.	41	41	10	12.20	6.51
1st Sept.	41	41	2	2.44	
4th Sept.	41	41	11	8.94	

Table XXXVIA

Balanus balanoides : animals fed : 15°C

8 hrs. light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	17	11.33	
26th July	50	50	7	7.00	8.44
28th July	50	50	7	7.00	
31st July	49	50	9	6.00	
2nd Aug.	48	49	8	8.16	7.44
4th Aug.	48	48	8	8.16	
7th Aug.	48	48	20	13.89	
9th Aug.	48	48	9	9.38	10.18
11th Aug.	48	48	7	7.29	
14th Aug.	48	48	13	9.03	
16th Aug.	48	48	10	10.42	8.57
18th Aug.	48	48	6	6.25	
21st Aug.	48	48	11	7.64	
23rd Aug.	48	48	8	8.33	9.14
25th Aug.	48	48	11	11.46	
28th Aug.	48	48	10	6.94	
30th Aug.	48	48	19	19.79	11.34
1st Sept.	48	48	7	7.29	
4th Sept.	48	48	16	11.11	

Table XXXVIIA

Balanus balanoides : animals fed : 15°C

12 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	17	11.33	
26th July	50	50	9	9.00	8.78
28th July	50	50	6	6.00	
31st July	50	50	17	11.33	
2nd Aug.	50	50	9	9.00	8.78
4th Aug.	50	50	6	6.00	
7th Aug.	50	50	18	12.00	
9th Aug.	50	50	9	9.00	9.33
11th Aug.	50	50	7	7.00	
14th Aug.	50	50	11	7.33	
16th Aug.	50	50	3	3.00	4.44
18th Aug.	50	50	3	3.00	
21st Aug.	50	50	18	12.00	
23rd Aug.	50	50	6	6.00	6.33
25th Aug.	50	50	3	3.00	
28th Aug.	50	50	11	7.33	
30th Aug.	50	50	10	10.00	9.44
1st Sept.	50	50	11	11.00	
4th Sept.	50	50	12	8.00	

Table XXXVIIIA

Balanus balanoides : animals fed : 15°C

18 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	7	4.67	
26th July	50	50	9	9.00	6.56
28th July	50	50	6	6.00	
31st July	49	50	13	8.67	
2nd Aug.	49	49	14	14.28	10.37
4th Aug.	48	49	8	8.16	
7th Aug.	47	48	14	9.72	
9th Aug.	47	47	10	10.64	8.91
11th Aug.	47	47	6	6.38	
14th Aug.	47	47	4	2.84	
16th Aug.	47	47	3	3.19	3.78
18th Aug.	47	47	5	5.32	
21st Aug.	47	47	11	7.80	
23rd Aug.	47	47	13	13.83	10.76
25th Aug.	47	47	10	10.64	
28th Aug.	47	47	11	7.80	
30th Aug.	47	47	10	10.64	7.21
1st Sept.	47	47	3	3.19	
4th Sept.	47	47	14	11.35	

Table XXXIXA

Balanus balanoides : animals fed : 15°C

24 hrs light

Date	No. animals	Mean no. animals	No. exuviae	No. exuviae 100 animals/ day	Weekly mean values
21st July	50				
24th July	50	50	14	9.33	
26th July	50	50	4	4.00	5.78
28th July	50	50	4	4.00	
31st July	50	50	16	10.67	
2nd Aug.	50	50	11	11.00	10.89
4th Aug.	50	50	11	11.00	
7th Aug.	50	50	15	10.00	
9th Aug.	50	50	9	9.00	10.00
11th Aug.	50	50	11	11.00	
14th Aug.	50	50	8	5.33	
16th Aug.	50	50	6	6.00	6.78
18th Aug.	50	50	9	9.00	
21st Aug.	50	50	15	10.00	
23rd Aug.	50	50	10	10.00	10.00
25th Aug.	50	50	10	10.00	
28th Aug.	50	50	16	10.67	
30th Aug.	50	50	13	13.00	12.22
1st Sept.	50	50	13	13.00	
4th Sept.	50	50	13	8.67	