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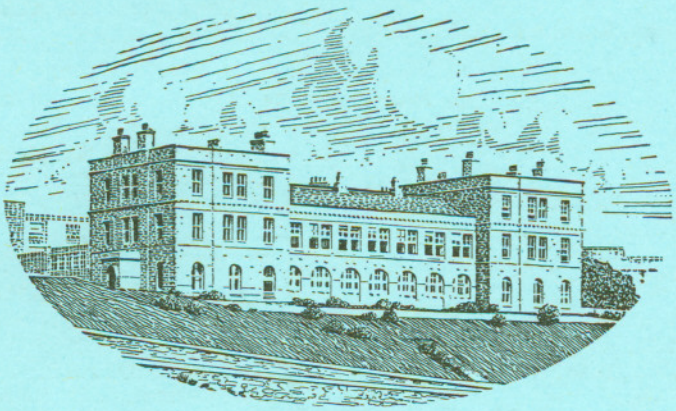

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THE BREEDING OF *ARENICOLA ECAUDATA* JOHNSTON AND *A. BRANCHIALIS* AUD. & EDW. AT PLYMOUTH

By EVE C. AND A. J. SOUTHWARD
The Plymouth Laboratory

(Text-figs. 1–9)

The two 'tail-less' lugworms, *Arenicola ecaudata* Johnston and *A. branchialis* Audouin and Milne-Edwards (= *A. grubii* Claparède), live in gravel and under stones, unlike the 'tailed' species which are found mainly in sandy beaches. Both are local in distribution compared with the wide-spread caudate species. *A. ecaudata* is known to occur from Iceland to northern Spain, and thus has a rather more boreal distribution than *A. branchialis* which ranges from the west coast of Scotland to Morocco, the Mediterranean and the Black Sea. (Ashworth, 1912; McIntosh, 1915; Fauvel, 1927; Rioja, 1935; Wesenberg-Lund, 1951.) Neither species is known from the western side of the Atlantic.

Since the two species can often occur side by side and it appeared that they were adapted, not for life in different habitats, but for different temperature régimes, it was suggested to us by Prof. G. P. Wells, F.R.S., that an investigation of their breeding seasons at Plymouth might help in understanding their distribution and specific separation. It happened that we had already made some preliminary investigations in the Isle of Man, and we were therefore pleased to adopt Prof. Wells's suggestion.

Mature specimens of both species have been noted by previous workers. Ashworth (1912) found mature gametes in *A. ecaudata* at Port Erin in April and at Plymouth in August. Fauvel (1899) recorded mature worms at Cherbourg from March to October, while Hentschel (1930) believed spawning occurred at Plymouth in February–March and August–September. It thus seemed possible that *A. ecaudata* spawned during the warmer half of the year. *A. branchialis*, on the other hand, was considered to be mature in September–October in Britain (Ashworth, 1912) and in winter at Naples (Lo Bianco, 1909). Samples of both species examined by ourselves at Port Erin showed no such difference in periods of maturity. Apparently mature gametes of *A. ecaudata* were found during the winter and of *A. branchialis* in the spring. It was therefore clear that it might be necessary to sample both species for more than a year to determine the breeding period with certainty.

The work at Plymouth has confirmed that both species may be breeding at the same time, but has shown significant differences in the breeding cycle. The

results help to explain certain aspects of the behaviour, development and systematics of the species.

We are indebted to Prof. G. P. Wells for ideas on which the work was based, and for encouragement and advice. One of us was in receipt of a D.S.I.R. senior research award during part of the work.

MATERIAL

A. ecaudata and *A. branchialis* are fairly common on most shores around Plymouth, in gravel or sandy gravel under stones. According to Fauvel (1927) the distribution of these species on the shore, in France, is as follows: *A. ecaudata* is found 'à un niveau assez bas', while *A. branchialis* lives 'jusqu'à un niveau découvrant à toutes les marées. A un niveau plus bas elle est souvent mélangée à *A. ecaudata*.'

Some of our samples were collected at Drake's Island, in Plymouth Sound, by Mr A. C. Briggs, but most of the material used was collected by the authors at Wembury Beach. Here a small sandy beach separates two masses of rock, Church Reef and West Reef, each with numerous gullies filled with gravel and boulders. The two species of *Arenicola* occur together in these gullies, from about mid-tide level to extreme low water mark. Between October 1955 and August 1956 we kept separate our samples from different tide levels. On Church Reef 201 worms were collected between L.W.S. and L.W.N.; of these 177 were *ecaudata* and 24 *branchialis*. Between L.W.N. and M.T.L. the total was 210, of which only 97 were *ecaudata*. The L.W.S. to L.W.N. level of the West Reef produced a total of 244 worms, including 197 *ecaudata*. These results agree in the main with Fauvel, except that *ecaudata* was found mixed with *branchialis* at all levels, not only towards low water.

Although the figures suggest that there were about equal numbers of the two species between L.W.N. and M.T.L., *branchialis* retreats rapidly into its burrow and more were present in the gravel than were collected in our samples. Thus *branchialis* may form rather more than 50% of the population above L.W.N. Above M.T.L. and below M.L.W.S. we found a few specimens of both species, but in too small a number to determine the real proportions.

Apart from this difference in abundance with tide level, we noted that at all inhabited levels *A. branchialis* was the commoner in patches of sandy gravel which were more exposed to wave action, while *A. ecaudata* was often found alone in sheltered hollows where the gravel contained much sea-weed debris and decaying organic matter. However, both species have been found at wave beaten places such as Rame Head, where they occur side by side in gullies of coarse gravel; both species can also be found in places where the 'black layer' (Bruce, 1928) lies close to the surface. There thus seems to be no constant correlation with type of deposit or organic content in either species. When both species are considered together and compared with the only other species of

Arenicola present in the British Isles (*A. marina* L.) there is a clear separation of habitats. At Plymouth and the Isle of Man we have found *A. marina* together with either of the other species only twice in samples totalling over 2000 worms.

METHODS

The investigations began in May 1954 and sampling was continued at irregular intervals through 1954 and 1955. Experience gained from these samples enabled a more regular series of samples, from November 1955 to January 1957, to be sexed and classified with greater accuracy. The samples were collected during spring tides, at monthly or fortnightly intervals during this period.

We planned to examine between twelve and twenty specimens of each species at a time, but owing to the irregular nature of the habitat, and the variable proportions of the species, some samples contained fewer than twelve specimens of each. We have omitted from our results samples of less than ten *A. ecaudata* and less than seven *A. branchialis*. The latter was always more difficult to find and it was necessary to search the same areas each time. However, there was no marked decline in abundance, and it is assumed that the vacant niches were filled up by migration of worms from surrounding areas.

In the laboratory the worms were first cleaned of gravel and mucus and sorted into species. For this purpose we relied on the differences in the number of abranchiate setigerous segments (Fauvel, 1927).

In the second series of samples the size of each worm was measured as volume (to the nearest 0·1 ml.) of sea water displaced, but this was not done before November 1955.

Measurement of volume was followed by an examination of a drop of coelomic fluid from each worm.

In *A. ecaudata* most of the development of the gametes takes place in the gonads and the sex-products do not appear in the coelomic fluid until they are nearly mature. Thus, in this species, if the coelomic fluid contained no gametes the gonads were examined, and one of them removed for measurement of the oocytes. Our ability to sex immature and spent specimens of *A. ecaudata* increased during the survey, and this probably accounts for the absence of worms of unknown sex from the 1956 samples.

In *A. branchialis* the gonads are very small (see Fauvel, 1927; Downing, 1909, 1911) and the gametes undergo most of their development in the coelom. It was therefore a simple matter to sex and classify this species by examining some of the coelomic fluid under the microscope.

Males and females were grouped into the developmental stages shown in Table 1, and the largest oocytes were measured with an eye-piece micrometer. The divisions of this micrometer (17μ with $\frac{2}{3}$ in. objective) were used to define

the eleven oocyte stages in this species and the ten stages in *A. ecaudata* (see Table 2). In both species the oocytes are slightly oval in shape and the greatest diameter was measured.

TABLE 1. DEFINITIONS OF BREEDING STAGES

	Arenicola ecaudata	
Male	Empty (E)	Sex determinable from shape of gonads, no sex products visible in gonads or coelom
	Developing (D)	Sex products in gonads, none in coelom
	Mature (M)	Coelom full of tailed sperm plates or free spermatozoa
	Spent (S)	A few sperm plates or spermatozoa in coelom, gonads empty
Female	Empty (E)	As male
	Developing	
	(1) in gonads (Dg)	Oocytes developing inside gonads, none in coelom
	(2) in coelom (Dc)	Coelom contains oocytes, of which less than 90 % have reached 0·17 mm in diameter
	Mature (M)	Coelom full of oocytes, of which at least 90 % are 0·17 mm or more in diameter
	Spent (S)	Gonads still large but empty of oocytes, occasionally a few large oocytes in the coelom
Sex unknown	Empty (E)	Gonads very small or absent, no sex products visible
	Arenicola branchialis	
Male	Developing (D)	Coelom contains sperm morulae and/or sperm discs, of which less than 90 % are tailed
	Mature (M)	Coelom contains sperm discs of which at least 90 % are tailed
	Spent (S)	A few tailed discs or spermatozoa in the coelom
Female	Developing (D)	Coelom contains oocytes, of which less than 90 % have reached 0·17 mm in diameter
	Mature (M)	Coelom full of oocytes, of which at least 90 % have reached 0·17 mm or more in diameter
	Spent (S)	Coelom contains a few large oocytes, with often a few very small
Sex unknown	Empty (E)	No sex products discernible

TABLE 2. DEFINITION OF OOCYTE SIZE-GROUPS

Group	Diameter of largest oocyte present	Group	Diameter of largest oocyte present
0	Ovary distinct but no oocytes visible	6	0·086–0·102 mm
1	Up to 0·017 mm	7	0·103–0·119 mm
2	0·018–0·034 mm	8	0·120–0·136 mm
3	0·035–0·051 mm	9	0·137–0·153 mm
4	0·052–0·068 mm	10	0·154–0·170 mm
5	0·069–0·085 mm	11	0·171–0·187 mm

ARENICOLA BRANCHIALIS

The sex of *A. branchialis* could not be determined from the gonads, so that when there were no sex products in the coelomic fluid the specimen was classified as 'of unknown sex'. The developing gametes, whether male or female, were shed from the gonads at an early stage in their development, and appeared in the coelomic fluid a few at a time over a period of months; gametes

at all stages of development might be found in the coelom of one worm. After the production of gametes ceased maturation continued until all were full sized.

The development of sperm discs from balls of spermatogonia has been described by Downing (1911) for several species of *Arenicola*, including *branchialis*. In the females the oocytes were shed from the ovaries at a diameter of 17μ or even less. They grew steadily in size to a diameter of 0·17–0·187 mm when mature. At a diameter of about 0·12 mm a thick vitelline membrane was formed. The mature oocytes have been described and figured by Ashworth (1912). After spawning, a few mature oocytes remained in the coelom of the female, often being mixed with the first small oocytes produced by the gonads as the cycle began again.

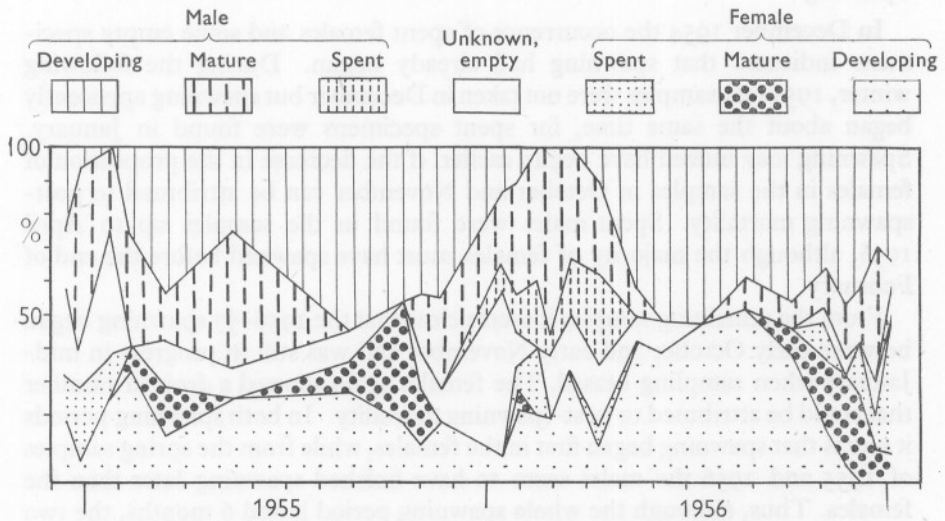

Fig. 1. *Arenicola branchialis*. Percentages of breeding stages (see Table 1) in samples from Wembury, December 1954 to January 1957.

Annual cycle

The results of 2 years' sampling are shown in Fig. 1, the developmental stages being those of Table 1. Samples were few in 1955 and included some small specimens; from 15 November 1955 specimens 1 ml. or under in volume have been omitted from the figure, since we believe that young specimens may develop at a different rate, and there seems to be a fairly steady recruitment of young specimens into the breeding population throughout the year.

The general pattern of development and spawning seems to have been the same in 1955 and 1956. The gametes developed throughout the spring, summer and autumn, and spawning took place over a period of 4–6 months centring

on the winter. The 1954–55 spawning did not finish before the end of January, and may have gone on longer. Spent specimens were not found after the end of January, but mature females and males remained in the population until at least March and May, respectively. Development began again soon after spawning, while many individuals still had a few residual gametes in the coelom. Development of the females continued until the end of October 1955, when more than half of them were mature, though apparently all the males were still developing. Fig. 2 shows the development of the oocytes of the female population throughout 1955 and 1956. Clearly, growth of oocytes continues slowly and steadily up to midsummer; thereafter, most of the population is mature for 3 or 4 months before spawning.

Spawning

In December 1954 the occurrence of spent females and some empty specimens indicated that spawning had already begun. During the following winter, 1955–56, samples were not taken in December but spawning apparently began about the same time, for spent specimens were found in January. Spawning may indeed have begun earlier, if the decrease in the proportion of females in the samples in October and November can be attributed to post-spawning mortality. Spent males were found in the samples up to April 1956, although the majority of females must have spawned before the end of February.

From the remaining samples it seems clear that the 1956–57 spawning began between early October and early November and was still in progress in mid-January when sampling ceased. The females again showed a drop in number that could be attributed to post-spawning mortality. In both spawning periods it seems that spawning began first in the females, while from the spring samples of 1955 and 1956 the males seem to have finished spawning later than the females. Thus, although the whole spawning period lasted 6 months, the two sexes apparently spawned together for only 4 months.

In *A. branchialis*, as in other species of *Arenicola*, spawning takes place through the nephridia and not through a breakdown of the body wall. Nevertheless, the sudden variations in the ratio of the sexes during the spawning season suggest that a number of worms of both sexes die after spawning, as in *A. marina* (Newell, 1948). We have not had spawning specimens of both sexes in the same sample, and have not seen naturally fertilized eggs, nor have any of our artificial fertilizations been successful. The larva remains unknown, but the post-larva has been described by Ashworth (1912); his specimens were collected in Ireland, among *Laminaria* roots, by Southern who also described them (1914). Post-larvae have not been recorded from the Plymouth area. The smallest specimens found by us among the gravel with the adults were 2–3 cm long and had all segments and branchiae.

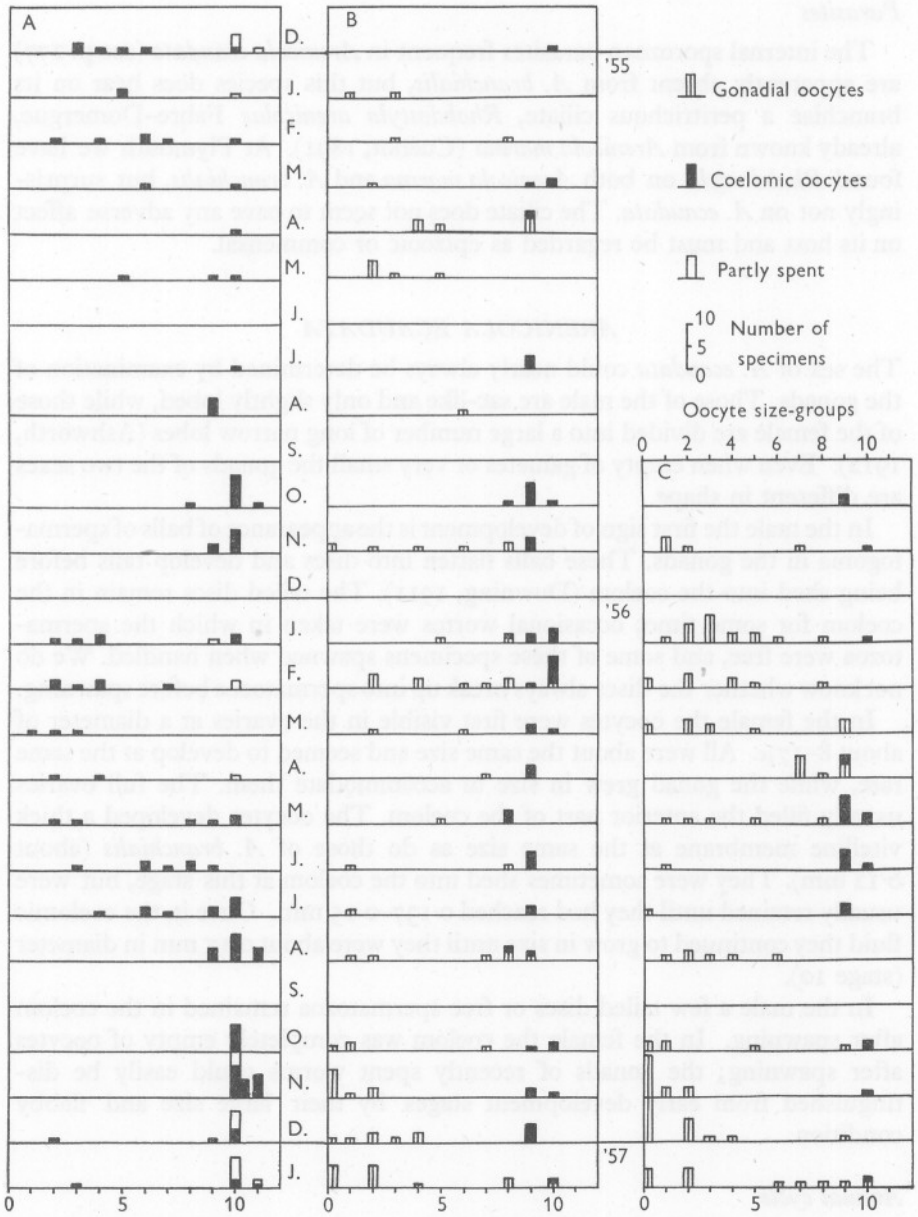

Fig. 2. Oocyte sizes. Number of worms containing oocytes of each size group (see Table 2), each month. A, *Arenicola branchialis*, Wembury; B, *A. ecaudata*, Church Reef, Wembury; C, *A. ecaudata*, West Reef, Wembury.

Parasites

The internal sporozoan parasites frequent in *Arenicola ecaudata* (see p. 277) are apparently absent from *A. branchialis*, but this species does bear on its branchiae a peritrichous ciliate, *Rhabdostyla arenicolae* Fabre–Domergue, already known from *Arenicola marina* (Cuénot, 1891). At Plymouth we have found *Rhabdostyla* on both *Arenicola marina* and *A. branchialis*, but surprisingly not on *A. ecaudata*. The ciliate does not seem to have any adverse affect on its host and must be regarded as epizooic or commensal.

ARENICOLA ECAUDATA

The sex of *A. ecaudata* could nearly always be determined by examination of the gonads. Those of the male are sac-like and only slightly lobed, while those of the female are divided into a large number of long narrow lobes (Ashworth, 1912). Even when empty of gametes or very small the gonads of the two sexes are different in shape.

In the male the first sign of development is the appearance of balls of spermatogonia in the gonads. These balls flatten into discs and develop tails before being shed into the coelom (Downing, 1911). The tailed discs remain in the coelom for some time; occasional worms were taken in which the spermatozoa were free, and some of these specimens spawned when handled. We do not know whether the discs always break up into spermatozoa before spawning.

In the female the oocytes were first visible in the ovaries at a diameter of about 8–17μ. All were about the same size and seemed to develop at the same rate, while the gonad grew in size to accommodate them. The full ovaries usually filled the anterior part of the coelom. The oocytes developed a thick vitelline membrane at the same size as do those of *A. branchialis* (about 0·12 mm). They were sometimes shed into the coelom at this stage, but were usually retained until they had reached 0·137–0·15 mm. Once in the coelomic fluid they continued to grow in size until they were about 0·17 mm in diameter (stage 10).

In the male a few tailed discs or free spermatozoa remained in the coelom after spawning. In the female the coelom was completely empty of oocytes after spawning; the gonads of recently spent worms could easily be distinguished from early development stages by their large size and flabby condition.

Annual cycle

The results of 2 years' sampling of the Church Reef population are shown in Fig. 3, together with 1 year's results from the West Reef and Drake's Island populations. The two Wembury populations are shown separately, unlike those of *A. branchialis*, not only because the samples were sufficiently large,

but because there seems to be some difference between them. The small specimens have been omitted from the figure, on and after 15 November 1955, as with *A. branchialis*. Before this date the classification 'sex unknown' probably included small undeveloped worms as well as spent and empty adults.

At all three sites, Church Reef, West Reef and Drake's Island, mature males of *A. ecaudata* were present practically all the year round. It is difficult to determine any definite cycle, although the least proportion of such males was found in late winter, the greatest in summer. This trend is best shown by the West Reef samples. From the minimum in January and February 1956,

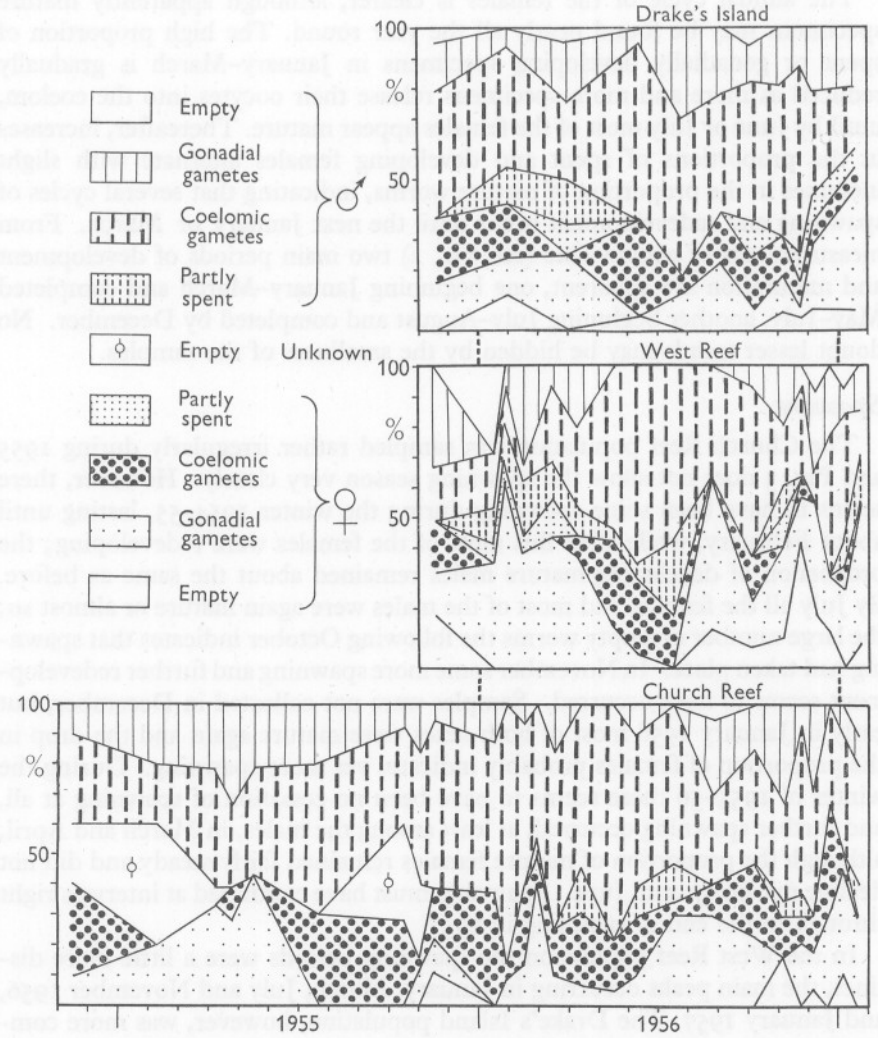

Fig. 3. *Arenicola ecaudata*. Percentages of breeding stages in samples from three localities.

when most of the males were either spent or developing gametes in the gonads, the number with coelomic gametes increased steadily to May, when over half the samples consisted of mature males. The proportion declined from June to August and at the same time the proportion of spent males increased. There was another increase in mature males up to September, after which the proportion declined, most of the males being in the gonad developing condition. A further peak of mature males occurred in December 1956, followed by another decline in January 1957. This suggests that several spawnings occurred during the year.

The annual cycle of the females is clearer, although apparently mature specimens may be found nearly all the year round. The high proportion of spent or gonadially developing specimens in January–March is gradually reduced as more and more specimens release their oocytes into the coelom, until by June or July most of the females appear mature. Thereafter, increases in the proportions of spent and developing females alternate with slight increases in the proportion of mature worms, indicating that several cycles of spawning and redevelopment occur until the next January or March. From measurements of oocyte sizes (see Fig. 2) two main periods of development and maturation are apparent, one beginning January–March and completed May–July, another beginning July–August and completed by December. No doubt lesser trends may be hidden by the smallness of the samples.

Spawning

The Church Reef population was sampled rather irregularly during 1955 and Fig. 3 does not show the spawning season very clearly. However, there seems to have been some spawning during the winter 1954–55, lasting until about February, while after this most of the females were redeveloping; the proportion of developing/mature males remained about the same as before. By July all the females and most of the males were again mature or almost so; the large number of empty worms the following October indicates that spawning had taken place. In November some more spawning and further redevelopment seems to have occurred. Samples were not collected in December, but early in January 1956 most of both sexes were mature again and the drop in the proportion of females probably indicates yet more spawning. During the winter of 1955–56 there seems to have been no cessation of spawning at all, and further spawning occurred, at least among the males, in March and April, although the proportion of mature females remained fairly steady and did not decline markedly until June. Spawning must have continued at intervals right through to the end of sampling in 1957.

In the West Reef population the spawning periods were a little more distinct, the main peaks occurring in January, March, July and November 1956, and January 1957. The Drake's Island population, however, was more comparable to that of Church Reef, in that spawning seemed continuous, although

the main peaks were indicated by the presence of spent females in the samples.

It is worth noting that, in general, the deposit at the Church Reef and Drake's Island sites was richer in decaying sea-weeds and other organic matter than that at West Reef. The average size of the worms was greater at these two sites, and it is possible that the apparently continuous breeding activities there may have been due to the presence of more abundant food.

Effect of parasites

Two sporozoan parasites have been recorded from *Arenicola ecaudata* at Plymouth (Cunningham, 1907; Goodrich & Pixell-Goodrich, 1920), and both were found again during the present survey.

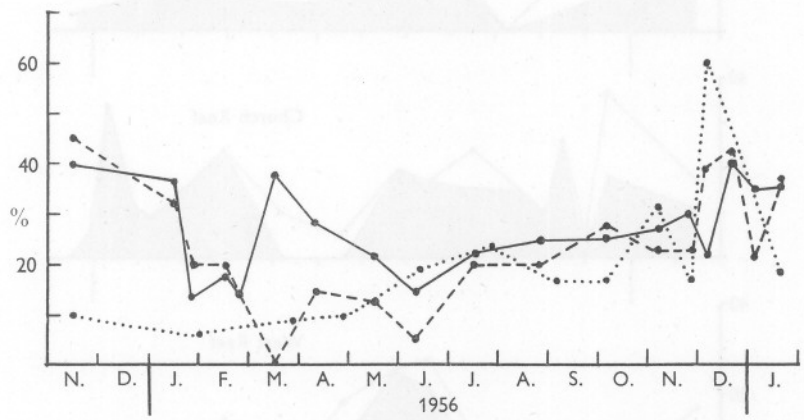

Fig. 4. Percentage *Arenicola ecaudata* containing cysts of *Gonospora arenicolae*, in each sample from three localities: ——, Church Reef; – – – –, West Reef; . . . ., Drake's Island.

The trophozoites of *Gonospora arenicolae* (Cunningham) live attached to the nephridia of both sexes of *Arenicola ecaudata*, dropping off into the coelomic fluid when mature and uniting in pairs or in groups to form gametocysts (Cunningham, 1907). These cysts are white spheres, about 1 mm in diameter, and obvious to the unaided eye. We did not examine the nephridia of every specimen but believe that most were infected with trophozoites (cf. Hentschel, 1930). We recorded the presence of gametocysts in the coelom and show in Fig. 4 their occurrence during 1956. There was a winter maximum in all three populations, coinciding more or less with the winter spawning. In the Church Reef population there was a second peak in March, just before the April spawning (see Fig. 3), but in the others there was a steady rise in rate of occurrence from February to November, with a rapid rise about December. Except in a few cases of extremely heavy infection, when the coelom was almost full of trophozoites and cysts, this parasite did not apparently affect the

rate of development of the gametes, although, by using part of the available food supply, it must have reduced their numbers.

Gonospora minchini Goodrich & Pixell-Goodrich has trophozoites which attack the oocytes of *Arenicola ecaudata*, entering them sometime after they have been released from the gonads. The growing trophozoite enlarges the oocyte membrane enormously and destroys its contents. The trophozoites emerge from the membranes when full grown and unite in pairs or groups, as

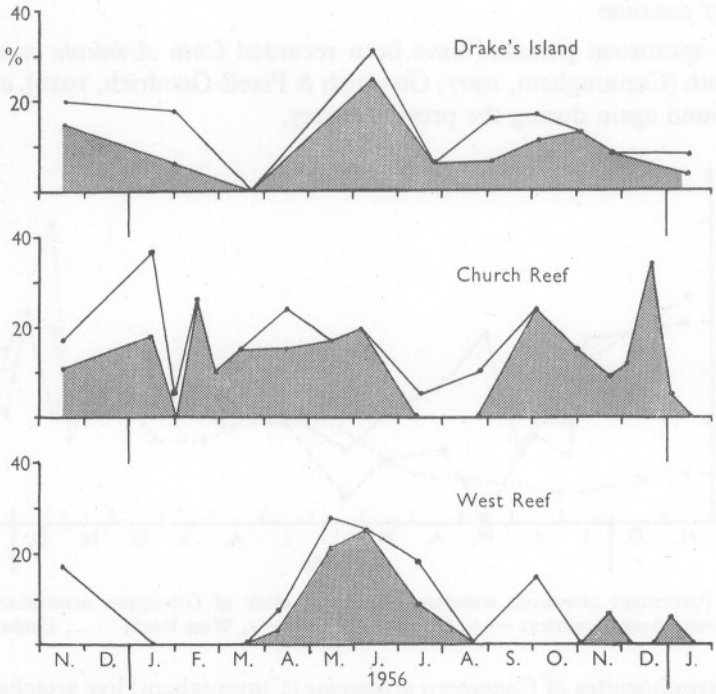

Fig. 5. Percentage *Arenicola ecaudata* containing coelomic oocytes in each sample from three localities. Percentage infected with *Gonospora minchini* stippled.

in *Gonospora arenicolae*. They form cysts which are, to us, indistinguishable from those of *G. arenicolae*. Since both parasites may be present in one worm, some of our records of *G. minchini* in the gametocyst stage may include *G. arenicolae* and vice versa.

G. minchini apparently never attacks the oocytes while they are in the ovaries. We have not been able to discover whether there is some other site of infection in the worm during the development of the ovaries, but well over 50% of the worms with coelomic oocytes had this parasite in them. Fig. 5 shows the proportion of these females infected in each sample during 1956. Some of these were only lightly infected (1–10% of the oocytes attacked) but others had up

to 50% of the oocytes destroyed. Though the presence of the parasite does not appear to affect the rate of development of the unattacked oocytes, it has a marked effect on the fecundity of the host.

Larval development

On two occasions (16 November 1954 and 2 November 1956) specimens of *Arenicola ecaudata* spawned in the laboratory and produced fertilized eggs, a few hours after the samples had been brought in from the shore. Hentschel (1930) noted that the worms spawned inside their transparent tubes, moving about as they poured out their genital products. We found the eggs embedded in these transparent tubes, which the worms always form when kept in the laboratory. The tubes, after being washed, were placed in finger bowls of filtered sea water and kept at a temperature of approximately 15° C.

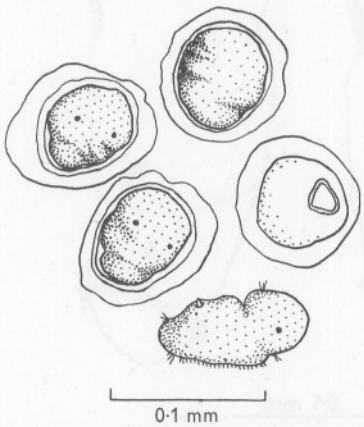

Fig. 6. *Arenicola ecaudata*. Group of larvae ready to hatch; with one already hatched and an empty egg membrane.

In the first case hatching was not observed, but the larvae were crawling about the dish on the eighth day. In the second case hatching took place on the eighth day, and before hatching the larvae were seen to twist about inside the egg membrane for some time. They finally emerged through a small hole (Fig. 6), crawled about the bottom of the dish and formed small transparent tubes; they were not seen to swim at any time.

The newly hatched larva (Figs. 6, 7A) had two circular bands of cilia with a ventral band between them (as in *A. marina* (Newell, 1948)), a pair of dorsal limbate setae and a pair of red eye-spots with dorsal lenses. During the next 2 or 3 days a second notoseta and an uncinate neuroseta appeared on each side; these were followed by the first limbate notosetae of the second setigerous segment (Fig. 7B). Table 3 shows the rate of development of the larvae. The second and third setigers developed in the same way as the first, but after this

the first notoseta to appear on each side was uncinate and the limbate setae developed later (Table 4; Fig. 7c); this also occurs in other species of *Arenicola* (Ashworth, 1912).

The 1954 larvae developed only to the 4-setiger stage, but lived a further 14 days without any more growth. The 1956 larvae reached the 6-setiger stage and lived a further 40 days without further growth or development, although various algal cultures (kindly supplied by Dr M. Parke) were provided as food and were eaten by the larvae.

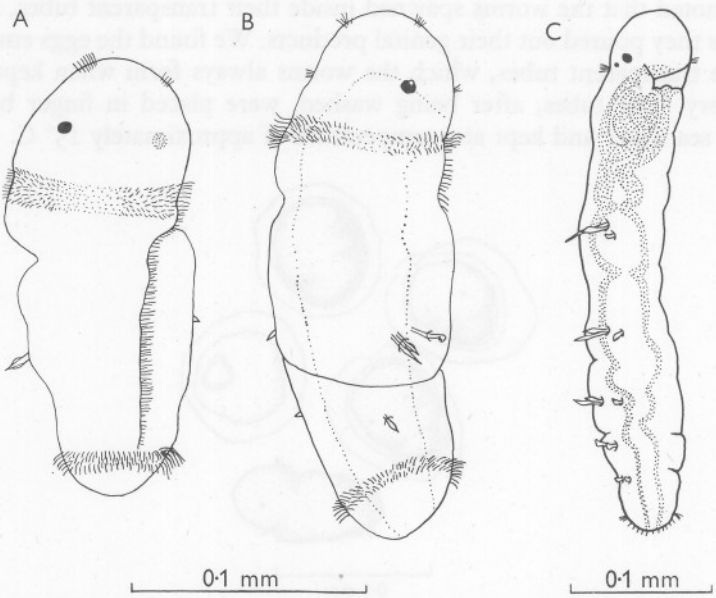

Fig. 7. *Arenicola ecaudata*. A, larva with one setigerous segment, showing ventral ciliated band; B, larva with two setigerous segments, side view; C, larva with five setigerous segments, side view (alimentary canal stippled). From camera lucida drawings of living larvae.

Behaviour of larvae

The larvae started feeding at the 4-setiger stage when the gut was completely formed and the mouth and anus open. The pharynx was ciliated and not yet eversible, though it was capable of some movement. The food taken was bacteria and small fragments of detritus, the remains being ejected as faecal pellets about $34 \times 17 \mu$. A second pair of eyespots appeared, and, as the 5th setiger developed, yellow pigment was laid down in the prostomium, around the anus, and in the wall of the mid-gut. The proboscis grew larger and became eversible. By the 6-setiger stage it was well developed, and the larvae were feeding actively, picking up bacteria, algae and detritus from the bottom of the dish. The proboscis was everted in two stages, as shown in Fig. 8. By this time the larvae had developed a fairly definite pattern of behaviour, which

consisted basically of backward and forward movements in the tube, which was about twice the length of the larva. When watched under the microscope, at 15–20° C, the larva moved back and forth once or twice a minute.

TABLE 3. RATE OF DEVELOPMENT OF *ARENICOLA ECAUDATA* LARVAE

Date of fertilization ...	16. xi. 54	2. xi. 56
Stage reached	Age in days	
Hatching (1-setiger)	8	8
2-setiger	11	10
4-setiger	17	20
5-setiger	—	28
6-setiger	—	34

TABLE 4. SETAE OF 6-SETIGER LARVA OF *ARENICOLA ECAUDATA*

Setigerous segment	Dorsal	Ventral
1st	2 limbate	1 uncinate
2nd	2 limbate	1 uncinate
3rd	2 limbate	1 uncinate
4th	1 uncinate 1 limbate	1 uncinate
5th	1 uncinate	1 uncinate
6th	1 uncinate	1 uncinate

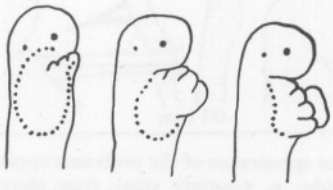

Fig. 8. *Arenicola ecaudata*. Two stages in eversion of the proboscis in a 6-setiger larva. Sketches of living larva.

During feeding the proboscis was extruded at the end of a forward movement, and there were up to twenty of these extrusions, at the rate of five to ten per minute, before the larva moved back. The next forward movement was followed by further extrusions of the proboscis, and so on for up to 15 min. Between periods of feeding activity there were periods of back and forth movement alone, lasting an hour or more, during which one or two faecal pellets were produced. Each of these was ejected at the end of a backward movement so that a heap of faecal pellets was built up a short distance behind each larva. The larvae would occasionally turn round in the tube and continue the same behaviour facing the other way.

Proboscis apparatus of the larva

The origin of the retractor sheath and gular membrane, or 'first diaphragm', of *Arenicola* has been the object of some speculation; in the adult the two structures are associated at their junction with the body wall and, while

Lillie (1905) considered that they originated separately, Wells (1952, 1954) suggested that they were derived from the splitting of a single septum. We have examined stained specimens of 1-, 2-, 4- and 5-setiger larvae of *A. ecaudata*, and find that in the last two of these the gular membrane and retractor sheath are already separate, although the membrane has not yet assumed its adult

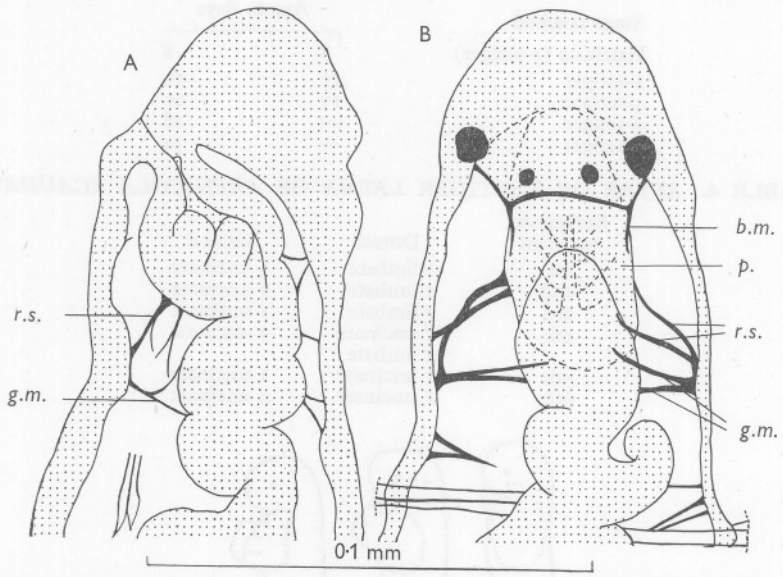

Fig. 9. *Arenicola ecaudata*. The appearance of the proboscis apparatus in stained whole larvae A, 4-setiger stage, from the side; B, 5-setiger stage, from above. Camera lucida drawings. *b.m.*, buccal membrane; *g.m.*, gular membrane; *r.s.*, retractor sheath; *p.*, pharynx.

form (Fig. 9). The 1- and 2-setiger larvae are rendered opaque by the yolk they contain. Any splitting of the larval septum must take place before the 4-setiger stage; to show it clearly, horizontal sections of the larvae would probably be required.

Later development

The post-larval and juvenile stages of *A. ecaudata* can be found towards L.W.S. among small algae and in *Laminaria* holdfasts. Ashworth (1912) recorded them from Ireland in September and McIntosh (1915) found them in the Hebrides in August. We have found juveniles (less than 2 cm long) in the Isle of Man in the following months: January, March, April, May, July, August and September. We have found them at Plymouth in October but have not looked for them at other times of the year.

The smallest specimens found in the adult habitat at Plymouth were 2–3 cm in length and had a volume of 1 ml. or less. These occurred in our samples throughout the year.

COMPARISONS AND CONCLUSIONS

Having described the breeding cycles of *Arenicola ecaudata* and *A. branchialis*, we can compare them with one another and with those of other species. The absence of resting phases from these cycles distinguishes them both from that of *A. marina*, the only other species thoroughly investigated (Newell, 1948).

In spawning habits *A. ecaudata* resembles most closely the Japanese *A. claparedii* (Okuda, 1938), although the breeding cycles are not very similar. The larvae of *A. ecaudata* are slow to develop, in comparison with those of other species of *Arenicola*, but the larvae of all species are otherwise very much alike.

The breeding cycles of animals and plants are usually dependent in some way on annual fluctuations in external conditions, particularly temperature and food supply (Orton, 1920; Thorson, 1946; Qasim, 1956; Crisp & Southward, 1958). At Plymouth the same temperature régime is experienced by both *A. ecaudata* and *A. branchialis* and the same food supply is available to each. The amount of algal detritus on the shore varies considerably with the weather conditions, but even when it is at its lowest it is still abundant in the habitat of these species, though *branchialis* can apparently tolerate a lower organic content than can *ecaudata* (p. 268). It appears that the food supply should always be sufficient, and so we must look to temperature for an explanation of the difference between their breeding cycles.

According to Orton (1920), cosmopolitan species should have a wide range of temperature within which breeding can occur; cold-water species near their warmer limits should breed in the colder months of the year; warm-water species near their colder limits should breed in the warmer months of the year. Many organisms have been shown to conform to these conditions, but the two ecaudate species of *Arenicola* do not.

The temperature requirements of any species vary during its life history and during its breeding cycle. The geographical distribution may be closely linked to the temperature tolerance of the adult, but the breeding cycle is affected by different temperatures at each stage and this also may affect the distribution of a species. *A. branchialis* is nearing its northern limit at Plymouth and if it is limited by low temperature and not by any other factor then this may take effect in one or more of the following ways. A lower winter temperature than that normally experienced on the west coasts of the British Isles may kill the adults. Since the gonads are capable of initiating gamete production during the coldest part of the year at Plymouth it seems unlikely that gametes could not be produced, in the warmer months at least, further north; however, growth and maturation of the gametes are slow at Plymouth, taking up to 10 months. Further north maturation might not be completed in the shorter summer. Spawning takes place at Plymouth as the sea temperature is falling, and the larvae must develop during the coldest part of the year;

farther north lower temperatures on the shore might be lethal to the larvae, if not to the adults. The distribution of *branchialis* south of the Mediterranean area is presumably limited by the effect of high winter temperatures on the spawning or the larvae, but little is known of the breeding cycle there.

A. ecaudata is nearing its southern limit at Plymouth but it extends further north than *branchialis*. Since gametes can be produced and matured all the year round at Plymouth, these processes should take place at least in the warmer months in the northern, and in the colder months in the southern part of its range. Both sexes apparently spawn at all times of year at Plymouth, and presumably larvae are produced, so a wide range of temperatures should be suitable for development. If low winter temperatures were lethal to larvae further north the spring and summer larvae should still be able to survive. Similarly, further south, the winter-spawned larvae ought to be able to survive. Thus, the northern limit of this species would appear to depend on the effect of low winter temperature on the adults, but there seems little reason for the position of the southern limit. As far as the breeding cycle is concerned *ecaudata* could presumably extend as far south as *branchialis*.

Other species of *Arenicola* do not show very clear correlation between breeding habits and geographical distribution; *A. marina* is a cold-water species which does spawn in the colder months of the year, near its warmer limits (Newell, 1948; Duncan, 1950), but *A. claparedii*, another cold-water species, appears to develop during the cold months and spawn in spring or early summer (Ashworth, 1912; Guberlet, 1934; Okuda, 1938). *A. cristata* is a warm-water species which spawns in summer in the cooler parts of its range, though the gametes develop throughout the winter (Downing, 1911). This suggests a breeding cycle rather like that of *A. branchialis*, with spawning a few months earlier. In the tropics, spawn of *A. cristata* has been reported at various times of the year (Okuda, 1938; Takahashi, 1938; Bhatti & Soofi, 1949), which suggests that, like many tropical animals, *A. cristata* may breed all the year round. Information on the breeding of the remaining species of *Arenicola* is not sufficient for comparison with their distribution.

Before we can come to any definite conclusions we need to know more about the breeding of all the species of *Arenicola*. Temperature can be only a partial cause of the limited distribution of *A. ecaudata*. The limits of *A. branchialis* seem, however, to show the effect of temperature on various stages of the life cycle. The boundary between these two species might be affected by the influence of temperature on competition between them. Although *ecaudata* is commoner than *branchialis* at Plymouth, the latter maintains itself by a short spawning period compared with the almost continuous spawning of *ecaudata*.

The effect of parasites must also be considered; it is possible that increasing temperature might favour the species of *Gonospora* at the expense of their host and that farther south *A. ecaudata* might be rendered completely sterile by heavy infections of these sporozoans.

From anatomical evidence Ashworth (1912) considered *A. ecaudata* to be the species of *Arenicola* nearest the ancestral form, and perhaps the most ancient of the existing species. If this be so, its geographical range might have been wider in the past and have become restricted by competition, parasites or other causes, in comparatively recent times.

SUMMARY

The two 'tail-less' lugworms, *Arenicola ecaudata* and *A. branchialis*, live in gravel and under stones, on the shores of western Europe. *A. ecaudata* has a more boreal range than *A. branchialis*, but both have a restricted geographical range compared with the remaining species of *Arenicola*, which live in sandy beaches.

At Plymouth *A. branchialis* is a winter-spawning species. Development of the gametes continues through spring, summer and autumn, and spawning apparently occurs over 4–6 winter months, usually November to February.

A. ecaudata, on the other hand, apparently spawns all the year round, mature males and females being found in all months. In 1954 and 1955 there was a fairly definite breeding cycle, with development of gametes in spring, followed by several periods of spawning and redevelopment until the end of the following winter. There seems to have been no cessation of spawning at all in the early spring of 1956; periods of redevelopment and spawning were practically continuous from winter 1955–56 to winter 1956–57.

Only *A. ecaudata* is infected with sporozoan parasites, which frequently reduce its fecundity.

Larval development of *A. ecaudata* was followed up to the appearance of the 6th setigerous segment, when the adult pattern of behaviour was already beginning to appear.

The distribution of *A. branchialis* may be controlled by the effect of temperature on various stages of the life cycle. The northern limit of *A. ecaudata* can likewise be explained by temperature limitation, but the restricted southern limit may be due to effects of competition, parasites or other causes; it is apparently not due to a direct effect of temperature on the breeding cycle.

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OBSERVATIONS ON THE GYMNOSOMATOUS PTEROPOD *CLIONE LIMACINA* (PHIPPS)

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(Text-figs. 1–3)

The Pteropoda Gymnosomata are fast-swimming, shell-less, carnivorous opisthobranchs. The single representative taken at Plymouth is usually regarded as the sexually mature dwarf form of *Clione limacina* (which replaces the typical boreal form of the species in warmer North Atlantic waters). It turns up frequently in the summer and was first shown by Lebour (1931) to breed in these waters. The material used in this account was taken alive near Station EI in August, 1953, and was supplemented by a collection very kindly made for me on the cruise of R.V. *Sarsia* in the Celtic Sea, in September, 1956, by Drs J. and Sylvia Gilpin-Brown to whom I am greatly indebted.

Workers on gymnosomes have suffered more than most from the difficulty of seeing well fixed and living material. It was, therefore, thought worthwhile briefly to examine some features of the morphology of this common pteropod that are not adequately known, and to give some notes on its habits in life. After Pelseneer's classic work (1888) came Meisenheimer's 'Valdivia' report (1905), our finest store of information on Gymnosomata. Later workers such as Tesch, Pruvot-Fol, Bonnevie and Massey have concentrated chiefly on the important task of taxonomy, and many details of the morphology and habits are necessarily passed over.

Plymouth specimens of *C. limacina* show well recognizable sexual development at 2·5 mm long. Lebour (1931) found eggs being laid by individuals of 5 mm. Like so many opisthobranchs, *Clione* is protandrous. Fig. 3C (p. 293) shows the size distribution of a sample of 338 specimens from Dingle Bay, west coast of Ireland, September 1956. The smallest size-group (2·5 mm length when fixed) already showed dividing spermatocytes and fully formed sperm in the ovotestis. Only a few undeveloped oocytes were present, as tiny inconspicuous cells at the periphery. Specimens of 3·5 mm length had most of the sperms mature and stored also in the hermaphrodite duct (see Fig. 3B), while in the largest of the September sample, oocytes were numerous and large (*ca.* 0·8 mm) but no eggs appeared ready to be shed. Even the largest specimens kept the posterior ciliated girdle, a characteristic feature of the dwarf race. The colour was yellowish white and the red patch of the gonad was less intense than in the typical 'northern' form. There is a further difference in the radula,

the dwarf form having fewer lateral teeth, as was shown by Lebour (1931) (see also M'Intosh, 1898).

C. limacina swims gracefully, either rising vertically or moving forward with the body horizontal. The locomotor organs are a pair of wings set transversely at the anterior fourth of the under side. They are narrowly attached at the

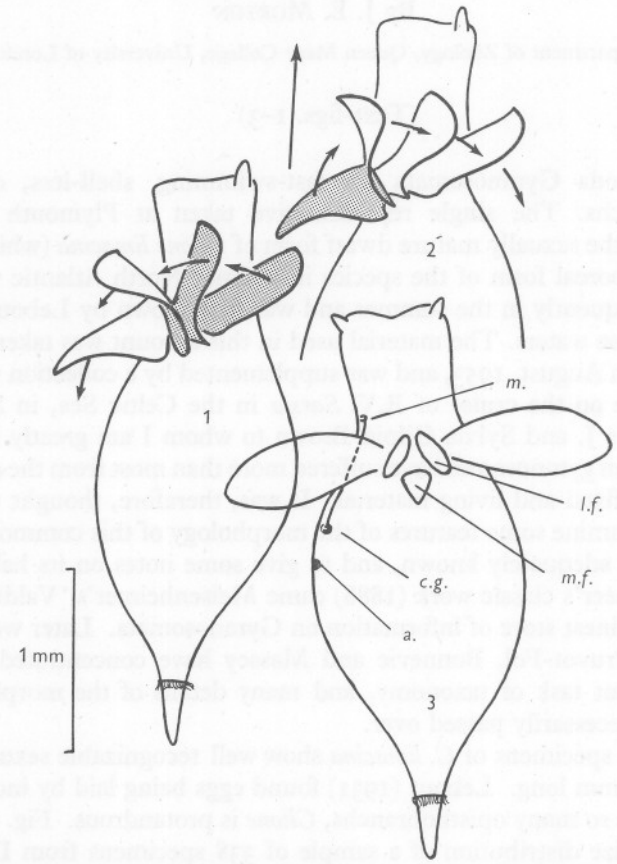

Fig. 1. Outline sketches showing the successive positions of the wing in downward (1) and upward (2) strokes (left-side view), and (3) ventral view. *a*, anus; *c.g.*, common genital aperture; *l.f.*, lateral lobe of the vesigial foot; *m.*, male aperture; *m.f.*, median lobe of the foot.

sides of the mid-line and expand to form broadly rounded flaps. As in all pteropods they are modified parapodia, or outgrowths of the side of the foot. The median part of the foot is vestigial, remaining near the centre in the form of one central and two lateral lobes (Fig. 1, *m.f.*, *l.f.*). Observations on swimming were made from Plymouth material, and I am particularly indebted to Dr J. Gilpin Brown, who observed living Irish specimens, for helping me with the diagrams in Fig. 1. The wings move synchronously dorsally and ventrally,

that is towards and away from the sides of the body. While the smaller thecosomatous pteropod *Limacina retroversa* (see Morton, 1954) uses its long wings as oars for rowing, *Clione* employs its shorter wings for sculling. At its narrow attachment to the body the wing makes a small twist after each stroke so that the leading anterior edge is directed relatively more strongly downwards in a down-stroke and upwards on the return. The effect of this is that the ventral surface of the wing faces posteriorly after a down-stroke and the dorsal surface is inclined backwards after recovery. Each stroke is thus given a backward component; while moving ventrally the wing has an upward as well as a forward thrust, compensated by a downward thrust as well as a forward thrust on moving upwards. The beating of the wings is much more rapid than in the thecosomatous pteropod *Limacina retroversa*, and movements are generally much faster, and the power of quick manoeuvre greater. With the ability to utilize both upward and downward strokes in a sculling action may be related the shorter, rounder wings, and the smaller wing surface found in gymnosomatous pteropods.

The body is perfectly streamlined and *Clione* has no external gills or excrescences of the skin. Respiration appears to take place through the general body surface, and the whole body cavity forms a wide, blood-filled haemocoele. The circulatory system is extremely simple. The heart, as first described by Meisenheimer (1905), is enclosed in a thin pericardial cavity lying at the posterior part of the visceral mass on the right side. The renal organ, which opens close to the anus, is a thin transparent sac; its walls are neither folded nor glandular.

Like most pteropods, *C. limacina* has the eyes reduced; I could find none at all in serial sections of Plymouth specimens. Gymnosomes, however, find no difficulty in catching zooplankton, and have been generally observed and assumed to feed on thecosomatous pteropods. *C. limacina*—as with both Plymouth and Irish specimens collected here—generally accompanies larger numbers of *Limacina retroversa*. The gymnosome buccal mass bears a fine array of hooks, spines, exsertile radular teeth, and adhesive tentacles (cephaloconi) or tentacles bearing suckers (acetabula). These pteropods thus as a group combine some of the efficiency of both cephalopods and chaetognaths. The Clionidae have no acetabula nor jaw, but possess cephaloconi, arranged in a circlet just within the mouth—in *Clione limacina* three at either side. There is also a pair of long hook sacs, one opening at either side of the pharynx, containing an invaginated cluster of chitinous 'hooks', in this species really slightly curved blades, about fifteen in number. Fig. 2B is from a specimen with its buccal armoury everted. So far as is known, the act of food capture has never been described. Observations on this and other habits of the fine gymnosomes of colder seas would be very rewarding.

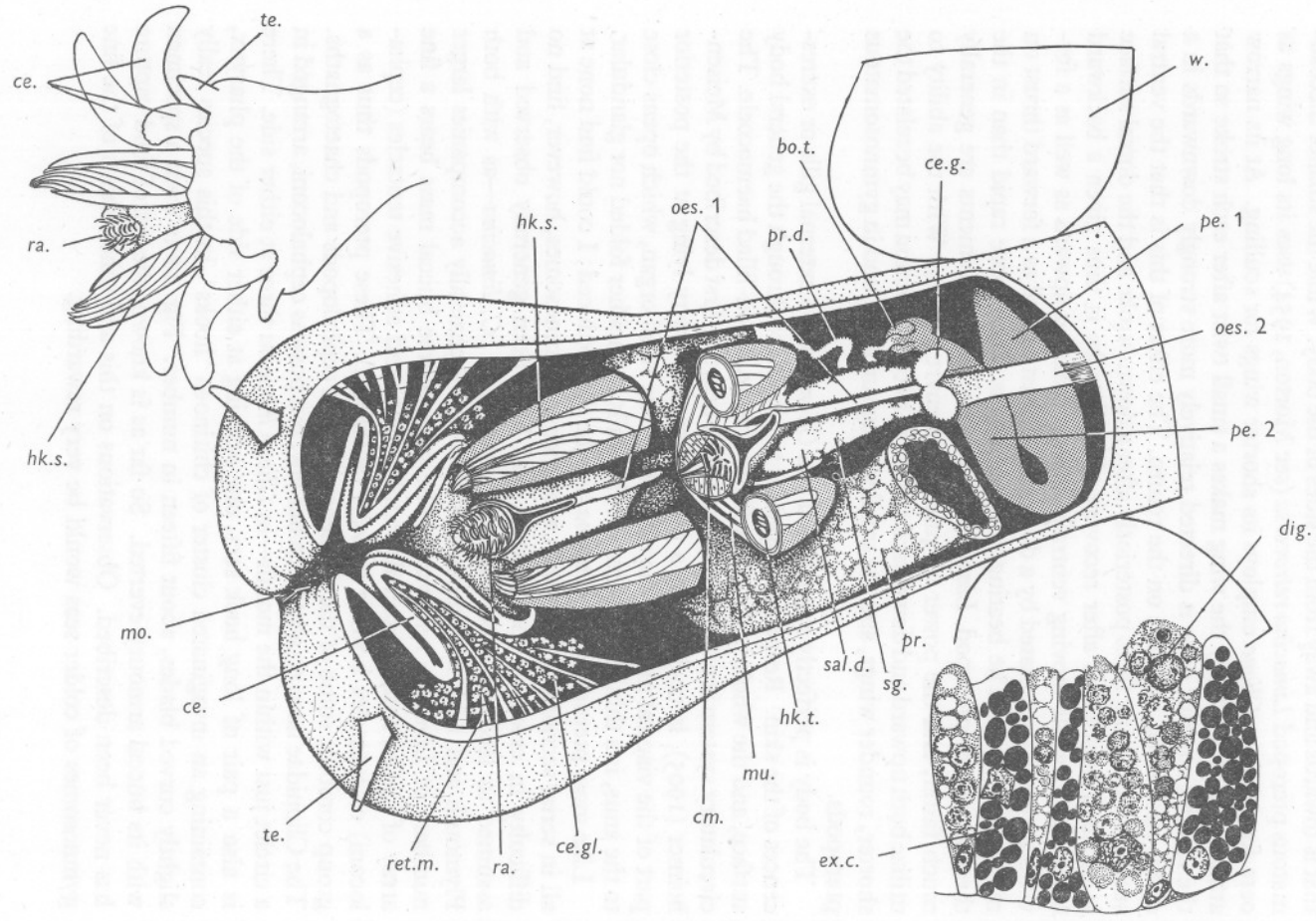

Fig. 2. For legend see opposite page.

ALIMENTARY CANAL

Fig. 2A explains the structure of the buccal mass. The mouth is a distensible vertical slit, and the prehensile organs—cephaloconi (*ce.*), hook sacs (*hk.s.*) and radula (*ra.*)—lie within it, and are everted, in that order. The three cephaloconi on either side are withdrawn into a recess at the side of the buccal cavity when out of use. The epithelium of the cones carries rod-like sensory cells, and their cavities and the adjoining cephalic haemocoele are filled with clusters of unicellular glands, having long ducts and staining blue in azan. Their secretion is assumed to be adhesive (see also Pelseneer, 1885). Slips of retractor muscle extend into the cones from the body wall, while they are evidently extruded by the pressure of blood. The hook sacs are ovoid to cylindrical tubes, longer than the pharynx in *Clione* and opening into it at the sides of the odontophore. Hooks of gradually increasing size are inserted on the mesial wall of the sac, each with a secretory cell underlying it, so arranged that all, including the shortest, protrude into the pharynx. The wall of the sac has a complex musculature, circular outside and longitudinal round the lumen. The hooks can evidently be everted and spread out rather more freely than can be appreciated from Fig. 2B, where the hook sacs themselves are probably extruded rather beyond the natural distance from the mouth. The radular sac runs back between and slightly below the hook sacs, lying in a groove above the two-rod-like cartilages which form the body of the odontophore. The teeth are well figured both by Pruvot-Fol (1926) and Tesch (1950). Slight differences in the serrations of the median tooth in the figures of various authors are to be attributed to different amounts of wear in the teeth at the front of the radula. The main prehensile teeth are those of the lateral rows, which can be strongly erected as the radula protrudes. The salivary glands (*sg.*) are long straps running forward to open into the pharynx at the sides of the radular sac. These pass with the oesophagus through the nerve ring. Their cells are filled distally with granules staining black in haematoxylin and red in azan. Subepithelial

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Legend to Fig. 2

Fig. 2. A. Stereogram of the head of *Clione limacina*, opened to show the buccal organs and the male genitalia lying within it The buccal cavity has been opened, and the oesophagus, radular sac and hook sacs cut across in transverse section. The distal part of the prostate gland has been removed, to display the penis sheath lying ventrally to it. Except for the cerebral ganglia, details of the nervous system are left out. × *ca.* 90. B. The buccal organs protruded from the mouth. Drawn from a preserved specimen. C. Histology of the digestive gland. *bo.t.*, 'bouton terminale', where prostatic duct opens into penis sheath; *ce.*, cephalocone; *ce.g.*, cerebral ganglion; *ce.gl.*, glands at base of cephalocone; *cm.*, radular caecum seen in section; *dig.*, digestive cell; *ex.c.*, excretory cell; *hk.s.*, hook sac; *hk.t.*, hook sac in section; *mo.*, mouth; *mu.*, transverse muscles between hook sacs; *oes.* 1, anterior part of oesophagus; *oes.* 2, posterior part of oesophagus; *pe.* 1, part of the penis sheath containing the penis; *pe.* 2, prostatic part of the penis sheath; *pr.*, prostate; *pr.d.*, duct of the prostate; *ra.*, radula; *ret.m.*, slips of retractor muscles of the cephalocones; *sal.d.*, salivary duct; *sg.*, salivary gland; *te.*, head tentacle; *w.*, base of swimming wing (cut short).

mucus glands open through the sides and floor of the buccal cavity in front of the odontophore.

The rest of the alimentary canal is extremely simple. The oesophagus is narrow, but with deep extensible folds, and leads back to a large stomach-like sac. This bag is formed by two massive brown or blackish coloured digestive diverticula; the posterior one is larger, and extends farther back, especially on the right upper side, and the smaller diverticulum opens from its deeper aspect as a pocket. The true stomach—as in most carnivorous opisthobranchs—is reduced, but to a greater extent in *Clione* than described anywhere else. It forms a mere funnel of ciliated epithelium on the right side of the digestive sac from which the short intestine runs forward to the body wall. The intestine is a narrow, strongly ciliated tube, and the relatively slight faecal waste is discharged without any mucus binding.

The digestive epithelium with which the 'stomach' is lined has two types of cell (Fig. 2C). The first is long and columnar, with the typical histology of molluscan absorptive-digestive cells. The cytoplasm contains vacuoles filled with particles of varying sizes staining purplish blue in azan, and especially concentrated near the broader free ends of the cells, where there is much diffuse material taken up from the lumen. The intact cell has a narrow striated border. Small spherules from it are constricted off at times into the lumen. The other type of cell, less than half as numerous, is filled with rounded spherules, jet black in haematoxylin, bright red after azan. On general grounds these cells are probably enzyme secreting; from the nature and bulk of the meal, there is obviously much extracellular digestion, and the gut has no other likely source of secretion. The only alternative function to be considered is excretion, since the kidney, a smooth oval sac lying on the right side, has lost all its glandular character. Amoebocytes, however, are distributed subcutaneously, and it may be that these—as in many other molluscs—are able to eliminate waste directly through the body wall.

REPRODUCTIVE SYSTEM

The genital ducts of *Clione* have never been described in detail. Fig. 3A illustrates the form and arrangement of the hermaphrodite reproductive system, which is laid out on the plan of primitive opisthobranchs. The ovotestis is a conical sac lying behind the other viscera. Its central part is chiefly a testis, and in older animals (3 mm and above) the periphery is crowded with small acini containing developing oocytes. A narrow hermaphrodite duct, which can be distended as a vesicula seminalis for outgoing sperm, leads forward to the glandular ducts, which fall into two parts. The first is the small, flattened albumen gland (*alb.*) into which the hermaphrodite duct opens directly, and through which both ova and sperm must pass. It is lined with large squarish cells, staining pink at the base and with the secretion spherules blue after azan.

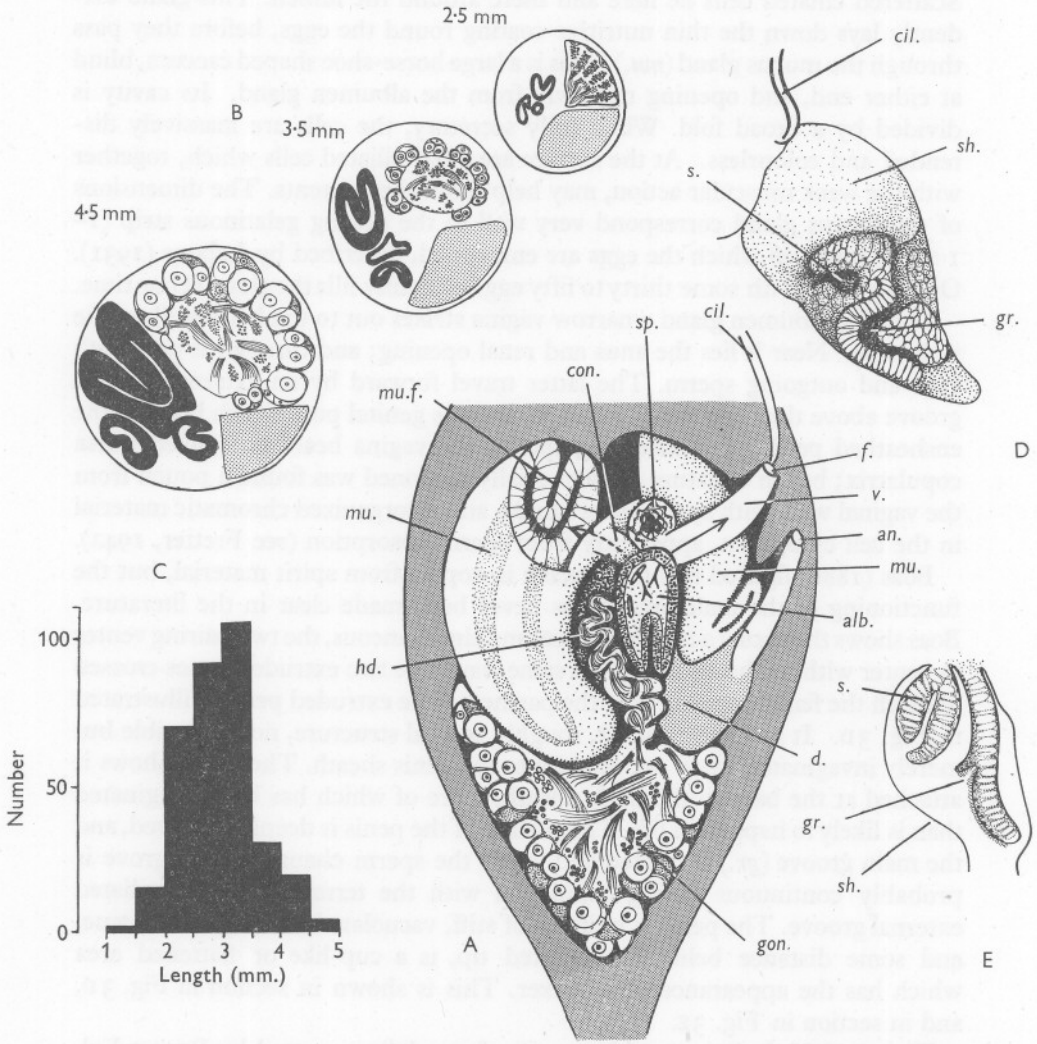

Fig. 3. A. Stereogram of the gonad and genital ducts, in relation to the outline of the body. The gonad and albumen gland are viewed as by transparency, and the mucus gland is cut across to show its internal fold. A more anterior portion of the gonad overlies the genital ducts and has been removed. × 10. B. Diagrammatic transverse sections showing the size and contents of the gonad in specimens of 2·5, 3·5 and 4·5 mm length. The genital duct is shown in black, the digestive gland stippled. C. Histogram showing size distribution of a September sample of 338 individuals from the Celtic Sea. The lengths were measured after preservation in formalin. D. Structure of the penis, everted together with part of its sheath. E. The penis within its sheath, in longitudinal section. *alb.*, albumen gland; *an.*, anus; *cil.*, ciliated seminal groove running to male aperture; *con.*, connecting duct between the albumen and mucus glands; *d.*, portion of the digestive gland, after removing the rest of the alimentary canal; *f.*, common genital aperture; *gon.*, gonad; *gr.*, seminal groove running along the penis; *hd.*, hermaphrodite duct, filled with sperm; *mu.*, mucus gland; *mu.f.*, internal fold of the mucus gland; *s.*, sucker-like appendage of the penis; *sh.*, penis sheath; *sp.*, pouch attached to the vagina, containing sperm; *v.*, vagina.

Scattered ciliated cells lie here and there around the lumen. This gland evidently lays down the thin nutritive coating round the eggs, before they pass through the mucus gland (*mu.*). This is a large horse-shoe shaped caecum, blind at either end, and opening narrowly from the albumen gland. Its cavity is divided by a broad fold. When fully secretory, the cells are massively distended and colourless. At the surface are a few ciliated cells which, together with the weak muscular action, may help move the contents. The dimensions of the mucus gland correspond very well to the oblong gelatinous strip (1–1·2 mm long), in which the eggs are embedded, described by Lebour (1931). One egg strip with some thirty to fifty eggs evidently fills the gland at one time.

Fom the albumen gland a narrow vagina strikes out to the body wall on the right side. Near it lies the anus and renal opening; and through it pass both eggs and outgoing sperm. The latter travel forward by an external ciliated groove above the right wing, to enter the male genital pore which leads to the ensheathed penis. For incoming sperm, the vagina bears no stalked bursa copulatrix; but in a mature female serially sectioned was found a pouch from the vaginal wall, with sperm in its lumen, and disorganized chromatic material in the cell cytoplasm, apparently from sperm absorption (see Fretter, 1942).

Boas (1886) figured two *C. limacina* in copula from spirit material, but the functioning of the male organs has never been made clear in the literature. Boas shows the sexual act as reciprocal and simultaneous, the two pairing venter to venter with the heads facing the same way. The two extruded penes crossed to reach the female apertures of the partners. The extruded penis is illustrated in Fig. 3D. It is a fairly robust, roughly conical structure, not inversible but merely invaginated when out of use into the penis sheath. The figure shows it attached at the base to the penis sheath, more of which has been evaginated than is likely to happen in life. The surface of the penis is deeply furrowed, and the main groove (*gr.*) is ciliated and forms the sperm channel. This grove is probably continuous during copulation with the terminus of the ciliated external groove. The penis is built up of stiff, vacuolated, cartilage-like tissue, and some distance behind its pointed tip, is a cup-like or flattened area which has the appearance of a sucker. This is shown in section in Fig. 3D, and in section in Fig. 3E.

Wagner (1885) gives an odd account of copulation, quoted by Pruvot Fol, in which he claims that an individual *B* takes semen from a partner *A* and hands it on to a third individual *C* (!). Fresh study of copulation in life is much to be desired, but even from careful anatomical study, it would seem that some of the things claimed in the literature are very unlikely. Eschricht (1838), followed by several others, figured the penis with a second branch, a long slender arm carrying a 'sucker' at its tip. This structure—which I have found everted in several preserved specimens—seems to be merely an artefact due to the over-evagination of the penis sac, carrying with it part of the prostatic duct. This duct opens into the penis sheath by a button-like sphincter called

by Kwietniewski (1903) the 'bouton terminale', which has been regarded as the 'sucker' when evaginated. It is unlikely that such evagination ever happens in life, and the whole 'arm' is thin and flaccid, certainly useless as a prehensive organ. If indeed the copulating animals must secure a firm hold on the smooth body of the partner, the shallow acetabulum on the body of the penis would be better adapted for this role.

The prostate evidently contributes its secretion to the semen by pouring it into the penis sheath, close to the base of the male organ. It opens there by a narrow, slightly coiled duct, discharging through the sphincter (*bo.t.*) called the 'bouton terminale'. The prostate itself is a massive strap-shaped appendage, extending forward from its duct, then transversely beneath the buccal mass, and finally backward on the left to fill all the space dorsal to the penis sheath. The gland was termed the 'foot-gland' by Pruvot-Fol (1926) but was correctly recognized by Kwietniewski. Its histology resembles very closely that of *Otina otis*, described by Morton (1955), the cells being very large and filled distally with large blue-staining (azan) aggregates of secretion.

AFFINITIES OF GYMNOSOMATA

Pelseneer (1888) first clearly distinguished the thecosomatous and gymnosomatous pteropods as separate stocks. From various evidence he held that the Thecosomata were specialized bullomorphs, a view that has commanded wide support ever since. The Limacinidae, with multi-spired sinistral shell and operculum, may well have arisen as pelagic neotenic bulloids (see Lemche's account (1948) of tectibranch larval shells). Pelseneer then demonstrated that swimming wings might have arisen by the enlargement of the parapodia seen in *Aplysia*—itself an inexpert swimmer—or in *Akera*, and suggested an aplysioid origin for the Gymnosomata. In his book of 1906 he abandons the Pteropoda as a formal grouping and places his six families of Gymnosomata in the Aplysiomorpha.

Later workers, such as Thiele (1931), Odhner (1939), and Hoffmann (1939) have revived the order Pteropoda, to include both the shelled ciliary-feeding Thecosomata and the naked carnivorous Gymnosomata. Thus, by overstressing the one resemblance in the possession of wings, very great differences are minimized and a wholly unnatural group erected in the Opisthobranchia. But the Gymnosomata, different though they are from thecosomes, are not readily acceptable as aplysiomorphs. The Anaspidea of Thiele (or Aplysiomorpha) are a small group with a well marked aspect and a rather narrow radiation, which—thanks to the work of Eales and others—we can now well recognize. They are bottom-dwelling herbivores, swimming temporarily by parapodia, and with the restoration of the Akeratidae (Morton & Holme, 1955) consist of two families. Of the aplysioid characters of the gut, with a well marked crop, double gizzard and caecum, there is not a hint in gymnosomes. Pelseneer

compared the spinose jaw of *Aplysia* with the deep hook sacs of gymnosomes, but of the acetabula, cephalocones and gymnosome radula the aplysioids offer no suggestion. The nervous systems and genitalia (see Eales, 1921, and Pruvot Fol, 1926) are likewise only comparable at the most general level.

Pelseneer placed much reliance of the ability on aplysioids to swim; but we now know that, if any accomplishment of the opisthobranchs can be credited to parallel evolution, it is this one—a specialization met with in primitive members of every group having parapodia (*Arthessa* and *Oxynoë* in the Sacoglossa, *Gastropteron* in the Bullomorpha, as well as in pleurobranchoids). *Akera* provides a model of how gymnosome swimming may have arisen, but denotes no close connexion. The gymnosomes are in short a specialized and very distinct group. It may be better to give both Thecosomata and Gymnosomata the status of separate orders, the course most recently followed by Pruvot Fol (1954). If we are to keep them together it must be a marriage of convenience liable to be broken up as soon as more compatible allies can be found.

SUMMARY

Some features of the digestive and reproductive systems, as well as the habits and swimming of the pteropod *Clione limacina* have been studied from specimens of the dwarf 'southern' race, which occurs at Plymouth. The animal swims rapidly by the sculling action of its two short rounded 'wings' or parapodia. Its buccal mass is characterized by specialized prehensile organs, consisting—as well as the radula—of adhesive tentacles (cephaloconi) and a pair of hook sacs. The rest of the gut is simple, consisting chiefly of a 'stomach' formed by two spacious digestive diverticula, that have replaced the true stomach. The lining includes absorbing-digestive cells and excretory cells. *C. limacina* is a protandrous hermaphrodite. The size distribution of a sample of 338 is shown. The youngest specimens are all males; in older groups developing oocytes are found, and in the largest are eggs not yet ready for shedding (September), as well as sperms. The genital tract is of the primitive opisthobranch form, with a sperm-storing hermaphrodite duct, albumen gland and mucus gland. An external seminal groove leads forward to the penial sheath in the head. The structure of the penis and prostate is described, and some previous views on the nature and functioning of these parts are criticized. Finally, the relationships of the gymnosomatous and thecosomatous pteropods are briefly reviewed, and the use of separate orders is recommended in place of the recently revived single group Pteropoda.

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NOTES FROM THE PLYMOUTH AQUARIUM. III.

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(Plates I and II)

CONTENTS

The present notes (see also Wilson, 1949, 1953) are mainly concerned with the breeding habits of two fishes commonly kept in marine aquaria. These habits may be familiar to keepers of other large marine aquaria, but I have failed to find any account of them in scientific literature other than a very brief and inadequate description by Raffaele (1898, p. 328) of the nesting of *Cantharus vulgaris* (= *Spondyliosoma cantharus*) in the aquarium at Naples. The scientific names of species mentioned follow the *Plymouth Marine Fauna*, third edition (Marine Biological Association, 1957).

THE BREEDING OF *SPONDYLIOSOMA CANTHARUS* (GMELIN)

The Black Sea-bream or Old Wife (*Spondyliosoma cantharus*) has never been common in the Plymouth sea area, but since 1951 occasional specimens have been obtained throughout the year. Earlier records are not numerous, and certainly for 25 years prior to 1951 no specimens were kept in the aquarium. Since that year a varying number, at present seven, have lived in the largest tank ($31 \times 9 \times 4\frac{1}{2}$ ft. deep) in company with more numerous *Pagellus centrodontus* with which they shoal, and with a variety of other fishes and with *Palinurus vulgaris* and *Homarus vulgaris*. Some of them have been in the tank for several years and since 1953 they have bred regularly. The present account is based on observations made over several years.

From early in the year onwards the males, from time to time, make nests until breeding is over, and occasionally afterwards. When nest-making begins the water is often at about its coldest (8–10° C); spawning takes place when the water is about 12–14° C. May is the usual month for spawning, but in 1957 the first spawning took place on 17 April, to be followed by others late in May. The winter had been mild and the water temperature of about 13° C was two to four degrees higher than usual for mid-April. In 1956 spawning was delayed to mid-June, although temperature during the earlier months of

that year had not been abnormal and spawning temperatures had been reached in May.

The nest is simply an area of the slate floor of the tank cleared of its usual covering of small pebbles. The male makes the clearing by swimming close above the bottom and exerting vigorous side-to-side movements of the tail, thus creating a backward current that sweeps away the pebbles. He remains upright, head more or less towards the centre of the patch as he clears it and travels thus around the edge of the clearing, not necessarily continuously but usually with pauses. In the early part of the year these nests, which are roughly circular, may be only a foot or so across, but as the breeding season approaches the bared patch reaches a larger size and may have a diameter of 3 or 4 ft. (roughly 1 m). Two or more males may each make a nest at the same time, a few feet apart. They will threaten and chase one another, sometimes facing mouth to mouth, nearly touching, fins erect and bodies flushed almost black with vertical white stripes. Little actual fighting between males has been seen.

Out of the breeding season males and females are practically indistinguishable. In the well-lit tank they are silvery, very pale violet on their upper sides and back, with broken horizontal stripes. It is only in the excitements of nest-making, threatening other males, mating and guarding the eggs that the males justify the common name of 'black'. In almost an instant they can when excited change from the normal pale coloration to an intensely dark violet, almost black, with a very prominent vertical white stripe on each side, just in advance of the anal fin (see Pl. I, fig. 1). There are often similar but paler vertical stripes before and behind the main one; another occurs about the level of the pectoral, and yet another at the base of the caudal fin. There are horizontal dashes of white along the lateral line and small whitish blotches more or less all over, but especially on head and shoulders. Broad horizontal banding may occur on the snout and gill-cover, below eye level. There is much variation between individuals, some never become as dark as others, and the same individual may vary from time to time. All individuals exhibiting this dark coloration and this colour pattern are judged by their actions to be male.

As the combined shoal of *Spondyliosoma* and *Pagellus* swim up and down the long tank, males with nests swim after and dart at others of their own species, assumed to be female, as they pass by. During the early months of the year there is no response from them and the males do not stay long on the nests they have made, soon leaving to shoal up again with the other fish. But every now and then the urge to nest-making drives them to repeat the performance.

When a female is ready to spawn she will accompany a soliciting male down to his nest. Spawning has always taken place very late in the evening or very early in the morning, possibly even at night though there is no evidence for

this. The actual spawning has rarely been seen; it has been watched once or twice at about 10 p.m., though not by a scientific observer and details are vague. I have seen, during the daytime, a female accompany a male, which had approached her, to the nest and stay there for some minutes testing the bared slate with her mouth and body. Meanwhile the male swam actively about her, not attacking but now and then nuzzling her ventral fin and her anal region. He was intensely black with prominent white stripes, and he repeatedly erected his dorsal fin. The female bore normal pale coloration. Unfortunately, there was no spawning and as the female swam away she was immediately followed by the male, fussing about her, though as far as could be seen she was not bitten by him.

On another occasion, the following year, a female which had accompanied a male to his nest darkened in colour, though not to the same extent as the male. On the side of her body, behind and above the insertion of the pectoral fin, there appeared a pale horizontal rectangular patch and there were a few irregular thin vertical stripes near the root of the tail. The horizontal pale patch on the female contrasted sharply with the vertical stripes in the same region of the almost black male. The latter became wildly excited and with all fins erect made rapid protrusions of the mouth. Unfortunately, the female stayed on the nest for only a minute or so and no eggs or milt were shed. The performance was repeated several times, for each time the female left she was chased by the male and induced to return, until finally she would go back no more.

The eggs are stuck in a single layer to the slate, although here and there a second layer covers the first. They form an irregular whitish patch a foot or so in diameter in the middle of the cleared area of slate, which is 3 ft. or so across. Each egg is about 1 mm in diameter, flattened underneath and flattened at the sides where it sticks to neighbouring eggs. The egg capsule is relatively tough. The developing embryo shows a single yellow oil globule about 0·25 mm in diameter. Hatching takes place in about 9 days at about 13° C. The larval fish retains the oil globule in its yolk sac and it swims right way up. The eye is darkly pigmented before hatching.

The eggs are guarded by the male until they have hatched. Whilst on guard he endeavours, especially during the first 2 or 3 days, to induce other females to add to them. In May 1954 a second spawning definitely took place on one nest within 24 hr. of the first, and presumably by a second female. There are less definite indications of double spawnings having taken place in other years. In May 1957 two males each had a nest with eggs at the same time, one lot of eggs being a day older than the other.

The first duty of the guarding male appears to be to keep the eggs clean and to prevent them from being silted over. At frequent intervals he swims slowly just above the eggs, wriggling his body and fanning vigorously with tail and fins. This action disperses silt, and also pebbles which have been scattered among them by such accidents as a ray, or flat-fish, settling down

close beside the nest and with flapping movements attempting to bury itself in the gravel. The male also goes around the borders of the nest, head towards the centre, and widens it by swishing with his tail, just as he does when making it originally. He also picks up pebbles in his mouth and drops them well to the side of the nest. This action is not pursued very effectively for the pebbles have never all been cleared away even when there were only a few on the eggs, and whilst swimming around with one in the mouth the fish occasionally let it fall back again on the eggs, especially when his attention was distracted by the approach of other fish.

The second duty of the male is to guard the eggs (Pl. I, fig. 1). In a tank with so many other fishes and invertebrates this is always an arduous task. Especially troublesome are the various species of *Raia*, the flat-fishes (*Pleuronectes platessa* and *Microstomus kitt*) and the rock-lobsters (*Palinurus vulgaris*). Large wrasses (*Labrus bergylta*), the shoals of common sea-bream (*Pagellus centrodontus*) and of mackerel (*Scomber scombrus*) give little trouble and neither do the pollack (*Gadus pollachius*), although if any kind of fish swims too low over the nest it is likely to be bitten. The most troublesome fish actually observed was a pout (*G. luscus*), of which more later. The reactions of a guarding male towards others of his own species varies with their sex. He attacks any male coming near and may chase after him for some distance; as already described he solicits any female and while following her deserts the nest for a short time.

With the exception of a small sting-ray (*Dasyatis pastinaca*) which on one occasion appeared to be feasting on the eggs (observation by Mr G. R. Forster) and was removed to another tank, none of the other fishes in the tank, not even the pout, were ever seen to attempt to eat the eggs. The offence of the rays and flat-fishes was to lie on the eggs, or on any part of the cleared area. They appeared to settle down on the nest just as they would on any part of the tank floor and seemed indifferent to the eggs. It was while the owner of the nest was away for a few minutes, chasing something else, that this was liable to happen. On his return the intruder was immediately attacked, often swooped down on from above and savagely bitten, on body, fins or even the eyes; this rarely failed to bring about a hurried departure. Once a small ray was seized beside the snout and towed away, not being released until well clear of the nest. Any fish approaching was rushed at and if it did not turn away, bitten. After a few days most offenders learned to avoid the nest and its immediate neighbourhood.

Encounters with rock-lobsters were always amusing to a human onlooker. When a *Palinurus* wandered over the nest the *Spondyliosoma* became very excited. There were several methods of dealing with it. One was to seize an antenna and pull the crustacean off the nest, small ones being lifted well clear of the ground and towed for several feet, until with a vigorous flap of its tail the *Palinurus* pulled itself free. Sometimes the *Spondyliosoma* approached

from behind and bit the upper surface of the abdomen, or the tail-fan, whereupon the rock-lobster hurried forward out of the nest. Sometimes the *Palinurus* was pushed from behind, a method adopted more particularly with large individuals with abdomens bent forwards under them, as with females in berry, although rock-lobsters with extended abdomens were pushed almost as readily. The fish applied its mouth to the abdomen of the crustacean and pushed as hard as it could, making very vigorous swimming movements. As it came up behind the *Palinurus* it would take obvious care to avoid the long backwardly directed antennae, coming into the attack between them; it appeared to the observer that it disliked being tickled by them. As far as could be seen only the lips of the fish made contact with the integument of the rock-lobster. The pushing of a large *Palinurus* off the nest entailed much effort.

Of the many fish attacked by the guarding *Spondyliosoma* only one was ever seen to retaliate. This was a pout (*Gadus luscus*) of approximately equal size and unknown sex. It was the only pout in the tank, where it had lived amicably with all the other fish for many months. This pout persisted in haunting the neighbourhood of the nest though not, apparently, with any intention of eating the eggs which did not seem to interest it. It was repeatedly attacked by the black sea-bream and bore on its body marks of the many bites it suffered. The pout often turned on its assailant; more often it would initiate its own attack and savagely bite the black sea-bream. On several occasions it was seen to rush at the bream while the latter was attacking a plaice or a ray lying on the nest; the bream then broke off its attack and fled before the pout. On one occasion the pout repeatedly prevented the bream from attacking a ray lying on the nest (Pl. I, fig. 2) and it was some minutes before the bream got a chance to bite the ray. On another occasion the two antagonists met unexpectedly face to face; for about a second they looked at one another, almost touching mouth to mouth, then they rushed at each other each trying to bite the other in the side. The fight, which was over in a few seconds, ended with the pout chasing the bream right down the tank.

This curious behaviour of the pout was observed both in 1953 and in 1954; by 1955 it was dead. As soon as nesting was over, and the male *Spondyliosoma* had shoaled up again with the other fish, attacks by both parties ceased and they again lived peaceably with one another. It is not known if the male black sea-bream which figured in this drama in 1953 was the same individual as that which played the part in 1954. In the latter year the nesting male was generally much more darkly pigmented than the male which nested in 1953; the two males therefore may not have been the same fish.

The events described took place during daylight hours and it is not known for certain what happened at night. On two occasions after dark the caretaker, Mr W. H. Gladwell, reported that when he had switched on the lights he saw that the male had reverted to non-breeding pale coloration and was swimming with the shoal up and down the tank. Several plaice were lying

over the eggs unmolested. Just before darkness had fallen the male had been very darkly pigmented and on guard.

In general, the pigmentation of a nesting male is darkest during the first few days. Later there is a lightening, but there are temporary intensifications of the darkly coloured areas during moments of special excitement, as when attacking intruders. When the male has rejoined the shoal, after the eggs have hatched, he can only be distinguished from others of his species by his frayed fins, evidence of the heavy work he has accomplished.

More fully illustrated accounts of these observations were published in *The Illustrated London News* for 28 August 1954, and in *Neptune* for April 1956.

THE SEXUAL DISPLAY OF *LABRUS OSSIFAGUS* L.

The Cuckoo Wrasse (*Labrus ossifagus*) is not uncommon on or near rocky grounds near Plymouth. Usually one or more specimens, male or female, are to be seen in the aquarium, some of them surviving for several years. Until recently breeding had never been observed, although males had fought one another.

In the spring of 1955 two males and three females shared the same tank. As was usual, the males were hostile but had tolerated one another for some time without undue disturbance. Early in May one of the males was found dead and was believed to have been killed by the other, which had become sexually active and was engaged in nest-building. The nest was merely a cleared area, a few inches across, of the slate bottom of the tank. The bottom was strewn with shell gravel and in order to clear his patch the male turned over on his side and flapped vigorously with his tail (Pl. II, fig. 1). This action should be contrasted with that of the male *Spondyliosoma* which remains upright during the same operation. In nature the fish may in this way clear patches of rock, or make saucer-shaped depressions in sand or gravel. In the tank two separate patches of slate were cleared and so the one male had two nests a foot or so apart. Nest-making took place on several evenings over a period of 1 to 2 weeks.

The nest, or nests, having been made, the male turned his attention to the females, darting swiftly at each in turn, sometimes not otherwise molesting them, but often biting and chivying them. His excitement was great and his colours unusually vivid, the white patch (see p. 305) on head and shoulders visible, though not of maximum prominence. The dorsal fin was fully erect during the attack. The colours of the females were also more pronounced than usual* and had been so for some days. Usually they did their best to avoid the attentions of the male and it was obvious that they were not in full breeding condition. Nevertheless, they were approaching it, for on several

* In the aquarium the colours of healthy but not breeding fish of both sexes are much paler than in living freshly caught specimens, or than in those recently dead.

occasions a female eventually followed the male to one of his nests. When this happened he became wildly excited and with all fins fully spread, showing the most vivid blue and orange colourings, he displayed himself to her, open mouthed (Pl. II, fig. 2). His head and shoulders were jerked from side to side, his body sometimes twisted into an S-curve. The most striking feature of the display was the blanching of a large patch of skin on top of the head and over the shoulders, the blanching extending a little up the base of the dorsal fin as far back as the sixth or seventh fin ray. From this large, almost completely white area the pigment appeared to have been drained away to leave only faint tracings of the irregular stripes normally present. It has already been mentioned that the whitish patch appeared, with varying degrees of prominence, during the attack on the females; it was also present, though not fully blanched, during nest-building (Pl. II, fig. 1). It completely transforms the normal appearance of the male and must to a ripe female be a visual excitation stimulating her to shed her eggs. Our females, not being ripe, responded only by staying for a few minutes beside the male on the nest, never more than one at a time, showing no excitement and with their colours only slightly heightened. Neither eggs nor sperm were shed, the females soon tiring and swimming away, promptly to be followed and attacked by the male. Occasionally this renewed attack would induce a female to return to the nest, but only for a few moments. Eventually the largest female had to be removed to another tank to save her from the persistent biting of the male. Two smaller females remained in the tank with him; they too were bitten but were not as severely treated as was the larger female. After nearly 3 weeks the latter was returned to the tank, (on 1 June) but almost at once was viciously attacked by the male and had to be rescued for the second time. This last attack took place in the morning, with no nest and with no appearance of the whitish head and shoulder patch, and therefore may not have been an attempt to mate.

Sexual displays always took place during the evening, after about 6 p.m. They occurred almost every evening during the first week of May, but were observed only every other evening in mid-May. They then became less frequent. The water temperature during May rose slowly from about 11° C at the beginning of the month to about 13° C at the end. The whitish head and shoulder patch was never visible until the evening; it appeared only when the male was sexually excited and then very quickly. During normal daytime activities the area of skin which it occupied could hardly be distinguished from that surrounding it.

On 9 June the male was suddenly attacked by numerous praniza larvae of *Gnathia* (both *G. maxillaris* and *G. oxyuraea* are known to occur in the tanks). The larvae attached themselves to fins and body and even inside the mouth. The fish became poorly and there was no more sexual activity. The praniza larvae also attacked the females, though to a lesser extent. The fish were

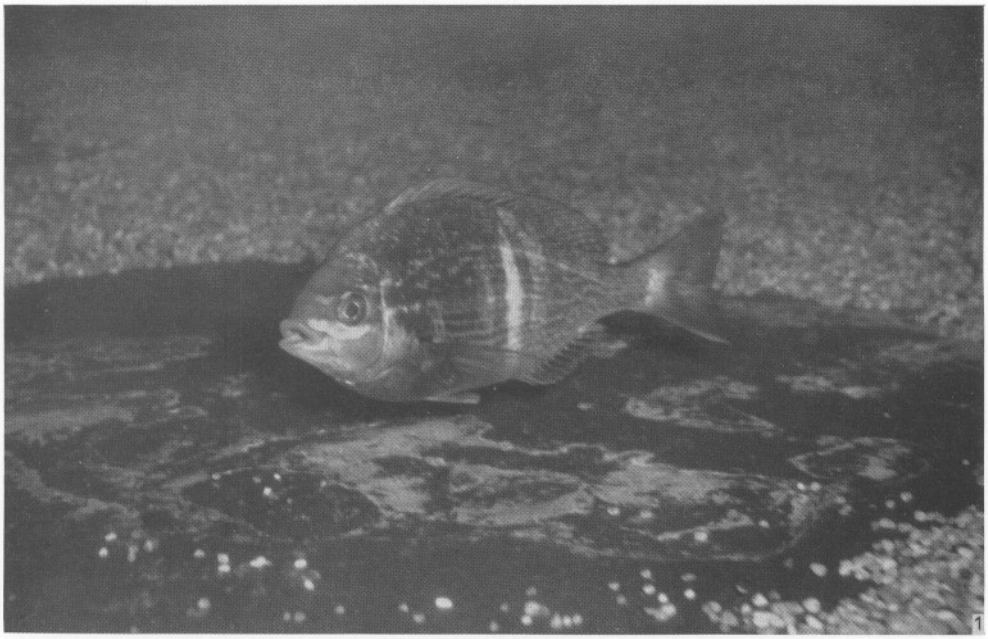
removed, freed from the pests, and their tank emptied and cleaned before they were put back in again. The male survived for about another year and the females for two. The trouble with praniza larvae reoccurred months later and in 1957 may have been largely responsible for the deaths of the females.

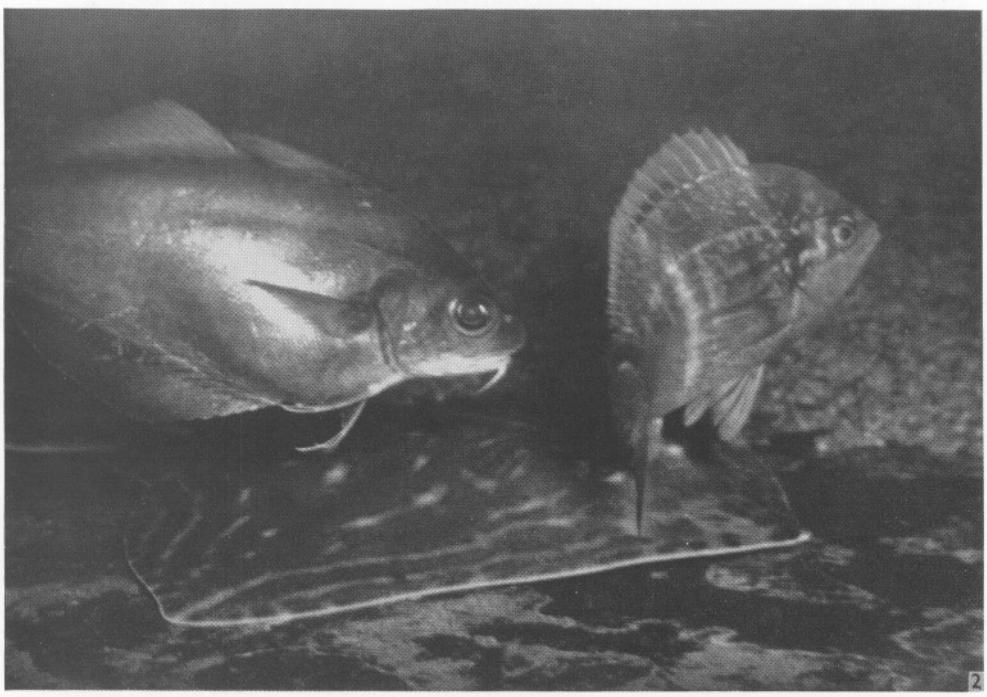
An account of these observations, illustrated by colour photographs, was published in *The Illustrated London News* for 26 May 1956.

LEARNING IN *ZEUS FABER* L.

The John Dory (*Zeus faber*) is predominantly a fish-eater, stalking its prey until sufficiently close to seize it with a sudden protrusion of the long extensile jaws. For very many years living small fishes, especially *Gobius minutus*, and occasionally living shrimps (*Crangon vulgaris*) when fish were scarce, were fed to the John Dories in the aquarium, and it appeared that they would not accept anything else. Dead fish were almost always ignored and, if perchance taken into the mouth, spat out again. Fish were always carefully scrutinized and a goby passively sinking was almost never seized until it made some movement, though some individual John Dories were less particular in this respect than were others. A few years ago one John Dory starved itself to death, for it would so rarely eat anything, though paying great attention to living gobies put into the tank and staring at one for minutes at a time, while its companions were greedily eating others. Pieces of fresh squid (*Loligo forbesi*), the staple food of the inhabitants of the aquarium, were never taken by the John Dories. Pieces of squid are pure white and are unlikely to be mistaken for fish. A dead goby looks unlike a living one, especially when sinking with silvery ventral side uppermost.

For many weeks after their arrival John Dories have always behaved in this way. Of recent years, however, they have been trained to eat squid as well as dead fish. The training takes a long time and says much for the patience of Mr W. H. Gladwell who is responsible for feeding the animals in the aquarium. After some weeks, when the newly arrived John Dories are feeding regularly on living fish, a few whole small squid or pieces of mantle cut into strips roughly fish-shaped, are dropped into the tank. As the strips sink they do so with an irregular motion, imparted by their shape, and to the John Dories may seem alive. They are scrutinized and eventually, sometimes after many such offerings spread over days or weeks, an odd strip or two will be seized. The diet of living fish can then be gradually stopped until finally only squid or dead fish is given. Mr Gladwell maintains that in giving dead fish the heads should first be cut off. At long last the John Dories feed regularly on squid and no longer need to be offered elongate pieces, square or any other shape will be accepted. At the time of writing (October 1957) six medium-sized John Dories, caught during the summer of 1956, are fed two or three times a week on portions of squid, which are taken eagerly.





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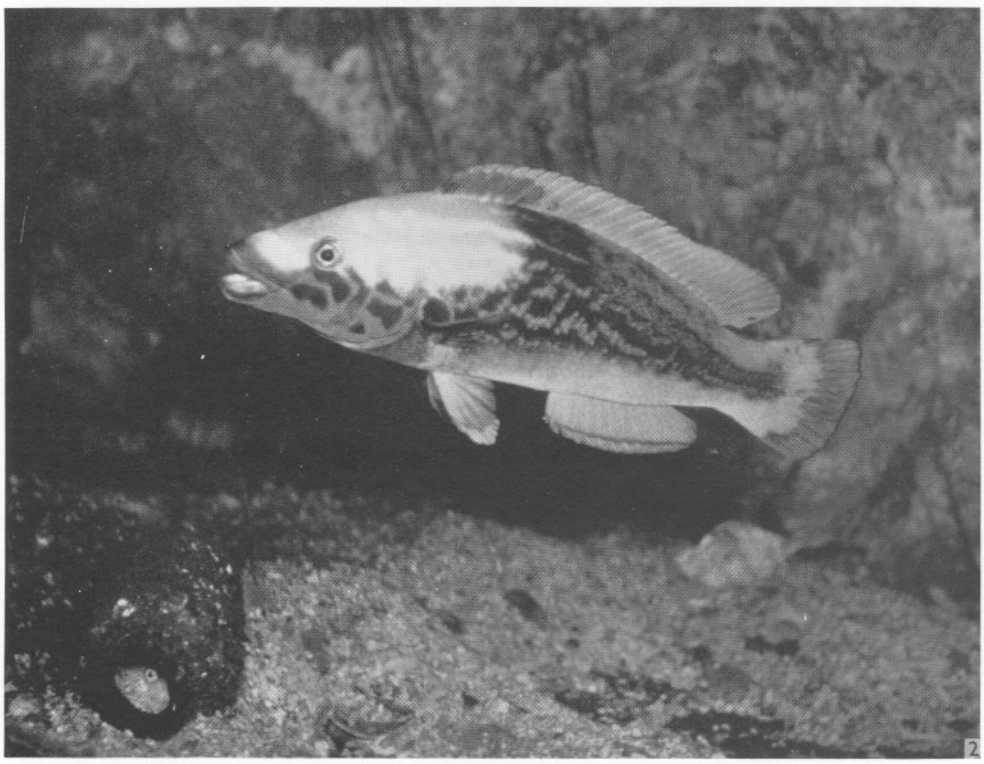
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EXPLANATION OF PLATES

PLATE I

Fig. 1. A male *Spondyliosoma cantharus* on guard over his nest (in May 1954) shows breeding coloration developed to about half full intensity. The nest is a cleared area of slate 3 or 4 ft. across; only a portion is shown in the photograph. Unfortunately, the slate bears the marks of old concrete (where rocks were formerly cemented to the tank floor) and the eggs, which when this picture was taken were hatching and losing their whiteness, are not readily distinguishable from this concrete.

Fig. 2. The male *Spondyliosoma* which was about to attack a *Raia microcellata* lying over the eggs, is being headed off by the *Gadus luscus* and it was sometime before the former shook off his pursuer and returned to his task of removing the ray.

PLATE II

Fig. 1. Male *Labrus ossifagus* making a nest by lying over on his left side and vigorously flapping his tail to wash away shell gravel, disturbed particles of which are seen behind him. The white area on the top of his head and shoulders is present but not fully blanched.

Fig. 2. Male *Labrus ossifagus* in full sexual display before a female; she is too far to one side to be included in the picture. All his fins are erect, his mouth is open, and the white area on top of his head and shoulders is fully developed. Near the lower right-hand corner about half of the nest is visible. The object in the left-hand corner is an old bottle of dark-coloured glass, with a *Blennius ocellaris* inside. This photograph, reproduced in colour from the original kodachrome, appears in *Marine Life of Coastal Waters* (*Western Europe*) by E. le Danois, translated and adapted by N. A. Holme (London: Harrap), p. 155.

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THE FECUNDITY OF CLYDE PLAICE

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(Text-fig. 1)

In early 1956 and again in 1957 small samples of mature female Plaice *Pleuronectes platessa* L. were collected for fecundity estimates to be compared with Simpson's (1951) results for the North Sea populations, and the data from elsewhere that Simpson summarizes.

The fish were collected on 22 and 27 February 1956 and 7 January 1957 by trawling off Mountstuart House, Isle of Bute, at *ca.* 40 m. The treatment, of the fish was similar to that of the Long Rough Dabs collected for fecundity estimation and which has been described in detail (Bagenal, 1957*a*). This paper on Long Rough Dabs should be consulted for details of the collection and treatment of the fish, and of the laboratory methods for the estimation of the egg numbers and also of the statistical analysis of the data.

I should like to thank the master and crew of M.F.V. *Calanus*, and Miss Sheila Morris who counted the eggs.

RESULTS

The data are shown in full in the Appendix, and summarized in Table 1. The relation of fecundity and length of the plaice is shown in the scatter-diagram (Fig. 1), in which the curves for Simpson's North Sea Southern Bight and Flamborough grounds, and the curve for Kändler & Pirwitz's (1957) Baltic plaice from the Bornholm area, are also shown. Kändler & Pirwitz unfortunately, do not give the raw data for each fish and the curve in Fig. 1 has been calculated from the mean values they give.

From Table 1 it can be seen that the fecundity adjusted to a common length was greater in 1957, but the 'condition' of the fish (as expressed as the expected weight of a 37 cm. plaice) was greater in 1956 (cf. Bagenal 1957*b*). However, neither the general level of fecundity nor the condition was significantly different. This is shown in Table 2 which summarizes the covariance analyses of the regressions of the logarithms of length and weight on log fecundity and also that of log length on log weight, for both sets of data. None of the regressions are significantly different and the data may be pooled. Comparisons with the results of other workers are shown in Table 3. It is clear that the Clyde plaice fecundity adjusted for length is considerably

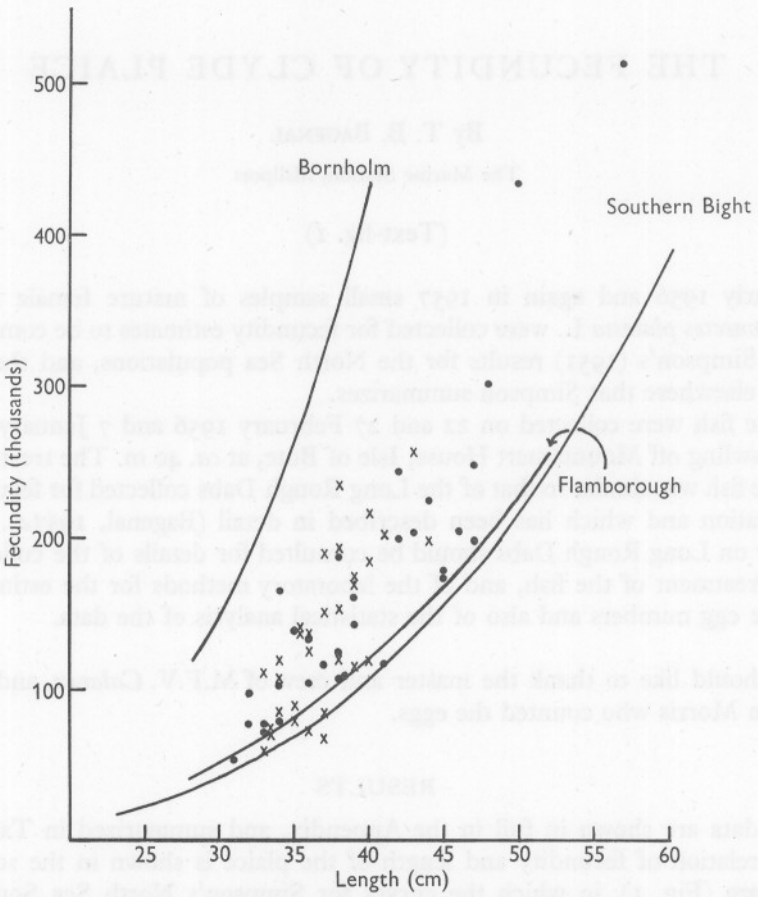

Fig. 1. Scatter diagram showing the relation of fecundity and length of plaice from the Clyde, and the calculated curves for Flamborough, Southern Bight and Bornholm fish (from Simpson, and Kändler & Pirwitz). ●, 1956; ×, 1957.

TABLE 1. SUMMARY OF CLYDE PLAICE FECUNDITY DATA GIVEN IN THE APPENDIX

Year	1956	1957
Number of fish	31	31
Mean length (cm)	39·76	36·91
Mean weight (g)	653·1	518·6
Mean age (years)	5·4	5·1
Mean fecundity	171,080	136,611
$\hat{W}$ for 37 cm	530·7	522·2
$\hat{F}$ for 37 cm	136,693	137,840

higher than that of the North Sea, and much lower than the Baltic plaice fecundity. These differences are statistically significant. Simpson (1957) has recently given a preliminary account of the fecundity of plaice from the Irish Sea; this appears to be similar to that of the Clyde fish given here.

TABLE 2. SUMMARY OF THE ANALYSIS OF COVARIANCE TESTING THE FECUNDITY-LENGTH, FECUNDITY-WEIGHT AND LENGTH-WEIGHT RELATIONS FOR CLYDE PLAICE IN 1956 AND 1957

Source	Fecundity on length	Regression of fecundity on weight	Length on weight
Due to total regression	**	**	**
Difference between means regression and average within subgroups regression	–	–	N.S.
Between adjusted subgroup means	–	–	N.S.
Between regression coefficients	N.S.	N.S.	–

**, indicates significance at 1% probability level.
N.S., indicates not significant at 5% probability level.
–, indicates mean square less than that against which it is tested.

TABLE 3. MEAN FECUNDITY ADJUSTED FOR FISH OF 37 CM, AND THE REGRESSION COEFFICIENTS OF THE REGRESSIONS OF LOG FECUNDITY ON LOG LENGTH

(From data given in this paper, by Simpson, and by Kändler & Pirwitz (1957).)

Author	Region	Date	$\hat{F}$ for 37 cm	Regression coefficient
This paper	Clyde	1956	136,693	3·11
	Clyde	1957	137,840	3·81
Simpson	North Sea Southern Bight	1947/48	82,996	3·13
	North Sea Southern Bight	1948/49	87,152	3·28
	Flamborough	1948/49	96,492	2·85
Kändler & Pirwitz	Kieler Bucht	1952/53	370,954	3·12
	Bornholm area	1952/53	322,771	3·58

Simpson (1951) showed that, among the 1947/48 and 1948/49 Southern Bight fish of the same length, there was only a negligible increase in fecundity with age, whereas for fish of the same age there was a considerable fecundity increase with length. He concludes: 'Thus age alone, apart from its relation to size, appears to have played an insignificant part in determining the fecundity of these fish.' This applied to fish of the same population. On the other hand, when comparing different populations (from the Southern Bight and Flamborough) he showed that the faster-growing Flamborough fish were more fecund. 'These observations lend weight to the view already expressed that fast growing, well fed fish have a higher mean fecundity than slow growing fish.' Age of course comes into this, since fast-growing fish are younger for a given length than slow-growing ones. In Table 4 the mean lengths and fecundities are given for each age group of the Southern Bight, Flamborough

and Clyde plaice. Here Simpson's remarks are confirmed and shown to apply to the Clyde fish also, which are faster growing and more fecund than even the Flamborough fish.

TABLE 4. THE MEAN LENGTH AND MEAN FECUNDITY (TO THE NEAREST THOUSAND) FOR DIFFERENT AGE GROUPS OF SOUTHERN BIGHT, FLAMBOROUGH AND CLYDE PLAICE

Region ...	Southern Bight			Flamborough			Clyde		
Age group	No.	Mean length (cm)	Mean fecundity (thousands)	No.	Mean length (cm)	Mean fecundity (thousands)	No.	Mean length (cm)	Mean fecundity (thousands)
II	4	24·4	23	1	31·1	47	—	—	—
III	19	28·4	32	3	32·1	66	3	34·4	84
IV	43	30·5	43	6	34·1	72	16	34·6	108
V	15	32·2	52	3	37·2	78	21	36·5	136
VI	11	35·5	61	2	37·0	87	6	39·9	176
VII	24	37·6	78	1	42·2	96	5	41·6	182
VIII	31	40·2	106	5	42·9	162	2	43·6	209
IX	23	42·9	127	4	45·3	189	1	49·5	434
X	13	43·7	123	3	50·6	194	1	40·0	118
XI	16	42·6	112	3	45·0	129	—	—	—
XII	7	42·6	127	1	51·8	284	—	—	—
XIII	6	46·4	163	—	—	—	—	—	—
XIV	3	47·0	174	1	45·0	144	—	—	—
XV	4	47·7	172	—	—	—	—	—	—
XVI	2	50·3	142	—	—	—	—	—	—

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APPENDIX

TABLE 5. THE LENGTH, WEIGHT, AGE AND EGG COUNTS OF FEMALE CLYDE PLAICE

Fish no.	Total length (cm)	Weight (g)	Age group	Egg count 1	2	3	4	Fecundity estimate
			22 and 27 February 1956					
1	33·8	443	IV	396	400	382	402	79,000
2	34·4	412	IV	844	801	839	809	164,650
3	40·5	596	Illegible	523	573	639	606	117,050
4	38·2	603	IV	933	948	1028	849	187,900
5	33·4	389	IV	414	327	421	374	76,800
6	42·0	796	Illegible	1039	877	1067	1021	200,200
7	35·8	498	IV	532	481	517	552	104,100
8	39·2	607	VI	814	684	692	672	143,100
9	42·5	845	V	1063	959	1060	1004	204,300
10	46·3	853	VII	1010	1053	1019	1011	204,650
11	48·3	1056	Illegible	1601	1479	1490	1479	302,450
12	49·5	1328	IX	2236	2017	2230	2204	434,350
13	56·6	1743	Illegible	2460	2388	2581	2826	512,750
14	32·1	344	IV	544	443	447	503	96,850
15	38·1	503	V	492	464	540	644	107,000
16	35·4	465	IV	791	609	695	689	139,200
17	31·7	321	IV	486	363	316	378	77,150
18	45·1	792	Illegible	828	921	917	918	179,200
19	37·9	529	IV	671	652	527	622	123,600
20	38·1	535	V	634	696	543	594	123,350
21	46·5	930	Illegible	988	1083	1056	858	199,250
22	39·3	575	VI	871	793	841	707	160,600
23	44·2	812	VII	1060	1105	1189	1150	225,200
24	44·9	772	VIII	975	847	819	843	174,200
25	37·1	491	IV	689	531	595	499	115,700
26	30·5	243	V	283	260	328	206	53,850
27	33·8	365	IV	527	508	486	511	101,600
28	46·7	870	Illegible	1249	1144	1137	1423	247,650
29	42·2	786	VIII	1192	1366	1179	1147	244,200
30	32·8	314	X	378	292	370	375	70,750
31	35·6	431	V	670	622	731	634	132,850
			7 January 1957					
1	38·9	545	V	866	841	894	858	172,950
2	34·8	445	V	633	739	682	678	136,600
3	39·9	743	V	1205	1041	1041	1039	216,300
4	34·7	418	V	368	404	378	427	78,850
5	36·5	416	V	512	401	386	379	83,900
6	36·5	518	V	705	744	733	837	150,950
7	37·0	490	III	300	354	277	402	66,650
8	36·2	470	VII	336	369	355	381	72,050
9	33·3	367	III	364	347	402	395	75,400
10	34·2	395	V	550	587	517	482	106,800
11	36·4	480	V	689	628	664	514	124,750
12	38·6	598	V	872	850	789	932	172,150
13	36·4	478	V	704	763	652	644	138,150
14	37·1	594	V	917	968	943	971	189,950
15	33·0	339	III	565	564	514	571	110,700
16	37·8	585	VI	1094	1184	1232	1189	234,950
17	32·7	337	IV	338	340	249	246	58,650
18	32·9	339	IV	360	404	320	333	70,850
19	35·0	447	IV	664	732	658	737	139,550
20	40·0	615	X	633	587	543	587	117,500
21	38·4	544	VI	526	547	618	684	118,750
22	38·7	567	IV	702	583	504	516	115,250
23	38·4	625	VII	681	825	793	755	152,700
24	39·8	684	V	984	974	939	809	185,300
25	34·5	470	V	414	430	429	512	89,250
26	33·8	404	IV	427	427	391	458	85,150
27	33·6	394	V	579	561	562	670	118,600
28	43·0	774	VII	1246	1214	1301	1386	257,350
29	37·7	557	V	871	1146	933	934	194,200
30	43·8	786	VI	1025	943	1090	900	197,900
31	40·7	652	VI	959	1097	1058	943	202,850

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TUBE FORMATION BY *POMATOCEROS TRIQUETER* (POLYCHAETA)

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(Text-figs. 1–18)

The serpulid polychaete *Pomatoceros triqueter* Linnaeus, 1758, with its white calcareous tube, is common on all rocky coasts around the British Isles. Normally 2–3 cm in length, and exceptionally 6 cm, the tubes are found attached to rocks, stones, and shells. Occasionally specimens obtained by dredging in offshore waters have tubes which, although still attached, grow away from the substratum.

This paper is the third in a series on serpulid tube formation. Earlier accounts (Hedley, 1956*a*, *b*) describe the calcium-secreting glands of four British serpulids, together with an account of the organic component of the tube of *P. triqueter*.

DESCRIPTION OF THE TUBE

After a free-living larval stage *P. triqueter* settles on the substratum and secretes a semi-transparent tube (Segrove, 1941). Initially the tube is shorter than the worm and only after a rapid increase in tube length is the animal completely enclosed. Tubes at this stage have thin walls which according to Dons (1927) are non-calcified and which are found by Segrove (1941) to consist of mucus and calcareous matter. Subsequent growth by the addition of calcareous material to the anterior end of this fragile tube results in the heavily calcified tube of the adult worm.

The adult tube is invariably curved in one or more directions and although straight tubes do occur they are extremely rare. On the upper surface there is a keel (Fig. 1), which varies slightly in size and position and which may project as a point beyond the anterior opening. Two other parts of the tube are referred to here as the lateral surface deposit and the anterior surface deposit (Fig. 3). The former is found on each side of the tube mass and the latter is a thin deposit on the substratum at the anterior end of the tube.

A series of ridges on the outer surface of the tube (Figs. 1, 2) give a false and superficial appearance of a series of growth rings. Previously the tube has been thought of as a series of calcareous rings (Potts, *teste* Robertson & Pantin, 1938), a view probably influenced by the surface ridge appearance, and also by

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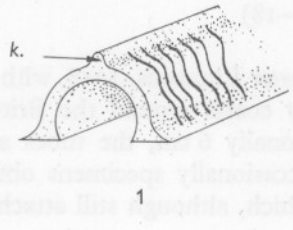
the fact that worms removed from their tubes produce hoop-shaped calcareous rings in the fold of the collar.

During early spring pure white additions to the anterior ends of the tube develop. These seldom appear in the winter, although if the worms are brought from the shore into the laboratory deposition quickly commences. As a result of such discontinuous deposition there are regions, at irregular intervals along the tube, which indicate cessation (and the beginning) of deposition phases. These are noticeable external features which occur perhaps two or three times



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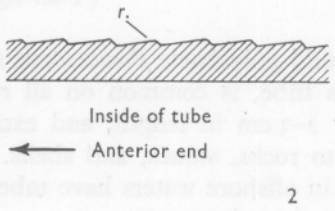

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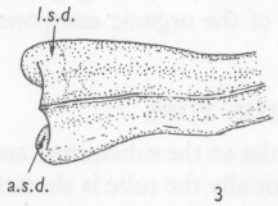

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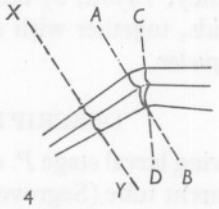

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Fig. 1. Diagram of the anterior end of a tube showing the position of the keel and of the ridges along the tube. *k.*, keel.

Fig. 2. Diagram of a section through the wall of the tube illustrating the ridges seen on the outer surface of the tube. *r.*, ridge.

Fig. 3. A top view of the tube. *l.s.d.*, lateral surface deposit; *a.s.d.*, anterior surface deposit.

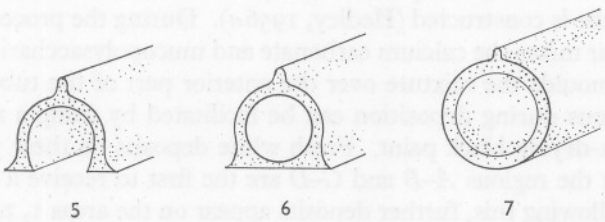
Fig. 4. Diagram to illustrate a process of tube growth which is discussed in the text.

along a 3 cm tube. The end of a deposition phase which begins at a level *X–Y* (Fig. 4) might be expected at a level *A–B*. This will occur only when both sides of the tube receive an equal amount of calcareous material. In many tubes one side develops to a greater extent than the other, for example, to a level *C–D*. At the onset of the next phase of deposition the tube will develop as shown only if an equal amount of calcareous material is deposited on both sides.

Tubes are usually incomplete in cross-section and appear as arches over the substratum (Fig. 5). Faouzi (1931) infers from the arrangement of the collar that the worm can never be completely surrounded by the tube. Apparently this is not the case as tubes are sometimes found which are complete in cross-section (Fig. 6). More convincing examples are provided by clusters of

P. triqueter, sometimes dredged in offshore waters, the tubes of which grow away from the substratum and are completely round in cross-section (Fig. 7).

Cavities in the thick lateral portions at the base of the tube (Fig. 9) have been observed and the resulting economy of tube material commented upon (McIntosh, 1923; Thomas, 1940). It has been suggested that other holes which penetrate the side of the tube may be used for the intake and outlet of water



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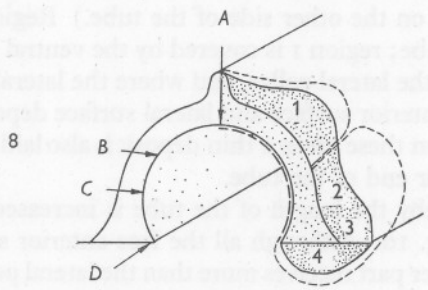


Fig. 5. Diagram of an attached portion of tube which is incomplete in cross-section.

Fig. 6. Diagram of an attached portion of tube which is complete in cross-section.

Fig. 7. Diagram of a portion of tube which has grown away from the substratum and which is completely round in cross-section.

Fig. 8. Diagram of the anterior end of the tube with the broken line indicating the region covered by the collar of the worm (drawn on one side of the tube only). Other areas which are labelled are explained in the text.

for respiratory purposes (Thomas, 1940). These holes, however, are not a regular feature of the tube, and when present, they are few in number and are confined to the early formed and thinner regions of the tube. They are in fact minor variations of the cavities just described, which occasionally are without an external covering and appear as holes in the side of the tube. Only on rare occasions is a hole of this kind continuous through the tube wall into the lumen.

THE MODE OF DEPOSITION

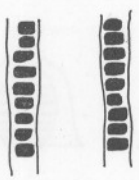
During deposition the worm's branchial crown and operculum project out of the anterior tube opening, and the ventral and lateral collars are folded back over the surface of the tube. In the fold formed by the collar two glands, one on each side of the peristomium, produce calcium carbonate granules, and these together with the secretion of mucopolysaccharide from the unicellular glands in the ventro-lateral peristomial epithelium constitute the material of which the tube is constructed (Hedley, 1956*a*). During the process of deposition the collar mixes the calcium carbonate and mucopolysaccharide, and concomitantly moulds the mixture over the anterior part of the tube.

Observations during deposition can be facilitated by using a tube painted with a quick-drying black paint. Fresh white deposits on these preparations indicate that the regions *A–B* and *C–D* are the first to receive a new deposit (Fig. 8). Following this, further deposits appear on the areas 1, 2, 3, 4, and on region *B–C*. (Fig. 8 is diagrammatic, and where reference is made to an area on one side of the tube it should be understood that a corresponding deposit is also being laid down on the other side of the tube.) Region *A–D* is the free anterior face of the tube; region 1 is covered by the ventral collar; regions 2, 3 and 4 are covered by the lateral collar, and where the lateral collar overlaps on the substratum the anterior surface and lateral surface deposits are found. In addition to deposits on these areas a thin deposit is also laid down on the inside surface of the anterior end of the tube.

The process whereby the length of the tube is increased can be described with reference to Fig. 10. Although all the free anterior surface is receiving new material the upper part receives more than the lateral parts, with the result that the upper part grows forward to a level *X–Y*. Meanwhile the lateral and anterior surface deposits become thicker, but do not advance beyond their limit *L–M* until the worm readjusts the position of the lateral collars. This readjustment is a definite move forward so that with subsequent deposition a very thin anterior surface deposit is found with a limit *O–P*. During this phase the upper part of the tube becomes thicker but not longer. This cycle is now repeated. The whole process involves a relatively rapid forward movement of the collar followed by a stationary phase during which the tube thickens and a small ridge appears on the tube surface. These are the ridges which have been described (Fig. 1).

The presence of cavities in the base of attached tubes (Fig. 9) is probably correlated with the precise arrangement of the lateral collars and the notopodial chaetae of the peristomium. The first sign of a cavity is in position *A* (Fig. 10), where, if direct observation were possible, one would expect to find the notopodial chaetae. These bristles normally move in and out of the chaetal sac and it is suggested that this constant movement prevents the formation of a deposit. With the thickening of the lateral and anterior surface deposits the depression

or hole *A* becomes deeper and surrounded by tube material. When the lateral collar moves forward the chaetae do likewise and are no longer associated with the hole just formed. The chaetae now prevent a deposit forming in position *B*, while the hole formed at *A* is covered over by a deposit being laid down on the inside of the tube. This explanation of the formation of the cavities remains speculative in the absence of direct observations.



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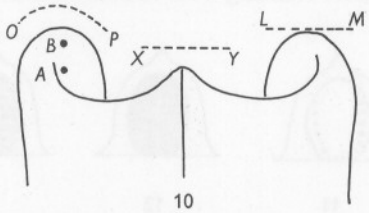

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Fig. 9. Diagram of part of a tube with the roof removed to show the cavities which are present in the base of each side.

Fig. 10. Diagram of a top view of the anterior end of a tube; for an explanation of the lettering see text.

THE POSTERIOR CALCAREOUS GRATING

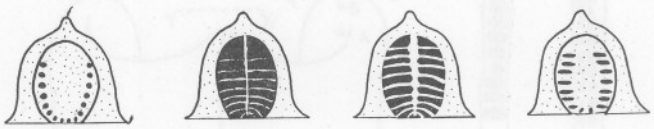
The tubes of *P. triqueter*, in the intertidal zone, are very often damaged at the posterior end. Almost invariably, in such cases, a single calcareous grating or plate (Fig. 11) is present inside the tube, just anterior to the damaged region (McIntosh, 1923; Thomas, 1940).

After damaging the posterior end of a tube, and removing the debris, a grating is formed after approximately 6–24 hr. The first stage is the secretion by the worm of a mucous septum inside the posterior end of the tube. This is followed by the appearance of a number of thin calcareous ribs in the mucous septum (Fig. 12) which become thicker and opaque, while the mucus between them becomes translucent and white (Fig. 13). Calcification continues (Fig. 14), and finally the typical grating is formed (Fig. 11) almost closing the posterior end of the tube.

The grating is a curved structure and this is correlated with the ventral flexure of the posterior end of the abdomen as the worm lies on its back in the tube (Fig. 15). The flexed region of the abdomen acts as a mould in which the calcareous grating is formed. The transverse calcareous ribs correspond in position to the intersegmental grooves (Fig. 16), while the main dorso-ventral rib corresponds to the mid-ventral ciliated groove of the abdomen. The holes in the grating correspond in position to the lateral swollen neuropodia of the abdominal segments (Figs. 16, 18) from which relatively long chaetae project and prevent the complete calcification of the grating.

Attempts have been made to locate the cells or tissues responsible for the secretion of the calcareous material required for the production of a grating.

Worms were fixed in a neutral solution, either ethyl alcohol or an alcohol-formalin mixture, and longitudinal (Fig. 17) and transverse sections (Fig. 18) of the posterior end of the abdomen were treated with the following methods. Staining with toluidine blue for the detection of mucous cells (Sylven, 1941); staining with purpurin (Lison, 1936) and gallamine blue (Stock, 1949) for the detection of calcium; and incineration to locate calcium ash. Negative results are obtained after staining with the lake-forming dyes purpurin and gallamine



11 12 13 14

Fig. 11. Diagram of a calcareous grating which is found inside, and almost closes, the posterior end of the tube.

Figs. 12–14. Diagrams of three stages in the formation of a calcareous grating.

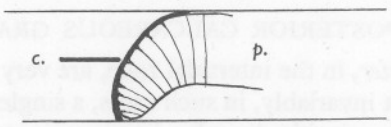
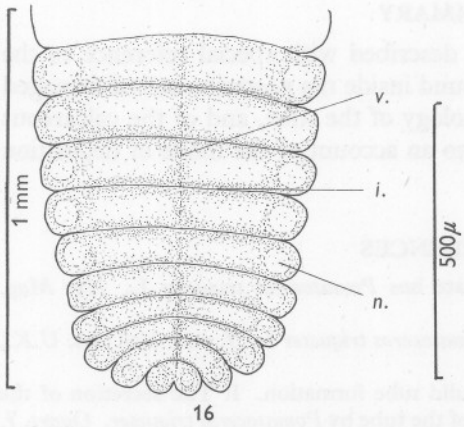


Fig. 15. Diagram to show the ventral flexure of the posterior end of the worm's abdomen and the position of the calcareous grating. *p.*, posterior end of worm; *c.*, calcareous grating.

blue. Other sections stained with toluidine blue and compared with alternate sections, which are incinerated, show that the ventral epithelial mucous cells contain a large amount of calcium. These cells are very numerous in the ventral epithelium compared with the dorsal epithelium where they occur only occasionally. Further comparisons of stained and incinerated sections were made in an attempt to find cells which do not contain mucigen and which have a high calcium content. Such cells were not demonstrated.

It is apparent from the position and form of the calcareous grating that the calcium originates from some tissue in the posterior end of the abdomen. It is most unlikely that the dorsal epithelium is involved (bearing in mind that a serpulid lies in its tube ventral surface uppermost), thus the ventral epithelium is most probably the source of the calcareous material. From the results obtained it appears that there are no obvious calcium-secreting organs in the posterior abdomen and it seems likely that the unicellular mucous glands of the ventral epithelium are responsible. It is worthy of mention that the rectum contains a large number of mucous cells and that the calcium-ash content of these is as great as that of the ventral epithelial mucous cells. The contents of

the rectum pass out via the anus and then along the ventral ciliated groove, and so the rectum may contribute some material towards the formation of the calcareous grating.



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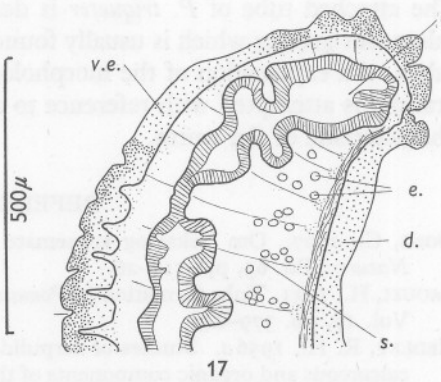

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Fig. 16. Diagram of the ventral surface of the ten most posterior segments of the abdomen. *v.*, mid-ventral ciliated groove; *i.*, intersegmental groove; *n.*, neuropodial swelling.

Fig. 17. Diagram of an oblique longitudinal section through the posterior end of the abdomen. The mucous secreting areas of the epithelium are more heavily dotted. *v.e.*, ventral epithelium; *r.*, rectum; *e.*, eggs, *d.*, dorsal epithelium; *s.*, intersegmental septum.

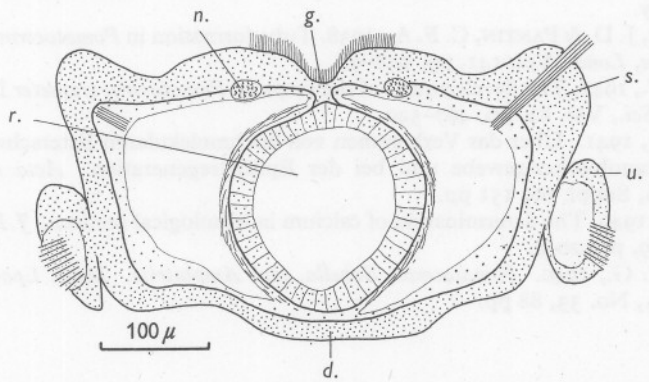

Fig. 18. Diagram of a transverse section through the posterior end of the abdomen. *d.*, dorsal epithelium; *g.*, ventral ciliated groove; *n.*, ventral nerve cord; *r.*, rectum; *s.*, swollen neuropodium with chaetae; *u.*, notopodium with uncinus.

This paper is an account of part of the work carried out during the period of tenure of a grant from the Department of Scientific and Industrial Research. The author wishes to thank Prof. A. D. Hobson for the facilities and help given while working in his department, and also Mr N. Tebble for suggestions at the manuscript stage.

Some of the work on serpulid tube formation was carried out at the Laboratory, Plymouth, and the author appreciates the facilities provided by the Director and his staff.

SUMMARY

The attached tube of *P. triqueter* is described with special reference to the calcareous grating which is usually found inside the posterior end of damaged tubes. An explanation of the morphology of the tube, and of the calcareous grating, is attempted with reference to an account of the mode of deposition characteristic of the worm.

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REQUIREMENT FOR THIAMINE AMONG SOME MARINE AND SUPRA-LITTORAL PROTISTA

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(Text-fig. 1)

It has been known for some years that a need for an exogenous source of thiamine exists among algal flagellates (Lwoff & Lederer, 1935; Provasoli & Pintner, 1953) and indeed the requirement coupled with one for B_{12} is quoted as characterizing auxotrophic algae (Provasoli, McLaughlin & Droop, 1957). Nevertheless, published data for marine species concerning thiamine are limited to a recent paper by J. J. A. McLaughlin (1958) which establishes an absolute requirement for thiamine on the part of *Prymnesium parvum*. The reason for this is partly due to interest in thiamine taking second place to interest in vitamin B_{12} and partly to the fact that thiamine-free media are not easily prepared, so that the requirement for the vitamin appeared as stimulatory rather than absolute (Droop, 1953*a*, 1957).

The following is an attempt to remedy this state of affairs as regards a few species maintained in bacteria-free culture at Millport.

METHODS

The strains used

In the first instance B_{12} requiring strains were chosen for the study, then two known to have no B_{12} requirement were added and finally some dinoflagellates on which there were no data (Table 1).

Culture media

Basal media were as follows: for *Skeletonema*, S 36 with thiamine omitted (Droop, 1955*b*); for *Hemiselmis*, S 46 with thiamine omitted (Provasoli *et al.* 1957); and for the remainder, excepting *Oxyrrhis*, S 50 with thiamine omitted. S 50 is given in full in Table 2. It differs from my previously published media chiefly in the composition of the trace metal mixture and the replacement of the pH buffer tris(hydroxymethyl)aminomethane by glycylglycine (which is very much less toxic to some species than TRIS) and glycine (which increases the buffering of the medium in the region of pH 9–10).

The media for *Oxyrrhis* are to be discussed fully in a paper on the nutrition of this species shortly to be published. Thiamine data are included here for the sake of completeness.

Both stocks and experimental cultures were kept in Pyrex test-tubes 15 × 150 mm. Oxo aluminium test-tube caps were employed to begin with, but were replaced by cotton wool when it was realized that contamination from this source was certainly less than 10 mμg/l.

TABLE 1. STRAINS USED

(Millport reference numbers in parentheses)

Chrysophyceae		
Monochrysis lutheri	(60)	Droop, 1953*a*, 1954
Prymnesium parvum Carter	(65)	Droop, 1954
Syracosphaera elongata	(62)	Droop, 1954, 1955*a*
Microglena arenicola	(72)	Droop, 1955*a*, 1957
Cryptophyceae		
Hemiselmis virescens	(64)	Droop, 1955*a*, 1957
Chlorophyceae		
Nannochloris oculata	(66)	Droop, 1955*a*
Bacillariophyceae		
Skeletonema costatum (Grev.) Cleve	(73)	Plymouth strain, Droop, 1955*b*
Phaeodactylum tricornutum Bohlin (syn. *Nitzschia closterium* f. *minutissima* (W. Smith) Allen & Nelson)	(14)	Finnish strain, Droop, 1953*a*
Dinophyceae		
Glenodinium foliaceum Stein	(47)	Finnish strain, Droop, 1953*a*
Peridinium trochoideum (Stein) Lemm.	(88)	From the Clyde
Oxyrrhis marina Dujardin	(18)	Finnish strain, Droop, 1953*b*

TABLE 2. MEDIUM S50

NaCl	15 g	Br	22 mg	Na_2EDTA	50 mg	Glycylglycine	500 mg
$MgCl_26H_2O$	2·5 g	Sr	3·8 mg	Fe	500 μg	Glycine	250 mg
KCl	400 mg	Al	28 μg	Mn	50 μg	KNO_3	100 mg
$CaSO_42H_2O$	500 mg	Rb	61 μg	Zn	5·0 μg	K_2HPO_4	10 mg
		Li	6·0 μg	Cu	5·0 μg	B_{12}	100 mμg
		I	20 μg	Co	500 mμg	Thiamine	1·0 mg
				Mo	500 mμg	H_2O to pH adjusted to 8	1·0 l.

Where possible, growth was measured optically, in which case it is expressed as percentage transmission or as OD × 100, $\left(= 100 \log_{10} \frac{100}{\%\ \text{transmission}}\right)$. It was necessary to count in other cases; growth is then expressed as cells per mm^3.

RESULTS

Serial transfer experiments

The concentrations over which growth is a function of thiamine concentration in other micro-organisms is 0–500 mμg/l. (Snell, 1951); and since my stock media contained up to 1 mg/l. of the vitamin, at least two transfers were necessary to reduce the concentration sufficiently in the blanks (cultures receiving no addition of the vitamin). This naturally depended on the size of the inoculum which was, therefore, made as small as possible. With *Monochryses*, *Prymnesium*, *Syracosphaera*, *Microglena*, *Nannochloris*, *Phaeodactylum*

and *Skeletonema* the inoculum could be reduced to 0·02 ml., thus allowing a 300-fold reduction at each transfer, so that the blanks of the second should have contained about 10 mμg/l. With *Hemiselmis* and the dinoflagellates, however, an inoculum of 0·2–0·3 ml. was advisable which only allowed a 20-fold reduction, with the consequence that four transfers were necessary to deplete the blanks to the same extent.

Transfers were made from the blanks. To the other cultures the vitamin was added initially in the form of the complete molecule (0·1 mg/l.), but later

TABLE 3. SERIAL TRANSFER EXPERIMENTS WITH THIAMINE

(−, blank; +, with 0·1 mg/l. thiamine. OD × 100.)

Transfer No.	Species													
	Nanno-chloris		*Phaeodac-tylum*		*Mono-chrysis*		*Prymnes-ium*		*Syraco-sphaera*		*Micro-glena*		*Hemisel-mis*	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+
1	66	68	57	70	122	110	80	92	44	41	35	52	32	70
2	92	92	89	100	30	140	0	80	16	39	11	48	7	110
3	110	100	92	89	4	150	2	80	27	59	22	43	2	105

TABLE 4. *SKELETONEMA COSTATUM*, SERIAL TRANSFER EXPERIMENTS

(OD × 100. standard errors in parentheses, $n = 5$.)

Transfer no.	Treatment		
	Blank	1·0 mg/l. Na_2S9H_2O	0·1 mg/l. thiamine
2	27 (5·4)	21 (9·0)	40 (3·7)
3	39 (1·0)	39 (0·8)	39 (0·7)
4	0·4 (0·4)	29 (1·25)	21 (3·4)
5	21 (0·7)	30 (1·05)	32 (1·1)

TABLE 5. DINOFLAGELLATE SERIAL TRANSFER EXPERIMENTS

(Numbers per mm^3.)

Transfer no.	Treatment				
	Blank	Thiamine	Thiazole + pyrimidine	Thiazole	Pyrimidine
	Oxyrrhis marina				
1	32	119	113	125	25
2	8	73	129	72	5
	Peridinium trochoideum				
2	9	8	13	13	7
3	6	8	6	13	3
4	9	7	8	6	9
	Glenodinium foliaceum				
2	34	26	27	30	24
3	20	28	27	33	28
4	—	33	39	28	31
5	28	21	26	26	25

the molecule was 'split' into its components, 4-methyl-5-β-hydroxyethyl-thiazole and 2-methyl-4-amino-5-aminomethyl-pyrimidine, and the following treatments given: (*a*) blank, (*b*) complete molecule, (*c*) thiazole and pyrimidine in equal amounts, (*d*) thiazole alone, and (*e*) pyrimidine alone.

The results of the transfer experiments are shown in Tables 3–5.

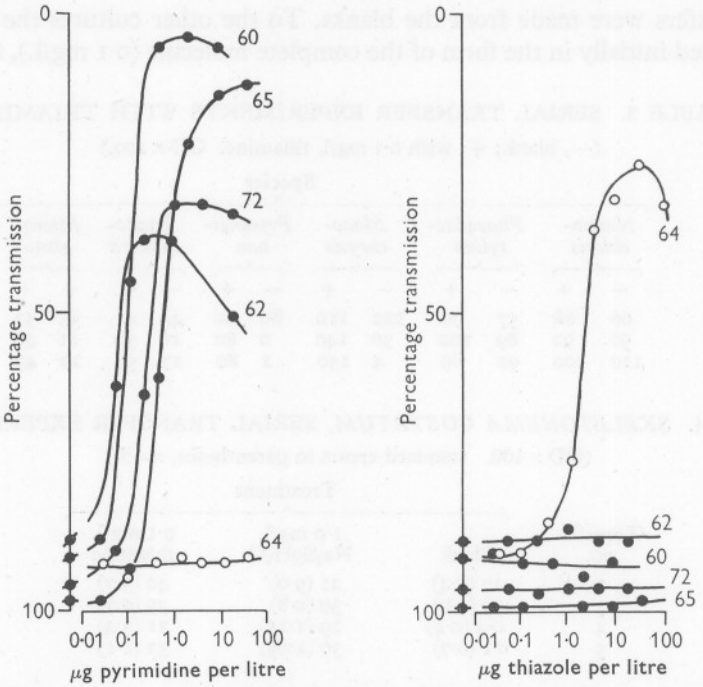

Fig. 1. Response to pyrimidine (left hand) and thiazole (right hand). 60, *Monochrysis lutheri*; 62, *Syracosphaera elongata*; 64, *Hemiselmis virescens*; 65, *Prymnesium parvum*; 72, *Microglena arenicola*.

Dose/response experiments

Where time and the material allowed the transfer experiments were confirmed by dose/response experiments in which was measured yield in response to graded doses of thiamine and its component parts. Response curves for the chrysomonads and *Hemiselmis* to pyrimidine and to thiazole are set out in Fig. 1.

DISCUSSION

Table 6 summarizes the results and includes data of other requirements, notably that for B_{12}. Previous reports regarding *Phaeodactylum*, *Nannochloris* and *Skeletonema* are confirmed (Peach & Drummond, 1942; Hutner, 1948; Droop, 1955*a*, *b*). All but two of the species under discussion in this paper

are of littoral or supra-littoral origin, the pelagic strains being *Skeletonema* and *Peridinium trochoideum*: neither had an absolute requirement for thiamine.

Skeletonema has been rather difficult to work with in defined media, because the vigour of cultures seems to depend to a large extent on such factors as pH, oxygen tension and the state of the inoculum—whether, for instance, it is in the exponential or stationary phase of growth, and its relation to the auxospore cycle. Under favourable conditions growth is quite independent of thiamine, and at such times thiamine does happen to be beneficial (as in the fourth transfer, Table 4) it can always be replaced by inorganic sulphide;

TABLE 6. SUMMARY OF RESULTS, WITH DATA ON OTHER HETEROTROPHIC TENDENCIES

	Requirement for thiamine	Portion of vitamin required	Requirement for vitamin B_{12}	Other heterotrophic tendencies
Chrysophyceae				
Monochrysis, *Prymnesium*, *Syracosphaera*, *Microglena*	+	Pyrimidine	+	Can utilize some amino acids as N source (Droop, 1955*a*)
Chlorophyceae, *Nannochloris*	–	–	–	Can utilize some amino acids as N source (Droop, 1955*a*)
Bacillariophyceae, *Phaeodactylum*	–	–	–	Can utilize some amino acids as N source (Droop, 1955*a*)
Skeletonema	–	–	+	Organic compounds sometimes stimulatory in an unspecific way (Droop, 1957)
Cryptophyceae, *Hemiselmis*	+	Thiazole	+	Amino N obligatory (Droop, 1957)
Dinophyceae, *Oxyrrhis*	+	Thiazole	?	Amino N obligatory; acetate as C source; phagotrophic in Nature; other B vitamins (unpublished data)
Glenodinium, *Peridinium*	–	–	+	?

consequently, it is unlikely that the vitamin is functioning here in this same way as in other organisms. Incidentally, neither *Hemiselmis* nor *Oxyrrhis* could utilize inorganic sulphide in place of thiazole. The significance of sulphur-containing compounds in diatom growth has been discussed by Harvey (1939, 1955); Lewin (1954) and Droop (1957)

The results appertaining to two photosynthetic dinoflagellates, *Peridinium trochoideum* and *Glenodinium foliaceum* (Table 5) are not completely satisfactory owing to the lightness of growth normally obtained with these two species. It is noted, therefore, simply that no requirement was demonstrable even after four transfers. On the other hand, a requirement for vitamin B_{12} became apparent on the first transfer and it was not possible to carry either species through more than two transfers in B_{12}-free media.

The four chrysomonads all responded to the pyrimidine half of the vitamin, whereas *Hemiselmis* and *Oxyrrhis* responded to the thiazole half (Fig. 1).

Possibly this represents a phyletic difference between the Chrysophyta and Pyrrophyta, though it is early to tell. With the exception of *Oxyrrhis*, all the species which proved to need thiamine had already been found to have a requirement for vitamin B_{12} (Droop, 1957), but the reverse was not the case.

The Chrysomonads are quite indifferent to the presence or absence of thiazole in the medium, so their response to pyrimidine can be regarded as simple. *Hemiselmis* and *Oxyrrhis*, on the other hand, appear to obtain some benefit from pyrimidine: pyrimidine does not support growth and, in the presence of thiazole, affects yield only slightly, nevertheless, *Hemiselmis* bleaches more quickly in its absence and *Oxyrrhis* fails to produce the fine pink colour characteristic of heavy healthy cultures.

It is not possible to arrive at more than a rough estimate of the magnitude of the thiamine requirement from dose/response curves based on optical measurements (Fig. 1). Furthermore, the basal medium S 50 was developed to suit *Monochrysis* and was not necessarily that most suitable for the other species. Half-maximal growth of the pyrimidine-requiring organisms appeared to be given by 100–300 mμg/l. pyrimidine or thiamine; half-maximal growth of both *Oxyrrhis* and *Hemiselmis* (requiring thiazole) by ten times as much, i.e. 2000 mμg/l., thiazole or thiamine. Such a difference in magnitude in thiamine requirement between pyrimidine and thiazole-requiring species has rather interesting implications should it prove to be real and of general occurrence. It suggests either that thiamine has a different function according to whether the requirement is met by one-half of the vitamin or by the other, or, alternatively, that the functions of pyrimidine and thiazole are divorced from each other and that the function of thiamine here is merely to supply either thiazole or pyrimidine as the case may be. Which alternative is biochemically less improbable it is difficult to say. On the other hand, it is possible that thiazole is merely more labile than pyrimidine.

SUMMARY

The requirement for thiamine was examined in eleven marine protists of littoral, supra-littoral or neritic origin. Six were found to have an absolute requirement for the vitamin.

The thiamine-requiring Chrysophyta responded to the pyrimidine half of the vitamin, whereas the two thiamine-requiring Pyrrophyta responded to the thiazole half.

All the species requiring thiamine were auxotrophic with respect to at least one other factor (usually vitamin B_{12}).

For half-maximal growth, species responding to pyrimidine required 100–300 mμg/l. pyrimidine or thiamine and species responding to thiazole 2000 mμg/l. thiazole or thiamine.

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BIOLOGICAL DIFFERENCES BETWEEN SEA WATERS: EXPERIMENTS IN 1954 AND 1955

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The experiments described and discussed below were made during the 1954 and 1955 breedings seasons of *Echinus esculentus*. They were a continuation of those made in previous years (Wilson, 1951; Wilson & Armstrong, 1952, 1954) and were designed to obtain more information concerning the factors responsible for biological differences between sea waters. In spite of the indefiniteness of many results it is considered desirable to publish them now because it does not seem likely that they can be supplemented in the near future, and because they do contain information which may influence further work in this field.

We should again like to express our thanks to the Marine Station at Millport, especially to Mr E. Latham, for co-operating with us in the collection of water samples.

THE EXPERIMENTS

Methods did not differ in any material respect from those already described in our earlier papers, with the exception of the sea water extracts used in Expt. 8. In making these sea water was passed slowly through a Berkefeld candle and then through a column of active carbon (copper-free). The carbon was then washed with a little distilled water, dried at room temperature, and extracted with successive small portions of cold pyridine until no further coloured material was removed. The extracts were combined and evaporated *in vacuo* at room temperature; waxy brown solids were obtained which dispersed readily in water. A 'blank' extract was also made from the untreated carbon. 18·0 l. of E 1 water gave 0·0547 g of material which was dispersed in 18 ml. water. 18·5 l. of Clyde water gave 0·0441 g, which was dispersed in 18·5 ml. water. The blank extract was dispersed in 18 ml. water. For the experiments 8·0 ml. of these concentrates were added to 2 l. of artificial sea water.

EXPERIMENT 1

Celtic water collected 12. iii. 54. Ship: R.V. *Sarsia* with Dr L. H. N. Cooper, position 51° 11′ N., 06° 13′ W. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters. A sample of bottom water, 118 m, and a sample of bottom mud, 123 m, were collected with a chromium-plated Nansen Petterson water bottle. The mud with some water was

kept for 5 days in a glass container and then filtered on a fine paper (previously well washed with sea water from the same station). The mud filtered off was dried and weighed; it amounted to 7·2 g dry matter per litre of sea water. The filtrate is referred to below as 'mud extract'.

E 1 *water* collected 12. iii. 54. Ship: M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters.

Clyde water collected 12. iii. 54. Small boat with Mr E. Latham. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters.

The waters collected in the carboys were further filtered through Doulton candles immediately before use.

The sea-urchins were trawled on 17 March and kept overnight under circulation, the fertilization being made at 4 p.m. on 18 March. Many urchins were opened, almost all had well-filled gonads, few seemed to have spawned. It was with some difficulty that a female with ripe ova in apparently good condition was found. The fertilization was made in a mixture of all three natural waters and the eggs were immediately divided equally between three beakers, and each part washed with six changes of one of the natural waters at room temperature (approx. 15° C—throughout the experiment the room temperature remained very uniform). The fertilization membranes were well elevated but 1¼ h after fertilization the cytoplasm of all the eggs examined (from all three waters) assumed an irregular indented shape, rounding up again shortly before first cleavage. The irregular shapes assumed were varied and striking; such well-marked irregularities were not observed in any of the fertilizations for the later experiments in 1954. After first cleavage eggs were distributed in approximately equal numbers to the various dishes comprising the undermentioned sets. Eggs in Sets IV–VI were further washed two changes in their respective presumed sterile waters and it was calculated that only about 0·3 % of the carboy-collected waters, in which the eggs were first washed, would remain in these dishes. Eggs supplied to Sets XIII–XV were taken in equal numbers from all three natural waters in which they had been washed since fertilization, and were further washed in three changes of artificial water before being distributed to the dishes of these sets. Sets XVI and XVII were supplied with eggs washed in E 1 water.

Set		No. of dishes comprising set
I	Celtic water from carboy	4
II	E 1 water from carboy	4
III	Clyde water from carboy	4
IV*a*	Celtic water from Winchester A (proved sterile)	2
IV*b*	Celtic water from Winchester B (proved sterile)	2
V*a*	E 1 water from Winchester C (proved sterile)	2
V*b*	E 1 water from Winchester D (not sterile)	2
VI*a*	Clyde water from Winchester E (not sterile)	2
VI*b*	Clyde water from Winchester F (not sterile)	2
VII	Celtic water + 10% Celtic mud 'extract'	4
VIII	E 1 water + 10% Celtic mud 'extract'	4
IX	Clyde water + 10% Celtic mud 'extract'	4
X	Celtic water + 10% Celtic bottom water	4
XI	E 1 water + 10% Celtic bottom water	4
XII	Clyde water + 10% Celtic bottom water	4
XIII	Artificial sea water	3
XIV	Artificial sea water + 10% Celtic mud 'extract'	3
XV	Artificial sea water + 10% Celtic bottom water	3
XVI	Celtic mud 'extract' undiluted	1
XVII	Celtic bottom water undiluted	1

The fertilization was not quite 100% successful and therefore in every dish there were on the bottom a small number of unfertilized eggs which eventually decayed. The experiment was continued for 7 days. Until 22 March the larvae in all the dishes of any one set were identical as regards swimming vigour, structure and range of variation in structure of the plutei (a proportion were mis-shapen), and in the numbers dead. After that date some variation within a set was observed in some instances, but mostly such differences as did occur were between the larvae of one set and those of another.

The carboy waters from the three localities (Sets I–III) gave almost identical rearings. Not until 22 March was there any definite distinction; on that day the larvae in the Celtic water were rapidly failing in all four dishes. They had begun to fail also in the E 1 and Clyde waters but not to the same extent. The difference was more marked the next day, although by that time there were differences between dishes within a set. The Clyde water proved to be slightly better for the larvae in these later stages than the E 1 water. The results with the dishes were confirmed by comparing the larvae left in the wash beakers.

Of the water samples filtered through Doulton candles at the time of collection only three proved to be sterile when the Winchesters were opened just before use. Two of these were from the Celtic sea, the other from E 1. In these waters (Sets IV *a* and *b*, V *a*) the larvae did not do quite so well as in the carboy waters (Sets I–III) but the difference was not great, especially in the E 1 water (Set V *a*), where the larvae were only just perceptibly inferior to those in Set II. The three remaining water samples filtered at the time of collection were not sterile. In that from E 1 (Set V *b*) the larvae were at all times closely similar to those in Set II. In the Clyde water (Sets VI *a* and *b*) the larvae were inferior to those in the carboy water (Set III), markedly so in Set VI *a*, slightly so in Set VI *b*. These results with both sterile and non-sterile water collected through candles are not entirely satisfactory. The candles were delivered by the maker only the day before the apparatus had to be got ready for use and it is possible that the soaking and washing they were given was insufficiently prolonged to ensure the removal of all soluble substances.

The addition of approximately 10% Celtic bottom water to the three natural sea waters and to the artificial (Sets X–XII, and XV) had little or no effect on the larvae reared in them for the first few days, but on and after 22 March a slight though definite improvement was to be seen, the larvae in the waters with the addition failing less rapidly than in those without. On the other hand, the addition of Celtic mud 'extract' while at first slightly stimulating in that on the third day, in all waters to which it had been added (Sets VII–IX and XIV), the larvae were swimming a little more vigorously towards the surface than in the waters without, in the later stages the larvae failed a little more rapidly than in these latter. In the dishes of undiluted Celtic mud 'extract' and Celtic bottom water (Sets XVI and XVII) the larvae were in poor condition even on the second day and in the mud 'extract' they were quickly killed. There is thus shown a graded series: the undiluted mud 'extract' poisonous; the undiluted bottom water harmful but not quite poisonous, the larvae survived in it abnormally for several days; the addition of a small volume of mud 'extract' at first slightly stimulative, but the larvae failed more quickly than in water without; the addition of a small portion of a small volume of bottom water without at first apparent effect, but the larvae survived a little longer than in the waters without.

EXPERIMENT 2

The difficulty experienced in obtaining good ova for Expt. 1 led to a fresh batch of sea-urchins being trawled on 19 March 1954 and a new fertilization made as soon as they were landed. As before, many ovaries contained irregular fragments of ova.

A female which looked better than the rest was chosen. After fertilization the eggs were divided into three equal portions and washed in the three kinds of sea water. A sample of the eggs was repeatedly examined until first cleavage, but this time the cytoplasm did not indent irregularly. The only tests made were of the three waters collected in carboys and filtered through Doulton candles just before use.

Set		No. of dishes comprising set
I	Celtic water from carboy	3
II	E 1 water from carboy	3
III	Clyde water from carboy	3

The results were closely similar to those obtained with Sets I–III in Expt. 1. The plutei were on the whole more even in structure with fewer mis-shapen individuals than in Expt. 1, but they never attained the fine development of plutei in the best rearings in the Celtic and Clyde waters of 1949, 1950 and 1952. Five days after fertilization most of them went to the bottom and lost their arms. As in Expt. 1 the larvae in all three waters were closely similar, but they failed a little earlier in the Celtic water and at the end were a little better in the Clyde water than in the other two, though the difference was no more than perceptible.

EXPERIMENT 3

E 1 surface water collected 29. iii. 54. Ship: R.V. *Sarsia* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. Also one sample filtered through sterile Doulton candle into sterile Winchester. A sample of water at a depth of 70 m was collected with a chromium-plated Nansen Petterson water bottle after this bottle had been used for the collection of several samples for hydrographical purposes.

Shortly before use the water collected in the carboy was filtered through a Doulton candle. The water which had passed through a Doulton candle at the time of collection proved to be sterile when the Winchester was opened on 1 April immediately before the experiment began. The carboy water before filtration gave, on the other hand, a heavy growth of mixed organisms when bacteriologically tested at the same time.

The sea-urchins were trawled on 1 April and used immediately. Of the many opened only a few were spent. In general the ovaries seemed to be in better condition than a fortnight previously and there was less difficulty in selecting a female for spawning. Immediately after the fertilization was made in E 1 surface water the eggs were divided into four equal quantities and each quantity washed with six changes of either E 1 surface water, E 1 sterile surface water, E 1 deep water from 70 m, or four changes of artificial sea water. Before first cleavage took place the cytoplasm of some of the eggs became irregular in outline and slightly indented (but not as markedly as in Expt. 1), rounding up again before cleavage. After first cleavage equal quantities of eggs were distributed as follows. There were four dishes in each set, two in each half-set in Series IV and V.

Set		Eggs washed between fertilization and first cleavage in
I	E 1 surface water (carboy)	E 1 surface water
II	Sterile E 1 surface water	Sterile E 1 surface water
III	E 1 deep water (from 70 m)	E 1 deep water
IV	E 1 surface water + 50% deep water	(*a*) E 1 surface water (*b*) E 1 deep water
V	E 1 surface water + 10% deep water	(*a*) E 1 surface water (*b*) E 1 deep water
VI	Artificial sea water	Artificial sea water
VII	Artificial sea water + 10% E 1 surface water	Artificial sea water
VIII	Artificial sea water + 10% E 1 deep water	Artificial sea water

The duration of the experiment was about 1 week. There was little difference in the condition of the larvae between Sets I and II, the water sterile until the experiment began being slightly less favourable than the carboy water. By this time the Doulton candle had been well washed before use.

The deep water (Set III) proved much less favourable than either of the surface waters (Sets I and II); from an early stage the larvae in it were mainly abnormal and in 2 or 3 days many were dead or dying. The mixtures of surface and deep water are of particular interest because the results with them were strikingly different according to whether the eggs were washed between fertilization and first cleavage in surface water or in deep water. With those washed in surface water (Sets IV*a* and V*a*) the results were similar to those with surface water alone (Sets I and II) being only a little less favourable in the 50% mixture. But with eggs washed in deep water (Sets IV*b* and V*b*) the larvae were decidedly inferior to surface water Sets I and II and only a little better at first than those in deep water alone (Set III). It will be realized that these results with deep water (which are similar in some respects to those obtained with the bottom water and mud used in Expt. 1, but in that experiment eggs did not come into contact with the bottom water until after first cleavage) could be explained by faulty technique if the beaker in which the eggs washed with the deep water had contaminated that water. The beaker had, however, been well cleaned with hot strong sulphuric acid before use, but it was not a new beaker. It could also be explained if the chromium-plated water bottle had affected the water.

The larvae in artificial sea water (Set VI) were poor compared with those in surface water (Sets I and II) although better than those in deep water (Set III). An addition of 10% surface water affected a slight improvement, while the addition of 10% deep water had little or no effect. These were the poorest larvae so far reared in artificial sea water in the course of these experiments; it should perhaps be noted that in earlier experiments the eggs were not washed into artificial sea water quite so soon after fertilization as they were here.

EXPERIMENT 4

E 1 *surface water* collected 12. iv. 54. Ship: M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. The presence of *Phaeocystis* was noted. Also samples of both surface and deep water from 70 m collected in plastic-lined water bottle, and stored in Winchesters.

Before use all waters were filtered. That from the carboy was passed through a Berkefeld candle and was used for making the fertilization. The waters from the Winchesters were aerated after passing through the Doulton candles and were used for washing the eggs and for filling the experimental dishes. Thus in this experiment the waters compared had both been collected in the same way. Moreover, the use of the plastic water bottle instead of the chromium plated one used for the experiment would resolve doubts concerning the latter. The beakers used for washing the eggs were new ones, thoroughly cleaned in hot strong sulphuric acid.

The sea-urchins were trawled on 13 April and used at once. The ovaries seemed to be in better condition than last time and almost 100% after fertilization had well-elevated membranes and even cleavage. No shrinking of the cytoplasm was noted between fertilization and first cleavage. The eggs were divided into two equal parts immediately after fertilization and one lot washed with surface water and the other with deep water, six changes of each. After first cleavage approximately equal numbers of eggs were distributed to the following sets of dishes, four dishes to each set.

Set		Eggs washed with
I	E 1 surface water	E 1 surface water
II	E 1 surface water	E 1 deep water
III	E 1 deep water	E 1 surface water
IV	E 1 deep water	E 1 deep water
V	Mixture: 50% surface 50% deep water	E 1 surface water
VI	Mixture: 50% surface 50% deep water	E 1 deep water

In spite of the apparent excellence of the fertilization a considerable proportion of the eggs died during late cleavage stages before the formation of the blastula, and many blastulae were abnormal, only a small proportion appearing normal. That this was not due to the plastic water bottle in which the experimental waters were collected is shown by the fact that the eggs and blastulae were identical in a control Berkefeld filtered water from the carboy, these eggs not having come into contact with water from the plastic water bottle at all. Unfortunately, in this experiment no water other than that from E 1 was available for comparison, and no fertilization in this water of another batch of eggs and sperm. In spite of these aspects being uncontrolled and of the relatively poor condition of the best larvae the experiment gave a striking result. For the first 2 days after fertilization all dishes in all sets were alike as regards their larval content. The larvae if not already dead, were slow to swim up, most of them remaining on the bottom even as late as the gastrula stage. The majority showed some abnormality, the gastrulae on the whole being small and shrunken. A day later (16 April) almost all those in deep water (Sets III and IV) were dead and so were those which had been washed in deep water between fertilization and first cleavage, although then put into surface water (Set II—it must be noted that this water would contain about 7·5 % deep water, added to it with the larvae). Only those larvae which had never at any time been in contact with deep water (Set I) were still mainly alive and swimming well, even more vigorously than before and on the whole a little more normal-looking. Sets V and VI with mixed water, whatever water the eggs had been washed with, were in much poorer condition than these, but strangely not quite so bad as Set II with much less deep water mixed with it. This would be understandable for Set V (eggs washed in surface water) if the eggs are specially susceptible to damage between fertilization and first cleavage, and were damaged by the deep water, but would not explain why Set VI (eggs washed in deep water) was better than Set II which contained a much smaller proportion of deep water.

The experiment was not seen again until 20 March. Conditions on that day neither added nor subtracted anything from the result.

EXPERIMENT 5

Clyde water collected 26. iv. 54 in Largs Channel, tide falling. Small boat with Mr E. Latham. Strained into carboy through 200-mesh bolting-silk, which was discoloured brown by diatoms.

Artificial sea water. The salts used were the same as in 1953 except for the $CaCl_2$ which was a new batch.

A more elaborate experiment had been planned. Unfortunately, stormy weather prevented the collection of natural sea waters at Plymouth and it was only possible to compare the Clyde water with artificial sea water and to investigate the effect of washing the eggs between fertilization and first cleavage in both waters. The Clyde water was passed through a Berkefeld filter-candle before use.

The sea-urchins were trawled on 28 April and used at once; most of them were spent. The eggs selected gave an almost 100 % fertilization with well-elevated fertiliza-

tion membranes. Less than 15 min after adding the sperm the eggs were divided into one of the two waters. Each water was changed six times and after first cleavage the eggs were distributed to the following sets of four dishes each. It should be noted that each dish would receive with the eggs a little of the water in which they had been washed. It was calculated that this water would amount to about 8% of the total volume of water in the dish.

Set		Eggs washed with
I	Clyde water	Clyde water
II	Clyde water	Artificial sea water
III	Artificial sea water	Clyde water
IV	Artificial sea water	Artificial sea water
V	Mixture in equal proportions of Clyde and artificial sea waters	Clyde water
VI	Mixture in equal proportions of Clyde and artificial sea waters	Artificial sea water

In spite of the apparent excellence of the fertilization the blastulae were slow to swim up in all dishes and the majority took an unusually long time to reach the surface and most were beginning to gastrulate before they did so. They developed better in the Clyde water than in the artificial sea water, their development in the mixture being nearly as good, though not quite, as in the Clyde water alone. The kind of water the eggs were washed in between fertilization and first cleavage made no significant difference. In all waters plutei became abnormal by the third or fourth day, earlier and to a greater extent in the artificial sea water than in that from the Clyde water. It has been noted that the latter had a heavy growth of diatoms in it at the time of collection, but as no comparison was made with any other natural sea water it is impossible to decide whether the relatively poor conditions of the larvae at all times in this water is to be attributed to its constitution or to the condition of the eggs or sperm.

EXPERIMENT 6

Clyde water collected 4. v. 54, in Largs Channel approx. an hour before high tide. Small boat with Mr E. Latham. Strained into two Winchesters through 200-mesh bolting-silk; hardly any diatoms present. At the same time two further samples (A and B) were collected through Doulton candles into evacuated sterile Winchesters using the method described in Wilson & Armstrong, 1954, pp. 348–9. Particulars of artificial water as for Expt. V.

Continuing bad weather prevented the collection of natural sea water at Plymouth. This experiment embodied two separate investigations: (1) a further attempt to compare sea water strained only through bolting-silk at the time of collection into clean but non-sterile glass containers, with sea water collected at the same time through Doulton candles into sterile containers and tested for sterility just before use; (2) to investigate the effect of adding a quantity of sea water from which a variety of thick growths of phytoplankton organisms had filtered out. We are indebted to Dr Mary Parke for supplying a number of thick cultures of various diatoms and flagellates for this purpose. These cultures were filtered through Doulton candles. Dr Parke also supplied the sample of unused sterile culture medium ('Erdschrieber') for our controls.

We are indebted to Dr C. H. Jellard, Public Health Laboratory Service, for bacteriological tests of the Clyde waters A and B, these being sampled bacteriologically on 6. v. 54 shortly before the experiment began. At the same time bacteriological samples were taken from the Winchesters which contained the water filtered only through bolting-silk. These latter samples gave heavy growths of a number of different organisms, the waters A and B gave no visible growths for 2 days, but later each gave some growth of a single organism, in each case a slow-growing Gram-negative bacillus,

a different one in A from that in B. The control plates which had not been inoculated were sterile.

The sea-urchins were trawled on 6. v. 54. Almost all were spent, or partially spent. Only one female in reasonably good condition was obtained and a few males. A fertilization was made in Clyde water filtered from the carboy which supplied Expt. V. The eggs were immediately divided into four equal quantities; one washed with six changes of new Clyde water from the Winchesters (filtered through Doulton candles in the laboratory), two other quantities each with six changes of Clyde waters A and B, and one quantity with six changes of artificial sea water. The temperatures of all waters were adjusted to room temperature before use.

Almost all eggs fertilized and showed good membrane elevation; a few protruded cytoplasm through the membrane after about $\frac{1}{2}$ h. The remainder eventually produced mainly normal blastulae even in size and appearance. At about the time of first cleavage equal quantities of eggs were distributed, from the appropriate washing waters, to the following sets of dishes.

Set		No. of dishes in set
I	Clyde sea water not sterile	4
II	Clyde sea water (A) proved almost sterile until used	4
III	Clyde sea water (B) proved almost sterile until used	4
IV	Clyde sea water (as I) + 10% culture medium (control)	4
V	Clyde sea water (as I) + 50% culture medium (control)	2
VI	Clyde sea water (as I) + 10% culture filtrate	4
VII	Clyde sea water (as I) + 50% culture filtrate	2
VIII	Artificial sea water	4
IX	Artificial sea water + 10% culture medium (control)	4
X	Artificial sea water + 50% culture medium (control)	2
XI	Artificial sea water + 10% culture filtrate	4
XII	Artificial sea water + 50% culture filtrate	2

For the first 2 days there were no observable differences between any of the dishes, the larvae swam up strongly in them all. The subsequent development of Sets I, II and III will be considered first. Three days after fertilization there was a slight tendency for the larvae in the dishes of Set II to concentrate at the surface more than in any other set, but this was not observed the next day though it re-occurred on the sixth day. It must be emphasized, however, that this tendency was never more than barely perceptible. There were no structural differences between the plutei of Sets I, II and III at any stage. They were in fine normal condition on the fifth day, but on the sixth the living tissues of the arms shrank down the supporting rods, which broke away leaving the larvae armless. The following day they were very abnormal in all three sets and many were dead.

The addition of culture medium and culture filtrate to the Clyde water (Sets IV to VII) made little difference. Such differences as were observed indicated that culture medium, when present in fair proportion as in Set V, was on the whole disadvantageous but in lower concentration (Set IV) was very slightly beneficial. The culture filtrate had even less effect.

In the artificial sea water (Set VIII) many of the larvae, which in the early stages swam up strongly, sank to the bottom of the dish a little earlier than in the natural sea waters in Sets I–III, but they did not degenerate quite so quickly as did those in the natural sea waters, although on the whole there was little difference. The addition of culture medium made only a very slight difference, being on the whole disadvantageous in quantity (Set X) and very slightly favourable in smaller amount (Set IX). Culture filtrate made little or no difference.

EXPERIMENT 7

E 1 *surface water* collected 15. iii. 55. Ship: M.F.V. *Sula* with F.A.J.A. Dipped up with wooden bucket and strained through 200-mesh bolting-silk into glass carboy. Plankton thicker than usual.

E 1 *bottom water* (70 m) collected same time as surface water using Perspex-lined water bottle. Stored in glass Winchester but not filtered through bolting-silk.

Echinus *ground bottom water* (52 m) collected same day as E 1 bottom water and in same way. This ground is situated Eddystone N. 160°, Rame Head N. 55° E.

Clyde water (*surface*) collected 14. iii. 55, in Largs Channel. Small boat with Mr E. Latham. Strained through 200-mesh bolting-silk into glass carboy.

Artificial sea water. All waters were filtered in the laboratory through Berkefeld filter candles not more than 1 day before use.

The purpose of this experiment was to compare again E 1 and Clyde surface waters which of recent years had not been as strikingly different in their properties as they had been several years ago when the first experiments were made. It also was to investigate further the 1954 results in which it appeared that there was a difference between the surface and bottom water at E 1, and that the development of the *Echinus* eggs was affected by the water in which the eggs were washed between fertilization and first cleavage (Expts. 3 and 4). As the bottom water at E 1 had apparently been unfavourable to the development of the eggs it was decided to test bottom water from the *Echinus* grounds themselves. These are situated some miles away from E 1, (where the bottom is muddy and there are no sea-urchins) and the water bathing these grounds would be that into which the *Echinus* would spawn under natural conditions. It must in this connexion be remembered, however, that the water over the grounds would be changing and would not be static from day to day, and that it was impossible to know whether on the day chosen for taking the water any of the *Echinus* would be spawning naturally. If their spawning is affected by the particular type of water bathing them it is conceivable that only at certain times, when the water is favourable, do they actually shed their eggs and sperms.

The *Echinus* used were trawled on 18 March from beyond the Eddystone and not on the ground from which the *Echinus* bottom water had previously been taken and where *Echinus* is normally abundant. In March and April 1955, perhaps as the result of the severe gales earlier, *Echinus* proved to be almost absent from this ground and they had to be got elsewhere when needed in quantity. This fact was not known when the bottom water was collected. The fertilization was made as soon as the urchins had been brought into the laboratory; the majority of the urchins were immature but a moderately mature female was found. The eggs and sperms were shed into, and the fertilization was made in, artificial sea water. The same urchins were subsequently spawned into, and a control fertilization made, in E 1 surface water; this was successful but not particularly good. About $\frac{3}{4}$ h after fertilization the cytoplasm of the eggs became irregular in outline (rather as in Expt. 1), rounding up again 40 min later before first cleavage. Seven minutes after the fertilization in artificial sea water had been made the eggs from it were divided into five equal quantities into the five wash waters. Six changes of these were given and after the first cleavage eggs were distributed in equal quantities to the dishes containing the waters to be tested. There were twelve sets of these, three dishes to each set. The arrangement of the experiment will be clear from the headings in Table 1. Thus Set I comprised three dishes each containing 100 ml. of E 1 surface water and into them equal quantities of eggs were ladled out of the beaker in which they had been washed with E 1 surface water to free them from the artificial sea water in which the fertilization had been made. Set II likewise

comprised three dishes each containing 100 ml. of E 1 surface water, but into these were put equivalent quantities of eggs washed in E 1 bottom water (and with them about 8 ml. of the latter water from which they could not be separated), and so on through the whole series. The condition of the larvae on subsequent days is recorded in Table 1. First, their swimming vigour as shown by the relative numbers at the surface and bottom, and as evenly distributed in mid-water between surface and bottom, and secondly on the fourth day and thereafter the structural appearance of the plutei is briefly described, together with rough estimates of the numbers observed to have died. There were never any differences between the three dishes of any one set; in so far as they differed from those of another set they all differed alike. Not until after 23 March, on which day the experiment was considered to have ended, did any inconsistencies appear between dishes of the same set and then only to a slight extent. These later stages are not recorded for they were concerned only with farther degeneration and death and did not yield any more information about the waters.

There are several interesting features to be noted concerning the results. It was very noticeable that on the morning of the second day the blastulae swam up more strongly in the Clyde water (Sets VI–X) than in any of the others and in the artificial sea water (Set XII) not at all. On the third day they were swimming well in all dishes but most strongly in both the bottom waters (Sets IV, V, X and XI), while the larvae in the Clyde waters which previously had led now flagged a little, there being more on the bottom than in any of the others and slightly fewer at the surface also. This latter fact is not indicated in the Table for the numbers involved were too few to justify recording a lower category than that shown. By the next day the larvae in the Clyde waters had caught up again with the others, and as regards swimming vigour all dishes were equal except those containing artificial sea water (Set XII) where almost all the larvae, which were structurally abnormal, had sunk to the bottom. These larvae never formed normal plutei although retentive of life. On 21 March the larvae of one dish were transferred to Clyde water and one other to artificial sea water and Clyde water in equal proportions. The following 2 days more larvae swam up off the bottom of these dishes than did those in the remaining dish of artificial sea water only (which alone is recorded in Table 2 for 22 and 23 March), but they never showed any indication of developing into plutei. On 21 March it was observed for Sets I–XI that the larvae, which were early plutei beginning to grow their first four arms, were decidedly more advanced in development with longer arms and were healthier looking in the Clyde waters than in any of the others. The larvae in all other natural waters looked much alike, except that possibly those in the *Echinus* ground bottom water (Sets X and XI) were a trifle better than those in the E 1 waters, both surface and bottom.

The next 2 days, 22 and 23 March, showed some marked differences. It became clear that Clyde water (Set VI) was proving a better medium for the larvae than E 1 surface water, and to an extent similar to that which existed a few years ago. It was clear too, that whilst washing the eggs between fertilization and first cleavage had not affected them when the wash water was E 1 surface water (Set VII), it had had an adverse effect when this wash water had been either of the bottom waters (Sets VIII and IX), especially the *Echinus* ground bottom water. This latter water although at first promoting swimming vigour did not long support the larvae in health (Sets X and XI) and finished by being almost worse in this respect than any other natural water. It had also proved bad when used as a wash water for eggs placed subsequently in E 1 surface water (Set III). The E 1 surface water itself, had not, as has been seen, given good plutei at any stage, the eggs which had been washed with E 1 bottom water (Set II) doing slightly better than others washed differently (Sets I and III), whilst the E 1 bottom water itself was slightly better (Sets IV and V) than the surface water from

TABLE 1. EXPERIMENT 7, FERTILIZATION OF *ECHINUS ESCULENTUS* MADE IN ARTIFICIAL SEA WATER ON 18 MARCH 1955

Set		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Water in dishes		E 1 surface	E 1 surface	E 1 surface	E 1 bottom	E 1 bottom	Clyde surface	Clyde surface	Clyde surface	Clyde surface	*Echinus* ground bottom	*Echinus* ground bottom	Artificial sea water
Wash water		(E 1 surface)	(E 1 bottom)	(*Echinus* ground bottom)	(E 1 surface)	(E 1 bottom)	(Clyde surface)	(E 1 surface)	(E 1 bottom)	(*Echinus* ground bottom)	(E 1 surface)	(*Echinus* ground bottom)	(Artificial sea water)
19. iii. 35 (a.m.)		Beginning to swim up	As I	As I	As I	As I	Swimming up strongly	As VI	As VI	As VI	As I	As I	All on bottom
20. iii. 55	*S*	***	As I	As I	*****	As IV	***	As VI	As VI	As VI	*****	As X	****
	M	*****			***		*****				***		***
	B	***			**		****				**		***
21. iii. 55	*S*	****	As I	As I	As I	As I	As I	As I	As I	As I	As I	As I	*
	M	***											*
	B	***											*****+
		Fairly good, very few abnormal	As I	As I	As I	As I	Good, very few abnormal	As VI	As VI	As VI	As I	As I	Abnormal
22. iii. 55	*S*	*	***	**	****	As IV	*****	As VI	***	****	**	**	*–
	M	**	****	**	****		****		****	***	***	**	*–
	B	*****+	*****	*****+	****		**			***	*****	*****+	*****+
		Poor, often abnormal, few dead	A little better than in I, few dead	Poor, many abnormal, several dead	Moderate, fair number abnormal	As IV	Good, very few abnormal, very few dead	As VI	Moderate, good number abnormal, several dead	Fairly good, good number dead	Some moderate, many abnormal, several dead	As X	Very abnormal
23. iii. 55	*S*	o	*	o	*–	As IV	***	As VI	*	*	*–	o	o
	M	*–	**	o	**		***		**	*	*	o	o
	B	*****+	*****+	*****+	*****+		*****		*****+	*****+	*****+	*****+	*****+
		Very poor, all abnormal, good number dead	Poor, mostly abnormal, good number dead	All abnormal, dead or dying	Slightly better than II, but very similar	Poor, mostly abnormal, good number dead	Moderate, majority becoming abnormal, few dead	As VI	Slightly inferior to VI	Very poor, all abnormal, many dead	All abnormal, majority dead	Very abnormal, almost all dead	Very abnormal, several dead

B = bottom; *M* = mid-water; *S* = surface. o = none; *– = very few; * = few; ** = several; *** = fair number; **** = good number; ***** = many; *****+ = very many.

the same position. This is the reverse of the 1954 findings with E 1 waters (see p. 335). It must be emphasized that all these differences although definite were slight; they were not of the order of the difference between the Clyde water and all the others.

The results standing out from this experiment are: (1) the superiority of Clyde water over all the others, although the plutei were never as well developed or as long lived in it as had sometimes been the case in earlier experiments. This may have been a reflexion of the quality of the eggs or sperm but this point was not controlled. (2) The inability of the eggs to form normal larvae in the artificial sea water, in contradistinction with those reared in artificial sea water in previous years. It may be noted that the sodium chloride used in making up the artificial sea water came this year from a different batch than before. (3) The fact that every difference in the treatment of the eggs, the water they were washed with and the water they developed in had some influence, however slight, on the structure and health of the larvae developing from the eggs, and these differences affected all dishes of a set equally.

EXPERIMENT 8

Celtic sea surface water collected 9. iv. 55. Position 50° N. 10° W. R.V. *Sarsia* with Mr A. D. Mattacola. Strained through 200-mesh bolting-silk into glass carboy. At the same time two further samples (A and B) were collected through Doulton candles into evacuated sterile Winchesters using the method described in Wilson & Armstrong, 1954, pp. 348–9.

E 1 surface water collected 12. iv. 55. M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into glass carboy. At the same time two further samples (C and D) were collected through Doulton candles into evacuated sterile Winchesters.

Artificial sea water as used in Expt. 1.

As usual the carboy waters were filtered in the laboratory before use, Doulton candles being used. The waters filtered through similar candles at the time of collection were tested for sterility shortly before use and three of them, two being from E 1 and one from the Celtic sea proved to be sterile. We were again indebted to Dr C. H. Jellard of the Public Health Laboratory Service for making the bacteriological tests.

The urchins were trawled on 14 April and used as soon as they arrived in the laboratory. They had unfortunately proved scarce and there were therefore fewer than usual from which to select. The male and female chosen gave what appeared to be an almost 100% perfect fertilization with well-elevated membranes and perfect first and second cleavage. There was no crumpling of the cytoplasm before cleavage, as occasionally noted previously. The fertilization was made in artificial sea water and immediately divided into ten equal parts, each part in a small beaker, and each part was then washed with four changes of one of the waters to be tested, as recorded below. After first cleavage the eggs were distributed to the following sets of dishes, three dishes to a set. The eggs supplied to Sets XI and XII were washed between fertilization and first cleavage in a water other than that to which they were finally transferred, but were subject to further washing after first cleavage to avoid adding to the water in the dishes even a little of another sort as had been done in some earlier experiments.

In spite of the apparent excellence of the fertilization up to and including the second cleavage at 9 p.m. on the first evening, almost all the eggs died in later cleavage stages and by the next morning relatively few had survived to form normal blastulae. All natural waters (Sets I–VI and XII) were equal in these respects, but in the artificial waters (Sets VII–XI) only about half were dead, the remainder being living blastulae, many obviously abnormal. These never developed into plutei, as in artificial sea water in the previous experiment, and the addition of the extracts made no difference whatever.

Set	Water tested. Except where otherwise stated four washings with the same water were given between fertilization and first cleavage
I	E 1 surface water (carboy)
II	E 1 surface water proved sterile until used (Winchester C)
III	E 1 surface water proved sterile until used (Winchester D)
IV	Celtic sea surface water (carboy)
V	Celtic sea surface water proved sterile until used (Winchester A)
VI	Celtic sea surface water filtered at time of collection but not sterile when tested (Winchester B)
VII	Artificial sea water
VIII	Artificial sea water with added blank extract
IX	Artificial sea water with added E 1 extract
X	Artificial sea water with added Clyde extract
XI	Artificial sea water—eggs in Celtic water between fertilization and first cleavage, then washed with artificial sea water
XII	E 1 surface water (carboy)—eggs in artificial sea water between fertilization and first cleavage, then washed with E 1 surface water

With so few left alive in the natural waters definite results are hardly to be expected. At most it can be said that slightly fewer larvae swam up in the sterile Sets II and III than in Set I, and the same thing can be said of Sets V and VI compared with Set IV, although Set VI was not actually sterile. These differences were very slight. There were no distinctions between Celtic and E 1 waters and the best larvae were poor stunted plutei which never properly grew arms. On the fifth day all those living were very abnormal everywhere and the experiment was abandoned.

There seems little doubt that the fertilized eggs had been incapable of normal development; they did badly in all waters. The experiment is, however, of value in that it indicates that the new type of extract is not poisonous, as had previously been the case with carbon and acetone extractions (Wilson & Armstrong, 1954, pp. 351, 355), and this may in future prove to be a useful method of investigating the main problem. It may be useful, too, to put on record that the method devised for obtaining sterile sea water at the time of collection was again successful, and on this occasion three times out of four.

EXPERIMENT 9

It was impossible to repeat again Expt. 8 the same year on account of the organization needed and the fact that no ship would be available to do the necessary collecting until after the end of the breeding season of *Echinus esculentus*. Indeed, for the time being no more sea-urchins could be got and so recourse was had to using *Sabellaria alveolata*, the larvae of which had previously responded to water differences, though not as readily as *Echinus* larvae (Wilson, 1951, p. 14). It seemed desirable at least to test the Celtic and the E 1 carboy waters used in Expt. 8 to make as sure as possible that the failure of that experiment should be attributed to the condition of the eggs and not to the waters.

On 24 April 1955 *Sabellaria alveolata* was collected at Duckpool, near Bude. On 27 April a fertilization (several males and females) was made in a mixture of the same Berkefeld filtered Celtic and E 1 carboy waters used in Expt. 8. After fertilization the eggs were divided equally into four beakers and each given several changes of one of the following four waters.

Set	
I	Aquarium water (unfiltered)
II	Celtic water
III	E 1 water
IV	Artificial sea water

The following day in each beaker all larvae which had swum to the surface were decanted off from unripe eggs on the bottom, and approximately equal quantities of these larvae distributed to the experimental dishes, three dishes in each set.

Until 30 April no significant differences were observed between any of the sets. On that day the larvae in the artificial sea water, which was an unused quantity remaining over from Expt. 8, were seen to be swimming more slowly than in the other sets, and they were not so well developed or as large as the larvae in the natural sea waters. This difference persisted until the experiment was abandoned a week later, by which time larvae in the artificial sea water were losing spines and many were dead. Throughout this time no differences appeared between the other sets of larvae, they developed well for a time and until checked by lack of food.

From these observations it appears that the Celtic and E 1 carboy waters were almost certainly not responsible for the high mortality in Expt. 8. It is also interesting to note that whereas the *Echinus* eggs in the two previous experiments had in artificial sea water failed to produce anything resembling a normal pluteus, the *Sabellaria* eggs in the same artificial sea water developed into larvae structurally normal so far as could be observed, except that they were smaller than those in the natural waters and swam more slowly.

DISCUSSION

The factor which has hampered our experiments more than any other has been the weather. Much initial planning and much careful preparation has been wasted and several experiments have been largely ruined because storms at critical times have prevented the collection of water samples. Storms also had their effect on the collection of *Echinus*, and as in 1955 gales in the weeks preceding the breeding period may, or so it appears, have an adverse effect on the numbers of sea-urchins present on the local grounds and perhaps even on the maturity of those still left there. It was particularly unfortunate, in this connexion, that, when, as for Expt. 8, a number of bacteria-free samples of water had been successfully collected from two localities the fertilization, at first seemingly excellent, failed to produce good larvae in any water, apparently because the zygotes were incapable of development beyond early cleavage, it being unlikely that the natural waters themselves were to blame (see Expt. 9). In the face of such hindrances by natural forces progress in this type of work is likely to continue to be very slow.

It is perhaps not surprising that bottom and surface waters at the same station should affect the developing eggs differently, for it is normal for such waters to be distinguishable chemically. What is surprising is that bottom water should often have proved harmful, especially when the eggs were exposed to it for only a short period following fertilization. It is at this period that eggs would normally be in bottom water after having been shed. It may be that this effect is due to experimental conditions, such as rapidly substituting one water for another. It can hardly be a temperature effect because all waters used were brought to a uniform temperature. If it is real it is a pointer to the possibility that bottom animals may spawn only when the water

in which they are bathed is suitable for development. In one experiment the bottom water was as good as, if not better than the surface water.

These results and those with mud extracts may be linked with the need of the developing echinoderm egg and larva to absorb substances from the surrounding medium (Needham, 1931) and it may be necessary for some of these substances to be absorbed very soon after fertilization. The experiments do not indicate that there is anything in filtrates (which contained some natural sea water) from diatom or flagellate cultures, or from culture medium itself, which can supply those needs.

The most interesting criticisms (Walne, 1956, and private discussions with others) which have arisen in the course of our work concern the possible effects of bacteria on the waters collected during the inevitable storage period between collection and use, and of excessive bacterial growths in the experimental dishes during the course of the experiments*. Moreover, these criticisms seemingly always imply that such bacteria produce harmful effects and it does not appear to have been envisaged that they may sometimes be beneficial, if not essential. In saying this we do not wish to imply that we believe that we have any real evidence that bacteria are beneficial: we approach the problems posed by their presence with completely open minds. None the less we do believe that differing bacterial populations would be no more than another demonstrable difference between disimilar water masses and not necessarily in themselves the basic cause of those differences. For the basic cause we must look even further back, behind the bacteria.

In our experiments we have, as recorded in the preceding pages and in an earlier paper (Wilson & Armstrong, 1954), endeavoured to filter water free from bacteria at the time of collection and to compare larvae reared in such water with those reared in water collected at the same time in the usual manner, using as a filter nothing finer than bolting-silk. A modicum of success has attended these efforts but the results obtained are open to varying interpretation. However, so far as they go they do not support the view that bacteria in the water between collection and use make it less suitable for the larvae reared in it: on the contrary such slight differences as were shown between bacteria-free samples and their corresponding waters collected in the ordinary way (carboy waters) were usually in favour of the latter. It is possible that the slightly unfavourable results obtained with the bacteria-free waters are explicable by contact of the water with the previously heated rubber tubing through which it was passed and from out of which substances may have dissolved; also water stored in a Winchester has a larger surface area of glass in contact with it, volume for volume, than has water in a carboy. In future experiments these details should be equalized on both sides. Even

* See also Walne, 1958 (this Number, p. 415). Although Walne's latest paper was not seen by us until after this discussion had been written, we feel that it already answers the chief points raised by Walne.

as it was, the difference between the two samples of one kind of water collected in the two ways was always much less than the differences which have often been observed between waters from separate localities.

Our bacteria-free waters were, of course, free from bacteria only until they were poured out into the experimental dishes and the eggs put into them. Presumably, at that time they had a smaller population of bacteria than did the corresponding carboy-collected water, although perhaps not so much smaller because this latter water was itself passed through filter-candles not many hours before use. Walne (1956) has recently shown how in vessels each containing 1 l. of sea water twice filtered through filter-candles, and to which were added a few ml. of concentrated *Isochrysis* culture together with several hundred oyster larvae, the concentration of bacteria increased during the first 3 or 4 days of the experiments. Presumably more or less the same thing must have happened in our dishes, but no bacterial counts were made. Certainly if some of the larvae died after a few days the water in the dishes became slightly cloudy, but it is difficult here to distinguish between cause and effect: did the bacteria increase because larvae died and decayed, or did the larvae die because the bacteria increased? Walne found that in five of his six successful rearings the bacterial peak numbers were markedly lower than in his unsuccessful rearings, and suggests that the bacteria may have been influencing the successful development of his larvae, although there was no definite proof of this. He concludes that 'differences which have been observed in the value of different natural waters for rearing larvae may have their origin in the variation in the size of the bacterial flora which the water can support'. In other words the effect on the larvae of water differences is indirect via the bacteria, but it is none the less true that the waters themselves have different properties, though it may be that these only affect larvae in the confines of a dish where the bacterial population per unit volume rises higher than is possible in the open sea. Whilst this could be so it should be remembered that real changes of fauna have taken place in areas where there have been known changes of water masses and, in default of any more reasonable explanation, it would be rash to conclude that there is no real relation between the inability of water in a dish to support larvae and the larval supporting capacity of the same kind of water in the sea.

Some observations which may have a bearing on this question of the effect of excessive bacterial growths in dishes of developing larvae were mentioned in a previous paper (Wilson & Armstrong, 1954, p. 357). The antibiotics penicillin and streptomycin in various concentrations added to the water had no definite effects on the larvae, but as no bacterial counts were made it is not known whether they had any effect in reducing the bacterial flora. It may perhaps be assumed that they had, for Spencer (1952, p. 100) states that at the concentration of penicillin we used bacterial growth in peptone sea water was inhibited for many days.

Whether the bacteria are the directly responsible agents for the observed structural defects in larvae reared in unfavourable waters or not, it cannot be too strongly emphasized that the effects on the larvae are but a reflexion of real differences between the waters themselves, though of what those basic differences are we are still ignorant. In any experiment in which waters are compared, natural sea waters treated or untreated, or artificial sea waters, nothing is more striking than the uniformity of appearance in structure and movement of the larvae in all the dishes containing the same kind of water. Only on very rare occasions during the first days is there an odd dish not in conformity with the others of the same set. Larvae in another set of dishes containing a different sort of water may or may not look different from these, but if any one dish of that set is at variance so are all the rest and in the same way. Therefore we cannot but conclude that the larvae are affected structurally, and in other ways, by the water in which they develop and that we can use these larvae as test objects, though we do not as yet know what properties of the water we are testing with them, or how far we can safely apply our results to what happens naturally in the open sea.

Of the many experiments we have made we believe that the tests with mixed waters (Wilson, 1951, Expts. 5 and 8A; Wilson & Armstrong, 1952, Expt. 1) are especially significant. It will be recalled that when good and bad waters were mixed in approximately equal proportions the larvae developing in the mixtures were closely similar to those in good waters alone and much better than those in the bad waters. Any hypothesis advanced to explain our results must account for this.

SUMMARY

In 1954 and 1955 various natural, artificial and treated sea waters were tested with developing eggs of *Echinus esculentus*. The following are the main observations:

1. Sea waters filtered through Doulton candles at the time of collection and proved bacteriologically sterile until use were very little different from sea waters collected at the same time in the ordinary way and not sterile. (Expts. 1, 3, 8 and, with qualifications, 6.)

2. Undiluted Celtic sea bottom water and undiluted Celtic mud 'extract' were harmful to the eggs, but small volumes of both added to natural sea waters and to artificial sea water had a stimulating effect. (Expt. 1.)

3. In water from near the bottom at E1 eggs did worse, in 1954, than in surface water from the same locality. In 1955 they did slightly better in the deeper water. (Expts. 3, 4 and 7.)

4. Eggs placed immediately after fertilization in water from near the bottom at E1 (and from near the bottom at the *Echinus* ground) and transferred at first cleavage to surface waters were affected unfavourably. (Expts. 3, 4 and 7.) Artificial sea water had no such effect. (Expt. 5.)

5. The addition to natural and to artificial sea waters of filtrates from culture medium ('erdschreiber') and from thick cultures of diatoms and flagellates had no significant effects on the eggs developing in those waters. (Expt. 6.)

6. In 1955 surface water from the Clyde was a better medium for development than surface water from E 1. The difference was similar to that frequently observed several years previously. (Expt. 7.)

7. Extracts of natural sea waters made by a new process were non-poisonous, but the experiment in which they were added to artificial sea water was inconclusive owing to the eggs proving to be of poor quality. (Expt. 8.)

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SOME OBSERVATIONS ON *EVADNE NORDMANNI* LOVÉN

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(Plate I and Text-figs. 1–6)

Apstein (1910) found *Evadne nordmanni* to be the most widely distributed and in general the commonest cladoceran in the plankton of the sea areas around the coasts of Europe investigated by the International Council. It is known to occur predominantly in coastal waters and has been regarded mainly as a neritic species in the past, but considering recent records Wiborg (1955) suggests that it may also be able to establish populations in the open ocean.

Despite its importance in the marine plankton our knowledge of the biology of this species is meagre compared with that of the common freshwater Cladocera. Rammner (1930) has reviewed the earlier investigations on *E. nordmanni* and other marine species. More recent studies include those of Jorgensen (1933) and Cheng (1947), while Baker (1938) gives a detailed account of the taxonomy of marine Polyphemidae.

In view of the often inadequate material available to previous workers for the study of seasonal changes in one locality, it was decided to follow the population of *Evadne* at a station in the Clyde Sea Area during 1951 with particular attention to fluctuations in abundance, size and the mode of reproduction.

Evadne reproduces mainly by parthenogenesis, in which case the eggs are laid into the brood pouch and develop there into free-swimming young. Often some of the females carry no developing embryos but show the formation of usually one resting egg. Males are normally present in the population on these occasions and it has been generally assumed that resting eggs develop as a result of sexual reproduction. This is known to be ordinarily the case in freshwater Cladocera, with the exception of some arctic populations of *Daphnia* which produce unfertilized resting eggs.

MATERIAL AND METHODS

Quantitative plankton samples were collected at a station near the Little Cumbrae lighthouse, where the depth is between 90 and 105 m. The samples were taken with a Clarke–Bumpus plankton sampler (Clarke & Bumpus, 1940) in a series of horizontal tows, each of 10 min duration, at average depths of 1, 10, 20, 30 and 50 m. An additional tow at 40 m was made on some

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occasions. Unfortunately, it was not possible to tow at a constant depth, the angle of the towing wire varying between 30 and 45° to the vertical. The sampler was used fitted with a net of 200 meshes to the inch, until 7 May when it was replaced by one of 129 meshes to the inch. The volume of water filtered during a single haul varied between 2 and 4 m^3.

Additional tow-nettings were frequently taken off Keppel Pier at Millport using larger nets of the same mesh size, and data obtained from these samples have been used when *Evadne* were scarce towards the end of the year.

All samples were preserved in 5% formalin. When necessary samples were subsampled for examination and counts using the dilution method of Russell & Colman (1931).

All measurements were made with an eyepiece micrometer on which each division represented 9·7 μ. Embryo numbers were counted after dissecting the embryos out of each parthenogenetic female.

Baker (1938) has discussed the various points of reference which have been used for the measurement of marine Cladocera and has given diagrams to illustrate what she calls the morphological length. This dimension is useful to compare the size of different species, but demands some personal judgement to determine the anterior point on the margin of the head.

In this investigation lengths have been measured from the point of attachment of the antennal elevator muscles on the crown of the head to the tip of the claws on the caudal furca (see Pl. I). This measurement approximates to the greatest length of the body excluding the brood pouch. In a very small proportion of the preserved *Evadne* there was a slight depression at the point of attachment of the antennal muscles, and measurements were adjusted to correct for this by noting the body outline on each side of the depression. The short caudal furca is capable of a slight amount of movement, but is set at a nearly constant angle to the rest of the body in preserved specimens.

VERTICAL DISTRIBUTION

The investigations made by Wiborg (1940, 1944, 1954) on the plankton of Norwegian coastal waters have shown that *Evadne* is mainly to be found living near the surface.

This was also found to be true in the Clyde. All the samples were collected during the daytime and the numbers of *Evadne* at each depth are given in Table 1. Very few occurred in hauls taken at depths greater than 30 m and, with one exception, greatest numbers were found at either the 1 or 10 m levels.

SEASONAL DISTRIBUTION

In a survey of the Clyde plankton during 1923 and 1924 Marshall (1925) records that *E. nordmanni* was common in May and June, fairly common till September and frequent in October. From a study of tow-nettings taken in

the North Sea off the Northumberland coast during the years from 1921 to 1930 inclusive, Jorgensen (1933) found that the numbers of *Evadne* usually showed an early summer maximum followed by a less extensive one during September or later. She also noted the presence of males during these periods and that the numbers of resting eggs produced were roughly proportional to the numbers of individuals present.

TABLE 1. NUMBER OF *EVADNE* PER CUBIC METRE

Date 1951	1 m	10 m	20 m	30 m	40 m	50 m
9. iii.	17	9	—	—	—	—
13. iii.	6	7	0	—	—	—
29. iii.	29	27	3	0	—	0
13. iv.	36	269	30	0	—	0
19. iv.	89	85	15	1	—	0
25. iv.	91	232	31	0	—	0
7. v.	1380	64	16	0	—	0
24. v.	375	1325	269	19	—	30
29. v.	428	465	168	51	60	64
7. vi.	211	1636	672	150	—	0
14. vi.	402	359	29	0	—	—
20. vi.	422	766	177	44	—	3
29. vi.	49	764	122	26	—	1
2. vii.	511	143	25	0	—	0
11. vii.	269	278	244	45	1	2
19. vii.	209	131	10	2	0	0
24. vii.	121	126	27	0	0	0
1. viii.	91	252	67	9	0	0
7. viii.	60	138	5	0	0	0
17. viii.	111	130	350	36	4	0
3. ix.	15	4	0	0	0	0
13. ix.	0	1	0	0	0	0
17. ix.	5	0	0	0	0	0
25. ix.	5	1	0	0	0	0
11. x.	16	2	1	0	0	0
24. x.	3	2	0	0	0	0
7. xi.	0	0	0	0	0	0

— No sample taken.

Text-fig. 1 shows the average numbers of *Evadne* per cubic metre in the upper 30 m at the station in the Clyde during 1951 and also the percentages of sexual individuals in the population (i.e. males, and females showing the formation of a resting egg).

Evadne was first observed in the plankton at the end of February; quantitative sampling was begun on 9 March and continued until 7 November. The population increased reaching a maximum from May to early June and declined during the following months to a very low density in September. There was a slight recovery during October, but the species was absent from all the samples taken in November and December. The first appearance of sexual individuals coincided with the marked increase in numbers during May and sexual reproduction became most intense in October.

There was, therefore, no clear development of a second maximum and the percentage of sexual individuals showed no relationship to the population density.

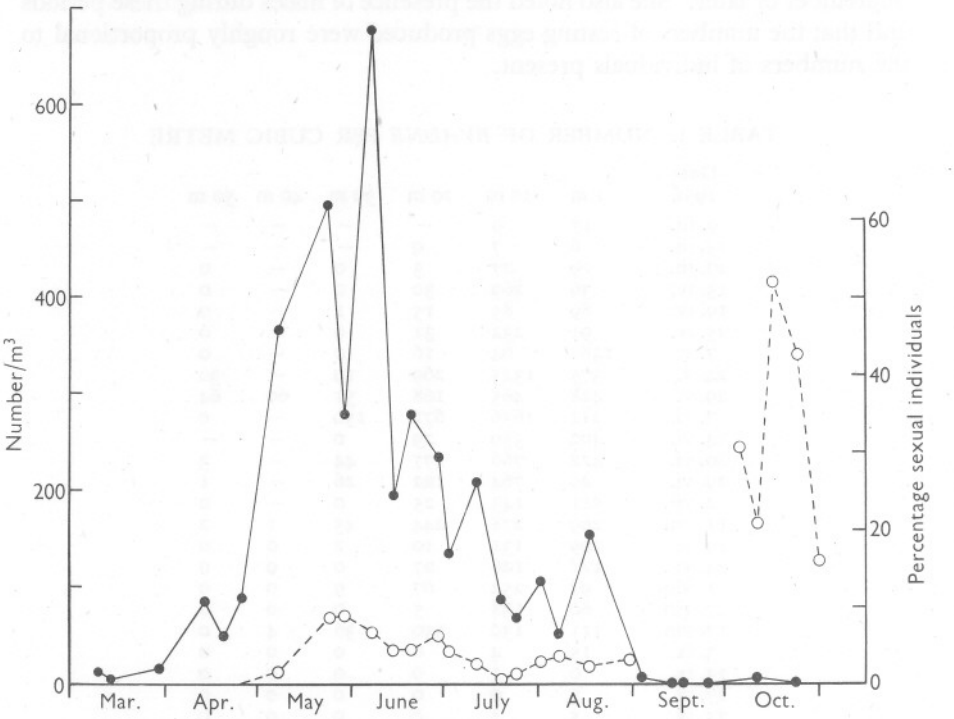

Fig. 1. Mean number of *Evadne* per cubic metre in the upper 30 m during 1951 (●——●), and the percentage of sexual individuals in the population (○– – –○).

GROWTH AND REPRODUCTION

Kuttner (1911) studied the development of the parthenogenetic eggs of *E. nordmanni* and found that when embryos reach an advanced stage of development and show the first traces of eye pigmentation, their own eggs mature and pass into the embryonic brood space. By the time the young are liberated from the brood pouch of the mother these eggs have segmented to a blastula stage with a large cleavage cavity. The young are in effect miniature adults.

Attempts were made to rear individual animals in order to determine the usual number of instars between birth and the liberation of the first brood of young, but proved unsuccessful. Two of the major problems are that *Evadne* tends to stick to any water-air surface film and since it is not a filter-feeder conditions suitable for feeding are difficult to produce.

In order to provide some information on the relation between size and

reproductive state two large samples of *Evadne* were examined. The length of each individual was measured and the stage of embryonic development in the parthenogenetic females noted. Two easily recognizable stages have been selected and are arbitrarily classed as early and late stages. They are defined as follows:

Early. Embryos roughly spherical, though sometimes distorted owing to being pressed together in the brood pouch. They show no signs of elongation or the appearance of the second antennal rudiment.

Late. Embryos at an advanced stage of development and showing at least the first traces of eye pigmentation. All the limbs are present and the dorsal pouch is developing so that the embryo lies on its side when removed from the brood pouch.

The results obtained from the two samples are shown as percentage size-frequency distributions in Text-fig. 2 and the stages referred to are illustrated in Pl. I.

Text-fig. 2A shows the size distribution of all parthenogenetically reproducing females and Text-fig. 2B the size distribution of those with embryos at the stages of development described above. Primiparae carrying early embryos can be clearly distinguished from older females with a second or subsequent brood of early embryos on the basis of size, shape of the carapace and position of the embryos within the dorsal pouch formed by the carapace (Pl. I, figs. 1, 4). In view of the considerable overlap in sizes it proved impossible to separate females with late-stage embryos (Pl. I, fig. 2) into primiparae and older individuals. The striking change in the outline of the brood pouch of females following the emergence of young is illustrated in Pl. I, figs. 3, 4.

Text-fig. 2C shows the size distribution of males (Pl. I, fig. 5) and females producing a resting egg (Pl. I, fig. 6), presumably after fertilization. When present, these sexual females are among the largest individuals in a sample; they fall within a higher size range than the parthenogenetic females with late-stage embryos, and more closely correspond in size distribution to females with second or subsequent broods of early-stage embryos. This is shown only in the sample collected on 29 May since, when sexual reproduction is intense as on 5 October, older females with early embryos are very scarce. At their birth young females are carrying developing embryos and it would appear that at least one brood of young is first liberated before a resting egg can be produced.

Rammner (1930) noted the large size of the sexual females, but since they differ from the parthenogenetic females in the nature of the brood-pouch epithelium and have a carapace opening, he was of the opinion that they must be large at birth and do not first produce a parthenogenetic brood.

Because of the size range of sexual females and the fact that all embryos nearing the stage of liberation can be distinguished either as males or parthenogenetic females with blastulae, Rammner's suggestion is untenable. A new

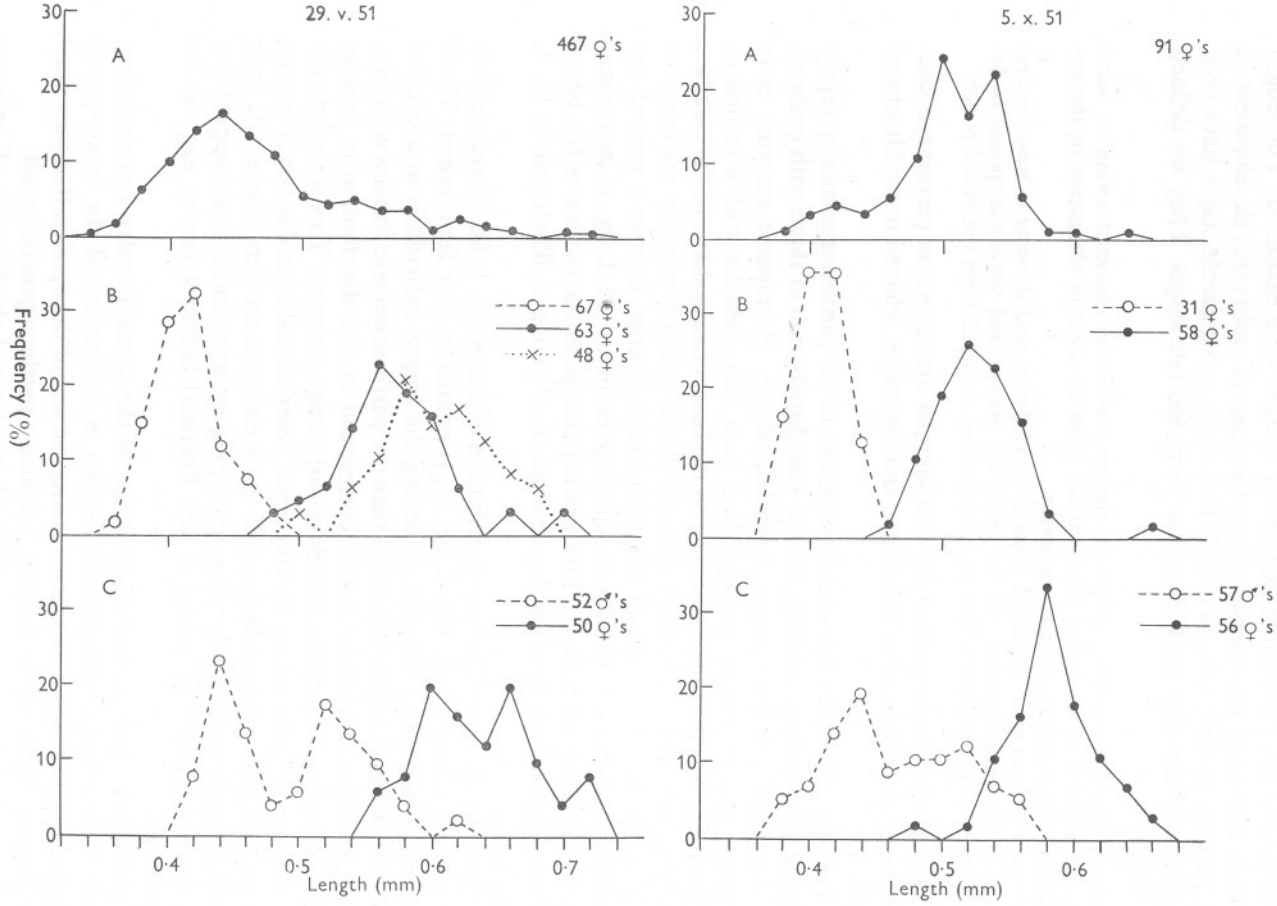

Fig. 2. Percentage size-frequency distribution of *Evadne*. A: parthenogenetic females. B: primiparae with early-stage embryos (○– – –○), females with late-stage embryos (●——●), and females with a second or later brood of early-stage embryos (× · · · · ×). C: males (○– – –○), and females showing the formation of a resting egg (●——●).

carapace is formed at the liberation of a brood of young as shown in Pl. I, fig. 3, and the carapace opening might possibly appear at this time. The shape of the carapace of females showing the first signs of resting-egg formation is also very similar to that of females with second or following broods of early embryos.

The fate of mature resting eggs can only be surmised. Jorgensen (1933) noted cast exoskeletons containing resting eggs in the plankton on several occasions, but these were never observed in the Clyde plankton. Nor, in fact, were any females with mature resting eggs ever found showing signs of an incipient moult. It therefore seems possible that the majority sink to the bottom after the death of the parent.

EMBRYO NUMBER AND SIZE

Cheng (1947) found that larger female *Evadne* generally have more embryos than smaller females. His results cannot, however, be accepted as conclusive, since he groups together individuals from several samples collected over a considerable period. Increased egg production with an increase in body size has been demonstrated in several species of *Daphnia* and other freshwater Cladocera by Green (1954, 1956).

In Text-fig. 3 the number of embryos is plotted against maternal length in two large samples. The means, standard deviations and ranges for both length and embryo number of females with embryos at the two stages defined are also shown.

The mean embryo numbers in the sample collected on 29 May are roughly constant in the lower size-groups, which represent only primiparae and increase in the higher size-groups, since these include older females with early-stage embryos.

In the sample taken on 9 March the embryo number shows a decrease with increase of size, individuals with late-stage embryos having fewer than the primiparae with early-stage embryos. This is at least partly due to a proportion of the embryos present at birth failing to develop, since disintegrating embryos and undifferentiated patches of tissue could be clearly seen in the brood pouches of some females. In preserved specimens these abnormal embryos are darker in colour, very fragile and occasionally recognizable as being at an earlier stage of development than normal embryos in the same brood pouch. As in other Polyphemidae the eggs of *Evadne* contain very little yolk, development depending on nourishment supplied by the mother through the fluid bathing the embryos in the closed brood pouch (Kuttner, 1911; Rammner, 1930). The loss of embryos could therefore be due to the effect of adverse conditions on the parents.

The apparent disintegration and resorption of embryos was observed in the samples collected 9 March, 13 April, 14 June and 30 October. Text-fig. 5B

shows that in many of the samples collected during the year the brood number of females with late-stage embryos was smaller than that of the primiparae with early embryos, despite the fact that the former group will include a small and unknown proportion of individuals producing a second or even later brood.

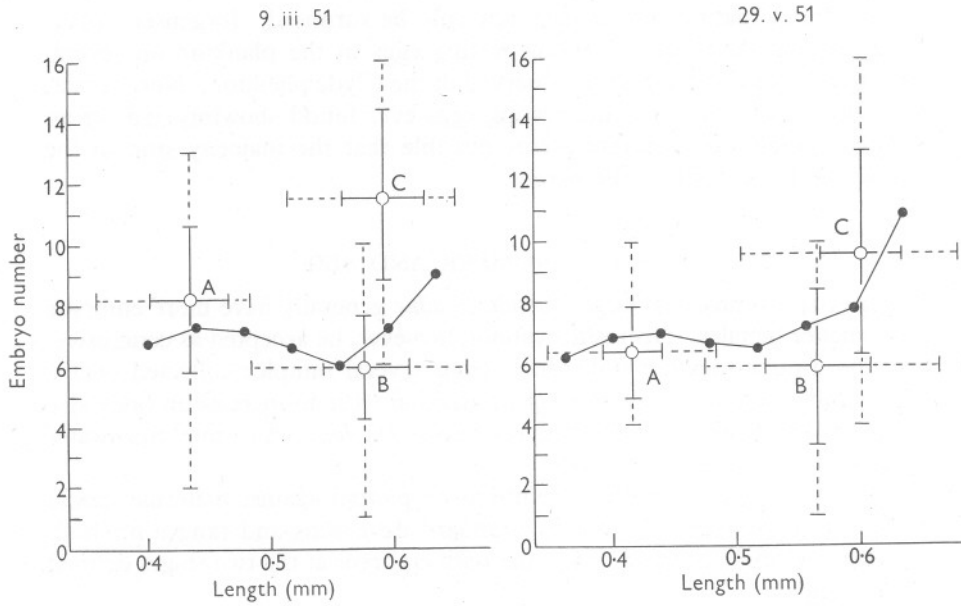

Fig. 3. Embryo number and size. Also shown are the means, standard deviations and ranges for embryo number and size of primiparae with early-stage embryos (A); females with late-stage embryos (B); females with a second or later brood of early-stage embryos (C).

Females with second and subsequent broods of early-stage embryos were very scarce in some of the samples and insufficient were obtained for the embryo numbers to be plotted on a seasonal basis. Their embryo numbers do, however, tend to fluctuate with those of females with late-stage embryos (Text-fig. 4).

It is clear that the best measure of the average production of young per brood can be obtained when only those individuals with late-stage embryos are considered.

Although the number of late-stage embryos is very variable and the range of sizes is rather small, larger individuals tend to produce larger broods of young. This is shown in Table 2 for several samples selected to cover a wide range of reproductive capacities.

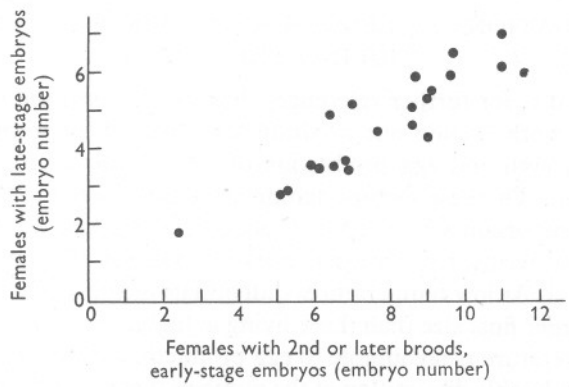

Fig. 4. Scatter diagram showing the relation of the mean embryo number of females with late-stage embryos and the mean embryo number of females with a second or later brood of early-stage embryos for each sample.

TABLE 2. SIZE AND EMBRYO NUMBER OF FEMALES WITH LATE-STAGE EMBRYOS

Date 1951	Length (mm.)*	No. examined	No. of embryos		
			Max.	Min.	Mean
9. iii.	0·49	4	6	2	4·0
	0·54	25	8	1	5·2
	0·59	36	10	3	6·4
	0·64	9	9	5	7·2
13. iv.	0·54	4	4	2	3·0
	0·59	20	6	2	3·4
	0·64	18	6	2	3·5
	0·69	6	5	1	3·7
	0·74	2	5	1	3·0
29. v.	0·49	8	8	2	5·1
	0·54	24	9	1	5·2
	0·59	27	10	1	6·5
	0·64	2	10	5	7·5
	0·69	2	10	7	8·5
7. vi.	0·49	11	5	1	2·1
	0·54	28	8	1	3·2
	0·59	5	6	2	3·8
	0·64	1	—	—	5·0
5. x.	0·44	1	—	—	2·0
	0·49	22	7	1	3·7
	0·54	31	7	3	5·1
	0·59	3	8	6	7·0
	0·64	1	—	—	5·0
31. x.	0·49	14	4	1	2·7
	0·54	18	5	1	3·2
	0·59	6	6	1	4·1
	0·64	2	6	5	5·5

* Class mid-point.

FLUCTUATIONS IN LENGTH AND EMBRYO PRODUCTION DURING THE YEAR

Green (1956, q.v. for further references) has summarized the main results of experimental work carried out to study the effect of environmental factors influencing growth and egg production of several species of the freshwater genus *Daphnia*. Of these factors, temperature and food supply are likely to be the most important which affect Cladocera living near the surface of the sea. In general terms, experimental work on cultures of *Daphnia* has shown two features: (i) At low temperatures individuals increase in size more slowly, but reach a larger final size than those living at higher temperatures. A certain range of temperature is favourable to egg production, above and below which there is a considerable diminution of the number of eggs produced. (ii) Starvation both decreases growth and reduces egg production. Green (1956) gives evidence that it also reduces egg size, which in cladocerans that do not secrete a nutrient fluid is directly related to the size of young at birth.

The samples of *Evadne* from the Clyde Sea Area during 1951 show that there were considerable fluctuations in size and the production of young. Text-fig. 5 shows the changes in the mean length and embryo number of females with late-stage embryos and primiparae with early-stage embryos. The mean lengths of these two classes of females show similar fluctuations and there is a notable fall from the end of May to late July. During this same period the mean number of late-stage embryos showed initially a sharp decline followed by a more steady rise. In early August, shortly after the recovery of embryo production, there was an increase in mean lengths, though they never attained their former level.

The seasonal variation in length of the two classes of females indicates that the size at which females had embryos nearing the stage of liberation was mainly dependent on their own size at birth, and that there were not usually any pronounced changes in the growth increment. A possible explanation of the relationship between fluctuations in the length of females and numbers of late-stage embryos produced, is that both are to a certain extent influenced by the same environmental conditions, but that changes in the size of young at birth are more gradual and lag behind fluctuations in the number of young produced per brood.

The fluctuations described can be correlated with surface sea temperatures at Keppel Pier, Millport, shown in Text-fig. 5c. In March, April and May females were larger than at any other time during the year. This may possibly be related to the lower sea temperatures during the spring, though otherwise there are no clear connexions since a partial recovery of size occurred during August when the temperature was about at a maximum for the year.

Both length and embryo number were better correlated with the amount of microplankton caught by the net, suggesting that food supply may be

the more important factor. The greatest quantities of microplankton occurred from 13 April to 24 May and again from 24 July to 17 October. Details of the relationship to food supply will be discussed in a following section.

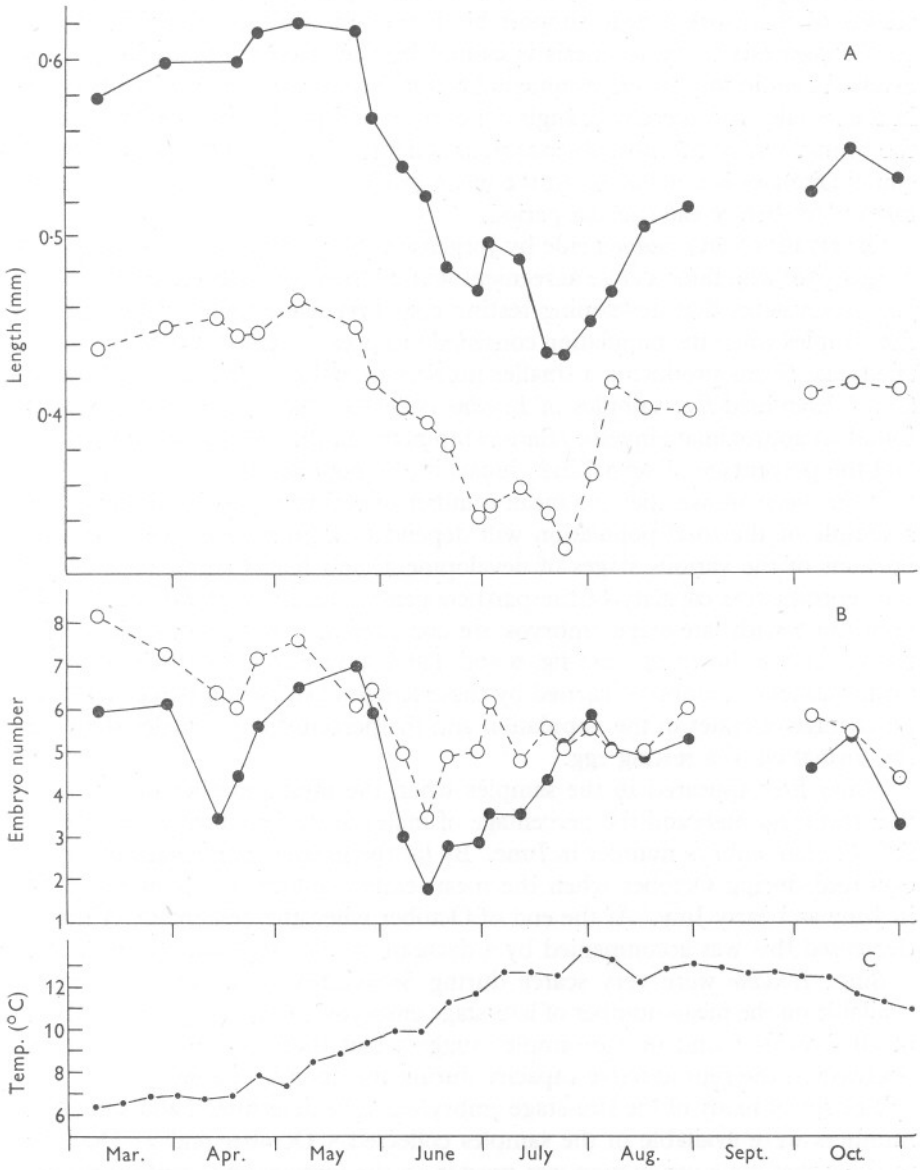

Fig. 5. Fluctuations in mean length (A), and mean embryo number (B), of primiparae with early-stage embryos (○- - -○), and females with late-stage embryos (●——●) (C). Surface sea temperatures at Keppel Pier, Millport, as 8-day means.

PARTHENOGENETIC AND SEXUAL REPRODUCTION

Berg (1931) carried out a detailed study of changes in the mode of reproduction of several species of *Daphnia* both in natural populations and cultures. The results of this work clearly support his hypothesis that the transition from parthenogenesis to gamogenesis is caused by the influence of unfavourable external conditions, the environmental factors producing a state of depression in the females and thereby changing the mode of reproduction and the sex of the young. One of the most obvious signs of depression shown by the parthenogenetic females is a reduction in the mean number of eggs produced and this takes place before each sexual period.

Observations on *Evadne* made by Jorgensen (1933) have been discussed by Berg (1936) who finds a close agreement with his own on freshwater daphnids. Jorgensen notes that developing resting eggs first made their appearance in the samples when the population consisted to a great extent of less bulky more triangular forms producing a smaller number of embryos than usual. Cheng (1947) examined five samples of *Evadne* collected during two summers and found an approximate inverse relationship between the mean embryo number and the percentage of sexual individuals in the population.

It has been shown that the mean number of embryos per female found in a sample of the total population will depend to a great extent on the proportions of the various stages of development, and that a better measure of the reproductive capacity of the parthenogenetic females can be obtained if only those with late-stage embryos are considered. The results obtained in the Clyde are shown in Text-fig. 6 and Table 3, in which fluctuations in the mean number of embryos carried by these females can be compared with the percentage of males in the population and the percentage of females showing the formation of a resting egg.

Males first appeared in the samples when the mean number of embryos was relatively high and the percentage of males decreased during the drastic fall of mean embryo number in June. By far the greatest percentage of males occurred during October when the mean embryo number was higher than in June and early July. At the end of October when the percentage of males decreased this was accompanied by a decrease in the mean embryo number.

Since *Evadne* were very scarce during September no adequate data are available on the mean number of late-stage embryos, but the embryo numbers of all females found in the samples suggest that there was no pronounced decrease in the reproductive capacity during this month (Table 3).

The sex of many of the late-stage embryos can be determined and sufficient numbers were available in the samples collected 5 October and 31 October to show that male production was greater on the former date, confirming the evidence of the different percentage of males in the two populations (Table 4).

These results do not admit a simple explanation on the basis of Berg's

depression hypothesis. It is possible, however, that the first appearance of males in the samples is related to a period of depression indicated by the lower mean embryo numbers found in previous samples during April. Later fluctuations in the percentage of males might also be accounted for if it was considered that moderate depression favours greater male production than severe

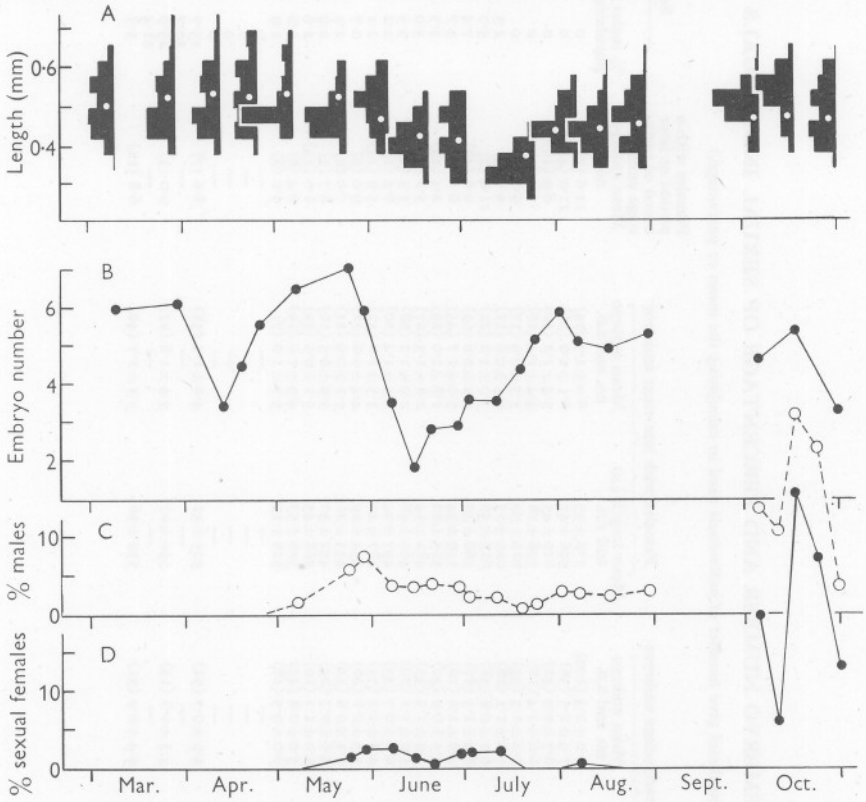

Fig. 6. A: percentage size-frequency distribution of females on some of the dates of sampling. The open circles within the histograms indicate the intermediate size between the means for primiparae with early-stage embryos and females with late-stage embryos. B: fluctuations in the mean number of late-stage embryos. C: percentage of males in the population. D: percentage of females showing the formation of a resting egg (sexual females).

depression. To explain the findings of Stuart, Cooper & Coady (1933) that under conditions of severe depression female *Moina* become what they term 'sex fast' and produce female young only, Berg (1936) suggests that severe depression might have a different effect from moderate depression. It is not, however, known whether sex-fast females occur in natural populations of freshwater Cladocera.

Fluctuations in the percentage of females showing the formation of a resting egg follow those of the males, but will give a distorted picture of

TABLE 3. LENGTH, EMBRYO NUMBER AND PERCENTAGE OF SEXUAL INDIVIDUALS

(Figures in parentheses give number of individuals used in calculating the mean or percentage.)

Date 1951	Parthenogenetic females. Mean embryo no.	Primiparae with early-stage embryos		Females with late-stage embryos		Females with a second or later brood of early-stage embryos. Mean embryo no.	Sexual individuals	
		Mean length (μ) and S.D.	Mean embryo no. and S.D.	Mean length (μ) and S.D.	Mean embryo no. and S.D.		% males in population	% females with a resting egg
9. iii	7·1 (344)	436 ± 31	8·2 ± 2·4 (102)	578 ± 35	6·0 ± 1·7 (74)	11·6 (38)	0	0 (800)
29. iii	7·6 (97)	449 ± 22	7·3 ± 1·5 (36)	598 ± 41	6·1 ± 2·0 (16)	11·0 (4)	0	0 (510)
13. iv	5·5 (95)	454 ± 29	6·3 ± 0·9 (32)	622 ± 45	3·4 ± 1·2 (50)	6·9 (10)	0	0 (303)
19. iv	5·7 (97)	443 ± 26	6·0 ± 1·4 (18)	598 ± 18	4·4 ± 1·4 (16)	7·7 (4)	0	0 (202)
25. iv	6·7 (133)	445 ± 28	7·1 ± 1·3 (33)	616 ± 30	5·5 ± 0·9 (17)	9·1 (6)	0	0 (344)
7. v	7·0 (77)	464 ± 22	7·6 ± 1·1 (28)	620 ± 27	6·5 ± 0·8 (11)	9·7 (4)	1·5	0 (198)
24. v	6·4 (119)	448 ± 23	6·1 ± 0·8 (38)	616 ± 36	7·0 ± 1·2 (22)	11·0 (2)	5·7	1·4 (846)
29. v	7·0 (467)	417 ± 24	6·4 ± 1·5 (67)	566 ± 42	5·9 ± 2·6 (63)	9·6 (48)	7·4	2·3 (876)
7. vi	4·4 (130)	404 ± 36	4·9 ± 1·2 (16)	539 ± 34	3·0 ± 1·7 (45)	6·1 (9)	3·7	2·5 (539)
14. vi	2·7 (99)	395 ± 17	3·5 ± 0·9 (30)	525 ± 22	1·8 ± 1·0 (23)	2·4 (14)	3·5	1·4 (736)
20. vi	4·6 (127)	382 ± 32	4·9 ± 1·3 (35)	482 ± 34	2·8 ± 1·3 (27)	5·1 (10)	3·9	0·4 (908)
29. vi	5·4 (90)	341 ± 27	5·0 ± 1·1 (20)	469 ± 48	2·9 ± 1·1 (28)	5·3 (3)	3·5	1·9 (693)
2. vii	5·7 (107)	348 ± 33	6·1 ± 0·7 (23)	497 ± 34	3·5 ± 1·4 (20)	6·5 (12)	2·2	1·9 (495)
11. vii	4·6 (113)	359 ± 38	4·8 ± 1·2 (33)	487 ± 40	3·5 ± 1·8 (31)	5·9 (14)	2·1	0·7 (418)
19. vii	5·7 (99)	344 ± 37	5·5 ± 0·8 (28)	434 ± 29	4·4 ± 0·8 (21)	9·0 (5)	0·7	0 (137)
24. vii	5·2 (91)	324 ± 22	5·1 ± 0·8 (31)	433 ± 26	5·2 ± 0·9 (21)	7·0 (2)	1·2	0 (264)
1. viii	5·7 (106)	366 ± 37	5·6 ± 0·7 (23)	452 ± 30	5·9 ± 0·5 (16)	8·7 (3)	2·9	0 (204)
7. viii	5·5 (103)	417 ± 43	5·0 ± 1·1 (19)	468 ± 45	5·1 ± 0·9 (33)	8·6 (13)	2·6	0·5 (188)
17. viii	4·9 (107)	403 ± 32	5·0 ± 0·8 (43)	506 ± 55	4·9 ± 1·0 (19)	6·4 (8)	2·2	0 (368)
30. viii	6·1 (126)	401 ± 24	6·0 ± 0·5 (18)	518 ± 19	5·3 ± 1·0 (7)	9·0 (8)	2·9	0 (177)
3. ix	6·0 (45)	—	—	—	—	—	0	0 (45)
13. ix	5·0 (2)	—	—	—	—	—	0	0 (2)
17. ix	5·8 (8)	—	—	—	—	—	0	0 (8)
25. ix	5·7 (12)	—	—	—	—	—	0	0 (15)
5. x	5·4 (103)	411 ± 16	5·9 ± 0·7 (31)	525 ± 31	4·6 ± 1·1 (58)	8·6 (7)	13·7	19·6 (490)
11. x	7·3 (28)	—	—	—	—	—	10·7	6·0 (56)
17. x	5·8 (102)	418 ± 13	5·5 ± 0·8 (15)	550 ± 27	5·4 ± 1·8 (27)	9·0 (3)	25·9	35·6 (216)
24. x	5·8 (17)	—	—	—	—	—	21·4	27·2 (28)
31. x	4·3 (107)	414 ± 20	4·4 ± 0·9 (31)	532 ± 36	3·3 ± 1·7 (40)	6·8 (11)	3·5	12·9 (136)

changes in the intensity of sexual reproduction, since from the size distribution of whole populations it is clear that there is a considerable variation in the number of primiparae present on the different dates of sampling and these do not produce resting eggs.

Changes in the percentage size distribution of the parthenogenetic females during the year are illustrated in Fig. 6A. From March to August major increases and decreases in the mean number of late-stage embryos tend to be followed by similar changes in the proportions of small individuals in the population. The preponderance of the larger size groups of females during October may be partly accounted for by the high production of males and resting eggs, since this will reduce the number of young females entering the population.

TABLE 4. THE SEX OF VERY ADVANCED EMBRYOS

(A single brood may contain both male and female embryos.)

Date 1951	No. of females with advanced embryos examined	No. of females with male embryos	Total no. of embryos	% of male embryos
5. x.	50	16	229	27·4
31. x.	33	4	111	9·9

The results described are at variance with the observations of Jorgensen (1933). The samples used by Jorgensen appear to have been taken with nets which would tend to select the larger size groups. She has noted that during the two periods of maximum abundance usually found during a year, the great increase in numbers was produced, as far as the samples were concerned, by a temporary influx of adults and states that no doubt immense numbers of young forms were present though not taken by the nets. It is possible, therefore, that populations consisting entirely of small individuals, similar to those found in the Clyde during June and July 1951, would not be adequately represented in the samples she examined.

The data obtained by Cheng (1947) is insufficient to be accepted as conclusive (see page 360). In Table 3 the mean embryo number of all parthenogenetic females can be compared with the percentage of sexual individuals in the population for each date of sampling in the Clyde during 1951. The values show no inverse relationship and therefore do not support Cheng's results.

FOOD AND FEEDING HABITS

Rammner (1930) states that *Evadne* is a predator, feeding on small organisms which are captured by means of its powerful raptorial legs, but notes that no direct observation had been made of the taking up of food by the living animal. He suggests that they may also feed on organic detritus. Previously Lebour (1922) had recorded the cells and spores of *Phaeocystis* from the guts of two *Evadne*.

The gut contents of both living and preserved *Evadne* were examined on several occasions, and though soft reddish or brownish debris was often present at the posterior end of the gut, no recognizable remains were found of any organisms eaten.

Some of the individuals examined were, however, found to be holding a variety of small organisms, either grasped between the endites of the second and third legs or pressed between the labrum and the mouth. Of all those measured and dissected from the plankton-sampler catches during the year, 2·1% were holding tintinnids, 1·4% *Peridinium* and *Goniaulax* species and 0·1% small copepod eggs. Tintinnids were most frequently present during the period from March to May and again during August and September, peridinians during June and July. Considerable quantities of *Skeletonema* and *Thalassiosira* were present in the plankton samples during the spring, but these diatoms were not found between the legs or mouth parts of the *Evadne*. During the investigation it became evident that the proportion of individuals holding microplankton organisms depended to a great extent on the treatment of the catch after hauling the net. On the whole, greater proportions were found in the non-quantitative samples which had been fixed immediately than in the quantitative samples which were not fixed for several minutes due to time spent in washing down the net. Living *Evadne* examined only a short time after collection were rarely observed holding any organisms.

A more detailed study of the feeding habits was made on two occasions towards the end of the year. *Evadne* were obtained from 10 min hauls with 50 cm medium-mesh tow-nets (48 meshes to the inch) taken at 4 h intervals through 24 h. Two nets were fished 25 fathoms apart on a trawl warp, to the end of which was attached a cable depressor. Rigged in this way, the upper net fished at 1–2 m and the lower at 33–38 m. These hauls were taken at a station just outside Tarbert, Loch Fyne. The catches were fixed in formalin as quickly as possible after hauling the nets. During the last series water samples were collected from a depth of 1 m and the number of organisms in the samples estimated by a method previously adopted by Marshall (1947).

On both occasions very few *Evadne* were caught by the lower net and there was no evidence of vertical movements to or from the surface throughout the 24 h. Results of observations are shown in Table 5, in which the number of *Evadne* per haul with the upper net and the percentage of these holding small organisms is given. From the marked difference between the percentages in the day and night hauls it is clear that small organisms were being captured almost exclusively during the hours of daylight. Since *Evadne* possesses a relatively large compound eye, this suggests that food organisms are normally taken by an act of capture depending on sight.

Table 6 shows the percentage composition of these organisms found held by *Evadne* and the percentage composition of the microplankton in the sea at 1 m on 17–18 October. In the series of samples taken on 15–16 August as

many as five tintinnids and peridinians were sometimes held by a single individual, but no *Ceratium longipes* (Bailey) were so found, although large numbers were caught by the nets. *C. furca* (Ehrenberg) was the most commonly held prey on 17–18 October, but not the most abundant dinoflagellate

TABLE 5. PERCENTAGE OF PRESERVED *EVADNE* FOUND GRASPING SMALL ORGANISMS IN SAMPLES COLLECTED AT 4-HOUR INTERVALS

(Figures in parentheses give the numbers examined which in each case was the total number found in the sample.)

Time (h) ...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
15 and 16. viii. 51	34·7 (121)	51·6 (250)	7·1 (294)	2·7 (150)	0 (152)	38·1 (134)	50·5 (198)
17 and 18. x. 51	19·6 (61)	25·9 (77)	0 (60)	0 (89)	0 (106)	27·3 (66)	20·4 (132)
			Hauls taken in darkness				

TABLE 6. THE PERCENTAGE COMPOSITION OF ORGANISMS HELD BY PRESERVED *EVADNE*

(The percentages for 17 and 18. x. 51 are compared with the percentage composition of the microplankton at 1 m.)

	Organisms held by *Evadne*		Microplankton
	15 and 16. viii. 51	17 and 18. x. 51	17 and 18. x. 51
Phytoplankton			
Diatoms	0·2	—	0·03
Peridinium spp.	18·2	20·9	3·0
Peridinium spp. fragments	2·7	1·6	—
Ceratium furca	0·2	56·4	34·6
C. furca fragments	—	15·0	—
Ceratium spp. less *C. furca*	—	—	55·1
Other dinoflagellates	—	1·6	6·6
Zooplankton			
Tintinnopsis spp.	73·2	1·6	0·06
Tintinnopsis spp. fragments	1·3	—	—
Small copepod eggs	1·3	3·2	0·5
Copepod egg membranes	0·7	—	—
Copepod nauplii	0·2	—	—
Larval lamellibranchs	0·9	—	—
Unidentified debris	0·9	—	—
Number of microplankton organisms examined	444	62	—
Mean number of organisms per litre	—	—	12,760

in the sea as estimated from the water samples. Peridinian plates and fragments of tintinnid cases were sometimes found near the mouth but never in the gut. The lack of any skeletons or hard parts of food organisms in the guts seems to indicate that only the cell contents are ingested and the hard remains later discarded.

The percentages of the various groups held by a sample of *Evadne* may not reflect the relative quantities of those eaten. Armoured organisms may be eaten more slowly than fragile or naked forms, while certain types may be rejected

after capture. There is also the possibility that more active prey if undamaged may escape during the fixation of the catch. However, the results suggest that *Evadne* is a selective feeder, shape and size being two of the factors determining suitable food organisms. The majority are fairly compact bodies with average dimensions of between 30 and 120 μ. *Ceratium furca* is an exception, but it differs from the other ceratia of the Clyde plankton in that the two posterior spines are rather short and point straight backwards—a factor possibly influencing its selection by *Evadne*. It is also noteworthy that most of the food organisms are motile forms.

DISCUSSION

Information obtained on the food and feeding habits of *Evadne* helps towards an interpretation of several other aspects of the biology of the species.

During the two 24 h series of tow-nettings feeding was limited to the hours of daylight which indicates that light may be necessary for the capture of food organisms. This would also account for the species being an epiplanktonic form and mainly living in the illuminated surface layers of the sea. Another implication is that in temperate regions during the winter, when most groups of microplankton are poorly represented, the time available for feeding and the depth at which prey can be seen and captured will also be limited. When these adverse conditions are considered, the ability to produce resting eggs appears to be a great advantage in ensuring that the population is carried through to the following spring.

Observations indicate that *Evadne* is a selective feeder and that peridinians and tintinnids are the most important food organisms. The results of several comprehensive plankton investigations show that fluctuations in the population density of *Evadne* can often be correlated with variations in the abundance of *Peridinium* spp. and dinoflagellates of a shape and size similar to those eaten by *Evadne* in the Clyde Sea Area.

The tables given by Dakin & Colefax (1933) to illustrate the cycle of plankton in the coastal waters of New South Wales during 1931, indicate that the periods of maximum abundance of each of the three species of *Evadne* recorded were also the periods when highest concentrations of *Peridinium* spp. and *Ceratium furca* occurred. Greatest numbers of *Evadne nordmanni* were recorded on the same date as the maxima for these dinoflagellates. Accounts of the zooplankton (Deevey, 1956) and phytoplankton (Conover, 1956) of Long Island Sound during 1952, show that *E. nordmanni* was only recorded within the period when *Peridinium* spp. were present in other than trace quantities, and that it occurred in greatest numbers towards the end of a flowering of these species. Lohmann (1908) gives complete tables for the plankton in Kiel Bay during 1905–6. The two species of *Evadne* recorded were always very scarce, nevertheless, each small peak in numbers followed

within 3 weeks of a maximum of *Peridinium* spp. excluding *P. triqueta* (Stein). This latter species was the dominant peridinian in Kiel Bay, but according to Lohmann's estimate of the average cell volume, smaller than any organism noted as captured by *Evadne* in this present investigation.

Although in all the reports of comprehensive plankton investigations examined significant increases in the population of *Evadne* could be correlated with flowerings of *Peridinium* spp., it is clear that factors other than food supply at times limit the population density. This is illustrated by the seasonal cycle of plankton at the Borkumriff Lightship station during 1910, described by Lücke (1912). Here the increase in numbers of *Evadne nordmanni* during April and May closely followed a flowering of *Peridinium* spp. After May numbers of *Evadne* declined and the species finally disappeared from the plankton in August, despite the fact that *Peridinium* spp. showed a second increase during July and remained fairly abundant until the end of October. A rather similar sequence of events occurred in the Clyde plankton during 1923, described by Marshall (1925). *Evadne nordmanni* was common during May and June and *Peridinium* spp. showed a maximum in May after the April diatom flowering. Fewer *Evadne* were recorded after June and there was no increase during or immediately after the second peak of *Peridinium* spp. in August.

All the investigations referred to reveal no clear relationship between the fluctuations in the numbers of *Evadne* and those of the Tintinnoinea when data on this group are available. Several recent studies, for example, that made by Digby (1953) on plankton production in Scoresby Sound, have, however, shown that tintinnids are often most abundant at about the same time as the peridinians, both groups being associated with the late phase of a phytoplankton outburst.

Only qualitative observations were made on the net-caught microplankton in the Clyde Sea Area during 1951. It is, however, possible to state that both peridinians and tintinnids were present in greatest quantities from early April to the end of May and from late July to mid-October. The first period of abundance coincided with the spring diatom flowering and the second with the period when ceratia were predominant. Fluctuations in both length and the mean number of late-stage embryos of *Evadne*, as shown in Fig. 5 (p. 359), may therefore reflect changes in the density of food organisms. Variations in embryo production might be expected to affect in turn the population density of the species as shown in Fig. 1 (p. 352). The rapid growth of the population during May took place when embryo production was highest and numbers decreased shortly after embryo production had declined. However, the population density was very low in September, although embryo production had recovered the previous month. Such a situation might be accounted for if during the summer and autumn the population of *Evadne* was being greatly reduced by predation. This appears to be a reasonable hypothesis, since at

Borkumriff during 1910 (Lücke, 1912) and in the Clyde during 1923 (Marshall, 1925), two cases when the population of *Evadne* did not respond to an increase of food supply during the summer, known zooplankton feeders: coelenterates, chaetognaths and larger crustacean larvae, were more abundant at this time than during the spring. In this connexion it may be relevant that *Evadne* is a slow and rather weak swimmer compared with the copepods, and consequently may perhaps be more easily caught by the larger predatory animals.

In the Clyde during 1951 there was no such clear relationship between the number of young produced per brood by the parthenogenetic females, and the occurrence of gamogenesis as that described in natural populations of *Daphnia* by Berg (1931) and Green (1955). It has been suggested that in the case of *Evadne*, moderate rather than severe depression, to judge by size and production of young, promotes gamogenesis. Comparing the development of the parthenogenetic and resting eggs in *Daphnia* and *Evadne* it does not seem unlikely that gamogenesis will be influenced by different degrees of depression in each case. The resting eggs of *Evadne* are large in relation to the size of the female producing them, and also *Evadne* does not have the capacity for fat storage in the ovary and surrounding tissues possessed by *Daphnia* species. Most of the nourishment necessary for the formation of the large and heavily yolked resting egg will, therefore, depend on the immediate food supply available to the parent during the period it is being produced. In view of the lack of food reserves it would seem advantageous to the species if resting egg formation took place before any drastic decrease in the level of nutrition, causing severe depression and possibly leading to the extinction of the population.

I wish to thank the Director and Staff of the Marine Station, Millport, for providing every facility required and for much assistance. I am especially grateful to Dr S. M. Marshall and Dr A. P. Orr for the encouragement and helpful advice they have given me during the course of this work. The research was carried out during the tenure of a Colonial Fisheries Research Studentship.

SUMMARY

During 1951 *Evadne nordmanni* was present in the Clyde plankton from late February to the end of October and was most abundant during May and early June. The bulk of the population was always found in the top 30 m. Reproduction is mainly parthenogenetic; sexual individuals first appeared in May and sexual reproduction was most intense during October.

The relation between maternal size and stage of embryonic development is described. Sexual females showing the formation of a resting egg are large and have previously produced at least one brood by parthenogenesis.

Of the females with embryos at an early stage of development the primiparae have smaller broods than larger and older individuals. During the development of embryos some may be resorbed and in the primiparae this results in a decrease of embryo number with increase of size. Larger individuals tend to produce larger broods of young.

There was a considerable decrease of size from June to July and a partial recovery in August. These fluctuations follow similar fluctuations in embryo production.

The reproductive capacity of the parthenogenetic females and the intensity of sexual reproduction did not show any clear relationship, though there are indications that the latter is favoured by moderate rather than severe depression.

Food organisms are captured only during the hours of daylight. *Evadne* appears to be a selective feeder the diet of which consists mainly of tintinnids and peridinians.

Several aspects of the biology of the species are discussed with reference to the findings on the food and feeding habits.

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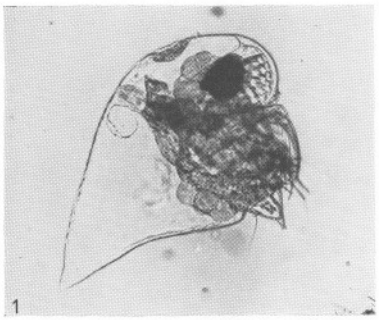
EXPLANATION OF PLATE I

(Magnification, × 66.)

Evadne nordmanni.

Fig. 1. Primiparous female with early-stage embryos.
Fig. 2. Female with a single late-stage embryo.
Fig. 3. Female about to moult and liberate a single late-stage embryo. Early-stage embryos are present in the newly formed brood pouch.
Fig. 4. Female with a second or subsequent brood of early-stage embryos.
Fig. 5. Male.
Fig. 6. Female with an almost fully developed resting egg.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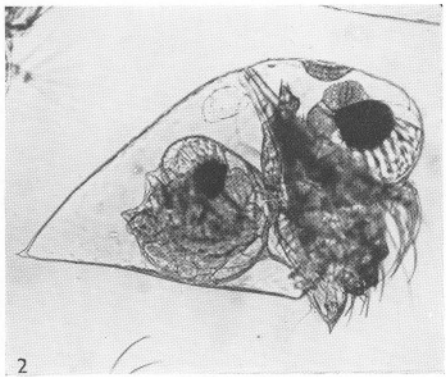
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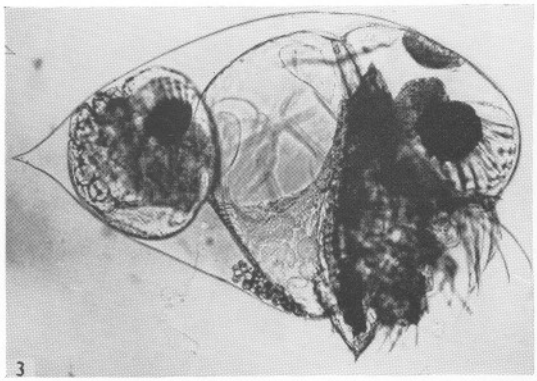
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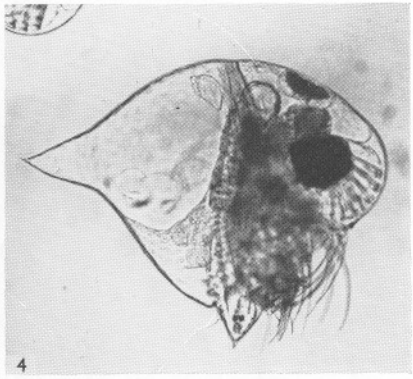
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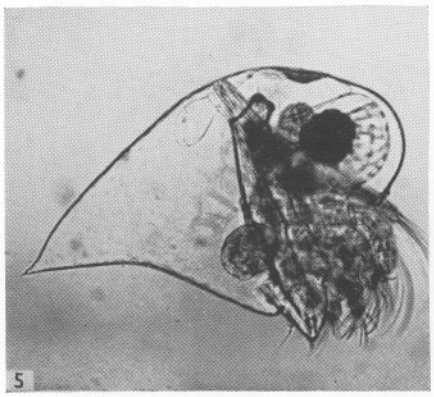
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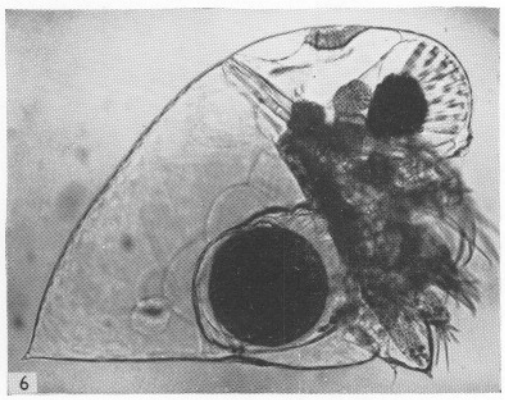
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(*Facing p.* 370)

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PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING 1956

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(Text-figs. 1–4)

Analyses of sea water collected during 1956 at the International Hydrographic Station E1 (lat. 50° 02′ N., long. 4° 22′ W.) are given here in the same form as in earlier reports (Armstrong, 1954, 1955, 1957). The methods of collection and analysis were substantially unchanged.

I am obliged to Lt.-Cdr. C. A. Hoodless and the crew of R.V. *Sarsia* and to Capt. W. J. Creese and the crew of R.V. *Sula* for assistance at sea. It is a pleasure to acknowledge the help given by Dr S. M. Marshall in making the counts of organisms listed in Table 3, and that of Dr T. Peirson, Medical Officer of Health for Plymouth, in providing access to the daily weather records of the city. Salinities were determined by the Government Chemists Department.

RESULTS

Temperature and salinity

The lowest surface temperature recorded was 8·7° C on 26 March, and the highest was 15·7° C on 23 July.

The vertical distribution of temperature during the year is shown in Fig. 1. It shows no sharp stratification until July; although on 11, 16 and 24 April there were small temperature gradients, the top of the water column being warmer than the bottom by 0·67, 0·28 and 1·12° C respectively. Temperature gradients then increased until July, when there was a sharp thermocline at 10–15 m, which persisted, with some variation in depth until September; it had broken down by 23 October.

Salinities throughout the year are given as integral means for the water column in Table 1. It is seen that there was a significant rise in March to 35·41‰ and that high values persisted until July. Such saline water is unusual at Station E1, figures of over 35·4 having been found only on six occasions out of sixty or so during the previous nine years. These unusual and persistent salinities give some grounds for supposing that during the spring months there may have been a consistent body of water at the station.

Phosphate

Vertical distribution is shown in Fig. 2, and integral mean concentrations in Table 1.

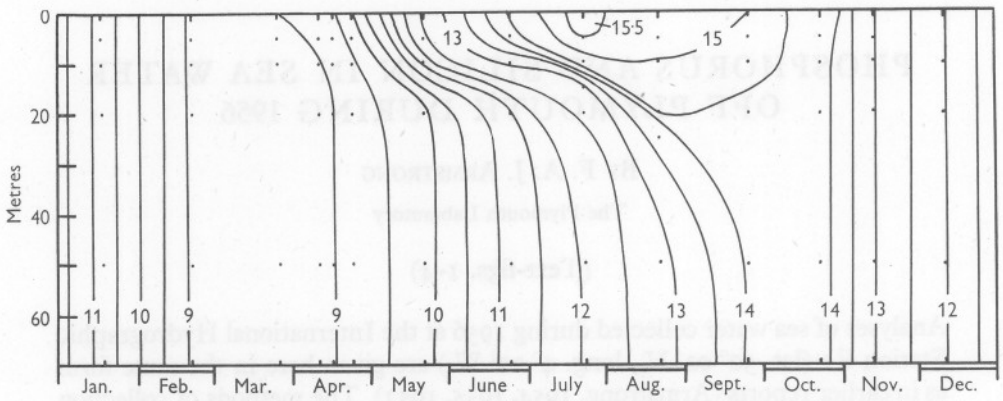

Fig. 1. Vertical temperature distribution at International Hydrographic Station E1, 1956. Contour lines at 0·5° C intervals.

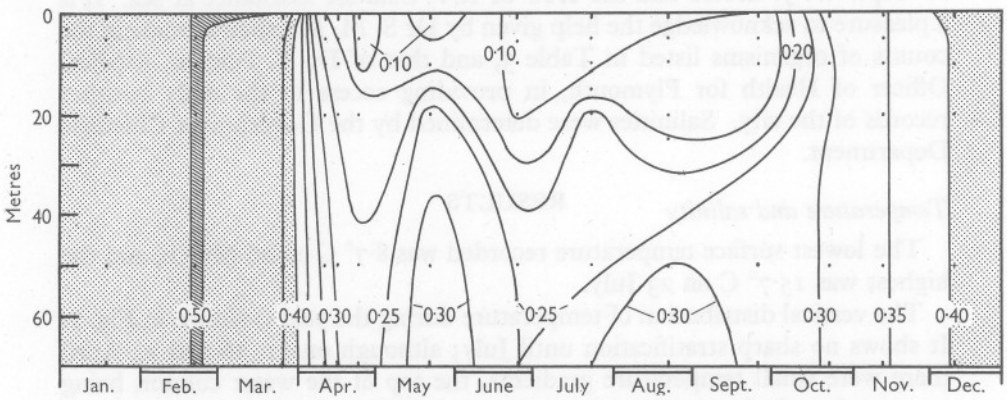

Fig. 2. Vertical distribution of phosphate at International Hydrographic Station E1, 1956. Contour lines at 0·05 μg atom P/l. intervals.

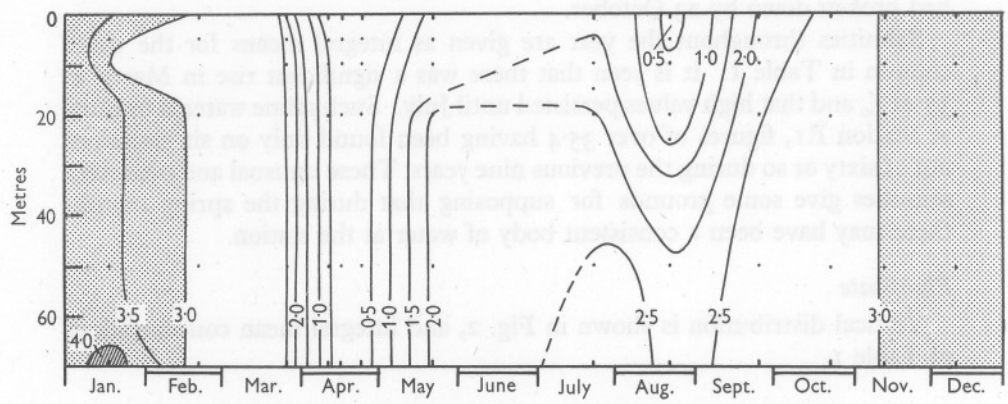

Fig. 3. Vertical distribution of silicate at International Hydrographic Station E1, 1956. Contour lines at 0·5 μg atom Si/l. intervals.

The maximum found was 0·50 μg atom P/l. on 21 February. This is about the usual value of recent years; the unusually high (0·58–0·59) ones of February and March 1955, were transitory. On 26 March, just after 2 days of bright weather, phosphate was 0·46 μg atom P/l. and phytoplankton was readily visible to the unaided eye in samples from all depths. Sunny anticyclonic weather continued, and while it lasted observations were repeated, on 11, 16 and 24 April, in order to follow chemical changes during the spring growth of plants.

TABLE 1. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN AT STATION E1

Date	Salinity (‰)	Phosphate-P (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)
17. i. 56	35·27	0·46	0·59	3·61
21. ii. 56	35·32	0·50	0·60	2·87
26. iii. 56	35·41	0·46	0·58	2·52
11. iv. 56	35·43	0·25	0·51	0·61
16. iv. 56	35·42	0·20	0·49	0·08
24. iv. 56	35·43	0·17	0·50	0·27
22. v. 56	35·42	0·27	—	1·90
25. vi. 56	35·43	0·16	—	—
23. vii. 56	35·43	0·21	—	2·29
22. viii. 56	35·37	0·22	—	1·41
25. ix. 56	35·30	0·19	—	2·51
23. x. 56	35·30	0·31	—	2·72
13. xi. 56	35·34	0·34	—	3·05
10. xii. 56	35·37	0·41	—	3·08

TABLE 2. SUNSHINE ON PLYMOUTH HOE (22 MILES FROM STATION E1)

		1956		1893–1952, mean for 60 years	
Period	Days	Total sunshine (h)	Mean per day (h)	Total sunshine (h)	Mean per day (h)
26 Mar.–10 Apr.	16	111·1	6·9	81·5	5·1
11–15 Apr.	5	17·8	3·6	28·6	5·7
16–23 Apr.	8	87·6	10·9	53·6	6·7
Total for period	29	216·5	7·5	163·7	5·6

Table 2 shows total and mean daily hours of sunshine on Plymouth Hoe, about 22 miles from Station E1, for the period 26 March to 23 April 1956, and also the mean figures from the daily records, for the same period, for 60 years from 1893 to 1952. There was more sunshine in 1956. It was noticed that only 4 days (29 March and 12, 13 and 14 April) had less than 3 h of daily sunshine.

Integral mean concentrations fell to 0·17 μg atom P/l. during the period, the change being greatest in the upper layers and less marked below 20 m, as shown in Fig. 4. By themselves these phosphate observations are not particularly significant and resemble changes in earlier years. They are of interest, however, if taken together with the silicate analyses below.

Afterward, phosphate remained low in the upper layers (minimum 0·05–0·06 μg atom P/l. on 25 June) until September. By 23 October, when the water column had become isothermal, phosphate had also become uniform vertically, and increased until the end of the year.

Total phosphorus

Determinations were discontinued after April. The maximum integral mean value was 0·60 μg atom P/l. on 21 February. The March and April values show that to some extent the element is conserved during the spring outburst of plants.

Silicate

Vertical distribution is shown in Fig. 3, and integral mean concentrations in Table 1.

The maximum integral mean concentration of 3·6 μg atom Si/l. was found on 17 January, although the vertical distribution was not uniform, being 4·2 at 70 m and 3·0 at 10 m, increasing again to 3·5 at the surface. The surface was very slightly warmer (0·04° C) than the bottom. A decrease to 2·9 μg atom Si/l. had occurred by 21 February, temperatures then being uniform. Possibly the water mass was changing in January, though there is no other evidence for this.

Changes during the period 26 March to 24 April are shown, with the corresponding phosphate changes, in Fig. 4. During the first 21 days silicate decreased almost linearly, and on 16 April most of it had been removed from the water at all depths. Even at 50 and 70 m there remained only 0·08 and 0·09 μg atom Si/l. This is well below the photosynthetic zone, and the 'compensation point' as defined by Jenkin (1937) could hardly have been deeper than 40 m. After a further 8 days silicate, particularly in the deep water, had increased again somewhat, though phosphate had continued to fall.

During the summer months silicate concentrations fluctuated rather, mostly in the top 20 m. Vertical uniformity was re-established by 23 October, after which silicate increased slightly as is usual.

Integral mean concentrations

These are shown in Table 1 (and have already had mention). The decreases representing consumption of nutrients in the spring may be taken as: phosphate 0·34 μg atom P/l., and silicate probably 2·78 μg atom Si/l. if the January figures be excluded.

Plankton

Samples from 10 and 50 m taken on 16 and 24 April were preserved and were examined by Dr S. M. Marshall, who made duplicate counts of 10 ml. and gave the results listed in Table 3. She commented that only the 10 m

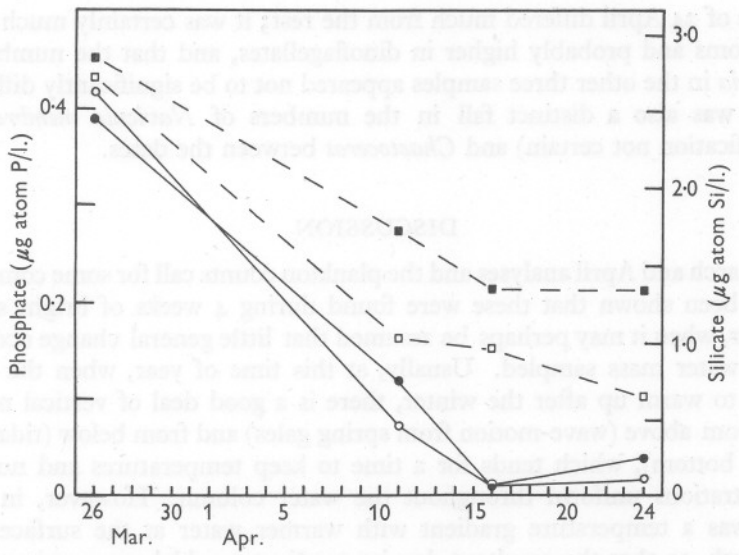

Fig. 4. Phosphate and silicate at 5 and 50 m at International Hydrographic Station E1, 26 March to 24 April, 1956. Phosphate at 5 m, □; at 50 m, ■. Silicate at 5 m, ○; at 50 m, ●.

TABLE 3. ORGANISMS IN 20 ML SEA WATER CENTRIFUGED

April 1956. Station E1

	16. iv. 56		24. iv. 56	
	10 m	50 m	10 m	50 m
Paralia sulcata	35	—	—	1
Coscinodiscus sp.	1	1	—	—
Thalassiosira sp.	2	2	—	15
Lauderia	3347	2720	980	3631
Stephanopyxis	5	7	1	13
Rhizosolenia				
Straight needle type	57	55	48	46
R. stolterforthii	77	119	30	126
Other *affines* types	108	57	43	114
Chaetoceros				
Phaeoceros	283	152	10	93
Hyalochaete	450	320	90	90
Biddulphia	3	—	—	—
Cerataulina	23	23	4	10
Eucampia	2	19	—	20
? *Navicula membranacea*	319	411	58	177
? Naviculid	2	2	1	1
Totals	4714	3888	1265	4337
Prorocentrum	—	—	1	1
Dinophysis and *Phalacroma*	1	1	6	—
Gymnodinian	—	—	2	—
Peridinium various spp.	13	11	28	20
Ceratium fusus	—	—	1	—
? Cyst	—	—	2	—
Distephanus	1	1	—	—
Tintinnid	—	—	—	4
Naked ciliate	—	—	1	—
Phaeocystis colonies	—	—	—	4
Temora nauplius	1	—	—	—
Oithona nauplius	1	—	—	—

Much detritus in all samples.

sample of 24 April differed much from the rest; it was certainly much lower in diatoms and probably higher in dinoflagellates, and that the numbers of *Lauderia* in the other three samples appeared not to be significantly different. There was also a distinct fall in the numbers of *Navicula membranacea* (identification not certain) and *Chaetoceros* between the dates.

DISCUSSION

The March and April analyses and the plankton counts call for some comment. It has been shown that these were found during 4 weeks of bright settled weather, when it may perhaps be assumed that little general change occurred in the water mass sampled. Usually, at this time of year, when the water begins to warm up after the winter, there is a good deal of vertical mixing both from above (wave-motion from spring gales) and from below (tidal drag on the bottom), which tends for a time to keep temperatures and nutrient concentrations uniform throughout the water column. However, in 1956 there was a temperature gradient with warmer water at the surface after 26 March, so that the resultant density gradient would have restricted that part of vertical mixing due to wave-motion turbulence. Indeed, in April, the phosphate figures also show a gradient, increasing toward the bottom. It is likely that less restricted vertical mixing took place between 11 and 16 April, which included three dull rather cold days with winds of force 5. The temperature gradient decreased, and so also did that of phosphate.

In these otherwise stable conditions silicate concentrations fell sharply, and by 16 April were very low, not only in the euphotic zone, but at the bottom. During 3 weeks the ratio of silicon to phosphorus removed from solution was 2·44:0·26 by atoms or just over 8:1 by weight. The silicon:phosphorus ratio in diatoms is found, by chemical analysis, in the range 16:1 to 50:1 (Vinogradov, 1953). Since the counts show that in size and numbers diatoms predominated over other organisms in the water, this suggests that they were deficient in silicon.

There seem to have been considerable numbers of diatoms in the deepest water; the counts show that this was so at 50 m. Some diatoms no doubt are carried out of the illuminated zone by turbulence. There is evidence too that at times they may lose buoyancy and sink rapidly (Marshall & Orr, 1928; Riley, 1941; Steele, 1956). Jenkins (1955) has shown that at Station E1, in March 1953, there was more chlorophyll in material filtered from bottom water than from the layers above. The counts of 24 April suggest either that diatoms in the upper water have been removed by some selective agency or that some have fallen into deeper water.

It is possible that diatoms in the deep water, out of the illuminated region, had continued to absorb silicate up to 16 April even if unable to make normal growth. Afterwards, many may have been eaten and voided by copepods, or

may have died quickly, so that some silicate was returned to solution by 24 April.

The continued uptake of silicate, in the dark, by diatoms deficient in silicon, was observed by Lewin (1954), with cultures of *Navicula pelliculosa*.

SUMMARY

The results of analysis of sea water from the International Hydrographic Station E 1 during 1956 are presented in graphical form and as integral mean values for the water column of 70 m. The seasonal variation is shown; it appears that consumption of phosphate in the spring outburst of plants was 0·34 μg atom P/l., and that of silicate 2·78 μg atom Si/l. The spring outburst was followed more closely than usual in the period 26 March to 24 April during 4 weeks of rather bright weather. Nearly all the silicate in the water was taken up, and some phosphate left, and it seems that the rapidly growing diatoms, which predominated at the time, were deficient in silicon. It is suggested that in the deep water they continued to absorb silicate, although not receiving enough light for growth and division.

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FURTHER OBSERVATIONS ON PROPRIOCEPTORS IN CRUSTACEA AND A HYPOTHESIS ABOUT THEIR FUNCTION

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(With Plate I and Text-figs. 1–5)

The observations on proprioceptors in the legs of Crustacea recorded recently (Alexandrowicz & Whitear, 1957) have shown that in these animals there are several receptor organs in the coxal region and that there is a difference in their number in the thoracico-coxal articulation. Whereas in some species, e.g. *Homarus* and *Astacus*, there are two receptor units running side by side, termed for short muscular and elastic receptor, in others, e.g. the Brachyura, only one, of muscular type, is present. It was stated, moreover, that in the latter case the arrangement of the sensory elements of this receptor exhibit certain features differing from those in the organ of the *Homarus* type. But the question was left open whether this particular pattern of innervation is an inherent feature of the muscular receptor when it is not associated with a second organ, and, if this is so, what inference concerning its mode of function could be deduced therefrom. It seemed, therefore, worth while to investigate the receptors in such species as *Eupagurus bernhardus* and *Palinurus vulgaris* in which, as some preliminary observations had shown, only one receptor unit is present. In the present paper are reported the results obtained with *Eupagurus*, which had the advantage of being easily obtainable.

METHOD

The nerves were stained with methylene blue and the method used was the same as previously recorded. The organs in *Eupagurus* stain readily so that a complete failure is never experienced, although the staining, as is usual with this method, is uneven. The main difficulty consists in exposing the organs in such a way that the methylene-blue solution can have free access to them and to keep all the parts of the preparation in the desired position. The usual method is to attach them to paraffin plates. Paraffin wax, however, has the disadvantage of being soluble in xylol. This is of no importance when the preparations are of such size and shape that after being stained and fixed they can be detached from the plates and remain flat up to the final stage of mounting. When, however, such objects as fine fibres pass through alcohol and xylol they usually become bent or deformed in various ways. In handling

such preparations polyethylene ('polythene') plates proved very serviceable, since the tissues attached to them with the finest pins can be transferred into xylol and remain perfectly stretched in it. I am indebted to Mr F. A. J. Armstrong for this suggestion and for providing the plates. This method can be also used when embedding easily deformable objects; they can pass unaltered from the moment of fixation through all media, to be detached only before being transferred into the paraffin bath or even shortly before embedding. Polyethylene, however, is not yet an ideal material for similar purposes since it is affected by xylol, although very slightly. It is to be hoped that with so many kinds of plastics now being manufactured, or being invented, some of them will prove to be satisfactory in every respect.

OBSERVATIONS

In the coxal region of *Eupagurus bernhardus* the following receptor organs have been found: (i) the muscular receptor spanning the thoracico-coxal articulation; (ii) two innervated strands associated with the muscles levator and depressor basipoditis; and (iii) the coxo-basipodite receptor.

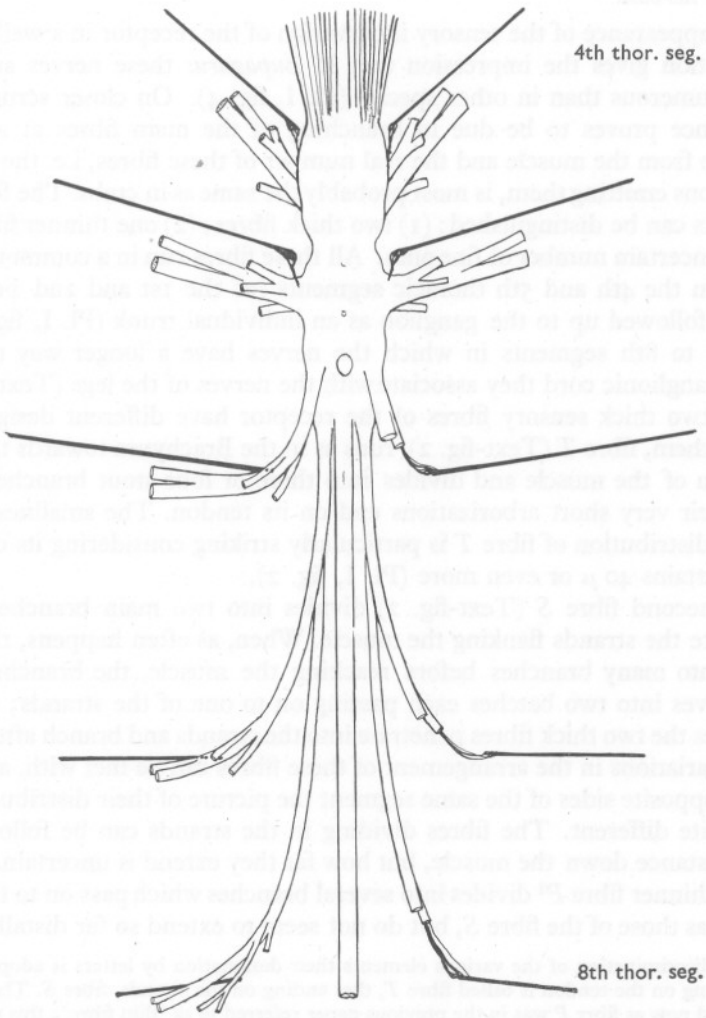
Muscular receptors

The muscular receptors are present, one on each side, in all five peraeopods. They have their proximal attachments in the vicinity of the ganglionic cord, but their position in relation to this cord and its main nerve trunks is different in each segment owing to the coalescence of the thoracic ganglia and various directions of the course of the trunks (Text-fig. 1). In the 4th–6th thoracic segments the receptor muscle has its origin on the posterior surface of the endosternite plate limiting anteriorly the respective segments. In the 4th segment it is near the border of the ganglionic mass and ventral to it; in the 5th segment, in which it is more easily accessible, it is situated laterally to the ganglion and in front of the main nerve trunks (Pl. I, fig. 1); in the 6th segment it is covered by thick nerves of the limb. In the 7th and 8th segments, in which the endophragmal skeleton is greatly reduced, the receptor muscles originate on small chitinous plates and are also covered by main nerve trunks running to the legs.

The endosternite plates do not exhibit any special projections at the point where the muscles arise. In this respect *Eupagurus* differs from all decapods examined in which special receptor rods are present in several segments (Macrura) or at least in one of them (Brachyura).

At the proximal end of the muscle its myofibrils pass into fine, short (*ca.* 100 μ), connective tissue fibres which thus constitute a tendon by means of which the receptor is attached to the membrane lining the calcified chitin of the endosternite. Despite its insignificant appearance this tendon, as will be discussed later, plays an important role. From this point of origin the muscle

runs in the direction of the anterior hinge of the basipodite to insert near the rim of the coxa. The muscle fibrils end here without passing into a tendon like that at their proximal attachment. They do not reach the calcified chitin of the coxa but insert near to it into the tissue of the articulation membrane. Alongside the muscle run connective tissue fibres which form two wider strands proximally, as in the Brachyura. Near the distal end of the muscle some of the fibres deviate sidewards to pass into the membrane of the articulation (Pl. I, fig. 3).



Text-fig. 1. *Eupagurus bernhardus*. Topography of the thoracico-coxal (muscular) receptors in relation to the ganglionic cord and its main nerve trunks seen from the dorsal side. On the right side parts of the nerve trunks covering the origin of the receptors have been removed.

Nerves

Motor nerves.

The motor fibres innervating the muscle run near the sensory nerves but do not associate with them more closely and often, especially in the posterior segments, are distinctly separated from them (Pl. I, fig. 4, *mot.*). The distribution of their branches follows the same pattern as in the receptors of other crustaceans.

Sensory nerves.

The appearance of the sensory innervation of the receptor in a well-stained preparation gives the impression that in *Eupagurus* these nerves are much more numerous than in other species (Pl. I, fig. 4). On closer scrutiny this abundance proves to be due to branching of the main fibres at a greater distance from the muscle and the real number of these fibres, i.e. the number of neurons emitting them, is most probably the same as in crabs. The following elements can be distinguished: (1) two thick fibres; (2) one thinner fibre; and (3) an uncertain number of fine ones. All these fibres run in a common bundle which in the 4th and 5th thoracic segments (of the 1st and 2nd peraepod) can be followed up to the ganglion as an individual trunk (Pl. I, fig. 1). In the 6th to 8th segments in which the nerves have a longer way to travel to the ganglionic cord they associate with the nerves of the legs (Text-fig.1).

The two thick sensory fibres of the receptor have different designations. One of them, fibre *T* (Text-fig. 2), runs as in the Brachyura towards the point of origin of the muscle and divides into three or four stout branches which with their very short arborizations end on its tendon. The smallness of the area of distribution of fibre *T* is particularly striking considering its diameter which attains 40 μ or even more (Pl. I, fig. 2).

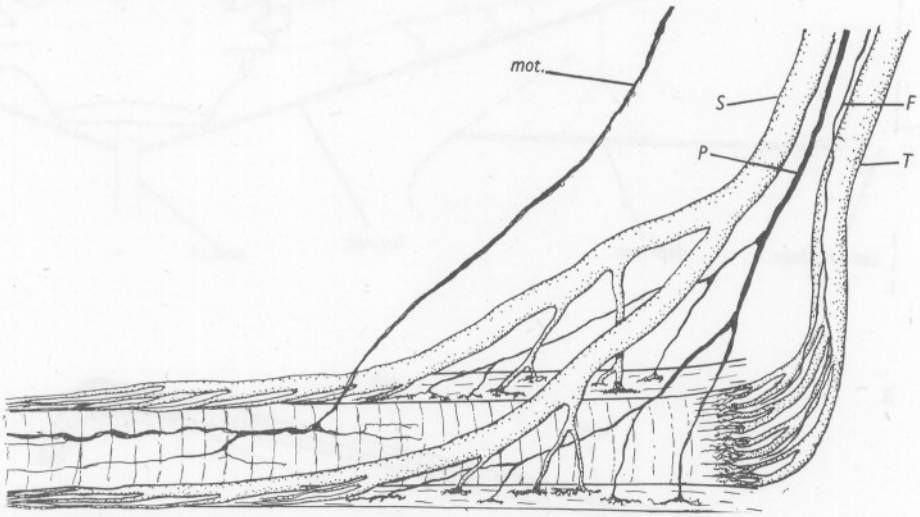
The second fibre *S* (Text-fig. 2) divides into two main branches which innervate the strands flanking the muscle. When, as often happens, this fibre splits into many branches before reaching the muscle, the branches range themselves into two batches each passing on to one of the strands; in other instances the two thick fibres penetrate into the strands and branch afterwards. Many variations in the arrangement of these fibres can be met with, and even on the opposite sides of the same segment the picture of their distribution can look quite different. The fibres dividing in the strands can be followed for some distance down the muscle, but how far they extend is uncertain.

The thinner fibre *P*[1] divides into several branches which pass on to the same strands as those of the fibre *S*, but do not seem to extend so far distally as the

[1] For discrimination of the various elements their designation by letters is adopted. The fibre ending on the tendon is called fibre *T*, that ending on the strands, fibre *S*. The element designated now as fibre *P* was in the previous paper referred to as 'thin fibre'; this term does not seem to be appropriate as this fibre is not very thin and there are others which are thinner, called now fibres *F*. Such terms as 'accessory' or 'small' fibres have been avoided as they have been applied to definite elements in other instances.

latter. Their endings are not so clearly delimited as in crabs, but there is ground for assuming that their areas of termination are independent of those of the branches of fibre *S*.

The most elusive elements are the fine fibres *F* which can sometimes be seen running with the fibre *T*, as has also been noticed in other species. They are here distinguished as a special sort, but the possibility that they are of the same kind as the fibre *P* or even branches of the same neuron is not excluded. As always when many nerves run side by side, there are many doubts whether a given fibre is an individual element or is only a branch of another fibre travelling in the same bundle.



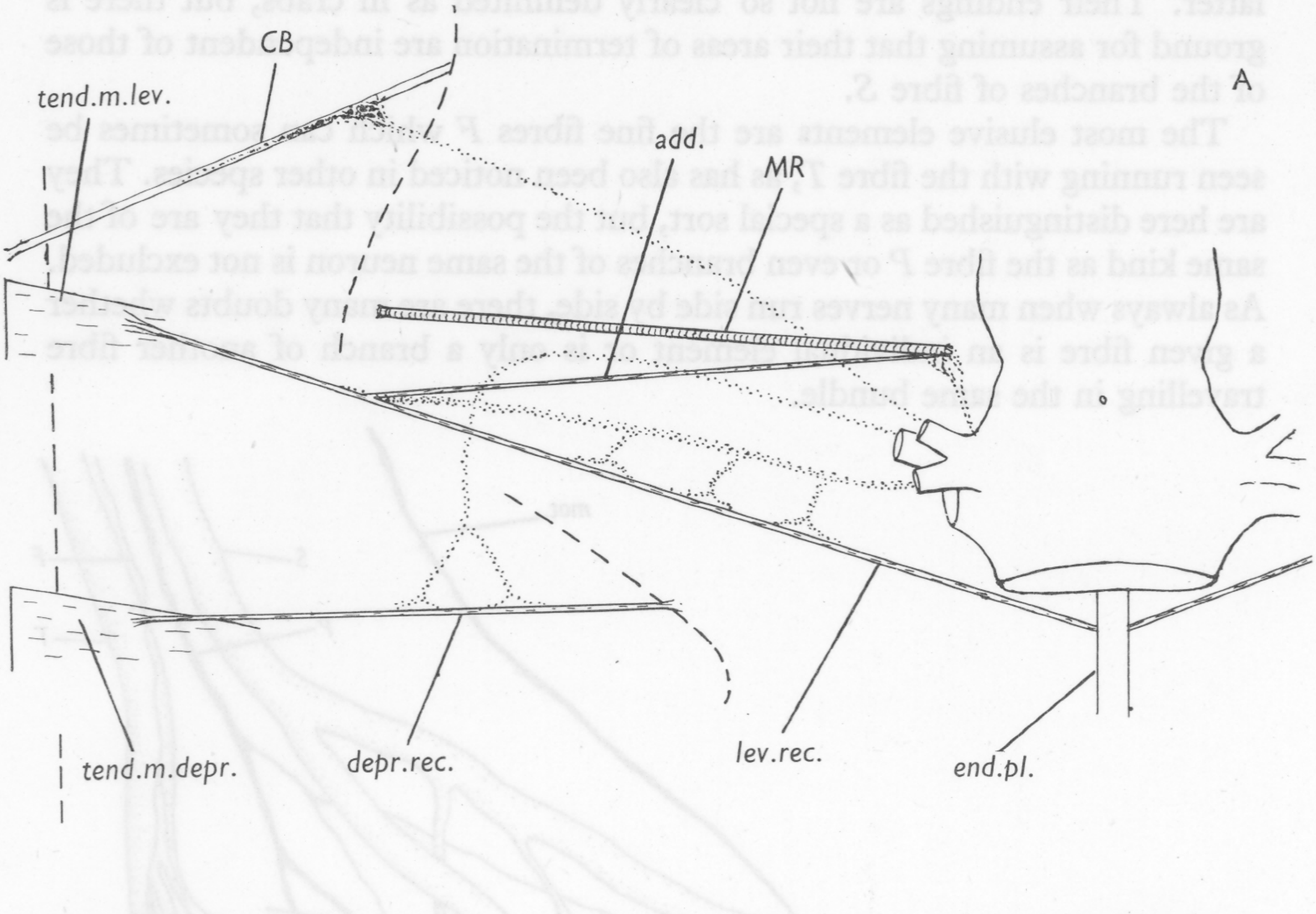
Text-fig. 2. *Eupagurus bernhardus*. Proximal part of a thoracico-coxal receptor. *T*, fibre ending on the tendon of the receptor muscle; *S*, *P*, fibres innervating the strands flanking the muscle; *F*, fine fibres accompanying the fibre *T*; *mot.*, motor nerves of the receptor muscle.

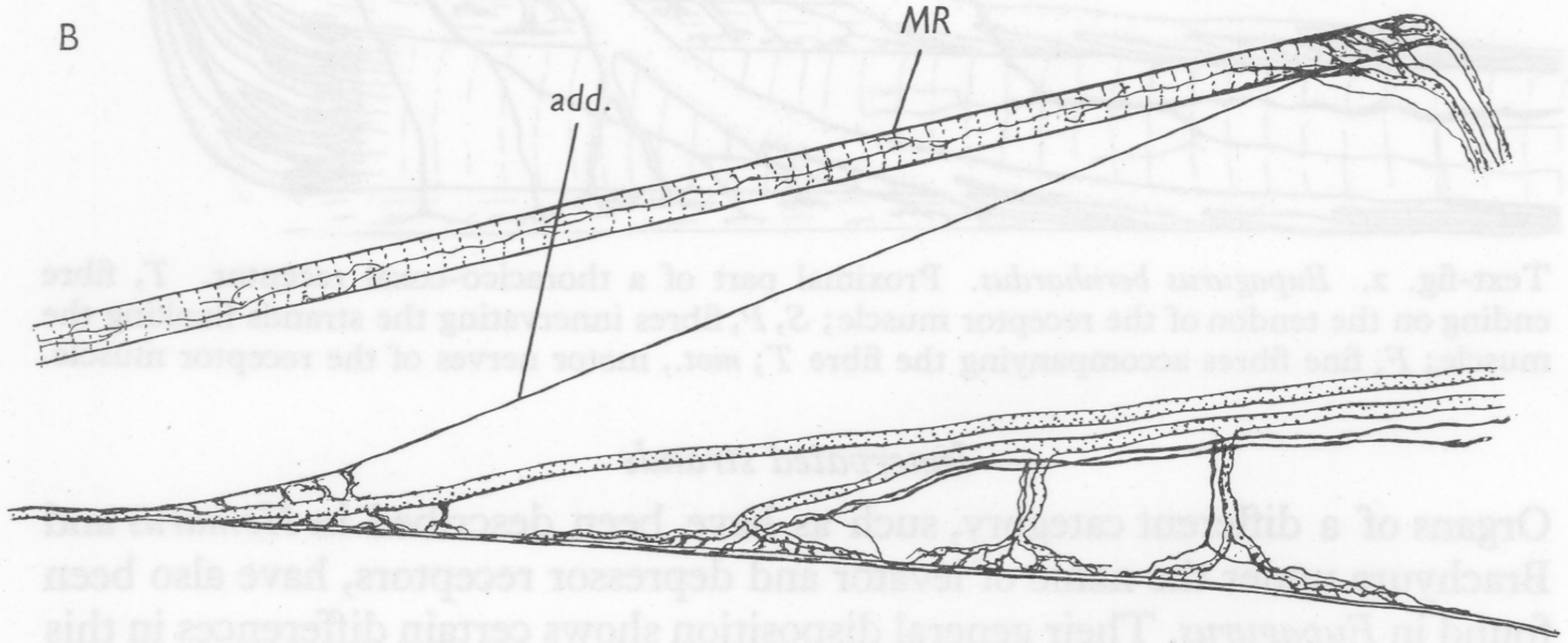
Innervated strands

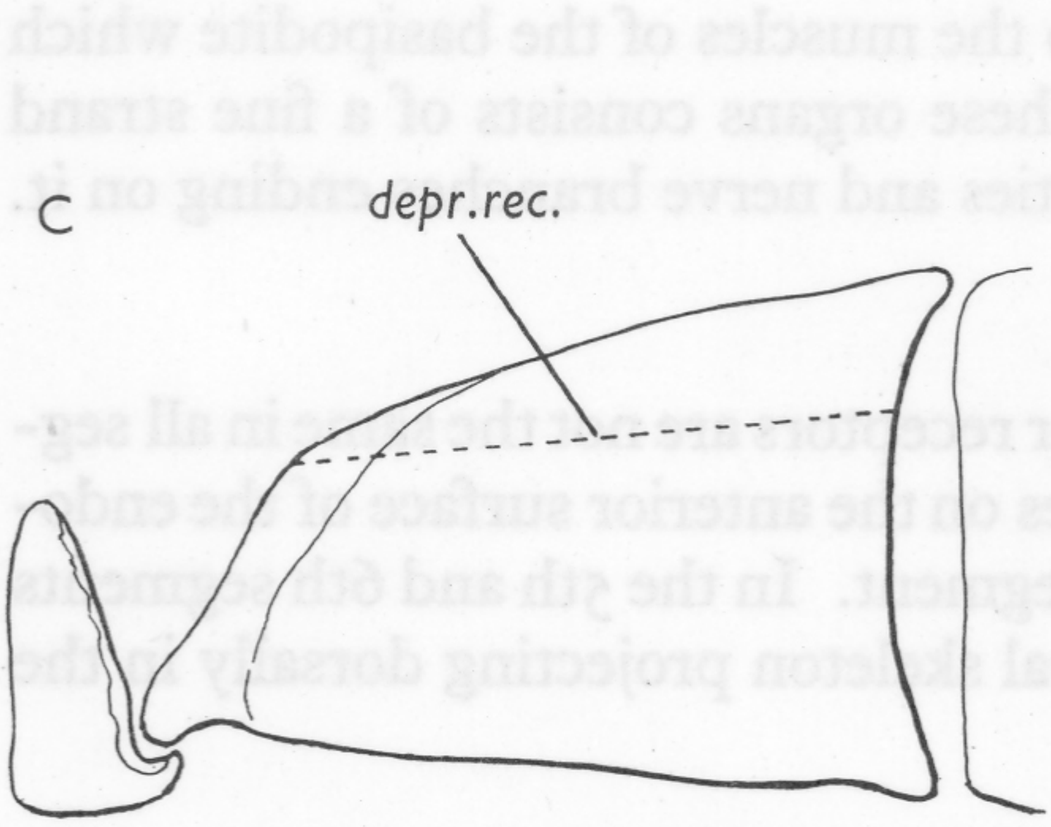
Organs of a different category, such as have been described in *Homarus* and Brachyura under the name of levator and depressor receptors, have also been found in *Eupagurus*. Their general disposition shows certain differences in this species, but they have similar relations to the muscles of the basipodite which gave rise to their designation. Each of these organs consists of a fine strand of connective tissue having elastic properties and nerve branches ending on it.

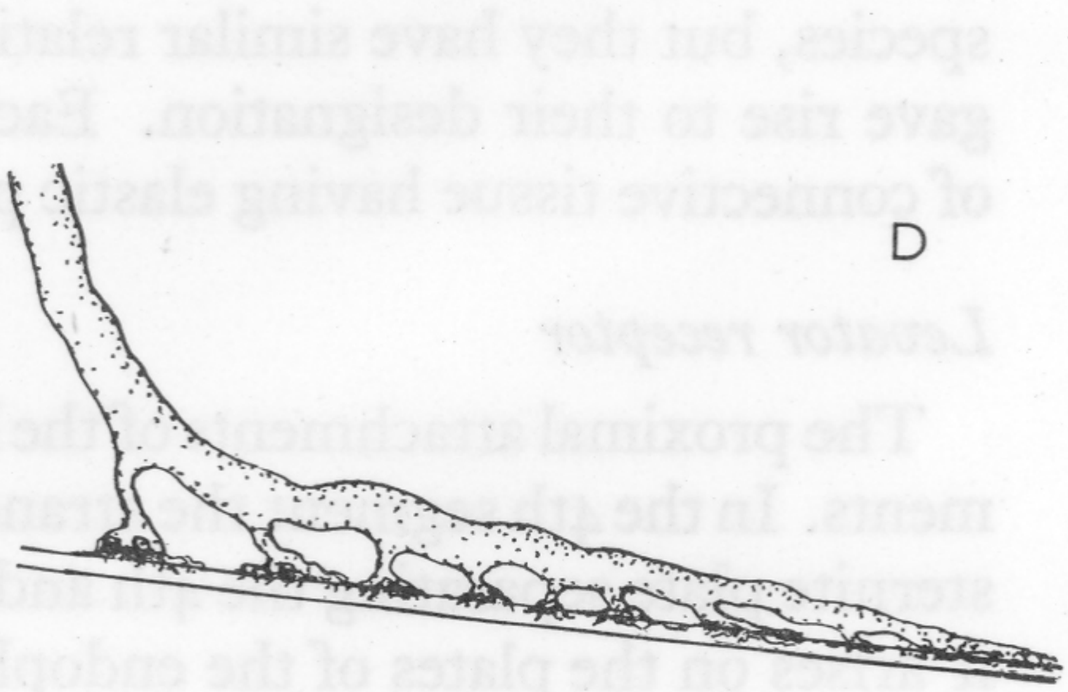
Levator receptor

The proximal attachments of the levator receptors are not the same in all segments. In the 4th segment the strand arises on the anterior surface of the endosternite plate separating the 4th and 5th segment. In the 5th and 6th segments it arises on the plates of the endophragmal skeleton projecting dorsally in the









Text-fig. 3 For legend see opposite page.

mid-line in each of the 4th to 6th thoracic segments; the points of attachments of the strands of the opposite sides lie thus very near to each other (Text-fig. 3A). In the two posterior legs they are again at a distance from one another.

In all legs the strand runs on the bundles of the ventral portion of the m. levator basipoditis near to its anterior margin. Approaching the tendon of the muscle it splits into very fine twigs which insert on the tendon between the muscle bundles.

In the distal half of its course this strand is joined by an additional strand which arises close to the muscular receptor and, after crossing obliquely the nerve trunks of the limb, unites with the main strand at an acute angle (Text-fig. 3A, B; Pl. I, fig. 7). The length of the main strand in the 2nd and 3rd peraeopod of a larger specimen is about 12 mm and its diameter varies from about 30 μ in the middle part to some 10 μ and less towards its distal end. The additional strand has a more uniform thickness in the range of 5–10 μ.

The main strand is supplied by no less than three and often four nerve branches given off by a nerve trunk which also carries motor fibres for the ventral portion of m. levator. One of the nerve branches reaches the strand always at the point where it is joined by the additional strand and the other three proximally to it and at a distance from one another (Text-fig. 3B; Pl. I, figs. 5, 7). The three proximal branches (or two if there are three in all) consist of one thick and one or more thin fibres (Text-fig. 3B, Pl. I, fig. 8); some of the thin fibres can sometimes be seen to run separately to the strand. In the most distal branch the thin fibres have not been noticed. The number of neurons taking part in this innervation could not be ascertained as the fibres run towards the ganglion in a common bundle with other nerves; it seems probable that the thick fibres belong to two neurons; as to the thinner it can only be said that they appear to be emitted by more than one neuron.

The photographs (Pl. I, figs. 7, 8) give an idea of the striking disproportion between the calibre of the nerve fibres (*ca.* 50 μ) and that of the strand on to which they pass not far from one another. They give off short branches which almost immediately resolve into numerous arborizations (Text-fig. 3D). Of the details of their terminations little can be seen with the ordinary microscope: in methylene-blue preparations they show irregular outlines and appear

Legend Text-fig. 3

Eupagurus bernhardus. (A) Diagram of the proprioceptors in the coxal region. *MR*, thoracico-coxal (muscular receptor); *lev.rec.*, *depr.rec.*, levator and depressor receptors; *CB*, coxo-basipodite receptor; *end.pl.*, plate of the endophragmal skeleton; *tend.m.lev.*, *tend.m.depr.*, tendons of the muscles levator and depressor. The nerves are drawn in dotted lines and the interrupted lines indicate the margins of the coxopodite. (B) Innervation of a levator receptor of the left side. Three of its nerve branches are represented as belonging to one neuron and the fourth, the most distal one, to a second neuron (the most probable disposition); *add.*, additional strand with its proximal attachment at the origin of the muscular receptor *MR*. (C) Coxopodite of the 2nd left peraeopod from below showing the projection of the depressor receptor *depr.rec.* (D) Mode of branching of a thick nerve fibre on the strand of a levator (or depressor) receptor.

confluent with one another. It is to be hoped that examination with the electron microscope for which these organs seem to be a particularly suitable object will show interesting features of the relations of the nerve fibres to each other and to the tissue of the strand.

To the question how much of the whole length of the strand is innervated no satisfactory answer can be given. The fibre entering the angle between the two strands can be followed for some distance until it fades out; it seems probable that the regions near the attachments have no innervation, but where its limit is is uncertain.

The additional strand is supplied by very short branches of the nerve fibre near its point of junction with the main strand (Text-fig. 3B) and no other nerves in the additional strand have been noticed. It may be recalled that in *Homarus* the additional strand is innervated by a fibre entering its proximal end. No such fibre could be seen in *Eupagurus*; if there is one it could be only a fine filament which does not show in my preparations.

The levator receptors of the two posterior legs are very tiny elements and it is more difficult to expose them without damaging some of their parts. That of the 4th leg has an additional strand while in the 5th leg this appears to be missing. The number of nerve branches in the 5th leg seems to be reduced to three or two.

Depressor receptor

The depressor receptor has been found in the three anterior peraeopods; whether or not it is present in the two posterior ones is a doubtful point. It is situated at the ventral wall of the coxal segment and extends from the proximal border of the coxa to the tendon of m. depressor basipoditis into which it inserts not far from its distal end. The position of its strand seen by transparency is shown in Text-fig. 3C. It runs near the anterior border of the short head of the depressor muscle arising on the rim of the coxa. Close to the bundles of this muscle is the attachment of the receptor strand the fibres of which pass here on to the tissue continuous with the membrane of the coxal articulation. It should be added that the short head of the depressor muscle is covered by longer bundles of another portion of the depressor muscle originating on the endophragmal skeleton; the receptor strand is therefore situated under these longer bundles.

The nerve carrying the fibres of this receptor is one of the branches innervating the depressor muscle. The branch of the receptor runs near the antero-ventral wall of the coxa and usually bifurcates at a distance from the strand (Pl. I, fig. 6). The two branches of this division, each containing two fibres of different calibre, pass on to the strand and behave in the same way as in the levator receptor. No adequate information about the number of fibres taking part in this innervation could be obtained; it is, however, evident that this organ has a poorer innervation than the levator receptor.

Coxo-basipodite receptor

The coxo-basipodite receptor is present in all five peraeopods. It consists of a connective tissue strand and numerous bipolar nerve cells ending on the fibres of the strand. It looks exactly like those described in *Homarus* and its position is the same, viz. it originates near the dorsal hinge of the coxa and runs towards the basipodite to insert near the tendon of m. levator (*CB*, Text-fig. 3 A). The axons of the cells unite into a bundle joining the nerve which arises from the ganglion dorsally from the thickest nerve trunk of the limb and which supplies the dorsal part of the coxa.

The disposition of all receptor organs in the coxal region is shown in Text-fig. 3 A. Their longitudinal dimensions and their situation in relation to the mid-line of the body are drawn to scale, but they are represented diagrammatically as if lying in one plane while in fact they occupy different positions in relation to the axis of the leg.

Hints on dissection

The proprioceptors in the coxal region have to be exposed from the dorsal side. After removing the carapace and the digestive organs, the dorsal parts of the endophragmal skeleton and the muscles should be cut away until the ganglionic cord becomes exposed. The muscles covering the nerve trunks in the coxa should be removed, care being taken not to damage the ventral portion of m. levator on which lies the levator receptor. It is not advisable to do much cleaning of the organs until the staining makes the tissues better visible. The difficulties in finding the muscular receptor in *Eupagurus* are aggravated by their topography, which differs in each segment. That of the 2nd peraeopod is better taken first because it is more easily accessible. Once the organ is spotted one can proceed further with cutting out parts of the muscles, removing the blood clots, arteries, etc., but all this work must be done by stages.

The receptor of the chela is less accessible because it lies more ventral and nearer to the ganglionic mass. The organs in the 3rd to 5th legs are covered by nerves and these must be pulled aside or cut out if the receptors are to be exposed. The frequent mistakes can be corrected when dissecting the opposite leg. The muscular receptor of the 5th leg, although apparently most hidden of all, proved to be quite convenient for staining and all other manipulations, as the preparation can be more easily spread and pinned down to paraffin or polyethylene plate than those from other segments. To reach the receptor all the overlying muscles must be removed until the big nerve trunks are free. Beneath them, as shown in Text-fig. 1, is the origin of the muscle. In male animals this point lies directly in front of and not far from the end portion of the vas deferens. The receptor muscle runs close to the anterior bundles of a thin layer of the ventral portion of m. depressor. To expose it parts of the nerves as well as the artery covering it must be removed. When the receptor is spotted it can be freed from overlying tissues along its whole length, which is 4 mm in the largest specimens.

The levator receptor can easily be found in the 2nd and 3rd peraeopod provided it has not been torn away during dissection. It can be located even before the strand itself becomes visible by the characteristic branching of its nerves. These nerves stain readily but their stain fades out in a comparatively short time.

To find the depressor receptor it is necessary to remove the muscles lying dorsal to

that part of the coxa where the receptor strand is attached (Text-fig. 3C); great care must be taken not to damage it when cutting out the muscles in that region. It is therefore better when arriving near the receptor to pull the muscles aside, fix them with pins and observe the progress of staining until the receptor can be distinguished.

To obtain preparations of the coxo-basipodite receptor the dorsal parts of the coxa and of the basipodite should be cut out and the adhering muscles pulled aside so that the staining solution has access to the region in which this receptor is situated. When it is visible, the unnecessary tissues can be cut out to expose it better. It is also possible to get preparations with this organ *in situ* still connected with the central nervous system. For this purpose, in a preparation made as for staining of the muscular receptor, the posterior part of the coxa and of the basipodite should be removed and their anterior parts held in such a position that the progress of the staining of the coxo-basipodite receptor can be followed.

Comparison of the proprioceptors in Eupagurus *with those in other Crustacea*

The observations described above have shown that the proprioceptors in the coxal region of *Eupagurus* occur in the same number as in *Carcinus* and *Cancer*, and that they have similar histological structures the most remarkable feature of which is the location of the cell bodies of sensory neurons in the thoracic ganglia. The muscular receptor proved to have the same nerve elements as those in the two crabs and their disposition is basically the same; the differences in the general appearance of innervation is due to the mode of branching of nerve fibres and also to the fact that one of the elements ending on the strands flanking the muscle, the thinner fibre *P*, appears to have in *Eupagurus* a greater share in innervation, for it has more numerous and longer branches than in *Carcinus*. Major differences have been found in the topographical relations of the muscular, levator and depressor receptors, the origins of which in crabs are close together, while in *Eupagurus* they lie far apart. A complete isolation of the depressor receptor, as in *Eupagurus*, has not been hitherto seen in other species in which it has either a common attachment with the muscular receptor (*Carcinus*, *Cancer*, *Palinurus*), or is connected with it by an additional strand (*Homarus*). The levator receptor in *Eupagurus* resembles this organ in *Homarus* and *Palinurus* in that it has a main and an additional strand, but such disposition of nerves as is found in *Eupagurus*, viz. with four branches approaching the strand at a distance from one another, has not been observed before in any species.

THE PROBLEM OF FUNCTION OF THE RECEPTORS IN THE COXAL REGION

The elucidation of the functions of the receptor organs lies in the field of physiology, and hypothetical speculations about them can easily go astray if not verified by experiments. However, these organs in the legs of the Crustacea exhibit such remarkable structural differences that it seems worth while to analyse those features of their anatomy from which their function may be inferred.

Summing up all evidence available, the following categories of proprioceptors in the legs of Crustacea can be distinguished: (1) rows of nerve cells ending on connective tissue strands; (2) muscular receptor spanning the thoracico-coxal articulation; (3) innervated strands associated with the levator and depressor muscles; (4) Barth's organ.

All these receptors are in all probability concerned with the movements of leg segments, with the possible exception of the organ described by Barth (1934), the function of which is obscure and will not be discussed here. Some of them may, moreover, be responsible for the reflex of the compensatory eye movements (Dijkgraaf, 1956).

The organs of the first category, of which there are at least seven in each peraeopod may show some differences in the arrangement of their elements, but all are made on the same principle. They are evidently stretch receptors, and being situated at the articulations are presumably designed to register the position of the adjoining segments in relation to one another. The problem why in some joints there is only one organ and in others—e.g. mero-carpopodite and carpo-propodite joints—there are two, and what are their different functions, awaits solution. In view of the ubiquity of these organs and the relation of some of them to the muscles, the hypothesis of Burke (1954) attributing to this type of receptor in the propo-dactylopodite articulation the additional function of perception of vibrations is open to doubt.

Muscular receptor

The muscular receptor in the thoracico-coxal articulation, which consists chiefly of contractile substance, must work on a different principle. In trying to understand its function the fact should be taken into consideration that it can occur either alone, or accompanied by a second receptor which by its structure belongs to the first category mentioned above.

Let us first examine the latter type in which, as in *Homarus*, two receptors run side by side: the one, a muscle with sensory elements ending on its tendon, and the other, a strand of connective tissue fibres with many sensory nerve cells ending on them. In the former, stimulation of sensory endings can evidently be brought about by the pulling on the tendon, and it must therefore take place each time that the muscle contracts and its myofibrils draw on the tendon.[1] The contraction of this muscle occurs in all probability when the leg is moved forwards (Text-fig. 4A). This can be assumed from its position on the

[1] It appears to be a general rule that the sensory elements in all the receptors known in Crustacea end not on the muscle itself, but on the connective tissue fibres. In contradiction to this Florey & Florey (1955) stated that in the abdominal receptor organs in *Astacus fluviatilis* no tendinous region is present and that the processes of sensory cells end directly on the muscle. This is a mistake. These organs in *Astacus* have intercalated tendons at the same place as in *Homarus* and *Palinurus*, i.e. where the nerve cells distribute their arborizations. Only a small number of myofibril bundles pass uninterrupted through this region in the same way as they are known to do in some segments of *Homarus*.

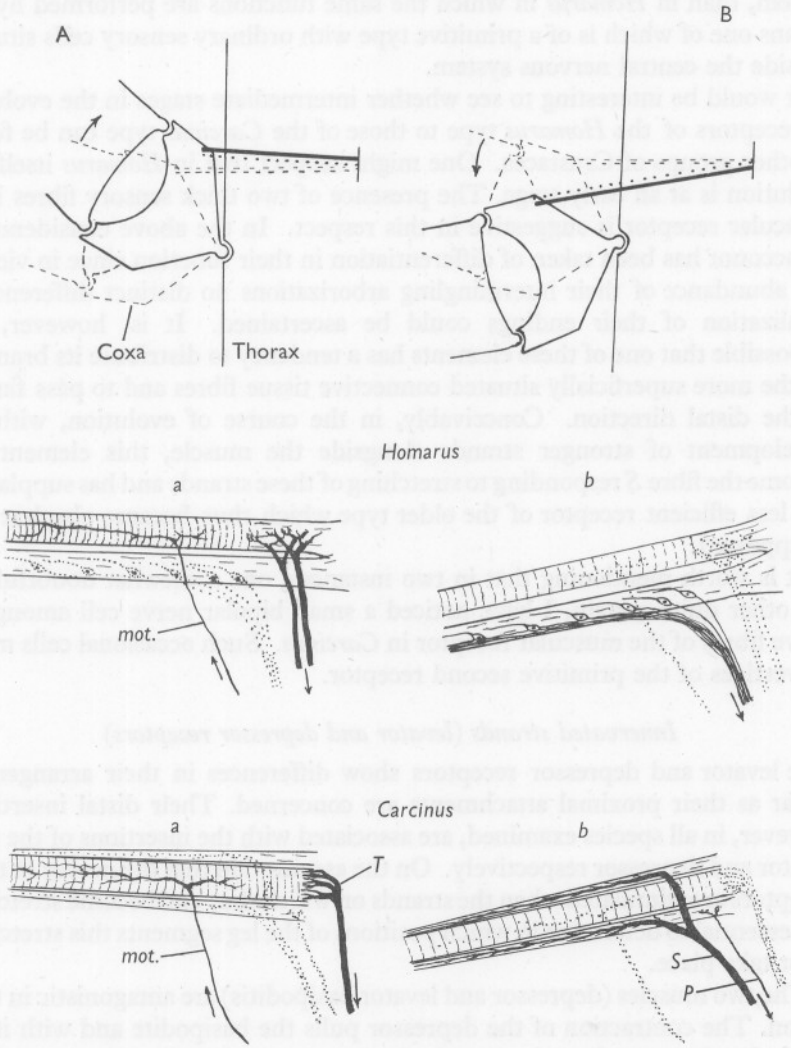
antero-ventral wall of the coxa in the vicinity of m. promotor coxae the name of which indicates its action. It has been moreover observed in some instances that the same fibre which takes part in the motor innervation of the receptor muscle sends its branches to m. promotor indicating the simultaneity of action of these two muscles.

The second receptor, which runs close to the muscular receptor and has similar attachments, consists of non-contractile elements and consequently can respond only when being passively extended. As can be easily demonstrated, its stretching occurs during the backward motion of the leg (Text-fig. 4B). It may be therefore conjectured that the two receptors respond to the movements of the leg in the horizontal plane the muscular receptor being stimulated when the leg is moved forwards and the elastic receptor when it is pulled backwards (Text-fig. 4, *Homarus a*, *b*).

It cannot be overlooked that when the leg is moved backwards the distance between both ends of the muscular receptor is increasing and its tendon might be affected by the passive stretching of the muscle. It is, however, conceivable that this muscle may have a certain degree of slackness so that the pull on the tendon in the backward position is negligible and unable to stimulate its nerves. This view seems to be more probable than the assumption that the two organs are stimulated at one and the same time.

When the muscular receptor alone is present it is found to have a special arrangement of its nerve elements which may be called the *Carcinus* type, as it was first observed in this species; besides, being less complicated as in *Eupagurus*, it is also more suitable for diagrammatic representation. The most remarkable feature of this arrangement is the separation of the areas of termination of the two main sensory fibres. One of them, fibre *T* (Text-fig. 4, *Carcinus a*), clearly shows that it is destined to respond to contractions of the muscle and should therefore be regarded as conveying impulses during the forward motion of the leg. There is no reason to suppose that the second fibre *S* is affected at the same time for it passes into the strands flanking the muscles which have proximally only a loose relation with it and which in any event would be slackened when the muscle contracts. When, however, the leg moves backwards and the receptor becomes extended these strands must take up the strain and their stretching stimulates the endings of fibres *S*, *P* (Text-fig. 4, *Carcinus b*). It must be assumed that here too the muscle is so adjusted that during the backward movements of the leg no response is elicited in its fibre *T*. It may be recalled that the flanking strands pass distally into the sheath surrounding the muscle and thus can give protection to the latter.

If this interpretation is correct the receptor of this type would be a remarkably effective organ capable of controlling the actions of two antagonistic muscles, viz. mm. promotor and remotor coxae. It would represent a higher stage of evolutionary development, in which only one organ is present with all its sensory neurons having their cell bodies within the central nervous



Text-fig. 4. (A, B) Diagrams of the movements of the legs in the thoracico-coxal articulation. The axis of these movements is represented as being perpendicular to the plane of the drawing and that of the coxo-basal articulation in this plane (actually these axes in different species can be inclined at various angles to the horizontal and vertical planes and can more or less deviate from being at right angles to one another). The drawings of the proximal parts of the thoracico-coxal receptors of *Homarus* type and *Carcinus* type show in *a* and *b* the nerve elements entering into action during the movements indicated in the diagram above in the same column. The elements out of action are drawn in dotted lines. The two strands flanking the receptor muscle in *Carcinus* are represented in diagrams by one line only.

system, than in *Homarus* in which the same functions are performed by two organs one of which is of a primitive type with ordinary sensory cells situated outside the central nervous system.

It would be interesting to see whether intermediate stages in the evolution of receptors of the *Homarus* type to those of the *Carcinus* type can be found in other groups of Crustacea. One might suspect that in *Homarus* itself this evolution is at an early stage. The presence of two thick sensory fibres in its muscular receptor is suggestive in this respect. In the above considerations no account has been taken of differentiation in their function since in view of the abundance of their intermingling arborizations no distinct difference in localization of their endings could be ascertained. It is, however, not impossible that one of these elements has a tendency to distribute its branches on the more superficially situated connective tissue fibres and to pass farther in the distal direction. Conceivably, in the course of evolution, with the development of stronger strands alongside the muscle, this element has become the fibre *S* responding to stretching of these strands and has supplanted the less efficient receptor of the older type which thus became obsolete and disappeared.

It is worth mentioning that in two instances, one somewhat doubtful but the other quite certain, I have noticed a small bipolar nerve cell among the nerve fibres of the muscular receptor in *Carcinus*. Such occasional cells might be vestiges of the primitive second receptor.

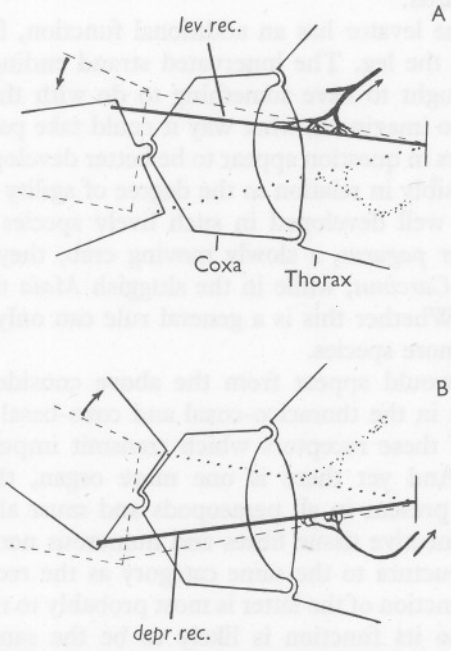
Innervated strands (levator and depressor receptors)

The levator and depressor receptors show differences in their arrangement as far as their proximal attachments are concerned. Their distal insertions, however, in all species examined, are associated with the insertions of the mm. levator and depressor respectively. On the assumption that the nerves of these receptors are stimulated when the strands on which they end become stretched, it is essential to determine in which positions of the leg segments this stretching must take place.

The two muscles (depressor and levator basipoditis) are antagonistic in their action. The contraction of the depressor pulls the basipodite and with it the whole leg downwards and when the legs are pressed against the ground the animal is able to lift its body. In this position of the basipodite, as shown in diagram (Text-fig. 5A), the dorsally situated strand of the levator receptor must become extended, and consequently whenever the animal stands up and walks its levator receptors are likely to be in action.

The ventral strand, inserting on the tendon of the depressor muscle, will become relaxed when the latter contracts, but is extended when the basipodite is pulled upwards (Text-fig. 5B). It is, however, not so obvious as with the levator receptor when precisely during this movement the receptor nerves can be stimulated, because when the body is actively lifted the ventral strand

is more slackened, compared with its state in the resting animal, and from this state it is unlikely to respond immediately to the contraction of m. levator. It should, however, be borne in mind that as the animal walks the leg movements in the dorso-ventral plane are combined with those in the horizontal plane, and as the movements in the latter may exert some tension on the receptor strands they can contribute in bringing the leg segments into such a position that the depressor receptor can come into play. Thus, according to this interpretation the two innervated strands would be designed to regulate



Text-fig. 5. Diagram of the movements of the legs in the coxo-basal articulation. The plane of this drawing is at right angles to that in Text-fig. 4. The position of the basipodite in the resting animal is indicated by interrupted lines. (A) Contraction of m. depressor basipoditis. (B) Contraction of m. levator basipoditis.

the action of the depressor and levator muscles, and it should be noted that the *levator* receptor would respond to the contraction of the *depressor* muscle and vice versa. The terms designating these organs relate therefore to their topographical connexions and not to their function. It is not proposed to change these names since they have been used in the description given previously, and moreover their anatomical relation is a fact whereas the assumptions concerning their function are as yet hypothetical.

Other hypotheses could also be put forward, as, for instance, that each of these receptors responds to variations of contraction of that muscle with which it is associated. There are two objections to this idea: first it implies that the

stimulation of the receptors would not occur when the receptor strand is stretched but when it is slackened, which seems less likely; and secondly, it would not explain why the levator receptor has a richer innervation and in some species like *Eupagurus* and *Palinurus* appears to be a much more conspicuous organ. This feature fits the conception that the levator receptor controls the depressor muscle which has the important function of keeping the body above the ground as long as the animal is on the move, while the task of the levator muscle is only to lift the leg and keep it lifted when it is being shifted forwards.

It is true that the levator has an additional function, for it is the muscle which autotomizes the leg. The innervated strand ending with this muscle might thus be thought to have something to do with the autotomy. It is, however, difficult to imagine in what way it could take part in this process.

The two receptors in question appear to be better developed in some species than in others, possibly in relation to the degree of agility of the animals. In fact they are very well developed in such lively species as *Eupagurus* and *Carcinus*: in *Cancer pagurus*, a slowly moving crab, they appear to be less developed than in *Carcinus*, while in the sluggish *Maia* they are apparently absent altogether. Whether this is a general rule can only be established by a special study of more species.

To sum up, it would appear from the above considerations that every possible movement in the thoracico-coxal and coxo-basal articulations elicit stimuli in some of these receptors which transmit impulses to the central nervous system. And yet there is one more organ, the coxo-basipodite receptor, which is present in all peraeopods and must also play some role. Being made of connective tissue fibres and numerous nerve cells, it belongs according to its structure to the same category as the receptors in all other joints. Since the function of the latter is most probably to register the position of the segments, so its function is likely to be the same. Its apparently superfluous occurrence in the same articulation in which other receptors are present can perhaps be explained on the supposition that it responds to the movements of the leg when the animal is not walking and when no complicated co-ordination of leg movements is needed. When, however, adjustments of the locomotory mechanism are required, a finer apparatus must come into action.

The superiority of this finer apparatus lies evidently (*a*) in the shifting of the cell bodies of all its neurons into the central nervous system, thus offering similar advantages as in the nervous system of vertebrates, and (*b*) in the considerable thickness of the afferent fibres facilitating the speedy transmission of the impulses in the reflexes co-ordinating the movements of the legs.[1] The

[1] The situation of the cell bodies of the receptor neurons in the thoracic ganglia could not yet be ascertained. The direction of the course of the thick sensory fibres of the muscular receptor traced in *Homarus* leads to a group of smaller cells, but not the smallest ones, situated laterally to the largest cells in the postero-ventral region of the ganglion.

abundance and variety of these fibres is indicative of the complexity of their functions and is further evidence of a far advanced evolutionary stage of the coxal receptors compared with those consisting of rows of sensory nerve cells. In the latter it is only the variations in size of these cells and in thickness of their axons which point to some differences, probably of graded character, of their function, whereas in the three receptors in the coxa there are several nerves and the role of each of them poses a problem which can only be solved by physiological experiments. Structural differences only occasionally afford some clue. One point concerning the thinner fibre *P* in the muscular receptor is worth mentioning. In crabs it can be seen that this fibre is confined to a small area of the same strand into which the thick fibre *S* penetrates (Text-fig. 5, *Carcinus b*), and as far as could be ascertained the end-filaments of the two fibres do not mix. Such a disposition would indicate that this fibre *P* is unlikely to have an inhibitory or excitatory character and that it probably responds to the variations of tension of the same strand as the fibre *S*, but transmits it at a lesser speed. In the levator receptor the thick and thin nerve fibres also appear to have independent areas of termination, but the pictures of their distribution are less distinct owing to the abundance of various nerve branches. Moreover, fibres which look much alike can certainly be physiologically different. Since, for instance, the thick fibres of the levator receptor, each of them much thicker than the strand on which they end, are at least two in number, it seems unlikely that they would have an identical function.

Thus, as far as the histological evidence goes, it can be stated that if experiments could show that a proprioceptor in the coxal region is capable of transmitting impulses to several neurons in the central nervous system, to excite some of them and inhibit others, and to do it at different speeds, there would be no reason to question whether it has enough nerve elements for all these various functions.

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SUMMARY

In the coxal region of *Eupagurus bernardus* the following receptor organs have been found: (1) a muscular receptor spanning the thoracico-coxal articulation, its innervation, in which several neurons take part, being arranged on a similar pattern as in *Carcinus*; (2) two innervated elastic strands running along the bundles of mm. levator and depressor basipoditis respectively and inserting into the tendons of these muscles; (3) a coxo-basipodite receptor consisting of connective tissue strand with numerous bipolar nerve cells ending on it.

With the exception of the coxo-basipodite receptor, all sensory neurons of these organs have their cell bodies located in the central nervous system.

It is suggested that these receptors convey impulses elicited by the movements of the legs and some hypotheses concerning the role of each of them are put forward.

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EXPLANATION OF PLATE I

All photographs were made from preparations of *Eupagurus bernhardus* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar. Figs. 1, 3, 5 and 6 are made with the same magnification but the respective preparations were obtained from animals of various sizes.

Fig. 1. *MR*, thoracico-coxal receptor of the 2nd left peraeopod in connexion with the thoracic ganglion. The thin fibre crossing obliquely the nerve trunk in the left lower corner is the strand of the levator receptor (displaced).

Fig. 2. Proximal part of the thoracico-coxal receptor of the 2nd left peraeopod. *T*, fibre ending on the tendon.

Fig. 3. Thoracico-coxal receptor of the 2nd left peraeopod, showing its distal attachment.

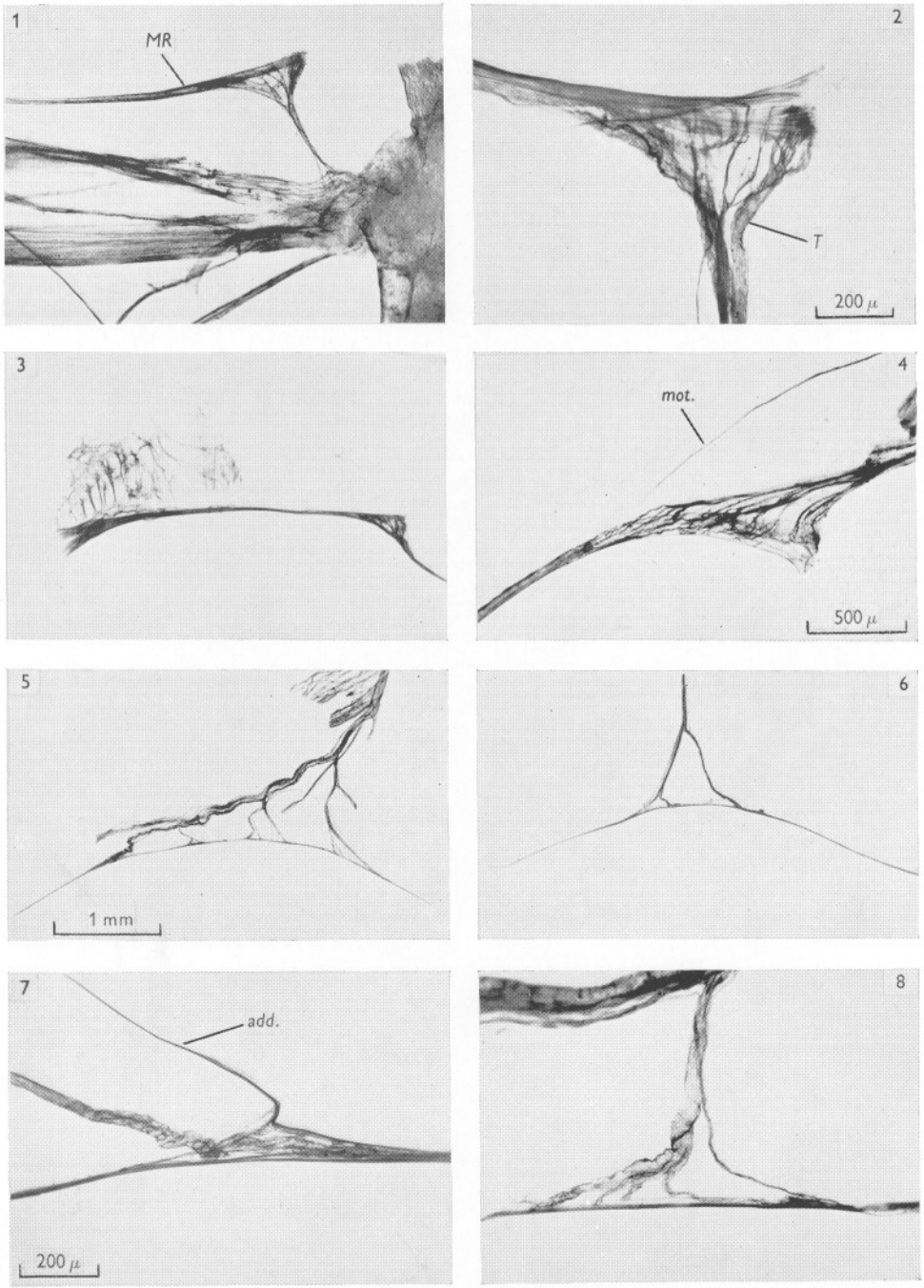
Fig. 4. Thoracico-coxal receptor of the 5th left peraeopod with nerve fibres branching before reaching the muscle. *mot.*, motor nerve.

Fig. 5. Levator receptor of the 2nd left peraeopod with four nerve branches ending on the strand; cf. Text-fig. 3B.

Fig. 6. Depressor receptor of the 3rd peraeopod. This preparation was made from a much larger animal than that shown in fig. 5.

Fig. 7. Part of the levator receptor of the 3rd right peraeopod showing the nerve fibre at the point of meeting of the main and additional strands.

Fig. 8. Thick and thin fibre ending on the strand of the levator receptor. Same magnification as fig. 7.



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STUDIES ON THE GROWTH OF MARINE PHYTOPLANKTON

I. *ASTERIONELLA JAPONICA* GRAN

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(Text-figs. 1–14)

Although the basic role of planktonic algae in the economy of the seas is well recognized, knowledge of their requirements for growth is still meagre. Besides work in which certain of these organisms are used as convenient objects for specialized physiological study there is need for more general investigations on the effects of different chemical and physical factors on the growth of species representative of the main groups of phytoplankton. Apart from their value for the understanding of the growth of natural populations, investigations of this latter type are essential for assessing the possibilities of the mass culture of planktonic algae for economic purposes. The object of the investigations to be described in this and subsequent papers was to obtain general information on the growth of single representatives of each of the three main classes of marine phytoplankton. The first of these to be considered is the diatom *Asterionella japonica* Cleve & Müller ex Gran.

Previous work on the growth of marine diatoms has been summarized by Harvey (1955), and most of the important references on their nutrition are given by Provasoli, McLaughlin & Droop (1957). Among studies particularly concerned with the growth rates of these organisms that of Braarud (1937) is noteworthy as having been the first to have been made with controlled temperature and illumination. In a subsequent paper Braarud (1945) has recorded the relative growth constants of many species grown in unialgal culture under such conditions. Recent studies on the effects of a factor of particular importance for diatom growth, viz. silica supply, have been made with marine and freshwater diatoms (Jørgensen, 1953, 1955, 1957; Lewin, 1957). The photometric technique for measuring growth has been used by Gross & Koczy (1946) and by Spencer (1954).

DEFINITIONS

In this work 'growth' has been taken primarily to mean increase in cell numbers. However, because it is more conveniently determined and leaves the culture intact, optical density rather than cell density of algal suspensions

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has usually been used as the direct measure of growth. Optical density depends on cell size, pigment content and other factors as well as on cell numbers, but a means of obtaining at least approximate values for relative growth constants from optical density determinations has been devised.

The term 'optimum' as applied to nutrient concentrations and the level of physical factors has often been used loosely. Rodhe (1948) distinguished between a potential optimum level realized only under ideal conditions and an actual optimum depending on other factors. The latter can be found not only for the rate of growth but also for the final population. The two may not be identical under certain conditions, that is the range of concentration of a nutrient allowing the maximum growth rate may not extend to concentrations high enough for the attainment of maximum cell crop. As the magnitude of the latter is dependent on many factors it seems convenient to determine and express nutrient levels in terms of requirement per cell so that limitations by each factor can be readily calculated. The number of cells produced in response to the addition of a particular amount of a nutrient has been termed the 'cell crop'. This has been taken as the difference between the final cell concentration in the control cultures without the addition and that in cultures to which the nutrient was added.

METHODS

Glassware was cleaned by soaking in chromic acid followed by thirty complete rinses with tap water and three with distilled water. Steaming was found unnecessary. Pyrex glass culture vessels were used, either 50 ml. boiling tubes or 100 ml. conical flasks, plugged with non-absorbent cotton wool. They were maintained in a constant-temperature water bath, usually at 20° C, with underwater fluorescent lighting providing 5000–9000 lux.

The system used for aerating boiling-tube cultures was a modified form of that used by Spencer (1954). Compressed air from a diaphragm pump passed through the sintered-glass discs of three wash bottles of distilled water to a manifold of twelve needle valves. Through each valve it passed to a cotton-wool filter which was heated to prevent condensation (Fogg, Smith & Miller, in the press). This was attached by rubber tubing to the arm of a culture tube. This consisted of a normal boiling tube with a fine-bore glass tube sealed into the side, about 3 cm from the top, and passing down the inside against the wall to end in a jet at the bottom. This culture vessel was plugged with cotton wool and placed in the constant temperature tank.

The three media used for experiments on *Asterionella* are shown in Table 1. To avoid precipitation phosphate was added after autoclaving. Aseptic techniques were always employed. Media were sterilized by autoclaving at 15 lb. for 15 min. The media used for testing for contamination were those of Spencer (1952) made up with undiluted natural sea water.

When necessary, inocula were washed by aseptic centrifuging. The culture used was pipetted into a sterile centrifuge tube and a cotton-wool plug tied on. After centrifuging the supernatant was drawn off with a sterile pipette and replaced with sterile medium. After repeating the process, if necessary, the cell suspension was used as an inoculum.

TABLE 1. THE COMPOSITIONS OF THE MEDIA USED FOR *ASTERIONELLA*

	AK In sea water	AR_N In sea water	AR_A In distilled water
KNO_3	2·0 mM	2·0 mM	2·0 mM
K_2HPO_4	0·2 mM	0·2 mM	0·2 mM
$FeCl_3$	10 μM	5·5 μM	5·5 μM
$MnCl_2$	1·0 μM	—	—
Na_2SiO_3	20 μM	100 μM	100 μM
$ZnSO_4$	—	2·5 μM	2·5 μM
$MnSO_4$	—	20 μM	20 μM
$CoSO_4$	—	0·05 μM	0·05 μM
$CuSO_4$	—	0·02 μM	0·02 μM
H_3BO_4	—	550 μM	970 μM
NaCl	—	—	401·5 mM
$MgCl_2.6H_2O$	—	—	50·2 mM
Na_2SO_4	—	—	27·6 mM
$CaCl_2$	—	—	10·9 mM
KCl	—	—	8·91 mM
$NaHCO_3$	—	—	2·28 mM
KBr	—	—	0·806 mM
$SrCl_2.6H_2O$	—	—	0·150 mM
NaF	—	—	0·0714 mM
EDTA	—	0·342 mM	0·342 mM
Tris	—	8·25 mM	8·25 mM
Soil extract	20 ml./l.	—	

Growth was estimated by cell counts alone or in combination with optical density measurements. For cell counts a 1 ml. sample was removed from each culture and kept in a stoppered tube with solid iodine which killed and stained the cells and increased their specific gravity. The sample was shaken vigorously, which broke up the colonies to some extent, and the cells counted with a haemacytometer. Between 800 and 1000 cells were counted from each sample. Counts on twelve groups of 800 cells from the same sample gave a standard deviation of ±6·0% of the mean. For optical density (OD) determinations a Unicam absorptiometer UIC no. 6117 was used. Culture tubes were selected to fit the compartment and marked so that they were always placed in the same way round. Appropriate blanks were used. Absorption was measured at the chlorophyll absorption peak, 680 mμ. The OD was found to be directly proportional to cell concentration up to 2500 cells/mm³. The units used for OD were arbitrary.

For comparison between cultures it was necessary to express growth rates in terms of cell numbers. It was observed that the OD/cell varied according to the conditions under which the cells were grown and according to their ages and could therefore change during the course of an experiment. The initial cell concentration and also that at or near the end of the logarithmic

growth period was determined by direct counting. The logarithmic rate of increase in OD was known, but if the OD/cell had changed this would not be equivalent to the logarithmic rate of increase in cell concentration. If it is assumed that the change in OD/cell occurs progressively during division an approximate way of calculating the relative growth constant k for cell numbers is to extrapolate the straight line on the graph of $\log_{10}$ OD to the levels of OD at the time the cell counts were made, and take the corresponding time as that needed to produce the observed change in cell concentration by exponential growth. This is shown diagrammatically in Fig. 1. The relative growth constant k is then given by the usual formula:

$$k = \frac{\log_e N_T - \log_e N_0}{t} = \frac{2{\cdot}3}{t}(\log_{10} N_T - \log_{10} N_0).$$

Where N_T = the cell concentration at time T; N_0 = the cell concentration at time 0; and t = the time in days.

There was sometimes considerable variation of the relative growth constant from experiment to experiment due to variations in the age of the inoculum (p. 409). This did not affect the results within each experiment as controls were always included. Comparisons between separate experiments have been made only when the inocula were similar.

Cultures were invariably prepared in duplicate or triplicate, Student's t-test being used to test the statistical significance of the differences in growth observed in the experiments.

EXPERIMENTS

Isolation

Asterionella japonica was isolated into unialgal culture from a tow-net sample from the Hamble River, Hampshire, in October 1953.

Attempts to free this diatom of bacteria were unsuccessful. It failed to grow on a solid sea-water agar medium, and could thus not be separated from the bacteria by plating techniques. The use of antibiotics also proved unsatisfactory. The diatom was grown in concentration ranges of penicillin and streptomycin similar to those used by Spencer (1952) and also in a range of solutions of chloramphenicol. The cell concentrations after about 10 days' growth are shown in Fig. 2. Before testing for contamination the algae were subcultured to ensure sufficient dilution of the antibiotic for the development of any bacteria present. The results of the tests showed that certain colonies of bacteria were unaffected by concentrations of any of the antibiotics tolerated by the diatom. It is possible that further exposure or a mixture of the substances might have been effective. The curious increase in cell concentration with the rise of penicillin from 60 to 3000 μg/ml. will be discussed later. Finally, small colonies were washed by the method described by Pringsheim (1946). As it seemed possible that the diatom was dependent on bacteria for

organic growth substances, the washed cells were inoculated into a variety of organic media which contained small quantities of soil extract, peptone, liver extract, amino acids and vitamins (including cobalamin). Growth of *Asterionella* took place only in medium AR without organic addition and then there was bacterial contamination. Most of the remaining media were contaminated. It seems likely that this was due to adherence of bacteria to the diatom frustules.

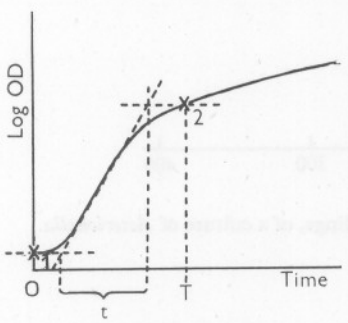

Fig. 1

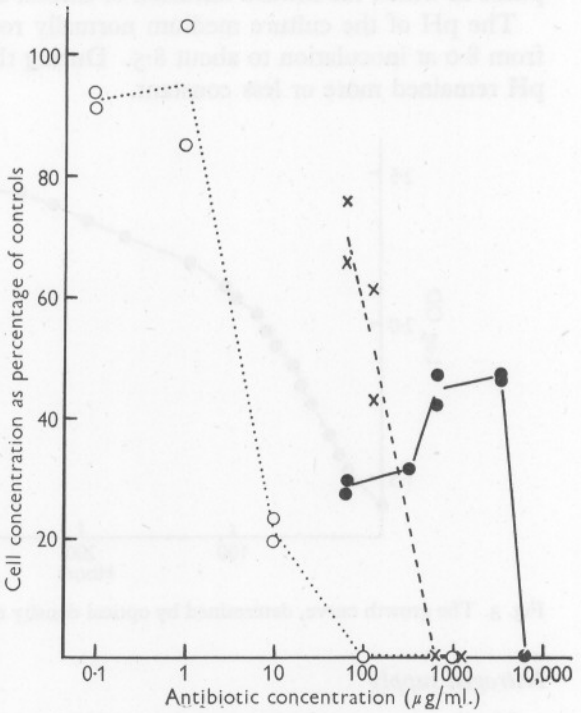

Fig. 2

Fig. 1. Diagram of the method of calculating the relative growth constant. The cell counts were made at times o and T, when the optical density readings were X_1 and X_2, respectively. The time interval t was used in the usual formula for the calculation of the relative growth constant k.

Fig. 2. Cell concentrations of *Asterionella* after 10 days' exposure to various antibiotic concentrations, expressed as percentages of those in controls without antibiotic additions. —●—●—, penicillin; –×– – –×–, streptomycin; ··○···○··, chloramphenicol.

With the failure to obtain a bacteria-free culture of *Asterionella*, studies were made of the nutritional requirements of the alga in unialgal but not pure culture. Most of the studies were on mineral nutrition and the effects of physical factors, so that complications due to the presence of bacteria were probably minimal, and identical in all the cultures of a single experiment with equal inocula.

Growth

An example of the growth curve of *Asterionella* in stagnant medium in boiling tubes is shown in Fig. 3. There was a lag period of 0–20 h followed by an exponential phase with a relative growth constant usually of $k = 0{\cdot}7–1{\cdot}2$. This gave way, after less than 100 h from inoculation, to a more or less linear phase in which the inward diffusion of carbon dioxide was evidently limiting.

The pH of the culture medium normally rose during logarithmic growth from 8·0 at inoculation to about 8·5. During the phase of slower growth the pH remained more or less constant.

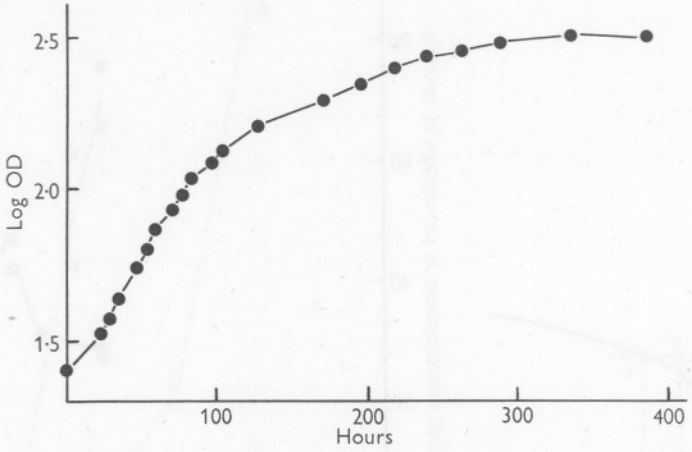

Fig. 3. The growth curve, determined by optical density readings, of a culture of *Asterionella*.

Nitrogen supply

Media were prepared containing added concentrations of potassium nitrate in artificial sea water of 0, 0·1, 0·3, 1·0, 3·0 and 10·0 mM. They were inoculated with cells that had been washed in nitrate-free artificial sea water. The cultures were aerated. The growth was followed by means of OD measurements and cell counts made when it had ceased.

Variation in nitrate concentration from 1 to 10 mM had no statistically significant effect on the relative growth constant. There was a marked difference in the OD/cell, however, that at 10 mM being three times that at 0·1 mM.

The cell crop is shown plotted against nitrate concentration in Fig. 4. The figure also shows the quantity of nitrate added to the medium divided by the crop obtained from it. This should be constant for nitrate-limited cultures. It is apparent that the two lowest concentrations were limiting. The mean quantity of nitrogen in these was 0·255 $\mu\mu$g atom N/cell. This can be regarded as the minimum required for these particular cells, since the method of

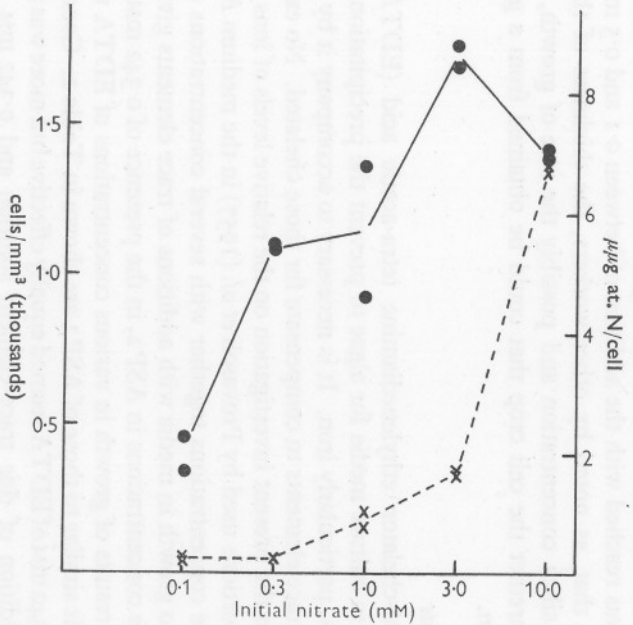

Fig. 4. The cell crop and nitrogen per cell of *Asterionella* in relation to concentration of potassium nitrate. —●—●—, cell crop; –×–––×–, nitrogen/cell.

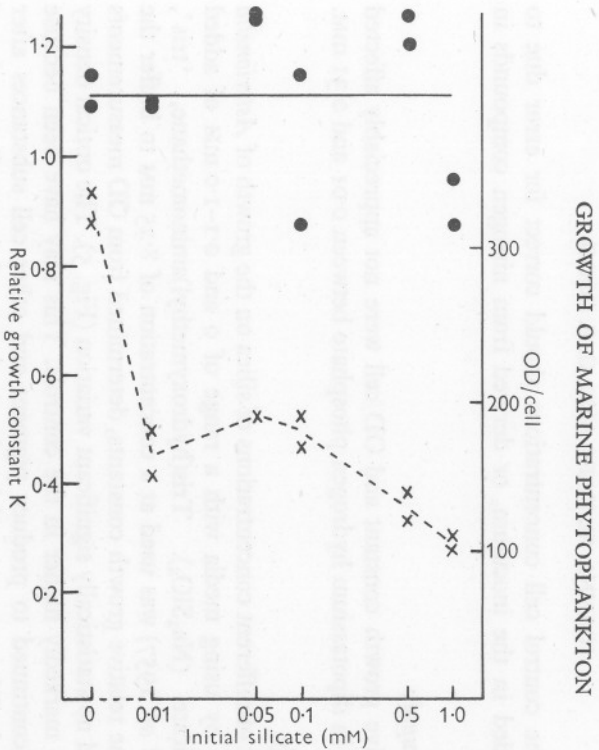

Fig. 5. The relative growth constant and final optical density per cell of *Asterionella* in relation to concentration of sodium silicate. —●—●—, k; –×–––×–, OD/cell.

deducting the control cell concentrations should correct for error due to nitrogen added in the inoculum, or derived from nitrogen compounds in the air.

Phosphorus supply

The relative growth constant and OD/cell were not appreciably affected by variation in dipotassium hydrogen phosphate between 0·01 and 0·31 mM.

Silicon supply

The effect of different concentrations of silica on the growth of *Asterionella* was studied by using media with a range of 0 and 0·1–1·0 mM of added sodium silicate (Na_2SiO_3). Tris(hydroxymethyl)aminomethane, 'tris', (Provasoli *et al.* 1957) was used at a concentration of 8·25 mM to buffer the medium. The relative growth constants, determined from OD measurements only, showed no statistically significant variation (Fig. 5). The optical density per cell was markedly higher in the controls. This may have been because the diatom continued to produce pigment and other cell substances after cell division had been prevented by the depletion of the small quantity of silica from the glass and the inoculum.

The final cell concentrations in this experiment showed that the maximum population was reached with the addition of between 0·1 and 0·5 mM silicate. It was clear that, as noted by other workers, the thickness of the frustule varied with silica concentration and possibly the rate of growth, making it difficult to predict the cell crop that could be obtained from a given silica concentration.

Trace elements

The metal-chelator ethylenediamine tetra-acetic acid (EDTA) is now widely used in culture media for algae to prevent the precipitation of certain essential ions, particularly iron. It is necessary to accompany it by additional amounts of trace elements to compensate for those chelated. No experiments were made in the present investigation on the relative levels of ions necessary, but the proportions used by Provasoli *et al.* (1957) in the medium ASP 2 were tried in three concentrations together with several concentrations of EDTA. There was no growth in media with additions of trace elements giving 10 and 100 times the concentrations in ASP 2, in the presence of 0·342 mM of EDTA or less. The results of growth in various concentrations of EDTA with trace-element levels similar to those of ASP 2 are shown in Table 2. Concentrations of 0·0342–0·342 mM of EDTA seemed equally effective but more was inhibitory.

As the addition of this trace-element solution and 0·342 mM of EDTA proved successful it was considered a convenient way of providing minor elements and was used thereafter, in media AR_N and AR_A.

TABLE 2. GROWTH OF *ASTERIONELLA* IN MEDIA WITH VARIOUS EDTA ADDITIONS

	EDTA				
	0·0342 mM	0·171 mM	0·342 mM	1·71 mM	3·42 mM
	First experiment				
Cells/mm³ at 485 h	2660	2110	3320	—	—
	2820	2400	1180	—	—
	Second experiment				
Cells/mm³ at 340 h	—	—	1067	657	363
Relative growth constant *k*	—	—	0·967	0·801	0·773
	—	—	0·912	0·801	0·690

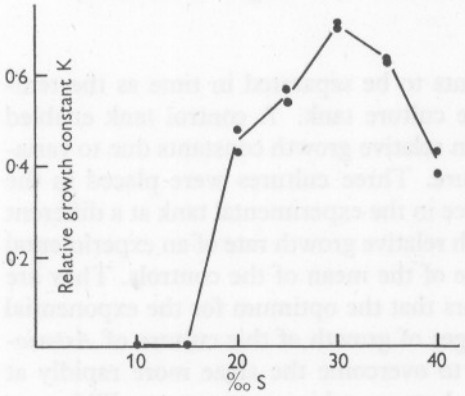

Fig. 6

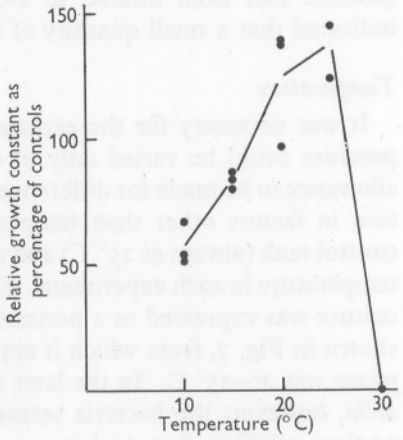

Fig. 7

Fig. 6. The relative growth constant of *Asterionella* in media based on sea water of various salinities.

Fig. 7. The relative growth constant of *Asterionella* at different temperatures (see text for further explanation).

Salinity

Asterionella was grown in natural sea water of various salinities produced by appropriate dilution or evaporation, with the usual nutrient additions. The relative growth constants, derived from cell counts only, are shown in Fig. 6. The optimum was between 30 and 35‰ S. The only salinities in which no growth took place were 10 and 15‰ S. It appears that *Asterionella* is fairly tolerant of salinities of 20 to 40‰ S or more. The relative growth constants at the extremes could possibly be increased by acclimatizing the cells to the different osmotic pressure.

Artificial sea water

Most of the experiments were made with natural sea water as a base. Although the principal constituents are remarkably constant there are several

components of biological importance which may vary. It was therefore desirable to use artificial sea water if the medium was to be defined as closely as possible. A solution of the principal constituents found by Lyman and Fleming (Harvey, 1955, p. 137) in their analysis was used in place of natural sea water. When this, with the addition of nitrate, phosphate, silicate, EDTA and trace elements (i.e. medium AR_A) was compared as a medium with natural sea water with the same additions (i.e. medium AR_N), growth was identical. This result is equivocal, however, as the inoculum introduced 5% of natural sea water. In a later experiment normal growth was obtained in artificial sea water after the inoculum had been washed in such a way that the original medium had been diluted to 0·02%. Nevertheless, further subculturing indicated that a small quantity of natural sea water might be necessary.

Temperature

It was necessary for the experiments to be separated in time as the temperature could be varied only in one culture tank. A control tank enabled allowance to be made for differences in relative growth constants due to variation in factors other than temperature. Three cultures were placed in the control tank (always at 25° C) and three in the experimental tank at a different temperature in each experiment. Each relative growth rate of an experimental culture was expressed as a percentage of the mean of the controls. They are shown in Fig. 7, from which it appears that the optimum for the exponential phase was 20–25° C. In the later stages of growth of this culture of *Asterionella*, however, the bacteria seemed to overcome the algae more rapidly at 25 than 20° C so that the latter was a better working temperature. This may have been associated with progressive inhibition with time, as noted by Rodhe (1948). After 7 days at 30° C the diatom cells did not recover when returned to 20° C.

Light

The relative growth constant and optical density per cell was determined in various intensities of fluorescent ('white') light. Light intensity was measured with an Everett Edgcumbe autophotometer. The relative growth constants in three experiments under different conditions of temperature and aeration are shown in Fig. 8. The results were variable but it appears that there was saturation at 4000 lux and no inhibition at nearly 10,000 lux. The final OD/cell was almost unaffected by light intensity.

Aeration

As the length of the exponential growth phase of *Asterionella* seemed to be limited by the partial depletion of the carbon dioxide in the medium, an attempt was made to prolong the initial growth rate by artificial aeration. The result of this is illustrated in Fig. 9, in which the means of OD readings of

aerated and stagnant cultures over the first 100 h are shown. Not only was the level to which exponential growth continued raised but the initial relative growth constant was increased. This latter could be due to keeping the cells in suspension or to the provision of carbon dioxide at a faster rate. These effects will be discussed in a later section.

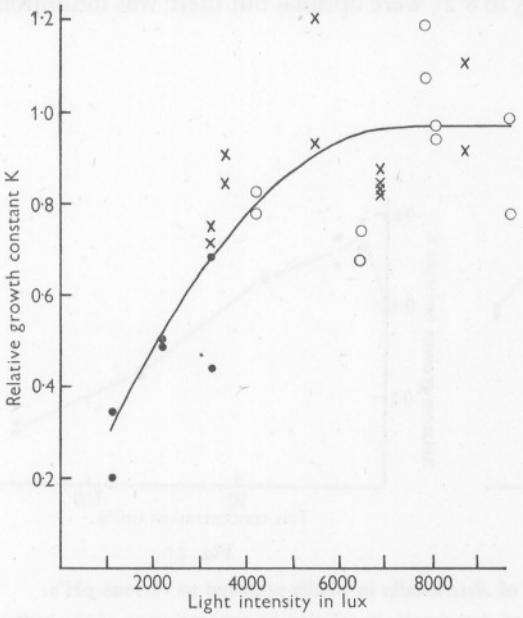

Fig. 8

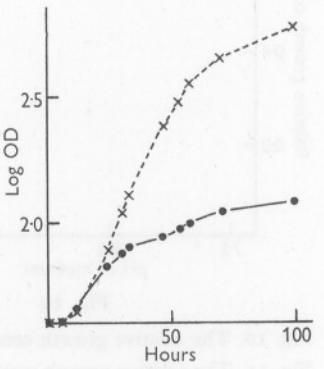

Fig. 9

Fig. 8. The relative growth constant of *Asterionella* grown under various conditions in different intensities of fluorescent light. ●, stagnant at 18° C; ×, aerated at 18° C; ○, aerated at 25° C.

Fig. 9. The growth curves, determined from optical density readings, of *Asterionella* in stagnant and aerated culture. —●———●—, stagnant; –×– – –×–, aerated.

Hydrogen-ion concentration

The apparent importance of the effect of pH on the growth of *Asterionella* necessitated a more detailed study of this factor. In the first place a method was devised to control the pH of the medium during the growth of the diatom. Two series of vessels were prepared, an 'experimental' and a 'dummy' series. Each of these consisted of duplicate cultures at six different pH's. The two series were treated in the same way except that no attempt was made to keep the dummies sterile. After inoculation and at intervals during growth the dummy tubes were opened and the pH of each medium adjusted. The quantity of acid or alkali needed for this adjustment was then added aseptically in a sterile form to the experimental series. Growth determinations were made on the latter.

In the first experiment of this kind no buffer was included in the sea-water medium. It was found that the removal of carbon dioxide altered the adjusted pH so rapidly that it was impossible to control it by this method. The buffer tris was therefore added to the medium at a concentration of 41·2 mM. The relative growth constants at the different adjusted pH's are shown in Fig. 10. It is apparent that pH's from 7·5 to 8·25 were optimal but there was inhibition at 8·5 and 8·75.

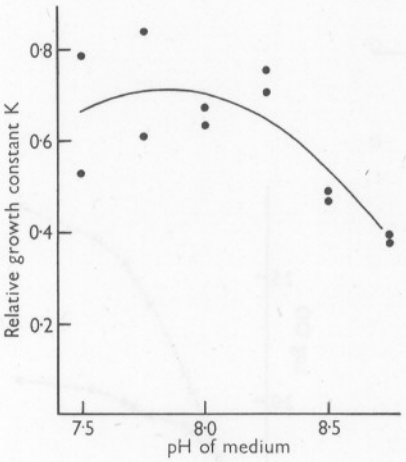

Fig. 10

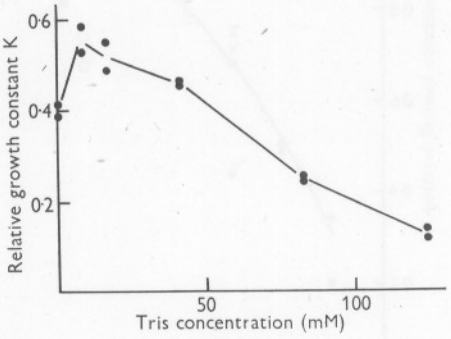

Fig. 11

Fig. 10. The relative growth constant of *Asterionella* in media adjusted to various pH's.

Fig. 11. The relative growth constant of *Asterionella* in relation to concentration of the buffer tris.

TABLE 3. GROWTH OF *ASTERIONELLA* IN MEDIA WITH AND WITHOUT TRIS

(Relative growth constant k (in triplicate).)

Without tris	0·380	0·437	0·366
With 8·25 mM tris	0·658	0·564	0·564

In using this buffer it was considered desirable to find the highest concentration that the diatom would tolerate. The relative growth constants were therefore determined in media with various additions of tris. These are shown in Fig. 11. There was stimulation at 8–16 mM, followed by inhibition with increasing concentration. Tris of greater purity caused a similar stimulation, as is shown in Table 3, the difference being statistically significant at the 1% level. The possibility that tris stimulated growth through its buffering action seemed the most likely and was studied further.

The effect of the growth of *Asterionella* on the pH of stagnant medium has already been mentioned. In order to follow the rise under different conditions the growth of two cultures was studied in detail in each case, while further

cultures under the same conditions were used successively for pH determinations. In this way the change in the pH of the medium was followed in the presence and absence of 8·25 mM of tris in stagnant culture and also in the absence of the buffer in aerated culture. The pH rose steeply during exponential growth in stagnant culture, but remained constant once this phase had ended. In the absence of tris it rose from pH 8·0 to 8·55 but in its presence only to 8·35–8·40. In aerated culture although the growth was much greater there was a negligible rise in pH. Thus, although the buffer was partly effective in preventing the pH change, it was not as effective as aeration.

TABLE 4. GROWTH OF *ASTERIONELLA* IN STAGNANT AND AERATED CULTURE WITH AND WITHOUT TRIS

(Relative growth constant k (in triplicate).)

	Stagnant			Aerated		
Without tris	0·695	0·619	0·663	1·18	1·16	1·17
With 8·25 mM tris	0·906	1·16	0·983	1·06	1·05	1·02

Arrows indicate a statistically significant difference at the 1% level.

If tris were stimulating growth solely because of its buffering action its effect would be masked under aerated conditions where little rise in pH took place in any case. An experiment was therefore made comparing the relative growth constants in media with and without tris, in stagnant and aerated culture. The results are shown in Table 4. It is apparent that instead of stimulating growth when the culture was aerated tris inhibited it, though only to the rate of stimulated stagnant growth. Aeration and tris thus had a similar action in accelerating growth. As in stagnant culture the cells settled to the bottom of the vessel, it would seem likely that this action was to prevent the pH in the vicinity of the cells from becoming inhibitory.

Unidentified inhibitory factor

The variation between relative growth constants from experiment to experiment was observed to be large, and it was therefore important always to have controls. In order to investigate the possibility that this variation might be associated with the size or age of the inoculum, identical culture media were inoculated with different amounts of inoculum from cultures of three different ages. The relative growth constants were calculated in the usual way and are shown plotted against the size of the inoculum in Fig. 12. There was evidence of a decrease in the constant both with increase in age and in increase of size of the inoculum. The extent of the variation with age was more than sufficient to account for the differences between the relative growth constants in different experiments. The decrease with increasing age may have been due to an increasing proportion of non-viable cells in the inoculum. The effect of size cannot be explained in this way. The relative growth constant should be the

same for a given proportion of viable cells with any inoculated number of these. A possible explanation might be that some inhibitory substance was carried over in the medium with the inoculum.

In an earlier experiment cultures were prepared with only half freshly autoclaved medium. The other half was Seitz-filtered sea water in three of the cultures and Seitz-filtered old *Asterionella* culture medium in another three. The relative growth constants (from OD measurements only) are given

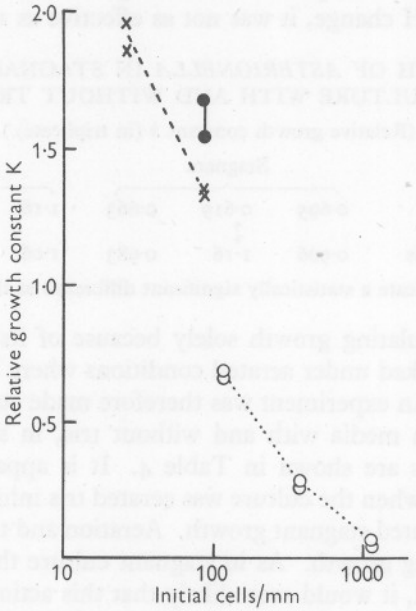

Fig. 12. The relative growth constant of *Asterionella* in cultures inoculated with different amounts of inoculum from cultures of three different ages. —●——●—, inoculum 37 h old; –×– – –×–, 190 h old; ··○····○··, 890 h old.

TABLE 5. GROWTH OF *ASTERIONELLA* IN PARTLY SEITZ-FILTERED MEDIUM

(Relative growth constant k (in triplicate).)

Half medium of sea water	0·442	0·529	0·465
Half medium of aged culture medium	0·331	0·412	0·343

in Table 5 and were significantly lower at the 2·5 % level in the cultures with the old medium. This seemed to indicate the presence of an inhibitory substance in the old culture.

Further experiments on this factor were inconclusive. It is possible that if an inhibitor was produced at all, it was produced only under certain conditions.

Unidentified stimulatory factors

It has already been suggested that *Asterionella* may require organic substances for growth and that it normally obtains these from bacteria in crude culture.

In the experiment on the range of penicillin tolerated by the diatom it was found that there was a stimulation of growth with increasing concentration of antibiotic within the range of 60–3000 μg/ml., although in general penicillin was inhibitory (Fig. 2, p. 401). It is likely that the inhibition at 60 μg/ml. was due to the effect of the antibiotic on the bacteria, inactivating them and thus slowing down the production of a substance necessary for diatom growth. Increasing quantities of penicillin caused stimulation either by providing the required substance or substances as chemical impurities, or by releasing them into solution from the bacterial cells as a result of autolysis. This might be taken as an indication that one or more organic substances stimulated the growth of *Asterionella*.

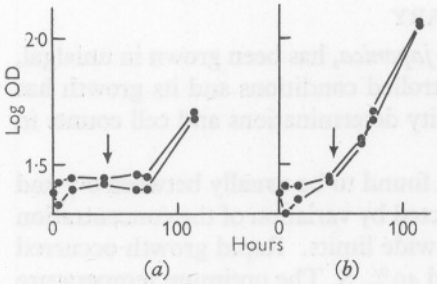

Fig. 13

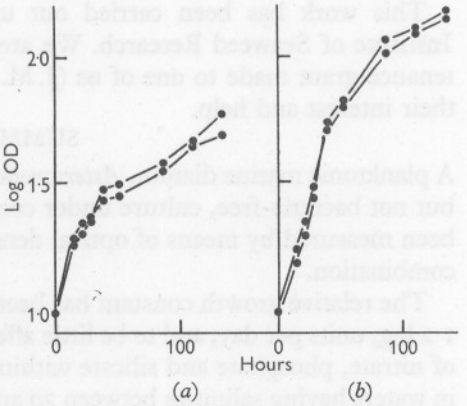

Fig. 14

Fig. 13. The growth curves of *Asterionella* in a synthetic medium, (*a*) without the addition of old medium, and (*b*) with the addition of 1 ml. of the supernatant of an aged culture. The arrow indicates the addition to each of 1 ml. of natural sea water.

Fig. 14. The growth curves of *Asterionella* in a natural sea water medium, (*a*) without addition, and (*b*) with the addition of 1 ml. of the supernatant of an aged culture.

Further evidence for this came from an experiment in which the inoculum was washed in artificial sea water for two successive subcultures in synthetic medium. The washing involved aseptic centrifuging and many of the bacteria would have remained in the supernatant and been discarded. Thus the proportion of bacterial to algal cells would have been reduced. Four of these cultures were grown, two as controls and two with the addition of 1 ml. of the supernatant of an aged medium. The growth curves are shown in Fig. 13. Growth was clearly stimulated by the presence of the aged medium, but only after the addition of 1 ml. of natural sea water to each culture. This may have been coincidental, though a lag of that length had not previously been observed.

In the next experiment the medium was based on natural sea water and an addition made from the same aged medium. The growth curves are shown in Fig. 14. Growth was immediate in all the cultures, but considerably better in those with the aged medium addition than in the controls. The absence of lag suggested that some substance, possibly cobalamin, present in natural sea water had to be present before growth could take place in the previous experiment. But it is clear that some further substance was derived from the aged medium. In a further experiment an addition of the same aged medium was made, in both autoclaved and unsterilized forms. The unsterilized addition was clearly more effective than the sterilized. Thus, some thermolabile factor is stimulatory to the diatom, but it cannot be said whether this is produced by *Asterionella* itself or by bacteria.

These results will be discussed together with those for other species on a later occasion.

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SUMMARY

A planktonic marine diatom, *Asterionella japonica*, has been grown in unialgal, but not bacteria-free, culture under controlled conditions and its growth has been measured by means of optical density determinations and cell counts in combination.

The relative growth constant has been found to be usually between 0·7 and 1·2 $\log_e$ units per day, and to be little affected by variation of the concentration of nitrate, phosphate and silicate within wide limits. Rapid growth occurred in waters having salinities between 20 and 40‰ S. The optimum temperature for growth was 20–25° C and the optimum light intensity from 4000 to 10,000 lux. The relative growth rate was affected most markedly by variation in hydrogen-ion concentration. This was manifest in stagnant cultures in which the rise of pH above 8·3, caused by absorption of carbon dioxide, resulted in the inhibition of growth. This could be countered by increased aeration or by the addition of the buffer tris(hydroxymethyl)aminomethane.

Evidence has been obtained which suggests that *A. japonica* requires a thermostable substance present in natural sea water and that a thermolabile substance stimulating its growth is produced in culture.

Final yields of the order of 4000 cells per mm^3 were obtained and, within limits, were dependent on the amounts of nitrate and silicate supplied. The nitrogen requirements per cell was determined as 0·255 $\mu\mu$g atom. Indications were obtained of the presence of a growth-inhibiting substance in the filtrates from old cultures.

The optical density of the cells was increased at high nitrate concentrations and when exhaustion of silicate prevented cell division.

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THE IMPORTANCE OF BACTERIA IN LABORATORY EXPERIMENTS ON REARING THE LARVAE OF *OSTREA EDULIS* (L.)

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Observations made by the writer during the summer of 1955 suggested that the density of the bacterial flora which develops when oyster larvae are reared in small glass vessels is inversely correlated with the number of spat settling (Walne, 1956). From the nature of the observations it could not be determined whether the bacteria were affecting the development of the larvae or whether the properties of the sea water which resulted in a dense bacterial population were unfavourable to larval growth. This was clearly a matter which required further investigation. Investigators rearing marine larvae have commonly found that when a standard rearing technique is employed over a period, considerable differences occur in the proportion of different broods of larvae which grow to metamorphosis. There has been no satisfactory explanation for this phenomenon, although it has been assumed to be due to the presence or absence of minute quantities of various organic compounds in the water (Wilson, 1951; Loosanoff, 1954). The observations recorded in this paper suggest that a partial explanation could be that batches of sea water differ in the number of bacteria which they will support when confined in laboratory vessels, and these bacteria in turn affect the growth of the larvae.

EXPERIMENTAL METHOD

To test the hypothesis, it is necessary to rear larvae in an environment where the bacterial density is low, and to compare the number of larvae which settle as spat with that in control experiments where the normal bacterial population develops. Sea water can be freed of bacteria by various methods. Filtration is perhaps the most satisfactory as the chemical composition of the water is less likely to be altered than by methods which employ heat, but Wilson & Armstrong (1954) have shown that it is very difficult to collect large samples of bacteria-free water by this method. However, although experiments could be set up with bacteria-free water, the larvae could not be freed from bacteria. At the rearing temperature (20° C) marine bacteria divide frequently, and even if only a few are present initially a dense population soon develops. It was therefore decided to use antibiotics, since these would either partially or completely control the bacterial population, without removing

any substance from the sea water which might be required by the larvae. Oppenheimer (1955) has used antibiotics to good effect in hatching marine fish eggs. Some experiments in which lamellibranch larvae were grown for a time in the presence of antibiotics have been reported by Davis & Chanely (1956).

A series of experiments was made in 1956 in which the success of cultures of larvae grown under standard conditions was tested against identical experiments to which penicillin G had been added. The results were promising and a more searching series of experiments, which also included the use of streptomycin and chloromycetin, was made in 1957.

Broods of larvae were obtained from oysters kept in running sea water heated to 20–22° C and enriched with cultures of *Phaeodactylum*. The larvae were liberated naturally and were removed by filtering the water through bolting-silk. After washing in filtered sea water, the larval density was determined by counting samples of an even suspension of larvae. The appropriate volume of larval suspension was then dispensed into hard glass beakers, each containing 1 l. of sea water which had previously been passed through filter-candles to remove silt and the plankton, with the exception of a few bacteria. The average number of larvae added was 1300 per l. The larvae were fed on the flagellate *Isochrysis galbana* Parke, which had been cultured in standard 'Erdschreiber' medium (Gross, 1937). Sufficient was added to give an initial density of about 50 cells per mm^3. Further culture was added daily to keep the food density at about this figure.

In 1956 all experiments of a series stood together in the same water-bath, the temperature of which varied between 20 and 23° C. In 1957 the beakers stood on a bench in a laboratory, the controlled temperature of which varied between 21 and 22° C. All were gently stirred by air bubbling from a glass jet held near the bottom of the beaker. When eyed larvae were observed, spat collectors, in the form of clean mussel shells which had been strung on stainless-steel wire, were suspended in the beaker. When the spatfall was complete, generally 20–30 days after the beginning of the experiment, the total settlement on the mussel shells and the sides and bottom of the beaker was carefully counted. Samples of the larvae which had failed to metamorphose were measured.

The bacterial flora was studied at intervals by plating either 1·0 or 0·1 ml. samples on ZoBell's medium No. 2216 (ZoBell, 1946), and incubated at 22° C for 48 h. When the flora was dense, ten microscope fields were counted on each plate. The size of the field was adjusted where possible so as to count ten to twenty colonies per field. At very low densities (< 1000 per ml.) the colonies were counted directly on the whole plate. The low density populations (0–20,000 per ml.) have thus been estimated fairly accurately; the higher densities, because of the crowding of the colonies on the plates, less precisely.

RESULTS

In 1956 ten series of experiments were made, each series using a different brood of larvae. In each series two treatments were tested. A control treatment, set up with filtered sea water, larvae and food, was compared with a second treatment which was identical, except that sufficient of the sodium salt of penicillin G was added at the commencement to give a concentration of 50 i.u. per ml. In eight of the ten series of experiments made, the two treatments were duplicated. In 1957 a further five series of experiments were made. In the first two series the treatments were triplicated and in the other three quadruplicated. The concentration of penicillin G was selected by reference to the work of Cviic (1953) who showed that which would control many species of marine bacteria. Spencer (1952) had already shown that it was relatively harmless to *Isochrysis* at this concentration.

There was, as expected, a marked difference in the density of the bacterial flora between penicillin experiments and the controls (Table 1). In 1956 the bacterial flora was estimated at the end of the first 24 h and every other day thereafter. In the controls the mean density was about 19,000 per ml. after 24 h, but in the penicillin experiments the density was less than 1000 per ml. On the third day the mean density was 48,000 per ml. in the penicillin experiments, whereas in the controls it had declined slightly to about 16,000. This increase of the bacteria in the penicillin experiments may have been due either to the penicillin breaking down and losing its bacteriostatic properties, or to the development of penicillin-resistant bacteria in the experiments. By the fifth day the numbers in the two treatments were approximately equal.

In the 1957 experiments bacterial estimations were made when the experiments were first set up, but before the addition of any antibiotic, and then every other day (except for series 4 where the initial count was omitted). The water used generally contained a fairly high bacterial population, probably acquired in the day or so which it took to pass through the filtering unit (water was generally used the day after filtering). The average initial bacterial population was 24,000 per ml. in series 1, 2, 5; in series 3 it was abnormally high (230,000). The average of all five series of experiments on the second day was 75,000 in the control, and < 1000 in the penicillin experiments. Thereafter, the density of bacteria declined rapidly in the controls. In the penicillin experiments, the numbers rose a little but not so markedly as in 1956. A general picture of the changes in bacterial density in this type of experiment was given by series 5, 1957 (Table 1), where the bacterial flora was observed daily for the first 6 days. The initial population was fairly high (45,000). In the controls it rose to 88,000 on the first day, and thereafter declined by about half each day, reaching an average of about 2000 per ml. on the sixth day. The penicillin was added after 24 h; the effect was to reduce the bacterial population, which had had time to build up, to only about half that found in the controls.

Experiments were made in 1956 to see whether alterations in the dosage of the penicillin would control this outburst of bacteria. No success was obtained and no improvement in the growth and metamorphosis of the larvae was observed; in many cases the reverse was the case. Apparently many of the bacteria which developed were penicillin-resistant species or strains. Two types of experiments were tried. In one 50 units per ml. were added at the beginning, and a further 50 units every third day. In the second type of experiment 100 units were added at the beginning and a further 50 units every third day. In many experiments of both series very dense bacterial populations gradually developed.

TABLE 1. SUMMARY OF THE BACTERIAL POPULATION IN THE EXPERIMENTS OUTLINED IN TABLES 3 AND 4

(The bacteria are recorded to the nearest thousand per ml. The concentrations of antibiotic used are shown in Tables 3 and 4.)

Day ...		0	1	2	3	4	5	6	7
				1956					
Series 1	Control	—	240	—	5	—	43	—	46
	Penicillin	—	2	—	26	—	39	—	47
2	Control	—	—	60	—	8	—	56	—
	Penicillin	—	—	1	—	24	—	135	—
3	Control	—	84	—	—	23	—	11	—
	Penicillin	—	23	—	—	127	—	19	—
4	Control	—	15	—	14	—	8	—	13
	Penicillin	—	3	—	57	—	3	—	10
5	Control	—	7	—	6	—	5	—	5
	Penicillin	—	1	—	57	—	7	—	24
6	Control	—	7	—	12	—	7	—	—
	Penicillin	—	0	—	95	—	37	—	—
7	Control	—	2	—	24	—	11	—	—
	Penicillin	—	1	—	46	—	129	—	—
8	Control	—	1	—	18	—	16	—	—
	Penicillin	—	0	—	43	—	8	—	—
9	Control	—	—	—	49	—	4	—	9
	Penicillin	—	—	—	51	—	4	—	46
10	Control	—	3	—	6	—	11	—	—
	Penicillin	—	1	—	15	—	30	—	—
				1957					
Series 1	Control	5	—	118	—	6	—	6	—
	Penicillin	5	—	<1	—	1	—	1	—
2	Control	23	—	115	—	6	—	<1	—
	Penicillin	23	—	<1	—	1	—	2	—
3	Control	231	—	77	—	9	—	117	—
	Penicillin	231	—	<1	—	4	—	<1	—
Penicillin and streptomycin		231	—	0	—	0	—	0	—
4	Control	—	—	11	—	<1	—	4	—
	Penicillin	—	—	3	—	<1	—	8	—
Penicillin and streptomycin		—	—	0	—	0	—	0	—
5	Control	45	88	55	20	9	5	2	—
	Penicillin	45	61	23	5	7	4	3	—
Penicillin and streptomycin		45	18	0	0	0	0	0	—

From these experiments three types of data about the larvae are available —the proportion of the larvae which metamorphosed and settled as spat, the maximum rate of growth of the larvae, and the size to which those larvae which failed to metamorphose grew before dying. These three sets of data will be considered in turn.

The yield of spat from an experiment is of primary interest, since settlement indicates that the larvae have completed their development in the conditions offered. In all, the settlement of fifteen broods of larvae has been compared in control experiments with those to which penicillin had been added. The mean number of spat per litre obtained with each of these fifteen broods with the two treatments is compared in Table 2. It will be seen that, on the average, more spat was always recorded in those experiments to which penicillin had been added and the significance of this difference has been calculated. Because the differences are log-normally distributed, it is necessary to transform the data into logarithms. A 't' test on the transformed data shows that these differences are significant at the $P = > 0{\cdot}001$ level.

TABLE 2. MEAN NUMBERS OF SPAT OBTAINED IN EXPERIMENTS WITH PENICILLIN

(The experimental medium contained 50 i.u. of the sodium salt of penicillin G which the otherwise identical control lacked.)

	1956										1957				
Series ...	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
Control	258	222	192	0	107	135	218	0	0	0	89	19	107	203	0
Penicillin	300	320	290	48	330	140	411	8	42	0	225	715	573	1155	45
Excess spat in penicillin experiment	42	98	98	48	223	5	193	8	42	0	136	604	466	952	45

Larvae were measured from time to time and their sizes noted. The mean size of the larvae in the different experiments cannot be calculated as it was not practicable to measure sufficiently large samples. The largest size present was, however, probably accurately estimated. There was no tendency for the larvae to be larger in the penicillin experiments than in the controls on the same day. This conclusion applies, of course, only to those controls which were not unduly affected by bacteria. A good example is given by the experiments in series 4, 1957 (Table 4). The mean sizes of about forty larvae measured on the fifth and eighth days are shown below:

Day ...	0	5	8
Control (μ)	183	217	235
Penicillin (μ)	183	217	241
Penicillin/streptomycin (μ)	183	218	243

Growth was slow in this series but the yield of spat from those treated with antibiotics was very good (Table 3). If the bacterial population becomes very dense, then the larvae will eventually stop growing.

An examination of the sizes (Tables 3 and 4) to which those larvae which failed to metamorphose grew before dying shows that in many cases the whole larval population was affected by the addition of penicillin. For conciseness, the sizes at which larvae died have been aggregated into four groups corresponding to the principal stages in larval development:

Group	μ	
1	160–200	Size at liberation, little or no growth
2	210–250	Substantial growth, D-shape of freshly liberated larvae lost
3	260–300	Size at which the anatomical changes which presage metamorphosis take place, including development of eye spots. Many larvae settle at this size
4	>300	Mature larvae. Most larvae settle before reaching this size

The significance of the differences in the proportions of larvae which grew into each size-group can be tested for each series of experiments by reference to the fourfold contingency tables published by Mainland, Herrera & Sutcliffe (1956). In one series (series 10, 1956), no larva grew out of the first size-group; these larvae were probably abnormal and can be disregarded. The difference in the proportion of larvae which grew into the 210–250 μ group between the penicillin experiments and the controls is significant ($P=0{\cdot}01$) in six of the eleven series of experiments with which we are concerned (series 4, 8 and 9, 1956, and series 1, 2, and 5, 1957). In all cases where a difference occurred, the larvae were larger when treated with penicillin. In the next size-group the differences are again significant ($P=0{\cdot}01$) in six of the eleven series of experiments (series 1, 4, 7, 8 and 9, 1956, and series 2, 1957). In one case (series 7, 1956), the larvae were larger in the control, but here many more larvae settled as spat in the penicillin experiments which probably reduced the proportion of larvae dying in the larger size-groups. The numbers in the fourth group, 300 μ, were generally small and considerably influenced by the number settling as spat.

As the larvae in the penicillin experiments were not observed to grow more rapidly than in the controls, the larger size at death of the larvae in many of the penicillin experiments must have been due to the larvae living longer.

In 1957 some experiments were made using streptomycin and chloromycetin. Cviic (1953) has shown that a mixture of penicillin and streptomycin is more effective for controlling marine bacteria than either antibiotic on its own. In series 1 and 2 (Table 4) a mixture of 50 units of penicillin G and 0·25 mg streptomycin sulphate per ml. was tried. This completely suppressed the bacteria for over 9 days but the effect on the larvae was variable. In the first series none of the larvae in the three replicates grew to more than 250 μ and they speedily died, which suggested that the streptomycin was harmful. In the second series, the larvae were killed by mistake in one experiment when they had grown to 280 μ. In the replicate there were 395 spat. The larvae, however, behaved abnormally in the early stages of the experiment. Their ciliary activity was extremely rapid and they did not close up as rapidly as

TABLE 3. SUMMARY OF THE RESULTS OF TEN SERIES OF EXPERIMENTS (1956) TESTING THE EFFECT OF ADDING 50 I.U. OF Na PENICILLIN G TO CULTURES OF OYSTER LARVAE

(C = control experiments. P = experiments to which penicillin was added.)

Series	Origin of parent oyster	Date started	No. larvae per litre	Type of expts.	No. of expts.	% of larvae which achieved each size group (μ) 210–250	260–300	>300	No. of spat
1	Isle of Lewis	19. iii. 56	1500	C	2	89	65	25	175, 340
				P	2	95	91	44	556, 43
2	1954, Brittany	4. iv. 56	1300	C	1	—	—	—	222
				P	1	—	—	—	320
3	Isle of Lewis	9. iv. 56	1600	C	1	—	—	—	192
				P	1	—	—	—	290
4	Brittany	3. vii. 56	1200	C	2	84	35	1	0, 0
				P	2	100	89	6	50, 45
5	1954, Brittany	3. vii. 56	1400	C	2	98	74	0	125, 89
				P	2	98	81	21	355, 305
6	River Colne	7. vii. 56	1500	C	2	93	73	16	75, 195
				P	2	91	58	12	230, 50
7	River Colne	7. vii. 56	1700	C	2	93	70	2	365, 70
				P	2	85	40	0	425, 397
8	1954, Brittany	7. vii. 56	1050	C	2	79	4	0	0, 0
				P	2	100	75	14	5, 10
9	River Colne	12. vii. 56	1300	C	2	80	12	0	0, 0
				P	2	96	32	0	8, 75
10	Brittany	5. viii. 56	1400	C	2	0	0	0	0, 0
				P	2	0	0	0	0, 0

TABLE 4. SUMMARY OF THE RESULTS OF THE 1957 SERIES OF EXPERIMENTS

(The parent stock was always from the river Colne. In series 5 the penicillin was not added until the experiment had been running for 24 h.)

Series	Date started	No. larvae per litre	Antibiotics added per ml. Penicillin G (i.u.)	Streptomycin (mg)	Chloromycetin (mg)	No. of expts.	% larvae which achieved each size group (μ) 210–250	250–300	>300	No. of spat
1	27. iii. 57	1100	0	0	0	3	86	26	—	111, 73, 82
			50	0	0	3	94	42	—	292, 61, 323
			50	0·25	0	3	28	—	—	0, 0, 0
2	29. iii. 57	1300	0	0	0	3	78	8	—	0, 0, 58
			50	0	0	3	93	45	—	809, 798, 538
			50	0·25	0	2	69	8	—	0,* 395
3	1. iv. 57	960	0	0	0	4	68	12	—	230, 2, 197, 0
			50	0	0	4	Not recorded			612, 629, 332, 720
			50	0·05	0	4	Not recorded			545, 282, 387, 394
4	3. iv. 57	1400	0	0	0	4	96	51	—	749, 14, 29, 22
			50	0	0	4	91	58	—	1091, 1211, 1137, 1182
			50	0·05	0	4	Not recorded			1259, 992, 1292, 1082
5	7. v. 57	1400	0	0	0	4	65	1	—	0, 0, 0, 0
			50	0	0	4	97	9	—	0, 0, 0, 179
			50	0·05	0	4	95	31	—	752, 183, 404, 74
			0	0·05	0	4	51	—	—	0, 0, 0, 0
			0	0	0·025	4	98	28	—	0, 0, 0, 0

* This experiment was terminated early by accident.

usual. This behaviour seemed to be characteristic of experiments to which streptomycin had been added. In the next three series of experiments a mixture of 50 units penicillin G and 0·05 mg streptomycin sulphate per ml. was tried. Once again the bacteria were completely suppressed for at least 9 days. In two of the three series of experiments (3 and 4) the larvae did well, much better than the controls, but not significantly different from those to which only penicillin G had been added. In series 5 the result from the penicillin/streptomycin mixture was much better than in the control, or in that to which penicillin alone had been added, both in respect of the number of spat produced and in the size reached by the larvae before death. It seems probable that when the bacterial flora is very vigorous, or perhaps when certain species are present, then the penicillin/streptomycin mixture will give better results than penicillin alone.

One experiment was tried using chloromycetin; this is a wide spectrum antibiotic. The larvae grew well until there was an outburst of bacteria apparently resistant to this antibiotic, when they died. Chloromycetin may not, therefore, be as useful as the penicillin/streptomycin mixture and, as it costs at least two-thirds as much again, it may not have a place in large-scale rearing of larvae.

DISCUSSION

The proportion of larvae which develops to metamorphosis differs, under standard conditions, in different broods. An example of this is shown by the results of series 6, 7 and 8, 1956. These experiments were all started on the same day with the same batch of water, but the three broods differed considerably in the proportion of larvae which metamorphosed. One of the factors affecting their survival is described in this paper, where it is demonstrated that the bacteria which develop when sea water is confined in the laboratory are sufficient to arrest the growth and development of otherwise healthy larvae. The almost complete suppression of bacteria by the penicillin/streptomycin mixture does not, however, make all larvae do equally well. Other factors in the larvae, and perhaps in the water itself, are still at work. At one time the writer thought that the glycogen food reserves at the time of liberation might be of importance, but the investigations of Collyer (1957) showed that there was little difference between various batches of larvae examined.

The way in which the larvae and bacteria interact is unknown but there seem to be two possibilities: either certain bacteria may cause a disease in the larvae, or some or all of the normal bacterial constituents of the sea water may irritate the larvae either directly by settling and multiplying on the shell or flesh, or indirectly by the secretion of 'external metabolites' into the water.

Bacteria are sparse in the sea because, amongst other factors, of the relative absence of solid surfaces. A surface immersed in the sea, unless protected in

some way, speedily becomes covered by bacteria. It has been suggested that because of the active physical properties of solid surfaces it is only at a surface that the dissolved organic matter, which is present in sea water in only very small quantities, reaches a high enough concentration to support a vigorous population (ZoBell, 1943). When the water is confined in a small vessel the area of surface becomes relatively large and the bacteria become very abundant. The smaller the containers the larger the bacterial population (ZoBell & Anderson, 1936). It is probable that many bacteria are associated with the larva both on the shell and perhaps on the surface of the living tissue. If the bacteria on the larvae are the important ones then this would give a mechanism whereby bacterial effects would be produced in the sea.

It can be seen from the results given in this paper and elsewhere (Walne, 1956) that batches of water differ considerably in the bacterial population which they will support when kept under standard conditions in the laboratory. If these differences are reflected in the bacterial population on the larvae in the field, then this could be a factor influencing the successful growth and development of marine larvae in the sea.

Whatever the mechanism, the results show a way by which the performance of a given batch of sea water can be improved in the laboratory. A number of workers in recent years have suspected that the dissolved organic matter in sea water plays an important part in the activity of marine animals. The observations of Collier, Ray, Magnitzky & Bell (1953) on the filtration of *Crassostrea* and of Loosanoff (1954) on rearing the larvae of *Crassostrea* and other species of lamellibranchs, may be cited. Of particular relevance are the papers of Wilson (1951) and Wilson & Armstrong (1952, 1954), in which the growth of *Echinus* larvae in waters of different origins are compared. If the results obtained by Wilson are viewed in the light of experiments reported in this paper, it seems possible that the bacterial floras, which waters from different areas will support, provide an explanation for Wilson's results. The flora will, in turn, depend on the nature and quantity of the organic compounds present in the water; little is known about these, but it is certain that they will vary considerably.

SUMMARY

Preliminary experiments had suggested that the extent of the development of the bacterial flora which develops when sea water is confined in small vessels is an important factor in the laboratory culture of oyster larvae. In the experiments reported in this paper the growth and settlement of oyster larvae was compared in controls of normal sea water with those in which the bacterial flora was controlled with antibiotics.

A concentration of 50 i.u. of the sodium salt of penicillin G per ml. suppressed bacterial growth for at least 2 days and, in a series of experiments using fifteen different broods of larvae, significantly ($P > 0{\cdot}001$) more spat

were obtained in those to which penicillin had been added than in the controls.

A mixture of 50 units of penicillin G and 0·05 mg streptomycin sulphate was tried in three series of experiments. This completely suppressed the development of bacteria for at least nine days. In all three series many more spat were obtained than in the controls, but in only one series was there more spat than in the comparable penicillin experiment. It is suggested that when the bacterial population is very vigorous, or perhaps when certain species are present, this mixture will be more useful than penicillin alone. Experiments with other concentrations of penicillin, and with streptomycin and chloromycetin alone were not successful.

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THE GROWTH RATE OF *VERRUCA STROEMIA* (O. MÜLLER)

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(Text-figs. 1 and 2)

The growth rate under both natural and experimental conditions of several littoral and sublittoral barnacles has recently been dealt with in some detail (Barnes & Powell, 1953, q.v. for earlier references; Barnes, 1952–53; Barnes & Barnes, 1954; Crisp, 1954; Barnes & Barnes, 1956; Barnes, 1956). No comparable data for *Verruca stroemia* are reported in the literature.

Although *V. stroemia* has been reported from the Red Sea (Darwin, 1854), the Adriatic (Kolosvary, 1947), and even the Indian Ocean (Nilsson-Cantell, 1938), the major area of its distribution is along the eastern Atlantic coasts of Europe from Spain to Finmark (for a review see Broch, 1924). In addition, Stephensen (1929, 1933) states that it is found in the coastal waters of Iceland, Greenland and the Faroes (see also Weltner, 1900, and Schaper, 1922). The species appears to be absent from the western Atlantic. Although recorded from a depth of 548 m by Weltner (1900), it is most common on relatively shallow banks and rough inshore grounds, and it extends into the lowest parts of the intertidal zone. According to Broch (1924) it is moderately euryhaline, but Schaper (1922) states that it does not occur in the Baltic Sea. In the Clyde Sea Area it is widely distributed on suitable stony ground from the lower littoral to deeper water and grows on crustaceans and molluscs. Although Broch considered the species to be lusitanian-boreal in character, with extensions into the Arctic, its breeding behaviour (as far as is known) suggests a cold-water origin; like many other boreo-arctic species, it has a single major brood each year that is liberated in the early spring.

THE MATERIAL AND METHODS

In previous studies on both *Balanus* and *Chthamalus* spp. the rostro-carinal axis along the base has been measured, but because of asymmetry this is less satisfactory with *Verruca stroemia*. The longest diameter at the base has, therefore, been measured and for brevity will be termed the length. For the small animals a binocular microscope with low-power objective and scaled ocular was used, and for the large, a measuring microscope with vernier attachment.

Animals were collected at an extreme low-tide level. For the estimation of annual growth under natural conditions large numbers of animals were measured and the results analysed with the help of probability paper. In order to estimate growth rates under conditions similar to those used for other species, stones bearing individuals well separated from each other were drilled

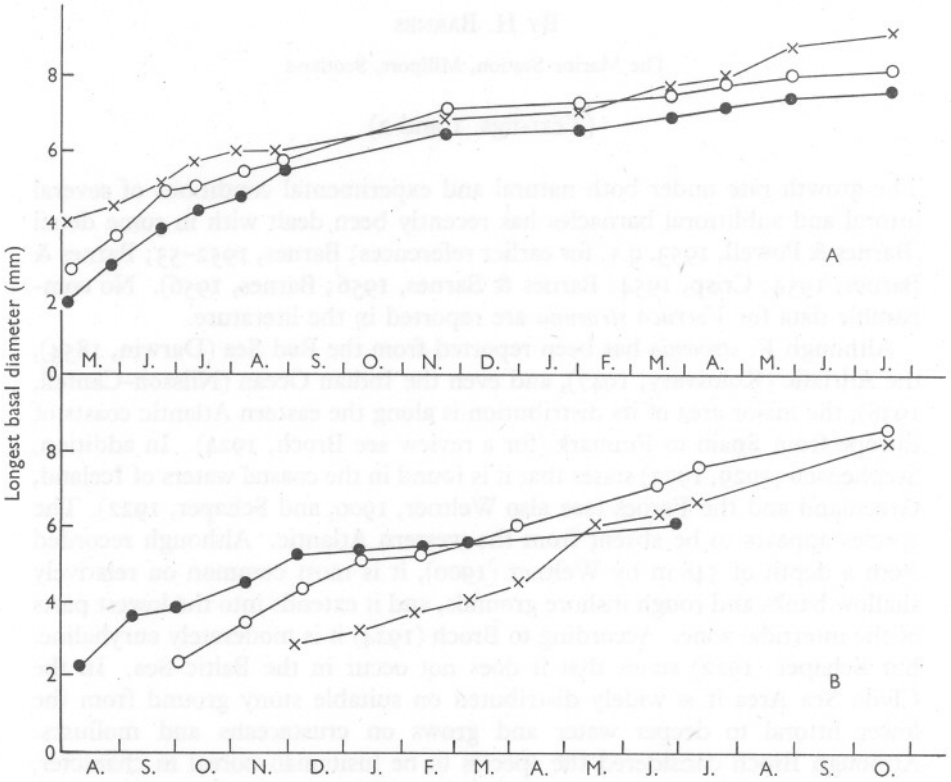

Fig. 1. Growth of *Verruca stroemia* over two fifteen-month periods under conditions of continuous immersion. Animals cleaned at each inspection. Exposures made throughout year. A, May 1955 to July 1956; ●—●, Series *a*; ○—○, Series *b*; ×—×, Series *c*. B, August 1956 to October 1957; ●—●, Series II; ○—○, Series III; ×—×, Series IV.

and fastened to plastic panels; these were exposed on a raft at a constant depth of about 3 ft. Several such series were put out at different times of the year. At appropriate intervals the material was brought in, the animals carefully cleaned, and each one measured. Growth was followed for several years.

The mean length of each size-group and the mean specific growth rates (increase in length per unit length per day) have been caculated and are plotted in Figs. 1 and 2. In some series a number of animals were lost, particularly during the later stages of exposure, a fact that introduces some bias into the comparisons.

THE RESULTS

The first series was put out early in May 1955; it consisted of three size-groups of mean length 1·9, 2·8, and 4·1 mm., equivalent to first, second, and third year-groups on the shore (see p. 431). In all these growth was rapid and continuous during the late spring and the summer months. By late August there was little difference between the mean sizes of the three groups, which were 5·5, 5·7, and 6·0 mm.

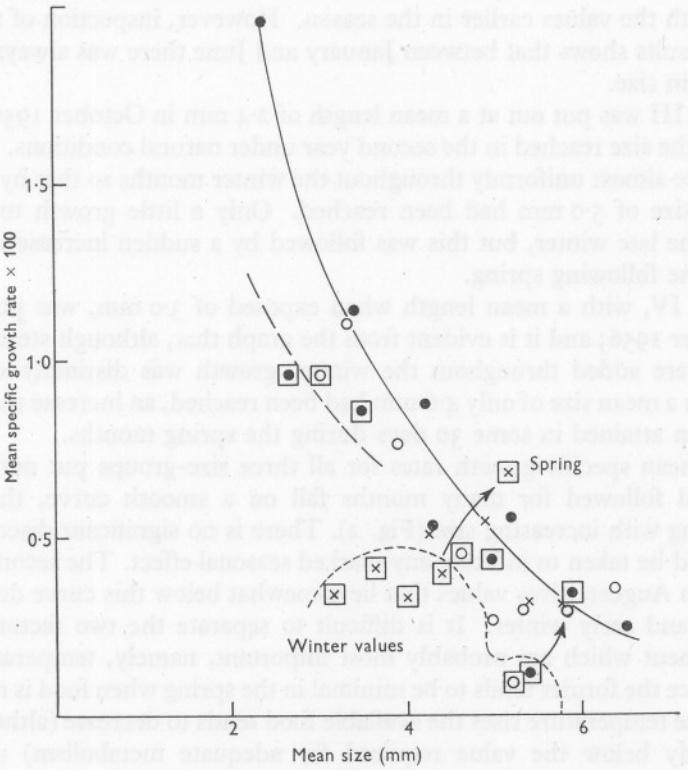

Fig. 2. The mean specific growth rate $\left(\frac{\Delta l}{l.n}\right) \times 100$, of *Verruca stroemia*, for all series: symbols as in Fig. 1, values from Fig. 1 B in squares.

During the following early winter growth was slow, but it must be remembered that the animals were approaching their maximum size. Between the end of November and February of the next year there was no increase in size in any of the groups, but growth was renewed in the spring of 1956 and continued very slowly during the following summer. By the end of the second summer all the groups had reached their maximum size of about 8 mm. It is evident that under the experimental conditions, namely, exposure on a raft near the surface and freedom from epiphytic growth and mud, this sublittoral

barnacle, which even extends into relatively deep inshore waters, grows rapidly. The maximum size is approached in only one season's growth—between settlement in the spring and the following November.

A second series, its mean size 2·2 mm (no smaller ones being available), was put out during the August of 1956. Growth took place rapidly and continued at about a uniform rate until early December when a size of 5·3 mm had been reached. During the following winter many of these animals were lost and the mean sizes are not representative of the whole series and cannot be compared with the values earlier in the season. However, inspection of the individual results shows that between January and June there was always a slight increase in size.

Series III was put out at a mean length of 2·4 mm in October 1956, that is again at the size reached in the second year under natural conditions. Growth took place almost uniformly throughout the winter months so that by January a mean size of 5·0 mm had been reached. Only a little growth took place during the late winter, but this was followed by a sudden increase in length during the following spring.

Series IV, with a mean length when exposed of 3·0 mm, was put out in December 1956; and it is evident from the graph that, although steady increments were added throughout the winter, growth was distinctly slow. In 4 months a mean size of only 4·6 mm had been reached, an increase that would have been attained in some 30 days during the spring months.

The mean specific growth rates for all three size-groups put out in May 1955 and followed for many months fall on a smooth curve, the values decreasing with increasing size (Fig. 2). There is no significant discontinuity that could be taken to indicate any marked seasonal effect. The second series, started in August, gives values that lie somewhat below this curve during the autumn and early winter. It is difficult to separate the two factors of the environment which are probably most important, namely, temperature and food, since the former tends to be minimal in the spring when food is maximal, and as the temperature rises the available food tends to decrease (although not necessarily below the value required for adequate metabolism) until the autumnal diatom outburst. The lower values in Series II, nevertheless, may be ascribed to inadequate food supply or, much less likely, to the deleterious effects of higher summer temperatures in the surface water as affecting particularly the smaller animals. The midwinter values of this series are only some 50% of the mean specific growth rates of Series I at the same size, and since the temperatures at this season are approaching those of the spring and early summer, it seems that the reduced growth rate if related directly to the environment is a result of inadequate food supply. This suggestion is substantiated by the fact that in the following spring a growth rate somewhat greater than that of the first series is found. Series III follows a similar pattern, the values for the autumn and early winter falling into line with those for

Series II (broken line); the midwinter values are again those of the first series and a return to the 'normal' is made in the following spring. Series IV was put out in December; the values are now initially very low, that is, throughout the winter, again supporting the hypothesis that food supply is a limiting growth factor at this time of the year. In the following late spring a much higher rate of growth was obtained at equivalent sizes than in Series I. This higher value in the spring and early summer in Series II and IV, and the fact that the effect is more marked in the smaller individuals, strengthens the suggestion that even the midsummer growth rates in Series I, although falling on a smooth curve, may be somewhat depressed by the higher summer temperatures or limited by food supply; however, loss of animals in the longer exposures tends to bias the results and the suggestion must be regarded as tentative.

The growth of individual animals was not followed on the shore. However, a large randomly taken population was measured during May and the frequency distribution plotted on probability paper. Three classes could be distinguished with mean lengths of 2·0, 2·8, and 4·0 mm. These seem to correspond to three consecutive year-groups—the first having settled in the early spring of the current year and having by May reached 2·0 mm, that is the size of the first exposures put out on the raft.

DISCUSSION

It is evident that *V. stroemia* follows a very similar growth pattern to several of the other species previously investigated. Settlement in the spring is followed by rapid growth; little increase in size takes place during the winter, but renewed growth is established in the following spring. The fact that animals exposed later in the year grow at almost the same rate as those put out in the spring at the same size suggests that there is no marked endogenous or seasonal rhythm *per se*. Growth appears to be directly related to conditions of environment, being rapid when there is abundant food. How far there is any seasonal change in growth rate in deep water, where the temperature range is small and food supply perhaps relatively so, is unknown. Indeed, it would be of considerable interest to have growth data from animals maintained in deep water under similar experimental conditions to the present series, in particular being kept free from encrusting organisms. Under these conditions any endogenous rhythm might be more easily recognized.

The rate of growth is very greatly increased under the experimental conditions and as with other species this may be ascribed in part to the greater availability of food in the surface layers and in part to the freedom from encrusting growths and mud—both common under natural conditions. The absence of the species from suitable substrata in shallow water cannot be ascribed to any deleterious effect of such an environment.

It is now possible to compare the growth characters of several species measured under the partially controlled conditions of raft exposure. For such a comparison the effect of size may be eliminated by using the specific growth rate and by employing its value at what may be termed the half life period, taken as half the maximum size attained by a species, we may expect to eliminate the effect of age. The values for several species are shown in Table 1.

TABLE 1

Species	Maximum size (mm)	Mean specific growth rate (× 100) at half size
Balanus balanoides	25	1·0
B. crenatus	25	1·3
B. balanus	43	0·9
Chthamalus stellatus	10	0·1
Verruca stroemia	8	1·0

The mean specific growth rates at half maximum size are, with the exception of *Chthamalus stellatus*, very similar, from which it may be assumed that the intrinsic metabolic activities are of the same order. Indeed, the apparent differences may well be the result of taking shell length as a measure of animal size and its rate of change. The value for *C. stellatus* is much lower than for the other species and while the above factor is again of importance a lower intrinsic metabolic rate is indicated. This agrees with the observations of Southward (1955) who found that over their optimal range the cirral beat of *C. stellatus* was slower than that of *Balanus balanoides*. However, unlike other species investigated, *Chthamalus stellatus* showed very little increase in growth rate when transferred to raft conditions; after an initial acceleration immediately following the change, the rate returned to a value little different from that on the shore (Barnes, 1956). Perhaps greater general stimulation—such as would be provided under natural conditions by wave action or experimentally by water currents—is necessary for the full metabolic activity of *C. stellatus*.

SUMMARY

Data are presented on the growth rate of *Verruca stroemia* under natural conditions and when exposed continuously and cleaned repeatedly. Several series exposed at different times of the year were followed.

Rapid growth takes place (under raft conditions) following settlement; the maximum size is virtually reached in one season's growth between spring and early winter. There is little growth in midwinter.

Differences between the mean specific growth rates of the various series can be ascribed to differences in the availability of food.

The question is discussed as to whether there is any seasonal rhythm; the evidence indicates that no marked rhythmic pattern of growth exists.

Observations on deep-water populations would be of value for comparison and to unmask any relatively weak rhythmic growth.

The mean specific growth rates at half their maximum size are compared for several species—*Balanus balanoides*, *B. crenatus*, *B. balanus*, *Chthamalus stellatus* and *Verruca stroemia*; it is similar for all species except *Chthamalus stellatus*. The high level barnacle may require stimulation such as is provided by wave action to elicit full metabolic activity.

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AN ADULT DIGENETIC TREMATODE FROM AN INVERTEBRATE HOST: *PROCTOECES SUBTENUIS* (LINTON) FROM THE LAMELLIBRANCH *SCROBICULARIA PLANA* (DA COSTA)

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(Plates I and II and Text-figs. 1 and 2)

Adult digenetic trematodes are typically parasites of vertebrates. Sexually mature trematodes occurring in invertebrate hosts are usually regarded as precocious last-larval stages, to which the term 'progenetic' is applied. These progenetic forms are often encysted, and can usually only contribute to the further life history of the species, and, in many cases, can only attain the definitive form of the adult trematode, after transference to a vertebrate host.

Specimens of the mud-burrowing lamellibranch *Scrobicularia plana*, collected from the region of the Thames estuary, were found to be infected with unencysted, sexually mature trematodes. These parasites occurred in the kidneys of the molluscs, and, as will be discussed later, there seems no reason to regard them as other than adult digenetic trematodes. They were identified as specimens of *Proctoeces subtenuis* (Linton, 1907) Hanson, 1950. This species has been recorded, hitherto, only as a parasite of the hind-gut of marine fishes belonging to the families Labridae and Sparidae, from the Red Sea, New Zealand, and the eastern seaboard of America.

In this paper an account is given of the occurrence, distribution, environment, morphology, and taxonomy of *Proctoeces subtenuis* from *Scrobicularia plana*, and the possible life history of this trematode in the Thames estuary is discussed with reference to the phenomenon of progenesis.

OCCURRENCE AND DISTRIBUTION

Specimens of *Proctoeces subtenuis* in the kidney of *Scrobicularia plana* were first observed in 1954 in animals collected from the mud flats at Chalkwell in Essex. During the subsequent three years nearly a thousand specimens of *S. plana* from this location were examined. Every single specimen was

infected. The degree of infection, estimated from examination of fifty-one hosts, varied from one to thirteen parasites per host, with an average of between four and five. There appeared to be no correlation between degree of infection and size of host.

The distribution of *S. plana* at Chalkwell has already been discussed by Freeman & Rigler (1957). The mollusc is plentiful at mid-tide level, but absent from the sandy deposits lower down the shore. It lies in a burrow 6–10 in. below the surface of the mud, maintaining contact with the overlying water through long, extensible siphons. Spooner & Moore (1940) record population densities of *S. plana* of up to 1000 per m^2 in the Tamar estuary at Plymouth, and Green (1957) gives 500–1025 per m^2 as the population density in the Gwendraeth estuary in South Wales. The impression derived from collecting at Chalkwell on numerous occasions is that similar population densities occur there also. No attempt has been made to delimit the occurrence of the parasite along the Thames estuary to the east and west of Chalkwell, but infected specimens of *S. plana* have been collected from stations over a distance of about a mile of the mud flats in the neighbourhood of Chalkwell. The total population of *S. plana* in this area must number many millions, and the evidence is consistent with the view that all of them are infected with the trematode. The only occurrence from a locality other than Chalkwell was at Whitstable in Kent where three specimens of *S. plana* of about 150 examined were infected. Specimens of *S. plana* have been examined from the Rivers Tamar and Tavy (*ca.* 80), the Gwendraeth (*ca.* 100), the Rivers Dart and Teign in south Devon (*ca.* 40), the Butley river in Suffolk (7), the Essex rivers Blackwater (*ca.* 50) and Colne (7), and Conway in North Wales (*ca.* 100), but no trematodes have been found. Specimens of the lamellibranchs *Macoma balthica* (L.) and *Mya arenaria* L., which occur alongside *Scrobicularia plana* in the mud flats at Chalkwell, have also been examined, but were found to be uninfected.

In addition to living specimens of *Proctoeces subtenuis*, the kidney of infected *Scrobicularia plana* was observed often to contain dead parasites. These varied in colour between light brown and almost black, and also varied in texture, the darker specimens being harder and even stone-like. These dead specimens clearly showed the external form of *Proctoeces subtenuis*, the oral and ventral suckers being evident, and, in some cases, the cirrus was extruded (Pl. I, fig. 1). The dead specimens occurred in about one in ten of all infected *Scrobicularia plana* examined, and as many as six were found in a single host. They always occurred together with living trematodes in the same host. It is known that some nematode parasites become calcified after death (von Brand, 1952), and a phenomenon comparable to that described above has been reported for another trematode by Macfarlane (1939).

ENVIRONMENT OF THE PARASITE

Proctoeces subtenuis has previously been reported as a parasite of the hind-gut of certain marine fishes. The present work is concerned with the occurrence of this trematode in the kidney of an estuarine lamellibranch. It is very probable that the conditions of the immediate external environment of the parasite in such different situations, in such different hosts, are widely dissimilar. A brief account will therefore be given of some aspects of the environmental conditions of a parasite within the lamellibranch kidney, with particular reference to the kidney of *Scrobicularia plana*.

The lamellibranch kidney consists essentially of a proximal, glandular, ciliated tubule (the organ of Bojanus) and a ureter which conveys the excretory products to the supra-branchial space, and thence to the outside world. The organ of Bojanus receives fluid through a ciliated coelomostome from the pericardium. This fluid is derived from the blood by ultra-filtration through the heart wall. The tubular filtrate is isotonic with the blood, but differs slightly from blood in its ionic composition (Robertson, 1949). Modification of the filtrate occurs in the tubule by the addition of nitrogenous excretory compounds, of which urea and the amino acids taurine and creatine have been identified in twenty-two lamellibranchs (Letellier, quoted by Delaunay, 1927). In the freshwater mussel, *Anodonta cygnea*, as much as 91·6% of the total nitrogen in the tubular fluid can be protein nitrogen, and this can represent a concentration of eight times the blood plasma protein nitrogen level (Florkin & Duchateau, 1948).

The infected population of *Scrobicularia plana* at Chalkwell is exposed to considerable seasonal variations in the tonicity of the overlying water, and these variations are accompanied by corresponding changes in the tonicity of the blood (Freeman & Rigler, 1957). Since the tubular filtrate in other lamellibranchs has been shown to be isotonic with the blood, it seems likely that the osmotic pressure of the tubular fluid will vary with changes in external salinity. This was examined by equilibrating specimens of *S. plana* to sea water and to 70% sea water, and, using the method described by Freeman & Rigler, comparing the depression of the freezing-point of fluid drawn directly from the organ of Bojanus with that of the external medium. No significant difference was found between the osmotic pressure of the tubular fluid and that of the external medium.

It seems certain, therefore, that the trematodes are exposed to an environment which is variable in osmotic pressure, and, in this respect, they resemble free-living estuarine turbellarians (Krogh, 1939) rather than adult trematode parasites in vertebrate hosts. It also seems possible that the fluid in which the trematodes live in the kidney of *S. plana* has an electrolyte composition similar to that of sea water, but has a high concentration of proteins and/or amino acids.

DESCRIPTION OF THE PARASITE

Size and shape.

Proctoeces subtenuis is generally cylindrical, but tapers at each end. The mouth is subterminal, and the ventral sucker is situated at about one-third of the total body length from the anterior end. The integument is without spines. Although living specimens are cylindrical, the general practice in studies of the Digenea of illustrating a well-flattened specimen has been followed here (Text-fig. 1). Some measurements, in mm, of ten such specimens, randomly chosen from a collection of permanent preparations, are given in Table 1. The first figure given is an arithmetic mean for the sample, and the figures in parentheses indicate the range for the sample. The egg capsule measurements were made on samples of five eggs from each of twenty adults, i.e. 100 eggs in all.

TABLE 1. MEASUREMENTS OF *PROCTOECES SUBTENUIS*

See text	Millimetres
Length	2·92 (1·52 – 4·80)
Maximum width	0·96 (0·56 – 1·40)
Oral sucker length	0·20 (0·14 – 0·32)
Oral sucker width	0·21 (0·14 – 0·30)
Prepharynx length*	0·03 (0·01 – 0·05)
Pharynx length	0·15 (0·10 – 0·19)
Pharynx width	0·15 (0·12 – 0·25)
Oesophagus length	0·22 (0·01 – 0·45)
Ventral sucker length	0·34 (0·24 – 0·57)
Ventral sucker width	0·39 (0·26 – 0·61)
Anterior end of body to anterior border of ventral sucker	0·90 (0·40 – 1·40)
Cirrus sac length	0·54 (0·42 – 0·70)
Cirrus sac maximum width	0·13 (0·10 – 0·20)
Anterior testis diameter	0·19 (0·10 – 0·28)
Posterior testis diameter	0·21 (0·12 – 0·32)
Germarium diameter	0·16 (0·05 – 0·27)
Egg capsule length	0·042 (0·026 – 0·073)
Egg capsule width	0·024 (0·015 – 0·030)

* Visible in only seven of the ten specimens in the sample.

When freshly collected specimens of *P. subtenuis* were transferred to dishes of sea water, they became quite active, but the muscular contraction and stretching was confined almost entirely to the region of the body anterior to the ventral sucker (Pl. 1, figs. 2A, B). In contracted specimens the genital pore was situated approximately midway between the oral and ventral suckers, but in stretched specimens it was that region anterior to the genital pore that was most elongated, so that the genital pore came to lie relatively nearer to the ventral sucker. This variation assumes special significance when it is considered that the position of the genital pore has sometimes been used as a diagnostic character of some digeneans.

Colour

Perhaps the most immediately noticeable feature of the specimens of *P. subtenuis* from the kidney of *Scrobicularia plana* is that they are red or pink. This coloration is not obviously restricted to any particular organ-system of the trematode but is generally distributed. The intensity of the colour varies with the size of the animal; even specimens about 1 mm long are noticeably pink, and large (3–4 mm) specimens are distinctly red. As is shown in Table 4, *Proctoeces* species have previously been described from the hind-gut of fishes on fourteen occasions, and in none of these descriptions was it observed that the specimens were red in colour. The name *Proctoeces erythraeus*, given by Odhner (1911) to a species from the Red Sea, is apparently a reference to the geographical location, rather than to the colour, of this trematode (cf. *Tomopteris erythraea* Caroli, 1928).

Red coloration in several other trematodes is known to be due to the presence of haem pigments (von Brand, 1952). Haem pigments react with certain nitrogenous compounds to form the appropriate haemochromogen, the absorption bands of which can be recognized by spectroscopic examination. Observations were made with a microspectroscope on specimens of *Proctoeces subtenuis* mounted in sea water in an attempt to characterize the pigment. No absorption bands could be recognized on examination of untreated specimens, but, after addition of Takayama's fluid (Hawk, Oser & Summerson, 1954), the characteristic absorption bands of pyridine haemochromogen were readily observed. The α-band at about 558–560 mμ was particularly evident, and a less well-defined β-band at about 525 mμ was also observed. Pieces of tissue taken from the host, *Scrobicularia plana*, including the wall of the kidney and the brown-coloured pericardial gland, gave no indication of the formation of pyridine haemochromogen when treated with Takayama's fluid.

The red coloration of specimens of *Proctoeces subtenuis* inhabiting the kidney of *Scrobicularia plana* is, therefore, due to a native haem pigment, whereas there is no evidence that members of the same species inhabiting the hind-gut of fishes are similarly pigmented.

It is not possible, on the basis of the present information, to suggest whether this pigment plays any part in the respiratory processes of the parasite, nor why it should be developed by specimens in the kidney of *S. plana* whilst apparently being absent in specimens in the hind-gut of fishes. It is, however, interesting to note that the only other adult platyhelminth parasite occurring in *S. plana*, the viviparous rhabdocoele *Paravortex scrobiculariae* (Graff), is red in colour (Freeman, 1957), whereas the closely related species *P. cardii* Hallez, occurring in cockles and some other lamellibranchs, is colourless. It is hoped in the near future to be able to determine whether this red coloration in *P. scrobiculariae* is also due to a haem pigment. A study of this assumption

of a red coloration by platyhelminth parasites of *Scrobicularia plana* might throw some light on the metabolism of this lamellibranch as well as on that of the parasites.

Alimentary canal.

The mouth opens into the oral sucker which is succeeded by a pre-pharynx, which in turn communicates with the pharynx. In whole mount preparations, the posterior end of the oral sucker often overlies the anterior end of the pharynx, thus completely obscuring the pre-pharynx (Pl. I, fig. 5A), but in sections the pre-pharynx is always readily evident (Pl. I, fig. 4). Posterior to the pharynx is the oesophagus, and the junction between these two regions receives the openings of a ring of gland cells. The oesophagus itself is provided with well-developed circular and longitudinal muscles, and, depending on the state of contraction of these muscles, the length of the oesophagus may vary very considerably. Thus, in some whole mount preparations the oesophagus may appear long (Pl. I, fig. 5B), and in others very short (Pl. I, fig. 5A), and so the presence or absence of the oesophagus, based on examination of whole mount preparations, is particularly unreliable as a diagnostic character. All the regions of the alimentary canal mentioned so far, namely the oral sucker, pre-pharynx, pharynx and oesophagus, are lined by a cuticle (Pl. I, fig. 4), which is continuous with that covering the external surface of the body. Posterior to the oesophagus the alimentary canal bifurcates into two simple intestinal caeca which reach almost to the posterior end of the body. This intestinal region is lined by an epithelium of columnar cells, each with a basal nucleus (Pl. II, fig. 4A). In living specimens the alimentary canal invariably contains an abundance of refractile globules (Pl. II, fig. 3) that readily take up fat stains and blacken with osmium tetroxide, but the kidney tissue of the host lacks such droplets.

Excretory system.

The most prominent feature of the excretory system is the Y-shaped bladder (Pl. II, fig. 1), the paired arms of which extend as far forwards as the posterior limit of the pharynx. Posteriorly, at about the level of the testes, these arms join to form the median stem that runs backwards as a relatively wide tube. Peristaltic waves were observed to pass posteriorly along this median stem of the excretory bladder. The median stem narrows posteriorly to join a cuticular-lined terminal duct (Pl. II, fig. 5), the junction receiving the ducts from a surrounding collar of gland cells (Pl. II, fig. 5). Similar glands have been illustrated in other trematodes, but their function appears not to be known. Except in its extreme posterior region, the Y-shaped excretory bladder lies in the dorsal half of the body in a plane immediately ventral to the alimentary canal. The anterior extremities of the bladder lie to the outside of the oesophagus, then, as they run posteriorly, they pass beneath the intestinal

limbs to appear on the inside of these structures at about the level of the germarium.

Numerous tributary ducts open into the anterior extremities of the arms of the Y, at least two prominent vessels on each side coming from the anterior region of the trematode, and one on each side from the posterior region. The tributary vessels were best seen in well-flattened living specimens (Pl. II, fig. 3) and were very difficult to observe in fixed specimens whether sectioned or mounted whole. Attempts were made to inject pigmented latex through the excretory pore in order to trace the tributary vessels but these attempts were unsuccessful.

In sections near to the anterior limits of the excretory bladder, the tributary ducts appear to have walls composed of 'fibrillar cytoplasm', i.e. they are non-cellular, and thus differ from the walls of the bladder itself, which are lined by a cellular epithelium (Pl. II, figs. 4A–C). The bladder epithelium, as seen in sections, consists of somewhat hemispherical or conical cells with large nuclei (Pl. II, fig. 4B). The cytoplasm often contains an abundance of refractile spherical bodies about 1–2·5 μ in diameter (Pl. II, fig. 4C), which readily take up haematoxylin, and, only a little less readily, acid stains such as eosin and Orange G. These spherical bodies are insoluble in xylene and do not take up Sudan dyes or blacken with osmium tetroxide. They may also occur freely in the lumen of the excretory bladder. The fibrous nature of the walls of the tributary ducts was described for *Proctoeces ostreae* by Fujita (1925), who also observed, 'la paroi de la vessie consiste en une très mince membrane pourvue de noyaux et de corpuscles d'excrétion, en particulier dans la paroi des rameaux'.

The excretory system of *P. subtenuis* (and also apparently of *P. ostreae*) assumes a special taxonomic significance in view of La Rue's (1957) recent classification of the Digenea based on the structure of the wall of the excretory bladder. La Rue divides the Digenea into two super-orders, which he characterizes as follows—Anepitheliocystidia: 'primitive excretory bladder retained, i.e. not replaced by cells from mesoderm, hence definitive bladder not epithelial'; and the Epitheliocystidia: 'primitive excretory bladder surrounded by, and then replaced by, layer of cells derived from mesoderm, hence definitive bladder thick walled and epithelial'. The Fellodistomatidae (which includes *Proctoeces*) is placed in the Anepitheliocystidia, its position there being thought to have been firmly established by the work of Cable. However, as described above and illustrated in Pl. II, fig. 4A–C, the excretory bladder in our specimens of *P. subtenuis* is lined by a well-developed epithelium.

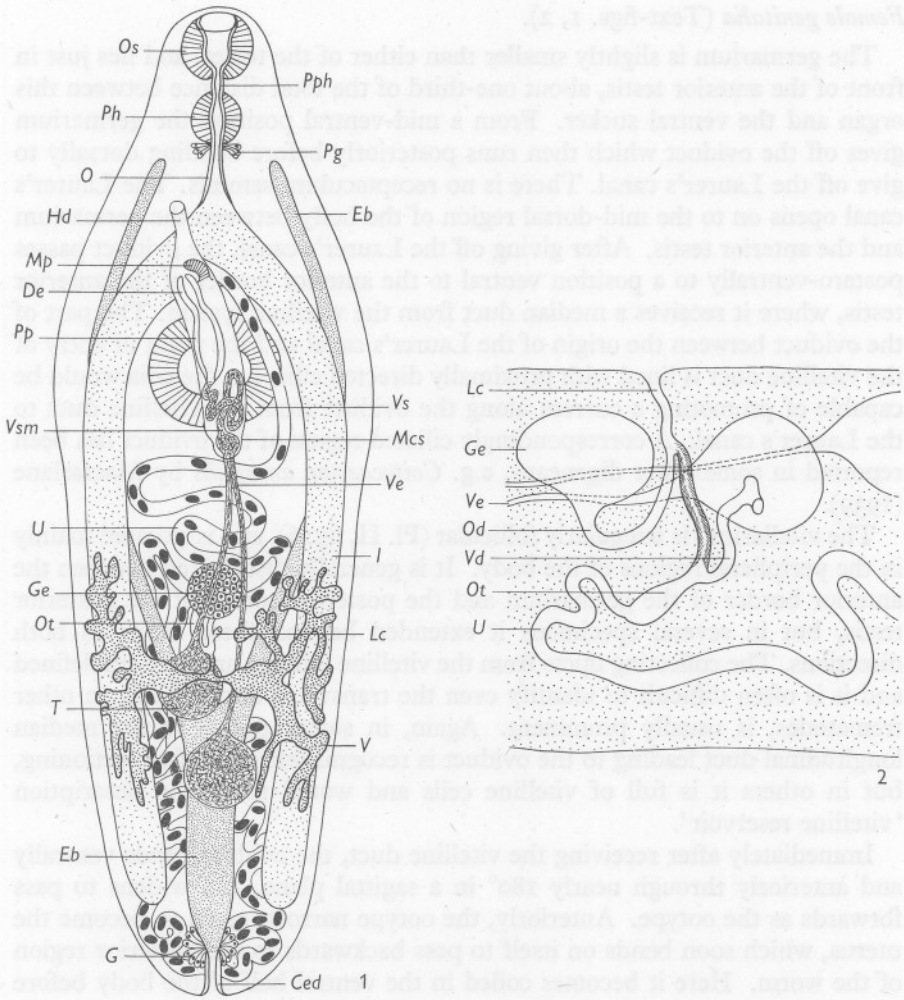
Occasionally the excretory bladder of *P. subtenuis* contains about five to twenty spherical bodies (Pl. II, fig. 2), each about 50 μ (35–66 μ) in diameter, and which in section may be seen to consist of several concentric, radially striated layers. These bodies are stained intensely by haematoxylin. Similar structures have been recorded from the excretory bladder of other trematodes (von Brand, 1952).

Male genitalia (Text-fig. 1)

The two spherical unlobed testes are situated in slightly oblique tandem in the anterior region of the posterior third of the body. Each testis gives rise anteriorly to a vas efferens, the two ducts running forwards fairly directly and joining with each other at their point of entry into the cirrus sac. When at rest this sac lies longitudinally in the body and overlies the ventral sucker, overlapping it both anteriorly and posteriorly, but pressure during histological processing may force it to lie entirely behind or in front of the ventral sucker. The posterior end of the cirrus sac is situated near the sagittal plane of the worm, but anteriorly it curves a little to the left to become confluent with the uterus to form the hermaphrodite duct.

The united vasa efferentia (there is practically no vas deferens) lead immediately to a much-coiled vesicula seminalis which may fill the posterior half of the cirrus. In ten of a sample of twenty flattened specimens the posterior region of the vesicula seminalis was somewhat conical as illustrated in Text-fig. 1, but in the remaining ten specimens such a conical region was not evident. It is possible that in living specimens a proximal conical region of the vesicula seminalis is demarcated, but that it may become obliterated during histological preparation.

The vesicula seminalis leads anteriorly to a 'pars prostatica' which is surrounded by unicellular glands, and which contains villous projections into its lumen. The pars prostatica leads in turn to a muscular ejaculatory duct, and this opens into the relatively narrow hermaphrodite duct. Active sperms were seen in the ejaculatory duct of pressed living specimens, even in specimens less than 1 mm long which contained no egg capsules. Lying sometimes alongside and sometimes anterior to the ejaculatory duct is a 'muscular papilla' (Pl. I, fig. 3A, B; Text-fig. 1). When lying alongside the ejaculatory duct, this papilla forms part of the wall of a pouch that is completed by the ejaculatory duct itself (Text-fig. 1). Such a muscular papilla has been reported in other species of *Proctoeces* (Fujita, 1925; Yamaguti, 1934, 1938), and a somewhat similar structure was described in the gasterostome *Bucephalopsis tylosuris* by Ozaki & Ozaki (1952) as a 'genital tongue'. By applying pressure to the cover-glasses of whole mount preparations of living *Proctoeces subtenuis*, the cirrus was sometimes caused to extrude, and, during this extrusion, the muscular papilla was seen to lie anteriorly to the muscular end of the ejaculatory duct. The muscular papilla preceded the cirrus proper in its passage out of the cirrus sac and was of such size as apparently forcibly to enlarge the normal diameter of the cirrus sac and hermaphrodite duct. The function of this muscular papilla is not known, but its topographical relationships and intimate association with the male intromittent organ suggest that it could play some part in the 'geometrical fitting' of co-copulants, as has been shown by Ullyot & Beauchamp (1931) to occur in certain turbellarians.



Text-figs. 1, 2. *Proctoeces subtenuis.* Text-fig. 1. Whole animal in dorsal view. (Diagram based on microprojections of whole mount preparations, with adjustments so that dimensions agree with the mean measurements included in Table 1.) Fig. 2. Proximal regions of the genitalia in side view. (Reconstructed from serial longitudinal sections.) *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Eb*, epithelial-lined excretory bladder; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Ge*, germarium; *Hd*, hermaphrodite duct; *I*, intestine; *Lc*, Laurer's canal; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *O*, oesophagus; *Od*, oviduct; *Os*, oral sucker; *Ot*, ootype; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, prepharynx; *T*, testis; *U*, uterus; *V*, vitelline follicles; *Vd*, vitelline duct; *Ve*, vas efferens; *Vs* ventral sucker; *Vsm*, vesicula seminalis.

Female genitalia (Text-figs. 1, 2).

The germarium is slightly smaller than either of the testes, and lies just in front of the anterior testis, about one-third of the total distance between this organ and the ventral sucker. From a mid-ventral position the germarium gives off the oviduct which then runs posteriorly before bending dorsally to give off the Laurer's canal. There is no receptaculum seminis. The Laurer's canal opens on to the mid-dorsal region of the body between the germarium and the anterior testis. After giving off the Laurer's canal, the oviduct passes postero-ventrally to a position ventral to the anterior border of the anterior testis, where it receives a median duct from the vitelline system. The part of the oviduct between the origin of the Laurer's canal and the point of entry of the vitelline duct is lined with proximally directed cilia, i.e. the cilia would be capable of promoting a current along the oviduct from the vitelline duct to the Laurer's canal. A correspondingly ciliated region of the oviduct has been reported in some other digeneans, e.g. *Coitocaecum anaspidis* by Macfarlane (1939).

The vitellarium is irregularly follicular (Pl. II, fig. 6), and is situated mainly in the peripheral regions of the body. It is generally distributed between the anterior border of the germarium and the posterior border of the posterior testis, but in several specimens it extended beyond these limits in both directions. The collecting ducts from the vitelline follicles are not well defined and it is often difficult to identify even the transverse duct, which, in other trematodes, is usually prominent. Again, in some specimens the median longitudinal duct leading to the oviduct is recognizable only after sectioning, but in others it is full of vitelline cells and would merit the description 'vitelline reservoir'.

Immediately after receiving the vitelline duct, the oviduct bends ventrally and anteriorly through nearly 180° in a sagittal plane, and widens to pass forwards as the ootype. Anteriorly, the ootype narrows again to become the uterus, which soon bends on itself to pass backwards to the posterior region of the worm. Here it becomes coiled in the ventral half of the body before eventually passing anteriorly, finally to take a fairly straight course alongside or beneath the cirrus sac, to open into the hermaphrodite duct ventral to the cirrus.

The hermaphrodite duct is provided with strongly muscular walls and leads to the genital pore, which, in fixed specimens, lies midway between the oral and ventral suckers to the left of the mid-line of the body (Text-fig. 1).

Egg capsules.

In a sample of 176 specimens of *Proctoeces subtenuis* from forty-one hosts, all but one of the trematodes had egg capsules in the uterus. This single specimen was very small, measuring 0·72 mm when flattened and fixed, and,

although no egg capsules were present, the vesicula seminalis contained active sperms. The larger specimens each contained at least one to two thousand capsules.

The egg capsules were oval, and about twice as long as wide ($42 \times 24\ \mu$). In some cases there was considerable variation in the size of capsules. Those measured for Table 1 varied in length from 26 to 73 μ, but there were only three longer than 52 μ. The capsules were light brown, with relatively thin, elastic walls which were capable of being stretched by muscular movements of a contained embryo. No operculum could be seen, even in capsules which contained active embryos and which had been freely liberated by the trematode. Furthermore, when capsules were pressed under cover-glasses until they burst, the lines of fracture did not reveal any incipient operculum. It was, however, possible to recognize a polarity in most capsules, a small part of the wall being thickened to form a disc at that end nearest to the posterior end of the embryo.

In a sample of twenty specimens of *P. subtenuis*, collected from hosts that contained more than one parasite per host, all twenty were found to have egg capsules enclosing active embryos. Only one of over 200 specimens of *Scrobicularia plana* examined contained only a single trematode, and, although this parasite possessed numerous egg capsules, they contained no embryos.

Attempts to obtain embryos freed from their capsules were unsuccessful. Capsules which had been freely liberated from the trematodes were kept in sea water for periods of up to 7 days, but no hatching took place. Pressure was applied to the capsules in an attempt to release the embryos, but, although the capsules were ruptured, the miracidia were always damaged. Therefore, it was possible to make observations on embryos only while they were still enclosed in their capsules. The embryos (Pl. II, fig. 7) were seen to be ciliated, and ciliary activity was sometimes manifested by a rotation of the embryo alternately to the left and right about the long axis, and also by the agitation of hyaline droplets, presumably residual yolk material, lying freely in the capsule, particularly around the equator of the embryo. The orientation of the cilia suggested that the posterior end of the embryo lay near to the thickened disc in the capsule wall. A constant feature to be seen at the other end of the embryo was a prominent refractile body. The only other internal structures that could be identified were two flame cells situated about mid-way along the length of the embryo. It is possible, however, that more flame cells may have been present.

Evidence was obtained that egg capsules are normally liberated by the trematodes while in the kidney of *S. plana*. Twenty-five hosts were carefully examined and egg capsules were found lying freely in the kidneys of three of them. Two of these hosts contained two capsules each, and the third over 500. A further twenty-five hosts were kept in separate dishes of filtered sea water and the contents of the dishes examined daily. Free egg capsules were

found in two of them. An estimate of the rate of egg laying was obtained by isolating freshly collected trematodes in filtered sea water at 19° C, and making daily counts of the egg capsules in each dish. The numbers of eggs laid on each of 5 days after isolation are given in Table 2. It is possible that eggs liberated immediately after removal of the trematode from the host were forcibly discharged as a result of mechanical stimulation involved in the act of removal, but the figures in the table show that eggs continued to be liberated for several days while the trematodes were left undisturbed.

TABLE 2. RATE OF EGG LAYING OF *PROCTOECES SUBTENUIS*

Days after isolation ...	1	2	3	4	5
Specimen A	123	9	10	5	8
B	17	0	Specimen died		
C	1	0	0	14	Died
D	37	8	0	0	7
E	82	3	51	4	5

It may thus be concluded that this parasite regularly produces large numbers of fertile eggs, and that these are liberated into the kidney and pass outside the body of the host. The ciliation of the embryo suggests the occurrence of a free miracidial stage, but there was no evidence of a special operculum to facilitate hatching. It is interesting to note the observation by Baer (1952, p. 127): Curiously enough, this ciliated coating is also found in miracidia that remain within the egg and hatch only when the latter is swallowed by the snail.'

The eggs are enclosed in a thin, light-brown capsule. The hardening of trematode egg capsules has recently been reviewed by Johri & Smyth (1956), and may be summarized as follows:

Dihydroxy-phenols are oxidized, through the agency of a phenol-oxidase, to the corresponding quinone, which combines with protein to form a tanned protein or sclerotin. The histochemical techniques used by Johri & Smyth have demonstrated that the vitellaria are the site of the protein, phenol, and phenol-oxidase involved. Stains such as malachite green have an affinity for the vitellaria and are believed to be specific for the protein component localised there. Diazo-reagents, such as Fast Red B, are specific for phenolic substances, and the localization of these phenols can also be demonstrated by use of ammonium molybdate. The presence of a phenol-oxidase acting on certain dihydroxy-phenols can be demonstrated by incubating alcohol-fixed specimens at 37° C for about 2 h in catechol, protocatechuic acid or dihydroxy-phenyl-alanine (DOPA).

These techniques were applied to specimens of *Proctoeces subtenuis*, and also, for comparison, to *Gastrocotyle trachuri*, a monogenetic trematode from the gills of the horse mackerel *Trachurus trachurus*. A specimen treated with Fast Red B is shown in Pl. II, fig. 6. The results (Table 3) refer in each case to the intensity of reaction localised in the vitellaria.

These show the presence in the vitellaria of *Proctoeces subtenuis* of the phenol and protein components of the egg tanning processes, but negative results were consistently obtained for techniques which, under identical conditions, demonstrated the presence of a phenol-oxidase in *Gastrocotyle*.

TABLE 3. EGG TANNING PROCESSES OF *GASTROCOTYLE TRACHURI* AND *PROCTOECES SUBTENUIS*

For explanation see text

	G. trachuri	*P. subtenuis*
Malachite green	+	+
Fast Red B	++	++
Ammonium molybdate	++	+
Catechol	++	o
Protocatechuic acid	++	o
DOPA	++	o

The use of catechol, protocatechuic acid, and DOPA as substrates will necessarily reveal only the occurrence of an enzyme acting on these dihydroxy-phenols, which may possibly be derived from tyrosine. Pryor (1955) has suggested the role of amino-phenols derived from some other source, probably tryptophan, in the hardening of some dipterous puparia. Perhaps a similar mechanism may be operative in *Proctoeces subtenuis*.

TAXONOMY OF THE PARASITE

Our description clearly identifies this trematode as belonging to the genus *Proctoeces* Odhner, 1911, of the family Fellodistomatidae, subfamily Haplocladinae. Yamaguti (1953) lists seven species of *Proctoeces*, together with a larva of which specific identification was not possible. Winter (1954) proposed another new species. Several of the species are based on very few specimens, sometimes only one, and their distinction rests on differences of relative body proportions and on egg size. We could not, however, refer our trematodes exclusively to any one of these species. Several hundred specimens were examined, and it is apparent that many of the characters thought to indicate specific differences probably represent intraspecific variations of the kind emphasized by Stunkard (1957). The present species are *P. maculatus* (Looss, 1901) Odhner, 1911; *P. erythraeus* Odhner, 1911; *P. subtenuis* (Linton, 1907) Hanson, 1950; *P. insolitus* (Nicoll, 1915) Dollfus, 1952; *P. ostreae* Fujita, 1925; *P. major* Yamaguti, 1934; *P. magnorus* Manter, 1940; and *P. macrovitellus* Winter, 1954.

Proctoeces was established by Odhner (1911) for *Distomum maculatum* Looss, 1901 and *Proctoeces erythraeus*. He distinguished *P. erythraeus* by its smaller ventral sucker (at least one-third smaller), its smaller eggs, and the shorter extent of the vitellaria. These differences are best illustrated by comparing the following measurements, in mm, taken from Odhner (1911):

	P. maculatus	*P. erythraeus*
Length	2·5	3·0
Ventral sucker	0·42 – 0·70 × 0·28 – 0·42	0·38 × 0·40 (about 0·3 in life)
Vitellaria	Extend behind posterior testis	Extend to anterior border of posterior testis
Eggs	0·072 – 0·079 × 0·027	0·045

Dawes (1946) lists *P. erythraeus* as a synonym of *P. maculatus* and says (p. 245) that only the difference of egg size is significant, 'but this is marred by the fact that only a solitary mature specimen was found'. *P. erythraeus* was reinstated by Manter (1947) who found six specimens in *Calamus* spp. at Tortugas, Florida. He gave the egg size as 46–53 × 19–24 μ and concluded that 'the extent of the vitellaria varied some but never reached past the posterior testis as it does in *P. maculatus*'.

Hanson (1950) collected two trematodes from *Calamus* sp. at Bermuda which agreed with *Proctoeces erythraeus* in sucker ratio, extent of vitellaria, and egg size, but she argues that Linton's (1907) description of *Distomum subtenue*, also collected from *Calamus* sp. at Bermuda, agrees with *Proctoeces erythraeus* sufficiently for them to be considered the same species. Linton's measurements, in mm, of two specimens of '*Distomum subtenue*' are:

Length	3·6	2·07
Ventral sucker	0·68	0·30 × 0·48
Eggs	0·05 × 0·02	0·042 × 0·015

He illustrates the vitellaria extending behind the posterior testis. Whereas Hanson's two specimens were said to agree with *Proctoeces erythraeus* in all three characters of egg size, sucker ratio, and extent of vitellaria, one of Linton's specimens has a considerably larger ventral sucker (the measurement of 0·68 mm was from a living specimen) and the vitellaria extend behind the posterior testis. Our material from *Scrobicularia plana* showed that the vitellaria could stop short of the posterior testis in some specimens while extending behind it in others. Examination of only a few specimens would permit a conclusion that would not have been true for all the specimens available. It seems best, therefore, to recognize the general similarity of *Distomum subtenue* and *Proctoeces erythraeus*, particularly because of identical hosts in Bermuda, and regard them as the same species, *P. subtenuis* (Linton, 1907) (syn. *P. erythraeus* Odhner, 1911), as was proposed by Hanson.

Comparison of *P. erythraeus* (as described by Odhner, Manter and Hanson) and *Distomum subtenue* (as described by Linton) illustrates the amount of individual variation within the same species. It also shows the difficulty of reaching any firm statement of differences between related species. On the evidence of size of ventral sucker and extent of vitellaria at least one of Linton's specimens agrees with Odhner's description of *Proctoeces maculatus*. This adds emphasis to Dawes's (1946) observation that only the difference of egg size is significant in separating *P. maculatus* from *P. subtenuis* (= *P. erythraeus*). The average egg dimensions (42 × 24 μ) of our trematodes agree with those of *P. subtenuis*, but the largest egg measured (73 × 30 μ) is within the size range given by Odhner for *P. maculatus*. While it seems generally true that the characters of sucker size, extent of vitellaria, and size of eggs are sufficient to distinguish *P. maculatus* and *P. subtenuis*, intraspecific variation may lead to some overlap in all three characters. Odhner, the only author

who had the opportunity of directly comparing *P. maculatus* and *P. subtenuis* (=*P. erythraeus*), considered them to be separate species. On the evidence available then it seems advisable to follow Odhner, but a critical re-examination of numerous specimens of these two species is obviously desirable.

Nicoll (1915) collected five trematodes from the rectum of *Sparus australis* and named them *Xenopora insolita* n.g., n.sp. This species was later placed in *Proctoeces* by Dollfus (1952). In egg size, ventral sucker size, and extent of vitellaria, *P. insolitus* agrees with *P. subtenuis*, but differs in the extension of the hermaphrodite duct a short distance behind the ventral sucker, the situation of the cirrus pouch entirely behind the ventral sucker, and in the vesicula seminalis being mainly external to the cirrus pouch. Odhner comments that in Looss's specimens of *P. maculatus* the cirrus sac was displaced behind the ventral sucker by pressure, the genital sinus (hermaphrodite duct) being stretched to twice its normal length. In some of our preparations of *P. subtenuis* the cirrus sac was similarly situated almost entirely behind the ventral sucker. Differences in extent of the hermaphrodite duct and the position of the cirrus sac are not in themselves, therefore, sufficient specific characters. The extension of the vesicula seminalis outside the cirrus sac is contrary to Odhner's diagnosis of *Proctoeces* and, as Yamaguti (1953) observes, this feature of *P. insolitus* requires confirmation. In all other respects *P. insolitus* agrees with *P. subtenuis*, but, pending further examination of the vesicula seminalis, it is felt that *P. insolitus* must remain a separate species.

The specific status of *P. ostreae* Fujita, 1925, has been discussed by Dollfus in his addenda to Fujita's paper. He concludes that it is a progenetic metacercaria that has not yet attained complete maturity nor definitive size, and declines to come to any firm conclusion whether it is a separate species or merely a metacercaria of *P. maculatus*. Its validity must therefore remain in doubt.

P. major Yamaguti, 1934, shows some resemblances to *P. maculatus*, but differs significantly in the size of the body, the trilobate shape of the ovary, and the size of the eggs. To these might be added the position of the genital pore to the right of the mid-line, not, as usually in *Proctoeces*, to the left.

The final species in Yamaguti's list is *P. magnorus* Manter, 1940, based on one specimen from Cerros Island, Mexico. Manter considered it most similar to *P. erythraeus* as described by Odhner (1911), but differing in having a larger oral sucker and smaller eggs. He says that it is also probable that *P. magnorus* differs from *P. erythraeus* in having shorter vitellaria, an acetabular stalk, a shorter oesophagus, a cirrus sac not reaching the ovary, and a longitudinal groove within the acetabular cavity. It is difficult to know how Manter reached these conclusions, since Odhner nowhere describes or figures the oral sucker, oesophagus, or cirrus sac of *P. erythraeus*. In view of the variability we have shown in extent of vitellaria and in egg size it would seem unwise to base a new species on such characters when only a single specimen has been

examined. *P. magnorus* must, therefore, be considered a synonym of *P. subtenuis* (syn. *P. erythraeus*).

P. macrovitellus Winter, 1954, was based on two specimens from the posterior part of the intestine of *Cymatogaster aggregatus* Gibbons from Southern California. This was the first record of *Proctoeces* from a fish of the family Embiotocidae, and also the first from the west coast of America. Many of the features of *P. macrovitellus*, as described and figured by Winter, are in disagreement with the original generic diagnosis by Odhner (1911) and the modified diagnosis by Yamaguti (1953). *P. macrovitellus* has the genital pore to the right of the mid-line at the level of the pre-pharynx, the vitelline follicles

TABLE 4. A REVISED LIST OF THE SPECIES OF THE GENUS *PROCTOECES* AND THEIR FISH HOSTS

Host	Family	Location	Authority
P. maculatus (Looss, 1901)			
Labrus merula L.	Labridae	Trieste	Looss, 1901
Julis pavo (L.) (= *Crenilabrus pavo*)	Labridae	—	—
Symphodus (= *Crenilabrus*) *griseus* (Gmelin)	Labridae	—	—
Blennius ocellaris L.	Blenniidae	Naples	Odhner, 1911
Milio (= *Sparus*) *macrocephalus* (Basilewsky)	Sparidae	Inland Sea	Yamaguti, 1934
Sparus sarba Forskål (= *S. aries*)	Sparidae	—	—
Pagrosomus auratus (Bloch & Schneider)	Sparidae	—	—
Epinephelus aka-ava Bleeker	Serranidae	—	—
Semicossyphus reticulatus (Cuvier & Valenciennes)	Labridae	Tarumi	Yamaguti, 1938
Crenilabrus sp.	Labridae	Black Sea	Wlassenko, 1931
Duymaeria flagellifera (Cuvier & Valenciennes)	Labridae	Hamazima	Yamaguti, 1953
P. subtenuis (Linton, 1907)			
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Bermuda	Linton, 1907
Bodianus rufus (L.) (= *Harpe rufa*)	Labridae	—	—
Iridio bivittata (Bloch)	Labridae	—	—
Lachnolaimus maximus (Walbaum)	Labridae	—	—
Calamus sp.	Sparidae	Bermuda	Hanson, 1950
Latridopsis ciliaris (Forster)	Latridae	Wellington, N.Z.	Manter, 1954
P. subtenuis (but described as *P. erythraeus*)			
Sparus (= *Chrysophrys*) *bifasciatus* (Forskål)	Sparidae	Red Sea	Odhner, 1911
Thalassoma lunare (L.) (= *Julis lunaris*)	Labridae	—	—
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Tortugas	Manter, 1947
C. bajanado (Bloch & Schneider)	Sparidae	—	—
P. subtenuis (but described as *P. magnorus*)			
Caulolatilus anomalus (Cooper)	Sparidae	Mexico	Manter, 1940
P. insolitus (Nicoll, 1915)			
Sparus australis (Günther)	Sparidae	North Queensland	Nicoll, 1915
P. major Yamaguti, 1934			
Pagrosomus auratus (Bloch & Schneider)	Sparidae	Tarumi	Yamaguti, 1934
Unknown species or species of doubtful validity; in molluscan hosts			
P. ostreae Fujita, 1925			
Ostrea gigas Thunberg	Ostreidae	Hiroshima	Fujita, 1925
Proctoeces larva, Yamaguti, 1938			
Brachiodontes senhausi (Reeve)	Mytilidae	Lake Hamana	Yamaguti, 1938

situated wholly in front of the testes, thick-shelled eggs, and the cirrus sac not parallel to the long axis of the body but acutely twisted in the first third of its length. Winter also describes and figures an ovoid receptaculum seminis, measuring 0·079 by 0·095 mm, situated immediately in front of the ovary. The presence of a receptaculum seminis would, in itself, exclude Winter's species from *Proctoeces* as defined hitherto. We consider that Winter's species cannot be included in *Proctoeces* without drastic revision of the limits of this genus as at present understood.

A revised list of the species of *Proctoeces*, based on the foregoing systematic considerations, with the modern names of their hosts, is given in Table 4. It is now possible to assign the trematodes from *Scrobicularia plana* exclusively to the species *Proctoeces subtenuis* (Linton, 1907).

DISCUSSION

There seems little doubt from their morphology that our specimens of *Proctoeces subtenuis* are adult digenetic trematodes. They exhibit, in all significant features, the same structure as other members of the species described from fish hosts, and the availability of very large numbers has, indeed, allowed a more detailed account of the structure and variability of this species than was hitherto possible.

All possible adult forms of this trematode have been found, from the small immature form, through larger sexually mature individuals, to dead specimens present in the same kidney with living individuals. Their unencysted condition in the kidney makes copulation and fertilization possible: the only specimen found singly in a host had undeveloped eggs. There is evidence that eggs containing active, ciliated miracidia are liberated from the trematode and passed out of the host. Such eggs were found free in the kidney and also in dishes in which infected hosts had been isolated. The eggs presumably pass from the kidney by the urinary pore into the supra-branchial space and reach the exterior by the exhalant siphon.

The very heavy infection of *Scrobicularia plana* at Chalkwell, and the isolated occurrences of the trematode at Whitstable, must have been initiated, and must be maintained, by larval stages developing in an invertebrate host. The life history of at least one member of the Fellodistomatidae is known (Palombi, 1934), and a cercaria undoubtedly belonging to this family has been described by Martin (1945) and redescribed, without reference to Martin's work, by Cable (1954). In both, the cercaria was non-ocellate and trichocercous, and developed in sporocysts in a marine lamellibranch. The life cycle studied by Palombi was of *Bacciger bacciger* (Rud.), subfamily Fellodistomatinae, and Martin's cercaria can almost certainly be referred to the same subfamily. *Proctoeces* belongs to the subfamily Haplocladinae, and Cable (1953) suggests that the cercariae of this subfamily may be furcocercous or

trichofurcocercous. We have found furcocercous cercariae developing in sporocysts in *Scrobicularia plana* at Chalkwell, but the fact that they occurred also in *S. plana* from the Gwendraeth and Tavy rivers where *Proctoeces subtenuis* was absent suggested that they were not related to this trematode. This impression that adult and cercarial stages of *P. subtenuis* did not both occur in *Scrobicularia plana* was confirmed by the negative results of numerous attempts to infect *S. plana* with these furcocercous cercariae, and the failure to find post-miracidial stages in *S. plana* into which fertile eggs of *Proctoeces subtenuis* had been introduced.

There is some evidence that *Proctoeces* in Japanese waters has an unencysted metacercaria in marine lamellibranchs. As already noted, the trematode described by Fujita (1925) as *P. ostreae* from the gonad of the edible oyster of Japan, *Ostrea gigas*, was considered by Dollfus to be a metacercaria of an undetermined species of *Proctoeces*, and similarly Yamaguti (1938) described a single immature stage of *Proctoeces* sp. from the digestive gland of the mytilid *Brachiodontes senhausi*. The normal life cycle of *Proctoeces* thus appears to be as follows: adult trematodes live in the hind-gut of labrid or sparid fishes; cercariae, which may be furcocercous, trichofurcocercous or trichocercous, develop in sporocysts in marine lamellibranchs; and unencysted metacercariae also occur in marine lamellibranchs. These metacercariae are almost certainly transferred to the fish which is the definitive host when it eats the lamellibranch (Yamaguti, 1938).

Our evidence indicates that this life cycle of *Proctoeces* must be modified for *P. subtenuis* in British waters. The apparent limitation of the species to the region of the Thames estuary, in a lamellibranch widely distributed in estuarine muds around the British Isles, strongly suggests that the introduction of *P. subtenuis* to this country is fairly recent. It is typically a parasite of fish of Asian, Australasian or eastern American waters. It could possibly have arrived through the accidental introduction of a normal fish host, or, more probably, in the marine lamellibranch that is the first intermediate host. The cercariae issuing from this mollusc could have entered *Scrobicularia plana* and, now being outside the range of the normal fish host, have achieved maturity in the kidney of this lamellibranch. It may be noted that a trichocercous cercaria has been found in the lamellibranch *Petricola pholadiformis* Lamarck at Whitstable (M. Duval, personal communication). *P. pholadiformis* is a recent introduction from the east coast of North America (Newell, 1954), and has a limited distribution in the southern and eastern parts of Britain, including the Essex coast of the Thames estuary (Cole, 1903). Work is now being done to determine whether the cercaria from *P. pholadiformis* is a stage in the life cycle of *Proctoeces subtenuis* in the Thames estuary. The evidence so far available suggests that the fish host has been eliminated from the life cycle. No sparids have been recorded from the Thames estuary, and only two members of the Labridae occur there, namely *Labrus bergylta* Ascanius (three specimens up to

1903 (Laver, 1903; Murie, 1903)), and *Crenilabrus melops* (L.) which is common. Any final statement on the life cycle of *Proctoeces subtenuis* in the Thames estuary must, of course, include consideration of the only common labrid in the area, but transference of the trematode from its last molluscan host to a fish must almost certainly be effected by the fish eating the mollusc, and there is no evidence that a lamellibranch such as *Scrobicularia plana* is eaten by *Crenilabrus melops*. The nature of the habitat of *Scrobicularia plana* makes it unlikely that it is readily available as food for fish, although shells have occasionally been found in the stomach of some flatfish (J. E. Forrest, personal communication). The limitation of *Proctoeces subtenuis* to the mouth of the Thames also suggests that a fish host is not involved in the life cycle. At Chalkwell, every specimen of *Scrobicularia plana* examined was infected, and yet the trematode appears to be absent from *S. plana* in the neighbouring Essex rivers, the Blackwater and Colne. It seems unlikely that mobile fish, acting as hosts to breeding trematodes, would not have produced a wider distribution of the parasite while the intensive infection of *S. plana* at Chalkwell was taking place.

If this view is correct then the trematodes in *S. plana* are directly comparable, structurally, functionally, and in their status in the life history, with the adults found in the hind-gut of fishes in other parts of the world. They enter the kidney of *S. plana* as immature stages, achieve maturity there, and eventually die there. They certainly differ from the stages of *Proctoeces* previously described from lamellibranchs. The single immature stage described by Yamaguti (1938) from *Brachiodontes senhausi*, and termed by him an adolescaria, had undeveloped female genitalia. Dollfus relegated Fujita's *Proctoeces ostreae* to the status of progenetic metacercariae on the grounds that only a few eggs were present, even these being abnormal, and that the animals did not appear to have attained complete maturity nor definitive size. In these respects our *P. subtenuis* clearly agrees more closely with previous descriptions of any of the adults of this trematode from fish hosts than with any metacercarial stage of related species. There seems no reason to modify the emphasis on their adult nature by invoking the phenomenon of progenesis.

The term progenesis was first used by Giard & Bonnier (1887) to describe the assumption of sexual maturity by male parasitic isopods while still in a larval condition. It has since been applied to numerous digenetic trematodes which become sexually mature as cercariae or metacercariae in an intermediate host. Many examples are listed by Wu (1938) and Dawes (1946). The only significant question from the point of view of this study is that posed by Dollfus (1929) in the title of his paper: 'Existe-t-il des cycles évolutifs abrégés chez les trématodes digénétiques?' This question is further discussed by Joyeux, Noyer & Baer (1930) and again by Wu (1938). Wu concludes that 'up to the present we still have no definite proof that there is an abridged life cycle or omission of a final host in the life history of a digenetic trematode'.

This proof was provided by Serkova & Bychowsky (1940). They exposed uninfected *Bithynia tentaculata* (L.) to members of the same species of snail which contained mature *Asymphylodora progenetica*, and obtained experimental infections. They identified rediae and also mature adults in the previously uninfected snails. In this abbreviated life cycle the rediae develop in one specimen of *Bithynia tentaculata* and the adults achieve maturity in another specimen of the same species. Serkova & Bychowsky assume that an alternative life cycle of *Asymphylodora progenetica* is possible, in which the parasite of the mollusc enters a fish and there attains sexual maturity. The importance of Serkova & Bychowsky's work, in relation to our study of *Proctoeces subtenuis*, lies in the precedent it established that abbreviated life cycles of digenetic trematodes are possible. More recently, Buttner (1955) has shown that *Ratzia joyeuxi* (Brumpt, 1922) and *Paralepoderma brumpti* (Buttner, 1950), both normally parasites of snakes, can show abbreviated life cycles in which the final host is omitted.

We suggest that the facts presented in this paper provide strong circumstantial evidence that *Proctoeces subtenuis* in the Thames estuary displays a similar phenomenon of a life cycle restricted to invertebrate hosts. A final decision on this question must, however, await the results of further investigations.

Most of the observations reported in this paper were carried out at the Plymouth laboratory and we are grateful to the Director and his staff for many kindnesses shown to us during the course of this work. We are particularly indebted to Dr J. S. Alexandrowicz for much assistance with the literature. We wish to thank Mr N. B. Marshall of the British Museum (Natural History) for help in compiling the list of fish hosts, and also Miss Emily Clay, Dr J. Green, Mr S. V. N. Casey and Mr B. W. Jones for sending specimens of *Scrobicularia plana* from localities we were unable to visit.

SUMMARY

The digenetic trematode *Proctoeces subtenuis* (Linton, 1907) is recorded from the kidney of the lamellibranch *Scrobicularia plana* (da Costa). At Chalkwell in Essex all of nearly a thousand *S. plana* examined were found to be infected, as were three of approximately 150 *S. plana* at Whitstable in Kent. The average number of parasites per host was between four and five. No *Proctoeces subtenuis* were found in *Scrobicularia plana* examined from other localities in Essex, Devon, Wales and Suffolk. The environmental conditions of a parasite in the lamellibranch kidney are discussed. It was found that the kidney fluid surrounding the parasites varies in osmotic pressure.

The anatomy of the parasite is described. The excretory bladder has a cellular wall, thus disagreeing with the inclusion of *Proctoeces subtenuis* in La Rue's superorder Anepitheliocystidia. The red colour of the parasite is due

to a native haem pigment. The egg capsules are hardened by a quinone-tanning mechanism, but incubation with catechol, protocatechuic acid, and dopa failed to reveal the presence of a phenol-oxidase.

The species of *Proctoeces* are reviewed, and some revisions suggested. *P. magnorus* Manter, 1940 is considered a synonym of *P. subtenuis*, and the synonymy of *P. erythraeus* Odhner, 1911 with *P. subtenuis* is confirmed. The differences between *P. insolitus* (Nicoll, 1915) and *P. subtenuis*, and between *P. maculatus* (Looss, 1901) and *P. subtenuis*, require re-examination. It is considered that *P. macrovitellus* Winter, 1954 should be excluded from the genus *Proctoeces*. A definitive host list of *Proctoeces* spp. is given.

The adult nature of *P. subtenuis* from *Scrobicularia plana* is discussed, and it is suggested that *Proctoeces subtenuis* in the Thames estuary shows an abbreviated life cycle restricted to invertebrate hosts.

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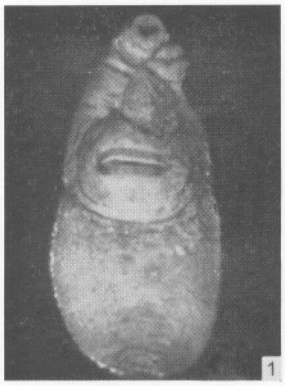
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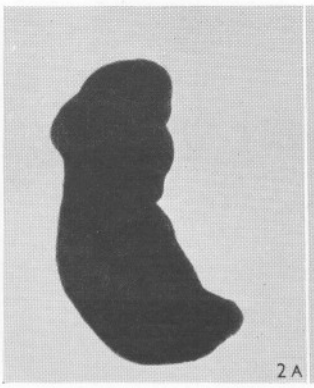
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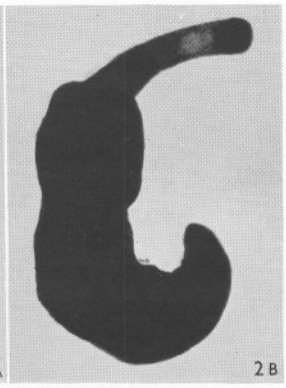
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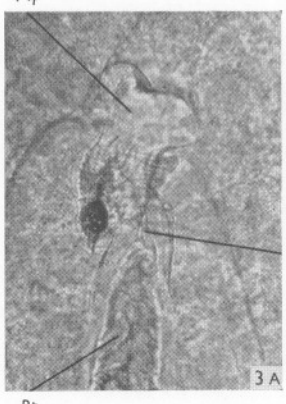
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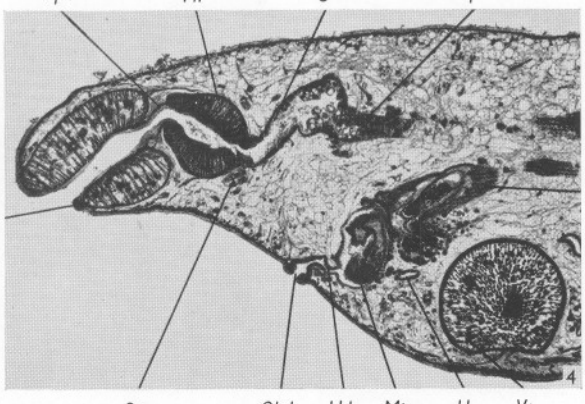
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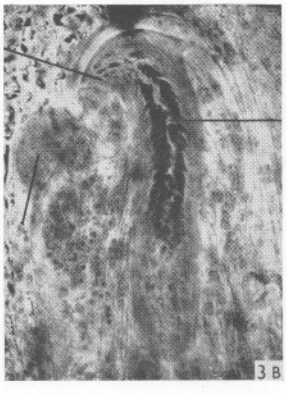
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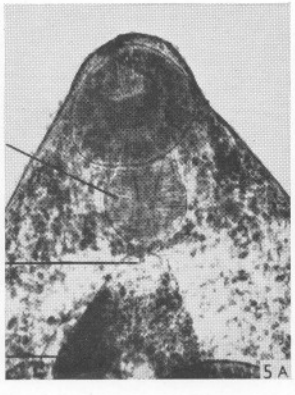
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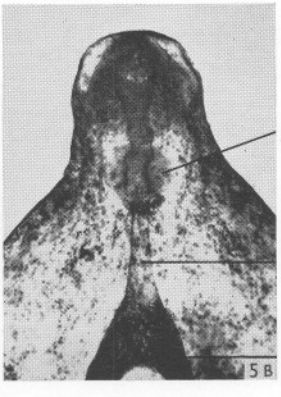
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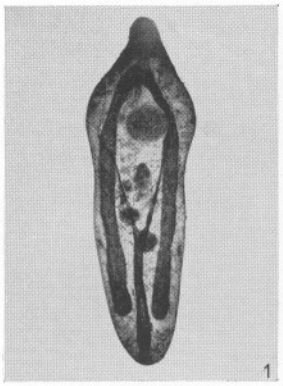
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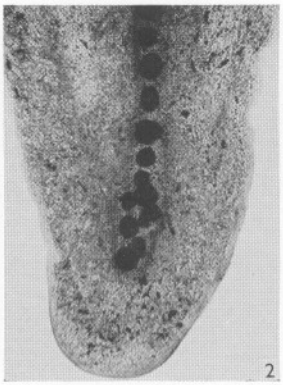
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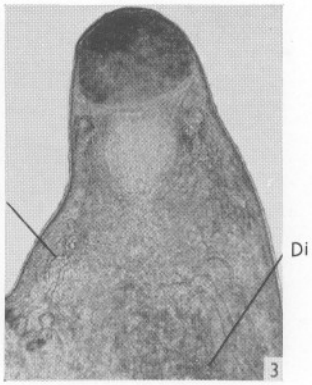
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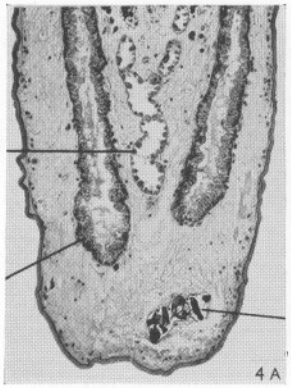
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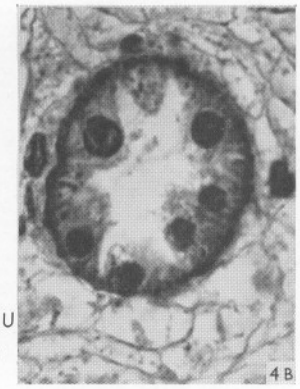
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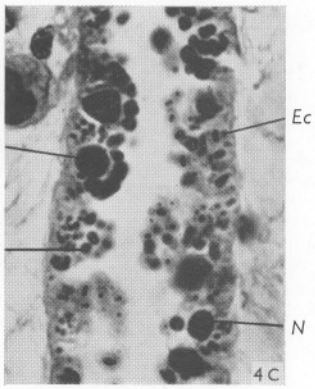
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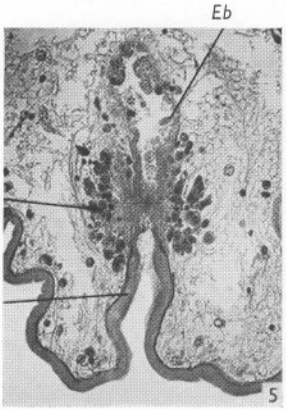
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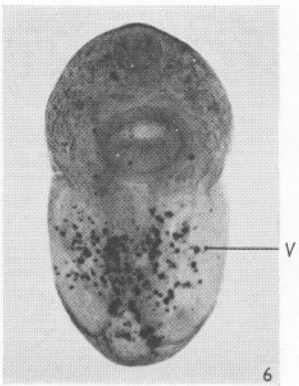
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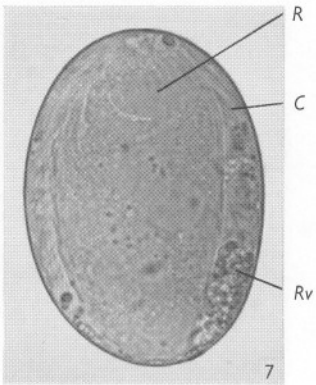
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EXPLANATION OF PLATES

Proctoeces subtenuis. Unless stated otherwise, all photomicrographs were made from fixed specimens mounted in Canada Balsam.

PLATE I

Fig. 1. Dead, 'petrified' specimen (unmounted). Fig. 2. (A) Contracted specimen (living). (B) Same specimen as (A), stretched. Fig. 3. Terminal part of cirrus, showing muscular papilla. (A) Living specimen. (B) Another specimen stained and mounted in Canada Balsam. Fig. 4. Vertical longitudinal section (slightly oblique) through anterior region. Fig. 5. Variation in length of the oesophagus in fixed preparations. (A) Contracted. (B) Stretched (different specimen from (A)).

PLATE II

Figs. 1–5. The excretory system

Fig. 1. The Y-shaped excretory bladder (from a preparation by Miss P. Milsom). Fig. 2. Concretions in the posterior region of the bladder (haematoxylin preparation). Fig. 3. Tributary canals in anterior region (living). Note also the droplets in the intestine. Fig. 4. The epithelial lining of the excretory bladder. (A) Horizontal section. (B) Transverse section. (C) Longitudinal section through epithelial cells charged with 'excretory corpuscles'. Fig. 5. Junction of epithelial-lined portion of bladder with terminal cuticular-lined portion, surrounded with gland cells (horizontal section). Fig. 6. The vitellaria. (Specimen treated with a diazo reagent to show phenolic substances.) Fig. 7. Egg capsule with ciliated embryo. (Living specimen, well pressed beneath cover-glass.)

Abbreviations

C, cilia; *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Di*, droplets in intestine; *Eb*, epithelial-lined excretory bladder; *Ec*, 'excretory corpuscles'; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Hd*, hermaphrodite duct; *I*, intestine; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *N*, nucleus; *O*, oesophagus; *Ohd*, opening of hermaphrodite duct; *Os*, oral sucker; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, pre-pharynx; *R*, refractile body at anterior end of embryo; *Rv*, residual vitelline material; *Td*, tributary duct of excretory system; *U*, uterus; *V*, vitelline follicles; *Vs*, ventral sucker.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

X. SEASONAL CHANGES IN OXYGEN CONSUMPTION

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(Text-figs. 1–5)

The oxygen consumption of *Calanus* has been studied by various workers. They give figures which, although not precisely the same, are of the same order of size. From these figures can be calculated the food requirements and these may be expressed as a percentage of its weight per day or as the volume of sea water of known richness which it must filter each day. However, a copepod does not feed continuously; when carrying out diurnal vertical migration it feeds chiefly during the hours when it is in the surface layers (Gauld, 1953). Again, the overwintering Stage V *Calanus* do not migrate vertically (Nicholls, 1933), but stay in deep water and at this time phytoplankton is very scarce. Altogether then, the volume of 72 ml., calculated as necessary for a *Calanus* to filter daily when the sea is moderately rich in phytoplankton (Fuller & Clarke, 1936), must be an underestimate for long periods of the year.

Many experiments have been made to find the volume which a *Calanus* actually does filter daily, with very varying results, from the 1 ml. of Fuller (1937) to the 250 ml. of Harvey (1937). From the decrease of diatom numbers between cruises in the North Sea, Cushing (1955) has calculated that a *Calanus* may filter as much as 1500 ml. daily.

Recently, using cultures labelled with radioactive phosphorus, we concluded (Marshall & Orr, 1955) that the volume is often below 80 ml. daily. Confronted with this gap between food required and food available, it seemed desirable to re-investigate oxygen consumption.

METHODS

In the earlier work two methods were used for the measurement of respiration. In the first (Marshall, Nicholls & Orr, 1935; Clarke & Bonnet, 1939) the *Calanus* were kept at constant temperature in dark bottles with 1–4 ml. of water to each copepod, and the oxygen consumed over a period of about 4 h measured by the Winkler method. In the second (Clarke & Bonnet, 1939; Raymont & Gauld, 1951) respiration was measured with a constant pressure manometer using *Calanus* in concentrations the same as, or higher than, in the first method. These high concentrations were necessary to obtain a suitable

reduction in oxygen in a time short enough for bacterial metabolism to be negligible.

The concentration in these experiments was high and the effect of crowding was tested by comparing the oxygen consumption of the same catch of ripe female *Calanus* in two parallel experiments, one using sixty *Calanus* in bottles of 60–70 ml. capacity for 4 h at 10° C and the other, single *Calanus* in bottles of about 30–40 ml. at 10° C. In the first (four bottles) the oxygen used, expressed as μl./*Calanus*/h, ranged from 0·336 to 0·392 and averaged 0·367; in the second (nine bottles) the range was from 0·220 to 0·348 μl./*Calanus*/h and the average 0·304. The crowding may therefore have affected the oxygen consumption slightly. On the other hand, when the concentration was changed from 10 in a 35 ml. bottle to 3, there was only a small difference in respiration. Another experiment comparing 100 *Calanus* in 160 ml. bottles with single *Calanus* in 60 ml. bottles for a longer time gave similar results.

By the use of antibiotics the bacterial population can be controlled so that an experiment may be allowed to last a much longer time. According to Oppenheimer (1955) penicillin is one of the most effective in sea water, but unfortunately it reacts with the iodine liberated in the Winkler method for oxygen determination and so it could not be used. We therefore used a mixture of equal parts of streptomycin and chloromycetin which were both found by Oppenheimer to be very effective in concentrations of 50 mg/l. With these, only slight changes in the oxygen content of a control sample of sea water occurred over a period of up to a fortnight. At the end of this time there was usually a sudden breakdown of the antibiotic effect and a correspondingly sharp fall in the oxygen content of control bottles. In some instances moulds also developed. Antibiotics are best added to the filtered sea water at least 10 h before an experiment since the oxygen content may fall appreciably during this period, thereafter remaining constant at the lower level.

The method eventually adopted was to keep three *Calanus*, which had been picked out from townettings taken the previous day, for 2 days in a bottle of 30–40 ml. capacity at 10° C, which is within the annual temperature range in the sea for this latitude. The water used was taken directly from the sea and passed through a membrane filter of about 1 μ average pore diameter and the antibiotics added (50 mg/l. of each). If sufficient numbers were available, ten experimental bottles were used for each stage and four or five controls.

Respiration was measured on Stage V, ripe and unripe females of both the *finmarchicus* and the *helgolandicus* forms, males, and young stages as each was available. In the early experiments Stage V and female *Calanus* were not distinguished as *finmarchicus* or *helgolandicus* but these forms were later (from October, 1956) tested separately and, from July 1956, ripe females were separated from immature, the intermediate stages of maturity being discarded. From July 1956 also the carapace length of all specimens used was measured. These measurements were made after the copepods had been exposed to alkali

and acid in the oxygen determination and, although comparable among themselves, may differ slightly from those of formalin-fixed specimens.

It was not possible to follow any stage throughout a complete year. From the end of October to the end of February ripe *finmarchicus* females were absent whereas during the summer months immature females were very scarce. The *finmarchicus* form is predominant throughout the spring and summer, the *helgolandicus* form during the autumn. Stage V copepodites of both forms were absent during March.

Smaller bottles and larger numbers of individuals were used for the younger stages. For nauplii and even for Copepodites I and II the bottles had to be very small (2–10 ml.) to get a measurable reduction in the oxygen and this was determined by the method of Fox & Wingfield (1938). On the suggestion of Dr M. R. Droop a slight improvement was made in the method. After the reagents are sucked up into the barrel of the pipette, a small bead of mercury is introduced by a half turn of the milled head. This acts as a 'stirrer' when the pipette is shaken and gives a more uniform and finely divided precipitate. Duplicate samples then give much closer results.

The early nauplii can be readily obtained from eggs allowed to hatch in the laboratory; they develop up to Nauplius III without requiring food (Marshall & Orr, 1956). The later nauplius stages, as well as the earlier copepodites, were picked out from the plankton but only small numbers were obtained.

One difficulty, particularly with the small individuals, was that if one animal only was used, the oxygen reduction was little beyond experimental error, whereas if several were used some were likely to moult and so give less reliable results. The figures from those bottles in which a significant proportion had moulted (except with nauplii) or died, were rejected.

Several hundred Nauplius I or II were used at a time but it was difficult to ensure that all those counted were actually put in the experimental bottle; some were always lost with the insertion of the stopper. The count at the end was therefore used for calculation. Since Nauplii I and II usually moult within 24 h, the majority moulted during the course of an experiment and the first point on the curve (Fig. 2) therefore represents Nauplii I and II.

EXPERIMENTAL WORK

It seemed possible that the metabolism of *Calanus* might be reduced in winter not only by the lower temperature but also by the decrease in food supply, and the winter be spent in a state of 'hibernation'. To test this, Stage V *Calanus* captured in winter were kept singly at constant temperature (usually 10° C) in bottles of about 70 ml. capacity. The *Calanus* were first kept in the laboratory overnight to avoid the fall in respiration which occurs during the first 24 h after capture (Marshall *et al.* 1935). A large number of bottles was set up and the oxygen consumption measured every 3 or 4 days by removing

eight or ten bottles and drawing off samples for analysis. The oxygen consumption varied considerably from one individual to another but on the whole it was decidedly lower than the earlier published results. Each of the experiments lasted for a little over 2 weeks but there was no indication that respiration decreased with time. The earlier figure taken for Stage V at 10° C was 0·25 μl./*Calanus*/h, but in these long-term winter experiments the average was 0·14.

Experiments were continued into the spring months and the respiration then began to rise, reaching a figure of 0·560 μl./*Calanus*/h for immature females at 10–14·5° C. At first it was thought that the high value might be caused by bacterial infection. In the following winter further experiments were made by the standard method using antibiotics and were carried on more or less regularly throughout the year. Once again one or two very high results were obtained in the spring and it then became clear that there was a seasonal variation in oxygen consumption.

TABLE 1. FALL IN RESPIRATION WITH TIME

1956			1957		
Total hours	O_2 μl./*C.*/h for total	Calculated μl./*C.*/h for period	Total hours	O_2 μl./*C.*/h for total	Calculated μl./*C.*/h for period
69·5	0·508	0·508	47	0·591	0·591
141·5	0·506	0·504	96	0·493	0·399
213	0·376	0·119	192	0·495	0·497
293	0·342	0·251	288	0·431	0·303
357·5	0·304	0·130			

The seasonal changes are shown in Fig. 1 and Table 2. In 1956 the respiration of females (Fig. 1B) was low at the beginning of April. There was then a rapid rise and values remained high until the middle of May. By June they had fallen to near winter values again. During spring and summer females were almost certainly *C. finmarchicus* and mostly ripe. In the following year the spring rise was earlier. It lasted from the beginning of April to the middle of June when there was a rapid fall.

During the spring of 1956, when respiration was near its peak, twenty-five female *Calanus* were kept singly in 70 ml. bottles and their oxygen consumption measured at 3-day intervals, to show if the high figure would be maintained. Control samples of sea water were analysed at the same time. Consumption remained high for the first 6 days and then fell. If allowance is made for the initial period of high respiration, it can be shown (Table 1) that the fall is considerable and that the respiration reached winter values by the end of the experiment. This was repeated with similar but less striking results in 1957.

The results for Stage V, immature female *C. finmarchicus* and males from October 1956 to late 1957 are shown in Fig. 1C. For males most values are

TABLE 2. OXYGEN CONSUMPTION OF ADULT AND STAGE V *CALANUS*

At 10° C in experiments lasting 48 h. The measurement in the second column represents length.

	Ripe females						Unripe females						Males			Stage V					
	C. finmarchicus			*C. helgolandicus*			*C. finmarchicus*			*C. helgolandicus*						*C. finmarchicus*			*C. helgolandicus*		
	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h
1956																					
18–20. vii.	12*	—	0·296	—	—	—	11*	—	0·241	—	—	—	—	—	—	—	—	—	—	—	—
24–26. vii.	28	2·31	0·336	—	—	—	26	2·30	0·197	—	—	—	—	—	—	—	—	—	—	—	—
9–11. viii.	14	2·22	0·263	—	—	—	15	2·21	0·179	—	—	—	—	—	—	—	—	—	—	—	—
31. viii.–2. ix.	15*	—	0·267	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11–13. ix.	9*	—	0·328	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3–5. x.	—	—	—	30	2·52	0·406	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9–11. x.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28	2·05	0·136			
23–25. x.	—	—	—	25	2·68	0·451	—	—	—	—	—	—	—	—	—	22	2·17	0·217	20	2·36	0·274
30. x.–1. xi.	—	—	—	—	—	—	13	2·35	0·172	5	2·70	0·359	—	—	—	—	—	—	—	—	—
6–8. xi.	1	2·43	0·360	26	2·60	0·477	20	2·41	0·204	7	2·65	0·356	—	—	—	18	2·16	0·222	16	2·40	0·255
4–6. xii.	—	—	—	—	—	—	7	2·22	0·118	2	2·70	0·335	—	—	—	29	2·04	0·142	30	2·08	0·140
12–14. xii.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	2·09	0·101	—	—	—
19–21. xii.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33	2·08	0·171	—	—	—
1957																					
9–11. i.	—	—	—	10	2·57	0·326	30	2·29	0·247	5	2·40	0·291	20	2·46	0·436	31	2·03	0·148	1	1·84	0·263
16–18. i.	—	—	—	—	—	—	29	2·35	0·332	12	2·47	0·415	28	2·51	0·412	25	2·07	0·188	23	2·00	0·338
17–19. i. Large	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19	2·31	0·209	—	—	—
17–19. i. Small	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18	1·99	0·151	—	—	—
30. i.–1. ii.	—	—	—	—	—	—	26	2·35	0·260	—	—	—	—	—	—	—	—	—	—	—	—
2–4. ii.	—	—	—	—	—	—	31†	2·42	0·200	—	—	—	—	—	—	—	—	—	—	—	—
12–14. ii.	28	2·44	0·316	22	2·57	0·305	7	2·54	0·261	1	2·21	0·193	8	2·54	0·259	4	2·27	0·153	1	2·21	0·279
12–14. iii.	32	2·42	0·347	28	2·52	0·383	11	2·40	0·238	—	—	—	—	—	—	—	—	—	—	—	—
2–4. iv. Large V	30	2·70	0·434	2	2·54	0·284	11	2·66	0·341	2	2·54	0·349	31	2·49	0·495	21	2·41	0·307	—	—	—
2–4. iv. Small V	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	30	2·23	0·276	—	—	—
18–20. iv. Deep	31	2·86	0·636	—	—	—	—	—	—	—	—	—	—	—	—	30	2·55	0·371	—	—	—
18–20. iv. Surface	30	2·74	0·736	—	—	—	—	—	—	—	—	—	—	—	—	21	2·49	0·387	—	—	—
23–25. iv. Deep	30	2·75	0·643	—	—	—	2	3·00	0·652	—	—	—	29	2·57	0·499	—	—	—	—	—	—
23–25. iv. Surface	30	2·72	0·569	15	2·75	0·454	—	—	—	—	—	—	8	2·56	0·453	—	—	—	—	—	—
10–12. v.	29	2·86	0·669	—	—	—	3	2·75	0·374	—	—	—	32	2·65	0·515	16	2·59	0·309	—	—	—
15–17. v.	10	2·82	0·591	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21–23. v.	31	2·76	0·632	—	—	—	10	2·74	0·337	—	—	—	31	2·66	0·456	22	2·46	0·364	—	—	—
4–6. vi.	28	2·67	0·566	—	—	—	—	—	—	—	—	—	15	2·66	0·377	24	2·51	0·327	—	—	—
6–10. vi.	10	2·73	0·545	—	—	—	—	—	—	—	—	—	—	—	—	12	2·67	0·310	—	—	—
6–10. vi.	—	2·75	0·578	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15–17. vi.	3	2·50	0·304	—	—	—	2	2·39	0·190	—	—	—	3	2·75	0·484	9	2·53	0·220	—	—	—
18–21. vi.	6	2·41	0·280	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9–11. vii.	27	2·35	0·391	—	—	—	2	2·59	0·246	—	—	—	8	2·24	0·380	18	2·27	0·239	—	—	—
16–18. vii.	31	2·31	0·304	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20–22. viii.	32	2·26	0·246	—	—	—	—	—	—	—	—	—	—	—	—	30	2·05	0·152	—	—	—
1–3. x.	30	2·35	0·268	—	—	—	—	—	—	—	—	—	—	—	—	30	2·17	0·182	—	—	—

* *C. finmarchicus* and *C. helgolandicus* not distinguished.

† At 7·0° C.

between 0·35 and 0·51 μl./*Calanus*/h and show little if any seasonal variation. The results for immature females resemble those for the ripe females before, but are decidedly lower during, the spring rise. At that time immature females were scarce and the results are based on only four experiments, each

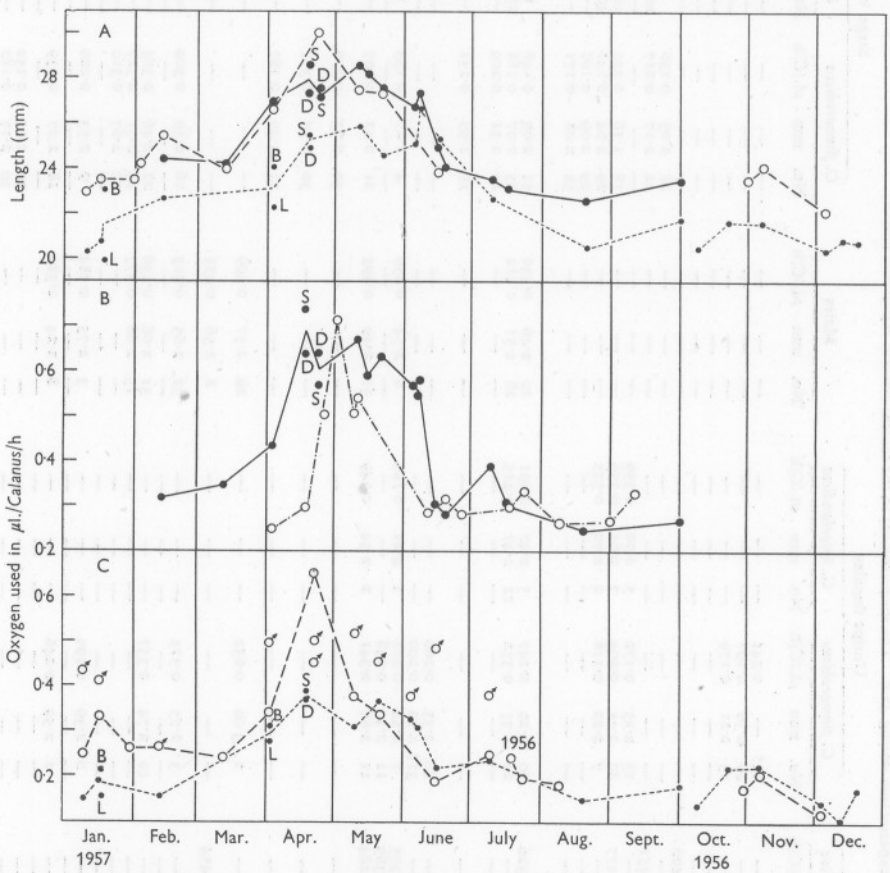

Fig. 1. Seasonal change in size and oxygen consumption. Continuous line, ripe female *Calanus finmarchicus*; dashed line, unripe female *C. finmarchicus*; dotted line, Stage V *C. finmarchicus*. (A) Length of carapace in mm. (B) O_2 consumption of ripe females; continuous line, 1957; dash-dot line, 1956. (C) O_2 consumption of unripe females, males and Stage V.

with only a few females. On an average, immature females use about 60% of the oxygen used by ripe females but unfortunately the highest value (23 April) is based on only two immature females, one of which was exceptionally large.

In Stage V (Fig. 1C) there is a slight increase in spring but, averaged over the high period, consumption is about 56% of that of ripe females, i.e. much the same as the immature.

When the results from all the experiments done with Dr A. G. Nicholls in 1930–32 were re-examined, it was found that as has already been suggested (Riley, Stommel & Bumpus, 1949; Gauld & Raymont, 1953), in them also there was a considerable seasonal variation. When those done at 10° C were arranged according to date, there was seen to be an irregular rise in the oxygen consumed from February to the end of April and several high values in the beginning and middle of May. The lowest values, those in February and March, were rather higher than those in 1956–57 and this might have been because of the method used.

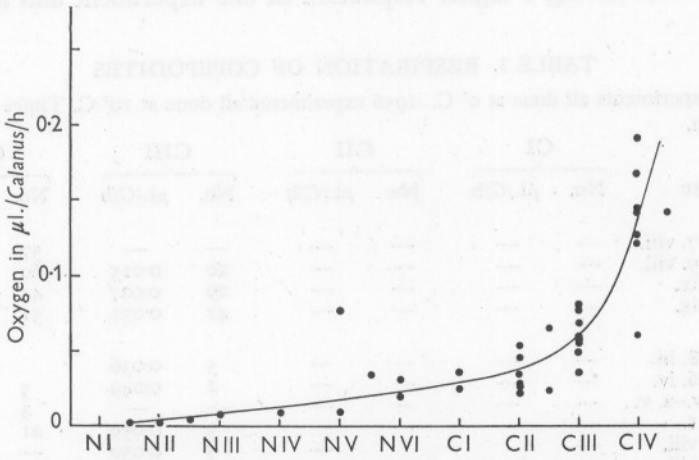

Fig. 2. Oxygen consumption of the early stages of *Calanus*. NI–NVI, nauplius stages; CI–CIV copepodite stages.

In the Clyde Sea Area the *helgolandicus* form is slightly larger than the *finmarchicus* and, as might be expected, the figures for respiration are slightly higher too. In spring 1957, however, when the two forms were almost the same size, the respiration of the ripe female *finmarchicus* was higher than that of the *helgolandicus* form. The *helgolandicus* form usually disappears before the first generation has developed and so there is no way of telling if they have a period of high respiration corresponding to that in *finmarchicus* (see, however, p. 467).

In both 1956 and 1957 *Calanus* were scarce during the summer and few early stages were obtainable. Their oxygen consumption was therefore measured mainly in April, May, and September to November. The experimental error of the determinations is relatively large and no clear seasonal cycle can be made out. The results are shown in Fig. 2 and Tables 3 and 4, and on the whole give a smooth curve ranging from 0·002 µl./*Calanus*/h for Nauplii I–II up to 0·15 µl./*Calanus*/h for Copepodite IV. There is no abrupt rise with the change from nauplius to copepodite.

There are several factors which might cause an increase in respiration during the spring months, and among these are increased light, increased food, increased temperature and increased size.

Light is a factor which has been found to have a marked effect in raising respiration in *Calanus*. It might therefore be expected that the first generation, which is often surface living, should have a higher respiration rate than other generations. On two occasions (see *D* and *S* on Fig. 1B, C) samples of first-generation *Calanus* were taken from deep and surface water at the same time and their oxygen consumption compared. The results were contradictory, the surface catch having a higher respiration in one experiment and a slightly

TABLE 3. RESPIRATION OF COPEPODITES

1955 experiments all done at 0° C. 1956 experiments all done at 10° C. Times vary from 19 to 48 h.

	CI		CII		CIII		CIV	
Date	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h
1955								
16–17. viii.	—	—	—	—	—	—	57	0·038
17–19. viii.	—	—	—	—	20	0·015	60	0·037
3–5. ix.	—	—	—	—	29	0·027	44	0·065
6–8. ix.	—	—	—	—	42	0·032	51	0·058
1956								
26–28. iii.	—	—	—	—	5	0·036		
24–26. iv.	—	—	—	—	2	0·049	3	0·141*
30. iv.–2. v.	—	—	—	—	—	—	3	0·167
7–8. v.	—	—	—	—	3	0·059	21	0·145
3–4. viii.	—		—	—	5	0·050	—	—
6–7. viii.	3	0·011	29	0·029	34	0·070	19	0·142
8–10. viii.	—	—	1	0·027	—	—	—	—
16–18. viii.	1	0·036	1	0·052	—	—	—	—
20–22. viii.	—	—	1	0·023	—	—	—	—
3–5. x.	—	—	—	—	6	0·057	—	—
9–11. x.	—	—	—	—	—	—	7	0·060
22–23. x.	—	—	3	0·054	7	0·081	10	0·127
29–30. x.	—	—	1	0·046	—	—	—	—
5–6. xi.	—	—	—	—	8	0·078	13	0·121

* Moulted to C V.

TABLE 4. RESPIRATION OF *CALANUS* NAUPLII

	Nauplii I–II		Nauplii II and II–III		Nauplius III		Nauplius IV		Nauplius V		Nauplius VI	
Date	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h	No.	µl./C/h
1956												
29–31. iii.	172	0·002	—	—	—	—	—	—	—	—	—	—
5–7. iv.	—	—	618	0·004	—	—	—	—	—	—	—	—
25–27. iv.	—	—	256	0·003	—	—	—	—	—	—	—	—
2–4. v.	548*	0·002	—	—	—	—	—	—	—	—	—	—
6–7. viii.	—	—	—	—	—	—	—	—	1	0·010	—	—
29–30. x.	—	—	—	—	7	0·008	2†	0·009	—	—	3	0·031
5–6. xi.	—	—	—	—	21†	0·008	2†	0·032	1	0·080	1†	0·033

* Some Nauplii II–III. † One or more moults.

lower one in the other. Bainbridge (1952), from observations while diving, has described a continuous interchange between the *Calanus* at the surface and those deeper down so that a clear difference between them should not be expected. This, however, would not exclude the possibility that it was the greater exposure to light of the surface-living generation which caused the increased respiration.

In the English Channel it is not the *Calanus* of the first but those of a later generation in July or August which are found at the surface (Russell, 1928), although the form is *helgolandicus*. The respiration of those from the surface and from deeper water was measured in August 1957 at Plymouth, and was found to be no higher than that of the *finmarchicus* form in the Clyde Sea Area at the same time although it was the same size. We may therefore conclude that the higher light intensity received by those living near the surface is not responsible for the increase in respiration.

The increase in respiration in the spring of 1957 began at the same time as the diatom maximum, but this was over by 22 April, whereas respiration remained high until the beginning of June. It then dropped suddenly and remained low in spite of a second but smaller diatom increase in the first part of June.

Attempts to starve ripe female *Calanus* in the laboratory over a long period were not successful. A few specimens died and eggs were laid providing some food for the rest. A comparison of the respiration of these with that of fed *Calanus* showed little difference. Tests were also made with the filtrate from diatom culture to find out whether it increased the respiration of a variety of small copepods (*Centropages*, *Temora*, *Acartia*); there was no effect.

Two thyroid derivatives which might be expected to have an effect on respiration were tested at the suggestion of Dr J. M. Dodd, St Andrews. Sodium L-thyroxine and triiodothyronine, each in a dilution of 10^{-6}, were used, again with negative results.

The increase of temperature in spring is much too slight to be the sole cause of the rise in respiration (Marshall, Nicholls & Orr, 1935; Clarke & Bonnet, 1939) and in any case it goes on rising when the respiration falls in June and July.

In 1930–33 a series of experiments was made to find the effect of temperature. Each series was done with the same set of *Calanus*, which involved subjecting the same individuals to a series of rising temperatures up to 20° C and repeating the lower temperatures to see whether they had been damaged. Almost invariably the second test at the same temperature (usually 10° C) showed a lower result than the first indicating that the *Calanus* had suffered somewhat. The result chosen for publication was that in which this fall was least marked. All the experiments were done in February or August and, together with one done in August 1957 by the modified method, are shown in Fig. 3. It will be seen that there is a considerable range of variation and

a tendency for the oxygen consumption to be higher at the same temperature in summer than in winter. The February females would be mainly unripe and the August ripe, which would account for the difference.

Another possible cause of the variation in respiration might be the variation in size. On two occasions Stage V *Calanus* were separated roughly by eye into a large and a small group (see *B* and *L* in Fig. 1A, C) and the respiration measured separately. There was some overlap both in lengths and in the oxygen values in the two sets of bottles but the oxygen consumption of the

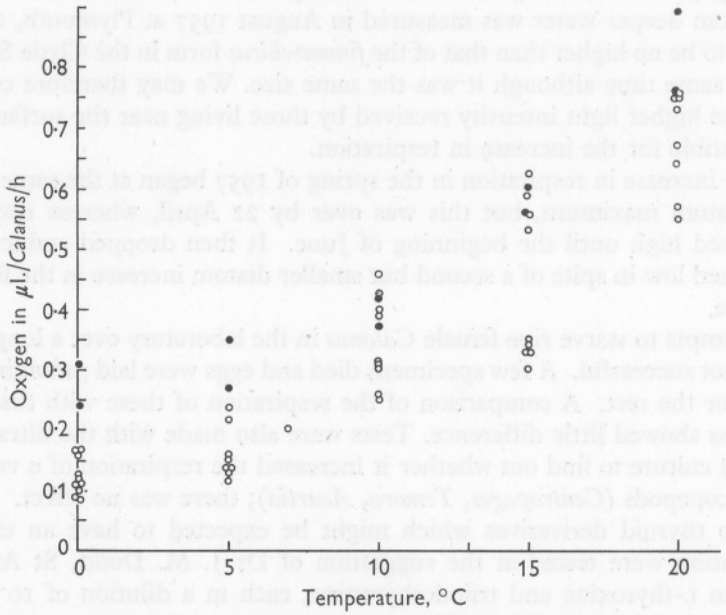

Fig. 3. Effect of temperature on the oxygen consumption of female *Calanus*. Open circles, February experiments; closed circles, August experiments.

large specimens was not so much greater as would be expected if size were the only cause of the respiration differences. In January, for instance, the average lengths were 2·31 and 1·99 mm and the oxygen consumption 0·21 and 0·15 µl./*Calanus*/h.

The largest *Calanus* are those of the first generation of the year, appearing as adults about the beginning of April. The overwintering stock gradually dies off and at the end of April and in May only large first-generation *Calanus* are found. After this the length decreases gradually and irregularly until the winter when it is at its minimum. It is therefore the large females of the first generation which have the exceptionally high oxygen consumption. It is noteworthy, however, that the unripe females of the first generation, although comparable in size with the ripe, have a much lower respiration (Fig. 1A, C).

Gauld & Raymont (1953) found differences in respiration between different generations of *Centropages* which did not depend on length.

If the length of the females is plotted against respiration (as can be done for some of the 1956 and all of the 1957 experiments), almost all the points for ripe *Calanus* lie well above those for unripe. The calculated lines (on a logarithmic scale) are shown in Fig. 4. The points for the ripe females of the first generation appear to be slightly above the upper line, which suggests that it is not length alone which accounts for their high respiration. This difference between the generations is not seen in unripe females.

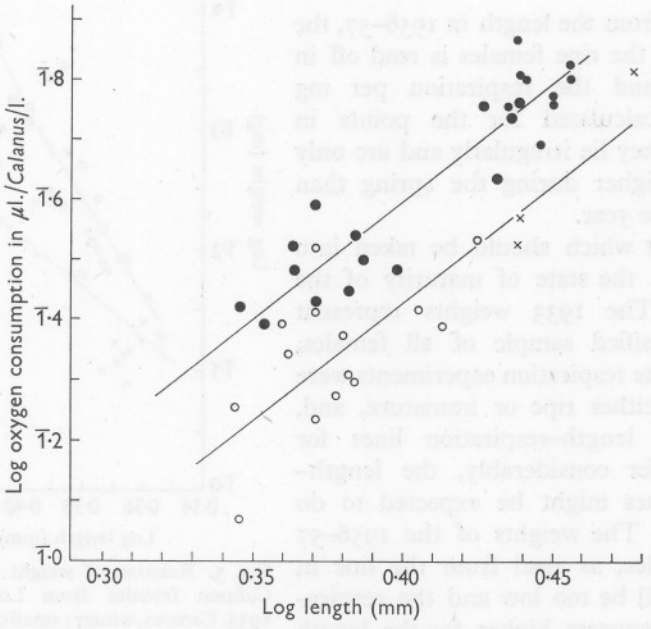

Fig. 4. Relation of length to oxygen consumption in *C. finmarchicus* females, 1956–57. Small closed circles, first-generation ripe females; large closed circles, all other ripe females; crosses, first-generation unripe females; open circles, all other unripe females. Lines calculated by the method of least squares.

The line for Stage V *Calanus* is not shown but lies between those for the ripe and the unripe. Not enough data are available for either males or for the *helgolandicus* form, although the relationship is not widely different.

A more useful measure than size might be the oxygen consumed per mg of *Calanus*. Unfortunately it is not possible to plot the respiration of the experimental animals against their own weight, but a series of weekly measurements of length and dry weight of male, female and Stage V *Calanus* is available from Loch Striven in 1933 (Marshall, Nicholls & Orr, 1934) and these may fairly be used.

When weight is plotted against length for the 1933 *Calanus* females, they fall into several distinct groups (Fig. 5). The points for the winter females are scattered and lie mainly on the lower part of the graph. These *Calanus* are the survivors of the late summer broods and the lower values show their loss of weight during the winter. Those of the first generation are, for a given length, lighter than those of the second and third. Thus although they have the maximum weight for the year, it is only because of their greater length.

When from the length in 1956–57, the weight of the ripe females is read off in Fig. 5, and the respiration per mg *Calanus* calculated for the points in Fig. 1B, they lie irregularly and are only slightly higher during the spring than later in the year.

A factor which should be taken into account is the state of maturity of the females. The 1933 weights represent an unclassified sample of all females, whereas the respiration experiments were done on either ripe or immature, and, since the length–respiration lines for these differ considerably, the length–weight lines might be expected to do the same. The weights of the 1956–57 ripe females, as read from the line in Fig. 5, will be too low and the respiration thus appears higher for the length than one would expect. The converse holds for the unripe females. The exceptionally high oxygen consumption of the ripe females of the first generation may be partly caused by the extra weight of the eggs.

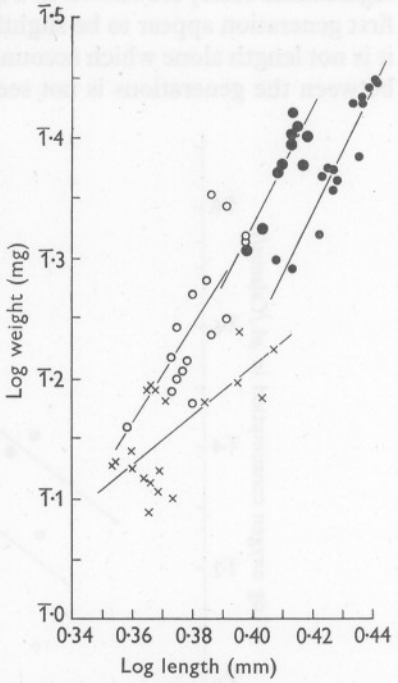

Fig. 5. Relation of weight to length in *Calanus* females from Loch Striven, 1933. Crosses, winter; small closed circles, first generation; large closed circles, second generation; open circles, third and later generations. Lines calculated by the method of least squares.

If the relationship of length to dry weight for the whole populations of males, females and Stage V *Calanus* in 1933 is calculated, the lines for males and females are not significantly different. Stage V is, however, much heavier per unit length and its line is significantly different from the others. In spite of this the respiration of the Stage V is lower.

The figures for Stage V can be analysed into broods as for the females in Fig. 5. They then fall into the same groups; a small and light overwintering generation, a large but relatively light first generation and a succession of

smaller but relatively heavier broods in summer. The overwintering Stage V show a fall in weight from autumn to January. If the respiration per mg dry weight is calculated from the 1933 figures, the seasonal changes are, as for females, very irregular but show a tendency to be lower for the first than for the overwintering generation. This is probably because of the much higher fat content of the former.

We should like to thank the Trustees of the Browne Fund for a grant which enabled us to work on *Calanus* in the English Channel. We are also grateful to the Director and Staff of the Plymouth Laboratory for their hospitality. Mr T. B. Bagenal gave helpful advice on statistical problems and Dr J. M. Dodd supplied us with thyroxine compounds; to them and to the Master and crew of the research vessels '*Calanus*' and '*Mizpah*' who kept us supplied with tow-nettings we should like to express our thanks.

SUMMARY

The seasonal changes in the respiration of *Calanus* are considerable and are, on the whole, related to size and therefore to weight. Length alone is not enough to account for the differences since ripe females, although the same length as unripe, have a markedly higher respiration. In addition, by taking samples of large and of small *Calanus* of a single stage, it was shown that the difference in respiration was small. Neither is weight by itself enough to account for the difference between groups. Stage V *Calanus* are, for a given length, heavier even than ripe females and yet their oxygen utilization is low. In this instance, however, an important part of the weight consists of fat which is a food reserve and not actively metabolizing. The difference of weight between ripe and unripe females is not known, but ripe females must be heavier and this will account for their higher oxygen consumption.

Although the oxygen and therefore the food required during the spring months is high, at that time the phytoplankton is at its maximum and is probably sufficient to fulfil all needs. Egg-laying depends on the food supply and it is then that *Calanus* starts breeding. In winter, on the other hand, the *Calanus* is present as Stage V and oxygen consumption is little more than half what earlier figures suggested. No 'hibernation' seems to take place but the population is living in an economical way for Stage V use little oxygen, live in deep water and do not undertake diurnal vertical migration.

At 10° C ripe female *Calanus* will require daily from 3·9–7·2% of their body weight as dry matter in summer and from 2·8–6·7% in winter. Stage V will require 2·3–3·1% in summer and 1·4–3·3% in winter. The higher values are for carbohydrate and the lower for fat.

It is difficult to believe that *Calanus* in winter will be able to find enough food by filtration alone. The fact that in the winter months it depends more on predation may account for its survival.

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UNDERWATER OBSERVATIONS ON THE FAUNA OF SHALLOW ROCKY AREAS IN THE NEIGHBOURHOOD OF PLYMOUTH

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The Plymouth Laboratory

(Text-fig. 1)

Since 1954 I have made many underwater explorations in the neighbourhood of Plymouth using aqualung equipment. Diving has been generally restricted to rocky areas within the 15-fathom line. In these areas, because of the difficulty of dredging, there has been little information available concerning the general pattern of the fauna; as Drach (1952) has pointed out, direct study with diving apparatus can not only give data on the depths at which various species flourish, but also the opportunity to collect the more secluded animals. It has not been possible to obtain such detailed results as Kitching, Macan & Gilson (1934), or Knight-Jones & W. C. Jones (1955), but these notes may provide an outline of the fauna as observed underwater in relation to the bottom topography.

To try to obtain as simple a picture of the fauna as possible, attention has been concentrated on places where the sessile animals are not intermixed with algae. In the shallowest water, therefore, the localities described are those with rock facies sufficiently shaded to exclude all but occasional algal plants.

Nearly all the best diving grounds are fully exposed to south-westerly winds; as these winds are generally prevalent it is frequently only possible to obtain sufficiently calm sea conditions once to twice a week even during the summer months. It is therefore very difficult to adhere to any fixed programme, and unless one is fortunate as I have been in having my own boat available at any time to take advantage of calm spells, opportunities for diving will be very restricted.

1. THE WRECK, WHITSAND BAY

The sunken hull of a 'Liberty' ship lies on the sand in 9 fm. (16 m) about $\frac{3}{4}$ mile offshore, towards the eastern end of Whitsand Bay. The one remaining mast, which is always uncovered, forms a convenient mark and mooring post.

On the remains of the top deck and superstructure, which are about 15 ft. (4·5 m) deep, there is some algal vegetation, including *Alaria* and rather scattered *Laminaria hyperborea* and *Saccorhiza* plants. Large numbers of small mussels (*Mytilus* sp.) occur around the top deck and, associated with them, numerous small *Asterias rubens*. Curiously enough, only small starfish

were seen both in 1955 and 1957, although the supply of mussels appeared amply sufficient to produce rapid growth. Possibly the *Asterias* population is considerably reduced by severe winter gales.

The two sides of the hull are both well colonized. On the west side in 1955 there were numerous *Ophiothrix fragilis*, various encrusting ascidians, especially *Botrylloides leachi*, sponges—particularly *Suberites domuncula* (= *Ficulina ficus* (L.))—and an abundance of *Metridium dianthus*. The *Metridium* flourish especially on the underside of any horizontal surfaces, such as occur at the openings on to the 'tweendecks. This is the only inshore locality outside the Sound where many *Metridium* have been found, showing that it is lack of a suitable habitat rather than inability to withstand wave action which normally restricts them to pier piles and dock walls.

2. QUEENER POINT

Queener Point is situated $\frac{1}{2}$ mile north of Rame Head at the east end of Whitsand Bay. From the point an area of submerged rocks runs out for more than $\frac{1}{4}$ mile. Except for Peader Rock, which dries at L.W.O.S.T.L., the depth varies from 4 to 7 fm. (7–13 m). Although the rock is the usual Dartmouth slate the strata run in the vertical plane and consequently the rock has been eroded in an unusual pattern—this area being notable for the large number of shallow gullies running roughly E.–W. in direction. These gullies are very roughly 6–8 ft. (2–2$\frac{1}{2}$ m) deep and 8–14 ft. (2·4–4·2 m) wide; their side walls provide a much more extensive area of vertical rock surface than is generally present on this part of the coast. In the narrower gullies the *Laminaria* vegetation is greatly reduced or absent, permitting the development of a moderately rich sessile fauna. It is not possible to do more than point out some of the commonest or most conspicuous species. Groups of the small orange ascidian *Stolonica socialis* are very common on the upper parts of the gully walls and in other situations where the *Laminaria* is not dense. Many of the small white anemone *Actinothoë sphyrodeta* also occur frequently in similar places. On the vertical rock walls there is a variety of sponges, ascidians and encrusting Bryozoa, together with scattered red algae especially *Delesseria sanguinea* which becomes covered, by the autumn, with a great abundance of the tiny white foraminiferan *Haliphysema tumanowiczi*. The case of this species is composed of sponge spicules. The encrusting sponges *Amphilectus fucorum* and *Hemimycale columella* are common, while tufts of the rare reddish horny sponge *Ulosa tupha* have been taken on one or two occasions. Colonies of *Alcyonium digitatum* are fairly common but not evenly distributed throughout the area, although no special habitat preferences were apparent. On the more sheltered or overhanging rock walls there are many cup corals, *Caryophyllia smithi*, some with the barnacle *Pyrgoma anglicum* growing on them. Also preferring sheltered conditions, colonies of *Bugula turbinatum* and other erect Bryozoa are fairly common. The finely branched

black tentacles of *Cucumaria normani* are frequently seen protruding from holes in the gully walls; similarly, one may find the large tubicolous polychaete worm *Bispira volutacornis* made conspicuous by its double whorl of branchial filaments. Some specimens of the beautiful red serpulid *Protula tubularia* have been seen, but since the opening of its calcareous tube is invariably set well back in a rock crevice this species is extremely difficult to collect.

In early summer the ascidian *Archidistoma aggregatum* is quite common but disappears by the end of July.

3. THE DRAYSTONE LEDGE

One dive was made about 20 yd. west of the Draystone Ledge Buoy off Penlee Point, depth about 6 fm. (11 m). The sea bed consists of low-lying reefs of slate not more than 2 or 3 ft. high and interspersed with patches of coarse gravel. Many *Holothuria forskali* were present, together with *Stolonica socialis* and red algae probably largely *Heterosiphonia* sp.

4. INNER PENLEE POINT

(Admiralty Chart no. 30, as Inner Broady Cove)

This is the only satisfactory position outside the breakwater where diving is possible with south-westerly winds. The rocks run out to depths of 4 or 5 fm. (7 or 9 m), sometimes as reefs with sand between or as a gently shelving slope fringed by the sand. Generally the rocks are thickly covered with *Laminaria* even though the water is usually muddy and turbid since the main tidal flow in and out of the Sound runs close by. In the sand adjoining the rocks there are sometimes numerous *Arenicola* castings but they do not appear to extend beyond depths of 4–5 fm. No sublittoral records of *Arenicola* appear in the 1957 Fauna List. It is, however, unlikely that the species generally penetrates more than a short distance below the lowest tidal level in the neighbourhood.

After the first few dives, this area was thought to have only a poor sessile fauna, limited to scattered pot-holes and overhanging ledges. The sponges *Tethya aurantium*, *Stelligera stuposa* and *Raspailia hispida* were found in these places together with a variety of sessile animals though without any species being noticeably common.

In 1957, however, an interesting gully with an extensive overhanging rock wall was found off the south side of Inner Broady Cove. The approximate dimensions and depths in this gully are shown in the sketch chart (Fig. 1). In a side branch from this gully large numbers of *Phoronis hippocrepia* live in a belt of dead *Pomatoceros* tubes about 1–2 ft. above the sand. The end of the side branch terminates in an overhanging wall about 4 ft. high; this provides a sheltered rock surface colonized by many *Caryophyllia smithi* together with a few *Balanophyllia regia*, Gosse's golden cup coral. In the main gully the

south wall, which varies in height from 6 to 10 ft., and is undercut in many places, has the better developed sessile fauna. Where the overhang is considerable there is a rich growth of the brightly coloured 'anemone' *Corynactis viridis*. *Stolonica socialis* is again common on the upper parts of the wall near

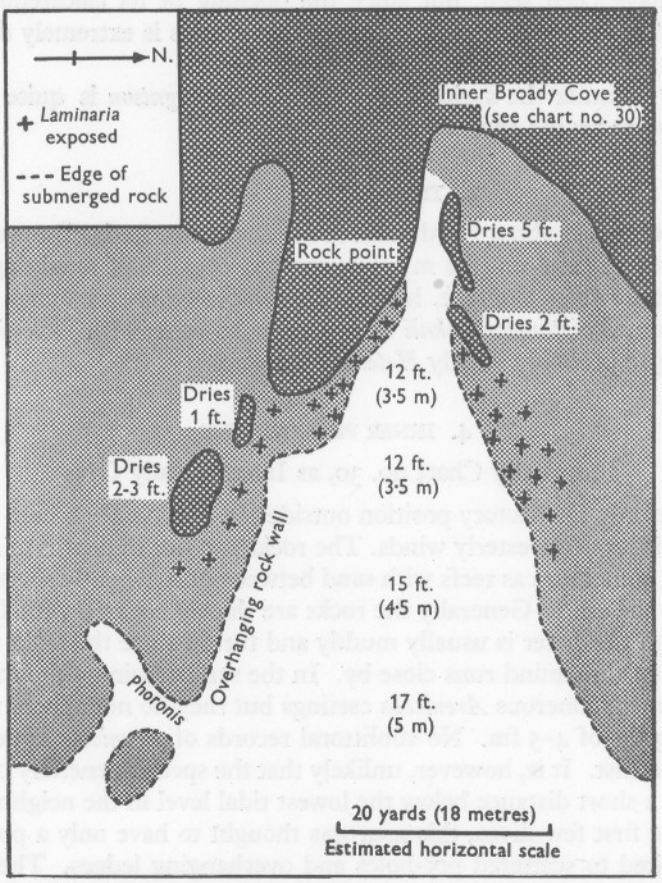

Fig. 1. Sketch plan of gully about ¼ mile north of Penlee Point. Rocks exposed at low tide shown by dark tint, submerged rock by light tint: sandy bottom untinted.

the *Laminaria* fringe. Other parts of the vertical rock surface bear a variety of encrusting animals, the bulk of which are sponges and ascidians; these have not yet been sampled as it is hoped that an intensive survey will be made sometime in the future.

5. THE TINKER SHOAL

This is a large shoal area south of the main breakwater. The bottom is slate rock often in the form of low reefs with occasional stony or gravel patches. No steep-walled gullies have yet been found but there are some ledges with

vertical rock walls of up to a height of six feet. A fine deposit of mud is generally present in the sheltered crevices of these ledges. The fauna is rather sparse, *Stolonica socialis* again being common together with the sponge *Myxilla rosacea*.

6. THE SLIMERS

A small isolated half tide reef at the west end of Wembury Bay, the Inner Slimers, lies about $\frac{1}{4}$ mile east of the Mewstone. On the north or landward side of the reef there is a projecting rise of rock just below L.W.O.S.T.L. which protects and shades the rock surface below it. The erect forms of Bryozoa (*Crisia* spp., and *Scrupocellaria reptans*) are particularly common in this sheltered niche. There are also various sponges forming a thin loose mat held between the stalks of the Bryozoan colonies; *Suberites epiphytum*, *Myxilla rosacea* and *Dysidea fragilis* are all common together with the firmer white crust of *Leuconia nivea*. From a rock sample of 501 cm^2, 131 small amphipods (chiefly *Parajassa*) were counted.

7. KITCHING'S GULLY, WEMBURY BAY

This gully lies 60 yd. east of the Tomb Rock shown on Admiralty Chart no. 95. The sublittoral fauna was surveyed in 1931 and 1932 by Kitching *et al.* (1934). It has been interesting to look for changes in the fauna which have taken place in the 25 years since this survey was made. The sponge *Halichondria panicea* is no longer common and there is no sign of a *Distomus–Halichondria* association. The only *Halichondria* found during 1956 and 1957 were a few patches at the shallow end of the gully remote from the area of the survey. The *Distomus variolosus* is still abundant, forming bright reddish patches through which the large siphons of *Polycarpa pomaria* may frequently be seen projecting. The large majority of the *Distomus* grow on the tough leathery tests of the *Polycarpa* which curiously enough was not taken by Kitching. *Corynactis* is rather more common now than would appear from their survey, particularly on the shaded area such as Kitching's position M p. 683, previously dominated by sponges. The south patch of *Stolonica socialis* still exists at the seaward end of the gully.

On the west side of the small rocky point, which forms the west side of Kitching's gully, there is a wide undercut ledge. On the underside of this ledge numerous small orange *Sagartia* sp. (probably *Sagartia elegans*) have been abundant during the last two summers. *Distomus* is still common but not so widespread as in the gully.

On the east side of the gully a small area of submerged rocks provides rather similar conditions; there are still small patches of *Distomus*, but in all shaded areas *Corynactis* is more abundant. Two hydroids, *Plumularia setacea* and *Sertularia operculata* are also very common in places.

8. THE STOKE POINT AREA

The fauna of a rock gully near Stoke Point Rock has already been described (Forster, 1954). On the latest copies of Admiralty Chart no. 1267 Stoke Point Rock has been renamed Hilsea Point Rock following the Ordnance Survey, but as Stoke Point is the name in general use it seems better to retain it for the present.

On the part of the shore lying about north-east from Stoke Point Rock several long narrow reefs run out in a south-westerly direction. Most of the gullies between these reefs have a purely algal covering, however one rather narrower gully was found with side walls at least 15 ft. high and an abundant sessile fauna. The position of this gully is 037° T from Stoke Point Rock, distance $3\frac{1}{2}$ cables. The depth at the bottom is about 4 fm. (7 m). The west side wall has a slight overhang; on it occur several species usually only found in much deeper water; there was one colony of the gorgonian *Eunicella verrucosa* and several of the ascidians *Phallusia mammillata* and *Diazona violacea*. There are numerous *Caryophyllia*, the feathery bryozoan *Crisia* spp. in abundance, together with the following sponges: *Dercitus bucklandi* in crevices, *Hemimycale columella*, *Pachymatisma johnstonia*, *Mycale macilenta*, *Leucosolenia coriacea* and one small patch of orange incrustation identified as *Antho involvens*.

About 600 yd. farther east along the coast, the three large Netton Rocks and submerged reef form a slight breakwater which makes it possible to anchor a boat close inshore. There are a number of gullies in this area (named 'Bloody Cove' on the $2\frac{1}{2}$ in. Ordnance Survey maps) roughly similar in proportion to those of Queener Point and Inner Broady Cove but with scarcely any sessile fauna. Instead the vertical rock walls are covered with algae in spite of the shading caused by overhanging *Laminaria* plants. The algae are often species more generally found in deeper water, e.g. *Dictyopteris membranacea* and *Halopteris filicina*. This increase in algal coverage is doubtless due to the lack of suspended material in the water, which is always much clearer than it is nearer the Sound. Secchi disk readings were twice taken at Inner Broady Cove and then as quickly as possible off Netton Rocks, the results were:

Inner Broady Cove	6–$6\frac{1}{4}$ m
Netton Rocks	11–12 m

A few narrow gullies not more than 3 ft wide did contain some sessile animals, encrusting Bryozoa and ascidians, but the algal coverage was still estimated to be over 50%.

9. SALCOMBE

Several deep clefts have been found in the submerged reef which runs for a short distance eastwards from the Great Mewstone off Bolt Head. These clefts have vertical walls over 20 ft. in depth with a rich sessile fauna. Although only one short dive has been made in this position, the ascidian *Distomus* was found to be clearly abundant. This is of interest as being the only position other than Kitching's gully in Wembury Bay where this ascidian is really abundant. *Corynactis* and *Crisia* spp. are also fairly common.

10. THE EDDYSTONE ROCKS

The Admiralty Chart no. 1267 shows a large-scale plan of the Eddystone rocks, which lie about 9 miles offshore. Several rocks are uncovered at low tide, the Southern Reef in particular forming a small natural breakwater on which landing is occasionally possible in extremely calm conditions. The area of reef within the 10 fm. line is a little over $\frac{1}{10}$ sq.mile. The water is generally much clearer than at any of the inshore positions except Bloody Cove. The rock is a very hard gneiss.

Several dives have been made on a line running about south-west from the mid-point of the south reef. From L.W.O.S.T.L. there is a narrow fringe of the brown alga *Alaria*, which is quickly replaced by large *Laminaria hyperborea* plants. Many brown tubes of the amphipod *Parajassa pelagica* are common in the space between the *Laminaria* holdfasts to a depth of about 7 fm. (13 m), penetrating much farther than under inshore conditions; their limit at Wembury being only 1–2 fm. (2–4 m). The lower limit of the *Laminaria* has not yet been ascertained, below 12 fm. (22 m), however, it becomes sparse. The reef slopes fairly steeply down to 10–12 fm. (18–22 m), although most of the surface is algal covered there are some ridges, clefts, and occasional very large boulders, with vertical or overhanging sides. Wherever the rock is sufficiently shaded *Corynactis* is particularly common, the different colour varieties forming red, green or brown patches. The light green variety appeared bright yellow at 4 fm. (7 m). In well-sheltered crevices from 7–8 fm. (13–15 m) downwards, besides *Corynactis*, towards the outside there is generally a rich growth of *Crisia* spp. giving the effect of a white moss. At slightly greater depths—around 11 fm. (20 m) the *Crisia* becomes more extensive. From 13 fm. (24 m) downwards the slope becomes gradual and these rocks are interspersed with patches of coarse shell gravel.

At all depths it is difficult to collect small stones or boulders as they are invariably very tightly packed. The few stones which have been examined have many *Caryophyllia smithi*, together with thin yellow encrusting sponges; notably *Spanioplon armaturum*, *Myxilla rosacea* and *Hymedesmia* spp.

On the east side of the Southern Reef it is possible to anchor within a few yards of the rock if there is a westerly wind and easterly going tide. There are

several rocks which appear dangerous in the clear water, but actually have 2–3 fm. (4–6 m) of water over them at L.W.O.S.T. These rocks form one side of a gully with a maximum depth of 9 fm. (16 m). Even the nearly vertical sides of the gully bear some *Laminaria* plants, but in a few overhanging places there are again fine *Corynactis* growths, and also numerous *Actinothoë sphyrodeta*; this species reaches a much greater individual size (disk diameter of nearly 1 in.) than it does inshore.

On the floor of the gully *Laminaria* plants are rather infrequent, being largely replaced by *Dictyota dichotoma* and *Heterosiphonia*. Several fine pinkish colonies of the ascidian *Clavelina* sp. have been observed, together with purple clumps of the sponge *Adocia cinerea* and the ubiquitous *Hemimycale columella*.

150 *yards north of lighthouse*

The depth at this position is a little over 10 fm. (18 m), and the bottom consists of large boulders and stones overgrown by tall *Laminaria* plants with here and there even larger plants of *Saccorhiza bulbosa*. The *Laminaria* stipes are well colonized by various red algae and various animals, chiefly hydroids and Bryozoa; *Sertularia operculata* is particularly common. *Echinus esculentus* is also abundant and has an estimated density of one sea urchin to about 5–10 m^2. In this area the *Echinus* are frequently observed climbing up the *Laminaria* stipes.

60 *yards north-west from old lighthouse stump*

The reef slopes rather steeply down from 5 to 12 fm. (9–22 m). There are again fine growths of *Corynactis* on several overhanging rock faces. Two specimens of *Lima hians*, a tunnel-building lamellibranch, were taken from underneath small boulders in this area.

11. THE HAND DEEPS

A misnamed submerged reef, the Hand Deeps, with a minimum depth of 4 fm. (7 m) lies $3\frac{1}{2}$ miles north-west from the Eddystone. The reef is composed of a micaceous schist evidently not so resistant to the waves as the Eddystone rock. In one position on the south side of the reef at 13 fm. (24 m) the rock slopes gently downwards and the bottom is largely strewn with boulders, the largest being 4 or 5 ft. in height. *Laminaria* was still present at this depth. Two dives have been made on the north side of the reef.

In the first dive a steeply sloping rock bottom was found with a gradient of about 1 in 2 from 9 fm. (16 m) downwards. There were no *Laminaria* plants after 12 fm. (22 m) and no other algae except *Lithothamnion* after 14 fm. (27 m). From 12 to 17 fm. (22–31 m) *Corynactis* was again abundant on vertical surfaces, while *Caryophyllia* and *Alcyonium digitatum* were common. There were also scattered hydroid colonies *Nemertesia* sp. in evidence. The second dive was made in a position probably near the north-east corner of

the reef. At 12½ fm. (23 m) there were scattered *Laminaria* plants growing on a gentle rock slope, these were replaced completely at 15 fm. (27 m) by *Dictyopteris* and various red algae. Numerous *Echinus esculentus* were also present.

12. THE EAST RUTTS AREA

One dive has been made on a rock pinnacle, depth 7 fm. (13 m), lying about 5 miles offshore in Bigbury Bay. This pinnacle is situated 1 mile to the east of the East Rutts. The fauna and flora at 8–9 fm. (14–16 m) depth are generally similar to those of Stoke Point Rock. There are large yellow clumps of the sponge *Cliona celata*, colonies of *Alcyonium digitatum* and numerous *Echinus* and *Holothuria*. In a small pit two fine colonies of the ascidian *Diazona violacea* were observed. This area is noteworthy for the presence of many clumps of the sponge *Halichondria panicea* in the unusual form shown in Bowerbank, Vol. 3, plate xl, figs. 2 and 5. Elsewhere in the Plymouth area *Halichondria* has not been found below about 2 fm. (4 m). Whether its abundance here can be related to the different rock substratum is not certain, but there can be little difference in the physical conditions compared with Stoke Point other than the nature of the rock.

DISCUSSION

In general the sessile fauna of submerged rocks off Plymouth tends to be rich in sponges, polyzoans and ascidians: compared with the low-tide area it is rather poor in actinians, and there are few gastropods apart from nudibranchs. The only common cirripede, *Verruca stroemia*, is mostly restricted to the underside of stones. Apart from *Corynactis* on the outlying reefs, no single species has been observed which could be regarded as dominant over an appreciable area. This situation contrasts with Bardsey Island, where Knight-Jones & W. C. Jones (1955) found various species of Polyzoa to be dominant, and differs also from Barn Pool in Plymouth Sound where the ascidian *Dendrodoa grossularia* covers all the stones at depths of 2–4 fm. (4–7 m) and forms a belt just below the *Laminaria*.

On the outlying reefs it would be reasonable to regard *Corynactis* as a dominant species on overhanging or shaded rock surfaces; but closer inshore, though still very common, it is generally mixed with other species, and for no obvious reason is virtually absent from the Queener Point area.

The general impression of the sessile fauna, particularly below 5 fm. (9 m), as it might be observed on a gully wall 3 or 4 m high is of a random dispersal of most species, with encrusting forms rarely managing to cover an area of $\frac{1}{20}$ m^2. A possible explanation of this effect is the browsing habit of the *Echinus esculentus*. A few *Echinus* will keep walls of an aquarium tank, 2–3 m^2 in area, almost completely free from sessile animals; since in most localities they are common below 8 fm. (15 m) the *Echinus* can be assumed to be

continually sweeping clear small areas of rock throughout the course of the summer and to some extent throughout the year. The places available for larval settlement at any one time would therefore be widely scattered. Predation of the youngest stages may be expected from decapod crustacea and asteroids; which would again tend to prevent the growth of any large group of a single species.

As Lilly, Sloane, Bassindale, Ebling & Kitching (1953) observe, many observations and experiments on small areas will be necessary before the effects of predation can be assessed.

Part of the diving gear was made available by a grant from the Royal Society. I am grateful to many people who have acted as diver's attendant and always managed to recover me safely.

SUMMARY

A brief description is given of the commonest sessile animals observed by diving from twelve positions near Plymouth, including three offshore reefs. The coelenterate *Corynactis viridis* is generally abundant on shaded rock surfaces. Many sessile species, even where common, tend to be dispersed in scattered patches or colonies; from this it is suggested that their distribution is affected by predation from browsing animals, particularly *Echinus esculentus*.

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APPENDIX

The numbers of dives made at the localities which have been described are tabulated. The period is from 1954 to 1957. The numbers include dives made specifically for collecting purposes when faunistic observation is very limited. The time spent underwater has not always been recorded, but shallow dives generally last at least 30 min, and for deeper areas the average would be about 20 min.

Area	...	1	2	3	4	5	6	7	8	9	10	11	12
No. of dives	...	3	18	1	11	3	3	6	6	1	9	3	1

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THE SPREAD OF *ELMINIUS MODESTUS* DARWIN IN NORTH-WEST EUROPE

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(Text-figs. 1–9)

The presence in northern waters of the Australasian barnacle *Elminius modestus* was first noted by Bishop (1947). In the course of examining some bakelite plates, which had been exposed to fouling in Chichester Harbour, he found a settlement of barnacles which differed from all indigenous species in having only four instead of six compartments. They agreed in every respect with Darwin's description of *E. modestus*. The settlement of many spat on this one plate indicated that the species was well established at least in Chichester Harbour. Crisp & Chipperfield (1948) stated that the species was present in 1947 along the whole of the south-east of England, that it had been present in the River Crouch since 1945, and had appeared also in South Wales. They also commented that the habitats favoured by the species in Britain were similar to those which it occupied in New Zealand (Moore, 1944). Knight-Jones (1948) reported that the prevalence of *Elminius* in the River Crouch constituted a further threat to the Essex oyster beds; he also recorded it from the Helford River in Cornwall. Fig. 1 shows the known distribution at the end of 1947, and at the time of writing (1956).

It is clear that the species must have occurred in Britain for several seasons prior to 1945 when it was present already at two widely separated places, viz. Chichester Harbour and the River Crouch. Stubbings (1950) has confirmed its prior occurrence in the Portsmouth area, based on examination of material collected before 1945, but it is not possible to give any precise information as to the year of its arrival there.

The earliest stages of colonization have therefore escaped notice, perhaps inevitably in view of the closing of the beaches of southern England during the war. It seems not improbable that the establishment of the species had some connexion with unusual conditions at the outbreak of war, in so far as they affected shipping from Australasia. It is, moreover, fairly certain that *E. modestus* was not prevalent at this time in many areas where it was very abundant in 1946.

Table 1 brings together several pieces of evidence to this effect, all based on the examination of material collected prior to 1940. This material consisted chiefly of shells and other substrata collected both from shallow dredge hauls

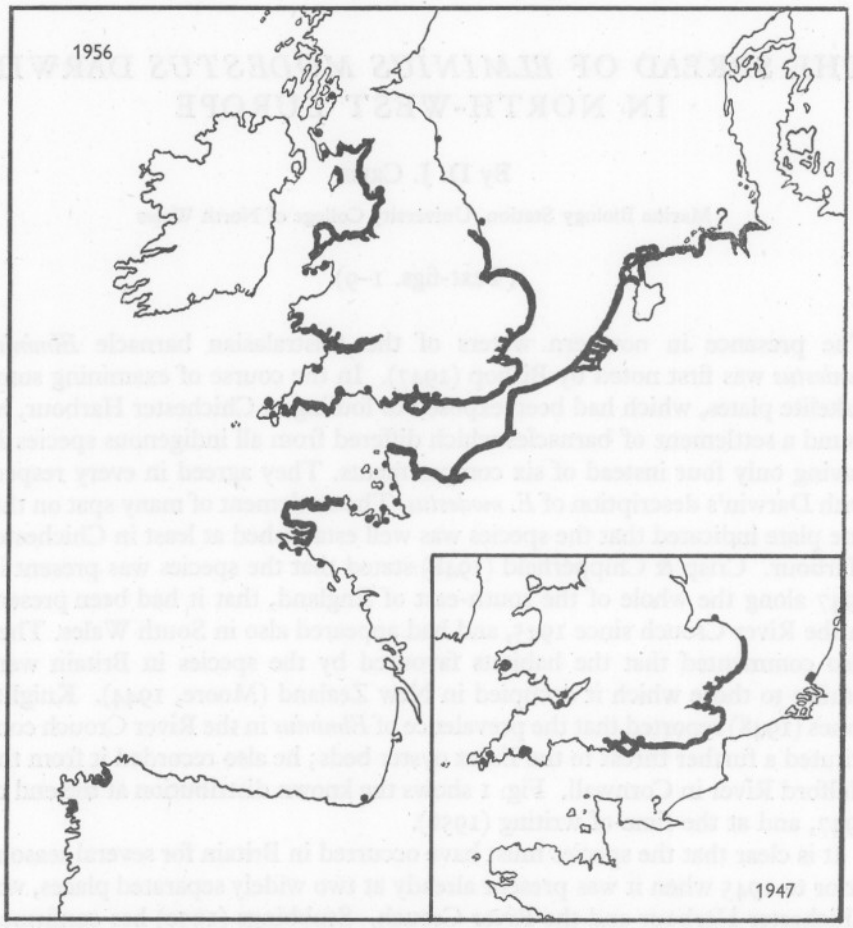

Fig. 1. Parts of the coast of Europe on which *Elminius modestus* was known to have been present in 1956, shown by a thickening of the coastline. Inset, same for 1947.

TABLE 1. OBSERVATIONS ON BARNACLE SETTLEMENT PRIOR TO 1940

Source of material	No. of barnacles found
River Alde, 1931. Shells and stones from low-water mark	*Balanus balanoides* 111 *B. crenatus* and *improvisus* 86 *B. porcatus* 1
Whitstable, 1937. Dredged shells	*B. improvisus* 1500
Tollesbury, 1939. Scrapings from piles	*B. balanoides* 33 *B. improvisus* 4
Steeple stone, River Blackwater, 1938. Scrapings from stones near low-water mark	*B. balanoides* 252 *B. improvisus* 2
Southend, 1938. Plankton hauls	*B. balanoides*, *B. crenatus* and *B. improvisus* larvae only

and from the shore, but also included a series of plankton samples taken from the Thames estuary at Southend throughout 1938 (Wells, 1938). In the plankton samples taken in the early part of the year, numerous larvae of *Balanus balanoides* and *B. crenatus* were identified, while in those taken during summer *B. improvisus* larvae were abundant (Jones & Crisp, 1954), but no *Elminius modestus* larvae were found in any of them.

Since 1946 a careful watch has been kept on the distribution of *E. modestus* in Britain. Quantitative records of its population density have been made repeatedly at numerous easily accessible stations along the coast of Great Britain, while visits have been made to those parts of the coast from which it has hitherto been absent as frequently as time and opportunity have permitted, in order to check its spread into new areas.

Changes are still going on. However, it seems unlikely that further changes will materially alter the general principles which follow from observations made up to the end of 1955. Nor is it likely that any new records will now be discovered to throw light on the early history of its arrival in British waters. It is therefore opportune to describe such changes as have been observed up to the present time.

The work reported in this paper was partly carried out during the period of my employment by I.C.I. Ltd. Paints Division, to whom I am indebted for access to my notes and records. I am also indebted to many colleagues who have from time to time given me information about the species, and whose records appear in the detailed appendix at the end of this paper. The work also constituted a part of a general investigation of British shores, for which generous grants were received from the Browne Fund of the Royal Society.

CHANGES IN DISTRIBUTION SINCE 1946

SOUTH-EAST ENGLAND

Records of the distribution of *Elminius* between the Isle of Wight and the Thames indicate no major change since 1946, as the species was already well established in the area at that time. Nevertheless, there is evidence that between 1946 and 1950 it was increasing steadily in abundance. This is demonstrated in Table 2A which gives the annual spatfall of *Elminius* during this period based on counts of numbers settling on regularly changed surfaces. The figure for 1946 is not entirely comparable with the others, since it was taken in the Blackwater, at West Mersea, a few miles to the north of the River Crouch where the rest of the observations were made. The Blackwater is probably not a more suitable river for *Elminius* than the Crouch, yet the records for 1946 in the Blackwater indicated a heavier spatfall than those for 1947 in the Crouch. This suggests that the severe winter of 1946–47 had an adverse effect. The lack of correspondence between the settlement on continuously

submerged panels on a raft and on plates exposed between the tide-marks, especially between 1949 and 1950, is difficult to account for. The intertidal exposures probably give the best comparisons year by year, as they were made at the same place and in the vicinity of previously settled individuals, which are known to encourage settlement (Knight-Jones & Stevenson, 1950; Knight-Jones & Crisp, 1953; Knight-Jones, 1953). The settlement on plates exposed on the raft may have varied with its location in the tideway (it was shifted slightly from time to time) and with the amount of settlement already on it. Indeed, the spatfall in different parts of the river may show considerable

TABLE 2A. TOTAL ANNUAL SETTLEMENTS OF *ELMINIUS MODESTUS* IN ESSEX AREA AS TOTAL SPAT RECORDED PER SQUARE CENTIMETRE

Year	Settlement at low water	Settlement on raft	Location
1946	97	—	West Mersea
1947	28	1·9	Burnham-on-Crouch
1948	165	3·9	,,
1949	531	59·4	,,
1950	284	421	,,

TABLE 2B. TOTAL ANNUAL SETTLEMENTS OF *ELMINIUS MODESTUS* BASED ON RECORDS GIVEN BY KNIGHT-JONES (1952)

Allowance has been made for the fact that his records do not cover the whole of the settling season. The figures below are therefore estimates only but probably correct in orders of magnitude. Units, number per square centimetre per season.

Locality	1947	1948	1949
Fambridge	0·2	42	160
Purleigh	—	55	53
Althone creek	—	4·7	—
Creeksea	—	27	410
Bush shore	72	270	470
Broadrakes	—	11	167
Shop laying	0·8	12	260
Pagglesham pool	—	3·1	—
Roach mud	—	0·75	9·8
Mean	24	47	219

variation, as demonstrated by Knight-Jones (1952). His observations from 1947–49 are given in Table 2B. They have been modified from the form given in his paper to make them directly comparable with those of Table 2A. Creeksea and Bush shore, about a mile, respectively, to the east and west of Burnham received the heaviest settlement indicating that this area, which is close to that on which Table 2A is based, represents the most heavily infested part of this river. It is interesting also to note from Knight-Jones's data that the proportionate increases in number settling, between 1947 and 1949, were greater in the upper parts of the river (e.g. Fambridge and Shop laying), where settlements were small, than in the more heavily infested part of the river nearer to its mouth. This suggests that the original colonies were

probably located near the mouth, and that the species spread upstream, towards Fambridge and Battlesbridge, as the substrata available near the mouth became fully covered. Intertidal observations indicated that there was an increase in density of adults corresponding to the increase in numbers setting during the same period. For example in 1947 the barnacle population on wooden piles was composed of 10–15% *Elminius* and 85–90% *Balanus balanoides* at mean tide level. On small scoured stones from which barnacles were constantly removed by abrasion *Elminius* already constituted about 90% of the population. By 1949 *Elminius* everywhere outnumbered *Balanus balanoides* several times, and was practically the only barnacle found on small stones on the foreshore.

Similar increases were noted at Whitstable and Ramsgate between 1947 and 1951. On the other hand, no significant changes were seen elsewhere on the south coast, although observations were made at a number of places such as Hastings, Bexhill, Eastbourne, Brighton and Bognor during 1947–48 and between 1953–54. In sheltered areas, particularly those with wood and stone substrata, *Elminius* was everywhere abundant, but on more exposed parts of the coast, especially on wave-cut chalk reefs, such as those near Beachy Head and Seaford, *Elminius* was present only in scattered groups and was less common than *Balanus balanoides*.

Elminius has spread northward along the east coast during the past 10 years, as shown in Fig. 2. In 1947 it was fairly common as far north as Lowestoft, with isolated individuals recorded on the shores of the Wash at Brancaster, Hunstanton and Skegness. During 1948 and 1949 extensive settlements occurred in the Wash, so that by the end of 1949 *Elminius* had become the dominant intertidal species. The rivers entering the Wash were also heavily infested. In 1950 *Elminius* was abundant at such places as Kings Lynn, Holbeach and Fosdyke. It continued to spread northward. In 1948 isolated individuals were already present on piles at the extreme end of the pier at Cleethorpes, though none were to be found on the foreshore at Mablethorpe and Sutton-on-Sea. In 1950–51 *Elminius* became common along the whole of the Lincolnshire coast, as far as the entrance to the Humber at Cleethorpes. A single individual was found at Hornsea in 1953, but a further search in 1955 failed to reveal any *Elminius* on the Holderness coast, save within the Humber itself, a small number having been found at Paull and Kingston-upon-Hull. The only record of *Elminius* north of Hornsea is of one individual on a mussel shell from Blythe (Bull, 1950). This was evidently only a transient settlement, since it has not been followed by any general invasion of the area.

The advance of *Elminius* has therefore been halted abruptly at the mouth of the Humber. Three possible factors may be put forward to account for the failure of the species to spread further. (i) The residual currents flow southward off the Holderness coast, thus opposing the dispersal of larvae northward (Tait, 1938; Edgell, 1943). (ii) There are no rock substrata, and little or no

artificial substrata suitable for barnacles for some 30 miles of coast north of Spurn Head. The abrasion on this part of the coast is moreover very severe. At Withernsea abrasion appears to have prevented anything but *Enteromorpha* sp. from attaching to the groynes even towards low-water mark. (iii) The Humber, unlike the Wash, is not a suitable area for *Elminius*, probably on account of

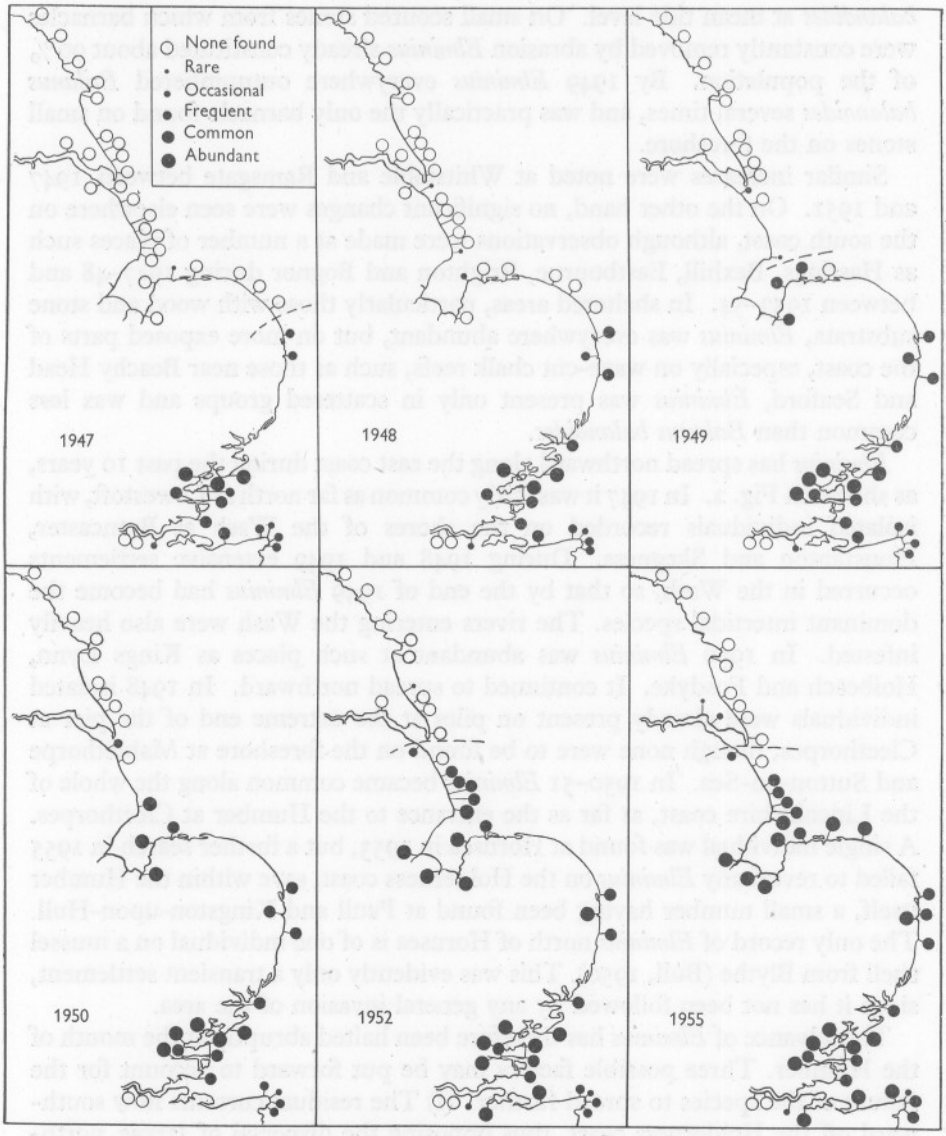

Fig. 2. Changes in the distribution of *Elminius* on the east coast from 1947 to 1955.

the pollution of the estuary. *Elminius* has not set up a dense population there, as it did in the Wash, and so may not be able to produce enough larvae to bridge the unfavourable Holderness coast.

It should be mentioned that many parts of the Yorkshire coast north of the Humber, particularly such places as Bridlington and Whitby, appear intrinsically suitable for *Elminius*, though sea-water temperatures north of the Humber are low, not usually exceeding 13–14° C in summer, and therefore unfavourable for rapid breeding.

SOUTH-WEST ENGLAND

In 1946 *Elminius* was common all along the south coast as far west as Poole Harbour. It was then rather rare at Swanage, and absent from the greater part of the Dorset coast, except for a possible centre discovered in 1947 at Weymouth (Fig. 3). By 1952 *Elminius* had become common at Swanage and Weymouth, and had appeared in smaller numbers in many of the intermediate anchorages, such as Kimmeridge Bay, Lulworth Cove, etc. Nevertheless, it has not spread westwards from Portland Bill. Apart from three specimens taken in West Bay in 1948, none has been found between Portland and Exmouth, despite repeated visits to the area. Whatever population once existed, none was found in West Bay in 1950 or in 1953.

Beyond the River Exe, *Elminius* is for the greater part confined to estuaries and drowned valleys, and absent from the open coast. Its first appearance in the area was at Plymouth in 1946. The following year an entirely separate colony was reported from the Helford River (Knight-Jones, 1948), and scattered individuals were found in Torbay late in 1947. It has since appeared in all the estuaries that have been examined between Helford and the Exe, but as it has not settled on the open coast, save as scattered individuals, it has spread somewhat erratically from one river estuary to another. For example, it became common to the west of Plymouth, in the rivers Fowey and the Looe in 1949, and to the east, in the Yealm (1948), Erme, Avon, Salcombe and Dart (1949). The Teign and Exe estuaries were colonized later, in 1949–50 and 1951, respectively. It therefore seems likely that Plymouth was the original centre of this dispersal, since the species appeared there as early as 1946 and achieved, by 1950, an average density of from 0·5–2·0 individuals per square centimetre on rocks and piles below mid-tide level in the rivers Plym and Tamar. Its colonization of these rivers may have been made easier by the widespread reduction in numbers of *Balanus balanoides* whose niche it has apparently filled (Southward & Crisp, 1952, 1954).

A significant feature of the spread into all the above rivers has been that the denser populations appeared first, and continued to increase, some distance upstream of the mouth. Gweek and Porth Navas in the Helford River and Greenway and Galmpton on the River Dart are good examples of sites where these early colonies developed, as shown in Fig. 4. The colonies in

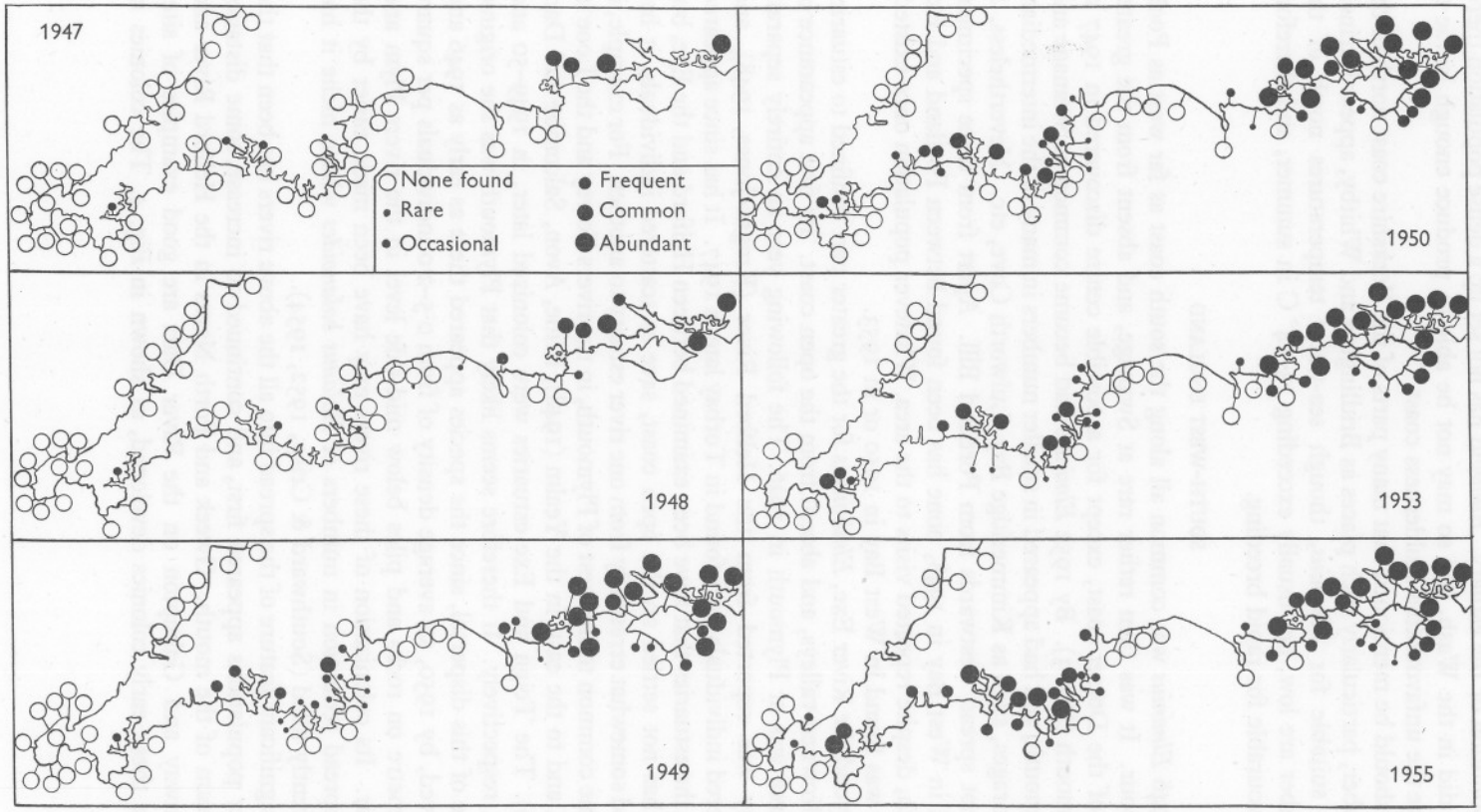

Fig. 3. Changes in the distribution of *Elminius* on the south-west coast from 1947 to 1955.

each estuary must therefore have become established and increased independently.

The spread of *Elminius* westwards from the Helford River into Penzance Harbour and on to St Ives occurred between 1951 and 1955, some years after it had first been found in the Helford River system.

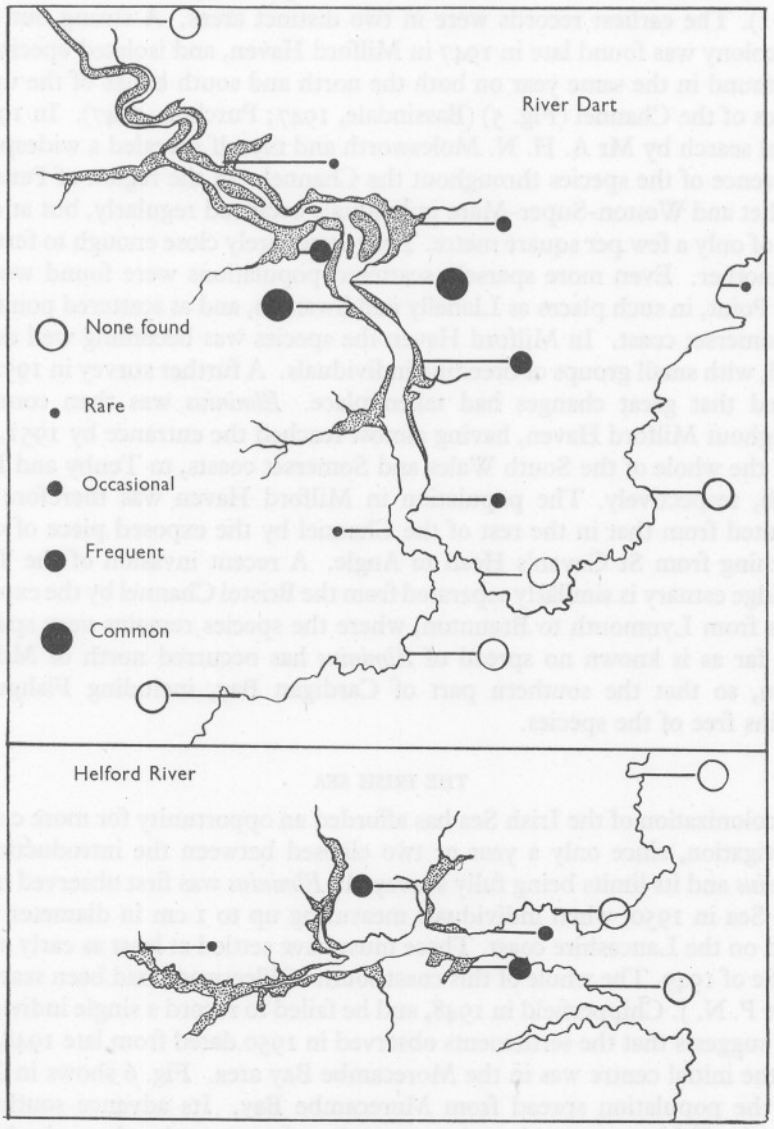

Fig. 4. The distribution of *Elminius* in the River Dart in 1949 (above) and in the Helford River in 1950 (below) to show where the first colonies develop in this type of estuary.

THE BRISTOL CHANNEL

The colonization of the Bristol Channel probably commenced at about the same time as that of Plymouth Sound. It arose as an independent population separated from those farther south by the unfavourably exposed coast of north Cornwall, which remains still for the greater part free of *Elminius* (Fig. 1). The earliest records were in two distinct areas. A strong but very local colony was found late in 1947 in Milford Haven, and isolated specimens were found in the same year on both the north and south banks of the upper reaches of the Channel (Fig. 5) (Bassindale, 1947; Purchon, 1947). In 1949 a careful search by Mr A. H. N. Molesworth and myself revealed a widespread occurrence of the species throughout the Channel. In the region of Penarth, Watchet and Weston-Super-Mare individuals occurred regularly, but at densities of only a few per square metre. They were rarely close enough to fertilize one another. Even more sparsely scattered populations were found west of Nash Point, in such places as Llanelly and Swansea, and at scattered points on the Somerset coast. In Milford Haven the species was becoming well established, with small groups of breeding individuals. A further survey in 1952–53 showed that great changes had taken place. *Elminius* was then common throughout Milford Haven, having almost reached the entrance by 1951, and along the whole of the South Wales and Somerset coasts, to Tenby and Lynmouth, respectively. The population in Milford Haven was therefore still separated from that in the rest of the Channel by the exposed piece of coast stretching from St Gavan's Head to Angle. A recent invasion of the Taw–Torridge estuary is similarly separated from the Bristol Channel by the exposed coasts from Lynmouth to Braunton, where the species remains very sparse.

So far as is known no spread of *Elminius* has occurred north of Milford Haven, so that the southern part of Cardigan Bay, including Fishguard, remains free of the species.

THE IRISH SEA

The colonization of the Irish Sea has afforded an opportunity for more critical investigation, since only a year or two elapsed between the introduction of *Elminius* and its limits being fully surveyed. *Elminius* was first observed in the Irish Sea in 1950, when individuals measuring up to 1 cm in diameter were found on the Lancashire coast. These must have settled at least as early as the middle of 1949. The whole of this coast south of Fleetwood had been searched by Dr P. N. J. Chipperfield in 1948, and he failed to record a single individual. This suggests that the settlements observed in 1950 dated from late 1948, and that the initial centre was in the Morecambe Bay area. Fig. 6 shows in detail how the population spread from Morecambe Bay. Its advance southward along the Fylde was rapid, and was continued westwards, along the North Wales coast, at a rate of some 30 km each year. Its advance northward along

the Cumberland coast was less rapid until it reached the Solway, whence it appears to be spreading westwards more rapidly. No doubt the extensive sands of the Fylde were beneficial in producing summer temperatures higher

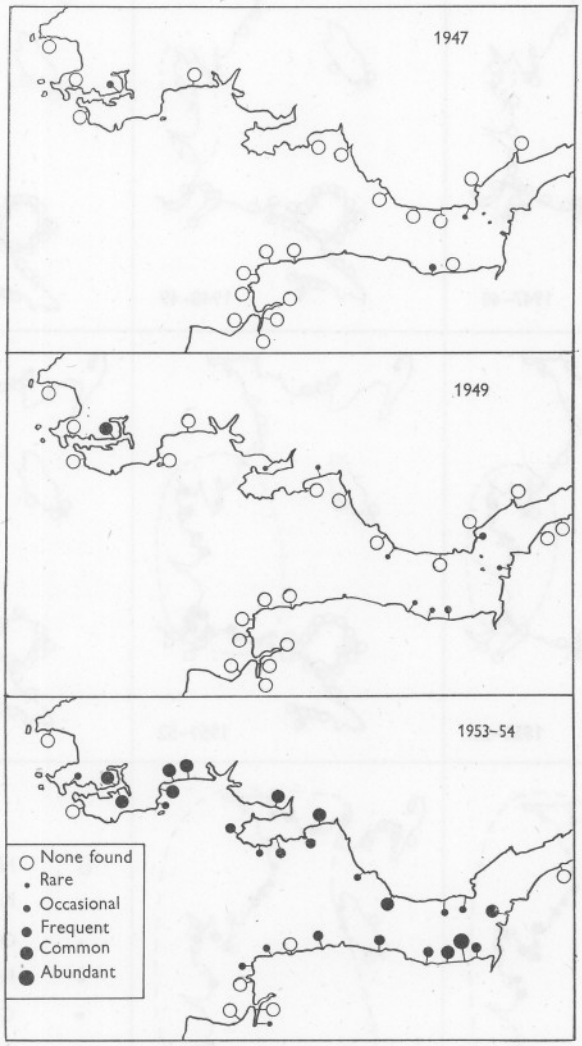

Fig. 5. Changes in the distribution of *Elminius* in the Bristol Channel from 1947 to 1954.

than those of the Cumberland coast, apart from the Solway. Perhaps more important, however, was the influence of residual drifts which are believed to flow southward along much of the Cumberland and Lancashire coasts and westwards along the Galloway and North Wales coast (Williamson, 1956).

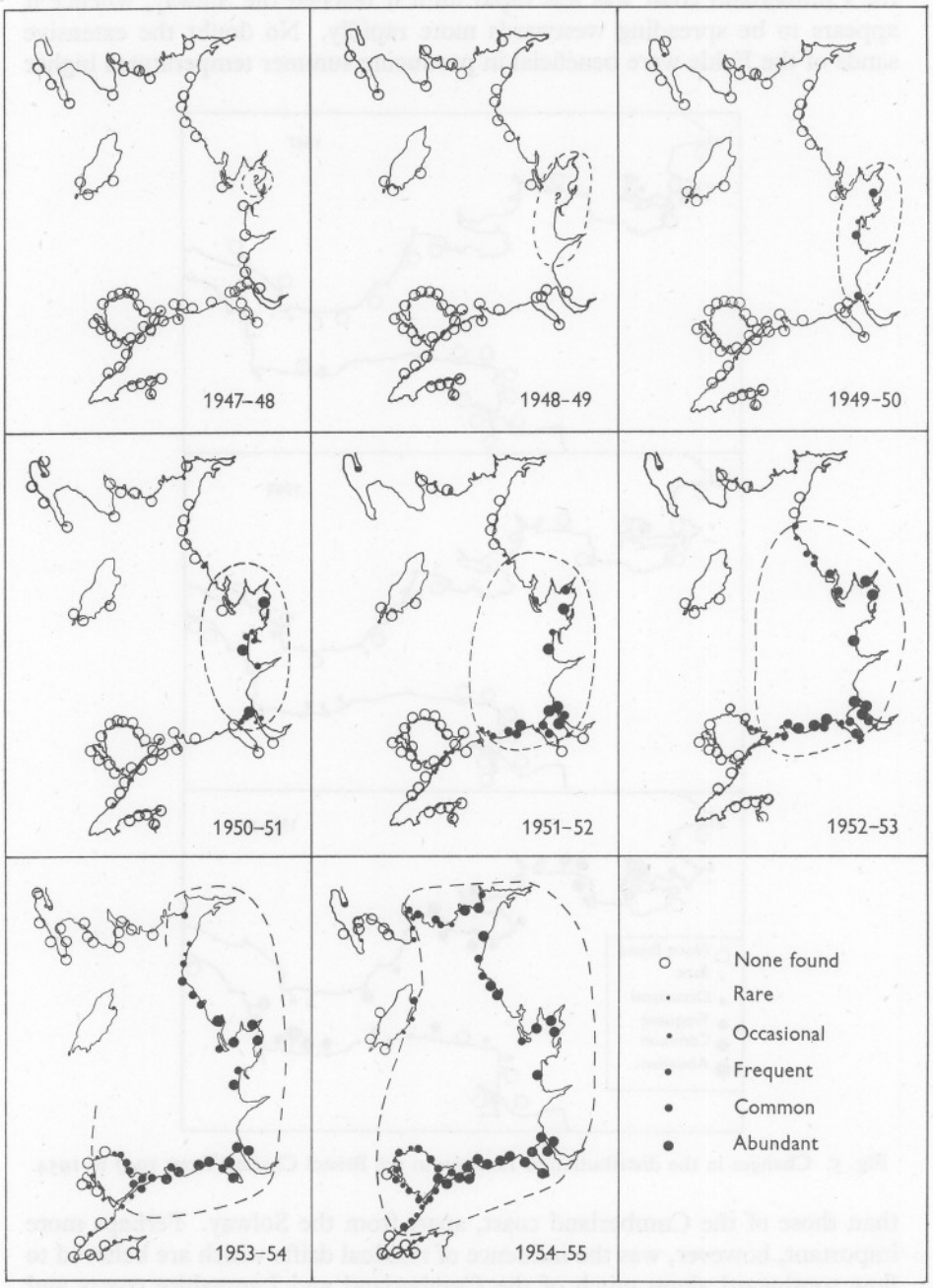

Fig. 6. Changes in the distribution of *Elminius* in the Irish Sea from 1948 to 1955.

Elminius has not advanced uniformly. Its advance has shown different patterns under differing hydrographical conditions. Along the north coast of Wales, from Rhyl to Bangor, the advancing population formed at any point of time a clearly marked front extending along a short stretch of the shore. Within a distance of about 20 km the density of the population fell from more than

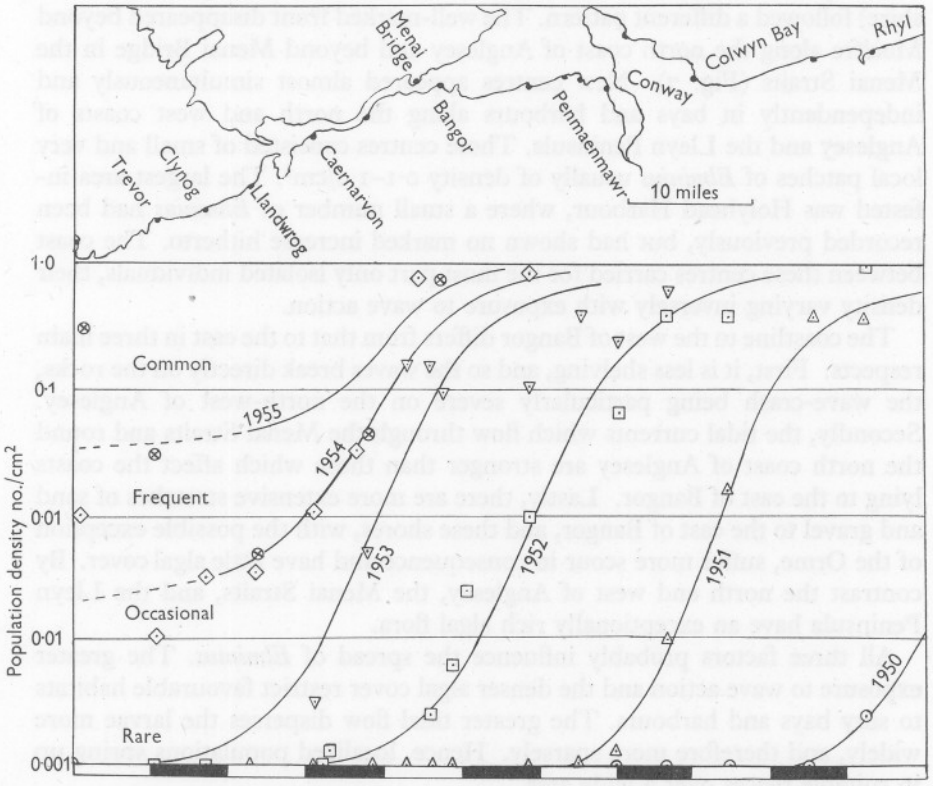

Fig. 7. The progress of *Elminius modestus* along the north coast of Wales. The upper part of the figure shows the coastal topography and the places at which regular counts were made. The lower part of the figure shows graphically the annual changes in the population density of *Elminius* (vertical axis) drawn to the same scale along the horizontal axis. Bottom scale represents intervals of 10 km.

one individual per square centimetre to only a few per square metre. Fig. 7, based on counts made on areas colonized by *Elminius*, shows how this front spread along the shore year by year. The proportion of young individuals was greater at the advancing edge of the front, particularly during August and September, when the spatfall often covered the greater part of areas left bare by other species during the summer. Thus, it was quite usual to find that where *Elminius* was present only as scattered adult individuals in one year, it formed

a complete cover on all bare areas during the following season. For example, early in 1951 none could be found on the pier or elsewhere at Colwyn Bay, but by the end of the season the pier surfaces had acquired a density of 0·04/cm² of adult *Elminius* surrounded by spat which grew to maturity the following spring and reached a density of 0·3/cm² in July 1952.

The spread of *Elminius* round Anglesey to the Lleyn peninsula (Caernarvonshire) followed a different pattern. The well-marked front disappeared beyond Moelfre along the north coast of Anglesey and beyond Menai Bridge in the Menai Straits (Fig. 7). New centres appeared almost simultaneously and independently in bays and harbours along the north and west coasts of Anglesey and the Lleyn Peninsula. These centres consisted of small and very local patches of *Elminius* usually of density 0·1–1·0/cm². The largest area infested was Holyhead Harbour, where a small number of *Elminius* had been recorded previously, but had shown no marked increase hitherto. The coast between these centres carried for the most part only isolated individuals, their density varying inversely with exposure to wave action.

The coastline to the west of Bangor differs from that to the east in three main respects. First, it is less shelving, and so the waves break directly on the rocks, the wave-crash being particularly severe on the north-west of Anglesey. Secondly, the tidal currents which flow through the Menai Straits and round the north coast of Anglesey are stronger than those which affect the coasts lying to the east of Bangor. Lastly, there are more extensive stretches of sand and gravel to the east of Bangor, and these shores, with the possible exception of the Orme, suffer more scour in consequence and have little algal cover. By contrast the north and west of Anglesey, the Menai Straits, and the Lleyn Peninsula have an exceptionally rich algal flora.

All three factors probably influence the spread of *Elminius*. The greater exposure to wave action and the denser algal cover restrict favourable habitats to silty bays and harbours. The greater tidal flow disperses the larvae more widely, and therefore more sparsely. Hence, localized populations spring up in suitable places over a wide area.

Since 1953 isolated individuals have been found from time to time on the north coast of Cardigan Bay. In 1955 this coast was carefully examined and the only incidence of the species in significant numbers was found at Pwllheli. It was present on stones in the harbour at a density of twenty to fifty individuals per square metre, some of them breeding. Some local fishing boats laid up on the beach had acquired a settlement of about the same density. It is not clear, therefore, whether these vessels introduced *Elminius*, or whether it has spread through Bardsey Sound by natural means.

By the end of 1955 the spread of *Elminius* westwards from the Solway along the south coast of Scotland had reached the Isle of Whithorn, and a single individual was found at Drummore near the Mull of Galloway. A small settlement which must almost certainly be regarded as a separate population has

persisted at Stranraer since 1950 (Crisp & Molesworth, 1950). In 1953 and 1955 *Elminius* was confined to the same part of Stranraer harbour; its numbers had dwindled, and nearly all the specimens were old ones with corroded shells. Subsequently, it was discovered that a ship-breaking yard which previously existed on the east bank of Loch Ryan had been out of operation since 1949. Possibly ships awaiting breaking up had liberated larvae, giving an initial settlement in the Loch, but these were never in sufficient density to give rise to an expanding population. A more recent report of a single specimen of *Elminius* from the Clyde (Connell, 1955) has not been followed by further reports of its presence in that area. Occasional individuals are found not infrequently some 50 or 60 miles from the main stocks. Whether they are carried there by ships or by exceptionally favourable eddies is not certain.

An isolated record of two individuals was made by Dr Southward at Ramsey in the Isle of Man early in 1952, but no further specimens were found there in 1953. However, by 1955 a centre of dispersal had clearly been established at Ramsey and was spreading southward; moreover, some of the individuals were probably at least a year old.

Ramsey is the most northerly point in the Isle of Man where suitable conditions exist for *Elminius*. Its arrival there, approximately 1 year after it had colonized the Solway, and simultaneously with its appearance at Whitehorn and Drummore, suggests that it had been carried by natural means across a sea barrier of some 25–30 miles. Crisp & Southward (1953) believed that this distance was near the critical limit for the spread of this species by normal water movements adjacent to a shoreline. At that time it was separated by a distance slightly exceeding 30 miles, and the numbers of larvae arriving at Ramsey were apparently not sufficient to start a colony.

INVASION OF THE MAINLAND OF EUROPE

Apart from records off the Dutch coast (den Hartog, 1953) the information regarding the colonization of the coasts of Europe remains scanty, and is insufficient for definite conclusions to be drawn.

The first record for the mainland of Europe was from the Kijkduin district in south Holland (Meulen, 1946). Its presence was subsequently reported by Boschma (1948). It has generally been assumed that the species was carried by ship from Britain and made its first appearance in the region of the Hook of Holland. It spread steadily northwards, reaching the southern end of the Zuider Zee by 1950, and the northernmost Friesian islands by 1951 (den Hartog, 1953). It was present in Cuxhaven in 1953 (Kühl, 1954). This migration, well documented by den Hartog, covered a distance of only 20–30 km in the first 2 years, but thereafter advanced probably at a steadily increasing rate of some 50–70 km a year, to reach Cuxhaven by 1953. The very rapid spread through north Holland and Germany is probably attributable to the residual

current flowing through the Straits of Dover and turning eastwards past the Friesian archipelago and the north German coast (Carruthers, 1930).

According to den Hartog (1953) *Elminius* spread even more rapidly from the Hook of Holland southwards across Belgium and France. In a subsequent publication (1956) den Hartog realized that the occurrence of *Elminius* in France was too widespread to be considered as having originated from Holland, and suggested that ships had carried the species to Normandy from England during the invasion of Europe. Bishop (1954) and Bishop & Crisp (1958) have described in detail the distribution of *Elminius* in France in 1953–54. They show that there were at least two distinct population centres, one in Brittany, and another, extending eastwards from the north coast of Cotentin, continuous with the Dutch population. In a more detailed consideration of the factors involved in dissemination, notably the influence of the residual current, Bishop & Crisp (1958) have arrived at the conclusion that the two French populations were established independently, and that the eastern population centre probably merged with the Dutch population on the Belgian coast approximately in 1949–50 (see Leloup & Lefèvre, 1952). A further centre has now developed in South Brittany between Concarneau and Lorient (Crisp, 1958), and another was discovered in 1955, at a great distance from all existing stocks, in north-west Spain (Fischer-Piette & Prenant, 1956).

MEANS OF DISSEMINATION

A species invading new territory may follow either of two possible courses. It may spread only at the boundary of the existing population, by natural processes of dispersal, or it may be carried a long distance by means of a vector and so establish a new centre of dissemination. It will be useful to refer to the former as marginal dispersal, and the latter as remote dispersal.

Elminius has clearly spread by both means. We shall therefore consider the limitations governing the two processes.

MARGINAL DISPERSAL

The process of marginal dispersal demands ideally a suitable coastline with closely spaced objects such as piers, breakwaters, and boulders available for settlement. Most of the north coast of Wales, between the Dee and Anglesey, fulfils these conditions. Fig. 7 shows the population density at various times, based on sample counts on various substrata. The vertical axis is given for convenience on a logarithmic scale. It can be seen that at any given time, the population density falls with increasing distance from the main stocks, sharply at first, then less steeply, and finally asymptotically to the horizontal axis. This pattern of distribution at the population boundary would be anticipated if dispersal were a random process, whether the population were a stable or an expanding one. Because the population extends at ever-decreasing density

beyond the obvious boundary, occasional records may be made at considerable distances, perhaps 40 or 50 miles in some instances, from the main population centre. Indeed over these wide areas where the animal is scarce records will depend as much on the diligence of the observer as on the density of the species. For this reason the practice of defining the boundary of a species as the point most remote from the main population at which an individual has been found is unsatisfactory. An objectively and accurately defined boundary can only be set by means of distribution contours (isopleths) such as those shown in Fig. 7. Any isopleth may be chosen, but the position of the boundary is determined most accurately where the isopleths are closest together and where the abundance of the species is sufficient to allow counts to be made. In Fig. 7 the choice is clearly between a density of 0·1 and 0·001/cm^2.

When measuring the rate of dispersal year by year the same isopleth must obviously be used for comparison. Due to irregularities in the distribution and in the environment, its position will not always be as clearly defined as in Fig. 7; nevertheless an estimate of the position of the isopleth should be made.

Table 3 summarizes existing information on the rate of progress of *Elminius* by marginal dispersal. The British records are based on the movement of the point of density 0·1/cm^2 on substrata favourable to the species. The table records only the results from areas where the limits were made certain from time to time by searches up to and beyond those parts of the coast known to have been invaded.

The rate of advance along different coasts, when taken over a number of years, is usually of the order of 20–30 km per year. The exceptions are confined to coasts influenced by strong residual currents. For example, the eastward drift along the Dutch and German coast accelerated the spread considerably; the southerly drift along the north-east coast of England appears to have arrested it altogether. With a strong and favourable drift, the relatively small numbers of larvae produced at first by an initial colony may be spread out so thinly and to such great distances that they escape unnoticed. As the population increases in density and in extent the larval output may become sufficient to supply the current with enough larvae to prevent their being unduly dispersed. An increasing rate of advance would then accompany the expansion of the initial population, as occurred off the Dutch coast.

REMOTE DISPERSAL

During marginal dispersal a fresh area is colonized from a large well-stocked area nearby, and becomes in turn a source of larvae for further dispersal. The supply of larvae is therefore practically unlimited; the chief factor limiting the spread of the species is the distance which, on average, a larva will be carried during its planktonic life. In remote dispersal, however, the number of larvae introduced is limited. Even under the best conditions, as, for example, when an old hull covered in barnacles is laid up, the actual numbers of larvae set free

must be very small compared with those which would be produced on a neighbouring well-stocked shoreline. The small number of larvae available in a given area is therefore a feature which in practice distinguishes remote from marginal dispersal.

TABLE 3. RATE OF PROGRESS OF *ELMINIUS* BY MARGINAL DISPERSAL

Region	Year	Estimated position of boundary	Distance covered (km)	Average rate of advance
North coast of Europe*	1946	Scheveningen	—	—
	1947	North of Oude Rijn	16	52 km/year (rate increasing sharply along Friesian archipelago)
	1948	North of Ijmuiden	33	
	1949	North of Petten	32	
	1950	Isle of Tessel	36	
	1951	Isle of Schiermonnikoog	92	
	1953	Cuxhaven	155	
East coast of England	1947	North of Yarmouth		33 km/year up to 1955. Advance ceased abruptly at the Humber
	1949	Hunstanton	75	
	1950	North of Skegness	26	
	1951	Cleethorpes	31	
	1951–55	No further advance	—	
West coast of England, southern front	1947	Morecambe Bay		30 km/year
	1949	Mersey estuary	73	
	1950	Point of Ayr	20	
	1951	Llandudno	36	
	1952	Red Wharf Bay, Aber.	25	
	1953	Holyhead, Port Dinorwic	30	
	1954	South of Porth Dinlleyn	25	
West coast of England, northern front	1947	Morecambe Bay		21 km/year (rate increasing as it spreads)
	1949	Barrow-in-Furness	17	
	1950	Millom	12	
	1951	Ravenglass	17	
	1952	Whitehaven	28	
	1953	Rough Firth	38	
	1954	River Cree	35	
South coast, eastward spread from Plymouth. (No clearly marked front, populations confined to estuaries and bays)	1947	Plymouth Sound		19·5 km/year
	1950	Rivers Dart and Torbay	60–75	
	1952	River Exe	35–20	

* From den Hartog (1953).

These few introduced larvae become dispersed by water movement, which will vary in magnitude with the topography, currents, tides and winds prevailing. Enclosed waters, especially long narrow estuaries carrying little freshwater drainage, will disperse the larvae less than open coasts with unrestricted lateral movement of the water by tides and winds. Thus, the introduction of the same number of *Elminius* into an area where the water exchange and dispersal is small will result in a more local and therefore denser spatfall. The tidal range is also of importance in controlling the amount of mixing of enclosed

water with the open sea (Bishop & Crisp, 1958) and so the ultimate density attained by an introduced population.

This density of the initial population is of critical importance as the following experiments indicate. *E. modestus* spat were artificially isolated at various distances from their neighbours, and allowed to mature in an area almost free of the species (Brixham Harbour, 1949). Individuals within only 3 cm of each other were fertilized at the normal rate, but those spaced out at a distance greater than 4 cm never became fertilized (Table 4). The distance of 3–5 cm is equal to that of the fully extended penis. There is a tendency for the larger size groups with correspondingly longer penes to fertilize over a greater distance. The experiments indicate that cross-fertilization is obligatory in this species (cf. Crisp, 1954; Barnes & Crisp, 1956) and that individuals separated

TABLE 4. EFFECT OF DISTANCE ON FERTILIZATION

The table gives, against the distance between individuals, the percentage which contained fertilized egg masses.

	Date and place of examination					
	Brixham transplants from Burnham-on-Crouch				Hunstanton natural population	Neyland natural population
Distance (cm)	Group A 1. v. 49 (%)	Group B 1. v. 49 (%)	Group C 23. v. 49 (%)	Group D 23. vii. 49 (%)	23. iii. 48 (%)	2. ix. 47 (%)
Controls, adjacent to each other	79	82	78	68	79	63
0 –1·0	70	80	83	—		55
1·0–2·0	72	22	67	—		50
2·0–3·0	50	0	75	27	69	20
3·0–3·5	30	0	30	73		0
3·5–4·0	0	0	25	20		
>4·0	—	—	0	0	0	0
Mean size (cm)	0·89	0·77	0·97	1·11	0·92	0·7

by a distance exceeding 5 cm will not produce any offspring. Observations in the field confirm this view (Table 4, columns 6, 7). Unless, therefore, the introduced cyprids, or those produced in the lifetime of the introduced individuals, can settle at such a density that a sufficient number of them are within 5 cm of each other, the colony will never establish itself. One may call this the 'critical breeding density'. If settlement were at random, and the conditions for maintaining the colony required that, say, 10% of the population reproduced, then, for this proportion to settle within 5 cm of each other, the critical breeding density would have to be somewhat in excess of ten per square metre. Owing to the gregarious tendency shown during settlement, a greater proportion will be in close proximity than would otherwise be expected, and this will improve the chance of successful colonization (Knight-Jones & Stephenson, 1950). Nevertheless, it is clear that the odds against an introduction must usually be very great. Success can only be achieved when large numbers are introduced into enclosed waters having little exchange with the open sea.

Dock areas, small harbours, and narrow estuaries with piston-like tidal movement are likely places for an introduction. Old ships brought from infected areas and laid up for some time or quantities of shellfish bearing spat would be the most likely sources of a successful introduction, since the *Elminius* thereby carried to the area will remain long enough to release successive broods of larvae (Crisp & Davies, 1955). Ships spending only a few days in the area are less likely to cause an introduction, for the actual numbers of larvae shed in so limited a time will be relatively small. Seaplane hulls and floats, though they occasionally become fouled by weed and are often treated with anti-fouling paint, rarely carry mature barnacles, and do not generally stay in one area for a long time.

There is no evidence that barnacles usually suffer harm when carried on ships, unless they have visited fresh or polluted water. On the contrary, the steady water movement provided by the slower classes of shipping is probably beneficial. Large gravid individuals can often be found on such vessels, indicating that growth has been rapid and breeding normal. Large ships from Australian waters also arrive from time to time fouled with *E. modestus*. Even when the species is absent outside the vessel, it is common to find it in the condenser boxes and ducts, in company with *Balanus amphitrite* and *Bugula neritina*, two other immigrant species to British waters.

BARRIERS RESTRICTING DISSEMINATION

We shall next consider the situation where parts of a coastline suitable for maintaining a population are separated by an unfavourable area. This barrier may be a rocky exposed section of the coast, or part of the open sea too deep for *Elminius*. Let us suppose one favourable area is already populated by the species but not the other. Fig. 8 illustrates this system qualitatively. The barrier is assumed to commence sharply at the termination of the existing population. The curve A shows the most probable form of the relation between the density of cypris larvae and the distance from the existing population from which they have been dispersed by water movements for the duration of their planktonic life. The situation is one which can be represented in its essentials by the equations of diffusion (cf. Skellam, 1955) in which the eddy diffusivity is responsible for random dispersal. It will be seen that at increasing distances from the parent stocks the density of larvae falls asymptotically to zero, as in the case of marginal dispersal (cf. p. 498 and Fig. 7). Since these larvae are ready to settle the potential settlement is represented also by the curve A. The maximum distance from the existing stocks, over which colonization is likely to occur, will clearly be determined by the distance at which the curve of potential settlement intersects the critical breeding density. This point is shown in the figure at A'. Fig. 8 also shows how the critical distance for colonization is influenced by the fecundity of the species K, the existing population density θ,

and the degree of water movement represented by the eddy diffusivity ϕ (Sverdrup, Johnson & Fleming, 1942). An increase in K (or θ) will not affect the shape of the curve A, but will increase the larval density proportionately throughout as shown by curve B. The effect of increasing the degree of water movement (ϕ) is shown by curve C. The number of larvae available (area under the curve) is equal to that in A, but curve C is flatter and the critical distance increased in consequence as shown by the position of the point C'. It should be noted that the idea of a critical colonizing distance does not imply a

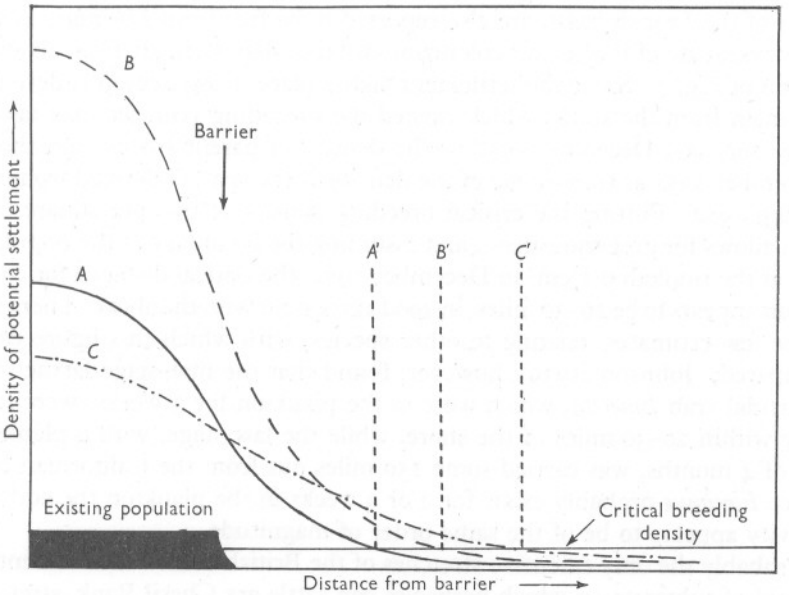

Fig. 8. Schematic representation of the influence of a faunistic barrier on dissemination of an animal with planktonic larvae. The existing population gives rise to larvae which, at the stage of potential settlement, are distributed according to diffusion theory as shown by curve A. Increasing the size or fecundity of the population will produce curve B, while increasing eddy diffusivity will modify curve A to curve C. The distance over which probable colonization may occur will be given by the intersection of curves A, B and C with the critical population level at which successful breeding can take place, that is at A', B' and C'.

clearly demarcated line beyond which colonization cannot occur under any circumstances. Owing to the assumptions involving probability in the above analysis, the effect of increasing the distance from the parent stocks is to diminish steadily the probability that a colony will be established. The critical colonizing distance is therefore definite only in relation to a given level of probability, and in the course of sufficient time increasing distances may eventually be bridged.

It is rare to find a stretch of coast of any great distance wholly unsuitable or devoid of any substrata on which the species can settle. Probably the open sea

is the most effective barrier, though even in the sea, fixed buoys may sometimes be present and provide regular oases. From the observations made in the northern Irish Sea area, it appears that the Isle of Man was colonized from a distance of some 20–25 miles away, but remained outside the critical range when the nearest *Elminius* were about 30 miles distant. This puts the critical distance for colonization in the order of 30 miles. Ireland, which is more than this distance from the coasts of England and Wales, had not been colonized by 1953, though a recent record shows that it has appeared in the south-west (Beard, 1957). The Channel Islands, separated by some 30 miles from infected parts of the French coast, are also reported to be free from *Elminius*. A more direct estimate of the critical colonizing distance may be made from an examination of Fig. 7. Since the settlement taking place in any season is derived in the main from the stocks which existed the preceding year, we may take the curve for, say, December 1951 as the density of parent stocks, and that for December 1952 as an estimate of the density of the spatfall derived from them during 1952. Putting the critical breeding density at two per square metre (this allows for gregariousness), and assuming the boundary of the population was at the isopleth 0·1/cm^2 in December 1951, the critical distance for colonization appears to be 29–30 miles, in good agreement with the above. There seem to be few estimates, relating to other species, with which this figure can be compared. Johnson (1939), however, found that the first-stage larvae of the intertidal crab *Emerita*, which were in the plankton for 3 weeks, were found only within 20–30 miles of the shore, while the last stage, with a planktonic life of 4 months, was carried some 150 miles out from the Californian coast. Since *Elminius* probably exists for 2 or 3 weeks in the plankton the eddy diffusivity appears to be of the same order of magnitude.

Probably the only extensive stretches of the British coast which are entirely devoid of substrata on which barnacles can settle are Chesil Bank, stretching from Portland to West Bay, and the Holderness coast of Yorkshire. There are, however, other places where *Elminius* has been temporarily or permanently halted; these are tabulated in Table 5. To what should the halt be attributed in these places?

Obviously the greater the proportion of unfavourable coastline, the smaller will be the numbers of larvae released and scattered along it, and the more likely will these larvae settle in unsuitable places or fail to reach the critical breeding density. Much of the Devon and Cornish coast is rugged and exposed, with few sheltered inlets. These conditions will diminish the rate of spreading of the species, for the majority of larvae produced will be wasted in unsuitable places. Another type of wastage may be important in such an environment, namely, the wastage of larvae offshore. Thorson (1950) draws attention to the calamitous wastage of larvae of littoral species from coasts where the prevailing winds and drifts are away from the land. Similar losses may occur at headlands which cause an offshore set of the current over a large part of the

tidal cycle (Crisp & Knight-Jones, 1955), or where the tidal streams are particularly strong, as at Portland and Cap de la Hague. Larvae in such areas may have little chance of returning to the shore to settle. This wastage, taken together with the high proportion of unsuitable shoreline, would account for the halts in the spread of *Elminius* at Portland Bill, Cap de la Hague, the Lizard, and the Pembroke peninsula.

TABLE 5. BARRIERS TO THE PROGRESS OF *ELMINIUS*

Barrier	Possible causes	Distance separating suitable habitats	Period over which advance was retarded
Holderness coast	Lack of substrata. Opposing residual drift, low temperature	Hornsea–Humber	1953–
Portland Bill and Chesil Bank	Lack of substrata. Rocky headland. Strong tidal races. Opposing residual drift	Portland Harbour–West Bay Harbour	1947 or earlier–
Lizard and Land's End peninsula	Rocky and exposed headlands	Helford River–St Ives Bay (except for a few small harbours, e.g. Porthleven, Penzance)	1947 or earlier–1953 approx.
Pembroke Peninsula	Rocky and exposed headland. Strong tidal races	Milford Haven–Fishguard (except for small harbours such as Solva, Porthgain)	1947–
Irish Sea	Sea barrier	Cumberland coast–Isle of Man	1949–53
	Sea barrier	Holyhead–Dun Laoghaire	1953–
St George's Channel	Sea barrier	Milford Haven–Rosslare	1947–
English Channel	Sea barrier	Cap de la Hague–Channel Isles	1950?–
Straits of Dover	Sea barrier	Dover Harbour–Calais	1945 or earlier–1949?
Cap de la Hague	Rocky headland. Strong tidal races. Opposing residual drift	Cap de la Hague–Carteret	1950?–

The conditions on rocky coasts will in general be such that marginal dispersal can scarcely operate, or will do so only slowly. Larvae from sparse and scattered populations will be dispersed over a wide area, many being lost out to sea. Thus there will be a few larvae everywhere, but probably in most places not in sufficient density to breed. A few larvae liberated from craft lying up in a small creek or from the few individuals settled close enough together to breed, may add sufficiently to those being dispersed from outside to pass the critical breeding density. The area would then eventually become populated. Thus, where *Elminius* has spread from one harbour to the next, without appearing on the neighbouring exposed parts of the coast, as in south Devon, the Bristol Channel and west Caernarvonshire, it is possible that both marginal

and remote means of dispersal have played a part. Where the distance traversed in one leap greatly exceeds 30 miles, however, it is reasonable to assume that the species has not spread naturally but has been carried by a vector.

PROBABLE HISTORY OF DISPERSAL

When first discovered in 1946 the species was already widespread in southern England. The two most likely areas for its original introduction from Australasian shipping would therefore be Southampton Water and the Thames estuary. Both are areas very favourable to *Elminius*, having high summer temperatures, and now support abundant populations. Of these two, Southampton is more likely to have been the original centre on the following grounds. First, Southampton Water is very enclosed, and because of the small tidal range it exchanges water only slowly with the sea outside. Secondly, there is little freshwater flow and less pollution there than in the Thames estuary. Thirdly, in the Thames all the docks are in areas too fresh or too polluted for survival of *Elminius* larvae, except possibly at Tilbury. In Southampton Water vessels are docked in sea water suitable for larval development. Lastly, though there are no continuous records for the Southampton area, we know that *Elminius* was still increasing in density in the Essex rivers from 1946–50.

No more than a guess can be made as to the time of arrival. The rate of spreading along comparable coasts of shingle and sand, such as north Holland, eastern England, and Liverpool Bay, lies between 52 km/year (with a favourable drift) and 30 km/year. There exists a general eastwards drift through the whole of the English Channel and Straits of Dover (Carruthers, 1930) and this probably extends to the shoreline as evidenced by the direction and movement of shingle bars by predominant wind and waves (Steers, 1946). A small contrary coastal eddy is shown by Edgell (1943) in Pevensey Bay. A rapid advance eastwards could therefore be assumed after the necessary time for establishment in Southampton Water. The many coastal defences in the area would also have assisted in providing substrata. Taking 50 km/year as a probable rate, then an introduction in 1939–40 would have reached the Thames estuary in 1945, and if the rate continued up the east coast it could have just reached Lowestoft by 1947. This timetable, however, appears only just adequate, and taking other factors into account an introduction by remote dispersal into the Thames estuary area probably in 1943 or 1944 seems more probable. In 1947 the species was more abundant in the area between the Thames and Harwich than on the Thanet coast, suggesting that the latter area was colonized after a population had been established in the Thames estuary. Furthermore, the Dutch coast was probably invaded as a result of remote dispersal between East Anglia and the Hook of Holland in 1946 (Bishop & Crisp, 1958). It is necessary, therefore, to assume that *Elminius* had been established in the Harwich area prior to 1946. Since the rate of advance northward from the Thames

would not be accelerated by residual drifts, but on the contrary retarded since the strongest winds are from the north-east, it is not easy to account for the spread of *Elminius* to Harwich by 1945, unless it was already established in the Thames by 1943 or 1944. Making this assumption, the probable history may be summarized as follows.

Shipping conditions were exceptional at the outbreak of war in 1939. As convoys assembled, large numbers of vessels were anchored just offshore. This may have allowed the barnacles on Australasian shipping time to liberate sufficient numbers of larvae to colonize Southampton Water. The large concentration of shipping which then occurred might have given *Elminius* a unique opportunity. The following summer (1940) was a warm one, and would have assisted in building up stocks above the critical level for maintaining a population; from Southampton *Elminius* spread rapidly eastwards aided by the residual drift, and more slowly westwards. In about 1943 or 1944 a new centre was established in the Thames estuary, probably by remote dispersal, and the East Anglian and Thanet coasts were colonized from this centre.

The westward extension along the coast from Southampton was halted at Portland, and no further westward spread has taken root in Lyme Bay. Several distant centres, however, were set up to the west of Southampton, between 1944 and 1946, presumably by remote dispersal. These were in the Helford River (1946 or earlier), Plymouth Sound (1946), Milford Haven (1947), and possibly in another place further up the Bristol Channel. These two centres in South Wales may have arisen simultaneously, though independently. The centre in Milford Haven has remained isolated up to the present time by exposed headlands, while *Elminius* has steadily colonized the remainder of the coasts north and south of the Bristol Channel. Perhaps, however, the original breeding stocks were present in Milford Haven, and larvae were carried by the prevailing surface drift to coasts higher up the Channel.

A major centre was next set up in Morecambe Bay in 1948, from which the coasts of North Wales, Lancashire, Cumberland and Galloway have now been populated. Small centres, probably arising by remote dispersal, have been set up in St Ives Bay (1951–54) and Tremadoc Bay (1953–54).

On the continental coast there appear to have been four, possibly five, main centres. The invasion of the coast of Holland began near the Hook of Holland about 1946. For reasons given elsewhere, another centre was probably established in the estuary of the Seine soon after the invasion of Normandy in 1944 (Bishop & Crisp, 1958). A third centre in the Brest area has been described by Bishop from which other areas in south Brittany have been colonized in a manner similar to that of the invasion of the coasts of south Devon (Crisp, 1958). Judging from the rather slow establishment on rocky coasts of this type, the centre in the Rade de Brest was probably established at least 3 or 4 years earlier than the date (1953) when Bishop first surveyed the area. The river estuaries in the Morlaix–Roscoff area may represent other separate centres established at

about the same time as that in the Rade de Brest. Much farther south yet another centre has been established in north-west Spain. Its discovery in 1955 (Fischer-Piette & Prenant, 1956) and its present rather limited area give it the appearance of recent origin, probably between 1950 and 1953. It may be distinct from recently reported settlements in Portugal (Fischer-Piette & Prenant, 1957).

The dates in which these centres have probably been established are shown in Fig. 9 .There can be seen to be some regularity in the order of their appearance, for the new centres have not sprung up at random. They seem to have arisen more readily in the vicinity of old ones than at great distances from them. Such is perhaps to be expected, since harbours are usually visited more frequently by craft from neighbouring localities than by craft from any other particular area. Local craft are also more likely to be allowed to accumulate fouling than are those which travel greater distances, and their shallower draught is more suitable for the settlement of an intertidal species. Larvae will thus be liberated continually into harbours once a neighbouring area is infected, to add to the numbers borne by water currents. It is therefore not easy to differentiate between remote and marginal dispersal in areas where suitable harbours are scattered and many local fishing vessels are operating.

ECOLOGICAL EFFECTS OF THE INTRODUCTION OF *ELMINIUS*

The introduction of a new species is bound to influence the balance existing between endemic species. A species occupying as dominant a place in its environment as *Elminius* may bring about profound changes. The main differences between *Elminius* and native barnacles are summarized in Table 6.

Elminius competes for space mainly with *Balanus balanoides*, which is the only indigenous intertidal barnacle on those parts of the British coast where *Elminius* thrives best, notably in south-eastern England. To a lesser extent it competes with *Balanus improvisus* and *B. crenatus* at low-water mark. *Elminius* ranges over a greater part of the intertidal zone than does *Balanus balanoides*, for small numbers grow at levels slightly above the highest *B. balanoides*, and it penetrates into the sublittoral, some 5 m below L.W.S. It is usually less common in the sublittoral, however, than *B. improvisus* and *B. crenatus*.

When *Elminius* first appears, the zone in which it settles is usually occupied by adults and spat of *Balanus balanoides*. Except in very muddy or brackish places, *B. balanoides*, which settles 2 months earlier than *Elminius*, initially covers the greater part of the available settling space, so that *Elminius* is often found attached to the upper parts of the shells of *Balanus balanoides* or even to the valves. As the spat of *Elminius* become more numerous they often form circlets of small grey barnacles round the apertures of large specimens of *Balanus*. Some *Elminius* settle regularly above the *Balanus balanoides* zone, almost as high as *Chthamalus* would be found if it were present.

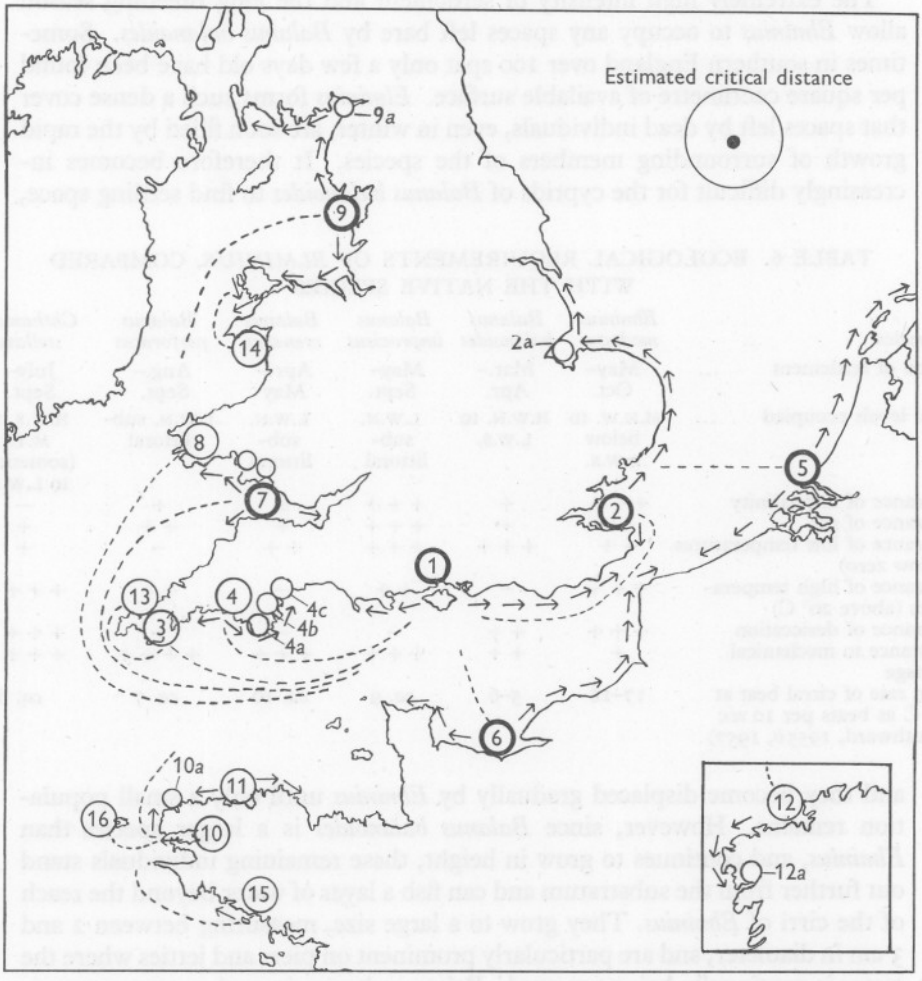

Fig. 9. Illustration of the probable order in which centres of dissemination of *Elminius* were established. Major centres responsible for the colonization of large areas of coast are shown by large dense circles. More localized centres also set up by remote dispersal are shown by large open circles. Subsidiary centres probably reached by marginal dispersal by small circles. Arrows show direction of marginal dispersal, dotted lines the probable routes of remote dispersal. Inset: north-west Spain

1, Southampton Water, ? 1940–43; 2, Thames estuary, ? 1943–44 [2*a*, the Wash, 1948]; 3, Helford River, ? 1944–46; 4, Plymouth Sound, 1946 [4*a*, River Dart, 1948; 4*b*, Salcombe, 1948; 4*c*, River Exe, 1951]; 5, South Holland, 1946; 6, Seine estuary, ? 1944–49; 7, Bristol Channel, 1946–47; [7*a*, Llanelly Bay, 1949; 7*b*, Swansea Bay, 1949]; 8, Milford Haven, 1947; 9, Morecambe Bay, 1948 [9*a*, Solway firth, 1953]; 10, Rade de Brest, ? 1944–52 [10*a*, l'Aber Wrach, l'Aber Beniot, ? year]; 11, Roscoff area, ? 1944–52; 12, north-west Spain, ? 1950–53 [12*a*, Noya, ? year]; 13, St Ives Bay, 1951–54; 14, Tremadoc Bay, 1953–54; 15, south Brittany, 1954–57; 16, Ile d'Ouessant 1956.

The extremely high intensity of settlement and the long breeding season allow *Elminius* to occupy any spaces left bare by *Balanus balanoides*. Sometimes in southern England over 100 spat only a few days old have been found per square centimetre of available surface. *Elminius* forms such a dense cover that spaces left by dead individuals, even in winter, are soon filled by the rapid growth of surrounding members of the species. It therefore becomes increasingly difficult for the cyprids of *Balanus balanoides* to find settling space,

TABLE 6. ECOLOGICAL REQUIREMENTS OF *ELMINIUS*, COMPARED WITH THE NATIVE SPECIES

Species ...	*Elminius modestus*	*Balanus balanoides*	*Balanus improvisus*	*Balanus crenatus*	*Balanus perforatus*	*Chthamalus stellatus*
Season of settlement ...	May–Oct.	Mar.–Apr.	May–Sept.	Apr.–May	Aug.–Sept.	July–Sept.
Tidal levels occupied ...	M.H.W. to below L.W.S.	H.W.N. to L.W.S.	L.W.N. sub-littoral	L.W.N. sub-littoral	L.W.N. sub-littoral	H.W.S. to M.T.L. (sometimes to L.W.N.)
Tolerance of low salinity	+ + +	+	+ + +	+	+	–
Tolerance of silt	+ +	+	+ + +	+	+ +	+
Tolerance of low temperatures (below zero)	+ +	+ + +	+ + +	+ +	–	+
Tolerance of high temperatures (above 20° C)	+ + +	–	+ +	–	+ +	+ + + +
Tolerance of desiccation	+ + +	+ +	–	–	+	+ + + +
Resistance to mechanical damage	+	+ +	+ + +	+ + +	+ + + +	+ + + +
Mean rate of cirral beat at 20° C as beats per 10 sec (Southward, 1955*b*, 1957)	17–18	5–6	*ca.* 9	*ca.* 10	*ca.* 7	*ca.* 6

and they become displaced gradually by *Elminius* until only a small population remains. However, since *Balanus balanoides* is a larger species than *Elminius*, and continues to grow in height, these remaining individuals stand out further from the substratum and can fish a layer of water beyond the reach of the cirri of *Elminius*. They grow to a large size, measuring between 2 and 3 cm in diameter, and are particularly prominent on piers and jetties where the water is continually being renewed. Being no longer in such severe competition with their own species they are individually large, healthy and successful.

The process of replacement of the *Balanus* population by *Elminius* is relatively slow; for example, at Hunstanton, *Elminius* was introduced late in 1947, and present in small numbers chiefly on the parieties of *Balanus* on the pier piles in 1948. By 1949 it was abundant, and smothered all existing individuals of *Balanus*, though the latter were still present to a density of 2–3/cm^2. In 1952 some reduction in *B. balanoides* was noticeable, and those remaining were no longer close packed and columnar, but were in the main isolated, their density being about 0·7/cm^2. They were rather larger than hitherto. By 1955 the density of *B. balanoides* had fallen further to about 0·15/cm^2, most of these being between 2 and 3 cm across the base, and about 1·5–2 cm in height.

In the less saline parts of an estuary and below low-water mark, *Elminius* may replace *Balanus improvisus*. There was a reduction in the numbers of *B. improvisus* settling on experimental panels between 1946 and 1950 in the River Crouch, and, judging from plankton samples taken in 1938 in the Thames, a reduction has taken place in the numbers of *B. improvisus* larvae in the plankton. It will be especially interesting to observe this interaction if *Elminius* spreads to the Baltic, where *Balanus improvisus* is the dominant species. The competition between *Elminius* and *Balanus crenatus* is probably less important as there is less overlap in their habitats. They are found together only at low-water mark where the salinity is fairly high.

The effect of the introduction of *Elminius* is negligible on rocky exposed shores of the west and south-west, where the dominant intertidal barnacle is *Chthamalus stellatus*. Nevertheless, in sheltered inlets and estuaries of these coasts *Elminius* is of importance. In the Plymouth estuaries *Elminius* had in 1950 largely occupied the situations from which *Balanus balanoides* had disappeared during its decline (Southward & Crisp, 1954). Though *B. balanoides* has begun to return to the outer parts of this coast it has not re-established itself in the estuarine regions where *Elminius* remains dominant (Southward & Crisp, 1956). Its effect on the indigenous fauna within these estuaries has therefore been mainly at the expense of *Balanus balanoides* as on the coasts of south-east England. In many of the estuaries in the south-west where *B. balanoides* is not very common, *Chthamalus* and *Elminius* are found together, *Chthamalus* being able to penetrate up the estuary beyond the seaward limit of *Elminius*.

Elminius is microphagous and behaves like other barnacles (Southward, 1955*a*) utilizing a wide range of particle sizes and taking in both animal and plant material. It grows rapidly, sometimes reaching maturity (diam. 6–7 mm) in 8 weeks. The cirral beat at temperatures from 15–25° C is faster than that of indigenous species (Table 6). It continues to feed vigorously after maturity, and produces broods of nauplii at regular intervals (Crisp & Davies, 1955). Under optimum conditions broods may be liberated every 10 days, each brood containing about the same amount of living matter as is present in the soft parts of the parent. A dense population of *Elminius* therefore removes during summer a great bulk of suspended food and transforms it into larvae. These larvae are extremely abundant, often forming the dominant component of the plankton in May, June and July, in estuaries of south-east England. The nauplii probably feed mainly on particles less than 10μ in size, as the diameter of the oesophagus is of this magnitude.

The replacement of a large proportion of the previously existing population of *Balanus balanoides* by *Elminius* may therefore have a considerable influence on other members of the marine fauna, for *Balanus balanoides*, unlike *B. improvisus*, is by no means the ecological equivalent of *Elminius*.

While both species as adults feed and so remove suspended food particles

during summer, *Elminius*, judging from its rate of cirral beat (Southward, 1955*b*), is probably more efficient than *Balanus balanoides* at the highest temperatures. Hence, more food may be diverted to barnacle growth and so made unavailable to other microphagous forms. But by far the more important difference is that whereas *B. balanoides* accumulates reserves for winter breeding, *Elminius* rapidly converts the food into nauplii which enter the plankton during summer. Moreover, the large number of *Elminius* cyprids which develop and settle (see above) is a clear indication of the success of *Elminius* nauplii in obtaining the necessary food in the face of competition by other larvae having a similar diet. Consequently, there has been a great increase in the total number of summer nauplii, following the introduction of *Elminius* which must adversely affect the growth of the planktonic larvae of other animals which breed in summer, such as *Balanus improvisus*, *Polydora ciliata*, *Littorina littorea*, *Crepidula fornicata* and *Ostrea edulis*. *Ostrea* is harmed also in the early stages of growth as spat by competing for space with *Elminius* (Knight-Jones, 1948). Little is known of the relative ease with which different barnacles may be browsed by predators such as *Nucella lapillus* or *Asterias rubens*. The more delicate and fragile shell of *Elminius* might make it more readily devoured than indigenous species, and so allow these predators to be more successful.

Elminius tolerates the presence of silt and pollution probably better than any other species of British barnacle, with the possible exception of *Balanus improvisus* and in warmer situations *B. amphitrite*. In dirty harbours and muddy rivers within the intertidal zone it may have few or no competitors. On the other hand, on clean and especially on wave-beaten shores, *Elminius* has not displaced either *Balanus balanoides* or *Chthamalus stellatus*, and is indeed often very sparse on these habitats. Mechanical explanations at first suggest themselves, for the species is more fragile and might suffer more from wave crash or pebble pounding. Perhaps, it might be supposed, limpets, which are rare or absent from the usual haunts of *Elminius*, destroy any settlement on exposed shores. But these explanations are not entirely satisfactory. Exposed rocks very close to stocks of *Elminius*—Point Lynas, Anglesey, for example—may have numbers of *Elminius* settled on them which from their eroded appearance seem to have survived for some time. There is no marked preponderance of young individuals and spat in these places. *Chthamalus*, the larvae of which are as small as those of *Elminius*, settles successfully in spite of limpet browsing. It seems more probable, therefore, that the species is uncommon on exposed coasts because its larvae do not settle there, rather than because of high mortality. There are no definite facts to indicate whether *Elminius* larvae could develop in the clearer waters that surround more exposed coasts, but it seems probable that the more turbid water of tidal estuaries contains particles of the appropriate kind to nourish them, while offshore water in general does not.

When considering the influence of other organisms competing with

Elminius for rock space, algae probably rank equally in importance with animals (cf. Barnes & Powell, 1953; Southward, 1953). Where the water is sufficiently clear and where, as in shelter, limpets are few, the growth of algae prevents barnacles from colonizing much of the illuminated surface of rocks. Hence, barnacles may appear to avoid insolation (cf. Moore, 1944) and to collect in dark crevices under piers, or beneath boulders and overhangs. Thus, there exist in temperate waters two extreme situations in which rock surfaces are available for barnacle settlement; one where the rocks are so covered in silt, scoured, or affected by low salinities that algae do not greatly flourish, and the other where algae are kept down by the grazing activities of limpets and by heavy surf. *E. modestus* and *Balanus improvisus* are adapted to the former habitat, *Chthamalus* to the latter. In adapting itself to these turbid estuarine conditions it is possible that it has become nutritionally dependent on the type of food particles found in silty estuaries and is ill adapted to survive in clear water.

SUMMARY

Material collected prior to 1940 indicates that *Elminius modestus* was not present on British coasts at that time.

Elminius increased in abundance in south-east England from 1946 to 1950 and extended its range as far as the Humber, where it halted.

Its advance westwards along the south coast was similarly halted at Portland, but by 1948 independent colonies had been established in several of the river systems of Devon and Cornwall, in Milford Haven, and in the Bristol Channel.

The first populations in the Irish Sea were in Morecambe Bay. From there *Elminius* spread rapidly south and west along the north coast of Wales, and more slowly north and west towards Galloway, eventually bridging the sea to the Isle of Man.

Detailed observations showed that *Elminius* advanced along the uniformly favourable north coast of Wales as a definite front moving at a rate of approximately 20–30 km per year. Around Anglesey where tidal currents were stronger it appeared simultaneously in many scattered centres.

A distinction is drawn between marginal dispersal taking place under the influence of normal agencies at the boundary of an existing population, and remote dispersal due to an artificial or freak transport over a long distance. In the case of *Elminius* the maximum distance that is likely to be bridged by marginal dispersal in the absence of strong residual drifts is about 30 miles.

Elminius probably first appeared near Southampton, and was introduced into the Thames estuary area probably by remote dispersal. Thence it spread along the east coast and was transported to Holland. Its extension into south Devon, the Bristol Channel, the Irish Sea, and to the French coast must also be attributed to remote dispersal.

The main ecological effects of *Elminius* result from competition for space with *Balanus balanoides*. Since *Elminius* breeds in summer, its dominance has a profound effect on the composition of the summer plankton, greatly increasing the number of barnacle nauplii, presumably at the expense of other larvae.

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APPENDIX

TABLE 7

Key: A Abundant. All available surfaces well covered to 30 % of area or more. Adults at 1·0/cm² or more. C Common. Covering less than 30 % of available area, the majority within 1–2 cm of each other. Density 0·1–1·0/cm². F Frequent. Readily found, but about half the population within 3 cm of each other and so just able to breed. Spat not usually in evidence. Adults 0·01–0·1/cm². O Occasional. Very local and must be searched for, very rarely close enough to breed. Density 1–100/m². R Rare. Only a few isolated individuals found in an hours' search. Density below 1/m². N. None found after 1 hr search in suitable area.

Records of Elminius modestus

Place	Date	Density	Notes*
Dunbar	vi. 55	N	—
Berwick-on-Tweed	vi. 55	N	—
Blyth	x. 47	N	P.N.J.C.
	1949	R	Specimen on mussel, but no subsequent records here. H.O.B.
Whitley Bay	ix. 49	N	—
Cullercoats	ix. 53	N	—
Runswick	vi. 55	N	—
Robin Hood's Bay	ix. 50	N	P.N.J.C.
	1954	N	E.A.S.
Scarborough	vi. 55	N	—
Flamborough Head	vi. 55	N	—
Bridlington	vi. 55	N	—
Hornsea	vi. 52	R	1 specimen only
	vi. 55	N	—
Aldburgh	vi. 52	N	—
	vi. 55	N	—
Withernsea	vi. 55	N	—
Kilnsea	vi. 55	N	—
Paull	vi. 55	O	—
Hull	vii. 48	N	—
	vi. 52	N	Area unsuitable from pollution?
	vi. 55	R	—
Grimsby	vii. 48	N	Few suitable substrata
Cleethorpes	vii. 48	R	Single specimen at far end of pier
	ix. 50	O–F	Just self-maintaining population
	vi. 52	F	Less increase than expected
Saltfleet	vi. 52	(N)	No suitable substrata
Mablethorpe and Sutton-on-Sea	vii. 48	N	—
	vi. 52	C	—
Ingoldmells and Chapel Point	vii. 48	N	—
	vi. 52	C	—
Skegness	vii. 48	O–R	5 specimens on pier
	ix. 50	C–A	—
	vi. 52	A	Much commoner than *B. balanoides*
Fosdyke	vi. 52	A	—
Sutton Bridge	vi. 52	A	—
	vii. 55	A	—
King's Lynn	vii. 48	N	P.N.J.C.
	xii. 49	C	On mussels. G.D.W.
	vii. 50	A	Adults 3·5/cm². Spat fall up to 60/cm²
	vi. 52	A	—
Hunstanton	viii. 47	N	—
	iv. 48	R–O	On pier piles only
	vii. 50	A	Heavy spatfall of 40–50/cm². Adults much commoner than *B. balanoides* and growing on them
	i. 53	C–A	A few damaged on pier after heavy storm, which caused widespread damage on east coast; much scouring of rocks and low substrata
	vii. 55	A	Only a few *B. balanoides* of large size (3–4 cm) on pier, 0·07/cm². Rest of area completely covered by *Elminius*
Brancaster	viii. 47	R	One small specimen only
	iii. 48	N	—
	vi. 49	F	Many small specimens on old wreck, off Scolt Head. A.H.N.M.
	ix. 49	C	Many small spat (up to 10/cm²) on stones near main creek. P.N.J.C.
	vii. 50	C–A	Settlement local, two or three times as abundant as *B. balanoides*. Spat 30/cm²
Wells	ix. 49	N	P.N.J.C.
	vii. 55	A	On mussels in creek
Sheringham	i. 48	N	Little suitable substrata. P.N.J.C.
	vii. 55	A	Confined to low water owing to scour
Cromer	i. 48	N	P.N.J.C.
Sea Palling	i. 48	N	P.N.J.C.
Great Yarmouth	i. 48	C	P.N.J.C.
Lowestoft	x. 47	F	On sheltered side of north jetty
	i. 48	F	P.N.J.C.
	v. 49	C	—
	xi. 51	A	—
	vii. 55	A	More restricted on open coast owing to scour. *Elminius* 98 % of population

* Initials refer to observers.

Place	Date	Density	Notes
Southwold	ii. 48	C	P.N.J.C.
	i. 53	C	—
Harwich	ii. 48	C	P.N.J.C.
Clacton	ix. 47	A	—
West Mersea	v. 46	C–A	Regular settlement throughout 1946
Maldon	ix. 49	A	—
Mayland	viii. 47	F	—
Steeplestone and Bradwell	viii. 47	A	—
Burnham-on-Crouch	i. 47	C	—
North Fambridge	viii. 46	C	On hull laid up above H.W. mark since July 1945
Hullbridge and Battlesbridge	ix. 49	C–A	—
Shoeburyness	ix. 47	C	—
Woolwich	ix. 47	N	Too much pollution
Erith	ix. 47	N	Too much pollution
Gravesend	x. 47	O	Limit of penetration of Thames estuary
Rochester	iv. 51	A?	Had been abundant, but nearly all dead
Whitstable	ix. 47	C	—
	iv. 51	A	—
Birchington and Margate	ix. 47	F	—
	iv. 51	C–A	Abundant on piers (5/cm²) fairly common on chalk (0·5–1·0/cm²)
North Foreland	ix. 47	O	—
	iv. 51	O	—
Broadstairs and Ramsgate	ix. 47	F	—
	iv. 51	C	—
Dover	ix. 54	C	—
Folkestone	viii. 56	F	Less common than *B. balanoides*
Hythe	viii. 56	O	On groynes, few barnacles present, much abrasion
Rye Harbour	ix. 48	A	—
Fairlight	viii. 56	F	—
Hastings	viii. 48	C–A	—
	vii. 53	C–A	—
Bexhill	ix. 48	A	—
	vii. 53	C	—
Eastbourne	viii. 48	C	—
	vii. 53	A	—
Seaford	vii. 53	O	Much abrasion by shingle
Brighton	ix. 47	F	—
	iii. 49	F	—
Portslade and Shoreham	ix. 47	A	—
	iii. 49	A	—
Worthing	xi. 48	A	—
Littlehampton	xi. 48	A	Abundant on training wall of River Arun
	vi. 53	A	—
Bognor	xi. 48	C	—
	vi. 53	A	—
Chichester Harbour	vii. 44	F?	Several spat on panel exposed for 3 weeks. H.G.S.
	vii. 45	A	About 30 spat/cm² of test surface. M.W.H.B.
	vii. 48	A	—
Portsmouth and Gosport	v. 44	?	2 specimens from boom defence vessel settled in 1943. H.G.S.
	1944	C?	Numerous specimens on ship's hull. H.G.S.
	iii. 49	A	A.H.N.M.
Southampton	vii. 49	A	Intense settlement covering all substrata
River Test, Redbridge	xi. 48	F	—
Cowes	vii. 49	A	—
Ryde	viii. 48	A	—
	vi. 53	A	—
Bembridge	vii. 49	A	—
Culver	vii. 49	F	Less common on chalk than elsewhere
Sandown	vii. 49	A	—
	vi. 53	C	Less common than *B. balanoides*
Shanklin	vii. 49	F	—
	vi. 53	C	—
Ventnor	vii. 49	F	—
	vi. 53	F	—
St Catherine's Point	vii. 49	N	—
	vi. 53	R	—
Brook and Freshwater	vii. 49	O	—
	vi. 53	O	—
Alum Bay	vi. 53	O	—
Totland and Colwell Bay	vii. 49	C	Up to 2·5/cm² in sheltered areas
	vi. 53	C	—
Yarmouth, Isle of Wight	vii. 49	A	—
Fishbourne	vi. 53	C–A	—
Lymington	vii. 49	A	Present on piles withdrawn from the sea in July 1947
Milford to Highcliffe	vii. 49	(N)	No suitable substrata
Mudeford	vi. 49	A	—
Hengistbury Head	vii. 49	O	—
Bournemouth	viii. 47	C	—
	vi. 49	A	—
Sandbanks, Poole Harbour	vi. 47	C	Settlement of spat at rate of 3–5/cm²
	iv. 48	C	10–15 % of population
	vi. 49	C	—
Studland	vi. 49	A	—
	vi. 53	A	—
Swanage Bay	iv. 48	O	Absent beyond Peveril Point
	vi. 49	F	Absent beyond Peveril Point
	vi. 53	F	Rare beyond Peveril Point
Kimmeridge Bay	xi. 48	N	—
	vi. 53	F	—
Lulworth Cove	v. 49	R	—
	vi. 53	N	—
Osmington Mills	vii. 49	O–F	—
	vi. 53	O	—
Weymouth	iv. 48	F	5 % of *B. balanoides* population
	vi. 49	F	No perceptible increase
Portland Bill	vi. 49	N	—
	vi. 53	N	—
West Bay	xi. 48	R	5 small specimens in inner harbour
	vii. 49	N	—
	vii. 54	N	—
Seatown	vii. 49	N	—
Lyme Regis	xi. 48	N	—
	vii. 50	N	—
	vii. 54	N	—
Seaton	vi. 47	N	—
	xi. 48	N	—
	vii. 54	N	—
Sidmouth	vii. 54	N	—
Budleigh Salterton	viii. 47	N	—
	vii. 54	N	—
Starcross	ix. 47	N	—
	iii. 49	N	A.H.N.M.
	xi. 51	R	—
	iv. 54	F	—
Dawlish	v. 53	O	—
Teignmouth	viii. 49	O	Confined to River Teign estuary
	vii. 50	F	One specimen from Teignmouth pier
Torquay	ii. 48	N	P.N.J.C.
	vi. 49	R	—
	vi. 51	O–R	—
Paignton and Broadsands	xii. 47	R	—
	vi. 48	R	—
	vii. 50	R–O	—
	iv. 54	O	—

APPENDIX (*cont.*)

Place	Date	Density	Notes
Brixham Harbour	vii. 48	N	—
	viii. 49	R	—
	vii. 50	R–O	A self-maintaining colony at foot of breakwater
	xii. 51	O	—
	iv. 54	O	—
St Mary's Bay and Mansands	iv. 48	R	P.N.J.C.
	viii. 50	R	—
Dartmouth	iii. 48	N	—
	x. 49	R	P.N.J.C.
	iii. 50	O	G.W.R. Ferry
	iv. 54	C–A	Higher Ferry
Greenway, River Dart	ix. 49	C	P.N.J.C.
Galmpton	v. 48	N	P.N.J.C.
	iii. 49	O	M.W.H.D.
Stoke Gabriel	v. 48	N	P.N.J.C.
	iii. 50	O	—
Blackpool, Devon	iv. 50	N	—
Torcross	iv. 50	N	—
Charleton Bridge	iii. 50	C	—
	iv. 54	A	—
Kingsbridge	viii. 49	O	—
Head of Frogmore Creek, Salcombe	iv. 56	A	*Elminius* only barnacle. A.J.S.
Salcombe Harbour	iv. 49	R	—
	iii. 50	F	—
Salcombe, south beach	x. 48	N	—
	iii. 50	N	—
	iv. 54	O	—
Bolt Head	vi. 49	N	P.N.J.C.
	iv. 57	N	—
Thurlestone	vi. 49	N	P.N.J.C.
	iv. 57	N	—
Bantham, River Avon	iv. 49	N	P.N.J.C.
	vi. 49	R	—
Ermemouth	viii. 49	R	—
	v. 55	F–C	A.J.S.
Steer Point, River Yealm	vi. 49	N	P.N.J.C.
Newton Ferrers	x. 48	R–O	—
	viii. 49	F	—
	iv. 57	C	—
Breakwater, Plymouth	ix. 51	F–C	A.J.S.
	xii. 54	F–C	A.J.S.
	ii. 56	C	Abundance confined to sheltered side of breakwater. A.J.S.
Rum Bay, Plymouth	viii. 48	N	P.N.J.C.
Tinside, Plymouth	v. 46	O	On mussels on old pier. P.N.J.C.
	xii. 46	O	G.W.R. dock
	xii. 54	C–A	A.J.S.
River Plym, Laira Bridge	iii. 50	C	—
River Tamar, near Saltash	viii. 49	F	—
	xii. 54	A	A.J.S.
Torpoint	viii. 49	C	—
Hole's Hole, Tamar	ii. 56	C	A.J.S.
Rame Head	i. 50	R	P.N.J.C.
	v. 52	R	J.H.O.
	x. 56	F–C	A.J.S.
Looe Beach	ix. 49	N	A.H.N.M.
	i. 50	O	None to east of Looe on exposed rock. P.N.J.C.
Looe River	viii. 48	N	P.N.J.C.
	ix. 49	F	A.H.N.M.
	i. 50	F	P.N.J.C.
Polperro	viii. 48	N	P.N.J.C.
Fowey	i 50.	F	—
Par	i. 50	O	—
	v. 55	F	—
Charlestown	i. 50	N	—
Falmouth	i. 50	N	—
	iv. 56	R	A.J.S.

Place	Date	Density	Notes
Malpas	vi. 49	C	E.W.K.J.
Meanporth and Durgan	v. 50	N	—
Helford Passage	1947	O	Well established but not common. H.A.C.
	v. 50	O	—
Porth Navas	v. 50	F	—
	v. 55	C	A.J.S.
Gweek	v. 50	R	—
	v. 55	A	A.J.S.
Coverack	vi. 51	N	—
Lizard	vi. 51	N	—
	v. 55	N	A.J.S.
Porthleven	v. 50	N	—
	v. 55	N	A.J.S.
Prah Sands	v. 50	N	—
Penzance	v. 50	N	—
	v. 55	O	A.J.S.
Scilly	v. 55	N	A.J.S.
Mousehole, Lamorna, Treen, Sennen, Morvah, Zennor	v. 55	N	A.J.S.
St Ives	v. 50	N	—
	v. 55	O	A.J.S.
Hayle	v. 50	N	—
Porthcothan	viii. 48	N	D.P.W.
Padstowe	viii. 48	N	D.P.W.
Port Isaac	viii. 48	N	D.P.W.
	v. 53	N	—
Porth Gaverne	v. 53	N	—
	v. 55	N	A.J.S.
Tintagel	v. 53	N	—
Boscastle	v. 53	N	—
	v. 55	N	A.J.S.
Bude	v. 53	N	—
	v. 55	N	A.J.S.
Clovelly	viii. 49	N	P.N.J.C.
	v. 51	N	—
Westward Ho!	vii. 49	N	E.N.
	v. 51	N	—
	viii. 54	N	—
Barnstaple	vii. 49	N	E.N.
Appledore and Bideford	viii. 48	N	—
	vii. 49	N	E.N.
	vii. 52	R	E.N.
Saunton sands	vii. 49	N	E.N.
	viii. 54	N	—
Woolacombe	vii. 49	N	E.N.
	viii. 54	O	—
Ilfracombe	vii. 49	N	E.N.
	viii. 54	O	—
Combe Martin	v. 54	N	A.J.S.
Lynmouth	viii. 54	F	—
Porlock	viii. 54	F–C	—
Minehead	ix. 49	R	—
Blue Anchor	xii. 47	O	C.M.H.
	i. 48	R	P.N.J.C.
	ix. 49	R	—
	viii. 54	F	—
Watchet	xii. 47	N	C.M.H.
	ix. 49	O	—
	viii. 54	C	—
Kilve	viii. 54	A	—
Lylstock	v. 54	F	A.J.S.
Weston-super-mare	iii. 50	R	—
	v. 54	C	A.J.S.
Clevedon	iii. 50	N	—
Portishead	iii. 50	N	—
	v. 54	N	A.J.S.
Newport (Mon)	viii. 49	N	A.H.N.M.
Cardiff	viii. 49	N	A.H.N.M.
Penarth	viii. 49	O	A.H.N.M.
Sully Point	viii. 49	N	A.H.N.M.
Barry	x. 47	R	G.D.W.
	vii. 54	O	—
Rhoose and Aberthaw	viii. 49	N	A.H.N.M.
	vii. 54	O–R	—
Stout Point	viii. 49	N	A.H.N.M.

Place	Date	Density	Notes
Nash Point	viii. 49	R	A.H.N.M.
	vii. 54	C	—
Dunraven Castle	viii. 49	N	A.H.N.M.
Porthcawl	vii. 54	O	—
Port Talbot	viii. 49	N	A.H.N.M.
Swansea	viii. 49	R	A.H.N.M.
	vii. 54	C	—
Mumbles	viii. 49	N	A.H.N.M.
	vii. 54	F	—
Oxwich	viii. 53	F	—
Port Eynon	vii. 54	O	—
Worms Head, Gower	vii. 54	F	Mostly towards L.W.M.
Llanelly	viii. 49	R	A.H.N.M.
	viii. 53	C	—
Pendine	viii. 49	N	A.H.N.M.
	viii. 53	C	—
Amroth	viii. 53	C	—
Saundersfoot	viii. 53	C	—
Tenby	viii. 49	N	A.H.N.M.
	viii. 53	O	—
Freshwater, west	viii. 53	N	—
Dale Fort	xi. 51	O	Just appeared at H.W.N. tide level. J.H.B.
Neyland	x. 46	N	—
	ix. 47	R–O	—
	iii. 49	C	A.H.N.M.
	viii. 53	C–A	—
St David's	viii. 53	N	—
Porthgain	viii. 53	N	—
Goodwick and Fishguard	iii. 49	N	A.H.N.M.
Newport	iii. 49	N	A.H.N.M.
Gwbert	viii. 53	N	—
Aberporth	viii. 53	N	—
Aberayron	viii. 53	N	—
Aberystwyth	viii. 53	N	—
	vii. 57	N	—
Borth	iv. 52	N	—
Barmouth	viii. 53	N	—
	v. 57	O	—
Mochras	viii. 53	N	—
Harlech	iv. 50	N	—
	iv. 56	N	—
Criccieth	vi. 52	N	—
	iii. 53	N	—
	iii. 54	R	E.W.K.J.
	v. 56	R	—
Afon Wen	v. 53	N	—
Pwllheli	v. 53	N	—
	viii. 55	O	E.W.K.J.
Llanbedrog	v. 53	N	—
	viii. 55	O–R	—
Abersoch	v. 53	N	—
	viii. 55	R	—
Trwyn Cilan	viii. 55	N	—
Hell's Mouth	viii. 55	N	—
Porth Oer	viii. 55	N	—
Nevin and Porth Dinlleyn	iv. 50	N	—
	viii. 55	C	None on exposed reefs, confined to bays
Trevor	v. 53	N	—
	viii. 55	F	—
Clynnog	iv. 50	N	—
	xii. 54	O	—
Llandwrog	xi. 51	N	—
	v. 55	O	—
Caernarvon	iv. 50	N	—
	x. 51	R	—
	xii. 52	N	—
	v. 53	N	—
	ix. 53	R	—
	viii. 55	F	—
Port Dinorwic	ii. 52	N	—
	v. 53	N	—
	v. 54	F	—
	vii. 55	F	—
Bangor	xi. 51	N	—
	ii. 52	N	—
	iii. 53	O–R	—
	xi. 53	C	Common, tubular bridge
	iii. 54	C	—
	iii. 55	A	—

Place	Date	Density	Notes
Llanddwyn	iv. 52	N	—
	v. 55	F	On mussel bed
Aberffraw	x. 51	N	—
	iii. 53	N	—
	xi. 53	N	—
	vi. 54	O	—
	viii. 55	F–C	—
Rhosneigr	iii. 52	N	—
	iii. 55	O	—
	viii. 55	F	—
Rhoscolyn	viii. 53	N	—
Porth Dafarch	v. 53	N	—
	viii. 55	N	—
Holyhead	iv. 50	R	—
	xi. 51	N	—
	v. 53	N	—
	viii. 55	F–C	Rather more common in inner harbour where there are fewer *B. balanoides*. Very common in inland sea
Church Bay	v. 53	N	—
	viii. 55	R	Little suitable substratum
Cemaes Bay	iv. 50	N	—
	vii. 53	N	—
	viii. 55	C	On walls, inner harbour
Bull Bay	iv. 50	N	—
	ii. 53	N	—
	vi. 53	N	—
	vi. 54	N	—
	viii. 55	F	—
Amlwch	iv. 50	N	—
	i. 53	N	—
	iii. 54	O	In submarine tunnel
Point Lynas Bay	xii. 52	N	—
	vi. 54	F	Occasional specimens on headland
Llys Dulas	ix. 52	N	—
Moelfre	xii. 52	O	—
Benllech	xi. 51	N	—
	vi. 52	N	—
	xii. 52	F	All of small size
	vi. 55	C	—
Penmon	iv. 52	N	—
Black Rock	viii. 52	O	—
	iv. 53	F	—
	iii. 54	C	—
Menai Bridge	vii. 51	N	—
	iv. 52	R	—
	iv. 53	O	Young spat frequent on pier, few elsewhere
	xii. 54	A	—
	vii. 55	A	—
Llanfairfechan	x. 51	N	—
	vi. 52	R	—
	xi. 53	F–C	—
	xii. 54	A	—
Conway	vii. 51	R	One specimen on a boat. E.W.K.J.
	x. 51	R	—
	ix. 52	O	On mussels in River Conway
	vi. 53	C	Settling in fair numbers, 2–3/cm²
	vii. 55	A	—
Llandudno	x. 47	N	P.N.J.C.
	iii. 50	R	—
	xi. 51	R–O	—
	vii. 52	F–O	—
	x. 54	C–A	Less common towards Orme's Head
Colwyn Bay	ix. 47	N	—
	iii. 50	N	—
	iii. 51	N	—
	xi. 51	F	0·04/cm² on pier piles, where it is most abundant
	vii. 52	C	Adults 0·3/cm², spat settling heavily
	ix. 52	A	—
Abergele	xi. 51	A	—
Rhyl	ix. 47	N	—
	v. 50	N	—
	xi. 51	A	Common in river, abundant on piles of pier

APPENDIX (*cont.*)

Place	Date	Density	Notes
Rhyl	vi. 53	A	—
Mostyn	xi. 51	O	—
	vi. 53	F	—
Greenfields	vi. 53	A	—
Connahs Quay	vi. 53	A	Survived high cyanide concentration during fish mortality of 1953, except in immediate vicinity of effluent
Heswall	xi. 51	C	—
West Kirby	v. 50	N	—
	xi. 51	C	—
	vi. 53	C–A	—
New Brighton	x. 47	N	P.N.J.C.
	v. 50	A	—
	xi. 51	A	—
	vi. 53	A	—
Liverpool	x. 47	N	P.N.J.C.
	v. 50	O	—
	xi. 51	C	—
Point of Air	ix. 55	N	Very unsuitable area, shingle scour
Ramsey	i. 52	R	A.J.S.
	ii. 53	N	A.J.S.
	ix. 55	F	A few 1–1½ years old
Laxey	ix. 55	O	Rare except at H.W near stream
Douglas	ix. 55	R	Two large individuals only
Castletown	ix. 55	N	—
Port Erin	ix. 55	N	—
Peel	ix. 55	N	—
Lytham St Anne's	x. 47	N	P.N.J.C.
	v. 50	O	—
Blackpool	v. 50	C	—
	ix. 53	A	—
Rossal	x. 47	N	P.N.J.C.
	v. 50	F	—
	x. 53	C	Heavy scour reduces numbers
River Wyre, Fleetwood	v. 50	O	—
Morecambe	vi. 50	A	Growing on parieties and valves of existing *B. balanoides* population
Grange-over-Sands	viii. 52	A	—
Bardsea	vii. 53	A	—
Barrow, Walney Channel	vi. 50	O	—
Barrow, Walney Island	vi. 50	N	—
	vii. 53	F–C	—
Millom	viii. 52	F	—
Ravenglass	vi. 50	O	On railway bridge, only 1 or 2 specimens
	viii. 52	C	On mussel beds, less common than *B. balanoides*
	vii. 53	C–A	Nearly as common as *B. balanoides*
	viii. 55	A	*Elminius* three times as common as *B. balanoides*. Spatfall 1·5/cm²
Seascale	vi. 50	N	—
	viii. 52	F–C	About 0·1/cm², patchy, much less common than *B. balanoides*
	vii. 53	C	About 0·25/cm² in groups
	viii. 55	A	About 1·5/cm², equal to *B. balanoides*
St Bees	vi. 50	N	—
	viii. 52	O–R	—
St Bees	vii. 53	C	As high as 0·2/cm² in patches
	viii. 55	C	Adults 0·06/cm², spat 1/cm²
Whitehaven	vi. 50	N	—
	vii. 53	F–O	Up to 0·04/cm²
	viii. 55	C–A	Abundant on loose stones in harbour
Harrington	vi. 50	N	—
	vii. 53	R	—
Workington	viii. 52	N	Much scour, not very suitable
Maryport	vi. 50	N	—
	viii. 52	N	—
	viii. 55	C–A	—
River Nith, Overton Merse	iii. 51	R	Found at H.W. where only suitable stones exist. P.N.J.C.
	viii. 55	F	—
Sutherness Point	x. 50	R	—
	viii. 55	A	Spatfall 25/cm²
Rockliffe, Rough Firth	viii. 55	A	—
Port Mary	viii. 55	O	Mainly at L.W. under stones
Manxman's Lake	viii. 55	F	—
Kircudbright Bridge	viii. 55	C	—
Fleet Bay	ix. 53	N	—
	viii. 55	C–A	All rather small, spatfall 10/cm²
Ravenshall Rocks	viii. 52	N	—
	ix. 53	N	—
	viii. 55	O	—
Creetown	viii. 52	N	—
	viii. 55	C	Much less common than *B. balanoides*
Garliestown	viii. 55	O	No spatfall seen
Isle of Whithorn	viii. 55	O	—
Monreith Bay	viii. 55	N	—
Luce Bay, Port William	ix. 53	N	—
	viii. 55	N	—
Auchenmalg Bay	ix. 53	N	—
	viii. 55	N	—
Sandhead	ix. 53	N	—
	viii. 55	N	—
Ardwell	ix. 53	N	—
	viii. 55	N	—
Drummore	ix. 53	N	—
	viii. 55	R	—
Port Patrick	i. 50	N	—
Corsewall Pt	ix. 53	N	—
Stranraer, Loch Ryan	i. 50	O	—
	ix. 53	O–R	All old specimens
	ix. 55	O–R	No change
Kirkcolm	ix. 53	R	—
Ballantrae	i. 50	N	—
	ix. 53	N	—
Lendalfoot	ix. 53	N	—
Girvan	ix. 53	N	—
Ayr	i. 50	N	—
Troon	i. 50	N	—
	x. 53	N	—
Ardrossan Saltcoats	i. 50	N	—
	x. 53	N	—
West Kilbride	x. 50	N	—
Fairlie	x. 50	N	—
Largs	x. 50	N	—
	x. 53	N	—
Dumbarton	x. 50	N	—
Millport, Isle of Cumbrae	x. 50	N	—
	1955	R	J.H.C.

Observers: J.H.B., Mr J. H. Barrett; M.W.H.B., Mr M. W. H. Bishop; H.O.B., Dr H. O. Bull; P.N.J.C., Dr P. N. J. Chipperfield; H.A.C., Dr H. A. Cole; J.H.C., Dr J. H. Connell; M.W.H.D., Mr M. W. H. Dowell; C.M.H., Miss C. M. Harrison; E.W.K.J., Prof. E. W. Knight-Jones; A.H.N.M., Mr A. H. N. Molesworth; E.N. Mr E. Norris; J.H.O., the late Prof. J. H. Orton; A.J.S., Dr A. J. Southward; E.A.S., Prof. E. A. Spaul; H.G.S., Dr H. G. Stubbings; G.D.W., Dr G. D. Waugh; D.P.W., Dr D.P. Wilson.

Careful searches have also been carried out on west, north and east coasts of Scotland, and on the coasts of Ireland, especially the ports Belfast, Dublin, Larne and Rosslare. Up to 1953 no *Elminius* was found north of Loch Ryan in Scotland, nor anywhere in Ireland.

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SURVIVAL OF ANAEROBIC PERIODS BY TWO INTERTIDAL POLYCHAETES, *ARENICOLA MARINA* (L.) AND *OWENIA FUSIFORMIS* DELLE CHIAJE

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Borden (1931) found that the lugworm *Arenicola marina* L. did not increase its rate of oxygen consumption after short (2 h) periods under anaerobic conditions, and concluded that there was no evidence that these animals could go into debt for oxygen after the supply of oxygen in the blood had been used. She calculated that there was sufficient oxygen in the blood to last the worm about 1 h, calculating that the oxygen capacity per gramme worm was 0·037 ml., and the oxygen consumption per gramme hour was 0·031 ml. As the period during the experiment when oxygen was excluded from the animals was 2 h, anaerobic respiration would have continued for about 1 h, which should have been sufficient to necessitate an easily measurable increase in the oxygen consumption on return to normal conditions if a debt for oxygen had been produced. In vertebrates, and some invertebrates, such a debt would be incurred by glycolysis, the lactic acid produced being reoxydized to glycogen in the presence of free oxygen, and thus producing an initially increased oxygen consumption on return to aerobic conditions.

The absence of an oxygen debt after a period of anaerobic conditions may be due to one or more of several causes. During an anaerobic period, a poikilothermic animal may (1) reduce its metabolic rate to a very low level, (2) metabolize glycogen to an end-product other than lactic acid, (3) excrete the lactic acid produced, or (4) utilize protein or oil rather than glycogen as an energy source. In polychaetes there is also the possibility that lactic acid could be accumulated in the coelomic fluid and only slowly reoxidized. In all these instances no marked increase in oxygen consumption would be expected on return to normal aerobic conditions.

The method of surviving anaerobic periods by polychaetes is interesting in view of the regular or occasional subjection of some species to such conditions, especially in the intertidal zone, and of their ability to survive such conditions in the laboratory. Borden (1931) found *Arenicola* to be unaffected by short periods, and Hecht (1932) found that lugworms would in fact survive for as long as 9 days without oxygen. *Owenia fusiformis* Delle Chiaje is even more resistant; von Brand (1927) found that this species could survive for 21 days under strictly anaerobic conditions. Packard (1905) found *Amphitrite* and

Nereis to survive a day; Jacubowa & Malm (1931) investigated the survival of several species of polychaetes, and found them to survive for periods of from 1 to 10 days. Neither Borden (1931) nor Hecht (1932) made glycogen or lactic acid determinations on *Arenicola*, but von Brand (1927) found 0·38% of the wet weight in the lugworm was glycogen, and 5% in *Owenia*; in an earlier paper (Dales, 1957*a*) I found 1–2% in *Arenicola*, and in the present work 2% in *Owenia*.

The long survival time of *Owenia* coupled with the high glycogen content reported by von Brand (1927) suggests that survival of long anaerobic periods may be made possible by metabolism of glycogen. Also the knowledge that *Arenicola* can survive anaerobic periods much longer than could be endured solely by utilization of oxygen in the blood, yet does not show an increased oxygen consumption on return to normal aerated conditions, indicates that if glycogen breakdown does occur, this either does not lead solely or mainly to lactic acid as in vertebrate glycolysis, or the lactic acid is wholly excreted.

Consequently, the glycogen content of various parts of the body of *Arenicola* and of *Owenia* has been measured in worms under normal aerobic, and under varying periods of anaerobic conditions. The oil content, lactate and pyruvate content have also been estimated, and the distribution of glycogen and oil studied histochemically. The results are discussed in relation to the actual conditions with which these worms may contend in nature. The *Arenicola* were mostly collected at Chalkwell, Essex, rather small worms being used for convenience in the experiments, but some duplicate experiments were done on Plymouth worms which were much larger. The *Owenia* studied were collected from Tor Abbey sands, Devon, and from near St Mawes in Cornwall.

Much of this work was done at the Plymouth Laboratory and it is a pleasure to acknowledge the continued interest and help shown by the Director and Staff. I also wish to thank Miss M. Weir for doing much of the histological work, and Dr J. Green and Mr R. F. H. Freeman for performing Winkler oxygen determinations on the water in the experimental vessels. I am grateful for a grant awarded by the University of London Central Research Fund, and for the use of the London Table at Plymouth; and to Prof. G. P. Wells, Dr E. D. S. Corner, and Dr J. Green for reading the typescript and for their helpful criticism.

METHODS

Worms were placed in a 'Thermos' flask with ice immediately on collection, and dealt with as soon as possible on return to the laboratory, always within 3–4 h. The glycogen content of animals described here as 'fresh', are those so treated and weighed immediately on reaching the laboratory.

In the experiments the weights of all the *Arenicola* refers to whole animals minus tails, coelomic fluid and gut. The weights of the *Owenia* refers to complete animals

including gut and coelomic fluid. In both cases wet weights were obtained after drying on filter-paper; although the *Owenia* were small, the body is smooth and cylindrical and the animal is easily and quickly dried.

The experimental animals were placed in 500 ml. conical flasks half filled with sea water, with three small *Arenicola* or twenty *Owenia* in each. The *Owenia* were not removed from their tubes but these were seen to be clean before being used; the *Arenicola* were washed before use in an experiment. Experiments were performed in a constant temperature room at 18° C. To obtain anaerobic conditions a stream of nitrogen from a cylinder was passed through alkaline pyrogallol wash-bottles into a trap from which a number of closable jets protruded, each of which could be connected to an experimental flask. A stream of nitrogen was maintained through the water until the oxygen content was negligible before the animals were introduced. Then a very slow but continuous stream was maintained for the duration of the experiment, the gas escaping through a bunsen valve in the stopper closing the flask. In a few instances the oxygen contents of the water during and at the end of an experiment was checked with Winkler's reagents, and found to be no more than would be expected in the reagents. The gas was therefore not passed over heated copper as well as through alkaline pyrogallol as recommended by von Brand (1946) as the cylinders of nitrogen used were sufficiently pure. In all the experiments the animals were fasting.

Glycogen was estimated by hydrolysis to glucose which was then determined by Hagedorn & Jensen's method (1923) as described in a previous paper (Dales, 1957*a*). Lactate was estimated by the method of Barker & Summerson (1941), the tissue being fixed by dropping the rapidly weighed tissue into ice cold 10% trichloroacetic acid. Pyruvate was estimated by Lu's method (1939) using 2:4-dinitrophenlyhydrazine and extracting with ethyl acetate. The final colour in both lactate and pyruvate determinations was measured with a 'Unicam' S.P. 500 spectrophotometer, the lactate at 570 mμ, pyruvate at 550 mμ, against standards of known purity.

Total oil was measured gravimetrically by the method of Sperry (Glick, 1955). The tissue was disintegrated in a Griffeth pattern tissue disintegrator and dried with acetone which was then blown off in a current of nitrogen, the sample being left to dry in a nitrogen-filled desiccator. The dried tissue was weighed and then extracted with 2:1, $CHCl_3$:MeOH in the cold, the extract poured off and the tissue re-extracted with three successive portions of solvent. The extract was poured through fat-free filter-paper into a flat-bottomed tube, and the paper rinsed through. The combined extract was then stood in a large volume of distilled water overnight, and the aqueous layer then removed (without flocculent interface) by suction. The solution was then transferred to a small flask and evaporated to dryness *in vacuo*, the oil redissolved in $CHCl_3$ and transferred to a small weighing bottle weighing rather less than 1 g. The $CHCl_3$ was evaporated and the sample left in a nitrogen-filled desiccator until ready for weighing. The weight of the oil was determined by difference after redissolving away the oil with $CHCl_3$. An aperiodic microchemical balance with a sensitivity of 0·1 mg was used.

Distribution of glycogen and oil was studied by the methods previously described (Dales 1957*a*). Glycogen was stained by Best's method after embedding in ester wax (Smyth & Hopkins, 1948), and oil with acetylated sudan black B (Casselman, 1954) in propylene glycol (Chiffelle & Putt, 1951), after formal-calcium fixation (Baker, 1946), frozen sections being cut from gelatin embedded tissue.

RESULTS

Arenicola marina

The results of estimations of glycogen content of the body wall of animals under various conditions are summarized in Table 1. It will be seen that the glycogen content falls under conditions of fasting when aerated, but that much more glycogen is used under anaerobic conditions. Although the glycogen content of the gut was found to be much lower than that of the body wall, the concentration in 'fresh' animals and those under anaerobic conditions was also measured. The fall is not statistically significant. To test whether the glycogen content would rise in fasting worms after an anaerobic period (as in vertebrate glycolysis) the glycogen content was measured at the end of a 40 h period under anaerobic conditions and after a further 6 h under fresh aerated sea water. The concentration did not, however, approach that of the fresh control worms.

TABLE 1. *ARENICOLA:* GLYCOGEN IN THE BODY WALL AND GUT

	No. of estimations	Mean (wet) weight (g)	Extreme weights	Mean total glycogen (mg)	Standard deviation total glycogen	Mean concentration glycogen (mg/g)	Standard deviation glycogen concentration
Body wall							
'Fresh'	40	0·398	0·116–0·975	6·80	3·85	16·82	4·57
Fasting, 40 h aerated	30	0·263	0·088–0·819	3·42	2·51	11·95	3·51
Fasting, 40 h under anaerobic conditions	19	0·310	0·093–0·655	2·55	2·01	7·03	3·90
Fasting, 40 h under anaerobic conditions; aerated 6 h	12	0·219	0·097–0·305	2·11	1·17	9·85	4·60
Gut							
'Fresh'	15	0·151	0·069–0·296	1·27	0·63	8·32	2·86
Fasting, 40 h under anaerobic conditions	15	0·078	0·028–0·138	0·52	0·81	7·11	2·54

Oil content of the body wall in 'fresh' worms and those subjected to anaerobic conditions for 48 h was also measured (Table 2), and there was found to be no change.

Concentration of lactate in the body wall was not significantly different in 'fresh' worms, and those subjected to anaerobic conditions for 42 h, aerated controls or those returned for 6 h to fresh aerated sea water (Table 3). As any lactate secreted might be broken down by bacteria in the medium, in order to test whether any secretion of lactate occurs under anaerobic conditions, one series of experiments was done under the same conditions as before, but using specially cleaned glassware and boiled and autoclaved sea water to which 100 μg/ml. of streptomycin sulphate had been added. A detectable, but negligible amount of lactate was found in the medium, after 40 h under

anaerobic conditions with three worms (total mean weight 10 g) in 250 ml. sea water (Table 3).

Pyruvate was not detectable in the tissues of worms after 48 h under anaerobic conditions.

TABLE 2. *ARENICOLA*: OIL CONTENT OF BODY WALL

	No. of estimations	Mean (dry) weight (g)	Extreme weights	Mean total oil (mg)	Standard deviation total oil	Mean oil concentration (mg/g)	Standard deviation oil concentration
Group A							
'Fresh'	15	0·3380	0·2152–0·7115	17·1	7·0	50·20	5·59
After 48 h under anaerobic conditions	14	0·3877	0·2174–0·7694	18·8	6·7	49·20	6·52
Group B							
'Fresh'	13	0·1775	0·1132–0·2968	7·6	1·8	43·90	5·55
After 48 h under anaerobic conditions	11	0·0951	0·0658–0·1420	4·2	1·3	44·46	6·52

Group A = Plymouth worms (August, large); B = Chalkwell worms (October, small).

TABLE 3. *ARENICOLA*: LACTIC ACID IN THE BODY WALL

	'Fresh'	Fasting, aerated 42 h	Fasting, under anaerobic conditions 42 h	Fasting, under anaerobic conditions 42 h; aerated 6 h	Concentration in the external medium after 40 h under anaerobic conditions
No. of estimates	9	12	15	10	10
Mean concentration (mg/g)	0·297	0·338	0·365	0·362	0·007
Standard deviation of mean	0·164	0·180	0·165	0·215	0·0036

Owenia fusiformis

The glycogen content of whole 'fresh' *Owenia* and those that had been subjected for 5 days and 9 days to anaerobic conditions are shown in Table 4. There appears to be no fall in glycogen content. Consequently, no measurements of lactate were made. Estimations of oil content (Table 5) of 'fresh' worms and those subjected to 5 days of anaerobic conditions showed no significant change in concentration. There was no significant fall in weight after 9 days under anaerobic conditions, nor did the green pigment in the intestine (Dales, 1957*b*) appear to decrease in quantity.

DISCUSSION

It is quite clear that in *Arenicola* metabolism of glycogen under anaerobic conditions leads to products other than lactic acid. This is consistent with the findings of von Brand (1946), who concluded that invertebrates generally metabolized glycogen to a mixture of acids; which acids are produced by *Arenicola* has not been determined. Thus, while an oxygen debt may be avoided, the resources may be wasted if normal activity is continued. Vertebrate glycolysis may be more economical of material, but this incurs an oxygen

debt which can only be cleared in a short time by an efficient respiratory mechanism. Even if true glycolysis existed in the lugworm, it might be that the less efficient respiratory mechanism would be unable to clear the debt within a reasonable period.

TABLE 4. *OWENIA*: GLYCOGEN CONTENT OF WHOLE WORMS

	No. of estimations	Mean (wet) weight (g)	Extreme weights	Mean total glycogen (mg)	Standard deviation mean total glycogen	Mean concentration glycogen (mg/g)	Standard deviation concentration
'Fresh'	17	0·059	0·027–0·104	1·215	0·53	21·0	8·8
After 10 days in laboratory (aerated)	10	0·055	0·033–0·073	1·064	0·30	19·9	4·5
After 5 days under anaerobic conditions	32	0·059	0·023–0·096	0·978	0·41	20·7	7·9
After 9 days under anaerobic conditions	34	0·053	0·025–0·089	1·048	0·45	20·2	6·4

TABLE 5. *OWENIA*: OIL CONTENT OF WHOLE WORMS

	No. of estimations	Mean (dry) weight* (g)	Extreme weights*	Mean total oil (mg)	Standard deviation total oil	Concentration of oil (mg/g)	Standard deviation of concentration
'Fresh'	10	0·1961	0·1647–0·2337	11·6	1·54	59·27	8·28
After 5 days under anaerobic conditions	12	0·1887	0·1370–0·2245	12·2	1·84	64·30	3·45

* Twenty worms weighed together.

It also seems likely that in nature anaerobic periods would be short, and the glycogen used could easily be replenished. Intertidally, the burrows are probably rarely uncovered for more than 9 h, and commonly for much less, although Hecht (1932) found some worms in a situation covered only by spring tides. Presumably aerial respiration (the trapping of bubbles of air in the burrow, described by van Dam, 1938, Wells, 1945, 1949) would play an important part in the lives of these worms, and anaerobic periods are more likely to be encountered where a layer of stagnant water remains over the burrow openings. But even if exposed for 9 h to completely anaerobic conditions, the glycogen concentration of the body wall would fall only by 2 mg/g, and the worm would lose 11% of its total glycogen. Probably lugworms are rarely subjected to such long periods, and even when they are, there is every indication that the activity of the animal would be reduced. In the experiments the worms became rather quiescent after some time without oxygen, though still showing spontaneous body-wall contractions and bursts of activity. This agrees with Wells' findings (1949). Dr J. D. Jones found no significant difference in lactate content in worms dug at the beginning and end of a 5 h intertidal period (private communication).

It is already well known (Barcroft & Barcroft, 1924; Borden, 1931; Wolvekamp & Vreede, 1941; Wells, 1949; Jones, 1954) that the oxygen dissociation

curve of *Arenicola* haemoglobin shows that oxygen may be taken up at low outside concentrations, lower than usually occurs in an exposed burrow after 5 h exposure which was higher than that of the interstitial water (Jones, 1954) perhaps owing to aerial respiration by the worm. Wolvekamp & Vreede (1941) have criticized Barcroft & Barcroft's original idea (1924) that the oxygen in the blood acted as a store for use by the animal when the burrow was uncovered by the tide, but maintained that it was of use during resting periods in the irrigation of the burrow. More recent writers (Wells, 1949; Jones, 1954) have also inclined to minimize the usefulness of the oxygen in the blood for surviving anaerobic periods. On the other hand, in view of Wells' (1949) observations on aerial respiration and the characteristics of the haemoglobin, do lugworms often have to contend with long anaerobic periods? Linke (1939) found the temperature of the surface water on an *Arenicola* beach to rise as high as 26·2° C, and to 21·3° C at 10 cm depth; Thamdrup (1935) found that the oxygen consumption of *Arenicola* falls above 20° C, and Wells (1949) that under such conditions the normal irrigation of the burrow would cease. Wells found that worms would withstand 7 h in his glass apparatus when this was closed and which, at the time of closure would contain about 0·2 c.c. oxygen. The behaviour was modified completely under such conditions, the worm making only occasional 'testing' movements, though responding at once when a fresh supply of oxygenated water became available. This suggests that, even though glycogen is used under anaerobic conditions, worms conserve their energies by becoming quiescent.

This is certainly so in *Owenia*, in which there was no significant fall in glycogen concentration after 9 days under anaerobic conditions. After a few hours the worm became quiescent, and after some days, coiled up tightly in the middle of the tube and showed no sign of activity. Nothing is known about the characteristics of the haemoglobin in this species. The reason why *Owenia* does not use any of its glycogen, whereas *Arenicola* does, though reducing its activity, may also be related to the site of the glycogen deposits which is mainly in the body wall in the lugworm, but in the coelomic cells in *Owenia*. It is unlikely that these cells constitute reserves to be drawn upon during periods of inanition (Dales, 1957*a*); further work on their function is in progress. Again, while *Arenicola* shows bursts of activity under anaerobic conditions, *Owenia* does not.

Von Brand (1946) found that the quotient of aerobic: anaerobic glycogen consumption was low, although he found a relatively high glycogen content, commenting: 'Whether this indicates an exceptionally great reduction of metabolic rate under anaerobic conditions, or a more pronounced participation of fat or protein in anaerobic degradation processes, or whether it is due to fermentative processes in aerobic conditions has not been established.' The rather large discrepancy between von Brand's figures (1927) for glycogen content of *Owenia*, and those obtained here, may perhaps be accounted for by

the difference in weight, his worms were six times heavier, weighing 300 mg each. Von Brand (1927) found that, under anaerobic conditions, 0·23 g glycogen/100 g worm/24 h was consumed. If consumption continued at this rate, all would have been used in 21 days, the survival time von Brand found. *Owenia* from Tor Abbey sands being much smaller may have relatively less in the body wall, so that the consumption of this could not be detected in the presence of the large quantities in the coelomic cells. This ability to reduce activity may well have adaptive significance as these worms may temporarily be buried by the shifting sand they inhabit.

The ability to reduce the metabolic rate may be general in other polychaetes. There seems to be no correlation between the survival time of different species under anaerobic conditions (von Brand, 1946) and the glycogen content (von Brand, 1927). Indeed those species with the highest values for glycogen are the more specialized tube dwellers, which are often provided with well-developed coelomic cells in which most of the body glycogen is stored, and which seem least likely to encounter anaerobic conditions in nature.

SUMMARY

Measurements of glycogen in the body wall of *Arenicola* indicate that glycogen is consumed during anaerobic conditions. Estimations of lactate and pyruvate show that neither is accumulated, accounting for the absence of an oxygen debt previously found by other workers, and suggesting that glycogen breakdown leads to other acids. In *Owenia* most of the glycogen is stored in coelomic cells and these deposits are not drawn upon during anaerobic periods, yet this species can survive long periods without oxygen, apparently by becoming quiescent. Oil content in both species has also been measured, and was found not to fall under anaerobic conditions. It is suggested that survival of anaerobic periods may be mainly due to an ability to suspend normal activity.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

BLASCHKO, H. & HOPE, D. B., 1957. Observations on the distribution of amine oxidase in invertebrates. *Arch. Biochem. Biophys.*, Vol. 69, pp. 10–15.

The tissues of a number of marine invertebrates have been examined for the presence of the enzyme amine oxidase. No enzymic activity was found in three species of tunicates examined. Tryptamine and β-phenylethylamine were oxidized by extracts of gut of a polychaete, *Chaetopterus variopedatus*. Enzymic activity was found to be present in many, but not all, of the species of molluscs examined; of particular interest is the finding that the digestive gland and the anterior retractor muscle of the byssus in *Mytilus edulis* contain the enzyme; the latter muscle is known to be highly sensitive to 5-hydroxytryptamine. Among the echinoderms, all asteroids and echinoids had amine oxidase activity but no activity was detected in holothuroids. The presence of the enzyme in echinoderms suggests that biogenic amines, hitherto not detected, occur in these animals.

H.B.

DARTNALL, H. J. A., 1957. The spectral variation of the photosensitivities of some visual pigments. *Symposium on visual problems of colour, held at Nat. Phys. Lab. Teddington, Middx., from 23rd to 25th September* 1957, Paper No. 3.

The photosensitive retinal pigments of the tench (*Tinca tinca*), the carp (*Cyprinus carpio*), the gurnard (*Trigla cuculus*) and the conger eel (*Conger conger*) were extracted by means of 2% digitonin solutions.

It was found that the bleaching kinetics of these four pigments could be described by the same equation as that previously developed for the well-known rhodopsin or visual purple of the frog. The photosensitivities of the pigments were measured as ratios relative to the frog pigment over the wavelength range 440–580 mμ.

The absolute values of the photosensitivities of the four pigments were obtained by multiplying the ratios by the known values for the absolute photosensitivities of the frog pigment.

H.J.A.D.

HANSON, JEAN & LOWY, J., 1957. Structure of smooth muscles. *Nature, Lond.*, Vol. 180, pp. 906–9.

The detailed structure of smooth muscles from various invertebrate (particularly molluscan) and vertebrate animals was investigated by electron microscopy and phase-contrast light microscopy. It was found that there are several very different types of fibres: there is no 'typical' smooth muscle. The dimensions and structure of the protein filaments are highly variable. They may be randomly arranged in the fibre or closely and regularly packed into distinct myofibrils. In the muscles of squid (*Loligo*) the myofibrils are helically arranged and are composed of two kinds of filaments which are cross-linked to each other and closely resemble those in the anisotropic bands of cross-striated myofibrils; but the smooth fibrils have this construction along the whole of their length. The mechanism of contraction is probably the same in these particular smooth muscles as in cross-striated muscles. Other muscles have neither cross-striations nor a double array of filaments. In vertebrate smooth muscles the filaments

are all very thin and look alike. In the white (slow) part of lamellibranch adductors the 'filaments' are very large and appear to be composed of ribbon-shaped subfilaments with the elaborate 'paramyosin' structure. J.L.

HOBSON, G. E. & REES, K. R., 1957. The annelid phosphokinases. *Biochem. J.*, Vol. 65, pp. 305–6.

Following the isolation of taurocyamine and glycocyamine from a number of annelid worms, it was suggested that these guanidine bases, when phosphorylated on the terminal amino group functioned as 'phosphagens'. We have confirmed that fresh body-wall muscles from a number of polychaete worms very often contain a mixture of glycocyamine phosphate with either taurocyamine or creatine phosphate. Furthermore, we have shown the presence in the same tissues of the appropriate phosphokinase enzyme systems, capable of building up the phosphates from the guanidine and adenosine triphosphate.

The existence of these active transphosphorylation systems is held as evidence for the products serving a new but typical 'phosphagens' *in vivo*, for certain of the annelids. Their distribution, however, appears to be completely arbitrary. K.R.R.

JONES, W. C., 1957. The contractility and healing behaviour of pieces of *Leucosolenia complicata*. *Quart. J. micr. Sci.*, Vol. 98, pp. 203–17.

Isolated pieces of the body wall, when left in sea water or in $MgCl_2$:sea water, undergo a process of reorganization whereby the tubular form of the olynthus is regained. The piece first curls with the choanoderm on the inner surface, and a membrane then spreads from the four edges, closing off a new spongocoel. The membrane consists of the two surface epithelia with a thin layer of mesogloea in between. Its spread is accompanied by a shrinkage of the area of the original piece.

When the internal epithelium has been brushed away, the pieces shrink rapidly and curl longitudinally towards the dermal side.

The experiments indicate that both epithelia are contractile. There is evidence that the porocytes are responsible for the contractility of the internal epithelium and are interconnected beneath the bases of the choanocytes. W.C.J.

NICOL, J. A. C., 1957. Observations on photophores and luminescence in the teleost *Porichthys*. *Quart. J. micr. Sci.*, Vol. 98, pp. 179–88.

Histology and physiology of the photophores of an inshore teleost, *Porichthys myriaster*, are described. Photophores lie in the dermis: each consists of a lens, photogenic and reflecting layers, and is supplied by a nerve. Luminescence in the living fish was evoked by electrical stimulation of the spinal cord, and by injection of adrenaline. Latency, following stimulation of the cord, was 7–10 sec; after intracardiac injection of adrenaline, 2 min. Electrical stimulation of the cord produced luminescence after arrest of the circulation. It is suggested that the photophores of *Porichthys* are under control of the sympathetic nervous system. J.A.C.N.

ROSS, D. M., 1957. Quick and slow contractions in the isolated sphincter of the sea anemone, *Calliactis parasitica*. *J. exp. Biol.*, Vol. 34, pp. 11–28.

This paper deals with the physiology of isolated marginal rings of the sea anemone, *Calliactis parasitica*, a preparation containing the marginal sphincter muscle which is responsible for the powerful closing movement of this animal. Besides this quick

contraction, so well known from the work of Pantin as a unique example of a facilitated response, the preparation shows slow contractions in response to stimuli at lower frequencies. On account of the relative absence of spontaneous movements in this preparation it was possible to obtain a good deal of information about relationships between the size and latent period of the slow contractions obtained and the number and frequency of stimuli applied. There is a threshold number of stimuli at each frequency which causes only a tiny response and there is an optimal frequency at which the biggest responses are obtained with fewest stimuli. In considering the possible causes of 'fast' and 'slow' activities in the margin of *Calliactis*, the general evidence is in favour of an explanation which imagines muscle fibres of the same type which can contract in two different ways according to the number and frequency of impulses reaching them, and possibly according to the route by which the impulses travel. D.M.R.

STOTT, F. C., 1957. Observations on the food canal and associated structures in the holothurian *Holothuria forskali* delle Chiaje. *Proc. zool. Soc. Lond.*, Vol. 129, pp. 129–36.

A simpler nomenclature is suggested for the parts of the holothurian gut. The rete mirabile of *Holothuria forskali* showed three regions, namely, a relatively unpigmented region attached to the dorsal edge of the stomach which connected by a single strand to a yellowish brown region alongside the intestine which had a brownish black edge to it filled with melanin granules. Feeding with saccharated iron carbonate showed that some amoebocytic ingestion took place in the stomach and intestine and clumps of amoebocytes containing iron appeared in the haemal canals, lumen of the rete mirabile and in the walls of the respiratory trees, where it was probably egested. Injection of trypan blue into the body cavity resulted in its phagocytosis by the agranulocytes and their clumping together with the granulocytes on the respiratory trees, gonads and intestine. Migration into the lumen of the latter was observed. Melanin granules were identified within granulocytes in the lumen and walls of the rete mirabile as well as in the cells of the rete wall. F.C.S.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. 27 (p. 761) and Vol. 31 (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

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Members of the Association have the following rights and privileges: they elect annually the Officer and Council; they receive the Journal of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

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