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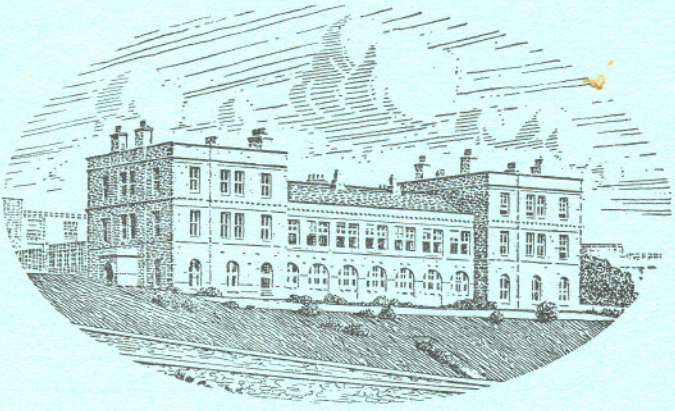

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STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. IV. THE POLYNOID GENERA *POLYNOË*, *LEPIDASTHENIA* AND *HARMOTHOË*

By Demorest Davenport

University of California, Santa Barbara College

The role of chemical substances released by hosts in governing the behaviour and determining the specificity of commensal polynoids has been demonstrated in previous investigations (Davenport, 1950, 1953; Davenport & Hickok, 1951). In all associations so far investigated, in which the host is an echinoderm, the commensals readily give a powerful positive response to their hosts either remotely or on contact, even after the partners have been kept under laboratory conditions for some while.

More difficulty has been experienced in demonstrating such a response in polynoids commensal with other annelids. *Halosydna brevisetosa*, commensal with *Amphitrite robusta* in Puget Sound, appears to be unable to discern the presence of its host at any distance but responds when it comes in contact with the host. Using *Gattyana cirrosa* and *Lepidasthenia argus*, commensal respectively with *Amphitrite johnstoni* and *A. edwardsi*, the author (1953) was unable with the Y-tube choice-apparatus of the 1950 and 1951 experiments to observe any indication of a sensitivity to water coming from aquaria containing hosts, even when a large number of fresh hosts were placed in the test aquarium. Using *Gattyana* which had been housed for some time with their hosts in glass U-tubes the author was unable to repeat previous observations made and reported to him by R. Phillips Dales, who, using freshly collected material, observed responses by these commensals to tentacle and body of their host, as well as to water drawn from the vicinity of the host's body by a pipette.

It became apparent that a thorough investigation of a terebellid-polynoid partnership required a continuous supply of fresh material, available regularly and not merely at the infrequent periods of low spring tides. The discovery of such a supply at Plymouth made the following experiments possible.

These investigations are a continuation of studies made during the winter of 1953 at the Plymouth Laboratory under a grant from the John Simon Guggenheim Memorial Foundation. The author is indebted to the Foundation and the Director and Staff of the Plymouth Laboratory for their support and co-operation.

Polynoë scolopendrina Savigny

Materials and Methods

On the Eddystone grounds great masses of intertwined *Chaetopterus* tubes may readily be dredged or trawled. The fauna occurring in and among these is rather diverse and worthy of detailed study and description. Here, making its rather characteristic tubes of sandy gravel mixed with bits of shell, between inhabited and in abandoned *Chaetopterus* tubes, occurs the handsome strawberry-coloured terebellid *Polymnia nebulosa* (Montagu). About 30% of these are accompanied by the commensal *Polynoë scolopendrina* Savigny, which, if large, often lies head-to-head along the body of the host or, if small, may be found wrapped about the extreme anterior region of the terebellid's body.

Although the *Plymouth Marine Fauna* (Marine Biological Association, 1931) gives no other host for this polynoid, Darboux (1899) states that at Wimereux the commensal is taken not uncommonly in the tubes of *Lanice conchilega* from deep water. McIntosh (1900) states that the polynoid occurs in the Channel Islands not only with *Polymnia* but also 'between chinks of rock (gneiss) in muddy sand in the tubes of' the eunicid *Lysidice ninetta*. It is of interest that in one locality, at least, the commensal occupies the tubes of two relatively distantly related annelids of different families, when it has not been recorded, so far, from more than two of the numerous terebellid species of Channel waters.

The technique of determining responses of the polynoids was very similar to that used in the analysis of the responses of *Acholoë astericola*. A number of commensals were placed in individual dishes in filtered 'outside' sea water. Materials to be tested were presented to the head of each commensal in rapid order and the response recorded. Between each series of tests the commensals were washed in clean sea water and the instruments in alcohol acetone.

At the very outset, brief experiments indicated that a commensal responded in a typical way if the host was brought in close proximity to, and in contact with, the commensal's head. The polynoid responded by orienting its head squarely in the direction of the host and by actively moving closer to it. If the body of the host was kept stationary and the commensal came in contact with an extended tentacle, it very quickly followed up the tentacle and brought itself alongside or underneath the host's body. If the body of the host were moved in any direction, the commensal would usually attempt to maintain contact no matter in what direction movement occurred.

Responses were recorded as follows. If the presentation of material at the head of the polynoid and subsequent movement of the material resulted in an immediate and active effort to follow it, the response was recorded as +. If the response was delayed or the activity produced was sluggish, this was recorded as (+). No response whatever after repeated presentation was recorded as 0.

Observations

Experiment No. 1. How specific is the response? Will the commensals respond to the presence of any polychaete if it is presented at their head? Members of four genera of polychaetes immediately available at the Laboratory were tested, with these results:

Worm	*Chaetopterus variopedatus*	*Arenicola marina*	*Myxicola infundibulum*	*Sabella pavonina*	Host control
1	o	o	o	o	+
2	o	o	o	o	(+)
3	o	o	o	o	+
4	o	o	o	o	o
5	o	o	o	o	+
6	o	o	o	o	+
7	o	o	o	o	+
8	o	o	o	o	+

It is clear that the commensals do not respond indiscriminately to polychaetes and that the host is 'recognized'. It is of interest that the commensals show no response whatever to *Chaetopterus*, with which in the particular environment from which the test sample came they are rather intimately associated.

Experiment No. 2. How specific is the response within the family Terebellidae, to which the host *Polymnia* belongs? A number of terebellids which were readily available at the time were tested:

Worm	*Amphitrite edwardsi*	*Terebella lapidaria*	*Polycirrus caliendrum*	Host control
1	o	o	o	+
2	o	o	o	o
3	o	o	o	+
4	o	o	o	+
5	o	o	o	+
6	o	o	o	(+)
7	o	o	o	+
8	o	o	o	+

Worm	*Amphitrite johnstoni*	*Amphitrite gracilis*	Host control
9	o	o	+
10	o	(+)	+
11	o	+	+
12	+	+	+
13	(+)	o	+
14	o	o	+
15	o	(+)	+
16	(+)	(+)	+

It would appear from these data that the response is not absolutely specific, since the *Polynoë* will occasionally respond to *Amphitrite johnstoni* and *gracilis*. Neither of these terebellids occur commonly in the same environment as the normal host.

Experiment No. 3. How do the commensals of *Polymnia* behave towards the two other polychaetes that have been recorded as their host, the terebellid *Lanice conchilega* and the eunicid *Lysidice ninetta*?. These were tested:

Worm	*Lanice conchilega* (Terebellidae)	*Lysidice ninetta* (Eunicidae)	Host control
1	o	(+)	+
2	o	(+)	+
3	o	+	+
4	o	+	+
5	o	+	+
6	o	o	+
7	o	(+)	+
8	o	+	+

The tests with *Lanice* were repeated with fresh material

Worm	*L. conchilega*	Host control
9	o	+
10	o	+
11	o	+
12	o	o
13	o	+
14	o	+
15	o	+

That the population from *Polymnia* does not respond to *Lanice* (recorded as an alternate host in France) seems to indicate that the attractants to which the *Polymnia* and *Lanice* populations are adapted to respond are not identical. It is possible, however, that the host-record for *Lanice* may be the result of a mistaken identification; *Lanice* is common enough in Plymouth waters but the association has never been observed there.

The significance of the strong response to the eunicid *Lysidice* is discussed in a further paragraph.

What can be determined concerning the manner in which the response is elicited and the nature and source of the attractant?

Experiment No. 4. *Polynoë* gives a marked response to host tentacle. Does simple physical contact with any terebellid tentacle initiate a response? Isolated tentacle from a form to which, when whole, *Polynoë* does not respond was tested:

Worm	*Amphitrite edwardsi* tentacle	Host tentacle
1	o	+
2	o	+
3	o	+
4	o	+
5	o	+
6	o	+
7	o	+
8	o	+

Contact with any tentacle does not initiate responses. Host tentacle is 'recognized'.

Experiment No. 5. In the above experiments it has been observed that the commensals are attracted by both the body and tentacle of their host. However, one may often find the *Polynoë* at some distance from the host within the host's tube. The tube of *Polymnia* is manufactured much as that of other terebellids by a secreted cement which knits together sandy gravel and bits of shell. Is the commensal 'bound' by the presence of attractant to the tube itself?

Bits of tube were presented at the heads of commensals:

Worm	Tube	Host control	Worm	Tube	Host control
1	(+)	+	9	o	+
2	(+)	+	10	o	o
3	+	+	11	(+)	(+)
4	(+)	+	12	o	+
5	+	+	13	+	+
6	(+)	+	14	(+)	+
7	+	+	15	(+)	+
8	(+)	+	16	(+)	+

These experiments indicate that enough attractant may be present in the host's tube to elicit sluggish or delayed and occasional full responses. Although care was taken in these experiments to remove bits of living tentacle from the pieces of tube, it is possible that bits were present and that responses were elicited by them, for occasionally pieces of tube which had unquestionably not long before been occupied by the host failed to elicit responses from any of eight commensals. Though these results are not entirely uniform, it appears that attractant may be present in fresh tube material, perhaps in reduced quantity or in an altered state.

Experiment No. 6. The body of the host is coated with transparent mucus. Is attractant present in it? When freed from its tube in a dish of clean sea water *Polymnia* produces mucus that may be richly taken up in cotton-wool. Such mucus-soaked cotton-wool was presented at the heads of commensals:

Worm	Cotton-wool plus mucus	Cotton-wool control	Host control
1	o	o	o
2	o	o	+
3	o	o	+
4	o	o	+
5	o	o	+
6	o	o	+
7	o	o	+
8	o	o	+

The data indicate that attractant is either not present in the mucus or that not enough is present in it to elicit even delayed responses.

Experiment No. 7. What can be determined concerning the source of attractant by dissection techniques? A large *Polymnia* was dissected and a number of tissues isolated and washed in clean 'outside' sea water. These were presented:

Worm	Host control	Gonad	Gut and blood	Gill	Ventral shield	Tip of abdomen
1	+	o	o	+	o	o
2	+	+	(+)	+	(+)	(+)
3	+	o	o	+	(+)	o
4	+	o	o	+	+	+
5	+	o	o	o	+	+
6	+	o	o	+	o	o
7	+	o	(+)	+	+	+
8	+	o	o	+	+	+

Exp. no. 4 has already shown that host tentacle elicits a powerful response. The above experiment indicates that gill is also very effective. The method of assay by means of responses is obviously not precise enough for comparisons between different tissues. However, it is clear that the internal organs gave little evidence of the presence of attractant (positive responses may have been the result of contamination in removal), while all the external tissues indicated the presence of enough attractant to elicit a number of full responses.

Experiment No. 8. In preliminary experiments to establish the regularity of response to host the commensals were occasionally observed to orientate their head toward the host when the host was not more than a mm. or so distant from it. In addition, Dales had reported (personal communication) that he had elicited responses from *Gattyana cirrosa* to water drawn with a pipette from a point close to the host's body. Can the presence of attractant in the immediate vicinity of the host be demonstrated consistently when contact with the host's body is effectively prevented?

An entire host was placed in a small sac of 200-mesh bolting silk and tied off. Great care was taken to prevent contamination of the outside of the sac by material from the host. The sac was presented:

Worm	Sac	Host control	Sac control
1	(+)	+	o
2	+	+	o
3	(+)	+	o
4	+	+	o
5	+	+	o
6	o	+	o
7	o	(+)	o
8	(+)	o	o

Evidence is presented by these data that a sufficient quantity of attractant diffuses a short distance from the host's body to be perceived by the commensal, since the small size of the mesh makes it unlikely that any direct contact with host tissue occurred.

Experiment No. 9. Can attractant be demonstrated in ground-up whole host? An entire fresh host was ground up in 3 ml. of clean 'outside' sea water and sand. Bits of cotton-wool soaked in this preparation were presented:

Worm	Preparation	Cotton-wool control	Host control
1	o	o	+
2	o	o	+
3	o	o	+
4	o	o	+
5	o	o	+
6	o	o	+
7	o	o	+
8	o	o	+

It thus appears that in the course of this preparation attractant is either destroyed or masked by the presence of injury substances.

The above experiments on the nature and source of the attractant indicate that the substance to which the polynoid responds is released and is present on the external surface of the host's body and may perhaps be involved in tube manufacture. That it is either unstable or very closely bound to other materials and hence released in such small quantity as to be rapidly diluted below the threshold of sensitivity of the polynoid is indicated by the fact that near contact is necessary for it to take effect. Destruction of the effect by grinding may be an indication of instability.

Work at present in progress indicates that it may be possible to learn more about the nature of some terebellid attractants by using tube material. *Polymnia* tube material, for some undetermined reason, does not always give constant results; attractant may be present in some parts of the tube and not in others. However, it has been possible to demonstrate that effective pieces of tube may retain their attraction for as long as 15 hr. after removal from the host if kept in cool circulation. Brief experiments have also indicated that temperatures as high as 60° C. destroy the attractiveness of tube material. One may be permitted to suspect that the attractant is a specific mucoprotein, but further investigations must obviously be carried out before this can be demonstrated.

Lepidasthenia argus Hodgson

From previous tests (Davenport, 1953) it was concluded that this handsome polynoid, commensal with the terebellid *Amphitrite edwardsi* which is its only known host, did not appear to be able to discern the presence of its host when tested in the Y-tube choice-apparatus of the 1950 experiments. However, experiments conducted on material freshly obtained with the aid of the Plymouth Easter Classes from the flats of Salcombe Estuary showed that these polynoids exhibit the same response when in close proximity to or

touching their host as does *Polynoë scolopendrina* to *Polymnia nebulosa*. How specific is this response?

Experiment No. 1. Two polychaetes were tested which may be taken in the same immediate environment as the partnership:

Worm	*Arenicola marina*	*Myxicola infundibulum*	Host control
1	o	o	+
2	o	o	+
3	o	o	+
4	o	o	+
5	o	o	+
6	o	o	+
7	o	o	+
8	o	o	+

Experiment No. 2. Tests were made with different members of the host's family, Terebellidae:

Worm	*Polymnia nebulosa*	*Terebella lapidaria*	*Amphitrite gracilis*	*Amphitrite johnstoni*	Host control
1	o	o	o	o	+
2	o	o	o	+	+
3	o	o	o	o	+
4	o	o	o	+	+
5	o	o	o	(+)	+
6	o	o	o	o	+
7	o	o	o	o	+
8	o	o	o	o	+

The response appears to be relatively specific within the family of the host. As might be expected, an occasional response was shown to *Amphitrite johnstoni*, which is closely related to the host. Such responses were exhibited by the large and active commensals as a movement directly to the body of *A. johnstoni*, followed by restlessness and a tendency to move away.

Both *A. edwardsi* and *A. johnstoni* occur at Salcombe. *Lepidasthenia* has never been taken with *Amphitrite johnstoni*, which does not appear to attract the polynoid strongly enough for an association to be established.

Harmothoë spinifera (Ehlers)

An investigation of the notes of the late Prof. J. H. Orton, F.R.S., now in the possession of Dr D. P. Wilson, disclosed that he had found a polynoid, which for want of accurate identification he labelled 'Polynoid B', in association with *Amphitrite gracilis* and *Polycirrus* (*caliendrum*?) at Rum Bay. The former association was re-discovered by the author during the 1953 Easter Class trips to Wembury and later at Mount Edgecumbe in Plymouth Sound. The animals can be obtained at medium low water by cracking apart the exposed soft layers of red shale; they appear to have somewhat the same ecological requirements as the eunicids *Marphysa* and *Lysidice*, often found near them. The polynoids were identified by Mr G. M. Spooner and the author as *Harmothoë spinifera* (Ehlers) in Fauvel (1923). With their hosts they have been preserved and placed in the Laboratory Museum.

Since the *Amphitrite gracilis–Harmothoë spinifera* partnership may be the most readily available intertidal terebellid-polynoid association in Plymouth waters, it seemed advisable to seek information concerning this polynoid's specificity of response.

Experiment No. 1. Do the commensals respond to the two polychaetes other than their host that occur commonly in the immediate red-shale environment? Two species were tested:

Worm	*Lysidice ninetta*	*Marphysa sanguinea*	Host control
1	o	o	(+)
2	o	o	+
3	o	o	+
4	o	o	+
5	o	o	+
6	o	o	(+)

Experiment No. 2. How specific is the response within the host family, Terebellidae? Four species were tested:

Worm	*Polymnia nebulosa*	*Terebella lapidaria*	*Amphitrite edwardsi*	*Amphitrite johnstoni*	Host control
1	o	o	(+)	(+)	+
2	o	o	o	o	+
3	o	o	o	o	+
4	o	o	(+)	(+)	+
5	o	o	o	o	+
6	o	o	(+)	o	+
7	(+)	(+)	o	o	+
8	o	o	(+)	(+)	+
9	o	o	(+)	o	+
10	o	(+)	(+)	(+)	+

Experiment No. 3. How does the commensal population from *Amphitrite gracilis* behave toward the alternate host *Polycirrus caliendrum*?

Worm	*Polycirrus caliendrum*	Host control
1	+	+
2	o	(+)
3	(+)	+
4	+	+
5	(+)	+

Repeated with a second test sample and control negative:

Worm	*Polycirrus caliendrum*	*Polymnia nebulosa* (control negative)	Host control
1	o	(+)	+
2	+	o	+
3	+	o	+
4	+	o	+
5	+	o	+
6	+	(+)	+
7	+	o	+
8	+	o	+

From these data we see that *Polycirrus caliendrum* (an alternate host of this commensal, according to Orton) elicits as strong responses from a population of the commensals taken from *Amphitrite gracilis* as the host, and indeed stronger responses than do other members of the host genus, *A. edwardsi* and *johnstoni*. The significance of this strong response to *Polycirrus* is discussed in a further paragraph.

Unfortunately, the author was unable to find a population of *Harmothoë spinifera* commensal with *Polycirrus* in order to make the logical cross-experiments. It would appear that Orton's 'Polynoid B' with *Polycirrus* was *Harmothoë spinifera*, for Mr G. M. Spooner has recently identified as *spinifera* a polynoid with *Polycirrus* taken in dredgings from Plymouth Sound.

Harmothoë lunulata (Delle Chiaje)

This form presents the most fascinating problem of all the polynoid commensals in European waters. It appears to have a number of variations that have been described under different names (Fauvel, 1923); it may be free-living or commensal. Table I, based on what appear to be well-established identifications, lists the hosts of this interesting species, with varietal names ignored. As can be seen, *H. lunulata* has been taken in commensalism with a limited number of species from four distantly related phyla, a situation

Table I. Hosts of *Harmothoë lunulata* (Delle Chiaje)

Chaetopoda	
Terebellidae	*Amphitrite edwardsi* Quatre. (Orton*; G.M.S., D.P.W.†)
	A. johnstoni Malmgren (Orton*; G.M.S., D.P.W., D.D.†)
	Polycirrus aurantiacus Grube (McIntosh, 1900; Fauvel, 1923; Orton*)
	Lanice conchilega Pallas (Fauvel, 1923)
Arenicolidae	*Arenicola marina* L. (Fauvel, 1923; G.M.S., D.P.W.†)
Eunicidae	*Marphysa sanguinea* Montagu (Marine Biological Association, 1931; Orton* and McIntosh, 1900: *H. marphysae?*)
Gephyrea	
Sipunculidae	*Phascolosoma vulgare* Blainville (Orton*; G.M.S., D.P.W.†)
	P. elongatum Keferstein (Orton*)
Ophiuroidea	
Amphiuridae	*Acrocnida brachiata* Montagu (Orton*; G.M.S., D.P.W., D.D.†)
Holothuroidea	
Synaptidae	*Leptosynapta inhaerens* O. F. Müller (Orton*; G.M.S., D.P.W.†)
	Labidoplax digitata Montagu (Orton*; D.P.W.†)
Asteroidea	
Astropectinidae	*Astropecten irregularis* Pennant (McIntosh, 1874‡)
Hemichordata	
Ptychoderidae	*Ptychodera minuta* [?] (Fauvel, 1923)

* From the collecting notes of the late Prof. J. H. Orton, F.R.S., in the possession of Dr D. P. Wilson at the Plymouth Laboratory.

† Records from the Salcombe and Yealm Estuaries; identification by G. M. Spooner and D. P. Wilson.

‡ A single record, but apparently well-established. McIntosh records its collection by Carrington at Southport *along with Acholoë* (!) from the ambulacral groove.

which somewhat resembles that of the commensals *Arctonoë pulchra* and *vittata* of the Pacific Coast of America (Davenport, 1950).

The difficulty of collecting enough commensals and hosts and of keeping all the animals alive at the same time has unfortunately allowed only the briefest experiments of the type conducted above. It is hoped that ultimately long enough series may be collected of the varieties of this species to permit a careful comparison of each population and its behaviour. However, with the co-operation of the 1953 Easter Classes it was possible to make preliminary investigations of the responses of three different populations of the commensal. At Salcombe, when a large group are digging, a reasonable number of *Harmothoë lunulata* may be collected with the ophiuroid *Acrocnida brachiata* (Montagu). Polynoids commensal with the holothurian *Leptosynapta inhaerens* (O. F. Müller) are not common but may occasionally be found. Likewise at the Yealm estuary *Harmothoë lunulata* is an occasional commensal with the terebellid *Amphitrite johnstoni*, whose more usual partner is *Gattyana cirrosa*.

The data that follow present the host responses of as large a number of these three commensal populations as could be collected by the classes, staff and author in two sets of trips to Salcombe and the Yealm during extreme spring

Worm	*Leptosynapta inhaer.*	*Amphitrite johnstoni* tentacle	*A. edwardsi* tentacle	*Polycirrus* (sp.?)	*Arenicola marina*	*Lanice conchil.*	*Phascolosoma elong.*	*Acrocnida brachiata*
			EXPERIMENT NO. 1. COMMENSALS (5–12 MM. LONG) WITH *ACROCNIDA*					
1	o	o	—	—	—	—	—	(+)
2	+	o	—	—	—	—	—	+
3	+	o	o	o	o	o	o	o
4	o	o	o	o	o	o	o	(+)
5	(+)	o	o	o	o	o	o	+
6	(+)	o	o	o	o	o	o	+
7	(+)	o	o	o	o	o	o	+
8	+	o	o	o	o	o	o	+
9	o	o	o	o	o	o	o	o
10	+	o	o	o	o	o	o	+
11	+	o	o	o	o	o	o	+
12	+	o	o	o	o	o	o	+
			EXPERIMENT NO. 2. COMMENSALS (29–31 MM. LONG) WITH *LEPTOSYNAPTA*					
1*	+	o	—	—	—	—	—	o
2	+	o	o	(+)	o	o	o	o
3	+	o	o	o	o	o	o	o
			EXPERIMENT NO. 3. COMMENSALS (16–32 MM. LONG) WITH *AMPHITRITE JOHNSTONI*					
1*	o	+	—	—	—	—	—	o
2	+	+	o	(+)	(+)	o	o	o
3	o	+	o	o	(+)	(+)	+	o
4	(+)	o	o	(+)	+	o	(+)	o
5	o	+	o	o	o	o	o	o
6	(+)	+	o	o	o	o	o	o

* First sample collected; untested alternate hosts not available.

tides. The collection and maintenance of the commensals and all the actual and reported hosts tested could quite obviously only have been accomplished with the help of the classes and of Mr G. M. Spooner and Mr P. G. Corbin, to whom the author wishes to express his thanks.

How did the commensals behave? Each population was tested against the actual and reported hosts available at the time (see Exps. 1–3).

If we examine the data of Exp. no. 1, an interesting fact emerges. It can be seen that *one* alternate host of the species, *Leptosynapta inhaerens*, has almost as strong an attraction for the commensals from *Acrocnida* as their own host. None of the other alternate hosts of the species do. However, if we examine the data of Exp. no. 2, keeping in mind that the sample of commensals tested is hardly large enough to allow any definite conclusions, we see that the three *lunulata* from *Leptosynapta* did not respond in return to *Acrocnida*, nor did they respond to any of the other alternate hosts of the species. The significance of these data will be discussed in a further paragraph.

The data from Exp. no. 3 are conflicting. These large active commensals from *Amphitrite johnstoni* are as difficult to work with as *Gattyana cirrosa*, for they are strongly affected by visual and tactile stimuli. They exhibit a powerful response to surfaces and some of their delayed and sluggish responses to alternate hosts (*Polycirrus*, *Arenicola*, *Phascolosoma*) may have been the result of other factors than chemical recognition. It is clear that they respond with the usual intensity to their own host, but a larger series must be assembled and perhaps a better experimental technique developed before an adequate comparative study can be made of their behaviour in relation to the various alternate hosts of the species.

Discussion

Studies on the specificity of responses have now been made in several genera of polynoids commensal with echinoderms and with other annelids. In the most extensive series of experiments (Davenport, 1953) conducted on echinoderm commensals (*Astropecten-Acholoë*), it has been shown that the polynoids respond to a number of starfish genera belonging to two taxonomic orders, which gives indication of some biochemical affinity among these rather distantly related starfish, at least as concerns the production of attractant. Perhaps here there is either a genetically determined (innate) or individually conditioned response to a substance which is produced by a number of different starfish, some of which are not the host of the polynoid. But in this type of association the specificity of response may not depend solely on chemical recognition of single factors acting as a bond between commensals. The possibility must be kept in mind that recognition-responses may be elicited by a particular pattern or sum of stimuli, part of which may not be chemical. Annelids are relatively highly organized and it has certainly

been demonstrated in many organisms with a fairly high level of nervous organization that recognition by chemical means alone may depend upon stimulation by a pattern or combination of substances. Responses of full intensity by commensals may, then, conceivably depend upon just the right combination of stimuli, a pattern produced only by the host. Any alteration of the pattern may dampen or abolish responses, so much so as to prevent the 'colonization' of species to which a delayed response may be given. Here, perhaps, the bond may simply not be strong enough to maintain the 'colonization'. In addition, many other ecological factors may prevent it.

However, the specificity of response of polynoids commensal with other annelids seems somewhat more strictly limited than that of the single echinoderm-annelid partnership the specificity of which has been investigated. In *Polynoë scolopendrina*, *Lepidasthenia argus*, and *Harmothoë spinifera*, responses, with one interesting exception, appear to be limited to members of the taxonomic family (Terebellidae) of the host from which the commensal population was taken. Responses to host species are far stronger than any that may be demonstrated to non-host members of the same genus or related genera. The tests with bolting-silk strongly suggest that the stimulus of primary importance in host recognition is chemical. It would appear, then, that these rather specific polynoid-terebellid associations may have in part evolved as a result of the development of innate or individually conditioned sensitivities to rather specific biochemical substances produced by their hosts (and perhaps at times in reduced quantity or in modified state by their host's relatives). That the attractants are relatively specific and differ to a recognizable extent from each other is indicated by the fact that *Polynoë scolopendrina*, *Lepidasthenia argus*, and *Harmothoë spinifera* will give constant responses of full intensity to their own hosts but not to each other's.

The tests in which *Polynoë scolopendrina* clearly exhibited a strong response to the eunicid *Lysidice ninetta* are of considerable interest. *Lysidice* may harbour this *Polynoë* (Channel Islands—McIntosh, 1900), but the association has not been recorded in Plymouth waters, where *Lysidice* is as relatively available as the normal host. At any rate the experimental population of *Polynoë* was taken in association with the terebellid *Polymnia nebulosa*, the usual host of the scale-worm at Plymouth. These commensals demonstrate no response whatever to members of three genera of terebellids which are certainly much more closely related to the host *Polymnia* than to the eunicid *Lysidice*.

Equally suggestive were the experiments in which *Harmothoë spinifera* taken with *Amphitrite gracilis* appears to respond more strongly to the alternate host *Polycirrus caliendrum* than to other species of *Amphitrite*.

Here for the first time evidence is presented that in single species which may occur commensally with unrelated hosts, the specificities, at least in some cases, may depend upon the production of similar attractants by the

unrelated hosts. Yet the experiments with the populations of *Harmothoë lunulata* are not quite in accord with this theory. The fact that *lunulata* taken from the brittle-star *Acrocnida* responded equally strongly to both *Acrocnida* and the alternate host holothurian *Leptosynapta inhaerens* seems in line with it, but were the attractants of these two host animals for their own commensal population in fact the same, then the population taken from *Leptosynapta* should have given an indication of a response to *Acrocnida*. Perhaps the *lunulata* from *Acrocnida* are adapted to a substance common to both the brittle-star and the holothurian, while those from *Leptosynapta* will respond to a different substance released by their host alone. Certainly the failure of the three commensal populations of *Harmothoë lunulata* to respond to the great majority of the alternate hosts of the species would argue that each of the three populations is adapted to respond to a substance not released by these alternate hosts.

Nevertheless, it would seem that if responses are the result of individual conditioning and are not innate, the attractants of such hosts as *Polymnia nebulosa* and *Lysidice ninetta* and of *Amphitrite gracilis* and *Polycirrus caliendrum* must be the same or very nearly so. On the other hand, if the response is innate and not individually conditioned (which seems to the author most likely, in view of the specificity and obvious long establishment of these associations), then the attractants may be the same or they may be different, under which latter circumstance one would have to admit the existence within a single commensal population of innate and highly precise responses to more than one attractant substance.

It is entirely possible that such multi-specific genetically determined responses may be important in governing the specificity of single commensal species that inhabit unrelated hosts. This again can only be determined by breeding experiments and the use of much larger samples of experimental material for behaviour studies than one has ordinarily available from restricted populations uncovered only rarely at extreme low tides. Certainly a deeper understanding of the mechanism of specificity of marine commensal associations can be obtained by a continued investigation of the behaviour of such species as *Harmothoë lunulata* existing as it does with such a diversity of hosts. If, as has been suspected (Wilson, 1951), the forms of *lunulata* which in ascending size respectively inhabit *Acrocnida*, *Leptosynapta* and *Amphitrite johnstoni* are nothing but different age-groups and not physiological races which can be separated on the basis of host-specificity, then one would have to hypothesize a different response-specificity for each age-group, determined perhaps by the state of development of the commensal. The behaviour studies on *Harmothoë lunulata* described above cast little light on this facet of the problem.

In an estuary such as Salcombe this *Harmothoë* occurs in association with so many animals that one may be tempted to conclude that it will occupy

'almost any convenient hole'. It is unquestionably true that the host specificity of marine commensals is 'superimposed' upon a number of other basic ecological requirements. Laing (1937) has ably demonstrated in the insects *Alysia* and *Mormoniella* that these parasites 'are attracted to an environment likely to contain their hosts by qualities of the environment itself, independent of the presence of hosts'. Whether this is also so in commensal polynoids, in which the early stages may have a long planktonic life, can only be determined by settlement and metamorphosis studies. At any rate, adequate data on the response-specificity of commensal populations must be assembled before such factors as the physical nature of the host's tube and the currents in it may be considered of primary importance in determining the specificity of the associations.

As a result of preliminary experiments with terebellid commensals (Davenport, 1953), the author stated that his observations indicated 'that a chemotaxis to the host may be of relatively minor importance in governing the behaviour of these commensals'. Clearly, this conclusion is no longer tenable; the observation of Orton & Smith (1935) that 'there is a tropic response on the part of the polynoid, causing it to enter an *Amphitrite* burrow whenever possible' was, although unsupported by data, correct.

Finally, all the studies so far conducted on echinoderm-annelid and annelid-annelid commensal partnerships indicate that normally commensals are restricted to their hosts by a positive chemotaxis to an unstable or closely-bound substance or pattern of substances. The adaptive significance of an unstable or closely bound attractant is clear; the function of the attractant could hardly be served if it were released in quantity and failed to break down or be rapidly destroyed or diluted in the environment of the partners.

Summary

The host-response of the polynoid *Polynoë scolopendrina* Savigny, commensal with the terebellid *Polymnia nebulosa* (Montagu), is relatively specific within the family Terebellidae.

Evidence is presented that an unstable or closely bound attractant is present on the outside of the host and perhaps in tube material, but apparently absent in the mucus secreted by the host. Efforts to demonstrate its presence in ground-up whole host resulted in failure.

The polynoid *Lepidasthenia argus* Hodgson demonstrates a similar specificity of response to its host *Amphitrite edwardsi* Quatrefages.

The polynoid *Harmothoë spinifera* Ehlers, commensal with *Amphitrite gracilis* Grube, responds to the alternate host *Polycirrus caliendrum* Claparède as strongly as to its own host, and more strongly than to other species of *Amphitrite*. Likewise, *Polynoë scolopendrina*, commensal with *Polymnia*, responds with greater intensity to the alternate host, the eunicid *Lysidice*

ninetta Audouin and Milne-Edwards, than to any non-host terebellid. This behaviour may be interpreted as evidence that the specificity of at least some single commensal species for hosts which are not closely related to each other may depend upon the production of similar attractants by these hosts.

The responses of three commensal populations of *Harmothoë lunulata* (Delle Chiaje), commensal respectively with the ophiuroid *Acrocnida brachiata* (Montagu), the holothurian *Leptosynapta inhaerens* (O. F. Müller), and the terebellid *Amphitrite johnstoni* Malmgren were tested against seven alternate hosts. Each gave maximum response to its own host. However, the population from *Acrocnida* responded as strongly to *Leptosynapta* as to its own host, while giving no responses at all to any of the other five.

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MOULTING HORMONES IN *LEANDER* (CRUSTACEA DECAPODA)

By D. B. Carlisle

The Plymouth Laboratory

(Text-fig. 1)

It is now well known that the initiation of the premoult in certain species of crabs and in the Astacidae is under the control of a moult-inhibiting hormone. Megušar (1912) observed that the removal of the eyes in *Astacus* led to an earlier moult. This was confirmed in the related *Cambarus* by Brown & Cunningham (1939). In the meantime, the same phenomenon had been observed in crabs of the genera *Uca* (Abramowitz & Abramowitz, 1938, 1940) and *Eriocheir* (Hanström, 1939). Kleinholz & Bourquin (1941) confirmed the finding for *Uca*, and Smith (1940) for *Cambarus*. Smith showed that this was not a result of injury alone. Brown & Cunningham (1939) followed eyestalk removal with implants of sinus glands and found that this prevented the early moult. They suggested that the sinus gland secreted a moult-inhibiting hormone whose absence allowed moulting to proceed. Kleinholz & Bourquin (1941) doubted this conclusion, but the work of Scudamore (1942, 1947) and Kyer (1942) on *Cambarus* went far to substantiate it. By 1947 it was generally accepted that the eyestalk secreted a moult-inhibiting hormone, and most workers believed that this originated in the sinus gland.

In 1951 a number of workers independently published papers which, while substantiating the hypothesis of a moult-inhibiting hormone, threw doubt on the suggestion that it originated in the sinus gland. Bliss (1951) working on *Gecarcinus*, Passano (1951*a*, *b*) on *Sesarma*, Frost, Saloum & Kleinholz (1951) and Havel & Kleinholz (1951) on *Astacus*, and Welsh (1951) on *Gecarcinus*, all found evidence which suggested that the true source of the moult-inhibiting hormone was the X-organ and that the sinus gland was merely a store. The story has been more fully worked out by Passano (1952*a*, *b*) and by Bliss & Welsh (1952). Briefly these authors believe that the moult-inhibiting hormone is formed in neuro-secretory cells of the X-organ, transported within the fibres of the sinus gland tract to the sinus gland, which consists merely of enlarged nerve endings, and there stored until finally released into the blood stream. Carlisle (1954*c*) has produced evidence that the same may be true of the ovarian-inhibiting hormone in the Mediterranean prawn *Lysmata*. Now the X-organ of most crabs and crayfish is specialized in that the two parts are united into a single organ, while in stomatopods, prawns and lobsters, hermit crabs and *Dromia* they are separated (Carlisle & Passano,

1953). Moreover, moulting in the species which have been investigated in the studies mentioned above seems to be characterized by its occurrence at specific seasons of the year—moulting seasons. *Cambarus*, for instance, moults in spring and autumn. Prawns, lobsters and some crabs, on the other hand, moult all the year round, and *a priori* we may suspect that moulting may never be directly inhibited in them. Travis (1951), indeed, failed to find any evidence of a moult-inhibiting hormone in the spiny rock lobster *Panulirus*, which has no definite moulting season and an intermoult period of 80 days under the condition of her experiments. The experiments reported here represent a failure to find any evidence for its occurrence in *Leander* (=*Palaemon*) *serratus*.

Experimental Data and Conclusions

Below 11° C. the rate of moulting of *Leander* is negligible, but it rises sharply above this temperature. All the observations recorded here were made at a temperature of $13{\cdot}5 \pm 1°$ C. The average intermoult period at this temperature was estimated by keeping 150 female prawns, between 55 and 70 mm. long (measured from the tip of the rostrum to the tip of the telson), through two or three successive moults, and taking the arithmetical mean of the individual intermoult periods. The prawns were kept singly in Breffit jars (4 lb. rock jars) in 1 l. of water each. They were fed twice weekly on squid flesh and the water was changed 3 hr. after feeding. Under these conditions the mean intermoult period was $34{\cdot}62 \pm 3{\cdot}19$ days.

Two hundred female prawns of the same size range were then selected, rejecting all which had recently moulted and were still soft. These were divided into two equal batches at random, using Fisher & Yates's (1943) table of random numbers for the purpose. Both eyestalks were removed from the individuals of one batch by electrocautery. The eyestalks were cut through at the base, at the level of the arthrodial membrane, using a red-hot platinum cautery needle. It was found that if the eyestalks were cut with scissors or with a knife and then cauterized the death-rate was much higher than if the whole operation was performed by cautery. This may be correlated perhaps with the complete absence of blood loss after direct cautero-ablation. These hundred operated animals were placed in a tank with the other hundred unoperated animals and left for 7 days; during this period they were fed twice *ad lib.* with squid flesh. Fifty from each group were then isolated in Breffit jars, one per jar, in 1 l. of sea water each. From then on until every animal in one group had either died or moulted the numbers which moulted and of those which died without first moulting were recorded each day in each group. The animals were fed twice weekly on squid flesh and the water changed 3 hr. after feeding. On the twenty-sixth day of this treatment the last animal, in the unoperated group, which had not already moulted, died. By that time only three animals in the operated group had neither died nor

moulted. The date on which each animal moulted was recorded on its jar, and after the twenty-sixth day those which were still alive, having already moulted, were continued under the same treatment until they either moulted again or died. In this way it was possible to determine empirically the intermoult period of some of the animals under these conditions. The figures are given

TABLE I

Operated	Unoperated controls
31	27
36	30
40	30
44	31
46	34
—	35
—	36
—	40
—	41
Mean *39·40*	*33·78*

Intermoult periods in days of those animals which survived through two moults.

in Table I. The average intermoult period calculated from these figures is 33·78 days for the unoperated controls and 39·40 for the operated animals. The difference is not significant ($P=0{\cdot}1$).

The numbers which died before moulting and of those which moulted during the earlier part of the experiment are given in Table II and the moult rate is illustrated graphically in Fig. 1. From these data we may obtain, by a calculation from the moult rate, an estimate of the average intermoult period in the animals of the two groups; in the unoperated controls this figure is 35·46 days and in the operated group 47·30 days. Statistical analysis of the data indicates that the difference in moult rate is not significant ($P=0{\cdot}5$). The method of analysis adopted was that used and described by Carlisle & Dohrn (1953).

There is no evidence from either of these tests in the one experiment that removal of the eyestalks results in an increased rate of moulting, a shorter intermoult period or earlier moulting; rather, in fact, the opposite. But have we used enough animals for this to be a fair test?—and has the experiment continued for long enough? The average intermoult period for unoperated animals at the temperature and under the conditions of the experiment has been calculated three times in two different ways; there is adequate agreement between the figures. The highest value of the three is 35·46 days. Now the first part of the experiment was concluded 7+26 days after the eyestalk ablation was performed—33 days afterwards. The removal of the eyestalks in crabs and crayfish initiates the premoult, or proecdysis. If the removal of the eyestalks in *Leander* in these experiments had initiated proecdysis, this phase of the moult cycle must be longer than 33 days as an average, for the moulting which terminates the premoult had not occurred *en masse* at the end of this

period. But the average intermoult period, including metecdysis and diecdysis as well as proecdysis, is only 35·46 days (taking the highest estimate—33·78 days if we take the lowest). This then only leaves 2·46 days (or 0·78) for the other stages of the intermoult besides the premoult, which is obviously out of the question. In other words, the first part of the experiment has continued long enough to test whether removal of eyestalks initiates proecdysis. Even more is this true for the second part, the continuation of the experiment.

TABLE II

	Operated		Unoperated controls	
Day	Dead	Moults	Dead	Moults
1	1	0	0	1
2	2	1	2	0
3	1	1	0	2
4	1	0	0	0
5	0	1	1	0
6	2	1	11	1
7	0	0	3	0
8	0	0	0	0
9	3	3	0	0
10	0	0	0	2
11	0	4	0	1
12	0	1	0	3
13	0	2	0	2
14	2	1	1	1
15	0	1	0	0
16	4	0	0	2
17	4	0	0	0
18	3	0	0	0
19	1	0	0	1
20	1	2	1	1
21	1	1	1	1
22	0	0	0	4
23	1	0	2	4
24	0	0	1	0
25	0	1	0	0
26	0	0	1	0
Totals	27	20	24	26

The numbers of animals which died without first moulting and of those which moulted on each day of the experiment.

From the data in the second part of the experiment we may calculate the probability that the mean intermoult period in the operated group is actually lower than that in the unoperated group, even though in our sample the reverse is true. The probability of this is about 5%, which is the usually accepted level of significance. This suggests that the number of observations made in the second part of the experiment is just adequate. Since more observations were made in the first part of the experiment the numbers were evidently adequate there also, and, moreover, the difference between the mean intermoult periods in the two groups is more pronounced in this first part.

It seems clear that whatever effect the removal of the eyestalks has had on the moulting of *Leander* under these conditions it has not produced an

abbreviated intermoult period, an earlier premoult or a higher moult rate. In fact there is an indication that the converse is true. Every experiment of this type that I have undertaken on *L. serratus* (=*Palaemon serratus*) or *L. squilla* (=*P. elegans*) has given a result similar to that found here—a lower moult rate and a longer intermoult period in the operated animals than in the unoperated controls, but with a difference that was never significant. These experiments took the form described above, but at varying temperatures and for varying lengths of time. None of them showed a significant difference in the moult rate. But when a compound probability is calculated for the difference in moult rate and intermoult period in the two groups (due attention

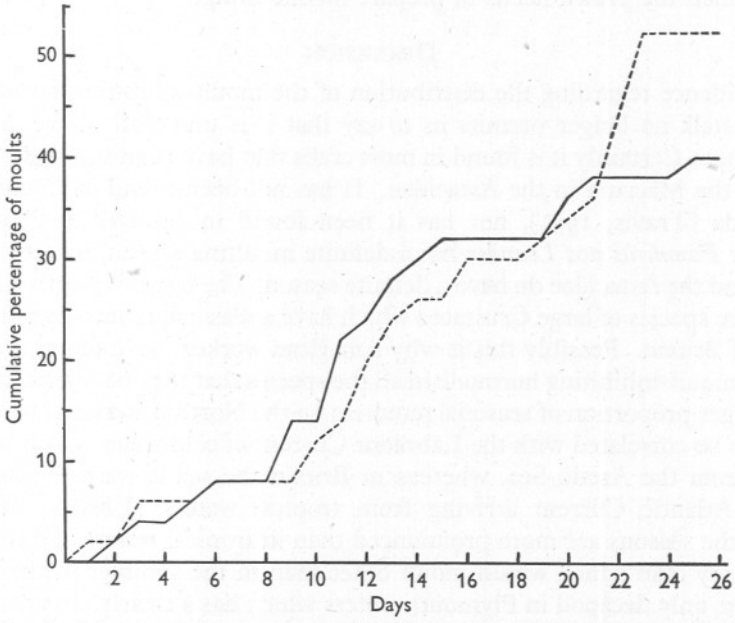

Fig. 1. Graph of the cumulative percentage of moults in the animals whose eyestalks had been removed (——) and in the unoperated controls (- - -). The number of days is measured from the date on which the animals were isolated, not from the date of the operation, which took place 7 days previously.

being paid to the sign of the difference) over the six experiments of this type which have been performed with these two species, the value of this statistic is less than 0·01. The average intermoult period was, as mentioned above, longer in the operated than in the unoperated groups. That is to say, far from demonstrating that removal of eyestalks leads to an increased moult rate and an abbreviated intermoult period, these experiments when taken together show just the opposite effect and the degree of the effect is significant. If we argue along the same lines as the workers who discovered the moult-inhibiting hormone in crabs and crayfish we shall conclude that in removing the eye-

stalks we are removing some organ which secretes a moult-accelerating hormone. The presence of such a hormone has already been demonstrated in the Mediterranean prawn *Lysmata* (Carlisle & Dohrn, 1953; Carlisle, 1954*a*) and it has been shown that it occurs in both parts of the X-organ (Carlisle, 1954*b*). Carlisle & Dohrn (1953) also showed that this hormone was present in extracts of eyestalks of *Palaemon* (=*Leander*) spp. when tested on *Lysmata*. Evidently, then, in removing the eyestalks of *Leander* we have reduced the amount of moult-accelerating hormone available to the animal (we have not removed the total supply, for it is produced in the thoracic and cerebral ganglia as well, see Carlisle, 1954*b*) and hence we have increased the length of time which the prawn needs to prepare for moulting.

Discussion

The evidence regarding the distribution of the moult-inhibiting hormone of the eyestalk no longer permits us to say that it is universal in the decapod Crustacea. Certainly it is found in most crabs that have been investigated and among the Macrura in the Astacidae. It has not been found in *Panulirus* in Bermuda (Travis, 1951), nor has it been found in *Leander* at Plymouth. Neither *Panulirus* nor *Leander* has a definite moulting season, whereas many crabs and the Astacidae do have a definite season. The coast of North America has more species of large Crustacea which have a seasonal moult than does the coast of Britain. Possibly this is why American workers have found evidence for the moult-inhibiting hormone in all the species that they have investigated. The larger proportion of seasonal moulters on the North American coasts may perhaps be correlated with the Labrador Current of cold water which streams down from the Arctic Sea, whereas in Britain the sea is warmer from the North Atlantic Current arriving from tropical waters. Clearly, in polar waters the seasons are more pronounced than in tropical waters and it would be a hardy crab which would moult other than in the summer in the Arctic Sea. The only decapod in Plymouth waters which has a clearly circumscribed moulting season, so far as I can ascertain, is *Maia squinado*, a northern form, which at Plymouth is towards the southern end of its range. It is possible, then, that the moult-inhibiting hormone is associated with seasonal moulting and with a cold water distribution, although it may well be present in other forms also which had perhaps a cold water ancestry.

Summary

Removal of the eyestalks in *Leander serratus* does not result in an earlier moult, a shorter intermoult period, or a higher moult rate. There is no evidence of an eyestalk moult-inhibiting hormone in this species. The evidence points to the existence of an eyestalk moult-accelerating hormone. The possible correlation of the presence of the moult-inhibiting hormone and seasonal moulting is discussed.

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SIZE VARIATIONS IN THE CYPRIDS OF SOME COMMON BARNACLES

By H. Barnes, D.Sc.
The Marine Station, Millport

(Text-figs. 1–4)

In descriptive works on cirripede larvae some of the dimensions of the various developmental stages are often quoted as an aid to identification, and data have already been given in this form for *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* (Pyefinch, 1948*a*; Bassindale, 1936; Runnström, 1925). There appear, however, to be no data on variations in size. The present account gives some observations on local variations, a discussion of which is a necessary preliminary to a study of regional variations and their significance.

The Collection and Examination of the Material

A number of different sampling methods have been used but most of the material has been collected by means of a Hardy Plankton Indicator (large model). The indicator was towed in the standard manner for a fixed distance (about 1 mile), the material being also collected for an investigation of annual changes in the barnacle population of this region. Such a method has the disadvantage that only one level is sampled, but other work has shown that the distribution of cyprids is fairly uniform between 0–20 metres. After completion of the tow the indicator was taken inboard, the plankton carefully washed off the silk into a bottle, and neutral formalin (5%) added. The whole sample was counted and measured, the measurements being made by means of a calibrated eye-piece micrometer. These hauls were taken regularly throughout the spring and were also supplemented by net hauls: later in the year a number of net hauls were made, and opportunity was also taken of using material collected by Mr V. Bainbridge during the course of his own work.

Attention has been largely confined to the cyprid stage, since this can be more easily and more accurately measured, but some nauplii have been measured. Results are presented for three species, *Balanus balanoides*, *B. crenatus* and *Verruca stroemia*, collected over the varying periods. The total length and the greatest width were measured with the cyprid lying on its side. Attention will primarily be concentrated on the lengths; but similar deductions can be made using the width measurements. In the stage VI total length and greatest width of the carapace were measured with the animal lying on its ventral surface. The scale of magnification was 50 eye-piece units = 1·02 mm.,

but it will be more convenient in the discussion of the results to refer to the arbitrary units. All the material was preserved in neutral formalin (5%), and strictly speaking the measurements refer only to animals preserved in this way.

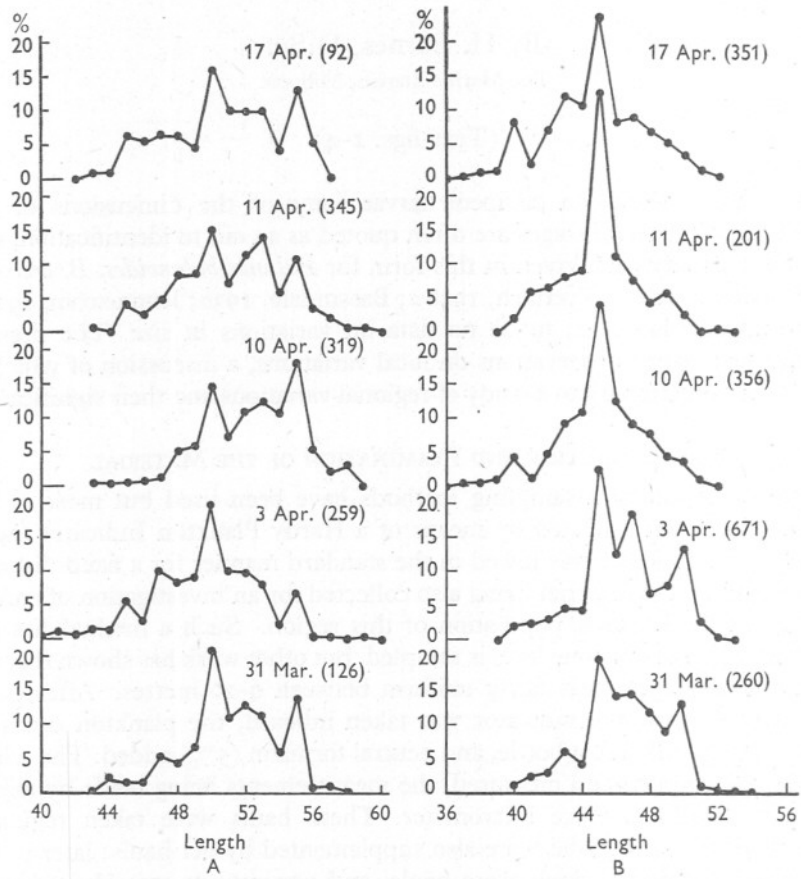

Fig. 1. Size-frequency distribution of *Balanus* sp. cyprids in a series of hauls through the spring. Length in eye-piece units. Dates and numbers in collections are given. A, *B. balanoides*; B, *B. crenatus*.

The Spring Outburst of *Balanus balanoides* and *Balanus crenatus*

The results for the cyprids of these two species are shown in Fig. 1, which gives the size-frequency curves together with the numbers of the animals measured, and the dates of collection. Even if the minor peaks are neglected it is clear that with both species these size-frequency curves exhibit a number of modes, which are to be presumed to correspond to a number of populations of the cypris stage each with its own mean size.

In *B. balanoides* the mode which is found at 50 units is present and well marked throughout the whole of the sampling period. A mode at 55 units is quite well marked in some of the samples, and there is a suggestion of other modes at intermediate points. In the earliest sample the 50-unit mode represents some 35% of the total frequency and is stronger than in the later samples. There is a tendency for the second mode at 55 units to be greater in strength later in the season. A study of the cumulative frequency curves supports the above deductions, but in view of the considerable overlap in the sizes they are not easy to analyse with certainty. It seems reasonable to suggest, however, that there are at any rate two distinct populations and that these are present throughout the season.

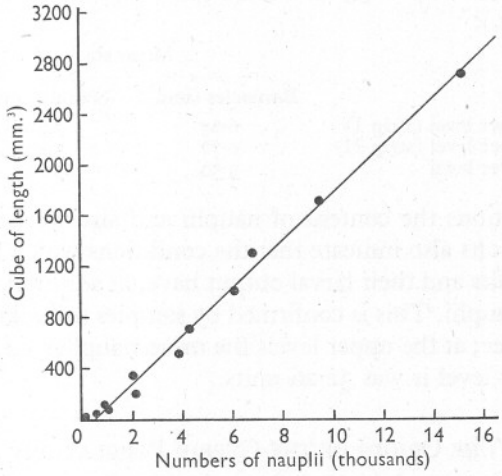

Fig. 2. Larval output and size of adults in *B. balanoides*. The total number of nauplii is plotted against the cube of the adult length. Grouped values are used.

In *B. crenatus* two modes are also present in the earlier samples, both quite strong, but as the season progresses the population giving rise to the higher modes disappears. This can only mean that one of the populations (larger size) concerned is produced only at the beginning of the season, whilst the other population is produced continuously throughout the season.

The Environment and Reproduction

Moore (1935) has shown that the number of larvae produced by a barnacle is a function of its size, a fact confirmed in this investigation. Fig. 2 shows a plot of the mean nauplii content for a series of size-groups against the cube of the length, which is approximately a linear function of the volume. It has also been shown (Moore, 1934; Barnes & Powell, 1953) that the size attained by the adult barnacle at any given time is a function of a whole complex of

conditions. However, since the conditions of the environment have such a marked influence on the growth-rate and on the reproductive capacity, it is important to examine whether they also affect the size attained by the nauplii at the time of extrusion. It has been possible to measure the size of ripe nauplii of known age at various levels on the shore. During his work on algal colonization Mr Powell had completely cleared a number of rocky strips of barnacles and other organisms, and a number of 1-year-old barnacles were collected from the upper and lower levels on these strips on which zonation was just developing at the time of the spring outburst. These adults were all ripe and the embryos were teased out under water, when every time some nauplii escaped from the egg cases. The total length of a number of nauplii was measured whilst in the egg cases; the values obtained were as follows (50 units = 0·423 mm.):

	Mean size	
	Barnacles (mm.)	Nauplii (units)
Upper level (strip I)	6·67	34·65
Upper level (strip II)	6·72	34·73
Lower level	3·50	33·61

In these collections the content of nauplii and size were again correlated, and the above results also indicate that the conditions which have affected the growth of the adults and their larval output have, in addition, affected the size attained by the nauplii. This is confirmed by samples (of unknown age-group) taken from the pier; at the upper levels the mean naupliar size was 34·94 units and at a very low level it was 32·26 units.

The Origin of the Cyprid Populations

Cyprid populations may come to be different in size from more than one possible cause. The populations may represent different races, that is, be genotypic in origin. In view of the extensive mixing of the cyprids in the surface waters during the planktonic phase and their ultimate distribution over the whole intertidal zone in *B. balanoides*, together with the necessity for cross-fertilization, it seems unlikely that distinct races of adults giving rise to populations of different-sized cyprids would be developed. Further, when the frequency curve for the length/breadth ratio is plotted it is almost unimodal, in marked contrast to those for the length or breadth, suggesting that the populations are only different in size and not in shape (Fig. 3). Again, the populations may exhibit growth differences as a result of the effect of the environment during the planktonic development. The persistence of the separate populations in *B. balanoides* throughout the spawning season and the existence of differences in the nauplii within the ripe adults from different levels tends to negate this suggestion.

A more plausible suggestion is that in *B. balanoides* the modes represent the

product of the different zones of the shore, for, in view of the above results, if the nutritive conditions are sufficiently well defined in the different zones there will be a tendency to produce a series of different-sized nauplii giving rise to different-sized cyprids which correspond to these levels with, of course, overlapping. Evidently, with *B. balanoides*, the upper and lower zones of the shore are sufficiently different to give this result, the environment and growth conditions at the two levels being sufficiently distinct. The growth-rate of *B. crenatus* also has been shown to vary according to the habitat, being far

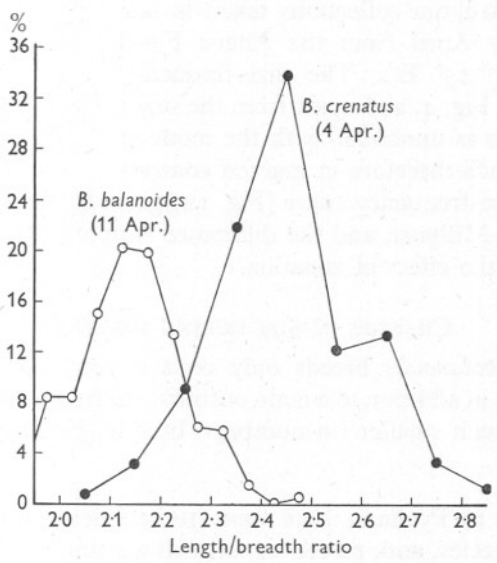

Fig. 3. Size-frequency of length/breadth ratio for *B. balanoides* and *B. crenatus*, for samples taken on 11 April and 4 April respectively. Grouped values are used. Compare with curves in Fig. 1.

greater under the more favourable conditions (Barnes & Bagenal, 1951; Barnes & Powell, 1953); but since this species is largely sublittoral in this region it is less clear why two distinct populations are produced. Perhaps those adults which just extend into the littoral and near-littoral zone are responsible for one population, whilst the deeper water populations produce the other. If this is so, since the major population of *B. crenatus* adults is certainly sublittoral, the near-littoral group must produce the larger cyprids, which constitute the smaller proportion of the total population (taken over the whole sampling period), and the sublittoral population must continue to spawn over a longer period, for the larger mode tends to disappear in the later collections (see Fig. 1B). It is, however, possible that the populations correspond to the products of the different year-classes of adults.

The Size Distribution of Swedish *Balanus balanoides*

The tidal rise and fall at Millport is of the order of 10 ft., sufficient to give large changes in environment with distinct algal zonation, and to give rise to at least two populations. On the west coast of Sweden the tidal rise is 1 ft. or less and on the preceding hypothesis only a single-sized population of cyprids might be expected. Through the kindness of Dr Höglund it has been possible to examine some plankton collections taken in late March and early April from the Altane Fjord (58° 45′ N., 11° 15′ E.). The size-frequency curve is given in Fig. 4, and apart from the slight break at 53 units is unimodal with the mode at 56 units. It stands therefore in marked contrast to the type of size-frequency curve (Fig. 1A) given by the same species from British waters at Millport, and the difference supports the hypothesis given above regarding the effect of zonation.

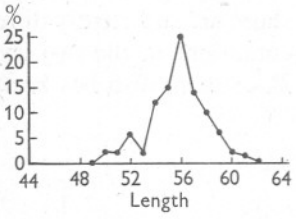

Fig. 4. Size-frequency distribution of Swedish *B. balanoides*. Length in arbitrary units.

Changes in Size during the Year

At Millport *B. balanoides* breeds only once a year, but *B. crenatus* and *Verruca stroemia*, in addition to a main outburst in the spring, produce several later although much smaller (in numbers) broods (Pyefinch, 1948).

Verruca stroemia

As pointed out by Pyefinch there seems to be a delay in the production of cyprids of this species, and, moreover, cyprids are never caught in quantities comparable with the nauplii, in surface or subsurface collections. However, small collections were obtained in both spring and autumn and have been examined. As far as can be determined from the smaller numbers (62 in each set, pooled samples from several days) the size-frequency curves for both collections are unimodal; and, since this is a genuine sublittoral species of fairly restricted habitat where conditions are uniform, this finding affords additional support for the previous hypothesis with regard to the other species. The mean lengths and their standard errors are given below for the spring and autumn cyprids:

	Mean length	S.E.	Ratio, L/B	S.E.
Spring	30·8	0·163	2·34	0·0196
Autumn	27·70	0·190	2·33	0·0185

Balanus crenatus

Only comparatively few (27) were obtained during the autumn, insufficient for adequate representation of a size-frequency curve. They have therefore been compared with the unimodal spring curves. Since it is the smaller-sized

mode that persists, any size decrease during the season will tend to be minimized:

	Mean length	S.E.	Ratio, L/B	S.E.
Spring	46·36	0·117	2·47	0·00695
Autumn	41·93	0·399	2·46	0·0267

In both these species, therefore, which produce more than one brood a year, there is a significant decrease in size in the cyprids of the later broods although, as far as can be judged from the length/breadth ratios, there is no change in shape.

Size variations in successive broods during a year have often been reported for copepods (see, for example, Marshall, 1933, 1949) and both temperature and food are considered to be important factors. In general, larger animals are found when development takes place more slowly at lower temperatures. However, it has often been difficult to reconcile the facts with the actual changes in temperature. It is suggested that the rate of development of the eggs or of the embryos within the parent may be as important a factor as the rate of development during the planktonic stages in determining the size of any given stage. This might explain to some extent the unexplained fluctuations found by Marshall in the sizes of copepod stages, since although water temperatures in general rise during the summer, if size is to some extent determined by the rate of development, temperature fluctuations acting over a much shorter time could be important.

In the case of *B. crenatus*, considered above, development of the spring brood of nauplii takes place during the autumn and winter months, October to March, from eggs fertilized in the autumn. Development of the embryos is therefore slow and occurs during the period when the sea temperatures are low. Development of the autumn brood is more rapid, affected by higher temperatures. The brood may even be produced, according to Pyefinch (1948*b*), from animals settled in the same spring; in consequence, on the above hypothesis, the nauplii and hence the cyprids so produced, would be expected to be smaller.

Thanks are due to Dr Höglund for providing the Swedish samples, to Mr V. Bainbridge for allowing the cyprids to be used from some of his collections, and to Mr H. T. Powell for providing the barnacles of known ages from his rock strips.

Summary

The sizes, length and breadth, of the cyprids of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* have been measured on material collected during the spring outburst and in the later part of the year.

The size-frequency curves for *Balanus balanoides* and *B. crenatus* show at least two distinct modes during the spring, equivalent to populations of two

distinct sizes. It is suggested that these correspond to populations developed in different environments. Measurements of the nauplii taken from adults at different points on the shore indicate that the size of the nauplii is dependent to some extent upon the conditions of development, the conditions also affecting growth and total nauplii output.

Swedish samples, in which the parents had developed under more uniform conditions, showed only a single population as regards size.

In *B. crenatus* and *Verruca stroemia*, which produce further broods during the year, the size decreases in these later broods. The rate of development of the eggs and embryos, probably dependent upon temperature, is suggested as the important factor.

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BIOMETRY OF THE COPEPOD *CALANUS FINMARCHICUS* (GUNN.) IN STAGES V AND VI

By H. Barnes, D.Sc. and Margaret Barnes

The Marine Station, Millport

(Text-figs. 1, 2)

There is much published information on the size of the several stages and its variation in the copepod *Calanus finmarchicus*. Marshall (1933), in the Clyde area, has found considerable variations in size even between the different generations developed throughout a single season, such variations extending to most of the copepodite stages. In general, the generations developed earliest in the season were the largest: further, the difference in size between the male and female stage VI copepodite was found to be considerable in the spring brood but almost negligible in a summer brood. Differences in size have also been reported with depth (Russell, 1928; Gardiner, 1933), and there are differences in the size of animals from distinct geographical regions. It has also been stated that the relative proportions of metasome and urosome vary. However, while some of these observations relate to the total length of the metasome and urosome taken together, most have been made on the metasome only: there appear to be no measurements of the individual segments and no consideration of the changes of size of the segments from one stage to the next. Further, the question is still open whether any sexual differentiation in size is evident in copepodite stage V.

The Materials and Experimental Methods

One of the difficulties in analysing the size variation in *Calanus* and other copepods arises from the fact that in most collections, even when obtained from a restricted area, there is often a mixture of two populations as a result of some overlap in the generations. Further, there is considerable spread, even in the sizes of individuals of a given stage from a distinct generation and this, together with the overlapping of the generations, has led to the use of overall means or modes when sizes are recorded. Certain features of the results have therefore been lost. Such an overlap of the sizes, although always likely to be present can to some extent be minimized by a choice of the time of collection.

In this work, therefore, all the results have been plotted as cumulative percentages, and in this way the presence of more than one population detected. Further, from the plots the means and standard deviations of the separate populations have been determined graphically.

The animals here referred to are *C. finmarchicus* in the sense given by Rees (1949).

The material used was collected in the Firth of Clyde at a constant depth of 100 m. with a tow-net hauled horizontally. As shown by a preliminary examination at the time of collection, late June, it consisted largely of stage VI, males and females (10·6 and 26·2% respectively), stage V (56·2%), stage IV (6·2%) and only a few of the early stages. It seems clear, from a comparison with the results of Marshall (1933) that this was the first summer generation and that the stage V population was giving rise to the population of stage VI, males and females, also present. This view is substantiated by the fact that the stage VI females showed, not only for total length but also for many of the other measurements, two populations, of which those with the larger mean size were present only to the extent of some 5%. This small population is no doubt a residuum from the larger-sized generation developed earlier in the year (Marshall, 1933). That only a single population of male stage VI was found is not surprising since, as Marshall has pointed out, the evidence indicates that these are shorter lived than the female stage VI.

The material was preserved in 5% neutral formalin as soon as possible after collection, and the measurements and any deductions derived therefrom can strictly speaking only apply to material treated in this way. The measurements were all made with the aid of a projection microscope, the image being projected on to a sheet of paper and the appropriate outlines drawn. The magnification for the metasome segments in the projected figure was $\times 103$, and for the urosome segments and the caudal rami was $\times 371$; the measurements were made from the drawings to the nearest $\frac{1}{50}$ of an inch with a precision steel ruler. One hundred animals of each of the copepodite stages to be considered, namely, stage VI male and female and stage V, were picked out at random from the sample and the following measurements made on the drawings from the projected images (Fig. 1).

From the dorsal side, the length of the head (including fused first thoracic somite) and the length of each of the five free thoracic somites (segments) were measured. The head measurement was taken from the anterior tip to the posterior border, and that of the free segments from the posterior edge of the preceding segment to the posterior border of the segment in question. On the last thoracic segment the length was taken to the centre of the posterior edge of the concave border. The drawings from which these measurements were taken were made with the animal lying flat on its ventral side, and, if necessary, any appendages which hindered keeping the animal horizontal were removed. The body shows a very slight curvature but no correction has been applied for it since tests showed that any correction was extremely small and did not affect the conclusions to be drawn from the results. Strictly speaking, however, the measurements are the lengths projected on to a horizontal plane. Also from the dorsal side the widths at the widest part of the posterior border

of all the metasome segments were measured; with the last free thoracic segment this was taken as the distance between the rounded tips. In addition, the head segment was divided into thirds and the width at each third was measured separately.

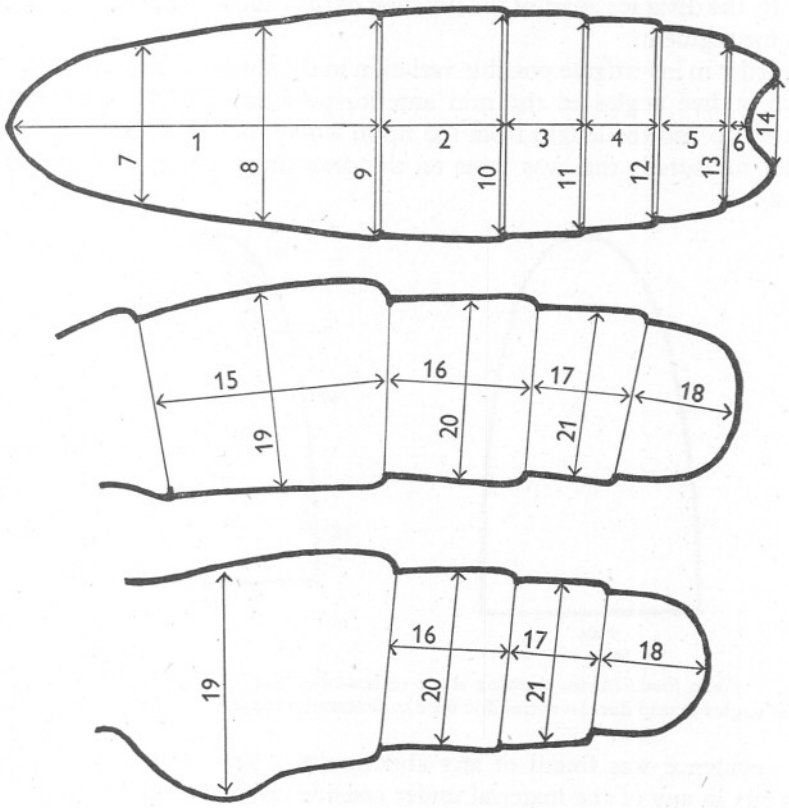

Fig. 1. To show the measurements taken on *Calanus finmarchicus* (Table I).

On preservation, the urosome often undergoes flexion, and all the measurements on this part of the animal were therefore made from drawings made with the animal lying on one side, again removing any appendages necessary in order to place the animal with its dorso-ventral axis horizontal. Even so the length of the first segment of the urosome cannot be accurately drawn in the intact animal, and it has therefore been omitted for all stages. The lengths of the other urosome segments were measured at the centres (posterior border to posterior border, again) so that there are four length measurements for stage VI male but only three for stage VI female and stage V. The depths (dorso-ventrally) at the widest part of each of these segments were also measured, and that of the widest part of the first urosome segment in the

female stage VI only (neglected in the length measurements), since here it could be clearly seen. The width of the terminal segment was not measured for any of the stages.

One further measurement was made with the animal on its ventral side, namely, the distance apart of the junction of the caudal rami with the terminal urosome segment.

In order to investigate possible variation in the shape of the head, lines were drawn at five angles to the mid anterior-posterior line from the extreme anterior tip and the length from the tip to where these lines cut the carapace border measured; this was done on the drawings from the dorsal side (see Fig. 2).

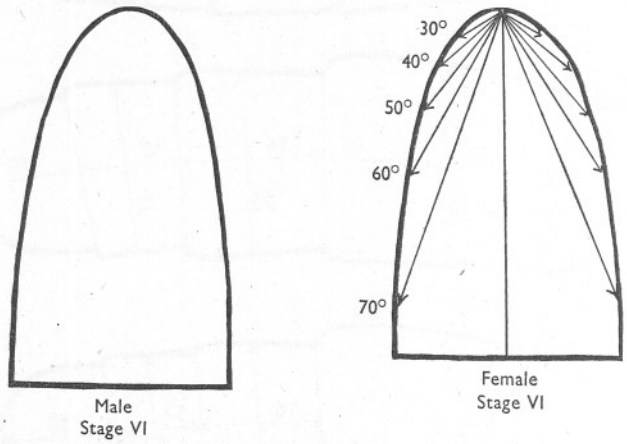

Fig. 2. *Calanus finmarchicus*, showing shape of head in stage VI male and female. Lines set off at angles to mid dorso-ventral line used to determine the shape (see text and Table II).

No evidence was found of any abnormalities such as telescoping of the segments in any of the material under consideration (Gurney, 1931).

In testing the significant differences between mean sizes, the *t*-test was used, the population means and standard errors being estimated from the graphs. For simplicity of discussion the actual units used in the measurements will always be quoted in the discussion of the sizes.

As already stated, a small larger-sized population was shown to be present in the stage VI females; only the smaller-sized and larger population (95 %) is considered in the following.

The Relations of Copepodite Stage VI Male and Female

The male and female stage VI at this time of the season do not differ in the size of the whole metasome obtained by adding together the separate segments (9·70 units males and 9·72 units females, Table I), and this is in accord with the observations of Marshall (1933) who found that the males, although

relatively smaller in the early part of the year, were in the summer brood about the same length (metasome) as the females. However, there are differences when individual segments are compared. The most striking difference is that of the head portion: this is much longer in the male (4·91 units) than in the female (4·56 units). With the exception of the first free thoracic segment in which the length of the female does not differ significantly from that of the male, the lengths of the other free segments are all slightly greater in the

TABLE I. *CALANUS FINMARCHICUS*. BODY MEASUREMENTS OF STAGES V AND VI

(In arbitrary units.)

	Stage VI		Stage V	
	Male	Female	(i)	(ii)
The metasome				
Length of metasome (by addition)	*9·700*	*9·720*	*8·990*	*8·590*
1. Length of head	4·910	4·560	4·300	3·900
2. Length of first free segment	1·550 (n.s.)	1·568	1·438	
3. Length of second free segment	1·052	1·140	1·047	
4. Length of third free segment	0·924	1·040	0·935	
5. Length of fourth free segment	0·822	0·908	0·810	
6. Length of fifth free segment	0·442	0·504	0·460	
7. Width of head one-third down	2·200 (n.s.)	2·230	2·020	
8. Width of head two-thirds down	2·720 (n.s.)	2·720	2·480	
9. Width of head posterior border	2·860 (n.s.)	2·880	2·670	
10. Width of first free segment	2·818	3·010	2·600	3·000
11. Width of second free segment	2·720	2·840	2·475	2·861
12. Width of third free segment	2·453	2·600	2·195	2·550
13. Width of fourth free segment	2·060	2·155	1·800	2·120
14. Width of fifth free segment	1·183 (n.s.)	1·170	0·920	1·300
The urosome				
15. Length of second segment	2·850	—	—	—
16. Length of antepenultimate segment	1·650 (n.s.)	1·635	2·100	
17. Length of penultimate segment	1·150 (n.s.)	1·160	1·420	
18. Length of ultimate segment	1·295	1·440	1·860	
19. Width of antepenultimate segment	2·600	2·950	—	
20. Width of penultimate segment	2·312	2·360	2·340	
21. Width of ultimate segment	2·215 (n.s.)	2·120	2·105	
22. Width apart of junction of caudal rami	1·000	0·716	0·620	

n.s., differences not significant.

female, and there is a suggestion of a relative gradient between the two sexes from anterior to posterior, the ratio changing from 0·928 in the head to 1·148 in the last free segment. It will be remembered that for the purpose of comparison within the head itself, the width was measured not only at the posterior edge but also at a distance of one-third and two-thirds its length from the anterior end. There is no significant difference between the male and female in any of these measurements, the head in both narrowing anteriorly.

In all the other segments the female is somewhat wider than the male, except in the width between the projecting terminal tips of the last free segment, where there is no significant difference.

The terminal segment of the urosome is significantly longer in the female

but there is no difference between the two preceding segments. There is no direct comparison available for the length of the second segment of the male, but it is much larger than the others. As might be expected, the width of the first segment (two fused somites) in the female, which bears the genital opening, is much larger than that of the male, although it should be remembered the comparison is between this and the second somite in the males. The widths of the penultimate and antepenultimate segments show also smaller differences, the former being slightly larger in the male (although not significant at the 0·02 level). The width apart of the caudal rami at their junction with the terminal segment (forming movable joints in the male) is much greater in the males. Indeed this, together with the difference in head length and width of the 'genital' segment of the urosome, are here the most distinguishing size differences in these male and female populations.

The Presence of Two Populations in Copepodite Stage V

The first question to be considered is whether there is any differentiation in the stage V animals in the sense that, as regards any of their size measurements, they can be divided into two distinct populations. There is a clear distinction into two populations of about 50% each in total length of the metasome (determined by adding together the separate segments), and this suggests that differentiation into potential males and females, at any rate as regards size, is evident in stage V. This separation into two distinct populations has been confirmed by the analysis of a large number of other catches taken at various times and by the analysis of published data of other workers: the lengths of the stage V metasome can almost always be separated into two populations each composing about half the total. This feature suggests sexual differentiation rather than lack of homogeneity in the material. Such a distinction, however, has not been found in copepodite stages earlier than stage V. It has been unobserved because of the considerable overlap in the sizes. A difference in the size of stage V *Calanus* has often been found in colder regions (Störmer, 1929; Bogorov, 1933), where the modes were quite distinct and has been observed by Ussing (1938) even for stage IV copepodites. In these northern regions, however, the proportions are not constantly 50/50, and the differences are probably due to lack of homogeneity, the two stage V populations having different developmental histories. Thus it has been suggested that the amount of food available at the time of production or even a 2-year development period for one population is concerned. The failure of Marshall (1933) to correlate large and small stage V with males and females in an experimental moult is due to the fact that only small numbers were used and that the differences noted above are population differences.

This differentiation in stage V does not, however, extend to all portions of the animal. It is well marked in the head length (4·30 and 3·90 units), and

this might be expected since the difference in the length of the head is the most marked feature in the metasome of stage VI males and females. There is no differentiation in the length of the other metasome segments in stage V, but the widths of all these segments except that of the head are clearly divided into two populations, of which the larger may be presumed to be the female population. There is no distinction in the lengths and breadths of the urosome segments.

Relation between the Sizes of Stage V and Stage VI Males and Females

There is no feature by which it can be known with certainty which population of stage V gives rise to the males and which to the females. There is considerable growth in the head segment from stage V to stage VI in both male and female; whereas the length of the other metasome segments (of which only one population was present in stage V) shows virtually no increase in the moult to males and only a slight increase in the moult to females. There is no difference in the widths of stage VI male and female heads and therefore it is not surprising to find only one population of these measurements in stage V and therefore the same increases must take place when passing from stage V to stage VI male or female. In the widths of the other metasome segments there are two populations in stage V, one of which is usually larger than the male stage VI and can therefore perhaps be presumed to correspond to the female population; in which case there is little change in the width on passing from stage V female to stage VI female and a moderate change in passing from stage V male to stage VI male. Only a single population was found in the stage V urosome measurements with hardly any change in width from stage V to stage VI. However, the length of all three segments measured in the urosome is much larger in stage V than the equivalent segment in stage VI male or female (as reckoned from the posterior segment). Apparently, therefore, considerable reorganization takes place in the urosome segments at moulting; but it should be remembered that the length of the first urosome segment was not measured.

The Shape of the Head in the Two Stages

The mean ratios of the lengths at 40°, 50°, 60° and 70°, set off as described previously, to that at 30°, are shown in Table II for the two stages considered, and in Fig. 2, where the 'average' head is drawn. This ratio, which increases with the angle, is always relatively smaller in the female, indicating that the head is rounder and less pointed than in the male. These lengths were plotted as for the others in stage V, and in each two populations were found present, the ratios derived from which are also shown in Table II. Clearly a round-headed

and a more pointed-headed population are present in stage V, and this further strengthens the view that there is differentiation into sexes in stage V. In the 'typical' stage V heads drawn from these figures only the narrow head is found to fit the longest head measurement; and, since the narrow head in stage VI is that of the male, the longest head in stage V may be inferred to belong to the male population.

TABLE II. *CALANUS FINMARCHICUS*. MEASUREMENTS ON THE HEAD OF STAGES V AND VI

	Stage VI		Stage V	
Ratio	Male	Female	(i)	(ii)
Length at 40°/30°	1·65	1·59	1·78	1·54
Length at 50°/30°	2·51	2·36	2·70	2·29
Length at 60°/30°	3·91	3·61	4·21	3·47
Length at 70°/30°	6·75	6·11	7·20	5·80

THE STATE OF THE ANTENNULES ON PRESERVATION

It is well known that the antennules always remain straight on preservation in formalin in stage VI males, but assume some amount of curl, often very characteristic, in the preserved stage VI females. Further, when stage V are preserved, some have curled and some straight antennules. However, it does not appear from the following observations that this can be used to differentiate the sexes in stage V. In the hundred of each stage, picked out at random, all the stage VI males had straight antennules, but so had also sixteen of the stage VI females. In the other females the antennules were curled to a varying degree. In the stage V animals 34% were straight. For all those measurements where analysis had shown that there were two populations in respect to stage V the means were separately calculated for those animals with straight and for those with curled antennules. These means were always the same; there was therefore no separation into two populations as would have been expected had the state of the antennules been correlated with sex, which in turn has been presumed to be the basis of the two population sizes.

Thanks are due to Dr S. M. Marshall for reading the manuscript and for making a number of helpful critical comments on it.

SUMMARY

One hundred each of stage VI male and female and stage V *Calanus finmarchicus* were picked at random, and, by means of a projection microscope, measurements made on a number of segments in both metasome and urosome.

The stage VI female population was mixed containing 95% of a small-sized population and 5% of a larger size-group. The males constituted a single population.

At this time of the season the stage VI male and female metasomes are the same length, but there are differences in the sizes of the individual segments, the most striking of which is that of the head.

The stage V animals in many of the measurements could be quite clearly separated into two size-groups of about 50% each and it is suggested that these are potential male and female populations. This separation at 50/50 has been noticed in numerous other collections of stage V, but it should be emphasized that the differences are population differences in regard to those measurements considered.

Evidence is presented which shows that the state of the antennules after preservation in 5% neutral formalin is unlikely in stage V animals to be a reliable guide to their sex in the next stage.

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PERITROPHIC MEMBRANES IN THE CARIDEA (CRUSTACEA DECAPODA)

By G. R. Forster
The Plymouth Laboratory

(Text-fig. 1)

Observations on the faecal pellets of various shrimps and prawns were begun after the discovery in 1949 of a thin transparent membrane surrounding the pellets of *Spirontocaris pusiola* (Krøyer). The presence of the membrane in the anterior part of the intestine showed that it could not be secreted by the hind-gut, which is normally very short in the Caridea. Moore (1932) states that the faecal pellets of the *Brachyura* have a thin investment of mucus while those of the Caridea and lower groups have a thin clear outer layer. Subsequently a peritrophic membrane has been described in *Daphnia* (Chatton, 1920) and in the barnacle *Pollicipes* (Batham, 1945). As the membrane found in *Spirontocaris* did not show the properties of mucus, a fuller investigation on its structure and function was made. A membrane has been found in all the Caridea so far examined. *Palaemon serratus*, the common prawn, has been used for many of the tests on account of its size and abundance.

Occurrence and General Observations on the Membrane

A membrane has been observed around the faecal pellets of the following Caridea: *Pandalus montagui* Leach, *Pandalina brevirostris* Rathke, *Hippolyte varians* Leach, *Spirontocaris cranchii* (Leach), *S. pusiola* (Krøyer), *Athanas nitescens* Montagu, *Processa canaliculata* Leach, *Palaemon serratus* (Pennant), *P. elegans* Rathke (= *Leander squilla* (L.)), *Crangon vulgaris* L., and *Philocheras trispinosus* Hailstone.

In *Palaemon*, *Hippolyte*, *Processa* and *Crangon* a membrane has been observed in the intestine as well as around the faecal pellets. The membrane is not always easy to find if the faecal pellets are packed with material, but there are usually small indentations in the faecal strand where it is visible, appearing as a transparent section of the edge of the pellet. When *Hippolyte* is starved for a few days the pellets contain less faecal material and the membrane is clearly visible. Before the animal moults very long pellets are often produced with only slight traces of faecal matter, indicating that the stomach is being completely emptied. To starve *Hippolyte*, and probably any other small caridean, is not at all easy because of its coprophagous habit. The faecal pellets must be frequently removed, as *Hippolyte* and especially *Palaemonetes*

can subsist, sometimes indefinitely, without any food by re-ingesting faecal pellets which contain large numbers of Protozoa.

With the larger pellets of *Palaemon* it has been possible to squeeze out the contents and observe the membrane in the form of a thin tube. But usually the slightest touch of a needle will cause the pellet to fragment. The membrane may also sometimes be obtained by itself if the pellets are washed in fresh water: the contents swell, and are slowly extruded from one end of the pellet, or else rupture the membrane. The process can be continued by washing first in dilute and then in more concentrated NaOH solution.

The Nature of the Membrane

Chemical tests have been made on the pellets of *Hippolyte* and *Palaemon* to ascertain the composition of the membrane.

Effect of Acids and Alkalis. The membrane is insoluble in either weak or strong alkalis, and withstands boiling in concentrated KOH solution. This shows that it does not contain mucins or mucoids as these are soluble in dilute alkalis (Lloyd & Shore, 1938).

Concentrated HCl dissolves the membrane rapidly, but a dilute solution has little or no effect.

Chitin Test. As the results given above conform to the properties of chitin (Yonge, 1932), the standard van Wissenlingh test for chitin (Campbell, 1929), critically discussed by Richards (1951), was carried out. This has given positive results for the membranes surrounding the pellets of *Hippolyte varians* and *Palaemon serratus*, both with naturally extruded pellets and with others dissected out from the anterior part of the intestine. A few of the chitin tests have not been positive but this has probably been due to insufficient hydrolysis with KOH. Owing to the small size and delicate nature of the test, pieces must not be allowed to boil, but heating for about 30 min. at 150° C. was found sufficient to obtain a good violet-red coloration with the iodine and sulphuric acid reagent. It was obviously difficult to be absolutely certain that the membrane itself, and not some of its contents, gave the positive reaction. On three occasions, however, pellets were obtained with clear sections of membrane that still gave the colour reaction satisfactorily. Moreover, when one pellet was treated with 3% acetic acid after boiling with KOH, the membrane dissolved as chitosan should, but the contents of the pellet remained. No precipitate of chitosan sulphate was obtained, but this is hardly surprising in view of the minute quantity of material in the test.

The Source of the Membrane

Fig. 1A shows the three regions of the gut. In numerous dissections a membrane has been found only in the intestine (mid-gut), and is assumed to be secreted by the epithelial cells. Sometimes the faecal thread and membrane projected a little way forward from the opening of the intestine, the anterior

end being occasionally twisted or rolled up into a coil. This was doubtless brought about by mechanical disturbance during dissection and by anti-peristaltic movements of the gut, as it was scarcely ever observed when the animals were first narcotized with urethane and the intestine separated from the stomach straightaway. Permanent sections were found unsatisfactory for

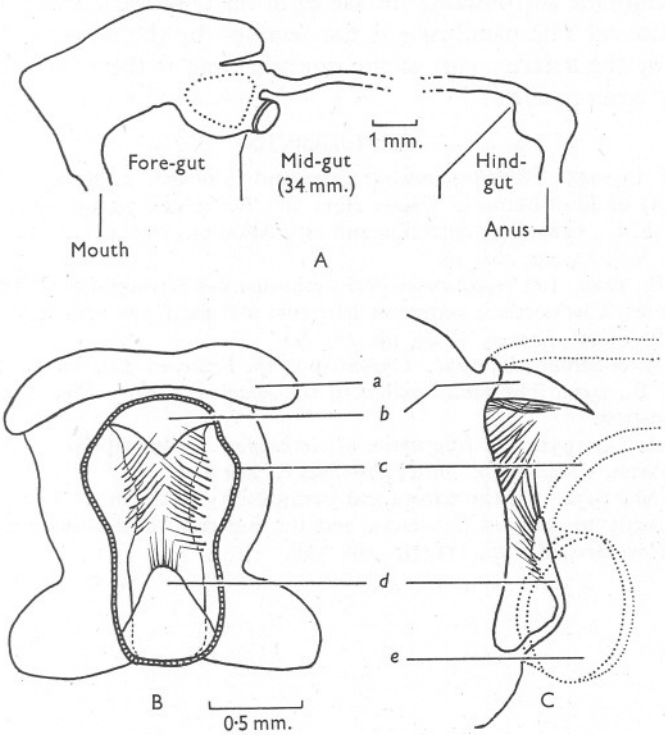

Fig. 1. A, the regions of the alimentary canal of *Palaemon serratus*. The dotted area marks the position of the pyloric filter press. B, the posterior end of the pyloric filter press, looking forwards. C, side view of B. *a*, dorsal caecum; *b*, dorsal lip or valve; *c*, extreme anterior end of the intestinal wall; *d*, chitinous flap (? ventral valve); *e*, opening of the digestive gland duct.

observations on the membrane, which owing to its extreme fragility was nearly always damaged or so tightly pressed against the faecal material as to be indistinguishable from it. Fig. 1 (B, C) shows the junction of the mid-gut and fore-gut. Although at first it was thought that the membrane might be secreted around the dorsal lip, this would scarcely be possible in the presence of large numbers of setae, and no indication of it has been found.

As the membrane is produced in the mid-gut region and contains chitin it may be considered a true peritrophic membrane (Wigglesworth, 1950). The

function is probably to protect the epithelium lining the intestine from abrasive material, such as fragments of shells, sponge spicules, and setae which are often found in the stomach-contents of prawns.

SUMMARY

A thin membrane surrounding the faeces of many of the Caridea has proved to be chitinous. The membrane is not secreted by the fore-gut, but almost certainly by the anterior part of the intestine, and is therefore a peritrophic membrane *sensu stricto*.

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THE SPAWNING BEHAVIOUR OF PLAICE

By G. R. Forster

The Plymouth Laboratory

On the evening of 20 February 1953, two plaice (*Pleuronectes platessa* L.) in the largest tank of the Aquarium were observed spawning. No information on the spawning behaviour of pleuronectids has been found in the literature; nevertheless, the observation may very well not be original, but is considered of sufficient interest to justify an account of it.

The two plaice were swimming in mid-water about 2 ft. 6 in. from the bottom, the female lying slightly diagonally across the back of the male their vents being close together. The female, considerably larger than the male, was quivering violently and emitting a rapid stream of eggs. Mr F. J. Warren who was also watching the tank saw a stream of milt coming from the male. After about 20 sec. the fish separated and settled on the bottom. The eggs were being eaten very rapidly by a shoal of sea-bream (*Pagellus centrodontus* de la Roche). The beginning of the spawning was not seen but the whole act did not take much longer than three-quarters of a minute as the tank had been under observation about half a minute earlier. When captured afterwards the female was found to be almost completely spent, but may have spawned previously. Fertilized eggs from subsequent spawnings by other plaice were taken in the tank for the next 3 days, between 6 and 9 p.m., though the actual spawning was never again observed.

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BLOOD AND URINE CONSTITUENTS OF *LOPHIUS PISCATORIUS* L.

By L. Brull and E. Nizet
with the collaboration of A. Dujardin

From the Plymouth Laboratory, and Institut de Clinique et Policlinique Médicales, Liège, Belgium

(Text-fig. 1)

The comparative physiology of glomerular and aglomerular kidneys has provided much valuable information which cannot be ignored in discussion of renal functions. Our knowledge, however, of the exact inorganic and organic metabolism of fishes, particularly aglomerular fishes, is still insufficient. *Lophius piscatorius* L. is the only aglomerular fish whose size permits thorough investigation of such problems. This is the reason why our research on the kidney of *Lophius* starts with an attempt to extend our knowledge in that field.

The habits of angler-fish observed in the Plymouth Aquarium have been described by Wilson (1937).

During a visit to the Plymouth Laboratory in July 1952, when a number of *Lophius* were available, we were able to attempt kidney perfusion. We also took the opportunity of analysing many urine and blood samples from these fish. The samples were taken either on board ship from freshly caught fish, or in the laboratory from fish which had just arrived or spent one night in the aquarium. Others were taken during perfusion experiments.

Nitrogen Constituents

Plasma proteins were found, by the Kjeldahl method, to be 39 g./l. in sample L28. A detailed cataphoretic analysis of the plasma of L48 and L49, made in Liège by Dr A. Nizet using Antweiler's microelectrophoretic apparatus, gave the following results: albumin, 6·7%; globulin 1, 14·3%; globulin 2, 46·1%; globulin 3, 32·9% (see Fig. 1).

It thus seems, subject to further confirmation, that the plasma of *Lophius* is not only poor in proteins, but that globulins are largely predominant, which is in keeping with a low osmotic pressure. According to Florkin (1944) the colloid osmotic pressure of teleostean fishes is usually below 20 cm. of water.

Figures in the literature for non-protein nitrogen constituents are very scarce. Denis (1913) gives the following data:

	mg. N in 100 ml.
Total non-protein N	40
Urea	8
Ammonia	3·6
Uric acid	0·9
Creatine and creatinine	5·0

In a pool of heparinized blood samples kept for 24 hr. in cold storage and centrifuged after being used in a perfusion experiment (L 18), we found in the trichloracetic filtrate:

	mg. N in 100 ml.		mg. N in 100 ml.
Total non-protein nitrogen (Kjeldahl)	21	Uric acid (Folin-tungstic filtrate)	0·17
Urea (urease in Conway box)	0·85	Creatine	0·21
Ammonia (Hoppe-Seyler, 1930)	0·32	Creatinine	0·28
Trimethylamine + trimethylamine oxide (Hoppe-Seyler, 1930)	11·34		

Sum of analysed constituents 13·17 = 63 % of the total

In a similar pool (perfusion L 28) we found *total non-protein nitrogen* to be 30 mg. N in 100 ml., of which urea was 1·5.

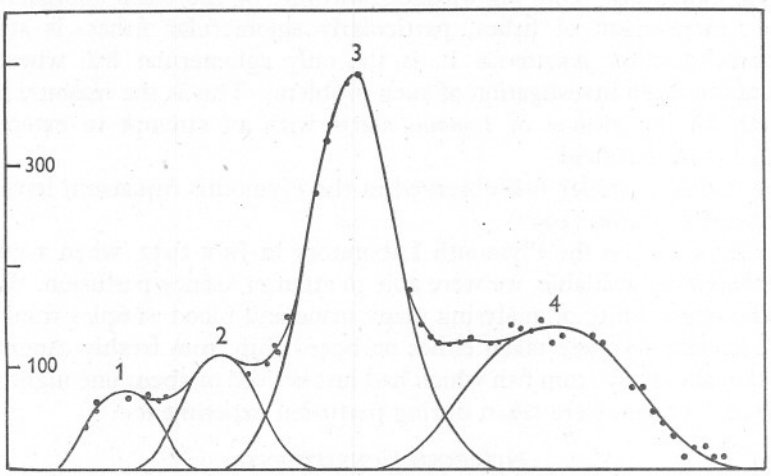

Fig. 1. Microelectrophoresis of *Lophius* plasma (Antweiler apparatus).

In a fresh sample of blood immediately centrifuged and deproteinized with trichloracetic acid (L 48 and 49 mixed), we found 16 mg. of total non-protein nitrogen and only traces of allantoin.

The haemoglobin content of *Lophius* blood is low, the cell volume being only about 17 %. We did not study the oxygen-carrying power of the haemoglobin, nor its absorption curve.

There are no figures in the literature of the amount of allantoin, trimethylamine and trimethylamineoxide in the blood of *Lophius*. In the pool of blood analysed, we found only negligible amounts of urea (much less than was accepted by Denis in 1913). There is very little ammonia, 0·32 (mg. N/100 ml.), as compared with the 3·6 of Denis. We have not yet tried to find whether this ammonia is preformed or not. Creatine and creatinine are not as concentrated as might be expected from the urine figures: 0·26 and 0·28 in plasma, and 51·2 and 4·3 in urine (Table II); therefore, they are highly concentrated by

the kidney. Trimethylamine and its oxide comprise 55% of the non-protein nitrogen in the plasma; they are much less concentrated by the kidney than creatin, up to only 28·4 mg. per 100 ml. urine, less than three times the concentration in the blood. Allantoin is practically absent from blood and urine (Table II). We did not have a sufficient volume of well-preserved blood and

TABLE I. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE (MG. N/100 ML.)

		Total non-protein N	Urea	NH_3	Trimethylamine. Total oxide	Uric acid	Creatine	Creatinine	Amino-acids
Denis (1913)	Composite sample of bladder urine, *c.* 1 hr. after death	83	12	1·2	—	0·1	14	0·7	—
Grollman (1929)	Woods Hole	180	1·2	1·0	—	0·8	50·2	7·3	25·8
	Caught in Atlantic	373	0·7	1·3	—	0·4	27·9	4·1	14·3
	Caught in Atlantic	337	0·6	1·0	—	0·2	156·0	8·0	18·0
	From aquarium	56	2·7	0·7	—	tr.	12·9	0·9	4·5
	From aquarium	—	2·7	1·3	—	—	23·0	1·5	8·0
Our determinations	Pool of bladder urines from 20 *Lophius*	148·4	1·6	19·2	28·4	0·03	51·2	4·3	4·5

Notes

According to Grollman, trimethylamine oxide constitutes the greater part of the remaining nitrogen.
According to Denis (1922), the percentage of amino-acids in urine and in blood is of the same magnitude.
Our determinations were from *Lophius* catheterized on arrival at the laboratory or after one night in the aquarium. The methods used were as for plasma, except that amino-acids were determined by a modification of Folin & Wu's method after elimination of NH_3 by permutit.

TABLE II. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE

Bladder urines from individuals, conditions as in Table I.

No.	Total non-protein N	Urea	NH_3	No.	Total non-protein N	Allantoin
L 3	21	2·5	—	L 19	245	—
L 10	82	—	—	L 20	280	—
L 12	54	2·3	2·2	L 21	89	—
L 13	290	—	—	L 22	194	—
L 14	346	5·3	2·1	L 23	140	—
L 16	140	—	—	L 27	56	0·13
L 17	91	—	—	L 36	75	0·15
L 18	89	—	—	L 37	50	—

Total non-protein N in bladder urines of individuals catheterized on deck: LE, 255; LC, 210; LK, 243.

urine samples for amino-acid determinations; according to Grollman (1929), their concentration would be of the same magnitude in urine and plasma. There still remains in plasma 37% of undetermined non-protein nitrogen, while in urine, where we found 4·5 amino-acid nitrogen, we failed to identify 30% of the non-protein nitrogen. There is still the possibility that an important nitrogen constituent of plasma and urine is as yet unidentified.

Mineral Constituents

In the following table we have condensed the figures for blood of Marshall (1930) and of Smith (1929), translated into milliequivalents per 100 ml. We reproduce only the limits of variations.

Marshall: Cl, 16·8–22·8.
Smith: Cl, 16·6–20·9; PO_4, 3·2–6·7; SO_4, 1·2–3·7; Na, 18·6–21·8; K, 6·9–9·9; Ca, 2·1–3·9; Mg, 1·0–1·4.

Table III shows the figures obtained in Plymouth in July 1952. Methods used were: Cl, St Russnyak (1926, p. 211); PO_4, Briggs (1922); Na and K, flame photometer; Ca, oxalic precipitation; Mg, colorimeter according to Harold Fister (*Manual of Standard Proced. for Spectr. Chem.*), a few gravimetric controls being made.

Table III. Minerals in Heparinized Plasma of *Lophius* (m.equiv./100 ml.) Plymouth, July 1952

Nature of sample	Cl	PO_4	Na	Mg	Ca	K	Δ
L 1+2 kept 15 hr. in aquarium under nembut. anaesthesia. Plasma separated after 5 days preservation of blood in cold storage	13·2	—	—	—	—	—	—
L 14 at end of kidney perfusion for 100 min.	14·0	—	—	—	—	—	—
Pool from L 16, 17, 18, separated after kidney perfusion 4 hr. after arrival	16·2	2·3	20·8	—	0·72	—	—
Pool from L 19, 20, 21, 22; 23, 24, 25, 26, 27, 28, 29 (2 days) kept in cold storage and after use for perfusion experiment, lasting 3.30 hr.	16·9	—	—	0·40	—	—	—
Pool from L 33, 34, 35, 36, 37, 38, 39, 40 all well alive 4 hr. after arrival and kidney perfusion	16·1	—	—	—	—	—	—
Pool from L 46, 47, 48 well alive—kept 24 hr. and after kidney perfusion 3 hr.	16·2	—	—	—	—	—	—
Plasma from L 48+L 49 (on arrival)	—	1·8	18	0·50	0·55	0·51	0·84
Plasma from L 30 and 31, arrived in good condition, bled and separated at once	19·6	—	21·7	0·58	—	—	—
Plasma L 32, arrived in good condition, bled and separated at once	19·0	—	21·3	—	—	—	—
Plasmas separated on board off Plymouth:							
L A	15·3	—	18·5	—	—	—	—
L D	15·3	—	—	—	—	—	—
L H	15·6	—	—	—	—	—	—
L E, F, +G	—	1·8	19·3	—	0·67	—	—
L	17·2	—	18·5	—	—	—	—

In one sample of pericardial fluid we found 17·9 m.equiv. chloride.

Our figure for Δ of the plasma, 0·84° C., is the same as the figure given by Smith (1929) at Woods Hole, while Bottazzi (1897) found 0·86 at Naples. In urine we confirm the figures of Smith, who found from 0·66 to 0·85. We found figures varying between 0·60 and 0·88 (Table V). We confirm the view of Marshall (1930) that urine of marine teleosts is either iso- or hypotonic to the plasma, never hypertonic.

Hypotonicity does not mean that certain, inorganic constituents are not

highly concentrated by the kidney (Table IV). Mg comes first. Our figures in plasma are about 0·5 m.equiv. per 100 ml. In urine the concentration varies from 22·6 up to 34·5 m.equiv., for *Lophius* catheterized on arrival. On board ship we found similar concentrations, except that two samples contained less, 14·6 and 15·8 respectively.

TABLE IV. MINERALS IN URINE OF *LOPHIUS PISCATORIUS*
(M.EQUIV./100 ML.)

Author	Cl	PO_4	SO_4	Na	Mg	Ca	K	Remarks
Denis (1913)	18	0·3	—	—	—	—	—	—
Sulze (1922)	2·1	—	0·7	—	2·5	0·36	—	Naples. Mixtures of urines from several *Lophius*
	2·3	—	1·4	—	3·2	0·42	—	
Grollman (1929)	5·6	0·8	—	—	3·8	0·2	—	Atlantic
	18·8	0·8	—	—	10·8	0·3	—	Aquarium
Smith (1929)	14·1	0·29	—	—	6·3	0·93	0·19	Aquarium (highest and lowest figures)
	20·9	1·47	—	—	10·0	1·52	0·76	—
Marshall (1930)	7·2	—	—	—	—	—	—	Aquarium (highest and lowest figures)
	22·9	—	—	—	—	—	—	
Smith (1929)	14·8	0·6	—	—	5·7	1·18	0·19	Starved in Aquarium
	20·9	0·7	—	—	6·3	1·38	0·27	
	16·1	0·28	—	—	6·4	0·93	0·19	Fed in Aquarium
	20·7	1·47	—	—	10·0	1·52	0·76	

So far as we know, Mg is the constituent of the urine for which the *Lophius* kidney shows its greatest power of concentration. Ca has been shown to be only slightly concentrated, as in man. But as one of us showed (Brull, 1930) there are three different physico-chemical forms of Ca in plasma, one of which is not excreted in urine (bound with proteins), one to a very small extent (ionized Ca), and one very fast and highly concentrated (complex Ca molecules with organic acids); therefore total Ca figures give no idea of renal concentration. The same is true for phosphates; the average plasma figure is below 2 m.equiv. %. In urine, we found from 0·15 to 9·0, which means deconcentration or concentration; but with biological methods and with the isotope ^{32}P, we demonstrated in our laboratory in Liège (Brull, 1927; Govaerts, 1947) that what is considered as inorganic phosphates in the plasma of the dog exists in at least two different physico-chemical forms, one of which only is excreted. It is possible that this phenomenon is interspecific. Potassium figures in plasma are about 0·50 m.equiv./100 ml., and the kidney seems to excrete very little potassium since concentrations in urine are lower than in plasma.

Chlorides in plasma range between 13·2 and 19·6 m.equiv. %; in the literature the variations mentioned are a little wider: 18·6–22·8; in urine, figures published range from 2·1 to 20·9. This might mean that chlorides may be diluted, never concentrated. Our figures for bladder urine range from 0·33 to 19·4, which would have the same significance. But our kidney perfusion experiments have shown that the kidney may slightly concentrate chloride

when the plasma concentration is raised. Under normal conditions, this either does not occur or is exceptional.

For sodium in the plasma, figures in the literature range from 18·6 to 21·8. We found the following limits: 18·5 and 21·7; according to Smith (1929)

TABLE V. MINERALS IN *LOPHIUS* BLADDER URINE. (M.EQUIV./100 ML.)

No.	Treatment	Condition	Cl	PO_4	SO_4	Na	Mg	Ca	K	Δ
L 3	Aq. 28 hr.	—	16·2	0·15	—	—	—	—	—	—
L 8	Arr.	—	18·1	—	—	—	—	—	—	—
L 10	Aq. 18 hr.	—	18·0	—	—	—	—	—	—	—
L 13	Arr.	D.	10·8	—	—	—	—	—	—	—
L 14	Arr.	H.	6·2	9·0	—	0·8	—	—	—	—
L 12	Arr.	D.	16·9	0·9	—	—	—	2·0	—	—
L 13	Arr.	D.	10·9	1·0	—	—	—	—	—	—
L 15	Arr. 4 hr.	—	17·0	—	—	—	—	—	—	—
L 16	Arr.	H.	18·4	0·1	—	—	—	1·2	—	—
L 17	Arr.	H.	18·8	0·16	—	—	—	—	—	—
L 18	Arr.	D.	11·6	—	—	—	—	—	—	—
L 19	Arr.	D.	11·7	—	—	—	—	—	—	—
L 20	Arr.	D.	11·1	—	—	—	—	—	—	—
L 21	Arr.	D.	18·7	—	—	—	—	—	—	—
L 22	Arr.	D.	18·1	—	—	—	—	—	—	—
L 23	Aq. 17 hr.	H.	16·9	—	—	—	—	—	—	—
L 24	Aq. 17 hr.	H.	18·8	—	—	—	—	—	—	—
L 25	Aq. 15 hr.	D.	18·0	—	—	—	—	—	—	—
L 27	Arr.	H.	16·4	—	9·3	—	31·0	—	—	0·68
L 33	Arr.	H.	17·0	—	—	—	—	—	—	—
L 38	Arr.	H.	—	—	7·6	—	30·5	—	—	—
L 43	Arr.	H.	13·3	—	—	—	—	—	—	—
L 14	Arr.	H.	4·0	9·0	—	0·8	—	—	—	—
L 32	Arr.	H.	19·4	—	—	—	32·4	—	—	0·74
L 47	Arr.	H.	16·2	—	—	—	—	—	—	—
Pool of bladder urines										
L 3–L 23	—	—	14·7	0·7	8·6	2·3	22·6	1·6	0·2	—
L 31	—	—	—	—	—	—	—	—	—	0·77
L 34	—	—	—	—	—	—	34·0	—	—	—
L 36	—	—	—	—	—	—	31·8	—	—	0·77
L 37	—	—	—	—	—	—	30·6	—	—	—
L 38	—	—	—	—	—	—	30·6	—	—	0·70
L 39	—	—	—	—	—	—	—	—	—	0·84
L 40	—	—	—	—	—	—	30·6	—	—	—
Catheterized on board										
L A	—	—	0·33	—	—	—	—	—	—	—
L D	—	—	7·2	—	—	—	32·5	—	—	—
L E	—	—	7·0	—	—	1·1	14·6	1·0	—	—
L F	—	—	14·8	—	—	1·5	—	—	—	—
L K	—	—	5·1	—	—	—	27·5	—	—	0·60
L I	—	—	7·5	—	—	0·4	19·0	—	0·17	0·88
L G	—	—	3·0	—	—	—	15·8	—	—	—

Aq. 28 hr.: kept in aquarium for 28 hr. Arr.: on arrival at the laboratory. Arr. 4 hr.: 4 hr. after arrival. H., healthy; D., dying.

figures for urine are the same, viz. 18·6–20·9; we find lower limits of variations, from 0·4 to 2·3. Sodium is not in line with chlorine. Sulphates in plasma vary from 1·2 to 3·7 according to Smith (1929); Sulze (1922) found from 0·7 to 1·4 in urine; we found higher figures: 7·6, 8·6 and 9·3. This means a concentration of at least three times, although much less than for magnesium.

Several authors suggested that the urine secreted by *Lophius* in the aquarium is not the same as that secreted by fishes living under normal conditions. Grafflin (1931) emphasizes that skin injury in toad-fish increases the concentration of chloride in plasma and urine, while normal urine is poor in chloride and rich in nitrogen. In *Lophius*, taken in 1 hr. trawl, he finds 13·2 and 13·9 m.equiv. %. Preliminary experiments made by one of us (E.N.) tend to show that the rubbed skin of *Lophius* is more permeable, *in vitro*, to crystalloids than normal skin. Grollman (1929) finds lower figures in urine of fish caught at sea, for instance 5·6 m.equiv. against 18·8 in the aquarium, 3·8 mg. sodium against 10·8; for phosphorus the figures are the same; for calcium, 0·2 against 0·3 m.equiv./100 ml. Our figures concern plasma and urine either collected on deck as soon as the fish was brought up, or on arrival at the laboratory after having been kept in running sea water on board.

If we consider the analyses of plasmas separated on board or on arrival, and only take into account plasma separated immediately, we find for chloride the following figures: on board, 15·3, 15·3, 15·6 and 17·2; on arrival 16·1, 19·6 and 19·0. There is therefore a tendency to increase which may explain a similar increase in urine. The same appears to be true for sodium: 18·5, 18·5 and 19·3 on board against 21·7 and 21·3 on arrival.

The Δ for urine of fishes examined on board give us the lowest figure: 0·60° C. and also the highest, 0·88° C., while on arrival they vary from 0·70° up to 0·84° (Table V); for chloride, figures on board vary between 0·33 and 14·8 m.equiv./100 ml., the mean of seven figures being 6·4, while urine from bladders catheterized on arrival in the laboratory gave figures from 4·0 up to 19·6, with a mean of 15·1. For sodium and calcium our figures are insufficient in number. A pool of twenty bladder urines taken on arrival in the laboratory gave 22·6 m.equiv. of magnesium, while the average of fifteen determinations on board was 20·2. Again, on board we have two of the lowest figures: 14·6 and 15·8. More results will have to be obtained before we can really assert that within a few hours after being caught in the trawl there is an increase in the urine inorganic constituents. Our values for chloride, sodium and magnesium tend to confirm the hypothesis of an increased absorption of minerals, at least of NaCl, presumably through the skin.

Summary

The blood of *Lophius piscatorius* is poor in haemoglobin, the volume of red cells being only 17%. The plasma contains less than 40 g. of protein/l., of which only 6·7% is albumin. This explains its low osmotic pressure. As is well known, it contains more crystalloids than mammalian blood, Δ (depression of freezing-point) being 0·84°, the same figure being found at Naples, Woods Hole and Plymouth. This rather high concentration is not due to organic constituents, that of total non-protein nitrogen being of the same magnitude as in mammals; it is mainly due to a high content of sodium

chloride. Chloride is at a concentration of 15·3 m.equiv./100 ml., sodium at 18·5, while in mammals they reach 9 and 15 respectively.

Total non-protein nitrogen concentrations in plasma are similar to concentrations in mammals; the main non-protein nitrogen constituents of plasma are neither urea, ammonia, uric acid or allantoin, but trimethylamine or trimethylamineoxide. Of our analysis 37% of non-protein nitrogen of plasma remain unidentified, so far as we can rely upon our chemical methods. The power of concentration of the kidney for non-protein nitrogen on the whole is not high; it varies up to fifteen times. But the degree of concentration by the kidney, small for most constituents, even for trimethylamine, seems to be very high for creatine which is the main representative of non-protein nitrogen. Of our urine analysis, 30% of non-protein nitrogen remain as yet unidentified.

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BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

By L. Brull, E. Nizet and E. B. Verney, F.R.S.,
with the collaboration of L. Wilsens

From the Plymouth Laboratory and Institut de Clinique et Policlinique Médicales, Liège, Belgium

(Text-fig. 1)

It is well known that the kidney of the adult *Lophius piscatorius* (L.) is practically aglomerular, some of the original glomeruli disappearing late in the young animal, as we have been able to confirm by the examination of fishes a few centimetres long. The kidney of *Lophius* receives only small amounts of arterial blood, its main supply being by venous blood from the caudal vein. Several attempts have been made by previous authors to investigate the secretion of *Lophius* kidney, and to these reference has been made in a paper in which the chemical composition of the blood and urine are dealt with (Brull & Nizet, 1953). We are not aware of publications on blood perfusion experiments on *Lophius* kidneys.

The aim of the present research was to study the secretion of the perfused *Lophius* kidney and its response to variations of venous pressure at the entrance to the organ, and to measure the blood and urine flows with a view to determining whether an aglomerular kidney is sensitive, and to what extent, to variations in its circulation. In a series of previous publications one of us, with his collaborators (Brull & Dor, 1940; Brull & Louis-Bar, 1950, 1953), has investigated the response of the glomerular kidney of the dog to wide variations of blood pressure. The main result of these experiments was that the blood flow through the dog's kidney in the whole animal is to a large extent independent of variations of blood pressure from 100 mm. up to 330 mm. Hg, while the urine flow responds regularly to these variations. Previous research on isolated perfused kidneys of the dog had regularly shown increases of blood flow in response to increases in perfusion pressure.

Methods

Several favourable conditions had to be realized in order to permit the organization of our experiments: a large supply of good-sized *Lophius*, brought in well alive (so allowing the gathering of sufficient amounts of heparinized blood for perfusion) and with kidneys of sufficient size to permit catheterization of the caudal vein and ureters, etc. These conditions were fulfilled

beyond our expectations at the Plymouth Laboratory; and we are much indebted to the Director and Staff of the Laboratory for the generous and efficient way in which they supplied us with *Lophius* in good condition and with any materials which proved necessary for our investigations.

As soon as the fish were brought in, they were killed by a blow on the head, and one of the branchial arteries was cannulated for bleeding into heparin. Several *Lophius* of all sizes up to 100 cm. long were bled, and the blood was kept in cold storage. As soon as more than 500 ml. of blood had been collected, a perfusion experiment was carried out, using a kidney of a large-sized fish. The blood was put in a glass bulb at variable and measured levels above the kidney. Rubber tubing and a glass cannula inserted into the renal end of the caudal vein carried the blood to the gland, while the effluent was collected in a funnel and returned to the bulb. By so doing, the perfusion was made at room temperature, except in one experiment where all the glassware was kept in ice in order that the kidney should be perfused at a temperature of about 5° C.

In Exp. 2 the blood was oxygenated and, after perfusing the kidney, reoxygenated in a large-sized spiral tube down which it flowed in a thin layer, while oxygen ran through in the opposite direction. The degree of oxygenation of the blood was not measured. In Exps. 1, 3 and 4 unreoxygenated venous blood was used.

Urine was collected from the ureter, and microdeterminations were made of total nitrogen (Kjeldahl), chlorine (St Russnyak, 1926, p. 211), and of magnesium (colorimetrically, as described by Fister, 1950).

Results

Experiment 1

Blood from several *Lophius* was collected the day before and heparinized. The perfused kidney (from L. no. 18, weighing 10·8 kg.) weighed 19 g. Perfusion was started 47 min. after the arrival of the fish. Bladder urine before experiment: 5 ml. with 414 mg. Cl/100 ml. The results are given in Table I. Perfusion pressures are in mm. of blood, blood flows in ml./g. of fresh kidney per minute, and urine flows in ml. per gram of fresh kidney per hour. Room temperature: 24·7° C.

This is the first renal blood perfusion experiment carried out in *Lophius*. We do not know the pressure in the caudal vein at its entrance to the kidney in the live animal, but considering the thinness of the wall, this pressure is likely to be very low. The urine flow began with a blood pressure of 20 mm., and raising this pressure progressively from 40 to 50 mm. up to 120 mm. had no influence on the rate of urine flow. This flow is abundant, and especially so when we consider that about 40% of the kidney is lymphoid and not renal tissue: the rate of flow (per g. fresh tissue) is, indeed of the same order as that in the living dog. The urine is acid (pH 5), and colourless. The kidney does not concentrate chloride, but concentrates total non-protein nitrogen more than five times in sample 3, and three times in sample 5. Magnesium is highly

TABLE I. EXPERIMENT 1

Time (min.)	Perfusion pressure, (mm. blood)	Blood flow (ml./g./min.)	Urine No.	Urine Volume (ml.)	Urine ml./g./hr.	Urine Cl (mg./100 ml.)	Urine Mg (mg./100 ml.)	Urine N (mg./100 ml.)	
0	20	0·36	—	—	—	—	—	—	Perfusion started (venous blood)
20	—			First drop collected					pH of urine: 5
41	15	0·20	—	—	—	—	—	—	—
42	40	0·48	—	—	—	—	—	—	Pressure raised
60	40	0·52	1	2·0	0·16	594	—	—	pH of urine: 5
73	50	0·65	—	—	—	—	—	—	—
90	50	0·55	2	2·0	0·20	598	—	—	—
93	50→65	0·55; 1·10	—	—	—	—	—	—	—
98	70	—	—	—	—	—	—	—	—
118	70	—	3	2·2	0·25	596	270	113	—
150	70→120	1·10→	4	2·0	0·20	584	255	—	Pressure raised
153	120	4·60	—	—	—	—	—	—	—
180	120	—	5	2·0	0·20	—	222	70	—
188	—	—	6	1·2*	—	—	180	—	Cl in plasma 576, N 23 mg./100 ml.

* Including vol. in catheter.

TABLE II. EXPERIMENT 2

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g.min.)	Urine No.	Urine ml./g./hr.	Urine Cl (mg./100 ml.)	Urine Mg (mg./100 ml.)	Urine N (mg./100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (oxygenated blood)
5	55	0·47	—	—	—	—	—	—
10	—	—	—	—	—	—	—	Ureter catheterized: flow starts
20	55	0·26	—	—	—	—	—	Spontaneous drop of blood flow
33	55	0·19	—	—	—	—	—	—
36	55	0·35	—	—	—	—	—	Spontaneous rise of blood flow
46	60	0·32	—	—	—	—	—	—
55	70	0·38	—	—	—	—	—	—
60	80	0·50	—	—	—	—	—	—
70	80→125	1·00	1	0·11	—	—	70	Pressure raised
75	160	1·10	—	—	—	—	—	—
85	190	1·50	—	—	—	—	—	—
108	210	1·40	—	—	—	—	—	—
130	228	—	2	0·13	595	279	—	—
135	228→340	—	—	—	—	—	—	Pressure raised
136	340	2·30	—	—	—	—	—	—
145	—	—	—	—	—	—	—	Blood sample I: Cl in plasma 595 mg., non-protein N 30 mg./100 ml.
160	360	2·40	3	0·16	—	291	—	3 ml. 5 M-NaCl added to the blood
175	—	—	4	0·14	—	—	86	—
180	385	2·50	—	—	—	—	—	—
205	380	—	5	0·14	970	384	—	Blood sample II: Cl in plasma 825 mg., non-protein N: 36 mg./100 ml.

concentrated seeing that *Lophius* plasma contains only about 6 mg./100 ml.: at the beginning of the experiment the urine Mg was about 45 times the

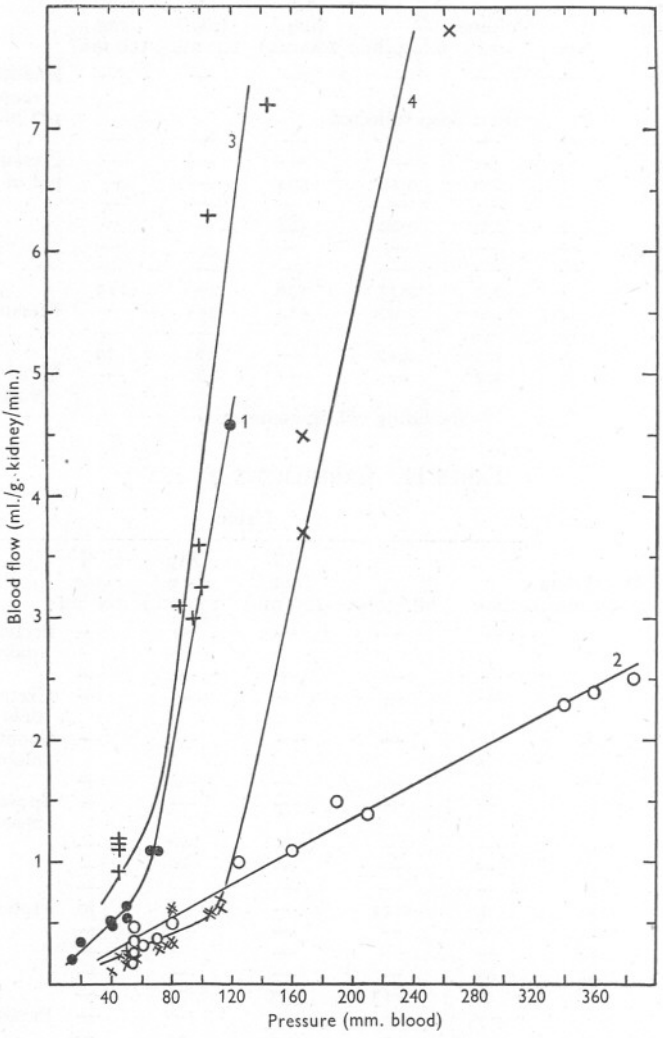

Fig. 1. The relation between blood flow and perfusion pressure in the four experiments described in the text. In Exps. 1, 3 and 4 the kidney was perfused with venous blood, in Exp. 2 with oxygenated blood. In Exp. 4 the perfusate was cooled, its inflow temperature being between 2 and 8° C (Table IV).

plasma Mg, and still 30 times at the end. Thus Mg secretion may be considered as one of the best tests for renal activity in this species. The blood flow, at perfusion pressures between 15 and 40 mm., varies from 0·2 up to 0·5 ml./g. of

fresh kidney per minute, and when the pressure is raised from 40 to 70 mm. the flow increases from 0·5 to 1·1 ml./min. With a higher pressure (120 mm.), the response is proportionally much greater, showing that the resistance of the vascular bed has diminished (Fig. 1).

Experiment 2

Blood was collected from several *Lophius* just before the perfusion, and the day before. The blood was oxygenated. Room temperature 24·2° C. The perfused kidney (from L. no. 28 weighing 7 kg.) weighed 17 g. The results of this experiment are given in Table II.

The renal blood flow shows spontaneous fluctuations, but follows the pressure more or less passively throughout (Fig. 1). The urine flow increases with perfusion pressure, but only to a very small extent: a 500% increase in pressure is associated with a 25% increase in urine flow. The kidney excretes Cl at the same concentration as it is present in the plasma until the plasma level is raised from 595 to 825 mg./100 ml.; from then on a slight concentration of Cl (970 mg./100 ml.) occurs. Nitrogen is concentrated twice, and this concentration continues throughout the experiment. Magnesium is highly concentrated, as in Exp. 1.

Experiment 3

270 ml. mixed heparinized venous non-oxygenated blood was used as the perfusate. Room temperature 21·7° C. The perfused kidney (from L. no. 36 weighing 7 kg.) weighed 12 g. Bladder urine before experiment: 20 ml. with Cl. 680 mg. and non-protein nitrogen 75 mg./100 ml. The results of this experiment are given in Table III.

The renal blood flow follows the perfusion pressure, but the variations of flow are proportionally very much greater than the variations of pressure (Fig. 1). In this experiment the urine flow is directly proportional to the perfusion pressure. Chloride in the urine is slightly concentrated from the beginning, and more so after the plasma chloride is raised. Non-protein nitrogen is high in the plasma and is not concentrated. Magnesium is again highly concentrated.

Experiment 4

550 ml. venous non-oxygenated mixed heparinized blood was used as the perfusate. The blood reservoirs were cooled in ice with sodium chloride. The kidney weighed 23 g. (from L. no. 45 weighing 10 kg. fished the previous day and kept alive in the aquarium). The results of this experiment are given in Table IV.

The blood flow is roughly proportional to the perfusion pressure up to a pressure of about 100 mm.; thereafter the variations of flow are proportionally very much greater than those of pressure (Fig. 1). After the kidney had been subjected to a pressure of 285 mm. of blood, the return of blood flow to its initial value when the pressure was lowered to its initial value (column 1, 290–300 min.) shows that the vessels had not lost their elasticity as a result of this operation. The urine flow, non-existent at the beginning, begins to be

appreciable only at a pressure of 70 mm., and at higher pressures there is no parallelism between perfusion pressure and urine flow. Nitrogen is concentrated three times at the most, no more than during perfusion at room temperature. Chloride is not concentrated. Magnesium is highly concentrated, but no more, or even less, than at room temperature.

TABLE III. EXPERIMENT 3

			Urine					
Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	No.	ml./g./hr.	Cl (mg./ 100 ml.)	Mg (mg./ 100 ml.)	N (mg./ 100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (venous blood)
10	50	0·7	—	—	—	—	—	0·2 ml. urine in cannula
15	45	1·15	—	—	—	—	—	Spontaneous rise of blood flow
30	45	0·92	—	—	—	—	—	—
50	45	1·10	—	—	—	—	—	—
62	45	1·20	—	—	—	—	—	—
70	45→85	—	1	0·10	—	—	—	Pressure raised
73	87	3·10	—	—	—	—	—	—
108	101	3·30	—	—	—	—	—	—
115	101→140	—	2	0·20	—	345	64	Pressure raised
120	145	7·20	—	—	—	—	—	—
125	—	—	—	—	—	—	—	Blood sample I: Plasma Cl 670 mg., non-protein N 87 mg./100 ml.
140	145→105	—	3	0·29	760	321	—	2 ml. 1 M-NaCl added to the blood. Pressure lowered
146	105	6·30	—	—	—	—	—	—
152	—	—	—	—	—	—	—	Blood sample II: Plasma Cl 787 mg./ 100 ml.
165	—	—	4	0·16	—	348	—	—
167	100	3·60	—	—	—	—	—	—
200	100	—	5	0·18	894	378	—	—
210	95	3·00	—	—	—	—	—	—
220	95	—	6	0·17	—	—	—	—
235	—	—	7	0·16	—	—	80	—

DISCUSSION

Lophius kidneys, perfused with heparinized blood, retain for several hours the power of producing urine. This urine is colourless but its chemical composition shows that the gland is actively secreting.

Among the constituents investigated, magnesium was concentrated up to 50 times by the kidney and reached the same degree of concentration as is found in the bladder urine of freshly killed fish. Total non-protein nitrogen is also concentrated by the perfused kidneys, but not so much as in the living fish. Some of the separate constituents are perhaps more concentrated than the figures for the total non-protein nitrogen would suggest: the small volume of the urine samples did not enable us to answer this question. The perfused

TABLE IV. EXPERIMENT 4

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	Blood T (° C) at kidney: In-flow	Blood T (° C) at kidney: Out-flow	Urine: No.	Urine: ml.	Urine: ml./g./hr.	Urine: Cl (mg./100 ml.)	Urine: Mg (mg./100 ml.)	Urine: N (mg./100 ml.)	
0	—	—	—	—	—	—	—	—	—	—	Perfusion started (venous blood)
5	40	0·11	5	15	—	—	—	—	—	—	—
25	40	—	—	—	—	—	—	—	—	—	—
35	40→46	—	5	14	—	—	—	—	—	—	Pressure raised
36	46	0·23	5	13	—	—	—	—	—	—	—
50	50	0·20	—	—	—	—	—	—	—	—	—
55	56	0·20	—	12	—	—	—	—	—	—	—
60	70	0·32	2	11	—	—	—	—	—	—	—
65	70	0·32	—	—	1	1·6	0·07	—	110	65	—
70	72	0·28	—	—	—	—	—	—	—	—	—
80	77	0·32	—	10·5	—	—	—	—	—	—	—
85	80	0·35	—	10·5	—	—	—	—	—	—	—
120	81	0·32	—	—	2	2·7	0·12	552	153	75	—
135	105	0·57	—	—	—	—	—	—	—	—	—
165	105	0·58	—	—	3	3·0	0·17	—	170	—	—
180	107	0·62	—	—	4	1·0	0·17	—	176	—	—
185	107	0·69	—	—	—	—	—	—	—	—	—
195	107→162	—	—	—	—	—	—	—	—	—	Pressure raised
200	165	—	2	9	—	—	—	—	—	—	—
205	168	—	—	—	—	—	—	—	—	—	—
215	168	3·70	—	—	—	—	—	—	—	—	—
225	160	—	—	—	5	2·4	0·14	525	170	—	Blood now looks well oxygenated
245	168	4·50	2	9	—	—	—	—	—	—	—
255	166	—	—	—	6	2·2	0·19	—	170	37	—
260	166→230	—	—	—	—	—	—	—	—	—	Pressure raised
270	265	7·80	—	—	—	—	—	—	—	—	—
280	285	—	8	8·5	—	—	—	—	—	—	—
285	—	—	—	—	7	1·8	0·15	—	170	—	—
290	285→45	—	—	—	—	—	—	—	—	—	Pressure lowered
292	45	0·20	—	—	—	—	—	—	—	32	—
300	45	0·16	—	—	8	1·2	0·19	—	196	32	—
	45→80	—	—	—	—	—	—	—	—	—	Pressure raised
305	80	0·68	—	—	—	—	—	—	—	—	—
325	80	0·60	—	—	—	—	—	—	—	—	—
330	—	—	—	—	9	1·2	0·11	508	202	—	Plasma at the end of perfusion: Cl 581 mg., non-protein N 20 mg./100 ml.

kidney excretes chloride usually at the same concentration as in plasma; but when the plasma chloride is artificially raised the concentration of chloride in the urine is higher than that in the plasma.

The activity of the kidney does not seem to be improved by oxygenation of the blood, which is not surprising seeing that venous blood is by far the main supply to such kidneys under normal conditions. Neither is this activity appreciably different at room temperature and at low temperatures close to those at which *Lophius* normally lives. Changes in perfusion pressure up to 100-150 mm. of water raised the urine flow; above 150 mm. there was no effect. This result is in marked contrast with the effects of such changes in a glomerular kidney.

The renal venous vascular net offers but little resistance to the flow of blood: at perfusion pressures between 20 and 40 mm. blood (about 1·5 to 3 mm. Hg) the flow is of the order of 0, 3 ml./g. fresh tissue/min., whereas in the dog the flow at arterial pressures of about 100 mm. Hg is of the order of 3 ml./g. fresh tissue/min. At low perfusion pressures the blood flow in *Lophius* is approximately proportional to the pressure. At higher pressures, however, the flow became proportionately greater in the three experiments in which venous blood was the perfusate, and this change in the slope of the pressure-flow relation occurred at a higher pressure in the experiment (no. 4, see Fig. 1) in which the temperature of the perfusate was lowered. In the experiment (no. 2, see Fig. 1) in which oxygenated blood was the perfusate the blood flow was approximately proportional to the pressure over a very wide range, viz. 50–385 mm. blood; and in both Exps. 2 and 4 the blood flows at a given pressure were lower than in the other two experiments. Further research will show whether the temperature and oxygen tension of the blood play a part in the tone of the kidney vessels of *Lophius* and in their responses to changes in perfusion pressure. In its blood-flow responses to changes in perfusion pressure, therefore, the kidney of *Lophius* behaves, too, in a manner which is in marked contrast with the behaviour of the dog's kidney.

Summary

Lophius kidneys perfused with the heparinized blood (venous) of the fish secrete urine in which total non-protein nitrogen is concentrated, magnesium highly concentrated, and chloride only slightly so or not at all. Oxygenation of the blood, or lowering the temperature of the perfusate from *c.* 20° to *c.* 5° C. does not appear to influence secretion. The blood flow through the kidneys increases with the perfusion pressure, the increase often becoming disproportionately large. The urine flow, on the other hand, above a certain critical level is largely independent of changes in perfusion pressure.

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DECREASED DISCRIMINATION DURING SETTING AFTER PROLONGED PLANKTONIC LIFE IN LARVAE OF *SPIRORBIS BOREALIS* (SERPULIDAE)

By E. W. Knight-Jones

Marine Biology Station, University College of North Wales, Bangor

(Text-figs. 1 and 2)

Many larvae of benthic invertebrates search actively for a place to settle, as the end of the planktonic stage approaches. During this process they show considerable powers of discrimination and they can postpone metamorphosis until their search is successful (Thorson, 1950; Wilson, 1952). Since their swimming is feeble and usually undirected, apart from a negative reaction to light in many species, their random explorations must often become very prolonged. Such prolongation will generally be dangerous because planktonic larvae are particularly vulnerable to predators, and because development cannot be long delayed without seriously weakening larvae such as those of barnacles and *Spirorbis*, which do not feed during the searching phase. It therefore seemed possible that searching larvae would become more prone to settle, and consequently less discriminating in their choice of environment, if the planktonic stage were prolonged. Wilson (1952, 1953) has suggested that *Ophelia* larvae behave in this way. Such behaviour would be of adaptive value, in avoiding the dangers of an excessively long planktonic stage. It would also agree with Lorenz's widely accepted idea of instinctive behaviour, as the expression of a conative urge towards a goal, which grows gradually stronger if attainment of the goal is delayed (Thorpe, 1948).

The gregarious reaction of *Spirorbis* larvae (Knight-Jones, 1951) offered a good opportunity for investigating this possibility. These larvae pass successively through a surface-swimming stage, searching stage, setting action pattern and cataclysmic metamorphosis. They were readily obtained by collecting adult *Spirorbis borealis*, epizoic on *Fucus serratus*, during the neap tide periods of larval liberation, and placing them without delay in troughs of sea water. As soon as larvae were liberated in sufficient numbers, they were distributed equally between the various series of experiments in progress, to ensure homogeneity of material. Possible effects, due to diurnal variations in activity, were guarded against by bright illumination at night and by collecting adults and starting experiments at intervals throughout the day and night. Parent *Spirorbis* did not readily liberate larvae at night, but bright illumination and changes of sea water induced some to do so.

Lowered Level of Selection after Prolonged Swimming

Larvae were removed immediately after liberation, and distributed, about twelve to each bowl, between four batches of glass bowls, each of which had been wiped with cotton-wool just previously, and filled with 250 ml. of sea water. *Spirorbis* larvae rarely attach themselves to freshly wiped glass surfaces, and these were kept for various periods without metamorphosis, until offered the choice of substrata described below.

The four batches of bowls were used in four series of experiments, one series involving freshly liberated larvae and the other three series involving larvae which had been kept swimming for 3, 6 and 12 hr. respectively. In each experiment the larvae were offered a choice between two similar pieces of freshly collected *Fucus serratus*, placed about 6 cm. apart. One piece of each pair, termed the 'previously colonized' piece, had already been exposed to larvae, which had attached themselves to it in numbers varying between 12 and 150, while the other was bare, having been kept under similar conditions, but without larvae. Variation in the numbers of larvae settled on the previously colonized pieces was difficult to control, but in this type of experiment variation within wide limits has been found to have no significant effect on the results, as indeed can be seen from close inspection of the figures in Table I.

A fifth series of similar experiments was carried out using larvae which had been kept from metamorphosis for 24 hr. The majority of such larvae were incapable of locomotion, so those used in this series were obtained by keeping large numbers of larvae in beakers for 24 hr., and then selecting those which still swam.

The numbers of larvae setting subsequently on the previously colonized pieces and on the bare control pieces in the course of each experiment are shown in Table I, together with the angular transformations of the fractions $\frac{\text{no. settled on control}}{\text{total no. settled}} = \sin^2\theta$ (Fisher & Yates, 1948). This transformation was used to render the variance independent of the mean, and so promote homogeneity in the statistical treatment. Analysis of variance of θ throughout the five series indicates a difference between series, which is significant at the probability level 0·05. Further analysis indicates a highly significant difference ($P = 0{\cdot}01$) between the results from freshly liberated larvae and those from all other larvae. There are no significant differences between the four series of experiments in which these other larvae were used, resulting from the increase in the period of enforced delay from 3 to 24 hr.

In deriving a mean value of θ for each series, it was found as a result of a χ^2 test that the individual larvae could not justifiably be treated as units, perhaps because all those in certain batches had been affected by small inequalities of lighting. Instead it was considered that the most efficient treat-

TABLE I. NUMBERS OF PREVIOUSLY SETTLED *SPIRORBIS* ON PREVIOUSLY COLONIZED/BARE SURFACES, PLUS NUMBERS OF LARVAE SETTING IN EACH EXPERIMENT

Angular transformations $\left(\frac{\text{no. setting on bare surface}}{\text{no. setting on both surfaces}} = \sin^2\theta\right)$ are given in brackets.

Larvae were kept without surfaces suitable for setting for				
0 hr.	3 hr.	6 hr.	12 hr.	24 hr.
69/0+ 8/0 (0)	32/0+ 3/1 (30·0)	32/0+ 1/4 (63·4)	97/0+6/1 (22·2)	42/0+0/0 (—)
28/0+ 6/1 (22·2)	12/0+ 3/0 (0)	83/0+ 2/2 (45·0)	57/0+3/3 (45·0)	132/0+0/0 (—)
25/0+ 7/1 (20·7)	25/0+ 5/2 (32·3)	21/0+ 2/1 (35·3)	53/0+3/3 (45·0)	15/0+2/0 (0)
52/0+ 5/5 (45·0)	57/0+ 5/3 (37·8)	39/0+ 2/0 (0)	25/0+8/2 (26·6)	83/0+1/0 (0)
41/0+12/2 (22·2)	49/0+ 2/0 (0)	78/0+ 2/0 (0)	51/0+2/2 (45·0)	45/0+1/0 (0)
19/0+14/6 (33·2)	30/0+12/1 (16·1)	30/0+10/2 (24·1)	57/0+4/5 (48·2)	53/0+1/1 (45·0)
30/0+11/0 (0)	12/0+14/3 (24·8)	39/0+10/2 (24·1)	56/0+1/8 (70·5)	67/0+7/2 (28·1)
32/0+11/2 (23·1)	40/0+10/8 (41·8)	26/0+ 5/6 (47·6)	77/0+6/6 (45·0)	83/0+4/3 (40·9)
74/0+12/0 (0)	35/0+ 7/6 (42·8)	61/0+ 7/1 (20·7)	62/0+2/7 (61·9)	39/0+0/1 (90·0)
130/0+ 9/4 (33·7)	87/0+ 5/5 (45·0)	154/0+ 7/5 (40·2)	89/0+9/2 (25·2)	93/0+2/1 (35·3)
51/0+13/2 (21·4)	75/0+ 6/9 (50·8)	31/0+ 1/5 (65·9)	43/0+6/4 (39·2)	27/0+1/1 (45·0)
91/0+ 7/8 (46·9)	72/0+10/6 (37·8)	80/0+ 4/5 (48·2)	57/0+5/3 (37·8)	87/0+1/1 (45·0)
81/0+ 7/3 (33·2)	37/0+ 8/6 (40·9)	49/0+10/9 (43·5)	54/0+8/2 (26·6)	56/0+2/0 (0)
52/0+13/0 (0)	51/0+13/3 (25·7)	44/0+ 6/2 (30·0)	65/0+2/1 (35·3)	38/0+2/3 (50·8)
92/0+ 6/7 (47·2)	49/0+ 8/3 (31·5)	43/0+ 5/3 (37·8)	85/0+1/1 (45·0)	30/0+2/1 (35·3)
45/0+13/1 (15·5)	52/0+ 4/8 (54·7)	54/0+ 8/3 (31·5)	80/0+3/4 (49·1)	59/0+3/0 (0)
33/0+10/3 (28·7)	44/0+ 2/6 (60·0)	52/0+ 2/3 (50·8)	30/0+0/0 (—)	44/0+0/0 (—)
24/0+11/3 (27·6)	34/0+ 3/7 (56·8)	44/0+ 2/2 (45·0)	32/0+1/0 (0)	35/0+0/0 (—)
24/0+ 9/3 (30·0)	70/0+ 3/9 (60·0)	39/0+ 0/6 (90·0)	30/0+1/1 (45·0)	34/0+0/0 (—)
40/0+ 8/5 (38·3)	50/0+ 5/5 (45·0)	27/0+ 1/0 (0)	21/0+0/1 (90·0)	44/0+1/1 (45·0)
Totals 192/56	128/91	87/61	71/56	30/15
248	219	148	127	45
Weighted mean θ with S.E. $\pm\Delta\theta$ (25·36 ± 3·35)	(39·31 ± 3·10)	(39·67 ± 3·90)	(41·45 ± 3·43)	(32·33 ± 4·42)
P from $\frac{45-\theta}{\Delta\theta}$ <0·001	>0·1	0·2	>0·2	0·05

ment was to take each experiment as a unit, but to weight the result according to the number of larvae involved, so as to minimize the effect of those batches which had large errors due to small numbers of larvae setting. The weighted mean for each series, with its standard error, is given in Table I. To ensure that this treatment was valid, a more rigorous treatment was also employed, in which each experiment was treated as a unit without weighting: the results were not significantly different from those given in Table I. It is therefore clear that freshly liberated larvae showed well-marked selection of the previously colonized pieces of *Fucus*, whilst larvae which had been kept swimming for 3 hr. or longer showed little evidence of discrimination. Indeed, in some of the series involving prolongation of the planktonic life, the evidence is consistent with random setting. The somewhat anomalous results, obtained from the 24 hr. series, may perhaps have been due to the fact that the larvae of this series had been selected for their vigour, whereas all the other larvae were unselected.

The decrease in numbers setting on the *Fucus*, in experiments which involved delayed metamorphosis (Table I), was due partly to prior setting on the glass of the dishes, but mainly to weakness of the remaining larvae. Their swimming became slower and was occasionally interrupted, until eventually they became entirely immobile. A few showed some of the changes of metamorphosis without becoming attached, but many retained their larval form for several days, remaining motionless and clearly doomed to die unmetamorphosed.

Percentages of Larvae Settled, in the Presence of Previously Settled Individuals and in Isolation, after various Periods of Swimming

Choice of substrata which bear previously settled individuals is probably associated with the fact that setting is delayed in isolation (Knight-Jones, 1951). It seemed that the lesser degree of choice exercised after prolongation of the planktonic life might be due to more hasty setting, in gregarious and in isolated larvae alike.

Two parallel series of experiments were set up, using equal numbers of freshly liberated larvae in small beakers, each of which had been wiped just previously and filled with about 50 ml. of sea water. The larvae in one series were distributed so that each beaker contained five, which may be termed 'associated' larvae. Those in the other series were isolated, each beaker containing a single larva. The associated larvae were offered small pieces of *Fucus*, which bore previously settled *Spirorbis*, whilst the larvae of the other series were offered similar pieces of bare *Fucus*. Each of the two series was made up of four similar batches, A, B, C and D. In A the larvae were offered *Fucus* immediately after liberation, but in B, C and D they were kept without *Fucus* until 3, 6 and 12 hr. respectively after liberation. After adding *Fucus*,

numbers of larvae metamorphosed were counted at hourly intervals. The experiments were repeated during the liberation peaks of several months, until 800 larvae had been used, comprising a hundred associated and a hundred isolated larvae in each of the four batches.

Fig. 1 shows the curves, representing percentages metamorphosed against time, which resulted from these experiments. In each batch the curve relating

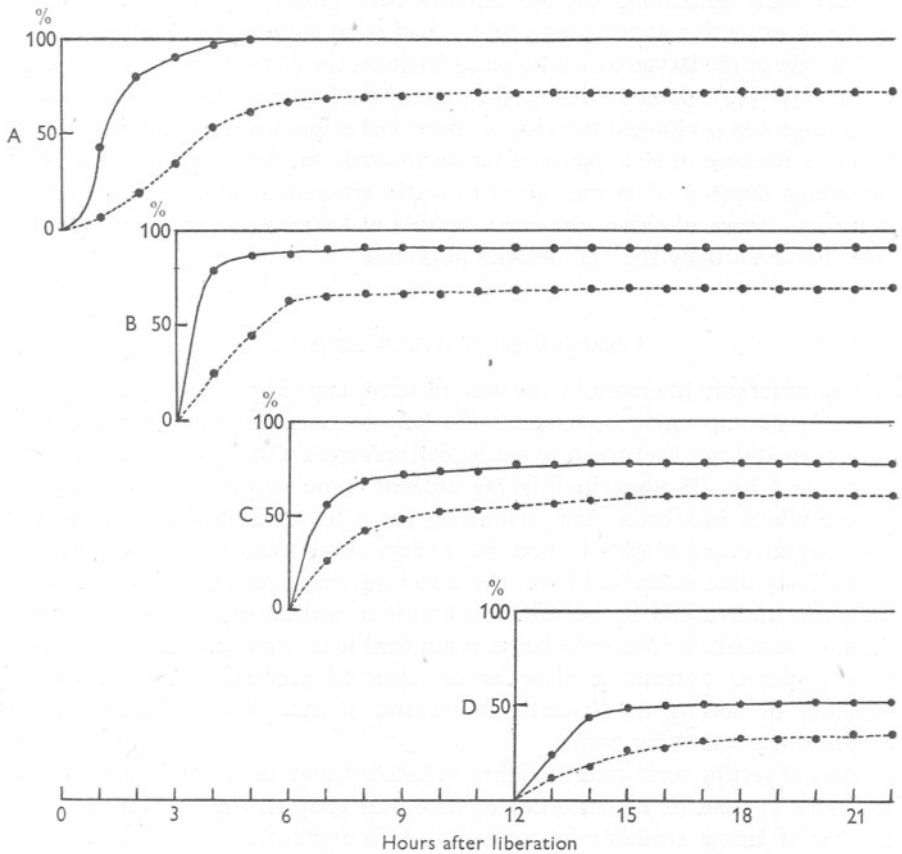

Fig. 1. Percentages metamorphosed, recorded at hourly intervals, in four similar groups of larvae. One group (A) was presented with *Fucus* without delay, whilst the others (B, C and D) were kept without substrata suitable for setting for periods of 3, 6 and 12 hr. respectively. Of the two curves drawn from each group, the continuous line relates to associated larvae, the interrupted line to isolated larvae.

to associated larvae is much steeper than that relating to isolated larvae. The difference between the two curves gives some indication of the degree of choice to be expected from each batch in gregariousness tests of the type described above. In batch A, for instance, eighty of the associated larvae settled within 2 hr., whereas only a quarter of that number of isolated larvae

settled within the same time. This agrees very well with the ratio between the numbers of larvae, which settled on previously colonized and bare *Fucus*, in gregariousness tests using freshly liberated larvae (Table I). In the remaining batches the curves are closer together, so the proportion setting in a given time on the bare *Fucus* is considerably higher. In gregariousness tests with similar batches of larvae (Table I) the ratio approached equality, probably because early settlement on the initially bare pieces of *Fucus* made these almost as attractive as the pieces which had been colonised previously.

The fate of the larvae which failed to settle on the *Fucus* (Fig. 1) is shown in Table II (p. 344). Some settled on the glass of the beakers, but when the planktonic stage was prolonged by 6 hr. or more, either because of delay in offering *Fucus* or because of reluctance of larvae to settle on bare *Fucus*, a gradually increasing number of larvae failed to settle altogether, apparently through weakness. Some of these remained capable of swimming for a considerable time, but eventually they all became immobile.

Comparative Rates of Setting

Setting in freshly liberated larvae was initially slow (Fig. 1 A) for larvae were generally photopositive throughout the first 15 min. after liberation and, if conditions did not lead them to settle, for recurrent short periods during the next 2 or 3 hr. Photopositive larvae did not come into contact with *Fucus*. Larvae which had been kept swimming for 3 hr. or longer were generally photonegative and alighted upon *Fucus* very soon after it was presented to them. They then embarked upon the searching phase proper, which involves crawling, interrupted by occasional periods of swimming. Previously they had not crawled, for *Spirorbis* larvae seem unable to crawl upon freshly wiped glass surfaces. Setting in these larvae occurred gradually, not because of difficulty in finding the *Fucus*, but because of individual variation in the length of the searching phase.

Rates of setting were clearly higher in batch B than in A, but seemed lower in C and D than in B. In order to take into account the reduction in the number of larvae available for setting as each experiment proceeded, a plot of the function $\frac{\log \frac{x}{x-N}}{t}$ was made, where x is the total number of larvae which finally settled on the *Fucus* and N is the number that had settled at time t (Fig. 2). The slope of this curve, $\frac{d \log \frac{x}{x-N}}{dt}$, gives an individual estimate of the rate of setting, which is independent of the number of larvae available. Considering the associated larvae first, the plot for batch A is a straight line,

which intercepts the base-line at about 15 min. after liberation (i.e. at the end of the entirely photopositive phase), and thereafter indicates a constant rate of setting of 0·44 of the population per hour. The plot for batch B is not a straight line, probably because the population is not homogeneous, for setting

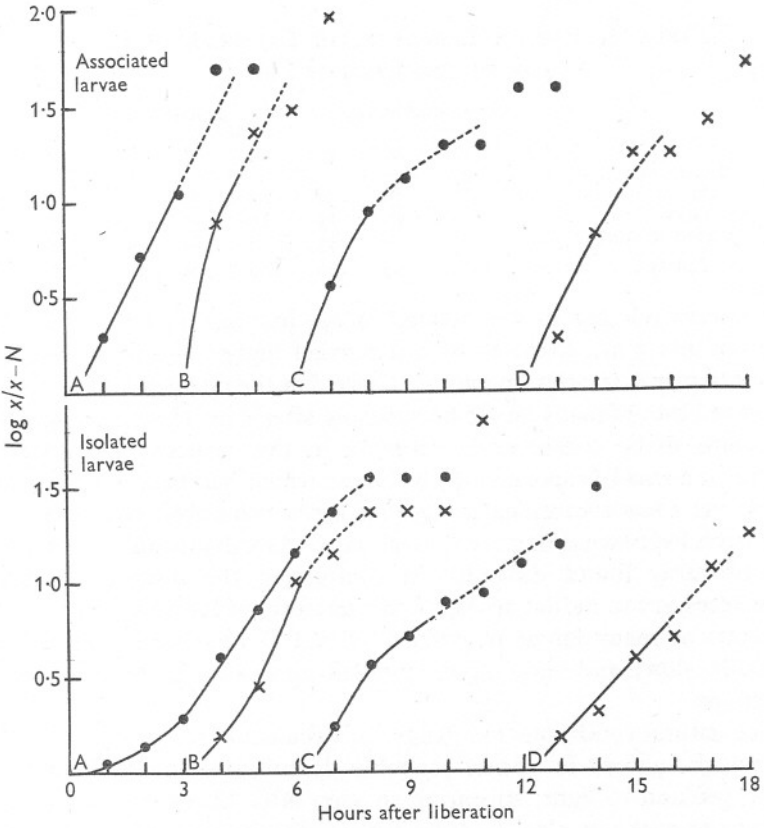

Fig. 2. The results recorded in Fig. 1, treated so as to obtain measurements of the rates of setting which are independent of the numbers of larvae available. A, B, C and D mark the curves relating to each batch of larvae. x = total number of larvae which settled on *Fucus* in each batch. N = number of these which had settled in a given time. The points where N approaches x are inaccurate, being based on small numbers of larvae, so these parts of the curves are dotted.

was delayed in some but not in others. The great majority of these larvae would have settled earlier, if they had had the opportunity, and it seems likely that these settled first, at about double the rate of batch A. The remainder corresponded to those individuals of batch A which were still swimming 3 or 4 hr. after liberation. Their setting was not delayed by the experiment, so it went on at the same rate as in batch A. In batches C and D the rate of

setting was secondarily lowered, probably because exhaustion, which fatally affected some individuals, was to some extent affecting the majority, but it remained somewhat faster than in A. In each of the curves C and D the initial rate of setting is faster than the ultimate rate, which would be expected if the more vigorous larvae settled first.

TABLE II. FATE OF LARVAE IN THE EXPERIMENTS WHICH FORMED THE BASIS OF FIG. 1

	Associated larvae				Isolated larvae			
	A	B	C	D	A	B	C	D
Settled on:								
Fucus	99	91	78	52	72	70	61	35
Glass	1	9	16	13	15	16	15	18
Failed to settle	0	0	6	35	13	14	24	47
Totals	100	100	100	100	100	100	100	100

The curves relating to the isolated larvae indicate a low rate of setting during the first 3 hr., followed by a somewhat higher rate which was maintained until 7 or 8 hr. after liberation. Thereafter the rate was lower. Probably the food reserves of many larvae become low after 8 hr. of swimming, though there seems to be considerable variation in this respect. Fatal weakness appeared in a small proportion of the larvae which had been kept swimming for 6 hr., yet a few exceptionally vigorous larvae were observed to settle after having been kept swimming for several days. As exhaustion approached the larvae probably found difficulty in completing the minimum searching routine necessary to induce setting, and therefore settled at a lower rate. The movements of many larvae in batches C and D had been observed to be abnormally slow, and they often spiralled aimlessly, or became stuck to obstructions.

Under natural conditions the danger of exhaustion seems slight, for most larvae which succeed in setting probably do so within a few hours. Their negative reaction to light, appearing so soon after liberation, will generally lead them to rocky or algal surfaces before they are carried away from the neighbourhood of the main parent stocks. This and gregariousness account for the fact that *Spirorbis* populations are well concentrated. The brief periods of surface swimming, which recur during the first few hours of searching, give larvae which descend into sandy or muddy coves, or creeks, repeated chances of being carried by currents over neighbouring rocks.

SUMMARY

Laboratory experiments showed that freshly liberated *Spirorbis* larvae settle on surfaces which bear previously settled individuals, in marked preference to bare controls, but that larvae which have been kept swimming for 3 hr., or longer, exercise less choice when setting.

When larvae are freshly liberated there is a great difference between the rate of setting gregariously, and the rate of setting in isolation; but after they have been kept swimming for 3 hr. this difference becomes less, owing to the larvae setting more rapidly both gregariously and in isolation. After swimming for about 8 hr. rates of setting fall off, and many larvae fail to settle, apparently through weakness.

I am greatly indebted to Dr D. J. Crisp for advice regarding this problem, and to him and Mr G. M. Spooner for much help with statistical treatment.

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A NEW APPARATUS FOR THE COLLECTION OF BOTTOM PLANKTON

By J. Wickstead

From the Plymouth Laboratory

(Text-figs. 1–5)

Introduction

Many workers have studied the vertical migrations of planktonic animals, and much knowledge has been gained of distribution at different levels at different times of the day. However, with the apparatus used by most of these workers, the plankton in the immediate vicinity of the sea-bottom has had to be neglected.

To sample this bottom plankton, various methods have been devised. Hensen (1895, Plates X and XI) designed a *Wagennetz* which was very complicated, and can have been of little practical use. Russell (1928), Reighard (1894), Beauchamp (1932) and Hardy & Lucas (personal communication) each designed simple and effective nets, but these have the very serious disadvantage of not limiting the samples to the bottom, and there is no way of telling at what level any particular specimen was collected.

The apparatus of Greze (1951) has apparently only been used in inland waters, its effectiveness in the sea being a matter of speculation. Here, precise workmanship is involved, and a finely adjusted mechanism. The latter is to be avoided if possible, since dragging along the sea-bed can so very easily disturb its accurate functioning.

The apparatus used by Bossanyi (1951) effectively restricts the haul to the bottom, but has some disadvantages, i.e. it is heavy and cumbersome, and can be used only in reasonably calm waters and in limited areas where the bottom deposit is suitable.

The apparatus about to be described has been devised to overcome these limitations. It is successful in that it is capable of sampling bottom plankton only; easy to handle, weighing only about 90 lb.; easy to shoot, the shooting not necessarily being restricted to calm weather; simple mechanically; independent of the sea-bed for operating; and self-contained, dispensing with messengers and throttling nooses; and it can be used for making accurately timed hauls.

The principles involved in the working are quite different from those of Bossanyi's net. The idea of using water pressure created over a plane surface caused by towing is not entirely new: Giesbrecht (1893) used it for a mid-water

closing net. However, it does not seem to have been used since, certainly not for a bottom plankton net.

I am very much indebted to Mr J. M. Smith, of University College, London; to Captain C. A. Hoodless, D.S.C., and the crew of R.V. *Sabella*; to the Plymouth Corporation, for permission to use their open-air swimming bath; and to Mr P. G. Corbin, whose suggestions and criticisms throughout have been invaluable.

The work was carried out at the Plymouth Laboratory while holding a Colonial Office Studentship.

Description

The apparatus consists of a net, with locks, etc., supported on a rectangular base, 5 ft. 3 in. by 3 ft. 7 in., and mounted on runners. The runners raise the net some 8 in. above the ground (Fig. 1C).

At the anterior part of the net is a box of thin sheet iron, 24 in. wide, 15 in. high, and 12 in. deep. A 4 in. bucket fits into the cod-end of the net proper. A hinged brass band secures the cod-end to the bucket, and the bucket is attached to the frame by a stout cord.

The net-box is central to the width of the frame, and is flush with the front cross-bar. (Attachment by bolting is used throughout to facilitate such alterations that may have been necessary.) The top of the net-box is supported by two steel strips, bent as seen in Fig. 1B, leaving a 3-in. gap between the sides of the net-box and uprights of the supports. The back 2 in. of the box is kept free of the supports for fitting of the net proper.

The bearings for the door-hinge are two $\frac{1}{2}$ in. holes in the anterior support, the centre of each hole being 10 in. from the top of the front cross-bar, and $1\frac{1}{4}$ in. from the leading edge of the support. Straight $\frac{1}{2}$ in. brass tubing serves as the door hinge, held in place by a washer and split-pin at either end. Two slots are cut in the sides of the box to accommodate the hinge.

The door is a sheet of 16-gauge aluminium, $23\frac{1}{2}$ in. by $14\frac{1}{2}$ in., and is bolted directly to the hinge. When in position and closed, it leaves a gap of $\frac{1}{4}$ in. around the edge, and is about $1\frac{1}{2}$ in. within the box (Fig. 1C). It is braced behind by stout aluminium angle, a piece from each bottom corner to centre top, and a piece from centre top to centre bottom.

During trials with a small experimental model, it was found important to have the door attached *behind* the hinge.

Outside the net-box two stout levers are attached to the door hinge. From the centre of the diameter of the door hinge to the lever/connecting-arm hinge is $7\frac{1}{2}$ in. About 4 in. of the levers are above the door hinge to act as a counter-weight. When the door is vertical, the levers project forward at about 20° from vertical (Fig. 2A).

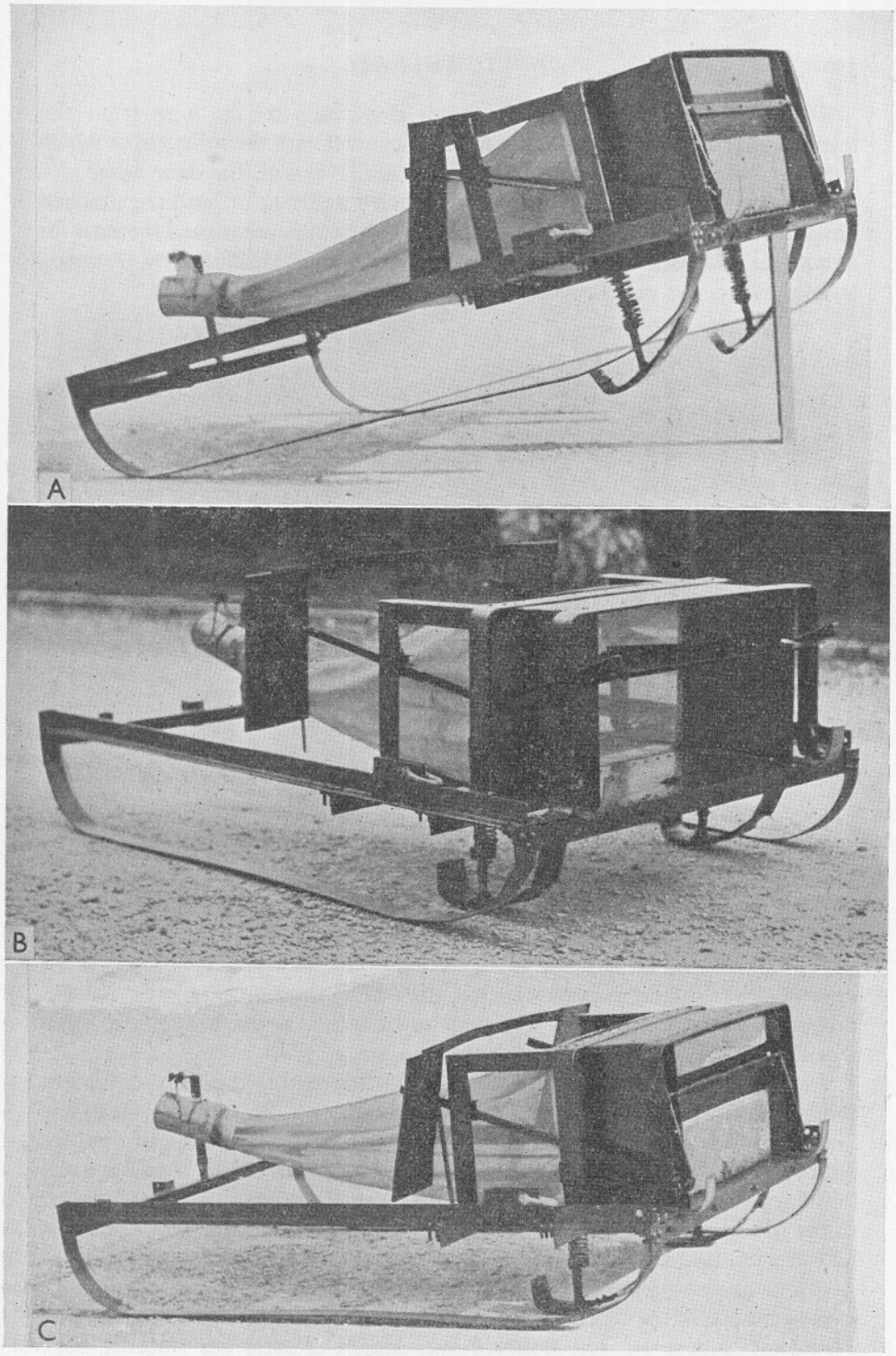

Fig. 1. A, the apparatus locked for lowering to the bottom; B, the apparatus open, as it is towed along the bottom; C, the apparatus locked at the end of the haul and ready for bringing to the surface.

The connecting-arms are $\frac{3}{4}$ in. angle-iron, each 25 in. long from the lever/connecting-arm hinge to the point of contact with the rollers over which they run (Fig. 2A). The tops of the rollers are level with the door hinge.

The vanes are of 16-gauge aluminium, each 7 in. by 14 in., and are attached perpendicularly to the connecting-arms. All the edges are turned forward for about $\frac{1}{4}$ in. to increase turbulence, and thereby to increase efficiency. A cross-strut prevents the vanes pivoting laterally on the connecting-arms.

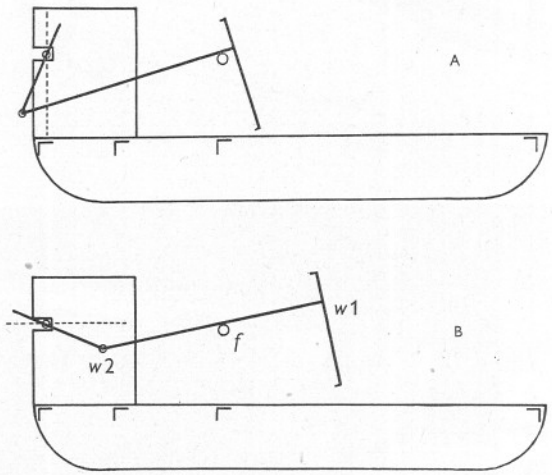

Fig. 2. Diagrammatic side-view of the net: A, closed; B, open. The dotted line represents the door. For explanation see text.

The principle of the apparatus is as follows. When towed through water, the pressure against the vanes and the lower part of the door pushes the vanes backwards. This pulls back the connecting-arms; the connecting-arms pull on the levers, causing the door hinge to rotate through 90°, thus opening the door (Fig. 2B). When stationary, the weight of the door plus levers causes the door to close, pulling the vanes back to the resting position (Fig. 2A). Attachments described below allow the door to be locked when lowered, to open when being hauled, and to be locked when bringing up.

Two small, thin sheet-metal, half-runners, attached to the front cross-bar, project some $2\frac{1}{2}$ in. below the main runners (Fig. 1A). Running up from, and hinged to, the free end of each half runner is a steel bar, bent and doubled back for about $2\frac{1}{2}$ in. at the top (Fig. 3). Guiding the steel bars is an open slot in the second cross-bar, and a closed slot in a small piece of angle steel bolted to the inside of the upright of the posterior net-box support. The steel bars, between the half-runner hinges and the second cross-bar, act as cores to two strong compression springs (Fig. 3). Bolts through the steel bars act as stops against the top of the cross-bar when the springs are at almost their maximum expansion (Fig. 3, *s*). The doubled-over length of steel bar (locking bar).

fits into a slotted piece of iron attached to each connecting arm when the spring expands. The connecting arms are thus locked with the door just sufficiently open to avoid the locking trips (Fig. 1A).

When the net is being prepared for shooting, the small half-runners are raised, the door fully opened, two strips of metal inserted beside the con-

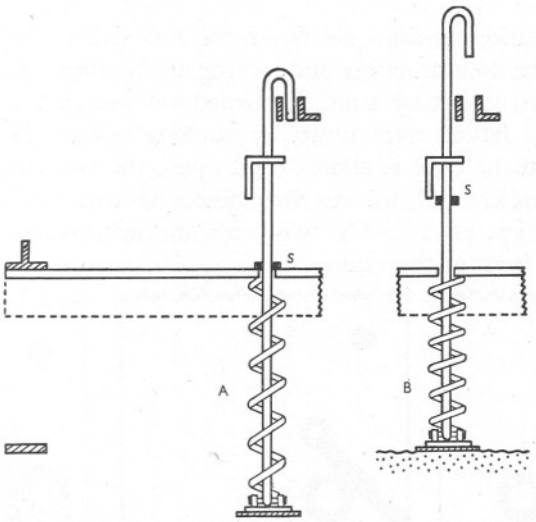

Fig. 3. Operation of the locking bar. For explanation see text.

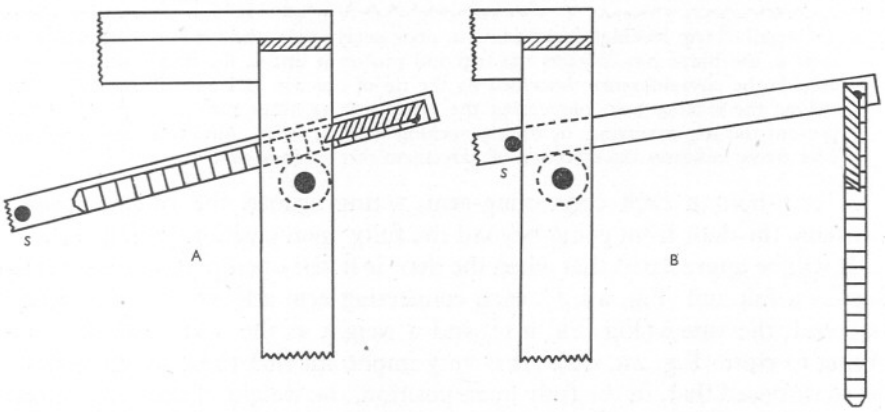

Fig. 4. Function of metal strips. For explanation see text.

necting-arms (see below), the door closed as far as possible, the small half-runners released, the springs pulling the locking bars into the slotted pieces of iron (Fig. 3A). This action locks the connecting-arms, and therefore the door. The net is shot in this state (Fig. 1A). On reaching the bottom, the whole weight of the apparatus rests upon the two small half-runners, com-

pressing the springs until the main runners take the weight. This compression raises the locking bars, thus freeing the connecting-arms, enabling the door to open (Fig. 3B).

Over the bottom of the two small half-runners some fairly stout sheet metal is fixed, to act as 'rubber soles', and prevent the small half-runners from wearing out.

When the connecting-arms are freed, the two strips of metal referred to above prevent the door dropping and locking at this stage. When they are inserted beside the connecting-arms, the turned over length at the end stops the connecting-arms falling right down by catching against the roller supports (Fig. 4A). When the door is almost right open, the two strips, freed of the support of the rollers, fall, leaving the connecting-arms free to close to their fullest extent (Figs. 4B, 1C). The two strips are each pivoted by a small bolt immediately in front of the vanes.

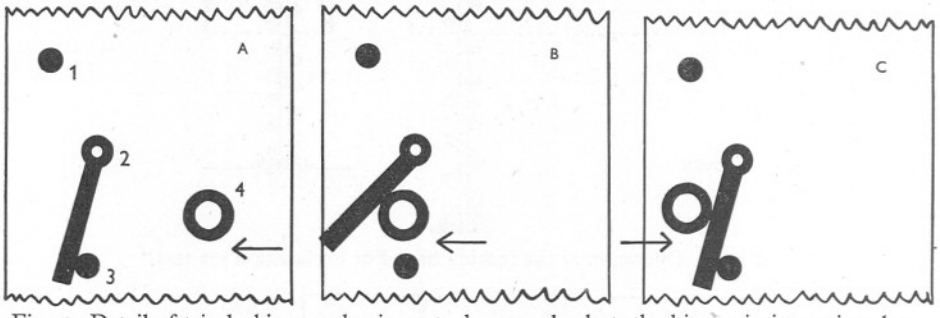

Fig. 5. Detail of trip locking mechanism. A, door nearly shut, the hinge pin is nearing the trip; B, the hinge pin contacts the trip and pushes it up; C, the hinge pin has passed outside the circumference described by the tip of the trip and the trip has fallen back against the locking bolt, preventing the return of the hinge pin. 1, stopping bolt, to prevent the trip reversing; 2, trip; 3, locking bolt; 4, lever/connecting-arm hinge pin. The arrow indicates the direction of movement of the hinge pin.

A stop-bolt in each connecting-arm, acting against the roller supports, prevents the door from going beyond the fully open position (Fig. 4B, *s*).

It will be appreciated that when the door is in the open position, each roller acts as a fulcrum (Fig. 2B, *f*), each connecting-arm a lever with a weight at one end, the vanes (Fig. 2B, *w*1), and a weight at the other end, the door trying to close (Fig. 2B, *w*2). It is very important that these weights should be so disposed that, in the fully open position, the weight of the vanes is only very slightly less than that of the door. If this is done, only the minimum of pressure is necessary to maintain the door fully open and steady.

During experiments in an open-air swimming bath, it was found that the door remained quite steadily open at speeds of less than $\frac{1}{2}$ m.p.h., or about 35 ft./min. This is important since it means that the door will remain fully and steadily open regardless of any irregularities in the towing speed, providing that the speed does not fall below this very low minimum.

When the net has finished its haul, it is stopped, causing the door to fall and engage three locking trips, one at the bottom centre of the door, and one at each lever/connecting-arm hinge (Fig. 1B), in the manner shown in Fig. 5A–C. The projecting lever/connecting-arm hinge pins engage the two outside trips, which are attached to the inside of the uprights of the anterior net-box support.

The importance of having the door bolted behind the door hinge is shown here, since this helps the door to fall beyond the vertical position, so that when the net is being hauled in, water pressure causes the door to be pushed back against the trips and held locked in a vertical position.

Shooting

The net can be shot by manhandling it over the side, but it is much more convenient to use a block and tackle. The method now adopted is as follows. The main warp from the winch is attached to the bridles via a ball-bearing swivel. A rope with a hook at the end is passed over a pulley attached to the mizzen boom. The rope is hooked to one of the swivel links. By means of this rope the net is lifted and swung outboard and lowered clear of the ship's side until the main warp takes the strain. This is to make sure that the small half-runners do not touch the side of the ship, thus releasing the connecting-arms. It is then a simple matter to lean over the side and detach the hook. The net is now lowered until it is some 10 or 20 ft. below the surface. For the above operations the ship is stationary. The ship now gets under way at tow-net speed. The net is observed to ensure that it is trailing the right way up, and is lowered. It is lowered quickly at first, but when it gets near the bottom, the warp is let out at about half the rate at which the ship is moving, until the necessary length of warp is out. The ship continues under way until the haul is finished, when a length of some 20 ft. of warp is let out as quickly as possible. This brings the net to a halt and enables the door to close and lock. The ship then heaves to and the net is hauled in. When the swivel is within reach, the rope is hooked on to it, and the last few feet of hauling is done by this. After the bucket has been detached, the net is unlocked and set for the next haul.

There has never been any difficulty in getting the apparatus to land the right way up. This is ensured by the low centre of gravity, the ball-bearing swivel, and having the towing shackles placed about a foot back from the front of the apparatus, near the point of balance, which corresponds with the third cross-bar (Fig. 1C). When being let out, the warp is at about 45° and the net is only about 15° out of horizontal.

The time taken for shooting is about 30 sec.

Discussion

From the outset it was decided that the design must be simple, effective, and self-contained, dispensing with messengers and throttling nooses. As can be seen from the description, all the working attachments are extremely simple, having no intricate mechanisms which may fail to function or function wrongly. Throughout the construction of the apparatus precise workmanship was never needed, and indeed, would have been a waste of time. The cost of construction is therefore very low.

The only part where any particular care is needed is in getting the right balance between the weight of the vanes and the weight of the door when in a fully open position, as described above.

The size of the aperture is a matter of choice for the maker; but the vane area must be increased or decreased accordingly. In the small model already mentioned, the optimum area of the vanes was found experimentally from tests with varying-sized vanes in the open-air swimming bath, where the results could be clearly seen. The vanes were then detached, and a length of cord was attached to the ends of the connecting-arms. This was led over a pulley and attached to various weights until the weight on the cord was sufficient to pull the door fully open. Knowing the area of the vanes, it was possible to calculate what weight of door would be opened by a certain area of vane. Then, by finding the weight necessary to open the door of the big net, the area of the vanes to be used could be worked out. The figure arrived at was $5\frac{1}{2}$ sq.in. for every ounce of weight (approx.). Of course, the pressure of water against the door itself will tend to open it, and this has to be taken into consideration, but the figure arrived at offers a good maximal starting-point.

The opening and shutting of the door rely upon two ever-present factors, i.e. water pressure and gravity. Because of this, the net can be operated on any type of workable bottom.

For quantitative work, hauls can be made of a specified duration since the net can be closed while it is still on the bottom.

There are no obstructions in front of the aperture of the net.

The apparatus can be used in quite rough weather.

All parts, except the lever/connecting-arm hinges, are protected by being within the area of the base frame. The lever/connecting-arm hinges are protected by 'bumpers' (Fig. 1 B).

Different metals are, as far as is possible, prevented from coming into contact by an application of bituminous paint.

In Fig. 1 B & C two attachments can be seen on the back cross-bar. To these can be fitted, when required, a small, coarse-silk net about 9 in. square. The purpose of this is to catch the small members of the benthic fauna which are disturbed by the runners. These are caught in the vortices

behind the right-hand vane and so are swept into the net. Small as the net is, it is surprising the numbers of animals that it catches, e.g. Amphipoda, Cumacea, etc.

Summary

A description is given of a new type of apparatus designed to sample plankton from the immediate vicinity of the sea bottom, and purely from this region. Details of the construction are given and the relative merits of the design are briefly discussed.

It could be used for quantitative hauls.

A small additional net serves to sample the smaller benthic fauna at the same time as bottom plankton is being sampled.

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AN EXAMINATION OF THE ORIGINAL SLIDES OF MARINE ACARI OF HODGE, 1863

By H. C. Fountain

(Text-figs. 1–3)

During work on Halacaridae collected in the Plymouth area it became apparent, in view of variations in diagnostic characters among certain species, that it would be useful and interesting to trace and examine as many as possible of the original specimens described and figured by the early British workers in this group of water mites.

Johnston (1836) described *Acarus basteri*, and Gosse (1855) described and figured three species of marine mites, *Halacarus rhodostigma*, *H. ctenopus* and *Pachygnathus notops*. In the same year Hodge (1855) figured a species of the genus *Pachygnathus* (namely *P. seahami*), and remarked that with those of Gosse 'to the best of my knowledge, these are the only recorded British species'. Hodge (1863) described and figured four new species: *Halacarus granulatus*, *H. oculatus*, *Pachygnathus minutus* and *Leptognathus falcatus*. By the courtesy of Mr G. E. Fisher, Curator of the Hancock Museum, Newcastle-upon-Tyne, I have been able to examine Hodge's original slides; these will be described later in this paper. Brady (1875) described four new species, namely *Trombidium fucicolum*, *Pachygnathus sculptus*, *Gamasus marinus* and *Cheyletus robertsoni*. The remaining mites dealt with in his review were *Pachygnathus notops*, *P. seahami*, *Leptognathus falcatus*, *Halarachne halichoeri* Allman, *Halacarus rhodostigma*, *H. granulatus*, *H. oculatus*, *H. ctenopus* and *Pachygnathus minutus*. Of these mites *Trombidium fucicolum* and *Scutovertex minutus* (=*sculptus* Mich.) are oribatids, *Gamasus marinus* is the mite *Halolaelaps marinus* (syn. *glabriusculus*) and *Cheyletus robertsoni* is *C. eruditus*. Of the others he placed *Leptognathus falcatus* in the genus *Raphignathus* Dugés, and Hodge's species *oculatus* and *granulatus* were made synonyms of Gosse's *Halacarus rhodostigma*.

In dealing with Hodge's *Pachygnathus minutus* Brady remarks 'I strongly suspect that it may prove to be an early stage of the following species'. This 'following species' he proceeds to describe as *P. sculptus* n.sp. As will be seen Brady was right in his suspicion and Hodge's *P. minutus* is the larval form of the mite now known as *Simognathus sculptus* (Brady). *Sculptus* therefore becomes a synonym of *minutus*. Brady's conclusion that *Halacarus granulatus* Hodge and *H. oculatus* are the same mite as Gosse's *H. rhodostigma* is not correct in the case of the first, but is true of *oculatus* (see Fig. 1B, C).

The complete collection of Hodge's slides (eight in number) lent to me by Mr Fisher will now be considered and some of them described and

figured. The transparency of the slides varies from perfectly clear and colourless to absolutely opaque. This last state was that of the Type slide of *Leptognathus falcatus*, but after treating it I was able to make a figure of the mite in some detail.

(1) *Pachygnathus minutus*—taken at Seaham. The mountant of the slide is discoloured but transparent. The mite is mounted to show the ventral aspect (Fig. 1A). The slide bears a label, in addition to Hodge's label, 'See Brady, *Proc. Z. Soc.* 1875, p. 305'. Etched on the slide is the legend 'On Coryne'. The surface (ventral) of the mite presents the appearance of shrinkage into the semblance of a fairly coarse network. The specimen is a larva having only six legs. There are several specimens of the diatom *Grammatophora marina* (Lyngb.) attached to the outside surface of the mite. The dotted lines in the figure indicate the shapes of the pre- and post-dorsal plates which can be seen by 'focusing down'. By the courtesy of Mr E. Browning of the British Museum (Natural History) I was able to compare this slide with one of *Simognathus sculptus* from the Canon Norman collection of Halacaridae in the British Museum, which comparison leaves no doubt as to the identity of *Pachygnathus minutus* with the *Simognathus sculptus* of Brady. The specimens on both Hodge's and Norman's slides were from the Durham coast littoral zone. Of *S. sculptus* Brady, André (1946, p. 138) writes: 'Cette espèce a été rencontrée sur les fonds rocheux de la zone littorale, entre 10 et 65 mètres de profondeur, dans la mer du Nord (Angleterre) et dans l'Atlantique (Le Croisic).'

Of the mite, Hodge himself says, 'Legs smooth, with the exception of the 5th and 6th joints, which have a few short stout hairs. Legs and rostrum irregularly dotted with minute granules; other markings in character. The posterior portion of the body minutely corrugated.... This curious species occurred on some stems of *Coryne eximia* from between tide marks, and from the specimen obtained possessing only three pairs of legs it would appear to be a young individual.'

Lohmann (1889, p. 333), in describing '*Aletes*' *minutus* (Hodge) says, 'Was Brady bewog, in dieser Larve ein Jugenstadium seines *Pachygnathus sculptus* zu vermuthen, weiss ich nicht. Anhaltspunkte dafür existieren jedenfalls nicht.' Lohmann evidently had not seen Hodge's slide.

(2) *Halacarus oculatus* (Fig. 1B)—taken at Seaham. The mountant[1] of this slide has become slightly brown. Like the previous slide it bears an added label, 'See Brady, *Proc. Z. Soc.* 1875, p. 310.'

[1] Lifting the label mentioned above on the '*oculatus*' slide reveals an inscription probably made by Hodge, scratched by a diamond on the glass, 'Deep Water 26.4.61 Deans Medium'. As already mentioned, the state of discoloration of the mountant varies from clear to opaque. Mr Fisher seemed to consider the mountant to be Canada Balsam, but from this inscription it may be that all are mounted in Dean's medium.

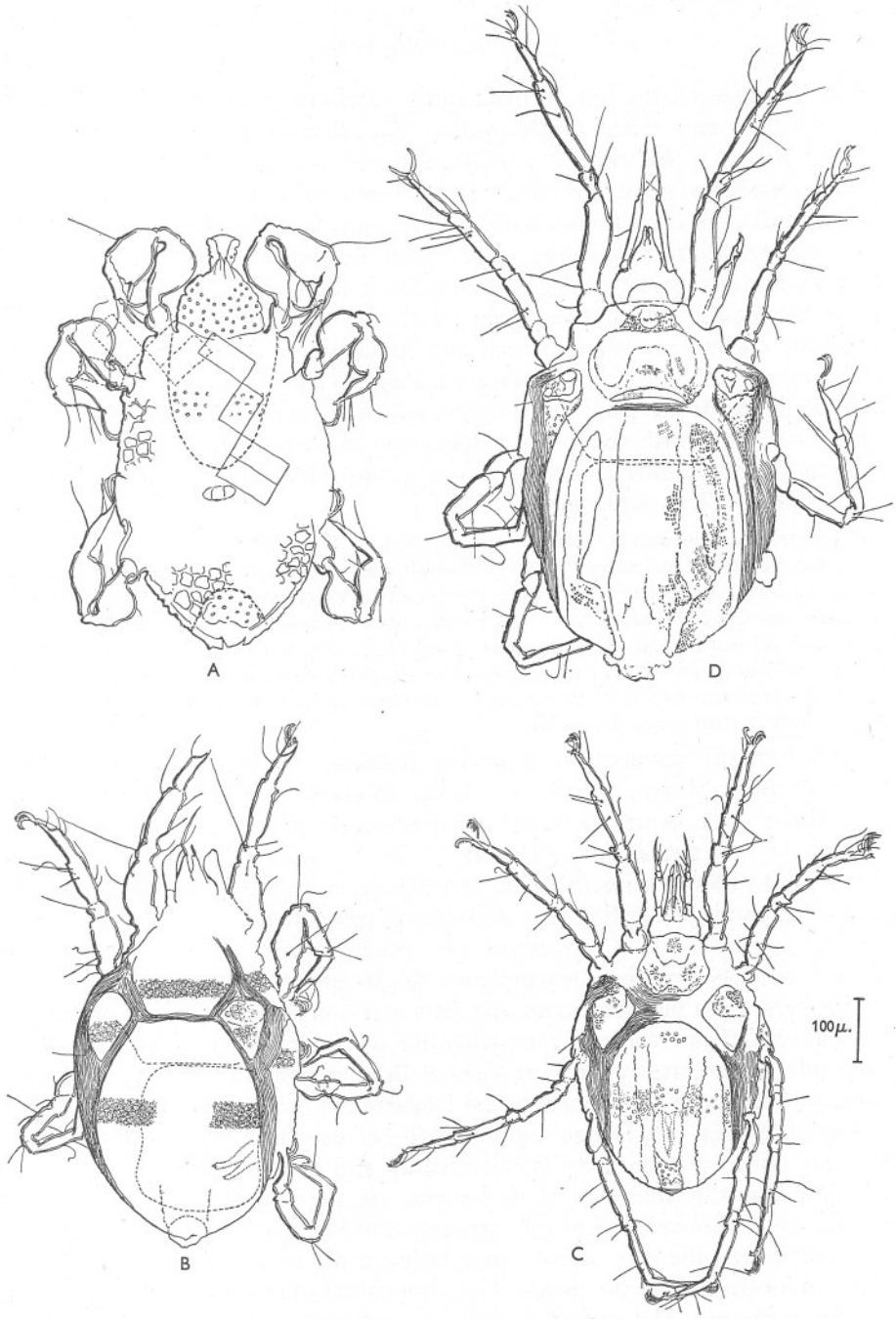

Fig. 1A. *Pachygnathus minutus*. The larval form of the mite now to be known as *Simognathus minutus* (Hodge, 1863). B, *Halacarus oculatus*. This is the mite known as *Copidognathus rhodostigma* (Gosse, 1855) and must be regarded as an error in identification by Hodge. C, *Halacarus granulatus*. This is the mite known as *Copidognathus glyptoderma*; it must now be called *C. granulatus* (Hodge, 1863). D, *Halacarus rhodostigma*. This is another error in identification and is the mite now known as *Copidognathus gracilipes* (Troues., 1894).

On examination the mite proved to be a definitely different animal from that which is now called *Copidognathus* (*Copidognathopsis*) *oculatus* (Hodge, 1863). Brady, in his review mentioned above, considers this, and the following (*Halacarus granulatus*, Fig. 1 c), to be synonymous with *H. rhodostigma* Gosse; and of Hodge's *oculatus* he says: '*H. oculatus* is, I think, without doubt only the young; and excepting some trivial distinction of surface markings, I cannot find out what Mr Hodge relied on to distinguish his supposed species.'

As the figure shows, the ocular plates do not possess the drawn-out posterior end by means of which the subgenus is at present diagnosed. Unfortunately the slide is not perfect enough to allow a view of the anterior margin of the dorsal plate. Careful examination reveals faint pectination of the claws. As regards this being a diagnostic character it will be well to take account of the following. Newell (1947, p. 150), in writing of *Copidognathus curassaviensis* Viets 1936, says:

> The presence or absence of combs on the claws of *Copidognathus* species is an undesirable primary key character, for although there are many species with clearly pectinate claws, and some which have completely smooth claws (but usually with an accessory tooth), there are many in which the comb is present but difficult to observe, even with oil immersion. In this latter group of species which are apparently in the process of losing the pecten, there are unquestionably some species which show individual variation (e.g. *C. acutus* n.sp.), some specimens having smooth claws, and others having faintly pectinate claws.

André (1946) has also the following footnotes on the same point. On *C. rhodostigma* (p. 90): 'Motas et Soarec ont observé un individu mâle de la mer Noire chez lequel, à toutes les pattes, les griffes sont pectinées et présentent une petite dent accessoire.' On *C. tabellio* (p. 94): 'Motas et Soarec ont observé un échantillon femelle de la mer Noire chez lequel, à toutes les pattes, les griffes sont pectinées et présentent une dent accessoire.' On *C. gracilipes* and *C. gracilipes* var. *quadricostatus* (p. 104): 'Motas et Soarec ont observé deux exemplaires de la mer Noire chez lesquels les griffes présentent non seulement une dent accessoire, mais aussi un peigne.'

I have had the privilege of comparing this specimen with a slide of *C. oculatus* kindly loaned me by Dr Karl Viets of Bremen and also with slides of the same sent to me by Dr Hobart of the Department of Agriculture and Forest Zoology, Bangor. In Hodge's slide it will be seen from the figure that the anterior border of the body is not distinct and the chief features used in deciding that this specimen is *rhodostigma* are the absence of the posterior elongation of the ocular plates, the strongly developed hair of the 3rd segment of the first legs, the very narrow space between the two dorsal plates and the even sculpturing of all the plates. The sculpturing of the ventral plates could not be made out. The size of the mite as mounted is length approx. 360 μ, breadth 230 μ. In a letter on the subject of these 'old' slides Dr Viets says: '...only the Hodge slides may be type specimens of his species, I think'. It seems that we must regard this specimen as wrongly identified by Hodge,

and Lohmann's (1889) description, which is clearly that of the *oculatus* of to-day, must be taken as the description of the type.

(3) *Halacarus granulatus* (Fig. 1C) taken at Seaham. This slide, as the two previous, bears a label, 'See Brady, *Proc. Z. Soc.* 1875, p. 310'. This specimen is undoubtedly the species now known as *Copidognathus glyptoderma* Trouessart. Trouessart's species must therefore sink and the type of the genus *Copidognathus* must be renamed *C. granulatus* (Hodge). The size of the mite is $480 \times 358\,\mu$. The sculpturing of the posterior dorsal plate, with its four longitudinal bands, is as shown in the figure of *C. glyptoderma* in André, while that of the anterior dorsal plate is a good approximation allowing for some slight deterioration of the mountant. The suggestion of three sculptured areas which André calls 'trois impressions saillantes disposées en triangle', and the subquadrangular shape of the anterior dorsal plate are also in agreement. Of the ocular plates André's description is 'subquadrangulaires, assez grandes, arrondies à leur angle antérieur et se prolongeant en arrière par une pointe bien marquée. Elles portent chacune une grande cornée antérieure et deux beaucoup plus petites, rudimentaires, en arrière de la précédente.' These two rudimentary cornea are not visible on Hodge's specimen, and although André mentions them they do not seem to be shown in his figure. The posterior dorsal plate is separated from the anterior dorsal plate by a band of dermal tissue about $2\frac{1}{2}$ times as broad as long (André says, 'deux fois plus large que haute'). The general oval shape, the very strong pectination of the claws and the minute denticles on the mandibles also agree with André's description, leaving no doubt that this specimen is the *C. glyptoderma* of Trouessart.

(4) *Halacarus rhodostigma*, 20 fathoms, 16 May 1861 (Fig. 1D). This specimen is, according to present-day diagnosis, wrongly identified, and is a specimen of the species known as *Copidognathus* (*Copidognathopsis*) *gracilipes* (Trouessart, 1889). Dr I. M. Newell (1947) has placed this species in a new subgenus, and on p. 34 writes:

> ...there is a group of species which formerly were placed in the subgenus *Copidognathopsis*, but which are distinct from all other species of *Copidognathus* in the number of ventral setae on tibia I. The other species of *Copidognathus* are remarkable for the apparently absolute constancy of the number (three) and arrangement (two ventro-medial; one ventral or ventro-lateral) of the ventral setae on the first and second tibiae. But in *C. arenarius* n.sp., *C. submarinus* n.sp., *C. gracilipes gracilipes* (Trouessart, 1889), *C. gracilipes quadricostatus* (Trouessart, 1894) and *C. gracilipes largiforatus* (Trouessart, 1889) there are two pairs of ventral setae on 1–5 in both the deutonymph and the adult. These five forms are more closely related to each other than to any species in the genus and therefore form a distinct and natural species group. All have a short, blunt, rostrum which extends only a little beyond the middle of P-2, and apparently all lack rosette pores on any of the plates. The above five forms are removed to

Arhodeoporus n.subg. The name indicates the absence of rosette pores, but this is not the most important character on which the subgenus is based. Furthermore, not all 'arhodeoporose' species belong here.

Concerning this new subgenus of Newell's, Viets (1950) makes the following remarks:

Newell 1947 trennt nun subgenerisch von *Copidognathus* (*Copidognathus*), nach ihm die Arten mit 3 (unpaaren) Ventralborsten an den I.B.5 umfassend, als *Copidognathus* (*Arhodeoporus*) die Arten ab, die 4, also paarige Ventralborsten an den I.B.5 tragen. Diese Unterschiede und andere unterschiedliche Merkmale (in der Plattenstruktur, p. 35 'apparently (sic!) all lack rosette pores' und in der basalen Breite und Länge des Rostrum relativ zur Palpenlänge) sind subgenerisch mindestens ebenso unbefriedigend, wie die immerhin messbaren Merkmale und Unterschiede in den Okularia. Da die ganz charakterischen Okularia der neben *Copidognathus* (*Copidognathopsis*) *gracilipes* (Trt. 1889) von Newell in *Arhodeoporus* gestellten Arten *arenarius* New. und *submarinus* New. typische Merkmale von *Copidognathopsis* zum Ausdruck bringen, vermag ich in *Arhodeoporus* New. nur ein Synonym zu *Copidognathopsis* zu sehen.

In the Hodge specimen the two pairs of ventral setae can be seen on the right 1–5, the short blunt rostrum is evident and a careful examination, using a 4 mm. apochromatic objective, fails to reveal any rosette pores. In all respects the mite also agrees well with André's description of *C. gracilipes*; the ocular plates and the dorsal plates with their distinctive sculpturing being especially convincing. The size of the specimen is $450 \times 280\,\mu$.

(5) *Halacarus rhodostigma* (Fig. 2). The mountant of this slide is dark brown in colour, but as the figure shows the mite is fairly visible and but for the faint pectination of the claws it agrees as far as it is possible to judge with the present *Copidognathus rhodostigma*. The view is ventral and the shapes of the dorsal plates are indicated by dotted lines. The specimen is a male, size $412 \times 211\,\mu$ and the sculpturing and general appearance agrees with André's description.

(6) This slide (not figured) is Hodge's slide of *Pachygnathus seahami* and is labelled 'Type'. Added inscription on the label is 'Rocks 1861'. There are six specimens on the slide and, although the mountant is perfectly clear and unstained, the specimens themselves are opaque for the most part. The terminal segments of most of the legs in each specimen are visible and are really the only characters it is possible to use for identification. Using these and the faint indication of the provisional genital area in some specimens I make them out to be:

	Length (μ)	
(1) Adult	425	All four medial claws large
(2) Adult	430	All four medial claws large
(3) Adult	475	All four medial claws large
(4) Deutonymph	340	Medial claws 3 and 4 small
(5) Deutonymph	370	Medial claws 3 and 4 small
(6) Deutonymph	335	Medial claws 3 and 4 small

All are the mite now known as *Rhombognathus* (*Rhombognathopsis*) *seahami*.

(7) This slide is also labelled *Pachygnathus seahami* and is of a very damaged specimen. There is only one specimen on the slide and the condition of the mountant is perfect as in the last slide. On the end of the glass slip is etched 'Deep Water 1860'. Using the same features as in the last slide I have no doubt of its being a true *seahami*.

(8) *Leptognathus falcatus* Hodge 1860. This mite (Fig. 3) is labelled 'Type'. When sending me this slide Mr Fisher said in his accompanying letter,

Fig. 2

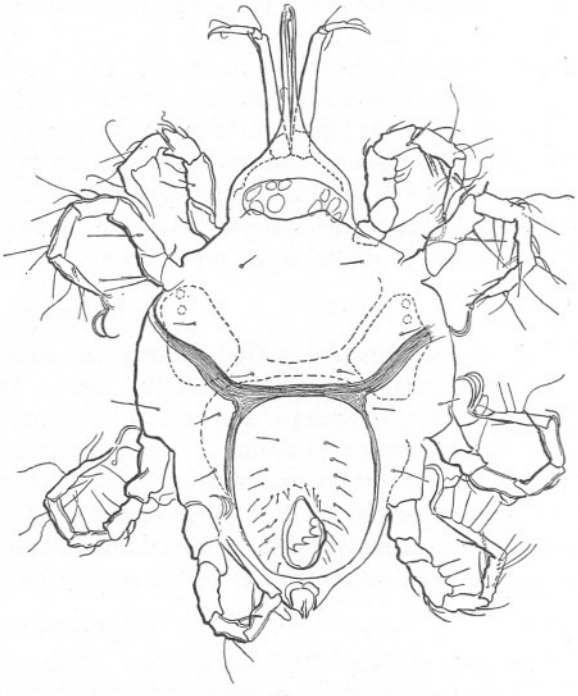

Fig. 3

Fig. 2. *Halacarus rhodostigma*. This mite was correctly identified by Hodge and the figure is given for comparison with Fig. 1D.

Fig. 3. *Leptognathus falcatus*. Hodge's *type* slide. This mite is now *Lohmanella falcata* (Hodge, 1863).

'. . . and *Leptognathus falcatus*, Seaham. Unfortunately the balsam of the latter slide has disintegrated so that the specimen will be of very little use.' A prolonged soaking in xylene had no effect on the mountant and a similar treatment with chloroform met with no more success. This seems to indicate that the mountant was *not* Canada Balsam but almost certainly Dean's Medium. The slide was then immersed in cellosolve and 'forgotten' for about 18 months. It was then possible to see the amount of detail shown in Fig. 3. Because of the danger of destroying the specimen by moving the cover-glass the drawing was made in instalments; the slide being returned to the cellosolve when

drying-up of the liquid was reaching the danger point. This is the condition of things at the time of writing. The legend 'Deep Water March 1861' is scratched on the slide by means of a diamond point. The details shown in the figure were obtained by the use of 'phase-contrast' methods and I think the figure is sufficiently detailed to confirm the identity of the specimen with the animal now known as *Lohmannella falcata* (Hodge). The aspect shown is as mounted (ventral) and as much of the dorsal plates and the ocular plates as could be made out is indicated by dotted lines in the figure. The specimen is a female, size $605 \times 345\,\mu$, and the genital opening is $66 \times 40\,\mu$.

For help and advice in writing these notes, as well as for material for comparison, I have to tender my grateful thanks to Dr K. Viets of Bremen, Dr J. Hobart of the Department of Agriculture, Bangor, and Mr E. Browning of the British Museum (Natural History, Arachnida). Especially have I to thank my friend Dr F. A. Turk for much guidance and for reading this paper, and also Mr Fisher of the Hancock Museum, Newcastle-upon-Tyne, for his patience and kindly replies to all my queries.

Summary

An examination of Hodge's original slides of marine Acari shows that three alterations in synonymy are called for: (i) *Simognathus sculptus* (Brady, 1875) to become a synonym of *S. minutus* (Hodge, 1863); (ii) *Copidognathus oculatus* Hodge, 1860 to become *C. oculatus* Lohmann, 1889; (iii) *C. glyptoderma* Trouessart to become a synonym of *C. granulatus* (Hodge, 1863). Observations were also made on the permanence of Dean's Medium as a mountant for small arachnids and some quotations and observations on the value of certain diagnostic characteristics of the genus *Copidognathus* are made.

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CHLOROCRUOROPORPHYRIN: A SIMPLE METHOD OF PREPARATION

By Gilbert Y. Kennedy

Cancer Research Laboratory, University Field Laboratories, Lodge Moor, Sheffield 10

Munro Fox (1926, *Addendum*, p. 219) described a method of preparation of chlorocruoroporphyrin from the blood of the marine polychaete worm *Spirographis*. This was based upon the very troublesome method of Nencki & Zaleski (1900), which is time-consuming and wasteful. Furthermore, *Spirographis* is not easily obtainable in this country. Lemberg & Parker (1952) started with protoporphyrin, and by careful oxidation with potassium permanganate in acetone obtained a solution which gave mixed crystals of chlorocruoroporphyrin and diformyldeuteroporphyrin esters. Purification was by fractional chromatography. The procedure to be described here is simple and rapid, and could easily be carried out in one day by a biologist requiring a small sample of the porphyrin. The two methods mentioned above would not come into this category.

This work was done at the Plymouth Laboratory, and I am very grateful to Mr T. R. Tozer of that laboratory for so kindly obtaining the animals for me and extracting the blood.

The polychaete worms *Myxicola infundibulum*, *Sabella pavonina* and *Branchiomma vesiculosum* are all readily obtained from the muddy shores of the Salcombe estuary, especially on the Salstone. Blood was taken directly from the vessels of about 100 assorted worms by syringe, and squirted as it was removed from the worms into a mixture of peroxide-free ether (3 parts) and 'Analar' glacial acetic acid (1 part). After all the blood had been added, the mixture was well shaken and allowed to stand in the ice-chest for 30 min. The acetic acid was then washed out with distilled water, the first few washings containing a little sodium acetate to avoid washing out of the pigment, and the remaining solution of chlorocruorohaematin dried roughly by filtering through ether-soaked paper into a distilling flask and evaporated to dryness. 100 ml. of a 50% solution of hydrazine hydrate in glacial acetic acid were then added, and the mixture heated on a water-bath at 90° C. for 10 min. During that time, the chlorocruorohaematin dissolved, and the solution became violet-red and red-fluorescent to ultra-violet light, indicating the formation of the free porphyrin. When cool, the solution was poured into a large separating funnel with 300 ml. peroxide-free ether, and the flask rinsed out with ether, the rinsings being added to the funnel. The acid and hydrazine hydrate were then washed out with distilled water, the first washings containing sodium acetate as before, and the ether solution of the porphyrin

shaken with 10 ml. lots of 5% (w/v) HCl (137 ml. conc. HCl/l.) until no more of the lower layers were fluorescent. The pooled acid extracts were then added to ether in a separating funnel, and the porphyrin driven back into ether by the addition of saturated sodium acetate solution—just *red* to Congo Red paper in the lower layer. The ether solution was washed with distilled water to remove salts and acetic acid, and dried by the usual method of filtering through ether-soaked paper. The ether was then distilled off on the water-bath to a volume of about 25 ml., and the remainder evaporated in a dish to dryness. The residue was dissolved in the minimum amount of dry chloroform and when hot, the porphyrin precipitated by the addition of light petroleum, B.P. 40–60° C. This is quicker than the crystallization method, but does not give so fine a product. The precipitate was centrifuged down and washed with petroleum ether, and dried in the incubator. Yield, 22 mg.

Absorption spectra were measured with the Hartridge Reversion Spectroscope:

Chlorocruoroporphyrin	I	II	III	IV
In pyridine (mμ)	645	571	553	517
		I	II	
In 5% (w/v) HCl (mμ)		615	556	

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EXPERIMENTS WITH RADIOACTIVE STRONTIUM (^{90}Sr) ON CERTAIN MOLLUSCS AND POLYCHAETES

By Vera Fretter
Department of Zoology, Birkbeck College, University of London

(Text-figs. 1–5)

Introduction

The use of radioactive strontium in the study of invertebrate metabolism is as yet not extensive; it has been employed successfully by several workers on the metabolism of bone. In both vertebrate and invertebrates calcium is the more important element and strontium, if present, is only in traces, except for the aberrant group of radiolarians, the Acantharia, in which the skeleton consists almost entirely of a salt of this element. Radiocalcium would be the ideal choice for these studies of metabolism, but the radioactive isotope of calcium, ^{45}Ca, has been available up till now only in small quantities and with weak activity. Chemically, and to some extent physiologically, however, strontium replaces calcium (McCance & Widdowson, 1939). Pecher (1941*a*, *b*) has shown that in mice, rats and man the metabolism of the two elements is similar qualitatively, though there are quantitative differences: both are concentrated in the skeleton, with very little in the soft tissues, but the uptake of calcium is greater than that of strontium. Resemblances in metabolism of the two elements in vertebrates are also demonstrated by Posin (1942) and by Erf & Pecher (1940). Thus it is because of their similar behaviour, and the availability of radioactive strontium which is carrier-free, that this element has been of interest as a tracer for the study of calcium metabolism in mammals. It is also employed by Swann (1950) to detect the glands which secrete the calcareous tubes of serpulid polychaetes. Exactly how strictly it follows the course of calcium in invertebrates is not known.

Spooner (1949) studied the absorption of radioactive strontium and yttrium by marine algae: comparing the decay characteristics of specimens of water in which the alga had been kept with control specimens, he was able to estimate the amount of strontium, as apart from yttrium, which was taken up by the weed. He suggests that the extraction of yttrium from the water involves adsorption by the surface of the tissue as well as ionic exchange, both processes being important with red algae. Autoradiographs of microtome sections of *Rhodymenia* have therefore been made to follow up Spooner's suggestion: they show, as he predicted, a surface adsorption layer of ions comprised mainly of yttrium, but also with some strontium. In animals,

specially in a form like *Mytilus*, on which preliminary experiments were carried out, such adsorption detracts considerably from the value of quantitative work, for the isotopes are not only adsorbed on any glass surfaces involved in the experiment, but also on the shell of the bivalve, the byssus threads which may be secreted during the experiment, the faeces, and any particle of detritus or micro-organism in the sea water or on the surface of the body. The decay-curves of pieces of *Mytilus* shell which have been immersed in activated sea water, then washed in distilled water, placed on a microscope slide and presented to a GM4 counter, resemble those of similarly treated *Rhodymenia* fronds (Spooner, 1949): about 90% of the activity is due to yttrium which is differentially concentrated.

^{90}Sr, the radioactive strontium which was used for the present investigation, was supplied by the Radiochemical Centre, Amersham. It has a half-life of 25 years; it breaks down by β-particle-emission to ^{90}Y. The yttrium is itself radioactive and has the comparatively short half-life of 63·4 hr., changing in turn to stable zirconium, ^{90}Zr. Tests made at Amersham show that 1 mc. of the ^{90}Sr would have a total weight of strontium within the range 10–70μg.; no inactive strontium was used for the extraction process, which was carried out using lead as carrier. Therefore, when sea water is activated by this ^{90}Sr to the extent of 100μc./l. there is an addition to the water of not more than 7μg. of strontium; the doses which were employed for marine organisms were kept within this quantity unless otherwise stated. Sea water from the English Channel contains 10 mg. Sr/l.: the addition of 7μg. means, therefore, an increase of only 0·07%.

The uptake of ^{90}Sr and ^{90}Y by the tissues of certain invertebrates was studied by means of autoradiographs. These detect the distribution of comparatively minute quantities of the tracer element, and are particularly useful in studying small invertebrates which have soft bodies, since autoradiographs can be made with sections of the intact animal and the concentration of the isotope by specific cells can be followed. For the preparation of such autoradiographs animals were fixed in absolute alcohol—a fixative which is useless for the preservation of cytological detail but advantageous in that, unlike acid fixatives, it does not appear to inhibit the sensitive emulsion of the stripping film in recording tracks of the β-particles which are emitted during the decay of the tracer. After fixation the tissues are cleared in xylol, embedded in paraffin wax, and sectioned to a thickness of not more than 10μ. Sections are floated on to slides which have been previously coated with 1% gelatine solution; later the wax is removed with xylol, the tissues hydrated, washed well in distilled water and covered with the fine-grain stripping film supplied by Kodak Ltd. The sensitive emulsion of the film is thus brought into intimate contact with the tissues, and this exposure may last from a few days to several weeks; during such a period the slides are stored in a light-tight box in a refrigerator. At the appropriate time the film, still covering the sections, is

developed in amidol developer; the slides may then be placed in haemalum to stain the tissues, and the stain differentiated with 1% HCl, which, at the same time, removes it from the film stripping.

The use of ^{90}Sr concerns two tracer elements, strontium and its daughter-product yttrium; it would be an advantage to have some method by which the activity derived from the strontium intake alone might be shown on an autoradiograph. Since ^{90}Y has a half-life of only 63·4 hr., over 99% of any quantity of this element will decay in 19 days. When an organism takes into its body both ^{90}Sr and ^{90}Y the activity due to this yttrium may be neglected if sections of the tissues be set aside for 19 days before they are covered with photographic film. The resulting autoradiographs can be compared with those made from adjacent sections of the same animal which were covered with film a few hours after the tissues were fixed: the latter will show as the nuclear track in the emulsion of the film the position of β-particles from both the ^{90}Sr and ^{90}Y.

Uptake of ^{90}Sr by *Arion hortensis* as compared with that by an Opisthobranch

The land pulmonate stores in the tissues of its body calcium salts which may be used for the growth and repair of the shell and perhaps in buffering the gut (Manigault, 1939; Robertson, 1941). The lime cell of the digestive gland is the main storage place; also around blood vessels in both slug and snail are cells filled with calcium spherules, and in the mantle are the glands which secrete the shell (Prenant, 1924; Barr, 1928). These terrestrial molluscs obtain all their calcium from food: this mineral element, one of the most important in their metabolism, is preserved in the tissues in large quantities when it is available; in contrast, marine molluscs have a ready supply of calcium ions at hand in the surrounding medium and their reserves may be relatively low. In order to see whether the divalent ions of strontium follow a distribution which is similar to that of calcium in the body of a terrestrial, soft-bodied animal there is some advantage in using the slug or snail: they feed readily in captivity, and their calcium metabolism has already attracted the attention of many workers so that the distribution of the element within the tissues is well known.

Arion hortensis was fed with lettuce on which had been evaporated a solution of ^{90}Sr: the average consumption of isotope was of the order of 4 μc./g. of body weight, though a considerable amount did not enter the tissues, but was lost in the faeces. The slugs were given from one to four meals, each at night; they were fixed immediately after eating the contaminated food, or taken from this and put on uncontaminated leaves for a day or two before being fixed. Autoradiographs of the tissues of an individual which has been given only one meal with ^{90}Sr, and fixed immediately after it, show the isotope in both types of cell of the digestive gland—the digestive cell and the lime

cell, more in the former. It would appear that both take up the ions directly from the lumen of the gland and they are scattered in the cytoplasm. Such absorption by the digestive cell is well known; but no investigation has proved that the lime cell gets its calcium by this route, yet if strontium enters thus, as well as ^{32}P (Fretter, 1952), it is highly probable that calcium would do likewise.

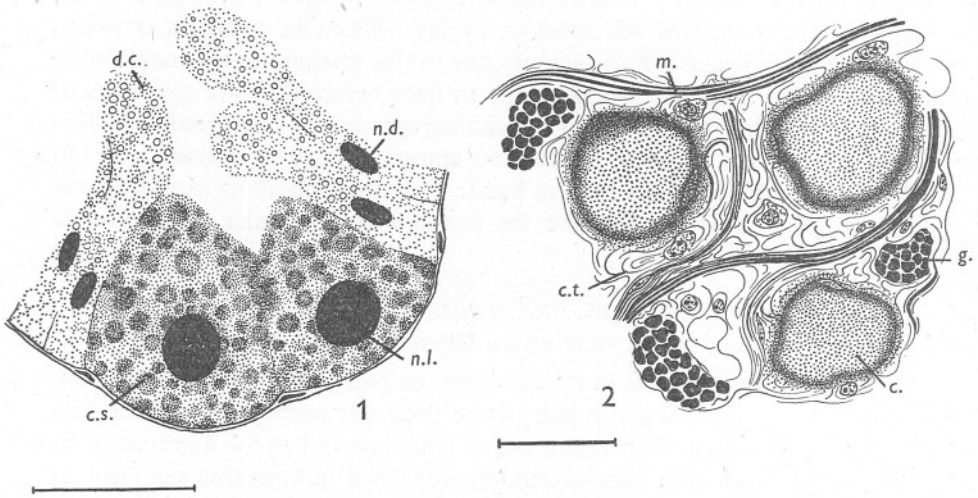

Fig. 1. *Arion hortensis*. Part of a transverse section through the digestive gland to show the distribution of ^{90}Sr. Stippling represents the superimposed autoradiograph. Fixed 3 days after commencing to feed on lettuce on which had been evaporated a solution of ^{90}Sr. *c.s.*, calcium spherule; *d.c.*, digestive cell; *n.d.*, nucleus of digestive cell; *n.l.*, nucleus of lime cell. Scale, 30 μ.

Fig. 2. *Acanthodoris pilosa*. Part of a transverse section through the periphery of the mantle to show the accumulation of ^{90}Sr. The specimen had been in activated, filtered sea water for 17 hr. and then in normal sea water for 12 hr. Stippling represents the superimposed autoradiograph. *c.*, calcium concretion; *c.t.*, connective tissue; *g.*, gland cell; *m.*, muscle fibre. Scale, 40 μ.

Slugs which have eaten larger quantities of the tracer over a period of 3 days show, in autoradiographs of their tissues, localization of strontium ions around the calcium spherules which fill the lime cells (Fig. 1, *c.s.*), and a general scattering in the rest of the cytoplasm of these cells; similar individuals, which have been kept on uncontaminated lettuce for several days before fixation, show that elsewhere in the digestive gland little of the isotope occurs for it is concentrated for storage in the one type of cell. Ions would appear to enter this cell from the haemocoel—into which the tracer passes from the digestive cell (*d.c.*)—as well as from the lumen of the gland; the two routes have already been described for the entry of ^{32}P (Fretter, 1952). The digestive gland offers the largest, yet not the only area of absorption from the gut: the intestinal

epithelium is also permeable to ions. The slug, which feeds at night, retains undigested matter in the intestine during the following day. On its slow course towards the anus this food loses ions which pass into the surrounding epithelium; they are not stored here, but are lost directly to the haemocoel. The anterior aorta traverses the haemocoel and is surrounded by a reserve layer of calcium to which passes some of the strontium: a slug which has been starved for a week after feeding on the isotope shows that the element is still retained here. The third type of calcium cell, that in the mantle, may contain the tracer within 12 hr. after its entry into the gut; these cells take the isotope directly from the blood.

The few experiments with *Arion* reveal that strontium, which is in the same group in the periodic table as calcium, though an unlikely constituent of the natural diet, is taken up by the gut and concentrated in the calcium stores. It is very probable, however, that there are quantitative differences between the uptake of the two elements by the storage tissues. Now in a marine animal, divalent cations may enter the tissues with the food, by way of the gut, or, as Bethe (1934) has shown for *Aplysia*, Ca^{2+} and Mg^{2+} may penetrate the integument from the surrounding water. Bethe's work was not extended to strontium which, present only in traces in sea water, is of much less importance. However, if a small specimen of *Aplysia punctata*, in which the buccal cavity is carefully ligatured without damage to the surrounding tissues, be placed in activated water for 8 hr., autoradiographs of the whole animal show the passage of strontium ions through the general surface of the body; the gill with its thin wall and active transport systems displays in its tissues the greatest activity.

A comparatively rapid accumulation of strontium from the surrounding water is seen in the nudibranch *Acanthodoris pilosa*. It is well known that dorids may have an unusually high amount of mineral matter which is concentrated in the mantle. An examination of the composition of the body tissues of *Archidoris britannica*, after removal of mucus and visceral mass, shows that calcium comprises 2% fresh weight, magnesium 0·93%, and strontium 0·08% (McCance and Masters, 1937); this proportion of strontium to calcium is higher than in sea water. In *Acanthodoris*, as in *Archidoris*, the mineral deposits scattered through the mantle give solidity to the thick covering of the body. McCance & Masters suggest that such nudibranchs, which in the course of evolution have lost the shell, never acquired the necessary excretory mechanism for dealing with ingested calcium and consequently accumulate it. If a specimen of *Acanthodoris* be placed in sea water which has been passed through a Berkefeld filter (KF grade) and activated with 90 μc. ^{90}Sr/l., autoradiographs of the tissues show that, after 10 hr., strontium ions which have passed into the body are most numerous in the mantle, gathering around the calcium concretions there; no such concentrations occur elsewhere (Fig. 2, *c*.). Some ions, though relatively few, are in the

lumen of the gut. This, as well as the outer surface of the body, may be the route by which they have entered. An individual which has been in the activated water for 36 hr., and then in normal sea water for a day, shows the strontium localized around the mineral concretions and in the cells which surround these (Fig. 4B). Some ions are leaving the body with waste matter from cells of the digestive gland. The radioactive strontium which has gathered in the mantle represents an exceedingly small amount, yet it indicates that in the nudibranch the mineral ions may be derived directly from the water, as well as by way of the food.

Uptake and Excretion of ^{90}Sr by *Mytilus edulis*

Marine lamellibranchs have attracted the attention of more scientific workers than any other group of molluscs, yet many problems of their metabolism are imperfectly understood: some of these concern the growth of the calcareous shell. This growth continues during periods of starvation and, since there is little storage of calcium in the tissues, points to the uptake of salts directly from the water. If uptake occurs it would appear to be to no slight degree: Chatin & Muntz (1895) calculated that 75 g. of shell formed by an oyster in 12 months correspond to about 27 g. calcium; this means an absorption of 0·52 g. per week. Orton (1935) puts the weekly absorption rate at about a third of this, and he states (1925) that the shells continue to grow in the absence of food. Later Galtsoff (1934), in studying the Virginia oyster, finds that from November to the end of April, when these animals are for the greater part of the time in a state of hibernation and have no food in the digestive tract, the weight of the soft tissues remains practically stationary whilst that of the shell increases at about the same rate as during the months of feeding. Such statements indicate that calcium from the water is taken up directly into the tissues of the bivalve: yet Robertson (1941) failed to detect direct uptake of calcium ions from sea water in certain common gastropods and lamellibranchs. It may be that the methods he employed were not sufficiently sensitive to detect minute quantities.

Fox & Coe (1943), in describing the biology of the Californian sea mussel (*Mytilus californianus*), state that an individual measuring 100 mm. in length accumulates in its tissues and shell 17 g. calcium in a year; the calcium is not available in such quantities in the food, and must, they say, be obtained from the water in particulate or soluble form. If the tissues are permeable to ions such a quantity of calcium can be readily obtained: Fox, Sverdrup & Cunningham (1937) have shown that a medium-sized mussel may pass water through its gill chambers at an average rate of 22,000 l. a year, provided filtration continues incessantly, and this volume of water would contain about 9·2 kg. of calcium as salts in solution or suspension; an individual need utilize only a very small quantity of this to obtain the required 17 g. Yet if the calcium in artificial sea water be cut down to one-eighth its normal value the organic

matter of the newly formed shell of *Pedalion* is deposited in the usual way (Bevelander & Benzer, 1948), but there is no calcification—the animals in this experiment may be influenced by factors other than the low calcium content of the artificial medium.

Korringa (1952) suggests that the oyster collects calcium ions from the sea in much the same way as it extracts and accumulates other positive polyvalent ions such as Fe, Cu, Zn, Fe, Hg and Mn, which are present in sea water only in small amounts: ions, if adherent to the mucous feeding sheets would be automatically collected and ingested. The use of autoradiographs presents an opportunity of finding whether this occurs, and may indicate which tissues accumulate specific ions. From results obtained by this method Bevelander (1952) concludes that both fresh-water and marine molluscs take up labelled calcium and phosphate ions. The experiments on which these conclusions are based concern only lamellibranchs: mantles and shells were removed and autographs made only of these parts; there was, apparently, no attempt to trace the course of the ions or their localization in any other part of the body.

Uptake

Small *Mytilus* measuring 1·0–1·5 cm. in length were chosen for the present study so that autoradiographs could be made of the entire body; most results were then verified on larger specimens. The activity of the sea water in which the experimental animals were placed varied from 30 to 100 μc./l.; sometimes this water contained a culture of dinoflagellates, at other times it had been passed through a Berkefeld filter. Doses up to 450 μc./l. did not appear to damage the tissues. Much of the yttrium and some strontium would be adsorbed on the vessel in which the animal was kept, and on its shell. The higher doses give an insight into the way in which the mussel may deal successfully with an unusual influx of ions into the body.

When dinoflagellates are present in the activated water a larger amount of tracer enters the body of the mussel, for the flagellates concentrate the ions to some extent and are readily collected by the feeding currents. It is seen from autoradiographs that after *Mytilus* has been in water with these flagellates for $2\frac{1}{2}$ hr. the stomach displays the area of greatest radioactivity, and the tracer has passed to the digestive gland where strontium and yttrium ions are most numerous in the distal part of the cytoplasm of the digestive cells, and are scattered proximally. Some activated food has by this time entered the intestine, and from it ions of both ^{90}Sr and ^{90}Y penetrate the epithelium of the intestine and enter the haemocoel. Absorption of ferric saccharate by the intestine of a marine mollusc has been described by Gabe & Prenant (1949) for chitons, though these authors do not mention the fate of the iron which is taken up in this way. Some preliminary work with autoradiographs of *Lepidochitona cinereus* and *Patella vulgata* shows that the intestinal epithelium in both of these molluscs is permeable to strontium ions which pass by this

route to the haemocoel. The long intestine in the herbivore is known to be concerned with the elaboration of faeces (Graham, 1932) and its permeability to ions may be of some importance; the food is slow to pass along it and the faeces are of considerable bulk. Yonge (1926*a*), working with *Ostrea edulis*, finds no evidence of absorption in the epithelium of the gut. He is not, however, concerned with passage of ions. It is easy to imagine that an epithelium may act as an area of ion absorption or exchange, yet be impermeable to the droplets of oil, blood corpuscles, or small diatoms which Yonge employed and which he finds are taken up by cells of the digestive gland and by phagocytes only. In *Mytilus* some strontium ions, though fewer, pass through the wall of the stomach.

In experiments with filter-feeders it is difficult to prevent the entry of particulate matter into the gut. Even if the shell of *Mytilus* be thoroughly cleaned and the animal kept in Berkefeld-filtered water (KN grade of filter) 3 days to clear the larger particles of detritus from the mantle cavity before the experiment, the results may occasionally show such particles in the gut. These preparations were made for the experiments which are cited below. MacGinitie (1945) has shown that in *Urechis* (Echiuroidea) particles between 36 and 90 Å. in diameter may be caught by the mucous feeding sheets and with them enter the alimentary canal; such food may form an important part of the diet of a microphagous feeder. In order to prevent entrapped particles from entering the mouth of *Mytilus* attempts have been made to block the oral opening without injury to any part of the body, but these did not meet with complete success.

If small *Mytilus* be placed in filtered sea water activated with 100 μc. ^{90}Sr/l. autoradiographs of the sectioned tissues show that after 2 hr. strontium ions are present in the lumen of the gut, in the digestive gland, and in the tissues of the gill filaments. Their occurrence elsewhere in the body will be neglected for the present. In some specimens ions may be adherent to the outer surfaces of the gill filaments and labial palps, also to secretion which may be in contact with these surfaces. The amount of tracer which has entered the body of an individual is greater than could have been admitted into the alimentary canal with occasional particles in the filtered sea water: tracer ions have been taken up directly into the tissues. Their entry may be by one of two routes, or by both of these. The extremely large surface presented by the single pair of ctenidia of a lamellibranch may offer an area for ionic exchange or absorption, even though, as Yonge (1928) has shown for *Ostrea*, the surface is impermeable to molecules of glucose. Ronkin (1950), using excised fragments of the gills of *Mytilus edulis*, traced a slow penetration of ^{32}P into the tissues from the artificial sea water with which they were irrigated: chemical and radioactive assay revealed that only 0·06% of the intracellular phosphate was exchanged after the tissues had been exposed for 140 min. Such an experiment cannot refer to uptake by the outer surface of the epithelium alone, since the cut ends

of the filaments were exposed to the external medium and this might penetrate through the open blood spaces. However, Ronkin assumes that ionic exchange takes place at the surface of the ctenidium, and most of this, he suggests, is for use in that organ. The filaments receive their blood from the kidney and other capillaries: their blood pressure is low and the flow through them too slow to serve as an efficient transport mechanism from their tissues to other parts. It is apparent from autoradiographs that strontium ions enter the body by this route. If a medium-sized *Mytilus* has the shell wedged open slightly to give free access to the mantle cavity, and is placed so that its posterior half is in activated sea water, after 40 min. some strontium ions are within the ciliated cells of the gill filaments. Since no activity can be traced within the gut, and since in so short a time it is unlikely that ions, which might have entered by this route, would have circulated in the blood stream and be picked out almost specifically by these cells, it is assumed that the strontium has made direct entry to the ciliated epithelium of the gills. However, such entry of ions into the body would not appear to be the major one. The primary function of the gills is feeding, and ions which adhere to the mucus secreted by them may automatically find entry to the mouth. Korringa (1952) thinks it possible that the electrical properties of both food particle and feeding sheet may determine whether or not particles are readily caught by a bivalve: the oyster takes up into its body the positive polyvalent ions which have been previously mentioned, though positive monovalent ions like Na^+ and K^+, and negatively changed ions, are not easily caught. If this be so then strontium ions should be readily collected. No *Mytilus* which has been sectioned shows the mucous feeding sheets; these, if present, were destroyed in the processing for autoradiographs. In occasional individuals secretion on the ctenidial surface and on the labial palps is present, and to this some ions adhere. *Mytilus* which have been left for varying periods—a few hours to 3 days—in filtered sea water activated with ^{90}Sr all suggest that the gut is the more important area for ingress of ions to the tissues; presumably they have been directed there by the feeding currents. Their concentration is greatest in the digestive gland.

If strontium enters so readily the tissues of the gills, and, more especially, the gut, there is no reason for believing that the divalent ions of calcium would not follow a similar course and so provide for the continuous growth of the shell even in starved individuals.

The digestive gland of lamellibranchs consists of one type of cell (Yonge, 1926*b*) which is concerned with ingestion and digestion. Strontium and yttrium ions which pass into the gland in *Mytilus* are not accumulated for storage in these cells: they enter the haemocoel and are circulated to all tissues of the body. Bevelander (1952) in his discussion on calcification in molluscs states that 'calcium ions present in the water are ingested by the organism and are localized in several regions'. Of these regions he mentions only the

mantle saying that the localization there is essentially the same in both freshwater and marine molluscs and he figures an autoradiograph of *Anodonta grandis* with tracer calcium concentrated in the periphery, slightly below the epithelium and in the epithelial cells themselves. In a similar position Trueman (1942) finds scattered calcium stores in *Tellina tenuis*. If strontium follows the path of calcium one might expect to find in experimental animals an accumulation of ions in these same parts which differentially select material for the production of the shell. Small *Mytilus* which have been in activated sea water up to 5 days and then in normal sea water for periods ranging from a few hours to 3 days show only a scattering of strontium in these mantle tissues and no pronounced localization. It may be that the experiments were carried on for too short a time or, rather, that the more abundant unlabelled calcium ions are preferentially selected. Actually in the mantle of *Mytilus* there is no heavy storage of calcium which might accumulate the divalent cations as a reserve comparable to that in the liver of a slug. If crystal filaments of shell be accidentally autographed with the soft tissues, however, a layer of adsorbed strontium ions is seen to encircle each. Normally strontium, if present in the lamellibranch shell, is only in very small amounts: Trueman (1942) has shown that in *Tellina tenuis*, *T. baltica* and *Donax vittatus* the shell contains traces of strontium which he suggests may contribute towards the formation of aragonite.

Excretion

Perhaps the most surprising results from the present experiments concern the rapidity with which the ions of both strontium and yttrium are accumulated for excretion, bearing in mind that the strontium, as displayed in autoradiographs, is an indication of the events of not more than 0·07% of the total quantity present in the sea water for concentrations of 100 μc./l. of water. Presumably a much greater percentage of unlabelled ions are following a similar course through the tissues of the mussel.

The excretory system of lamellibranchs consists of two separate groups of organs, the kidneys and the pericardial glands; to these may be added the digestive gland which, though with other major functions to perform, liberates to the intestine waste from its own metabolic activities. Takatsuki (1934) found that in *Ostrea edulis* these organs work in conjunction with amoebocytes which free the body of foreign or indigestible particles: they ingest these and remove them by way of the excretory organ, pericardium, surface of the auricle, rectum and mantle cavity; no such particles are taken up directly by cells of the excretory tubules. *Mytilus* which have been in activated water for 10 hr. show that cells of the pericardial gland are accumulating the tracer elements (Fig. 3A). These glands (*p.g.*) surround the auricles (*a.*) and so come into contact with the blood from which the ions are extracted; there is no indication that they have entered the cells of the gland exclusively, or even to any

appreciable extent, by means of amoebocytes transporting them there as described for the course of waste particulate matter in *Ostrea* (Takatsuki, 1934). As more tracer enters the body of the mussel, with longer periods of

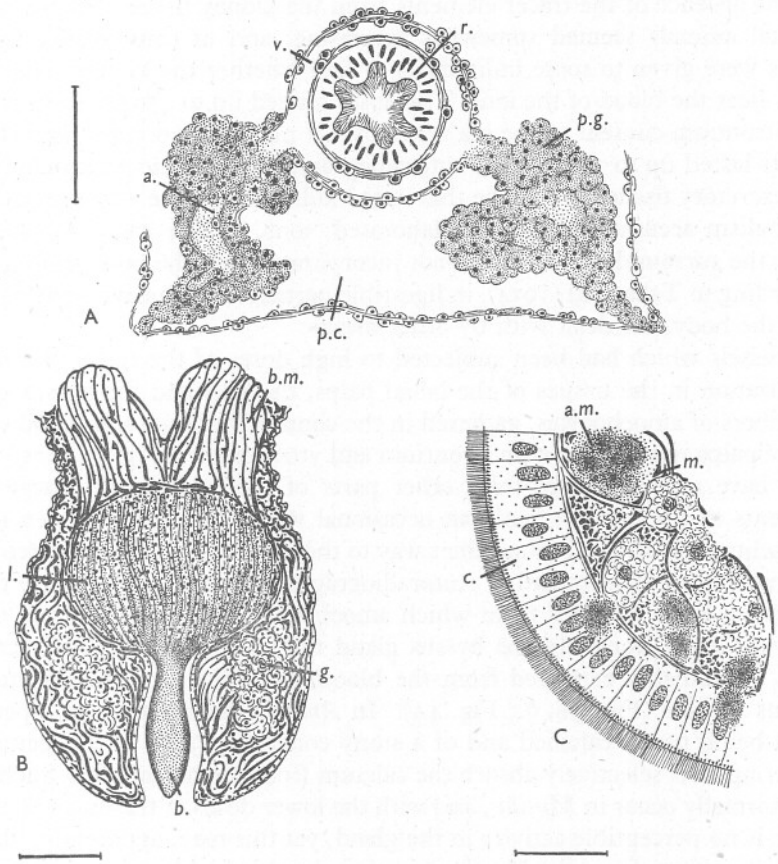

Fig. 3. *Mytilus edulis*. A, transverse section through the heart and pericardial glands. Stippling represents the superimposed autoradiograph. The mussel had been in activated sea water for 10 hr. Scale, 250 μ. B, transverse section through the foot, after the mussel had been subjected to a high dose of ^{90}Sr. Scale, 500 μ. C, part of a transverse section through a labial palp, after a high dose of ^{90}Sr. Scale, 25 μ. Stippling represents the superimposed autoradiograph. *a*, auricle; *a.m.*, amoebocyte; *b.*, byssus thread; *b.m.*, byssus retractor muscle; *c.*, ciliated epithelium; *g.*, byssus gland in foot; *l.*, glandular lamellae dividing byssogenous cavity; *m.*, muscle fibres; *p.c.*, pericardial cavity; *p.g.*, pericardial gland; *r.*, rectum; *v.*, ventricle.

exposure to the activated water, so a heavier accumulation of ^{90}Sr and ^{90}Y is found in the pericardial gland. Cuénot (1899) states that the phagocytic cells of the glands which capture particles may eventually transport them from the

gland: they deposit the waste in connective tissue or carry it from the body by way of the gills or palps. Alternatively, the phagocytic cells may discharge their contents directly into the pericardial cavity (Takatsuki, 1934), which is in communication with the lumen of the kidney by the renopericardial duct.

The absence of the tracer elements from the kidney tissue of these experimental animals seemed somewhat surprising, and in consequence heavier doses were given to some individuals to find whether the kidney cells might then clear the blood of the ions. The doses ranged up to 450 μc./l., increasing the strontium content of the sea water by not more than 0·315% and experiments lasted up to 3 days. Yet autoradiographs revealed no accumulation by the excretory tissue. It may be that in the kidneys only the waste products of katabolism accumulate and are elaborated: ions, as ^{90}Sr and ^{90}Y, taken up from the surrounding water and not incorporated into the body tissues, and, according to Takatsuki (1934), indigestible particles which have been injected into the body, are dealt with by other means.

Mussels which had been subjected to high doses of the tracer showed its localization in the tissues of the labial palps, the gills and the byssus gland. Numbers of amoebocytes, gathered in the connective tissue and blood spaces of the palps, were laden with strontium and yttrium ions (Fig. 3C, *a.m.*): they may have migrated here from other parts of the body to discharge their contents to the exterior; only an occasional wandering cell was seen in the epithelium—if any had found their way to the surface they had been removed on fixation, or subsequently. Autoradiographs gave a similar picture of the blood spaces of the gills from which amoebocytes pass to the mantle cavity carrying excess ions. In the byssus gland the tracers were in the secreting cells, presumably extracted from the blood, and they were passed into the byssus threads (Fig. 3B, *b.*; Fig. 4A). In *Anomia* the byssus is of a peculiar form being partly calcified and of a stony consistency, for in this genus the secreting cells selectively absorb the calcium from the haemocoel. Such does not normally occur in *Mytilus*, and with the lower doses of the tracer elements there is no perceptible activity in the gland, yet this may, apparently, liberate divalent cations from the blood when their level is high.

Mention must still be made of the excretory function of the digestive gland. The tubules comprise only one type of cell (Yonge, 1926*b*) which is active in the uptake of ions from the gut. After a period of feeding yellow concretions may be found to accumulate in a distal vacuole of the cytoplasm—presumably the waste products of cellular metabolism, including digestion. Concretions are expelled to the lumen of the gland and so reach the intestine: since a large number of digestive cells may excrete at any one time the volume of this faecal matter can be quite considerable. Mussels which have been in activated, filtered sea water from 3 to 5 days and then in normal sea water for a corresponding length of time, show strontium and yttrium ions leaving the body with waste from the gland, and the tracers can be found leaving the cells with

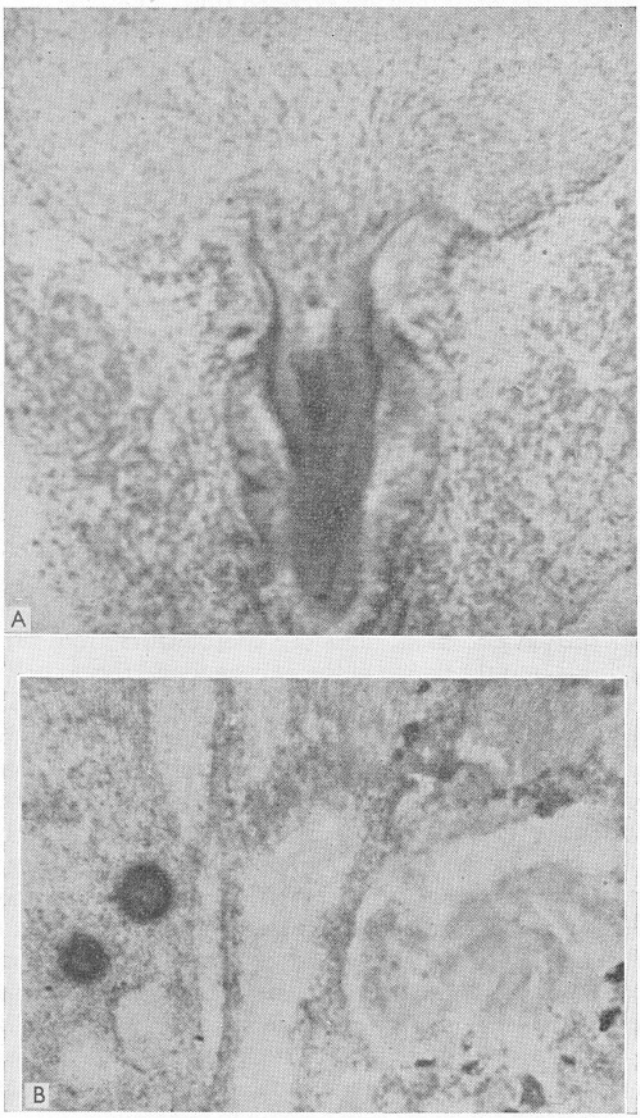

Fig. 4. A, *Mytilus edulis* subjected to a high dose of ^{90}Sr. Photomicrograph of part of a transverse section through the foot, with superimposed autoradiograph, to show ^{90}Sr and ^{90}Y in the byssus thread. B, *Acanthodoris pilosa*, photomicrograph of part of a transverse section through the mantle, kidney and hermaphrodite gland, with superimposed autoradiograph, to show ^{90}Sr localized around two mineral concretions in the mantle.

the yellow concretions. Some ions taken from the gut by the digestive cells may never leave them until they are excreted thus. This would not appear to happen to all, for there is evidence that ions which have circulated in the blood may eventually be taken back by the digestive cells to be freed from the body. Such evidence comes from animals which after taking up the tracer from filtered water are placed in normal sea water for a number of days: the digestive gland may show an accumulation of ions which is just as heavy after 5 days in normal sea water as after one, although during those 5 days excreta from the gland, carrying the tracer with it, have passed to the intestine.

Wandering Phagocytic Cells in *Calyptraea chinensis* and *Platynereis dumerili*

The importance of phagocytes in the metabolic activity of lamellibranchs has long been recognized (Cuénot, 1899; Yonge, 1926*a*, *b*). Occurring in large numbers they provide a source of digestive and excretory activity. In their cytoplasm are elaborated digestive enzymes to deal with such ingested particles as diatoms, and even blood corpuscles; their excretory function, at least in *Mytilus*, is extended to the uptake of excess ions, for which they may take the role of an emergency system. Less recognition has been given to the phagocytes of gastropods. Millott (1937) suggests that in nudibranchs they are of significance in excretion, ingesting effete matter from the haemocoel and discharging it into the lumen of the gut; he found this to be their activity with particles of iron saccharate. Prosobranchs may have phagocytes which are similarly excretory in function. If specimens of *Calyptraea* be placed in filtered sea water activated with ^{90}Sr to the extent of 100μc./l., ions pass into the body, and, circulated in the blood stream, they may be carried to all tissues. Autoradiographs of individuals which have been in activated water for 2 days show concentrations of strontium and yttrium in phagocytic cells. These may occur in various parts of the body: the tissues of the foot, the bases of the gill filaments, the wall of the pericardium and of the kidney. It is doubtful whether these positions can be connected with any nutritive function of the wandering cells. Whatever their action may be it is associated with an increase of strontium in the external medium of 0·70%: in the body tissues the increase could therefore be only slight. This suggests that the phagocytes are extremely sensitive to changes in their environment. Perhaps one of their important functions is in helping to maintain an ionic steady state within the body. The localization of ^{90}Sr is also marked in the hypobranchial gland. It elaborates large quantities of secretion, and in taking up constituents from the blood presumably abstracts the divalent cations of strontium, which are then freed from the body with its secretion.

Wandering amoebocytes occur in polychaete worms. Romieu (1923), referring to them as leucocytes, says that they may collect excretory matter

from the body and transport it to the epidermis, where they may be seen in *Dodecaceria concharum*. Similar phagocytosis by leucocytes occurs in nereids and *Aphrodite aculeata* (Fordham, 1925); the cells are particularly large and active in *Perinereis cultrifera* (Romieu, 1923). In polychaetes it is recognized that one type of leucocyte may deal with waste, another with food stuffs. The former, which phagocytose and eliminate noxious material, are called lymphoidocytes; the latter, which transport and release food into the blood and tissues, are trephocytes. Liebman (1946) describes and figures the two in *Amphitrite crinata*. Since in certain molluscs amoebocytes are active in the transport of ions it was thought that the investigation of a polychaete might show that in this class also they can be similarly employed. Autoradiographs of sections of *Amphitrite gracilis* and *Platynereis dumerili* which had been

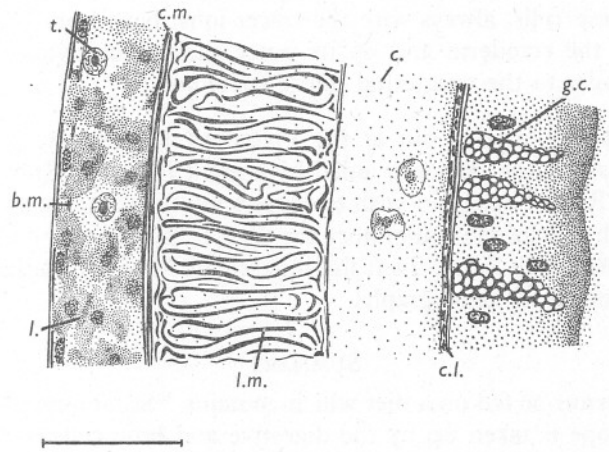

Fig. 5. *Platynereis dumerili*. Part of a transverse section. Stippling represents the superimposed autoradiograph. The specimen had been in sea water activated with ^{90}Sr for 3 days, and had taken activated food into its gut. *b.m.*, basement membrane of ectoderm; *c.*, coelom; *c.l.*, circular and longitudinal muscles of gut; *c.m.*, circular muscle of body wall; *g.c.*, gland cell in epithelium of gut; *l.*, lymphoidocyte; *l.m.*, longitudinal muscles of body-wall; *t.*, trephocyte. Scale, 250 μ.

subjected to the dose of 100 μc. ^{90}Sr/l. of sea water, showed no use of amoebocytes in dealing with tracer ions which have entered the tissues. *Platynereis* were then kept for 3 days with 3 times as much tracer in the water, some in filtered sea water, and others amongst small quantities of sand and detritus from the coralline pool in which they had been collected. Autoradiographs of those individuals revealed wandering cells laden with both strontium and yttrium ions (Fig. 5, *l.*). They were found most commonly beneath the ectoderm (*b.m.*) of the body wall, a position which suggests that they are lymphoidocytes ridding the body of the tracer elements. The greatest amount of radioactivity was found in a worm which had ingested a fragment of weed

together with some detritus; from these contents ions passed through the wall of the alimentary canal (*g.c.*) to the coelom (*c.*). The gut would appear not to be the only path by which ions enter the body, although they find entry here even in the absence of food: individuals kept in radioactive sea water for half an hour show little tracer in the alimentary canal and more in the integument, suggesting an absorption here. Lymphoidocytes with some ^{90}Sr and ^{90}Y are occasionally in the coelom: always those with the highest concentration are clustered between the basement membrane of the ectoderm and the underlying fibres of circular muscle (*c.m.*). Amongst them occurs an apparently less frequent type of cell with little or no tracer in its cytoplasm. This is presumably the trephocyte (*t.*) which is concerned with the transport of nutritive material. The subepithelial clusters of lymphoidocytes may become very pronounced if a worm be subjected to high doses of the tracer for several days. Some of these cells, always with the tracer ions, have been found between the cells of the ectoderm and on its outer surface, behaving in a fashion which is similar to the wandering cells of *Mytilus*.

This work was carried out at the Plymouth Laboratory of the Marine Biological Association, and was aided by a grant from the Browne Research Fund of the Royal Society. I wish to express my thanks to many members of the staff of the Plymouth Laboratory for their help, and to the Committee of the Browne Research Fund. I am also indebted to Mr F. C. Padley of Reading University for taking photographs.

Summary

If *Arion hortensis* be fed on a diet which contains ^{90}Sr, autoradiographs show that the isotope is taken up by the digestive and lime cells of the digestive gland. From the former it passes to the haemocoel; in the lime cells it is concentrated around the calcium spherules. Some of the tracer enters the body through the wall of the intestine. Calcium stores which surround blood vessels and calcium cells in the mantle also concentrate the tracer.

In *Aplysia punctata* ^{90}Sr from the surrounding water passes through the surface of the body, and especially the gill; in *Acanthodoris pilosa* ions which enter the tissues from the sea water accumulate around the numerous calcium concretions in the mantle. These marine molluscs obtain cations directly from the water as well as by way of the food.

There is a slow uptake of strontium ions by the ctenidia of *Mytilus edulis*, though, even in filtered sea water, the gut is the more important area for their ingress to the body. It is possible that they enter with the mucous feeding-sheets. They pass readily into the cells of the digestive gland. Some of the isotope taken in with the food is absorbed by the wall of the intestine; this also occurs in *Patella vulgata*, in which the intestine provides a much larger area, and in *Lepidochitona cinereus*.

Mytilus placed in filtered sea water which is activated with ^{90}Sr, so increasing the strontium content by 0·07%, show the tracer localized for excretion within 10 hr. Ions are aggregated in the pericardial glands, not in the kidney. If the strontium content of the water in which *Mytilus* are kept be increased by the tracer up to 0·315%, within 3 days amoebocytes with the element, and with its daughter product yttrium, are gathered in the connective tissue of the labial palps, and in blood spaces of the gills from which they pass to the mantle cavity. The tracers also leave the body with secretion from the byssus gland, but are not accumulated by the cells of the kidney. There is no storage of ^{90}Sr by the digestive gland which excretes the ions into the gut, nor are there high concentrations retained by tissues of the mantle.

Amoebocytes of *Calyptraea chinensis* accumulate the tracer element which enters the tissues from filtered sea water. One of their important functions may be in helping to maintain an ionic steady state within the body.

Two types of wandering cells occur in *Platynereis dumerili*: one of these, the lymphoidocyte concentrates the isotope in its cytoplasm and transports it to the outer surface of the body.

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STUDIES ON THE INTERRELATIONSHIPS OF ZOOPLANKTON AND PHYTOPLANKTON

By Richard Bainbridge

Department of Zoology and Comparative Anatomy, University of Oxford, and
Department of Zoology, University of Cambridge

(Plate I and Text-figs. 1–4)

CONTENTS

INTRODUCTION

It has in the past been thought that the marine plankton is more or less uniformly distributed. With more and more intensive sampling and observation, however, it has become apparent that the distribution of all forms of both animal and plant plankton is uneven in the extreme. Further, it has been demonstrated that generally, but by no means always, there is an inverse relationship between the quantities of zooplankton and phytoplankton taken in any one limited area. Attention was first strongly focused on this fact by Hardy (Hardy & Gunther, 1935), although earlier authors had remarked on its occurrence. Almost simultaneously, at that time, two hypotheses were put forward to account for this phenomenon.

Harvey (1934, and more fully in the joint paper of Harvey, Cooper, Lebour & Russell, 1935), recorded occurrences of the inverse relationship in the English Channel and proposed that grazing by the zooplankton could adequately

account for the observations. His hypothesis suggests that, in areas of high zooplankton concentrations, most of the phytoplankton would be grazed down; while in areas of low zooplankton concentration it would flourish. The effect of this would be to produce the observed inverse relationship.

Hardy (*loc. cit.*) showed how his own Antarctic results substantiated earlier records by Pearcey, Castracane and others of the inverse relationship, and proposed the hypothesis of 'animal exclusion' to explain it. This hypothesis supposes that high concentrations of phytoplankton are in some way 'distasteful' to plankton animals, which, during their diurnal vertical migrations, either refrain from coming up or come up for a much shorter time in areas rich in phytoplankton. The differing speeds and directions of currents at various levels then automatically segregate the concentrations of animals and plants and produce the observed inverse geographical distribution.

Both authors acknowledge that their hypotheses are not mutually exclusive and agree that either may be operative at any one time depending upon the circumstances. The possibility that a pattern may first be imposed upon the phytoplankton by grazing and may then be maintained by exclusion of the animals from the resulting areas of increasing phytoplankton density is mentioned by Hardy (p. 310).

The conception of a direct 'lethal' effect of phytoplankton concentrations upon zooplankton has been developed particularly by Lucas (1936, 1947). Such an effect could result in an exclusion without the mediation of vertical migration and could account for those occurrences of the inverse relationship in shallower waters where phytoplankton concentrations may reach right to the bottom, as in the North Sea. There is now considerable evidence (most usefully collated by Brongersma-Sanders, 1948) on the toxic properties of blooms of coloured water, but it seems likely that this is a specialized phenomenon restricted to certain species and localities. The possibility of a less marked, but nevertheless adequately operative lethal effect amongst other members of the phytoplankton, must, however, be borne in mind. Lucas (1949) has in fact drawn together much evidence on the importance of the external secretions of both animals and plants in the communal life of many organisms, and now looks upon 'animal exclusion' as one manifestation of a widespread system of ecological inter-relationships, which embraces both beneficial and harmful effects on the part of various reactants.

Steemann Nielsen (1937), while not minimizing the possible effect of grazing, maintains that the naturally different rates of development of phytoplankton and zooplankton populations are sufficient to account for the inverse relationships. Under favourable conditions a phytoplankton maximum can develop rapidly while a zooplankton maximum must necessarily lag behind. This and the frequent occurrence of phytoplankton concentrations in areas of up-welling water, which generally has a low zooplankton stock, combine to make phytoplankton and zooplankton concentrations occur in different regions.

There is yet little evidence unequivocally in support of any of these hypotheses. Mathematical work by Fleming (1939) and observations on rates of feeding by Fuller (1937) and Gauld (1951), among others, make it clear that Harvey's idea of grazing is well within the realms of probability. Lucas's (1936) work on zooplankton distribution in partially shaded diatom cultures, and Hardy & Paton's (1947) work have not produced the looked for evidence in support of the exclusion hypothesis.

Any attempt to resolve between the ideas of grazing and exclusion, or to determine which may be the more important, must necessarily include an experimental approach. The present steady accumulation of data on distribution can only substantiate the existence of the inverse relationship, or at the very best serve as an indication of the possible means through which it arose. A most intensive survey would be needed to provide a solution to the problem in the sea itself. Essentially the problem is that of the behaviour of zooplankton in the presence and in the absence of phytoplankton. If the whole phenomenon arises through mechanical means (Harvey or Steemann Nielsen), then there need not be special behavioural characteristics, while if it arises through an exclusion mechanism it should be possible to demonstrate, in the laboratory, those patterns of behaviour which result in the observed distributions.

Lucas (1936) interprets his results as showing an avoidance by animals of illuminated, active, phytoplankton. Elimination of the confusing effects of shading in this work is made possible by the technique of producing a gradient of phytoplankton in a long tube (Bainbridge, 1949). If animals introduced into this were to show any avoidance of high phytoplankton concentrations, then support for Hardy's hypothesis would be obtained, while if they fed blindly, remaining in even the highest concentrations of phytoplankton, then Harvey's hypothesis would be supported.

Hardy's hypothesis utilizes only the powers of vertical migration of zooplankton combined with water movements, and it would therefore be most desirable to investigate the movement of zooplankton in vertical gradients. The practical difficulties of preparing stable vertical gradients and of introducing animals into them are, however, considerable and this method of approach was rejected. Instead, the vertical migrational behaviour of animals in the presence and absence of phytoplankton in simple vertical tubes was studied. It is generally maintained that members of the plankton possess negligible powers of horizontal migration and make extensive journeys only in the vertical plane.[1] I believe this view to be erroneous and am led by experimental data and observation to the conclusion that those animals now

[1] The term 'zooplankton' is a very broad one covering what is not in fact a distinct group but rather a loose assemblage of animals with clearly graded powers of locomotion. Recent work (Hardy & Bainbridge, 1951) has shown that many of these animals can in fact swim much faster and for more sustained periods than has previously been thought. It is known that almost all perform great vertical migrations but any horizontal component there may be in their movements is generally thought to be random and undirected.

broadly grouped as planktonic fall naturally into two classes: one comprising most of the copepods, coelenterates, smaller and early larval stages, etc., which, if migrating at all, in fact only migrate vertically; and the other comprising some large copepods, the euphausids, mysids and cumaceans and larger larvae, etc., which, besides migrating vertically, can, and under suitable stimuli do, make horizontal migrations extensive enough to be of great consequence in the ordering of distribution. The experiments here described cater for these two classes of animals, the vertical tubes for the former and the horizontal tubes for the latter, while the results obtained serve to support the reality of this grouping.

I am deeply indebted to Professor A. C. Hardy, F.R.S., for his constant interest in this work and for having allowed me to do part of it while employed by him with a grant from the Leverhulme Trustees; to the Royal Society whose generous grant from the Browne Fund allowed it to be completed; to the Directors of the Plymouth and Millport Laboratories who most kindly provided facilities; to Messrs Siebe, Gorman and Co., who made a loan of diving equipment; and to Dr T. J. Hart and Dr C. E. Lucas for their helpful criticisms of the manuscript. Finally, I have been encouraged and advised by many friends, to all of whom I am most grateful. Most of the work described has been approved by the University of London for award of the degree of Ph.D.

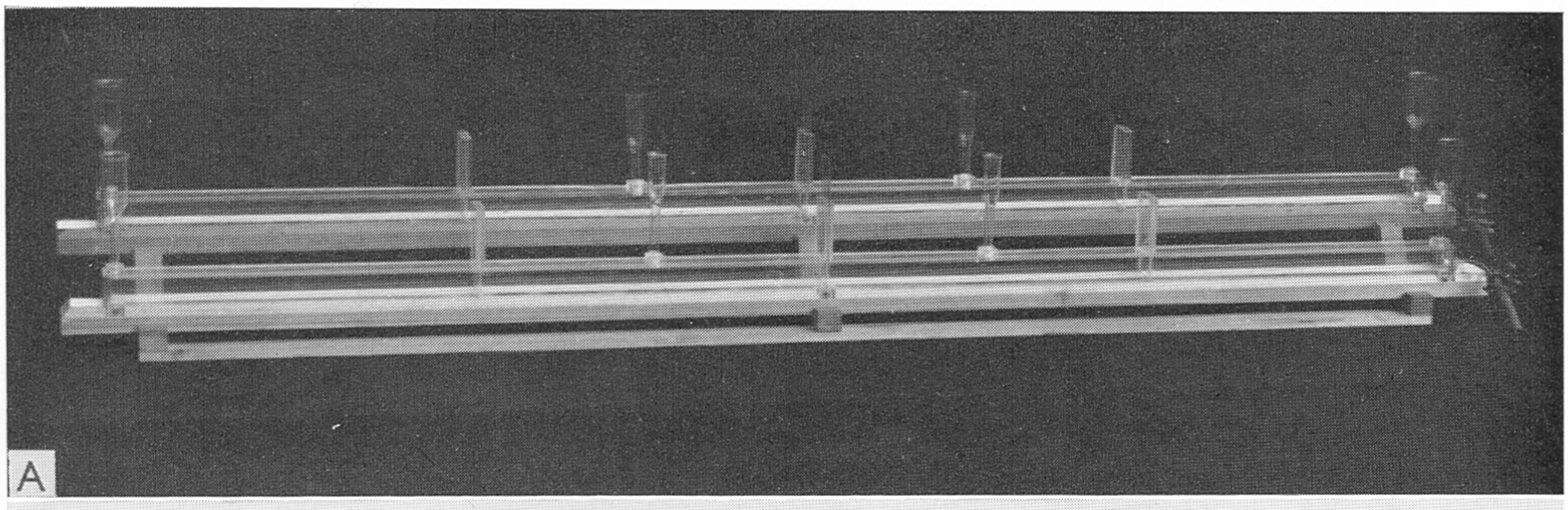
Apparatus and Methods

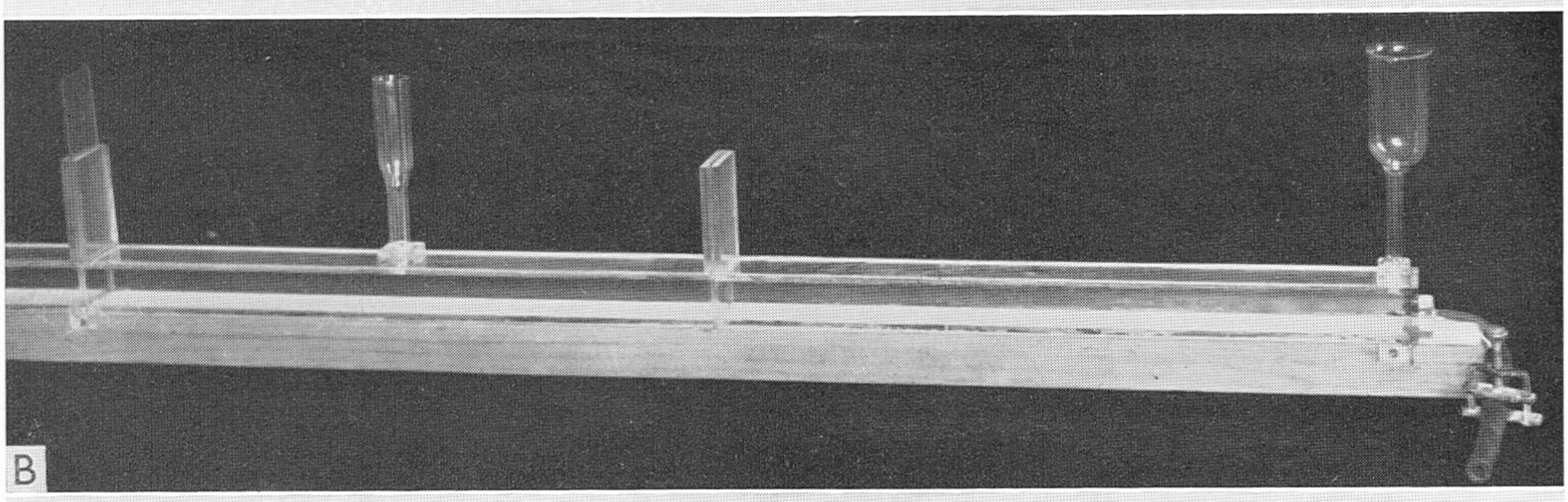
Horizontal Experiments

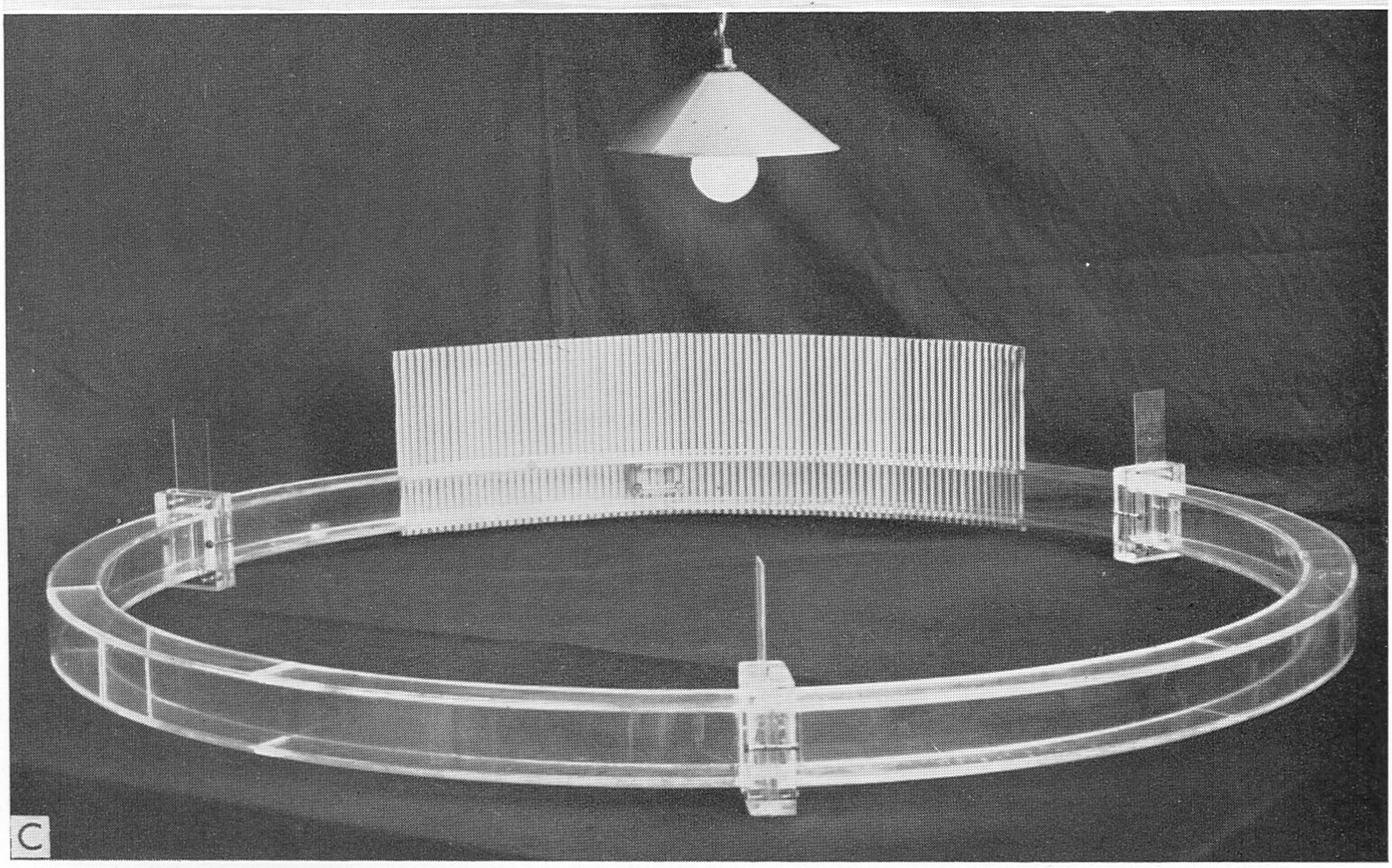
Straight Apparatus

Preliminary horizontal experiments were performed in the glass apparatus described in Bainbridge (1949). This apparatus was eventually replaced by one constructed entirely of Perspex. With this material it was possible to build up a tube of rectangular cross-section, thus eliminating those optical effects of a cylindrical tube which make the counting of small animals difficult and inaccurate. The second apparatus consists (Pl. I A, B) of a horizontal Perspex tube 48 in. long of internal dimensions 1 in. wide by $\frac{3}{4}$ in. deep. This is divisible into four equal water-tight compartments by sliding doors; funnels at the outer ends of the outer two compartments and at the centres of the two central ones allow for filling and the introduction of animals. Two of these pieces were made so that control or replicate experiments could be performed simultaneously.

In a typical experiment the central door is first closed and the apparatus filled: freshly taken ordinary sea water (hereafter referred to as 'normal'), or filtered sea water, being put into one side and water enriched with phytoplankton into the other. After a pause of 3 or 4 minutes to allow currents to cease, the central door is opened. Diffusion and mixing of the phytoplankton and normal water follows fairly quickly and within 5 minutes a visible







A. General view of second Perspex horizontal apparatus.
B. Detail of right-hand half of one tube of above.
C. Perspex horizontal circular apparatus, showing the three sliding doors, one section of a surrounding cardboard shield and the electric light.

gradient of phytoplankton exists in the central 10 in. or so of the tube, separating the clear water at one end from the slightly cloudy, enriched water at the other. For a satisfactory uneven distribution to be maintained it is necessary for the waters put into the two sides of the apparatus to be identical in temperature and specific gravity. Otherwise flowing occurs and results in the formation of two layers along the length of the tube. The enriched water is always of a lower specific gravity than sea water owing to the addition of a small amount of tap water during the preparation of the culture solution (see below, p. 391). Immediately before the experiment, therefore, the two lots of water to be used, after having been brought to the same temperature, are tested with a hydrometer and the specific gravity of the higher is brought down to that of the lower by the addition of drops of tap water. With the apparatus full, animals are pipetted into each side of it in equal numbers through the two central funnels. The distribution of animals throughout the length of the tube is now recorded at regular intervals. For this purpose the tube is divided into imaginary quarters delimited by the central door and the two outer doors. At the appropriate time the number of animals in each quarter is counted quickly and recorded in a table. When using the second apparatus, if a large number of quickly moving animals is involved, then all three doors are closed during the counting, which is thus carried out much more accurately. The doors are opened again to allow the experiment to continue.

Recording continues for the duration of the experiment and this varies from 1 or 2 hr. up to 3 days. During this time notes are made as to the stability of the uneven distribution in the tube and of the gradient. The simple criterion taken for the persistence of these is their continued visibility. This method is not always practicable, however, and in some instances water has to be removed from both ends by a pipette and the phytoplankton content assessed by counting. At the completion of an experiment the apparatus is emptied through the draining tube fitted at one end and is washed out. Occasionally a more thorough cleaning is required. The ends are then removed and the tube pulled through with a wad of cotton-wool on a string.

Circular Apparatus

One of the disadvantages of the straight apparatus is that animals consistently swimming in one direction come sooner or later to the end of the tube. If they have no reaction for turning and exploring in the opposite direction then they may continue swimming and butting into the end of the tube in an environment in which they would not have remained had they been able to sample that at the other end. To obviate this disadvantage some experiments were performed in a circular horizontal tube.

This apparatus (Pl. 1c) consists of a Perspex tube 12 ft. long and 2 by $1\frac{1}{2}$ in. internal dimensions, built up in the form of a circle 4 ft. in diameter.

The whole is divisible into three equal sections by sliding water-tight doors, and openings $1\frac{1}{2}$ by $\frac{1}{2}$ in. at these three points allow for filling and the introduction of animals. The apparatus is set up horizontally in a darkened constant-temperature room and illuminated by a 25 W. electric bulb suspended symmetrically about 2 ft. above it.

In a typical experiment two of the doors are closed and the segment between them is filled with water enriched with phytoplankton. The remaining two segments are filled with normal or filtered water and, after a pause, the doors are opened. Animals are introduced in equal numbers at the three openings. For the purpose of counting the whole tube is divided into imaginary sixths, three of the boundaries coinciding with the sliding doors and three placed symmetrically between them. Starting from a fixed reference point in relation to the room the animals in successive sixths are counted quickly and the numbers recorded in a table. This is repeated at intervals for the duration of the experiment. At its completion water is drawn out at the three openings and counts of the phytoplankton made to assess the stability of the uneven distribution in the tube.

Vertical Experiments

The vertical experiments were performed in an apparatus consisting of two parallel Perspex tubes 48 in. long and 2 by $1\frac{1}{2}$ in. internal dimensions. The tubes are held 2 in. apart by Perspex spacers and are closed at the top and bottom by removable Perspex covers. In the centre of each is a butterfly valve of 1 mm. thick Perspex which, in the horizontal position, divides the tube into upper and lower portions and, in the vertical position, presents only a very small obstacle to the movements of the animals. The valves are worked by levers, weights and strings.

In a typical experiment one tube is filled with normal or filtered water and the other with water enriched with phytoplankton. A number of animals are introduced into the top of each tube and the lids are bolted down. (The animals may be picked out and counted before use, when equal numbers are put into each tube; or roughly equal numbers may be poured in and, at the completion of the experiment, removed by filtration and counted at leisure.) The apparatus, when filled, is lowered to 6 in. below the surface of the water in the centre of a large (72×33 ft.) open tank and suspended, with the valves open, from a wooden boom. At intervals the doors are closed, the apparatus removed from the water and the number of animals in each of the upper compartments is counted and recorded in a table. The apparatus is then lowered back into the water and the doors opened. This is continued for the duration of the experiment, which may be from a few hours to several days.

Preliminary experiments were performed in this apparatus, but it soon became clear that the method had several disadvantages. First, rather small numbers swam up during the daytime owing to the high intensity of light

and this made assessment of any difference between the two tubes difficult; and secondly, the animals were undoubtedly disturbed when the apparatus was taken out of the water and when wind caused it to move about. To obviate these disadvantages the later and main series of experiments was performed indoors in an aquarium tank.

The apparatus was suspended close to the glass of a $4 \times 4 \times 5$ ft. tank with the tops of the tubes just above water-level. Lighting was oblique and chiefly from above and the intensity was a good deal less than that out of doors. The water circulating in the tank kept the apparatus at a temperature only a few degrees above that in the sea and the animals could be counted readily through the glass without any disturbance whatsoever. As a result of this it became unnecessary to close the doors, and these were removed and the apparatus split up to give two pairs of simple vertical tubes, each 2 ft. $\times$ 2 in. $\times$ $1\frac{1}{2}$ in. This enabled duplicate readings to be taken in every experiment.

Culture of Phytoplankton

Almost all the phytoplankton organisms used in the experiments were grown in pure cultures. Occasionally natural sea water at the time of a rich diatom outburst was used, and occasionally natural water was concentrated by filtration and the concentrate used. The cultures were first grown in jars of sea water enriched with Miquel solutions and sterilized by heating to 70° C. after Allen & Nelson's (1910) instructions. More satisfactory results, however, were obtained by a modification of the 'Erdschreiber' technique, and in the bulk of the work only cultures grown in this manner were employed.

A soil extract was made by bringing a mixture of 500 g. of garden soil and 1 l. of tap water to the boil and allowing to simmer for 40 min. This mixture was left to stand for a few days and the supernatant liquid was decanted, brought to the boil, and again left for a few days. This process of decantation and boiling was continued until a clear sherry-coloured liquid resulted. The sea water used for growing the cultures was filtered through coarse filter-paper, sterilized by heating to 70° C., and allowed to cool. For every litre of sea water 50 ml. of the clear soil extract were brought to the boil and 0·3 g. of sodium nitrate and 0·03 g. of sodium hydrogen phosphate dissolved in the hot liquid. This hot solution was then added to the cold sea water and the whole stirred. After standing for a day the enriched sea water was ready for use as culture solution.

The cultures were grown variously in flasks and breffit jars standing in a window with a north light. Most of the diatoms were picked out from sea-water samples that had been enriched by the addition of an equal volume of culture solution and allowed to stand for a week or so. This ensured that only healthy, growing cells were taken. Dense cultures grown from a single cell or chain of cells were obtained in this manner. The author is deeply indebted to Dr Mary Parke who provided initial samples of all the flagellates used. These

had themselves previously been picked out by the method described, although many had been in culture for some time.

Exploratory Toxicity Experiments (with Results)

The horizontal experiments are generally lengthy to perform and use large volumes of culture. It soon became apparent, when all the early experiments were giving positive migrations into the cultures, that if any were to be found producing a reverse 'exclusion' effect, then some quicker method must be used to give a preliminary indication of cultures that might produce this effect. A series termed the 'toxicity experiments' was therefore set up in order to test cultures before use in the horizontal experiments.

A number (generally twelve) of 50 ml. capacity jars with open tops were arranged on black paper trays. 20 ml. of the culture to be tested, either as grown or sometimes diluted with ultra-filtered water, was put into each jar and the concentration of cells assessed by counts on a haemocytometer slide. The animal chosen was *Hemimysis lamornae* and one of these was put into each jar and the whole batch covered with a glass plate. The animals were examined from time to time for a period of 3 days and notes were made of any that died, of the presence or absence of faecal pellets, food in the gut and other relevant observations. The trays stood on a bench in front of a window with a north light. No attempt was made to control the temperature.

The results of these experiments are summarized in Table 1, where it is seen that a total of sixty-eight different pure cultures of phytoplankton organisms was used. Control experiments, with normal sea water passed through a Berkefeld filter candle and containing no particulate matter, were used as a reference. The mean result for ten experiments with filtered water, each using twelve animals, is a total of 5·3 dead out of 12 at the end of 3 days. It will be seen that the great bulk of the cultures used gave a much better survival than this. Out of a total of seventy-one cultures tested (including three duplicates) twenty-four gave a complete survival and sixty-five gave five or less dead. These have all been assumed lacking in toxic properties although they may, of course, have very different nutritive values. Three cultures—Flagellate 12; *Gymnodinium* I and Flagellate One—each gave eight dead, *Chlorella stigmatophora* eleven dead, and *Gymnodinium* II twelve dead. These five have been suspected of having toxic properties of some kind.

In an attempt to demonstrate more clearly that these deaths were due to a toxic action and not to starvation, *Chlorella stigmatophora* was chosen for a series of experiments identical with those above, save that various concentrations of *Chlorella* were used. The results obtained from sixteen batches of jars (one with twenty-five jars, three with eight and the remainder with twelve jars) containing cultures ranging from 13,120 cells/mm.^{3} down to 585 cells are shown in Text-fig. 1A. The concentrations have been divided into five groups, the number of jars in each group adjusted to be the same,

TABLE 1. RESULTS OF EXPLORATORY TOXICITY EXPERIMENTS

Culture	Concentration (cells/mm.^{3})	Number out of 12 dead in 1st 24 hr.	2nd 24 hr.	3rd 24 hr.	Total
Flagellate 3	2,310	—	1	—	1
Flagellate 10	900	1	—	1	2
Flagellate 25	655	—	1	1	2
Flagellate K	3,760	—	—	5	5
Lancelot II Clone 8	200	—	—	1	1
L 4 (IV) Clone 3	310	—	—	2	2
L 4 (IV) Clone 6	410	1	—	—	1
Lancelot I Clone 4	905	—	—	1	1
L 4 (13/4) 2L Clone 1	1,480	—	—	—	—
L 4 (4/5) Clone 3	610	1	2	—	3
Chromulina pleiades	3,220	1	—	—	1
Chromulina pusilla	37,000	—	—	—	—
Pavlova gyrans	820	—	—	—	—
Coccolithus huxleyi	2,080	2	—	—	2
Isochrysis galbana	3,680	—	—	—	—
Dicrateria inornata	11,220	1	—	1	2
Pseudopedinella I	230	—	—	—	—
Flagellate One	**680**	**1**	**4**	**3**	**8**
Flagellate 5	2,120	1	—	—	1
Flagellate 22	8,800	—	—	1	1
Flagellate J	2,640	1	1	—	2
Flagellate A	1,600	—	—	—	—
Lancelot I Clone 1	1,760	—	1	—	1
Lancelot I Clone 5	830	—	3	—	3
Lancelot I Clone on 19/5	1,240	—	1	—	1
Lancelot II Clone 10	2,920	—	—	2	2
L 4 (13/4) 2L Clone 6	2,520	—	—	1	1
Flagellate 6	820	—	—	—	—
Flagellate 6*a*	3,840	—	—	—	—
Hemiselmis rufescens]	3,440	—	—	—	—
Flagellate 23	220	—	—	—	—
Flagellate 23*a*	36	—	—	2	2
Flagellate 14	4,320	—	—	1	1
Flagellate 14	2,860	—	1	—	1
Flagellate 16	555	—	—	—	—
Flagellate 19	420	—	—	1	1
Flagellate 20	230	1	1	—	2
Flagellate 21	135	—	—	—	—
Flagellate 29	500	—	—	—	—
Lancelot II No. 16 Clone 3	300	1	—	—	1
Lancelot II No. 16 Clone 3	250	—	—	—	—
Gross's μ flagellate	480	—	—	—	—
L 4 (13/4) 2L Clone 5	255	1	—	2	3
Exuviaella baltica	100	1	1	2	4
Exuviaella I	9	1	1	1	3
Prorocentrum micans	14	—	1	1	2
Prorocentrum triestinum	62	—	—	—	—
Flagellate 12	**220**	—	—	**8**	**8**
Oxyrrhis marina, etc.	2	—	1·5	1·5	3*
Gymnodinium I	**310**	—	**1**	**5**	**6**
Gymnodinium I	**200**	**3**	**3**	**2**	**8**
Gymnodinium II	**82**	—	**9**	**3**	**12**
Massartia rotundata	28	—	—	2	2
Peridinium trochoidium	50	—	1	—	1*
Chlamydomonas III	1,000	—	—	1	1
Platymonas apiculata	11	—	—	—	—
Pyramimonas sp.	235	—	—	—	—

TABLE 1 (*continued*)

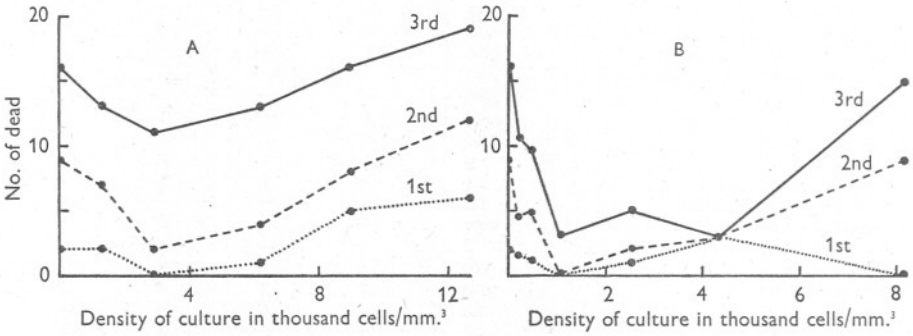
Culture	Concentration (cells/mm.^{3})	Number out of 12 dead in 1st 24 hr.	2nd 24 hr.	3rd 24 hr.	Total
Chlorella stigmatophora	**13,120**	**5**	**2**	**4**	**11**
Stichococcus sp.	570	—	—	—	—
Coscinodiscus concinnus	28†	—	—	—	—
Skeletonema costatum	35	—	—	—	—
Thalassiosira gravida	18	1	—	0·5	1·5*
Lauderia borealis	65	—	3	—	3
Ditylum brightwellii	92†	—	—	—	—
Eucampia zoodiacus	8	—	—	1	1
Chaetoceros decipiens	53	—	—	—	—
Licmophora lyngbyei	275	1	—	—	1
Naviculoid	2	—	—	1	1
Nitzschia closterium (min.)	*c.* 400	0·5	1·5	2	4*
Nitzschia closterium (normal)	73	1	—	—	1
Nitzschia seriata	12	—	—	—	—
Filtered water	—	1	8	3	12
Filtered water	—	1	—	4	5
Filtered water	—	—	2	3	5
Filtered water	—	—	2	2	4
Filtered water	—	2	3	2	7
Filtered water	—	—	—	1	1
Filtered water	—	1	1	6	8
Filtered water	—	—	6	—	6
Filtered water	—	1	—	—	1
Filtered water	—	—	2	2	4
Mean of filtered results	—	0·6	2·4	2·3	5·3

Note. The above organisms are in classificatory order as in Appendix I and not in the order of testing. Species considered toxic have been put in heavy type.

* Calculated from a total of 25.
† Cells/100 mm.^{3}.

Summary showing numbers of cultures resulting in various deaths:

No. of deaths	—	1	2	3	4	5	6	7	8	9	10	11	12
No. of cultures	24	20	12	6	2	1	1	—	3	—	—	1	1



Text-fig. 1. Results of toxicity experiments with cultures of various concentrations of (A) *Chlorella stigmatophora*, (B) *Nitzschia closterium*. Numbers of dead, out of thirty-two in A and thirty-six in B, on the 1st, 2nd and 3rd days.

and the figures expressed as numbers dead per thirty-two animals after 1, 2 and 3 days. It is seen that there is an optimum survival at a concentration of about 3000 cells/mm.^{3}. Below this the increase in deaths may be assumed due to starvation and above it to the toxic effect.

Lucas (1936) has put forward evidence for an optimum survival in *Nitzschia closterium* cultures of a certain strength, and an attempt was made to demonstrate this with *Hemimysis lamornae*. The results were treated as those for *Chlorella* and are depicted in Text-fig. 1 B. It is seen that survival is generally much better than with *Chlorella*, except at the lowest concentrations, but that again there is an optimum at a certain concentration, this time about 1000 cells/mm.^{3}. (This figure is higher than that found by Lucas who considered the optimum to lie between 25 and 100 cells/mm.^{3}, but he used *Neomysis vulgaris* and the two species may well react differently.) It may again be assumed that there is some sort of toxic effect at higher concentrations. This was not recognized in the earlier 'toxicity' series of experiments because a sufficiently high concentration of cells was not used. Such a concentration would not generally be met with in nature so the result must largely be of academic interest except, perhaps, in so far as it is an indication of what may, in lesser degree, be happening at lower concentrations and not detectable by this experimental method.

Treatment of the Results

In some of the experiments the behaviour of the animals in relation to the uneven distribution of phytoplankton in the straight or in the circular horizontal apparatus is very distinctive, but in general it is necessary to apply an appropriate statistical technique in order to determine which differences in the distribution of the animals are significant.

In the straight apparatus, for any given reading, this involves calculation of the deviation for the numbers in the left- and right-hand sides of the tube. The reading is taken to be significant if this exceeds twice the value of σ calculated for the particular number of animals in the tube. For the purpose of this work a significant difference is termed positive or negative depending upon whether it involves a movement into the phytoplankton-rich side of the apparatus or away from it.

It is not possible to apply the test for significance to more than one reading in a particular experiment and then attribute an independent and equal value to each answer, because each successive reading is to some extent dependent for its value upon those preceding it in the series. This invalidates the test used. The latter is therefore only applied to one reading in an experiment or only once to the mean of several readings. The single reading or the selection of readings averaged may be taken arbitrarily or may be chosen as typical of the experiment as a whole.

In the circular experiments the results are treated similarly. The numbers in the six sections counted cannot however be divided symmetrically about the third of the tube containing the enriched water. The number in the phytoplankton third is therefore compared with the number in the third opposite it across the circle and the numbers in the two sixths between these sectors are disregarded. This is a more rigorous test than the simple comparison of halves and in some border-line cases special counts were made of the two halves. The dividing line was then that diameter at right angles to the one passing symmetrically through the phytoplankton third. This special count was most valuable when there had been more than the normal spreading of the phytoplankton sector and parts of the disregarded sixths were rich in both phytoplankton and zooplankton.

In the vertical experiments such changes as occur in the vertical migrational behaviour in the two tubes have again to be treated mathematically in order to determine whether they differ significantly from expected chance variations. The test employed in this case is a test of independence and is applicable because the data fit a two by two table. The factor χ^2 is calculated for the reading to be tested and if it exceeds 3·841 then the difference in distribution in the two tubes is taken as significant. Again only one reading or the mean of several readings may be tested in this manner.

If, as in most of the experiments, the difference between the numbers swimming up in the pair of tubes is too small to satisfy this test for significance, it is possible to lump together the single readings from each one of a series of comparable experiments and perform the test on these larger figures. Reliance can only be placed on this 'pooled result' if the data are shown independently to satisfy a test for homogeneity.

EXPERIMENTAL RESULTS

The Horizontal Apparatus

The results of three typical experiments in the straight horizontal apparatus are given in Tables II–IV. The first comprises the first day's readings in a control experiment having both tubes uniformly filled with water freshly taken from the Plymouth Laboratory circulation ('tank water') and with ten *Hemimysis lamornae* introduced into each side of both tubes at 12.00 hr. It is seen that the distribution remains fairly uniform, and although two readings (12.30 and 13.00 hr. in tube II) show a significant departure from normality this is not maintained. Such significant clumpings in a uniform tube are referred to later.

Table III gives the readings in an experiment with water rich in the flagellate *Rhodomonas* sp. in the left-hand side of one tube and ultrafiltered water in the right-hand side; and *Rhodomonas* in the right-hand side of the other and ultrafiltered water in the left. Ten *Hemimysis lamornae*, previously

fed on diatoms and flagellates, were introduced into each side of both tubes at 10.15 hr. An immediate movement away from the flagellates occurred and this was maintained for the duration of the experiment.

TABLE II. EXPERIMENT DHT 30 (11 March 1952)

(Two horizontal tubes set up as described. Both completely filled with fresh tank water. Ten *Hemimysis lamornae* put in each side of both. Just taken from the aquarium tanks.)

	I				II			
Time (hr.)	A Normal	B Normal	C Normal	D Normal	A Normal	B Normal	C Normal	D Normal
12.00	—	10	10	—	—	10	10	—
12.30	8	1	1	10	4	1	2	13
13.00	7	1	1	11	5	—	4	11
14.30	8	2	2	8	8	2	3	7
16.00	8	1	3	8	12	—	1	7
17.30	8	2	3	7	11	4	1	5
19.00	8	3	2	7	10	4	2	4
20.30	—	12	7	1	1	11	7	1

TABLE III. EXPERIMENT DHT 31 (13 March 1952)

(Two horizontal tubes set up as described. I, *Rhodomonas* sp. culture in L.H.S. Ultrafiltered water in R.H.S. II, ultrafiltered water in L.H.S. *Rhodomonas* in R.H.S. Ten *Hemimysis lamornae* put in each side of both. Previously fed on a mixture of diatoms and flagellates.)

	I				II			
Time (hr.)	A *Rhodomonas*	B *Rhodomonas*	C Normal	D Normal	A Normal	B Normal	C *Rhodomonas*	D *Rhodomonas*
10.15	—	10	10	—	—	10	10	—
10.30	1	—	7	12	10	7	3	—
11.00	1	—	7	12	14	4	2	—
11.30	1	—	5	14	15	3	2	—
13.00	1	—	7	12	13	5	1	1
14.30	1	1	7	11	12	6	2	—

TABLE IV. EXPERIMENT DHT 32 (14 March 1952)

(Two horizontal tubes set up as described. I, *Thalassiosira gravida* culture in L.H.S. Ultrafiltered water in R.H.S. II, ultrafiltered water in L.H.S. *Thalassiosira* culture in R.H.S. Ten *Praunus neglectus* put in each side of both. Previously fed on a mixture of diatoms and flagellates.)

	I				II			
Time (hr.)	A Diatom	B Diatom	C Normal	D Normal	A Normal	B Normal	C Diatom	D Diatom
11.00	—	10	10	—	—	10	10	—
11.30	9	5	1	5	7	2	2	9
12.00	11	5	1	3	5	2	3	10
13.00	10	6	1	3	3	1	7	9
14.30	11	7	—	2	—	—	10	10
16.00	13	5	1	1	—	—	8	11

Table IV gives the readings in an experiment with water enriched with the diatom *Thalassiosira gravida* in one side of each of the two tubes, and filtered

water in the other, and with ten *Praunus neglectus* put in each side. A marked migration is again apparent, but in this case into the enriched water and rather slower in occurrence. All but the first reading in tube I and the first two in tube II are significant.

TABLE V. EXPERIMENT HCT 4 (16 October 1951)

(Horizontal circular tube set up as described. Completely filled with fresh tank water. Twelve *Hemimysis lamornae* put in at each junction. Previously fed on *Peridinium trochoidium*.)

	A	B	C	D	E	F
Time (hr.)	Normal		Normal		Normal	
12.30	6	12		12		6
12.45	4	4	7	8	8	5
13.00	6	2	8	7	8	5
14.15	3	2	7	7	11	6
16.00	3	2	11	7	8	5
16.30	2	3	12	6	8	5
17.00	3	5	8	6	9	5
17.45	4	3	11	5	7	6
18.30	1	5	10	4	11	5
19.00	2	6	8	6	8	6
20.45	3	4	3	7	11	8
21.30	7	4	5	6	6	8

TABLE VI. EXPERIMENT HCT 3 (15 October 1951)

(Horizontal circular tube set up as described. Water rich in *Peridinium trochoidium* in one third. Outside water in the rest. Twelve *Hemimysis* put in at each junction. Starved in filtered water for the previous 4½ hr.)

	A	B	C	D	E	F
Time (hr.)	Normal		Flagellate		Normal	
15.00	6	12		12		6
15.15	2	3	10	12	5	4
15.30	3	3	11	11	4	4
16.00	3	4	7	13	7	2
16.30	1	2	9	14	7	3
17.00	1	5	4	19	6	1
17.30	1	4	5	17	5	4
18.00	—	4	8	14	6	4
18.30	2	3	7	16	4	4
19.00	4	3	9	12	6	2
22.45	4	3	5	10	7	7

For comparison with these three experiments, the results of two performed in the horizontal circular tube are given in Tables V and VI. The first is a control experiment with the tube completely filled with tank water and twelve *Hemimysis lamornae* put in at the three entrances; and the second has one-third filled with water enriched with *Peridinium trochoidium* culture and the *Hemimysis* used had previously been starved in ultrafiltered water. Distribution in the control experiment in relation to sector CD departs significantly from normality in only one reading, that of 16.30 hr. In the other, however,

a clear movement into the enriched water sector CD takes place at once and is maintained for the greater part of the experiment.

These, and the remaining horizontal results, have been treated mathematically and the experiments put into one of the three categories—*positive*, *negative*, or *not significant*—depending upon whether a significant migration into, or away from, the phytoplankton takes place, or whether there is no significant departure from an even distribution. The experiments are classified first according to the animals used, and secondly, according to the phytoplankton organisms or the type of water. They are recorded in the following tables, together with details of the concentration of phytoplankton put in the one side of the apparatus and the state of the animal. This latter is taken as either starved or fed dependent upon whether the animals had been kept for some time in filtered water, or had been fed or just recently caught (when food is generally to be found in the gut).

The tables may best be considered according to the animals used and *Hemimysis lamornae*, with which the greatest number of experiments was performed, is taken first.

Hemimysis lamornae

Experiments involving Diatoms

Amongst the diatoms, clear migrations into water enriched with *Skeletonema costatum*, *Thalassiosira gravida*, *Biddulphia sinensis*, *Nitzschia closterium* and a mixed culture of diatoms were obtained. There were no negative migrations amongst these species but out of nineteen experiments performed five gave no significant result. The distribution of the positive results between fed and starved animals suggests a more marked migration by the latter, but the results do not satisfy a mathematical test for significance. Exp. DHT 51 has been disregarded, as later work showed animals to move away from ordinary water passed through the Berkefeld filter candle employed here. This is considered later (p. 407). Five experiments were performed with *Lauderia borealis*, of which three proved not significant, one positive and one just negative (four significant negative readings out of a total of eight). *Lauderia* was not used in any other experiments, but it may be that it is not particularly acceptable to *Hemimysis*. There was no special indication of a lethal effect in the 'toxicity experiments'. *Coscinodiscus concinnus* also gave two not significant results, but more experiments would need to be performed before it could be said not to be acceptable to the animals. Excluding these two doubtful species, the summarized results with diatoms reveal a substantial majority of experiments showing a positive migration. Even including them there is only one negative migration as compared with fifteen positive in a total of twenty-six.

Two of the experiments with *Skeletonema* and two with *Thalassiosira* utilized water from a growing culture which was centrifuged in order to

remove the bulk of the cells. Positive migrations were still obtained in three of these four cases suggesting that the attractive principle may reside in the water itself.

TABLE VII. *HEMIMYSIS LAMORNAE* AND DIATOMS

(In this and the subsequent tables the four columns represent: (i) the serial number of the experiment; (ii) the concentration, in cells/mm.^{3}, of the phytoplankton used; (iii) the state of the animal, F representing fed and S starved; (iv) the result of the experiment as a whole. If there should be insufficient significant readings to merit designating the whole experiment such, or if only a minimum number should be so, then this may be noted as e.g. (3 rds +ve) meaning three significant positive readings.) N.S.: not significant.

Skeletonema costatum

DHT 22	10	F	+ve	DHT 68	Centrifuged	F	+ve
...	10	F	N.S.	...	Centrifuged	F	+ve
DHT 23	10	S	+ve	[DHT 51	Filtered	F	−ve]
...	10	S	+ve	[...	Filtered	F	−ve]

Thalassiosira gravida

DHT 24	147	F	N.S.	HCT 18	55	S	+ve
...	147	F	N.S.	DHT 70	Centrifuged	F	N.S.
HCT 16	90	S	+ve	...	Centrifuged	F	+ve

Lauderia borealis

DHT 52	21	S	N.S.	DHT 55	2¼	S	N.S.
...	21	S	−ve*	...	2¼	S	N.S.
HCT 1	20/ml.	F	+ve				

* 4 rds.

Biddulphia sinensis

P 63	275	F	+ve	P 61	220	S	+ve
P 62	220	F	N.S.*	P 60	220	S	+ve

* In dark.

Coscinodiscus concinnus

2P 5	28/ml.	F	N.S.	2P 6	28/ml.	S	N.S.

Mixed diatoms

HCT 48	700	F	+ve	HCT 49	350	F	+ve *

* Halves.

Nitzschia closterium

P 64	3500	S	+ve

Summary distribution of results with *H. lamornae* and diatoms

	All species			Excluding *Lauderia* and *Coscinodiscus*			
	+ve	N.S.	−ve		+ve	N.S.	−ve
Fed	8	6	—	Fed	7	5	—
Starved	7	4	1	Starved	7	—	—

Experiments involving Flagellates

The independent evidence of a possible toxic action by some of the flagellates used is taken as the basis for a division of the remaining results involving phytoplankton and *Hemimysis* into two groups—those involving 'toxic flagellates' and those involving 'non-toxic flagellates'. The latter will be considered first.

As seen in Table VIII, significant migrations were obtained into concentrations of *Chlamydomonas* sp., *Peridinium trochoidium*, *Pyramimonas* sp., *Dicrateria inornata* and Flagellate K. Again a greater proportion of positive results was obtained with starved than with fed *Hemimysis*, but not significantly so. There was a greater number of not significant results even with the

TABLE VIII. *HEMIMYSIS LAMORNAE* AND 'NON-TOXIC FLAGELLATES'

			Chlamydomonas sp.				
2P 11	520	F	N.S.	DHT 46	42	F	N.S.
HCT 25	225	F	N.S.	...	42	F	N.S.
P 30	170	F	N.S.	DHT 48	30	F	N.S.
DHT 49	113	F	N.S.	...	30	F	N.S.
...	113	F	N.S.	2P 12	520	S	+ve
DHT 45	105	F	+ve	HCT 24	250	S	+ve
...	105	F	+ve	HCT 21	235	S	+ve
DHT 50	77	F	N.S.	P 29	170	S	+ve
...	77	F	N.S.	HCT 72	120	S	N.S.
DHT 47	62	F	N.S.	DHT 53	60	S	N.S.
...	62	F	N.S.		60	S	N.S.
			Peridinium trochoidium				
DHT 1	53*	F	+ve	HCT 3	18	S	+ve
DHT 2	28*	F	+ve	HCT 5	18	S	N.S.
...	28*	S	N.S.				
			* Mixed with *P. micans* and *P. triestinum*.				
			Pyramimonas sp.				
DHT 41	139	F	+ve	DHT 42	139	S	N.S.
...	139	F	+ve	...	139	S	N.S.
			Syracosphaera carterae				
HCT 51	68	F	N.S.	HCT 19	77	S	N.S.
HCT 70	8	F	N.S.	HCT 20	70	S	N.S.
			Exuviaella baltica				
HCT 40	2	F	N.S.	DHT 21	31	S	N.S.
			Dicrateria inornata				
2P 4	2760	F	+ve	2P 3	2040	S	+ve
2P 2	3130	S	+ve				
			Flagellate K				
HCT 57	230	F	+ve*	HCT 71	10	S	N.S.†
	* 4 rds.				† 3 rds −ve.		

Summary distribution of results with *H. lamornae* and non-toxic flagellates

	All species				Excluding *Syracosphaera* and *Exuviaella*		
	+ve	N.S.	−ve		+ve	N.S.	−ve
Fed	8	16	—	Fed	8	13	—
Starved	7	11	—	Starved	7	8	—

starved animals, and the totals are fifteen positive results to twenty-one not significant. Four experiments with *Syracosphaera carterae* and two with *Exuviaella baltica* all gave not significant results. A 'toxicity experiment' was not performed with *Syracosphaera* but there was no indication of a harmful

effect in the horizontal tubes; one performed with *Exuviaella* revealed no effect. It may be that these two forms fall into the same 'unacceptable' category as

TABLE IX. *HEMIMYSIS LAMORNAE* AND 'TOXIC FLAGELLATES'

Gymnodinium II

HCT 58	54	F	N.S.	DHT 69	Centrifuged	F	−ve
HCT 50	40	F	N.S.*	...	Centrifuged	F	−ve
HCT 47	14	F	+ve	DHT 71	Cent. (dil.)	F	−ve
HCT 46	7	F	N.S.	...	Cent. (dil.)	F	N.S.
[DHT 37	Filtered	F	−ve]	DHT 35	63	S	N.S.‡
...	Filtered	F	N.S.†]	...	63	S	N.S.
				2P 20	—	S	N.S.

* *Gymno.* I. † 2 rds −ve. ‡ 3 rds −ve.

Flagellate 12

DHT 18	98	F	N.S.*	[DHT 36	Filtered	F	−ve]
...	98	F	N.S.	...	Filtered	F	N.S.
HCT 53	37	F	−ve	DHT 19	98	S	N.S.
HCT 52	18	F	N.S.	...	98	S	+ve
HCT 37	6	F	N.S.	DHT 20	98	S	N.S.
DHT 64	Centrifuged	F	N.S.	HCT 66	29	S	N.S.
...	Centrifuged	F	+ve				

* 3 rds −ve.

Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*

HCT 31	20·5	F	+ve	DHT 20	80	S	N.S.
HCT 56	16·0	F	N.S.	DHT 25	60	S	−ve
HCT 35	15·0	F	N.S.	...	60	S	−ve
HCT 33	12·0	F	N.S.	2P 10	*c.* 4·0	S	+ve
P 33	11·0	F	N.S.	DHT 26	40	F	−ve
P 69	7·0	F	N.S.*	...	40	F	−ve
HCT 55	6·5	F	N.S.	DHT 27	23	F	−ve
HCT 54	5·5	F	N.S.	DHT 28	Aerated	F	N.S.†
2P 9	*c.* 4·0	F	N.S.	DHT 29	65	F	+ve‡

* +ve tend. † −ve finally. ‡ No *Rhodomonas*.

Rhodomonas only (*Oxyrrhis* filtered off)

DHT 29	200	F	−ve*	DHT 56	1	S	−ve
DHT 31	100	F	−ve	...	1	S	−ve
...	100	F	−ve	[DHT 27	Filtered	F	−ve]

* 4 rds.

Chlorella stigmatophora

DHT 33	3580	F	+ve	DHT 34	3800	S	+ve
...	3580	F	N.S.	...	3800	S	N.S.

Summary distribution of results with *H. lamornae* and toxic flagellates (excluding *Chlorella*)

	+ve	N.S.	−ve
Fed	3	17	12
Starved	2	7	4

Lauderia and *Coscinodiscus*. In none of this group of experiments was any negative migration obtained and, although there are more not significant results than positive, the positive bias is still most marked.

The 'toxic flagellate' group comprises the two cultures of *Gymnodinium* (I and II) and Flagellate 12, all showing lethal effects in the toxic experiments; and the mixed culture of *Oxyrrhis marina*, *Rhodomonas* sp. and *Nitzschia closterium* which, although not showing a toxic effect in the weak culture used in the test experiments, afterwards showed enough harmful effects in the horizontal tubes to merit its inclusion here. The position of *Chlorella* is anomalous both on account of its being a non-motile chrysophycaean and because the experiments performed with it were at the optimum concentration which showed little toxic effect. The results with it are therefore considered separately.

All but one of the *Gymnodinium* experiments involved *Gymnodinium* II, the species showing the more marked toxic effect. Out of eleven experiments one gave a positive result (with fed animals) and three gave negative results. The remainder were all not significant. The positive result was obtained with a low concentration of cells and the negative results with water from a growing culture which had been centrifuged to remove most of the cells. The centrifugate used in DHT 71, where only one of a pair was significantly negative, was previously diluted with ordinary water. The inference may be made that the repellent principle is situated in the water in which the flagellate has grown.

Of eleven results with Flagellate 12 the majority again are not significant. One is negative, involving fed animals; and two positive, one normal with starved animals and one with centrifuged water and fed animals. The latter would seem opposed to the idea of a toxic or repellent principle in the water, and in fact the form of the gut in most specimens (which fed freely) suggested some internal effect, but the evidence is rather tenuous. Unfortunately, the only cultures of Flagellate 12, which were at Plymouth, Millport and Bangor, died out at about the same time in the spring of 1952. The results will, however, be followed up if it proves possible to re-establish a culture from the sea. This organism is of particular interest as it is thought to be a primitive dinoflagellate, a group which embraces the gymnodiniums, *Oxyrrhis* and other forms such as *Goniaulax*, which are known to have toxic properties.

Three positive results were obtained with the *Oxyrrhis*, *Rhodomonas* and *Nitzschia* culture, but ten negative ones: all out of a total of twenty-three. *Oxyrrhis* is not an autotrophic flagellate and can only be grown if supplied with food. This particular culture provides a large *Nitzschia* and the autotrophic *Rhodomonas* as possible food and is of long standing. The *Nitzschia* content fluctuates a great deal but is generally low, while most *Rhodomonas* cells clump into aggregations about the size of a small tea leaf, and the *Oxyrrhis* swims freely. The culture was used just as grown in the early experiments when both positive and negative results were obtained. These led to an attempt to separate the component parts in order to assess their individual effects, and this was found to be relatively easily done by filtering

through fine silk. A growth was obtained with a low *Nitzschia* content, and after being left quiescent for some days to ensure a maximum clumping of the *Rhodomonas*, was passed through fine silk. Almost pure *Oxyrrhis* passed through and the *Rhodomonas* clumps were left on the silk. They were washed with culture solution and then resuspended and shaken vigorously in filtered sea water. This treatment gave a suspension containing a good many free cells. Consistent and very marked migrations away from such a suspension were obtained, while the almost pure *Oxyrrhis* gave a positive result. It seems clear that the variable results with the mixed culture derived from the opposing reactions to the *Oxyrrhis* and the *Nitzschia* on the one hand and to the *Rhodomonas* on the other. The two positive migrations with the mixed culture were both into samples with a low *Rhodomonas* content as compared with the *Oxyrrhis*.

The summary of results with the toxic flagellates shows the preponderance of negative migrations as compared with positive. These result largely from the effect of *Rhodomonas* and *Gymnodinium* II, both of which may be considered to be avoided because of their harmful properties.

Four experiments were performed with *Chlorella stigmatophora* at its optimum concentration for survival, two with fed *Hemimysis* and two with starved. One of each of these pairs showed a significant positive migration, the other being not significant.

Miscellaneous Experiments

An earlier experiment (HCT 9) used small zooplankton animals in place of phytoplankton. Some of these died and a concentration of bacteria quickly formed in the tube and was apparently avoided by the *Praunus neglectus* being tested. This led to a closer study of the effect of water rich in bacteria.

TABLE X. *HEMIMYSIS LAMORNAE* AND BACTERIA

DHT 3	Normal bacteria	F	−ve	DHT 8	Filtered	—	−ve
...	Normal bacteria	F	−ve	...	Filtered	—	−ve
DHT 9	Normal bacteria	—	−ve	DHT 4	Aerated (60 min.)	—	N.S.
DHT 14	Normal bacteria	—	N.S.*	...	Aerated (60 min.)	—	N.S.
DHT 7	Diluted to 50%	—	−ve	DHT 9	Aerated (5 min.)	—	−ve
...	Diluted to 50%	—	−ve	DHT 14	Aerated (45 min.)	—	−ve
DHT 65	Diluted to 25%	F	−ve	DHT 16	Aerated (45 min.)	—	N.S.†
...	Diluted to 25%	F	−ve	DHT 67	N_2 bubbled	F	N.S.
DHT 6	Diluted to 10%	F	N.S.	...	N_2 bubbled	F	N.S.
...	Diluted to 10%	—	N.S.	DHT 17	Bubbled over from bacteria	—	N.S.

* All dead in bacteria.

† Using new diffuser.

A suspension of bacteria was grown by leaving some small dead animal, such as a prawn, in a breffit of raw sea water for about 24 hr. This gave rise to a slightly cloudy water with a distinctive but not overpowering smell. No

attempt was made to identify the species producing this putrefaction, but on separate occasions the method of growth gave a culture consistent in appearance and presumably in composition, although it appeared at times more potent than at others.

As is shown in Table X this type of culture produced a marked and sustained avoiding reaction in the *Hemimysis* used. On occasions, as in DHT 14, when the culture was especially strong, deaths might even be caused. The negative migration persisted when the culture was diluted first to 50% strength and then to 25%; but when diluted to 10% no significant result was obtained. In an attempt to define more precisely the agent inducing this reaction a culture was passed through an ultra-filter, when a negative migration was still obtained. If a culture was aerated for 60 min. before use, however, no significant result was obtained, although aeration for a shorter time did not seem adequate to remove the noxious agent, until a much more efficient diffuser and method of bubbling were adopted. The destruction or removal of the material inducing the avoidance by bubbling with air, left the possibility of its being oxidized or perhaps carried away in the stream of escaping bubbles. A culture was therefore bubbled with nitrogen and then tested in the apparatus (DHT 67), when no significant result was obtained. It may thus be concluded that the substance avoided is a volatile, water-soluble metabolic or decomposition product of the bacteria. In the light of this conclusion an attempt was made to remove the substance from a culture by bubbling and to reabsorb it in filtered water which could then be used in the apparatus. No significant movement in relation to such water was demonstrated.

With the above evidence that some of the migrations in relation to bacteria and phytoplankton were perhaps mediated by substances dissolved in the water, attempts were made to determine the reactions of *Hemimysis* to one or two simple substances.

The first of these tested was carbon dioxide. Sea water was saturated with carbon dioxide by bubbling with gas from a cylinder (and washed before use). When such saturated water was put into the apparatus and animals introduced, those going into the carbon-dioxide-rich side went immediately into convulsions and died within a matter of seconds without being able to escape to the other side of the apparatus. Those animals put into the other side made no attempt to move into the carbon-dioxide-rich water. When the saturated water was diluted down to 10% with normal water and the animals introduced, then a rapid and sustained negative migration took place without any deaths. An equally clear reaction was got with a 5% dilution, but not with 1 and $\frac{1}{2}$%, when no significant movement resulted. The pH of the $\frac{1}{2}$% dilution was about 8·0, which is similar to that of the bacteria-rich water. The pH of the 5% dilution was about 7·2.

The second series tested migration in relation to degree of aeration. One

sample of water was supersaturated by shaking vigorously, and another deaerated by subjecting to the reduced pressure of a powerful filter-pump together with shaking. When these two lots of treated water were put into opposite sides of the apparatus and animals introduced, then no significant departure from a uniform distribution was obtained. Similar results obtained with water deaerated by this means in one side and normal water in the other. If the water was deoxygenated by bubbling with washed nitrogen from a cylinder for 45 min., then animals going into that side of the apparatus died within minutes but made no attempt to escape to the normal side. There were in this case no convulsions, and death was much slower than with

TABLE XI. *HEMIMYSIS LAMORNAE* AND MISCELLANEOUS SUBSTANCES

Carbon-dioxide-rich water

DHT 5	10% saturated	—	−ve	DHT 13	1% saturated	—	N.S.
DHT 11	5% saturated	—	−ve	DHT 12	½% saturated	—	N.S.
...	5% saturated	—	−ve	...	½% saturated	—	N.S.
DHT 13	1% saturated	—	N.S.				

Aerated and deaerated water

DHT 10	Aerated* and deaerated†	—	N.S.	DHT 61	28% N_2 bubbled	—	N.S.
...	Aerated* and deaerated†	—	N.S.	...	28% N_2 bubbled	—	N.S.
DHT 15	Normal and deaerated†	—	N.S.	DHT 66	25% of 20 min. N_2 bubbled	—	N.S.
...	Normal and deaerated†	—	N.S.	...	25% of 20 min. N_2 bubbled	—	N.S.
DHT 60	N_2 bubbled 45 mins.	—	N.S.‡				
...	N_2 bubbled 45 mins.	—	N.S.‡				

* Shaking. † Reduced pressure. ‡ Animals died at once.

Ammonia-rich water

DHT 62	6 drops of 10% NH_3/litre	F	+ve	DHT 63	12 drops of 10% NH_3/litre	F	+ve
...	6 drops of 10% NH_3/litre	F	+ve	...	12 drops of 10% NH_3/litre	F	+ve

Filter etc.

DHT 54	Mysid inhabited water	F	N.S.	DHT 58	Berkefeld filtered water	F	−ve
...	Mysid inhabited water	F	N.S.	...	Berkefeld filtered water	F	−ve
DHT 57	Berkefeld filtered water	F	N.S.	DHT 59	Membrane filtered water	S	N.S.
...	Berkefeld filtered water	F	−ve	...	Membrane filtered water	S	N.S.

carbon dioxide. All movement ceased almost at once and the animals lay on their sides and gradually became opaque. If they were removed within about 5 min. and put into well-aerated water, then a good proportion recovered completely. The nitrogen bubbled water was diluted down to 28%; and water bubbled for 20 min. was diluted down to 25% with normal water but no avoiding reaction towards either could be demonstrated, while no further deaths took place at these concentrations.

The possibility of ammonia being one of the bacterial decomposition products led to its being tested in the apparatus. 0·880 ammonia was diluted to 10% with sea water and six drops of this solution were added per litre of sea water, giving a pH of just over 8·8. This water was placed in one side of

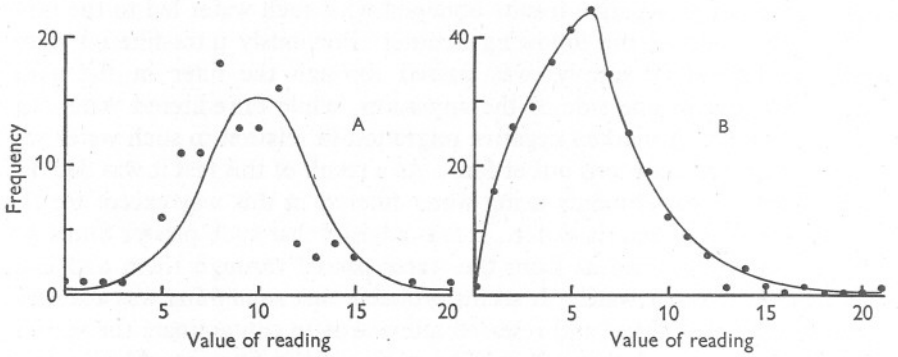
the apparatus and normal water in the other. The *Hemimysis* introduced moved immediately into the ammonia concentration and remained there. A precisely similar result was obtained using double the concentration of ammonia (12 drops per litre).

Miscellaneous tests included one on water in which mysids had lived for some time. No significant migration in relation to this was obtained. The experiments referred to earlier as involving filtered water from various cultures, used water passed through a coarse Berkefeld plaster filter candle. The water was drawn through by suction with a siphon about 5 ft. long. The high proportion of negative results obtained with such water led to the filter itself being tested in the following manner. Previously ultra-filtered water (from the laboratory supply) was passed through the filter in the usual manner and put in one side of the apparatus, while unrefiltered water was put in the other. A marked negative migration in relation to such water was obtained on three occasions out of four. As a result of this test it was decided to disregard the experiments using water filtered in this way except for the first one involving bacteria water. It was advised that such plaster filters are liable to absorb substances from the water passed through them and later give them up to other water. It seems probable that something was absorbed from the bacteria culture and released afterwards to contaminate the various types of water passed through it. The pores of the filter would also hold organic matter of various kinds from the diatoms, etc., and this could decompose and then contaminate other water. Water filtered through a membrane filter was tested and found to induce no significant migration. It did not prove practicable, however, to use this method for filtering cultures, as it was extremely slow. It was finally decided to use the centrifuging method already referred to in order to remove cells and leave the culture otherwise unaffected.

Besides the above experiments involving cultures and chemical substances, fourteen control experiments were performed with the apparatus uniformly filled with water of some kind. None of these resulted in a whole experiment being termed significant but occasionally, as already mentioned in DHT 30 and in HCT 4, a temporary distribution differing significantly from a uniform one was obtained. The frequency with which each particular reading occurred in the nine experiments done in the straight horizontal apparatus is shown in Text-fig. 2A. The form of the curve drawn through the points is typically that which would be expected were the distribution of the animals in the tube random. Such significant departures from a uniform distribution as do occur in the control experiments could perhaps be a manifestation of the shoaling habit which is common in these animals, but this tendency is not marked enough to appear in the figure. The second curve (Text-fig. 2B) shows the frequency with which each particular reading occurred in three control experiments done in the circular horizontal tube. This includes all the

readings for each of the six sectors, and is asymmetrical because the one or two high readings (to be expected by chance) each necessarily result in there being several low readings, since the remaining few animals have to be distributed amongst the other five sectors.

It may therefore be concluded from the control experiments that in the uniform tubes there is no tendency to aggregate in any particular section, and those significant departures from an even distribution which are found in the remainder of the experiments are due to the environmental conditions in the tubes.



Text-fig. 2. Summary of control experiments with *Hemimysis lamornae*. A, frequency of occurrence of readings in Exps. DHT 1, 30, 38, 39 and 40. B, the same in Exps. HCT 2, 4 and 17.

Other mysids

Experiments were performed with four other species of mysid—*Praunus neglectus*, *P. flexuosus*, *Neomysis integer* and *Mesopodopsis slabberi*. The behaviour of these species appearing to be similar, and rather few experiments having been done with each, it seems most convenient to consider all the results together.

Experiments involving Diatoms

As seen from the summary distribution of results (Table XII), out of twenty-four experiments involving various diatoms twenty gave significant positive migrations and four gave no significant result. Movement was most marked into *Nitzschia closterium* (the minute Plymouth strain) and *Thalassiosira gravida*; while neither of two experiments performed with *Eucampia zoodiacus* gave a significant result. One of the experiments with *Neomysis integer* was performed in the dark and gave no significant migration until the apparatus was moved into the light. The distribution then became positive. In one of the experiments with *Neomysis* the diatom culture was deoxygenated before use by subjecting it to reduced pressure combined with shaking and in another the diatoms were killed by heating to 80° C., washed on a filter and

TABLE XII. OTHER MYSIDS AND PHYTOPLANKTON, ETC.

(1) *Praunus neglectus*

Nitzschia closterium			
HCT 60	3000	F	+ve
HCT 65	440	F	+ve
HCT 63	2300	S	+ve*
Thalassiosira gravida			
DHT 32	115	F	+ve
...	115	F	+ve
Chlorella stigmatophora			
HCT 43	1210	F	N.S.
HCT 61	1840	S	+ve†
Eucampia zoodiacus			
HCT 69	1½	F	N.S.
Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*			
HCT 32	11	F	+ve
HCT 34	7	F	N.S.
HCT 62	34	S	+ve
HCT 64	17	S	+ve
Chlamydomonas sp.			
HCT 27	275	S	+ve
HCT 68	200	S	+ve*
Flagellate 12			
HCT 38	8	F	N.S.
HCT 41	11	S	+ve†
Flagellate L4 (13/4) 2L Clone 6			
HCT 44	650	F	N.S.
HCT 45	505	S	+ve
Exuviaella baltica			
HCT 42	5	F	N.S.
DHT 21	31	S	+ve
Zooplankton			
HCT 36	—	F	N.S.
HCT 28	—	S	N.S.
HCT 39	—	S	N.S.
Bacteria			
HCT 59	—	F	−ve†
Ammonia			
HCT 73	—	S	N.S.

* Halves. † 4 rds.

(2) *Praunus flexuosus*

Eucampia zoodiacus			
HCT 11	1·4	S	N.S.
Chlamydomonas sp.			
P 66	810	F	N.S.
Zooplankton			
HCT 9	—	S	−ve
Thalassiosira gravida			
HCT 13	120	S	+ve
HCT 14	100	S	N.S.
Bacteria			
HCT 12	—	F	−ve

(3) *Neomysis integer*

Nitzschia closterium			
H 9	1090	F	+ve
H 19*	1080	F	N.S.
H 25	1060	F	+ve
H 20	1060	(F)	+ve
H 27	1060†	F	+ve
H 23	1020	F	+ve
H 17	800‡	(F)	+ve
H 4	590	(F)	+ve
H 13	1600	(S)	+ve
H 10	1090	S	+ve
H 26	1060	S	+ve
H 22	1020	S	N.S.§
DHT 18*	800	S	+ve
DHT 28	Killed	S	+ve
DHT 12	400 (and alga)	(S)	+ve
Non-motile green alga			
H 14	1400	S	+ve
H 6	50	(F)	N.S.
Miscellaneous			
H 24	1060 *Nitzschia* and tinted	F	N.S.
H 5	830 *Nitzschia* and tinted	(F)	N.S.
H 7	830 *Nitzschia* and tinted	S	+ve
H 8	Filtered *Nitzschia*	(S)	N.S.
H 21	Filtered *Nitzschia*	(F)	N.S.
H 29	Kieselguhr	(S)	N.S.
H 11	Tinted water	(S)	N.S.

N.B. When the letters F and S are bracketed in this table the probable state of the animals is indicated.

* Half *N. integer* and half *Mesopodopsis slabberi*. † Culture deoxygenated.
‡ Cells resuspended. § +ve in light (performed largely in dark).

Table XII (*continued*)

Summary distribution of results with other mysids.

	Diatoms				Non-toxic flagellates		
	+ve	N.S.	−ve		+ve	N.S.	−ve
Fed	11	2	—	Fed	—	5	—
Starved	9	2	—	Starved	6	—	—

	Toxic flagellates		
	+ve	N.S.	−ve
Fed	1	2	—
Starved	3	—	—

resuspended in filtered water before use. In both these experiments marked positive migrations were still obtained. This whole series is distinctive for the high proportion of positive results, there being no negative ones and no apparent difference in behaviour between fed and starved animals.

Experiments involving Flagellates

Of eleven experiments with typically 'non-toxic' flagellates five results, all with fed animals, were not significant and six, all with starved animals, were significantly positive. These include two experiments with *Chlorella stigmatophora* rather below its optimum concentration, and two with an unidentified non-motile green alga which was probably a *Chlorella*. Of two experiments with *Exuviaella baltica*, which had induced no positive migration in *Hemimysis lamornae*, one significant positive result was obtained, this being with starved animals and a much higher concentration of cells than used previously. The other positive results were with *Chlamydomonas* and the flagellate L 4 (13/4) 2L Clone 6. Again there were no negative results.

Finally, of four experiments with the mixed *Oxyrrhis* culture and two with Flagellate 12, both in the 'toxic' group, four positive results were obtained, the two not significant ones being with fed animals. The Flagellate 12 concentrations used were lower than those used previously, and the proportions of *Rhodomonas* to *Oxyrrhis* were generally low so this perhaps may account for the absence of negative migrations amongst these experiments.

Miscellaneous Experiments

Amongst the various miscellaneous experiments reported in Table XII is the one involving *Praunus flexuosus* and a concentration of zooplankton which led to the series using bacteria. In this there was no evidence of any movement into the sector rich in zooplankton taken from a townet haul nor was there in three other similar experiments with *P. neglectus*, all of which gave not significant results. The negative movement in the *P. flexuosus* experiment must almost certainly be taken as being induced by the growth of bacteria which took place in the tube. This growth, although making the water

slightly cloudy, was not sufficient to kill any of the animals used. Two experiments with independently produced bacteria cultures confirmed the negative migration.

An experiment with the non-motile alga culture in one side and *Nitzschia* in the other showed a clear migration into the latter, despite the not unattractive nature of the alga as shown in an independent experiment. One involving a suspension of Kieselguhr (diatomaceous earth) and also six control experiments using various animals all gave no significant results, as did three with water tinted with water colours to resemble a diatom culture.

TABLE XIII. *ARTEMIA SALINA* AND DIATOMS AND FLAGELLATES

Nitzschia closterium				*Hemiselmis rufescens*			
P 42	3720	F	N.S.*	2P 19	3500	S	N.S.
2P 15	2380	F	N.S.	2P 18	2820	S	+ve
2P 14	2340	F	N.S.†				
2P 16	1800	F	N.S.‡				
DHT 44	480	F	N.S.§				
...	480	F	+ve	Flagellate 4			
DHT 43	43	F	N.S.	HCT 7	72	S	N.S.
...	43	F	+ve	HCT 8	70	S	N.S.
2P 17	1005	S	N.S.				
P 43	820	S	N.S.‖				

* −ve 2nd day.
† Poor gradient.
‡ −ve 2nd day.
§ 2 rds +ve.
‖ 3 rds +ve.

Summary distribution of results with *Artemia salina* and diatoms and flagellates.

	+ve	N.S.	−ve
Fed	2	6	—
Starved	1	5	—

Artemia salina

Fifteen experiments were performed using specimens of *Artemia salina*, the brine shrimp, which had been reared in captivity at Plymouth. Of ten of these involving *Nitzschia closterium*, two gave significant positive results (both with fed animals) and two had positive tendencies; the remainder, with the exception of two with negative tendencies on the second day, being not significant (Table XIII). Of two experiments with *Hemiselmis rufescens*, one was significantly positive and of two with Flagellate 4 both were not significant. Most of the results were thus not significant but again there were no negative results.

Decapod larvae

Twelve experiments with decapod larvae gave much more variable results. Of nine with cultures not considered toxic two positive and two negative results were obtained; and of three with the mixed *Oxyrrhis* culture, one positive, one negative and one not significant. These experiments were done with various kinds of zoea and megalopa larvae, but there did not appear to

be any correlation between the species used and the type of result obtained. There may perhaps not be enough experiments in the series for the usual trends to become apparent, but it seems more likely that a rather different type of behaviour is being encountered.

TABLE XIV. DECAPOD LARVAE AND DIATOMS AND FLAGELLATES

		Nitzschia closterium		
P 77	4400	S	N.S.	*Leander* larvae
P 26	2720	S	N.S.	Zoeas
		Thalassiosira gravida		
P 75	10	S	−ve	Zoeas
		Dicrateria inornata		
2P 7	3440	F	N.S.	*Carcinus* zoeas
2P 1	3130	F	+ve	*Carcinus* zoeas
P 74	4620	S	N.S.	Zoeas
P 16	2250	S	−ve	—
P 27	1280	S	+ve	Zoeas and megalopas
P 25	1220	S	N.S.	Zoeas
		Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*		
P 76	36	S	−ve	—
P 79	35	S	+ve	*Porcellana* zoeas
P 80	35	S	N.S.	*Porcellana* zoeas

Summary distribution of results with *Decapod larvae* and all diatoms and flagellates

	+ve	N.S.	−ve
Fed	1	1	—
Starved	2	5	3

Small copepods, etc.

The six experiments performed with small copepods of various kinds all gave significant positive migrations, one being with fed animals (Table XV). The three diatoms used in this series have all on other occasions seemed acceptable to animals, and the high incidence of positive results may perhaps depend upon this and the fact that many more animals than usual were used in each experiment so that a proportionally small movement into one side could more easily be designated significant.

Two other experiments, one with *Sagitta* sp. and the other with small medusae, are, for the sake of convenience, included in this table. No significant migration was apparent in either of them.

Calanus finmarchicus

Forty-seven experiments were performed with *Calanus finmarchicus* and various diatoms and flagellates. These, as Table XVI shows, gave very variable results. The twenty-eight with diatoms gave four positive results (all with starved animals) and three negative (one with fed and two with starved animals) and the eleven with non-toxic flagellates three positive

results (two with fed and one with starved animals); the remainder being not significant. This gives a total distribution of seven positive to twenty-nine not significant to three negative.

The results with toxic flagellates were perhaps more consistent—two negative and six not significant out of a total of eight; but considering the series with *Calanus* as a whole it would seem unwise to place any reliance upon them. Ten control experiments and one each with bacteria, oxygen and carbon dioxide all gave not significant results.

TABLE XV. SMALL COPEPODS, ETC. AND DIATOMS

		Copepoda		
		Nitzschia closterium		
H 3	800	—	+ve	*Eurytemora hirundoides*
P 68	2920	S	+ve	—
65	2840	S	+ve	*Tigriopus fulvus*
		Biddulphia sinensis		
P 72	198/ml.	S	+ve	—
78	860/ml.	F	+ve	*Tigriopus fulvus*
		Thalassiosira gravida		
P 73	10	S	+ve	—
		Chaetognatha		
		Hemiselmis rufescens		
P 18	1080	F	N.S.	*Sagitta* sp.
		Small Medusae		
		Nitzschia closterium		
P 6	800	S	N.S.	

Summary distribution of results with small copepods and diatoms

	+ve	N.S.	−ve
Fed	1	—	—
Starved	5	—	—

The most reasonable deduction to be made is that *Calanus* shows no significant migration in relation to the various phytoplankton organisms used, and that such departures from an even distribution as occur are the result of some swarming or shoaling behaviour and are independent of the distribution of phytoplankton in the tube.

THE VERTICAL APPARATUS

Most of the experiments using the vertical apparatus were done with the copepod *Calanus finmarchicus*. The first ten were performed in the open tank with the apparatus in its original form and these are considered first.

In each experiment the water in one of the tubes had previously been drawn, by siphoning, through a Berkefeld-plaster filter-candle; and the water in the other tube, after similar filtration, had been enriched with one

TABLE XVI. *CALANUS FINMARCHICUS* AND DIATOMS AND FLAGELLATES

Nitzschia closterium

P 51	3440	F	N.S.*
P 12	3060	F	N.S.
P 23	1900	F	N.S.*
P 5	500	F	N.S.
P 1	970	S	+ve
P 2	970	S	N.S.
P 3	970	S	N.S.
P 7	490	S	+ve
P 8	490	S	N.S.

* Medium *Nitzschia*.

Thalassiosira nordenskiöldii

P 58	20	F	N.S.
P 53	18	F	N.S.
P 54	18	F	N.S.
M 3	13	S	−ve
M 4	12	S	N.S.
P 67	10½	S	+ve

Biddulphia sinensis

P 57	222	F	N.S.
P 55	186	F	−ve
P 56	223	S	N.S.
P 52	39	S	N.S.
P 70	—*	S	+ve

* Culture used previously as food for *H. lamornae*.

Skeletonema costatum

P 10	23	F	N.S.
P 9	23	S	N.S.
P 11	23	S	−ve
P 15	Filtered	S	N.S.

Coscinodiscus concinnus

P 59	132	F	N.S.
P 45	84	F	N.S.
P 49	42	F	N.S.

Mixed diatoms

P 36	64	F	N.S.

Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*

P 32	22	F	N.S.
P 35	9	F	N.S.
P 28	4	F	N.S.
P 34	Filtered	F	−ve
P 31	22	S	N.S.
2P 13	3	S	−ve

Dicrateria inornata

2P 8	3220	F	N.S.
P 22	2060	F	+ve
P 4	3900	S	N.S.*
P 17	850	S	N.S.

* 2 rds −ve.

Phaeocystis pouchetii

P 50	—*	F	N.S.
P 40	51	S	+ve
P 37	35	S	N.S.

* Old disintegrating culture.

Cryptomonad

P 46	110	S	N.S.
P 47	110	S	N.S.

Gymnodinium sp.

P 48	120	F	N.S.*
P 39	75	F	N.S.

* 3 rds +ve.

Hemiselmis rufescens

P 14	1168	F	+ve

Nitzschia sp. and a flagellate

P 71	286	F	N.S.

Bacteria

P 24	—	F	N.S.

Oxygen

P 44	—	F	N.S.

Carbon dioxide

P 41	—	S	N.S.

Summary distribution of results with *C. finmarchicus*

Diatoms

	+ve	N.S.	−ve
Fed	—	13	1
Starved	4	8	2

Non-toxic flagellates

	+ve	N.S.	−ve
Fed	2	3	—
Starved	1	5	—

Toxic flagellates

	+ve	N.S.	−ve
Fed	—	5	1
Starved	—	1	1

of a variety of phytoplankton cultures. Twice, exactly 100 animals were picked out and counted before use, but otherwise only roughly equal numbers were put in and then accurately counted after the experiment. The number of *Calanus* swimming in the top compartment of each tube was counted at various irregularly spaced times during the day and night; each experiment lasting about 24 hr. Because of this irregularity it was decided to assess the results on, and apply the test of significance to, the mean of all the readings in each experiment. The percentages calculated from the mean number of animals swimming up in each experiment are given in Table XVII. It will be seen that these figures are small and variable, but that, in all experiments

TABLE XVII. RESULTS OF FIRST TEN EXPERIMENTS OUT-OF-DOORS WITH *CALANUS FINMARCHICUS* IN THE VERTICAL APPARATUS

Serial no.	Phytoplankton organism	No. of animals		Total mean % swimming up	
		Contl.	Exptl.	Contl.	Exptl.
ML 16	Mixed diatoms	58	60	0·2	1·5
ML 17	Mixed diatoms and flagellates	217	226	4·0	3·9
ML 23	Mixed diatoms and flagellates	55	59	1·3	3·8
ML 25	*Syracosphaera carterae*	73	82	0·3	3·6
ML 29	*Ditylum brightwellii*	100	100	0·8	4·4
ML 30	*Gymnodinium* sp.	99	119	1·2	5·5
ML 33	Water rich in *Mesodinium**	100	100	0·9	1·9
ML 34	*Peridinium trochoidium*	84	97	1·0	2·8
ML 36	*P. trochoidium* and *Gymnodinium* sp.	142	218	2·8	2·7
ML 37	*P. trochoidium* and *Gymnodinium* sp.	215	270	0·5	1·2

A summary of the χ^2 results confirming that these differences are significant is as follows:

	Degrees of freedom	χ^2	P
Interaction	9	4·98	0·85
Pooled readings	1	5·0	*ca.* 0·03
Total of individuals readings	10	9·98	

* This water was unfiltered and as taken from the sea during a rich outburst of the ciliate *Mesodinium* sp.

but two, there are more animals swimming up in the enriched water than in the filtered water. If the test of independence described earlier is applied to each experiment, in none is the difference greater than might be expected by chance, but if it is applied to the pooled results, then the difference in behaviour between the two sets of tubes is clearly seen to be significant at the 5% level ($P=0{\cdot}03$). The test for homogeneity confirms that this pooled result is reliable ($P=0{\cdot}85$) and is in fact made up of a series of small and almost uniform differences all in the same direction.

As the reaction of the animals to all these different phytoplankton organisms

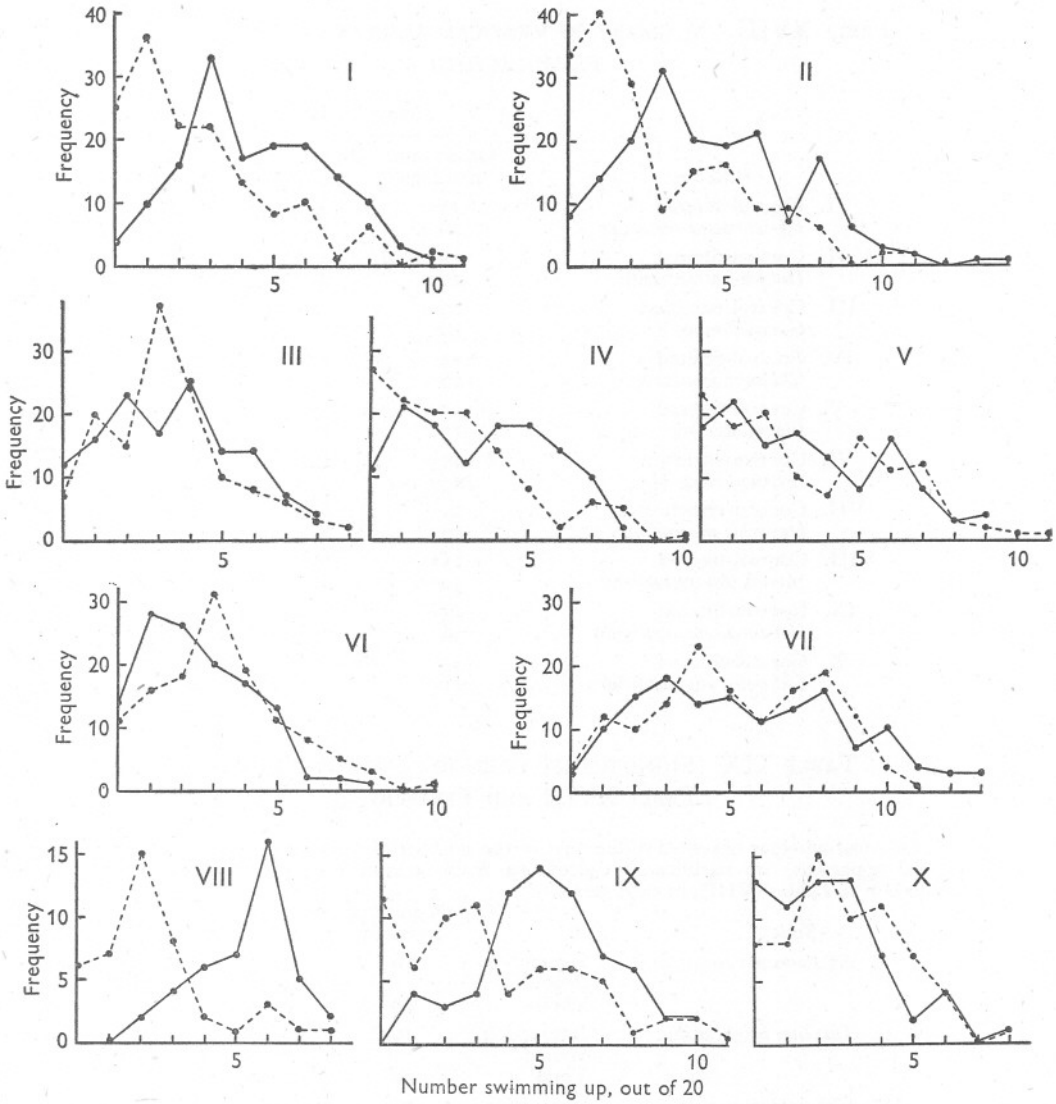
appears to be similar, it may be taken as permissible to calculate a total mean percentage of animals swimming up for the whole series of experiments. If this is done, 1·3% are found to swim up in the filtered water and 3·1% in the enriched.

After these preliminary experiments 103 were performed with the apparatus indoors in the aquarium tank. This whole series is more uniform in that readings were taken at regular intervals after the start of the experiment, and the same number of counted animals was used each time. The fact that each experiment involved two control and two experimental tubes gave a further check on the reliability of the results. The whole series falls into ten groups dependent upon the phytoplankton organisms used and may best be considered in this manner. Detail of the method of presentation of the results is given in the first group.

Group I: *Skeletonema costatum*

Ten experiments were performed with the pair of control tubes containing water filtered by suction through a Whatman no. 1 filter-paper and aerated before use by shaking, and with the experimental tubes containing similar water enriched with a culture of *S. costatum*. A small quantity of tap water was added to the water in the control tubes to make its specific gravity equal to that of the enriched water, the latter being slightly more dilute owing to the addition of the culture. The volume added was calculated, and was of the order of 5–10 ml. depending upon the amount of culture used. Twenty *Calanus finmarchicus* were picked out from a townet catch taken as short a time as possible before the experiment, and put into the top of each tube. The tubes were then suspended in the tank and the number of animals in the top compartment of each was counted from time to time. In order to economize in the use of cultures it was sometimes decided, especially when an experiment was of short duration, to use the water more than once. On these occasions it was poured out, filtered through coarse silk to remove the animals, and replaced in the tubes ready for use with a fresh sample of *Calanus*.

A satisfactory way of showing pictorially whether there was any difference in behaviour between the pairs of tubes was found to be as follows. The frequency with which each particular reading occurs throughout the whole group of experiments is determined, and this number plotted against the value of the reading. The result for all the experiments involving *Skeletonema costatum* is shown in Text-fig. 3 (I). The ten experiments gave a total of seventy-three readings, each in pairs (there being two control and two experimental tubes), and this provides 146 values for filtered water and 146 for enriched. It can be seen at once that there are more of the higher readings in the enriched water and in fact the curve for the latter bears a close resemblance to that for filtered water but is moved bodily to the right.



Text-fig. 3. Upward movement of *Calanus* in water containing various organisms, each graph representing the results of a series of comparable experiments with the organism stated. Each graph plots the frequencies of the different values, out of twenty, for the number of animals moving up—either in water rich in the experimental culture (continuous line), or in a control medium (broken line). The control was usually filtered sea water, but enriched water was chosen for series III, VI and VII. I, *Skeletonema costatum*; II, *Ditylum brightwellii*; III, *Gymnodinium* I; IV, *Chlamydomonas* sp.; V, Flagellate One; VI, *Gymnodinium* II; VII, *Oxyrrhis*, *Rhodomonas*, and *Nitzschia*; VIII, mixed phytoplankton; IX, *Coscinodiscus concinnus*; X, *Chlorella stigmatophora*. See Tables XVIII and XIX.

TABLE XVIII. SUMMARY OF VERTICAL RESULTS WITH *CALANUS FINMARCHICUS*

Series	Mean % swimming up: On 60 min. readings	On 1st four readings	On all readings
I. Control-filtered	16·0	16·4	10·5
Skeletonema costatum	27·8	23·9	17·5
II. Control-filtered	21·7	22·4	14·0
Ditylum brightwellii	29·5	29·2	22·5
III. Control-enriched	20·0	18·8	16·6
Gymnodinium I	20·2	18·9	17·4
IV. Control-filtered	14·4	14·8	12·9
Chlamydomonas sp.	20·2	19·3	16·9
V. Control-filtered	17·5	18·2	16·5
Flagellate One	17·2	18·1	16·3
VI. Control-enriched	15·9	14·2	16·4
Gymnodinium II	13·6	11·5	12·5
VII. Control-enriched	29·7	27·9	25·5
Oxyrrhis marina etc.	30·7	29·1	27·9
VIII. Control-filtered	15·0	15·0	12·2
Mixed phytoplankton	30·8	29·6	26·6
IX. Control-filtered	23·0	20·8	22·2
Coscinodiscus concinnus	36·0	29·1	26·3
X. Control-filtered	15·5	14·8	14·0
Chlorella stigmatophora	11·5	13·1	11·4

TABLE XIX. SIGNIFICANCE TESTS FOR DATA PRESENTED IN TABLE XVIII AND TEXT-FIG. 3

(These test whether observed differences in the number of *Calanus* swimming up (in the vertical apparatus) are significant. Calculation from 60 min. reading for all experiments (column 1 in Table XVIII), in each series.)

Series		Degrees of freedom	χ^2	P
I. *Skeletonema costatum*	Interaction	19	22·10	*ca.* 0·25
	Pooled	1	16·19	**<0·001**
	Total	20	38·29	
II. *Ditylum brightwellii*	Interaction	19	15·88	0·7
	Pooled	1	6·73	**0·01**
	Total	20	22·61	
IV. *Chlamydomonas* sp.	Interaction	23	31·97	>0·10
	Pooled	1	5·65	**<0·02**
	Total	24	37·62	
VI. *Gymnodinium* II	Pooled	1	0·95	*ca.* 0·35
VIII. Mixed phytoplankton	Interaction	5	1·69	*ca.* 0·9
	Pooled	1	8·51	**<0·01**
	Total	6	10·20	
IX. *Coscinodiscus concinnus*	Interaction	9	13·65	0·15
	Pooled	1	8·12	**<0·01**
	Total	10	21·77	
X. *Chlorella stigmatophora*	Pooled	1	1·37	*ca.* 0·25

To test whether the difference is significant, the test of independence can be applied either to one reading from each particular experiment or to the mean of a group or the mean of all the readings from each particular experiment. Examination of the results for one or two of the experiments revealed that the number of animals swimming up generally rose to a maximum at about 1–1½ hr. and then fell gradually for the rest of the time. It was decided consequently to apply the test to the reading taken at, or as close as possible to, 1 hr. after the start of the experiment and to use only this one reading from each experiment. When this is done for the *Skeletonema* results it is found that only four out of the total of twenty show significant differences. (The test is applied twice to each reading; each time one control tube being compared with one experimental tube, and this results in twenty values of χ^2 for the ten experiments.) When, however, the single readings are lumped together and the test applied to this pooled result the whole series is shown clearly to be significant and, further, satisfies the test for homogeneity. A summary of the values of χ^2 concerned is given in Table XIX.

It may therefore be concluded that a significantly greater number of *Calanus* swim up in the enriched tubes than in the filtered tubes. The total mean percentage swimming up, as calculated from the 60 min. readings, may be taken as a measure of the actual difference between the two tubes. This is 16·0% for the filtered water and 27·8% for the enriched. The average of the concentrations of cells in the enriched water used in the various experiments is 18·3 cells of *Skeletonema costatum* per mm.^{3}. This figure includes both single cells and cells in chains of various numbers.

Group II. *Ditylum brightwellii*

A series of experiments precisely similar to the above but using the diatom *Ditylum brightwellii* gave similar results. The graph in Text-fig. 3 (II) reveals a similar displacement of the curve for enriched water and that for the filtered water is almost identical with the one in the previous series. There is perhaps a little more irregularity in the enriched curve, and the whole is more flattened with more readings of higher value and fewer of more intermediate value. Only one of the twenty values of χ^2 calculated from the 60 min. readings is significant of itself, but the pooled result is clearly significant and the test for homogeneity is easily satisfied (Table XX). In fact there is less variation within this series than in the previous. A summary of the χ^2 values is given.

Significantly more *Calanus* therefore swim up in the *Ditylum*-enriched water. The total mean percentages swimming up being 21·7% in the filtered water and 29·5% in the enriched: both values a little higher than those with *Skeletonema*. The average concentration of *Ditylum* in the enriched water used was 527·3 cells/ml. or 0·53/mm.^{3}.

Group III. *Gymnodinium* I

Evidence of the effect of the two diatoms having been found, *Gymnodinium* I, one of the organisms suspected of having a toxic effect, was next tried. With the possibility, after the hypothesis of animal exclusion, that *Gymnodinium* might suppress vertical migration to some extent, the control chosen for this series was an enriched water and not a filtered one. Twelve experiments of this nature were performed. The water was first passed through a Whatman no. 1 paper and one lot was enriched with the *Gymnodinium* culture and the other variously with *Skeletonema*, *Chlamydomonas* and a culture of mixed phytoplankton organisms grown by seeding the normal culture solution with a little raw sea water taken at the time of a diatom outburst. This culture contained diatoms and flagellates of several kinds including *Skeletonema*, *Thalassiosira* and a *Nitzschia*.

As may be seen from the graph in Text-fig. 3 (III), behaviour of the *Calanus* in the two sets of tubes was almost identical and closely resembled that in the enriched water in the two previous series. No tests of significance were applied and the total mean percentage swimming up in the enriched control was 20·0% and in the *Gymnodinium*-enriched water 20·2%. The average concentration in the latter was 9·8 *Gymnodinium* cells/mm.^{3}: and in the former 47·4 cells/mm.^{3} of various kinds. It may be noted that the percentages swimming up are rather smaller than in the previous experiments.

Group IV. *Chlamydomonas* sp.

This series consisted of twelve experiments having filtered water in the control tube and water enriched with *Chlamydomonas* sp. culture in the experimental tube. Examination of the graph in Text-fig. 3 (IV) reveals the likelihood of some difference in behaviour between the two tubes, but this is by no means so clear as in the previous experiments. The control curve is similar in form to that obtained before, although it is somewhat flattened. Altogether there are sixty-two pairs of readings giving 124 values for each type of water. This is less than the numbers in groups I and II and may partly account for the difference in shape. The enriched curve lacks the definite peak characteristic of the previous curves, but nevertheless indicates a preponderance of higher readings.

When the test of independence is applied to the 60 min. readings, four out of the twenty-four are shown to be significant, but one of these has the greater number swimming up in the control tube. Despite this, when the results are pooled and the test applied to the totals, their difference is shown clearly to be significant (Table XIX). The test for homogeneity is just satisfied and hence it is possible to place reliance on this result. The readings are, however, very variable and the difference in behaviour is neither as clearly marked nor as great as in the diatom experiments. The mean percentage swimming up

in the control tubes was 14·4% and that in the enriched tubes 20·2%. The average concentration of *Chlamydomonas* employed was 73·7 cells/mm.^{3}.

Group V. Flagellate One

Ten experiments were performed using water enriched with a culture of Flagellate One. This is one of the organisms which the toxicity experiments indicated might be harmful, but it was still compared with a control of Whatman no. 1 paper-filtered water. The total number of pairs of readings is sixty-two, this being equal to the number in the previous group. Inspection of the graph in Text-fig. 3 (V) reveals almost complete identity of behaviour between the two lots of water. Further, both curves closely resemble the control in the previous group, being only slightly flattened and having a few more readings of higher value. The mean percentages swimming up are 17·5% in the control and 17·2% in the enriched tubes. As a result of this no test for significance was applied. The average concentration of Flagellate One used in the first eight experiments was 94 cells/mm.^{3}. In case this culture was not sufficiently dense to manifest any possible 'exclusion' effect the remaining two experiments were performed at a concentration of 209 cells/mm.^{3}. There was, however, no apparent difference in behaviour, and it may be concluded that the presence of Flagellate One in no way affected the number of *Calanus* swimming up. The average concentration of cells employed in the whole series was 117 cells/mm.^{3}.

Group VI. *Gymnodinium* II

Eleven experiments were performed with *Gymnodinium* II, another of the flagellates shown to be toxic. The chosen control was water enriched with a mixed phytoplankton culture, grown, as before, by seeding with raw sea water. This culture consisted of almost pure *Nitzschia closterium* of the normal large form with a little *Skeletonema* and a few flagellates. In both the control and the experimental tubes the water was paper-filtered before being enriched. In one of the experiments only one pair of the four tubes was used, and as a result there are fifty-nine pairs of readings and five odd ones giving a total of 123 values each for the control and the experimental curves. Examination of the graph in Text-fig. 3 (VI) shows the form of the enriched control curve to be very similar to those obtained previously, including again the characteristic peak. The experimental *Gymnodinium* curve is also similar but is displaced now to the left and almost in the position of previous control curves. Two of the twenty-one values of χ^2 show the difference between the two tubes to be significant. When the 60 min. readings are pooled, however, the χ^2 value does not even approach that required for significance (Table XIX). It seems therefore that, although fewer animals swim up in the *Gymnodinium* II enriched water, not enough stay down to give a significant difference, and the effect must be ascribed to chance. The total

mean percentage swimming up in the diatom-enriched control was 15·9%, and that in the *Gymnodinium*-enriched 13·6%. The average concentration in the former was 5·6 cells/mm.^{3} and in the latter 9·5 cells/mm.^{3}.

The mean percentages swimming up are later calculated on the first four readings in every experiment and also on all the readings. Examination of Table XVIII suggests that, with *Gymnodinium* II, the difference between the two tubes increases with time. The pooled χ^2 test was therefore applied both to the means of the first four readings and to the means of all the readings in each experiment, but neither of the values obtained showed a significant difference, although they were higher than for the 60 min. readings.

Group VII. *Oxyrrhis marina*, *Rhodomonas* sp. and *Nitzschia closterium*

Ten experiments were performed with the mixed culture of *Oxyrrhis*, *Rhodomonas* and *Nitzschia*, the *Rhodomonas* component of which is now thought to be toxic. The control was again chosen to be water enriched with mixed phytoplankton. This mixed culture consisted chiefly of *Skeletonema*, a naviculoid collected in chains, and *Thalassiosira*, with a little *Chaetoceros* and some flagellates. Altogether, seventy-one pairs of readings were taken giving 142 values for each of the cultures. The graph in Text-fig. 3 (VII) shows the behaviour of the animals in the two sets of tubes to have been almost identical. Both curves are typically those of enriched water but they lack the peak which is common in most of the earlier experiments and also include a greater number of higher values than previously. The curious dip in the centre of both curves at the value six is interesting. The total mean percentage swimming up in the enriched control was 29·7% and that in the *Oxyrrhis*-enriched water 30·7%. No tests were applied. The average concentration of diatoms in the enriched control water was 0·74 chain/mm.^{3}, a chain of average length being about four cells, together with some single cells. The concentration in the mixed experimental water was 6·4 cells of *Oxyrrhis*/mm.^{3}, together with a few *Nitzschia* and a very few *Rhodomonas* cells. This fact may account in part for the absence of any depressant action on the vertical migration of the *Calanus*.

Group VIII. Mixed phytoplankton organisms

No clear difference having been found between filtered and enriched water in the last four series, it was considered desirable to confirm that this difference could still be demonstrated. Consequently, three experiments were performed with Whatman no. 1 paper-filtered water in the control tubes and similar water enriched with a mixed phytoplankton culture in the experimental tubes. This culture was grown as before, but was rather older and contained the normal large *Nitzschia closterium*, various small and large flagellates, a heliozoan and a large ciliate. Twenty-two readings were taken, giving forty-four values for each of the curves. Because of this low number

the graph in Text-fig. 3 (VIII) is drawn to double the vertical scale used previously. A difference greater than any so far obtained between behaviour in the two waters is immediately apparent; and both curves closely resemble the early ones despite the higher numbers of animals swimming up. Even with so small a number of experiments the pooled 60 min. results, when the test of independence is applied, are seen clearly to be significant (Table XIX). Although none of the six single 60 min. readings are significant of themselves, the test for homogeneity shows them to be more uniform than any of the other series and the pooled result is therefore reliable. The total mean percentage swimming up in the filtered water was 15·0% and that in the enriched 30·8%. The concentration of phytoplankton in the enriched water was 2 cells/mm.^{3}.

Group IX. *Coscinodiscus concinnus*

The next five experiments used a culture of the large diatom *C. concinnus*. The control tubes contained Whatman no. 1 paper-filtered water and the experimental tubes water enriched with the *Coscinodiscus* culture. Twenty *Calanus* were again put into each tube. Thirty-three readings were made giving sixty-six values for each curve, and the graph in Text-fig. 3 (IX) is once more drawn to double the vertical scale. A preponderance of higher readings in the enriched water is again apparent, although the curve has a less pronounced and more rounded peak. This, however, accords well with the more flattened nature of the control curve (deriving from the greater number of higher readings) which, with a little extra irregularity to be expected from the smaller number of readings, otherwise closely resembles the early ones. It is particularly like the group IV and V control curves. Application of the test of independence to the 60 min. readings gives three out of the ten as significant, but when it is applied to the pooled figures the whole series is shown to be so (Table XIX). The homogeneity test is satisfied although there is more variation between different readings than in series VIII. The total mean percentage swimming up in the control tubes was 23·0% and that in the enriched ones 36·0%. Both these figures are the highest obtained for these two types of water in all the ten groups of experiments. The average concentration of *Coscinodiscus* in the enriched water was 22·4 cells/ml. or 0·02/mm.^{3}.

Group X. *Chlorella stigmatophora*

The last five experiments with *Calanus* utilized a culture of the non-motile alga *Chlorella stigmatophora* which has been shown to have toxic properties. The control chosen was paper-filtered water and thirty-two pairs of readings were taken, the graph in Text-fig. 3 (X) being drawn to the same scale as the previous two. The control curve is seen to be steeper and more like the first obtained, while the curve for the enriched water is very similar. The total mean percentages swimming up are 15·5% in the filtered water and 11·5%

in the enriched, suggesting the possibility of a significant difference in behaviour between the two. This is not verified, however, and the test of independence demonstrates significance neither on the individual 60 min. readings nor on the pooled figures. The average concentration of *Chlorella* in the enriched water was 226 cells/mm.^{3}. This is well below even the optimum found in the toxicity experiments and need not necessarily have any harmful properties.

The summary of the results in Table XVIII shows the mean percentages swimming up as calculated from (*a*) the 60 min. reading from each experiment, (*b*) the mean of the first four readings, and (*c*) that of all the readings in each experiment. It can be seen that, generally, in each series as time goes on fewer animals swim up. This gradual decline in the proportion swimming up has been mentioned earlier as the reason for using the 60 min. readings in assessing the significance and magnitude of the differences. It also emphasizes the importance of using animals caught as short a time as possible previous to the experiment, and it must probably be attributed to a general change in behaviour on being kept in the unnatural conditions of captivity.

The second change with time to be seen is that between successive series of experiments. There are considerable differences between what should be comparable figures in the different series. There are altogether seven series with filtered controls, and the proportions swimming up in these range from 23·0% in IX to 14·4% in IV. These particular experiments cover a period of just over 2 months and during that time, of course, considerable changes were taking place in the population of *Calanus* which was drawn on for the experimental animals. This factor, combined with the varying light and temperature conditions, probably accounts for this variation with time. Male, female, and stage V and occasionally stage IV *Calanus* were used just as taken in the townets; the plan being to test the reactions of a typical sample and not one particular stage. The stages and sexes present were determined from time to time, but the type of behaviour obtained could not be correlated with the composition of the sample. On account of this fluctuation with time it is perhaps unwise to make comparisons between the effects of different cultures. However, taking the percentage differences between the filtered control and the enriched experimental tubes in each of seven series, and the enriched control and experimental tubes in the other three, and arranging the figures in order of magnitude we obtain the scheme given in Table XX.

Each of the two columns in itself may be reliable in showing the relative degree to which the various cultures affect the number of *Calanus* swimming up. Relation of the one column to the other, however, presents difficulties. There are two alternatives. If the behaviour towards the enriched control water in each of the *Gymnodinium* I and II and the *Oxyrrhis* experiments is taken as identical with that in the mixed-phytoplankton-enriched experi-

mental water in column I (i.e. the top value of 16); then the three cultures should be assigned the positive values of 16, 15 and 14 as shown in brackets (i.e. 16 minus 0, 1 and 2 respectively). This puts them at the top of the whole series as regards degree of effect on *Calanus*. If, however, the differences invoked by the first five (significant) cultures in column I are averaged and this figure of II is taken as the common denominator, then the three cultures must be assigned the values of +11, 10 and 9 as underlined, and they should then be placed between *Skeletonema* and *Ditylum* in the whole series.

TABLE XX. COMPARISON OF THE EFFECTS OF DIFFERENT CULTURES IN THE VERTICAL APPARATUS

	% differences from filtered water		% differences from enriched water			
S	Mixed phytoplankton	16	*Gymnodinium* I	0	(+16)*	+11†
			Oxyrrhis	−1	(+15)*	+10†
			Gymnodinium II	−2	(+14)*	+9†
S	*Coscinodiscus*	13				
S	*Skeletonema*	12				
S	*Ditylum*	8				
S	*Chlamydomonas*	6				
	Flagellate One	0				
	Chlorella stigmatophora	−4				

( ) * Equating enriched control with mixed phytoplankton result.
— † Equating enriched control with mean of significant phytoplankton results.
S indicates those differences shown mathematically to be significant.

It is reasonable to suppose that different phytoplankton organisms may affect the behaviour of *Calanus* differently, but if this should be so it is not possible, with only the present evidence available, to state with any certainty the relative degree to which they act. It is clear, however, that more *Calanus* swam up in the presence of all of the phytoplankton organisms tested, except Flagellate One and *Chlorella stigmatophora*.

Besides the above series of experiments with *Calanus*, three each were done with decapod larvae and young *Thysanoessa inermis*. All but one of these were with a mixed phytoplankton culture similar to that used in group VIII above, the other being with *Chlamydomonas*. The control for each was Whatman no. 1 paper-filtered water. No significant difference in behaviour between the pairs of tubes could be detected in any of the experiments, either when tested individually, or when pooled. The number of animals swimming up was greater with the decapod larvae, but this derives from their marked positive reaction towards light. The total mean percentages swimming up in the three decapod larva experiments were 70·0% in the filtered control and 71·5% in the enriched water. In the three *Thysanoessa* experiments there were 17·9% in the control and 19·3% in the enriched water. Details of the individual results are given in Table XXI.

Finally, five experiments were done using ten young *Hemimysis lamornae* in each tube of the apparatus in its original form. Owing to the marked movement of *Hemimysis* away from light these experiments were done in complete darkness in a constant temperature room and the animals counted from time to time by aid of a dim red lamp. All had ultra-filtered water in the control tube, and in the experimental tube similar water enriched in three experiments with *Chlamydomonas* and in two with *Skeletonema* culture. The

TABLE XXI. RESULTS OF EXPERIMENTS WITH *CARCINUS* ZOEAS *THYSANOESSA* AND *HEMIMYSIS* IN THE VERTICAL APPARATUS

	Mean % swimming up	
	Control	Experimental
20 *Carcinus maenas* zoeas in each tube:		
Mixed phytoplankton 0·84 chains/mm.^{3}	50·8	63·3
Mixed phytoplankton 0·84 chains/mm.^{3}	62·8	64·4
Chlamydomonas sp. 99 cells/mm.^{3}	84·6	83·4
20 young *Thysanoessa inermis* in each tube:		
Mixed phytoplankton 3 cells/mm.^{3}	29·2	29·5
Mixed phytoplankton 3·4 cells/mm.^{3}	10·7	13·7
Mixed phytoplankton 4·4 cells/mm.^{3}	16·0	13·2
20 young *Hemimysis lamornae* in each tube:		
Chlamydomonas sp. 90 cells/mm.^{3}	6·1	20·8
Chlamydomonas sp. 92 cells/mm.^{3}	9·0	26·0
Chlamydomonas sp. 96 cells/mm.^{3}	3·0	25·0
Skeletonema costatum 26 cells/mm.^{3}	26·0	30·0
Skeletonema costatum 26 cells/mm.^{3}	25·0	60·0

Summary of χ^2 tests for *Hemimysis*:

	Degrees of freedom	χ^2	P
Interaction	4	1·57	*ca.* 0·8
Pooled readings	1	4·70	0·04
Total	5	6·27	

results have been assessed on the mean of all the readings in each experiment. When this is done no individual experiment is shown by the test of independence to be significant; but if all the results are pooled, the series is clearly so, and the test for homogeneity is satisfied (see Table XXI). The total mean percentages swimming up were 13·8% in the filtered and 32·0% in the enriched tubes. The average concentration of *Chlamydomonas* in the enriched water of the first three experiments was 93 cells/mm.^{3} and of *Skeletonema* in the last two, 26 cells/mm.^{3}.

UNDERWATER OBSERVATIONS

If the results of experimental work of the kind reported here are to be applied to a consideration of problems in the sea itself, it is important to know whether or not the behaviour of the animals in the various pieces of apparatus used

resembles their behaviour under natural conditions. Underwater observations on the behaviour of some of the animals used have already been described (Bainbridge, 1952). Ten more excursions were made in 1952, each involving descents in different places and giving a total of about 8 hr. underwater. In particular, the technique of swimming with foot-flippers and following animals while over deep water has been developed successfully. All the 1951 observations on the spring brood of *Calanus* have been verified, although the 'upper zone' of 12 in. has seemed to be very much shallower this year, and there have been fewer animals in the deeper 'zone of migration'. In particular, the marked cessation of motion when the sun is covered has been noticed repeatedly. The whole sea gives a peculiar impression of idleness on such occasions. Besides the almost universal vertical swimming up and down, a more erratic movement has been seen once or twice. This is distinct from the bouncing along on the under surface of the water (which has also been seen again) and results in little or no forward movement at all. It is a violent dashing about in different directions, and is so rapid that it is not possible to tell how the animal is orientated or what limbs are being used. It is perhaps most adequately described as a 'caper' and seems to be rare. On one occasion also *Calanus* was seen swimming horizontally, deep in the body of the water with an uncommon sort of jerky side-to-side motion. Apart from these exceptions the continued occurrence of straight up and down swimming makes it certain that this is the form generally indulged in. It should perhaps again be noted that the downward swimming is invariably head-first down. Some *Calanus* from a deep townetting, when taken below in a jar and released, swam head-first downwards out of sight.

The collection in small swarms or clusters was again noticed, although these appeared smaller this year with rarely more than six animals in a group. One stage V *Calanus* was seen swimming with three adults, all circling round and round each other.

There were again many small copepods and young stages, but these were harder to observe because of the large amount of detritus repeatedly found in the water. A random sort of motion was prevalent, and even those swimming upwards appeared to do so in a jerky and 'kinetic' manner and then bounce away similarly (often towards the sun) along the under surface of the water. None were ever seen to go downwards in this way and it may perhaps be assumed that they gradually sink downwards to go jerkily up to the surface again after a certain time. Many seemed to hang head-first downwards but it was not possible to decide whether they sank in that position or whether they were preparing for swimming down.

Perhaps the most interesting observations concern three decapod larvae. The first two of these were megalopas both seen on 23 May. They were pale in colour and were rather small and were both swimming rapidly in a perfectly horizontal straight line, one about 2 ft. below the surface and the other about

3 ft. I was able to follow one for about 12 ft. and the other for 24 ft. There was no sign of deviation whatsoever, and the movement appeared most purposeful. They were both swimming roughly at 20° to the right of the direction of the sun's rays. The third was a zoea seen on 3 July. This was large and probably *Carcinus*. It was about 4 or 5 ft. down and was swimming almost horizontally (slightly upwards) in a gently undulating line. (It is just possible that the undulation was the effect of my own movements, but this is very unlikely.) These three are the only decapod larvae so far seen.

Three young *Thysanoessa inermis* caught several days previously and used in one of the experiments were taken down and released separately from small jars. They all went down, one sinking, one swimming quite vigorously obliquely, and one head-first downwards.

Many ctenophores and medusae were seen on different occasions and these were usually quite randomly orientated. One large *Bolinopsis* was seen travelling horizontally, its large oral lobes streaming behind. Very many, roughly spherical, transparent bodies with six to ten small bubbles enmeshed in them were seen drifting along during one descent. One was taken in a breffit and proved to be *Oikopleura* in its house. Examined later in the laboratory, the house was found swarming with flagellates of all kinds and a few small diatoms and ceratia, etc. It seems likely that this enormous concentration of even the smallest flagellates was producing the tiny oxygen bubbles by photosynthesis. These must have buoyed up the house and affected the animal's movements in some way.

One descent was made purposely during rough weather. There was a great amount of wave action resulting in swirling about of medusae, ctenophores and a few small copepods. This movement went down to about 10 ft. but diminished gradually. It was possible to follow some of the animals, but they were all being swirled about the whole time and no directive movement was observed.

GENERAL DISCUSSION

Experiments in the Horizontal Apparatus

Considering first the results obtained with the horizontal apparatus it may safely be claimed that, under the conditions of the experiments, significant migrations by various animals into concentrations of phytoplankton have been demonstrated. This is particularly so with the mysids used, which swam into water enriched with cultures of *Skeletonema*, *Thalassiosira*, *Biddulphia*, *Nitzschia* and mixed diatoms and into cultures of the flagellates *Chlamydomonas*, *Peridinium*, *Dicrateria*, Flagellate K, and *Oxyrrhis*. No marked movement into the diatoms *Lauderia*, *Coscinodiscus* and *Eucampia* was observed nor into the flagellates *Syracosphaera* and *Exuviaella*, but a movement

away from *Rhodomonas* and *Gymnodinium* II was demonstrated. Various small copepods swam into cultures of the diatoms *Nitzschia*, *Biddulphia* and *Thalassiosira*, and positive results were also obtained with *Artemia salina*. It was not possible, however, to obtain consistent or intelligible results with either *Calanus finmarchicus* or with various decapod larvae.

There can be little doubt that these positive migrations are for the purpose of feeding; and, although this could not be demonstrated significantly, there was a greater incidence of them amongst starved animals than amongst fed ones. No experiments have so far been designed especially to throw light on the mechanism underlying the movement, but it seems likely that sometimes the effect of the phytoplankton may be mediated by substances dissolved in the water. This would not seem to be either concentration of carbon dioxide (which has the opposite effect), oxygen (which has no determinable effect) or pH (in which property the cultures do not differ appreciably from normal water), but more probably some kind of dissolved organic substance. Sometimes the actual physical presence of the diatoms is necessary.

Those flagellates invoking an avoiding reaction in the animals almost certainly do so through some substance dissolved in the water; this would seem both harmful and distasteful, although the deaths produced by Flagellate 12 seemed to result from the actual ingestion of the cells. The less consistent results with these cultures classed as 'toxic' are perhaps due to there being two conflicting reactions on the part of the animals—an avoidance of the water due to the distasteful substance in it and a movement into the actual concentration of cells for the purposes of feeding. If the feeding reaction is strong, due perhaps to starvation, a positive migration will result, if the avoiding reaction is strong, then a negative migration may. With the bacteria culture, of presumably little nutritive value to those animals tested, a consistent negative migration can be obtained: here clearly away from some dissolved organic substance.

In extent, both the positive and negative migrations seem to indicate an all-or-nothing reaction. The lumping together of those readings in any particular group which are classed as 'not-significant' but which might have been expected to be positive does not give any indication of a positive tendency but rather emphasizes the random nature of the distribution. It thus seems that if a positive or negative migration is to take place it does so without equivocation. In the mysids this may be connected with the shoaling reaction which they exhibit. The marked positive migrations into concentrations of ammonia are of particular interest on this account. Ammonia is one of the excretory products of these animals, and a tendency to congregate in water richer in ammonia would automatically act as an aggregating mechanism for them. Any small group of animals containing individuals circling round and round each other as they do, would excrete and develop its own cloud of ammonia round it. This would attract others and the whole would work on

a snow-ball principle, gathering more and more animals into a bigger and bigger cloud. Such a sequence could conceivably take place most quickly in the presence of food and could constitute part of the mechanism behind the migrations in the tubes. Those shoals of mysids seen in aquaria and tanks must almost always be brought into being by uneven lighting conditions, but this cannot generally be so in nature and especially in the open sea. The shoals of euphausids seen by many observers (Hardy & Gunther, 1935; Hart, see below), and the uneven distribution of small plankton animals reported by Barnes & Marshall (1951), and seen underwater by myself, cannot be dependent upon light. There may be many means by which animals keep together in swarms, such as sight or a rheotactic sense, but the possibility of some agent such as ammonia being involved in the mechanism in some species must be borne in mind.

Satisfactory and understandable results were not obtained with *C. finmarchicus* in the horizontal tubes and with the few samples of decapod larvae used. This would seem to be due to the different swimming habits of the various animals. As has been mentioned, it is not generally thought that members of the plankton possess powers of active migration of any consequence in the horizontal plane. The experimental results point to this being so with *Calanus* and it was for this reason that the vertical experiments were developed. This apparently fundamental tendency of *Calanus* to swim in a vertical plane is amply confirmed by the underwater observations.

This is not true of the other animals used in the horizontal tubes. The various mysids and *Artemia* appeared to behave perfectly naturally when indulging in their horizontal excursions. It is unfortunate that comparable animals could not be observed under water. The mysids are mostly nocturnal, when they cannot be seen, and in the daytime the euphausids are restricted to comparatively deep water. Those *Thysanoessa* released from captivity under water swam in straight lines with the long axis of the body pointing forwards but not horizontally, of course, as the initial response was to go away from the higher light intensity.

The decapod larvae that were seen were behaving exactly as might be expected if they are to fit into the group possessing powers of horizontal movement. The very variable results obtained with them are all the more puzzling. They may, of course, be due to the lack of any definite behaviour reaction towards the particular cultures used but they are nevertheless disappointing. It is interesting that six out of the twelve are significant one way or the other.

The surprising results with small copepods, where six out of six experiments showed the most definite movements into diatom cultures, may be accounted for by a third type of swimming behaviour. This is the random movement repeatedly observed under water and it is of course precisely such as would result in the rapid development of an uneven distribution of animals in the

tube if there should be a tendency on their part to remain in one particular environment. Seen in the sea it is a vigorous movement, and canalized as it is in the tubes, it could result in quite a rapid horizontal movement. The experiments, nevertheless, demonstrate a marked preference by the animals for water rich in diatoms.

Experiments in the Vertical Apparatus

Considering the results obtained with the vertical apparatus it may again safely be claimed that, under the conditions of the experiments, significantly greater numbers of animals are shown swimming up in the presence of cultures of mixed phytoplankton, *Gymnodinium* I and II, *Oxyrrhis*, *Coscinodiscus*, *Skeletonema*, *Ditylum* and *Chlamydomonas*; while Flagellate One has no effect on the behaviour of *Calanus*, and *Chlorella* appears to depress the numbers swimming up. The control in some of these experiments was ultra-filtered water, but generally it was water filtered through a paper of the normal pore size. This was especially intended to allow minute flagellates and diatoms to pass through but to stop all the larger cells. The behaviour of the animals in the enriched water was therefore being compared with their behaviour in water containing a low concentration of food and not in entirely sterile water such as would not be found in the sea. In all the indoor experiments the intensity of light was such as to allow an average of about 25% of the animals to swim up. Under these conditions roughly twice as many did so in the enriched tubes as in the filtered, but as mentioned earlier some of the phyto-plankton organisms used may have a greater effect than others.

The animals in the tubes appeared to resolve themselves into two distinct groups, the one swimming up and the other down. Those in the top compartment were generally in the upper half of this and, when *Calanus*, performed their 'hop-and-sink' movements there. Those in the lower compartment were generally in the lower half and some would be swimming head-first downwards and others doing the 'hop-and-sink'. It seems likely, therefore, that the short length of the tubes does not greatly affect the manifestation in the population they contain, of these two groups. There was, of course, interchange between the two but this was usually fairly rapid. In general, the swimming behaviour, especially of *Calanus*, bore a close resemblance to that seen in the sea.

The five experiments with young *Hemimysis lamornae* gave much greater numbers swimming up in the presence of *Chlamydomonas* and *Skeletonema*. Such young forms in nature are often found higher in the water and might be expected to show this sort of vertical movement in the tubes. The results with decapod larvae and *Thysanoessa* are more puzzling. The latter are very delicate and proved difficult animals from the experimental point of view. They are caught only in deep water and do not live at all well in captivity, especially in the unnatural confinement of the tubes. The decapod larvae

showed their definite reaction to light by swimming constantly at the top of the tube and at the side nearest the oblique source of light. From this observation and their behaviour in jars and bowls while being picked out, there is no doubt that, had they been free to do so, they would have continued to swim along, horizontally near the surface and towards the source of light. It is possible that this overriding reaction towards light masked any effect on them of the phytoplankton, but it is also possible that they have no specific reaction under these circumstances.

No experiments have yet been performed expressly to search for the mechanism underlying this increase in upward migration in the presence of phytoplankton. The presence of plant cells must reduce the light intensity in the experimental tubes. The possibility of this being the direct stimulus is, however, rendered unlikely by the fact that the reduction in intensity in the *Chlorella* and Flagellate One experiments must have been as great, if not greater, than that in the rest of the series; and that the difference was still encountered in those experiments performed in the dark.

Experimental results such as those so far described must only be applied with the greatest diffidence to the problems found in nature itself. There are, however, certain extenuating circumstances in the present case. Foremost, in considering the problems of the inverse distribution of zooplankton and phytoplankton in the sea, is the difficulty of obtaining any other insight into the mechanisms involved. The most intensive sampling of plankton and the collection of physical data over a prolonged period of time from the same body of water (which may in the course of the months necessary for the study move hundreds of miles) is finally the only certain way in which the problem can be resolved. Such an investigation presents almost insuperable practical difficulties and certainly insuperable financial ones. Under these circumstances, and in view of the fact that such underwater observations as have been made confirm that the behaviour of the animals under experimental conditions is essentially like that in the sea, we may proceed to a projection of the experimental results into the field. During the process, however, the propriety of this operation must constantly be under review.

The Size of Plankton Concentrations

Before discussing the consequences of the occurrence in the sea of such migrations as have been shown experimentally, it is necessary to consider both the sizes of phytoplankton and zooplankton patches and the density of cells in the former.

Phytoplankton

There is a great body of evidence, collected by naturalists and seafarers, concerning the size and shape of patches of discoloured water which have

been seen from on board ship. Scoresby, as early as 1820, records long bands and streams of water apparently discoloured by diatoms; Darwin (1839) refers to bands and lanes discoloured by *Trichodesmium*, and Hornell (1908, 1917) and Allen (1921, 1928, 1938) refer especially to patches of red water caused by various flagellates. These observations are generally accompanied by estimates of the size of the areas, and maps may even be given, as in Suffren (1951).

The second method of delimiting areas of high phytoplankton density involves intensive sampling with nets, and is very laborious. Savage (1930), Savage & Hardy (1935), and Savage & Wimpenny (1936) record the results of intensive sampling in the North Sea and give charts on which the geographical distribution and size of diatom patches are depicted. Hardy & Gunther (1935) and Hart (1934) give the results of sampling work in the Antarctic and reveal similar sized concentrations.

Thirdly, the size of patches may be estimated from records taken with the Hardy continuous plankton recorder (Hardy, 1936; Lucas, 1940; Lucas & Macnae, 1941).

These references make it clear that the occurrence of patches of both diatoms and flagellates is a normal state in the sea and that these patches, as was so aptly stated by Scoresby, are 'of very variable dimensions'. The lowest size estimate is 20 or 30 yards across but much longer, and the highest 200 miles by something of the order of 40 miles; while a general mean might be considered 3 or 4 miles by half a mile to a mile. There is abundant evidence that, although some patches are diffuse, they may often have quite distinct boundaries and may extend to considerable depths. The most interesting feature is undoubtedly their shape—almost invariably long and narrow. Even where precise dimensions are not given words such as 'bands', 'streaks', 'lanes', and 'stripes' frequently occur. It could most reasonably be expected that any patches would be roughly circular in shape or at any rate irregular; the former might arise from some one point of origin that was particularly well seeded with the species, and the latter in a more diffuse manner from several centres. The possible action of currents or of wind in influencing these shapes must of course be considered. Tidal streams and movements in coastal localities frequently result in the collection of flotsam and jetsam into lines, but this is unlikely to happen in the open sea. Wind action may be thought too superficial, but it is known that it can produce lines of convergence and upwelling orientated parallel with its direction (Langmuir, 1938; Woodcock, 1944) and that the circulations causing these lines may extend as far as 20 m. down. Such an effect on a sufficiently large scale could influence the shape of patches but as so far demonstrated it appears to be too limited in extent. The possibility that grazing may be the cause will be considered later.

Zooplankton

Although the evidence is far more limited, reference to Hardy & Gunther (1935), Hardy (1936), Wimpenny (1936), and Rae & Fraser (1941) makes it clear that patches of zooplankton of the same order of size as those of phytoplankton are to be found in the sea. While recently, Barnes & Marshall (1951) have shown, by a statistical analysis of the results of a large number of small samples, that a number of small zooplankton organisms are often to be found in small swarms or clumps which are discontinuous in three dimensions. These patches are of a much smaller order than any previously demonstrated.

The Density of Phytoplankton Concentrations

Estimates of the density of cells in patches of diatoms can be found in Johnstone (1908), Gran (1912), and Allen (1919), who give, amongst others, values of 6·0, 0·5 and 0·46 cells/mm.^{3} for different organisms; while Harvey *et al.* (1935) give 45 cells/mm.^{3} for *Skeletonema* in Loch Striven. Flagellates and nanoplanktonic forms were not measured so exactly until later, when their great value as a source of food came to be realized. Gaarder & Spärck (1932), Alvik (1934), and Gaarder (1938) give values for Norwegian oyster polls and fjords, and Cole (1939) values for water pumped into oyster tanks at Conway. These are of the order of 4–24 cells/mm.^{3}. The highest values for natural water appear to be those of Gross (Marshall, 1947), who records 2400/mm.^{3} of flagellates for the water of Sailean More, the arm of Loch Sween used as a control in some fertilization experiments. The comparable figure for diatoms is 1·2/mm.^{3}.

Figures for more open waters as in Savage *et al.* (1935–6) and Mare (1940) are generally lower, but Cole & Knight-Jones (1949) give 5–70 organisms/mm.^{3} as general in the open sea. Estimates may be made from the larger hauls reported in Hardy & Gunther (1935) and Hart (1942); these give figures of 0·50 and 0·42 cell/mm.^{3} respectively of diatoms, but in one instance 25 cells/mm.^{3} of *Chaetoceros socialis* in Deception Island Harbour. Davis (1948) records 60 cells/mm.^{3} of *Gymnodinium brevis* during a red-water bloom in the Gulf of Mexico.

It is thus clear that the density of phytoplankton organisms in the sea can, on occasions, almost equal the densities attainable in enriched culture media, but that generally it is much lower than this. At present our interest is in customary and regularly attainable maxima rather than average concentrations over wide areas. The former may be taken to be of the order of 10 cells/mm.^{3} for diatoms and 100 cells/mm.^{3} for flagellates, although much higher figures than these may be found, as for instance 31·6 cells/mm.^{3} for diatoms and 2400/mm.^{3} for flagellates. Such concentrations must, however, be considered localized and of limited duration. More usual maxima would be 0·5 cells for diatoms and 50 cells/mm.^{3} for flagellates.

Those experiments performed at concentrations much higher than these are of especial interest in connexion with the exclusion hypothesis; as any excluding effect might be expected to be more marked at such concentrations. Those performed at concentrations more comparable with the ones found naturally are of especial interest in connexion with the new suggestion of feeding migrations.

Conclusions

Accepting then that patches of phytoplankton, of a comparable order of concentration to those in the tubes, occur commonly in the sea and that they are of the sizes indicated, what would be the consequences if the animals behave in the sea as they do in the various pieces of apparatus?

First it may be deduced that the hypothesis of animal exclusion cannot be of such universal application as was originally envisaged. The excluding effect was thought to be due to any concentration of phytoplankton (particularly diatoms) and to be of general and constant occurrence; affecting the vertical migrations of the great majority of animals in direct proportion to the concentration of plants. None of the diatoms used in the experiments has shown any toxic or excluding effect (with the exception of the highest concentration of *Nitzschia*). Such an excluding effect as has been shown is restricted to fairly high concentrations of two kinds of flagellates. This has, however, involved an active avoidance in the horizontal tubes, and what would amount in the sea to a passive avoidance, in the vertical tubes. It has been suggested by Hardy and by Lucas (personal communications) that exclusion might be mediated by only some specialized diatoms or flagellates. If that should be so and the responsible organisms should be of the sort found in the present work, then, in the mixed concentrations found in the sea, the excluding effect of the few would constantly be pulling in the opposite direction to the attractive effect of the majority of phytoplankton forms. There is insufficient evidence available on the distribution of flagellates in the sea for it to be stated firmly whether or not the widespread inverse relationships found could result from the wide occurrence of some particular toxic flagellate but this would seem to be a very unlikely mechanism. Indeed, Hart (1942) makes it clear that nanoplanktonic forms are not nearly so abundant in the Antarctic plankton as elsewhere and that the latter may often be almost purely diatom.

It would seem most consonant with the results to suggest that the exclusion mechanism as a means of producing the inverse relationship is of much more restricted occurrence. It could well be envisaged as operating where there are intense, often monospecific, blooms of some toxic flagellate, as for example *Goniaulax*. Under these conditions vertical migration up into the patch could easily be suppressed and there could also be an active horizontal migration away from the area by such animals as are equipped to do this.

This denudation of animals would allow unsuppressed division of the flagellate and the development of an even more repellent and larger toxic area. The marked avoidance by mysids of the concentrations of bacteria is of interest in this context. One of the effects of toxic blooms is to cause a concentration of dead fish and other animals in the area (Gunther, Williams, Davis & Walton Smith, 1948; Hornell, 1917). These decompose and must result in bacteria-rich water at least as concentrated as that used in the experiments. These regions will then be avoided by zooplankton forms, and this reaction should be classed as an exclusion effect.

Pure blooms of toxic organisms are however a rather specialized case, and such flowerings as do occur must frequently contain an admixture of attractive forms. Under these circumstances the reactions of animals must depend upon both the relative concentrations of the different forms and upon the physiological state of the animals themselves, and may be quite variable. Harvey (1937) has demonstrated what is apparently a selection in the feeding of *Calanus*. If this should occur in the sea, and acceptable forms should be taken in preference to harmful ones in such mixed patches, then the proportion of toxic organisms to others would be automatically and gradually increased until, perhaps, an almost monospecific bloom capable of excluding animals resulted. Such selective grazing could not account for blooms of organisms other than toxic or unaccepted forms.

The most normal condition in temperate seas, however, must be the occurrence of patches of phytoplankton which, upon all the evidence available, will be both acceptable and attractive to the animals. If the principles of migration which have become apparent during the experimental work obtain also in the sea, then there must be a gradual accumulation of animals in areas rich in phytoplankton. This will be effected in two ways, that is by vertical and by horizontal migration.

Hardy has developed the conception of, as he terms it, 'planktonic navigation'. The basis of this hypothesis is a variable vertical migration. It is well known that the water layers at different depths travel at different speeds and a vertically migrating animal must, if altering its behaviour in different areas and depths of water, automatically alter its position relative to a fixed point on the surface. Animals can thus by swimming up more, or less, in particular areas collect in these or avoid them. This valuable idea is fully dealt with in Hardy & Gunther (1935, pp. 343–56). The principle does not require volition on the part of the animal and must, without any doubt, work continuously so long as the animals indulge in vertical migrations and so long as these alter according to the nature of the surrounding water. It is precisely such an alteration in behaviour which is demonstrated in the vertical experiments, but the alteration is directly opposite in sign to that originally postulated for the majority of animals by Hardy. The mechanism of navigation must still obtain, however, and must necessarily result in a gradual accumulation of

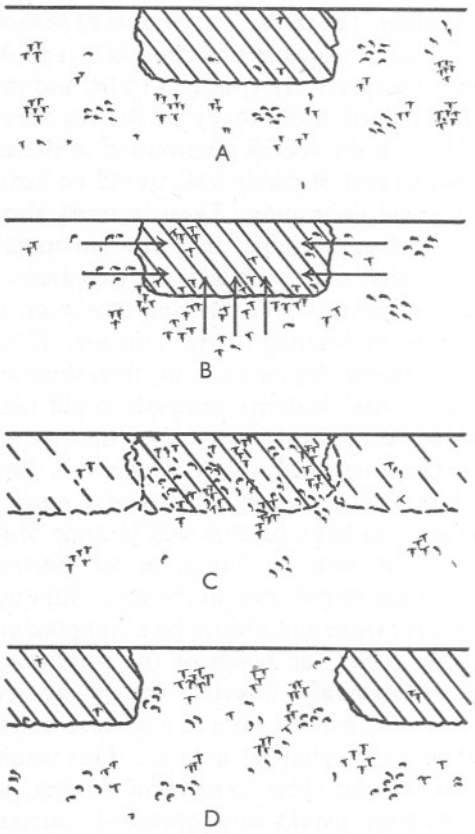
animals in diatom-rich areas and a denudation of the neighbouring less rich areas. Indeed three species—*Calanus simillimus*, *Drepanopus pectinatus* and *Antarctomysis maxima* are cited by Hardy as behaving in this opposite manner and being attracted towards phytoplankton. These he regards, however, as interesting but special cases.

This migration into phytoplankton patches will proceed also in the horizontal plane. It is known that *Meganyctiphanes norvegica* is able to swim vertically upwards at about 128 m./hr. for periods of several hours (Hardy & Bainbridge, 1951) and to be capable of bursts of 271 m./hr. (unpublished). These figures represent respectively 1 mile in 13 hr. and in 6 hr., and if such speeds should be maintained horizontally in the sea they must necessarily have considerable effect on the spatial distribution of the animals concerned. Certainly no more effort, and probably less, would be involved in horizontal as compared with vertical swimming. Those animals then, already named, which are able to travel appreciable distances horizontally will gradually move up any gradients that may exist into the rich areas. It has been suggested that such a mechanism could take place only on a very small scale because of the lack of any orientating factor in the sea. If it should depend on random horizontal movements by the animals, then their area of search must of course be greatly reduced and the principle could not be of much importance relative to some of the large patches that we know to exist. If, however, there is some orientating factor which will draw the animals on constantly in one direction, then they will cover a much greater area, and their movements relative to large patches will become of importance. Such an orientating factor may well be found, in the northern and southern latitudes, but not in the equatorial ones, in the sun. Although daily traversing the sky from east to west there will always be a component in its light which is predominantly from either the north or the south depending upon the hemisphere. Such a horizontally directive component, even if very small, could in the otherwise uniform sea, impose a general trend in one particular direction on the aimless excursions of animals. This would greatly increase their exploratory powers and their chances of finding, and remaining in, areas rich in phytoplankton would consequently be increased. The strange little piece of swimming behaviour observed in those small copepods that came up to the surface and then moved along horizontally may easily be a representation on a small scale of the sort of behaviour we are envisaging. In any case, whether directive or random movement be operative, there are patches of phytoplankton of sizes conveniently within the reach of all zooplankton forms.

This gradual accumulation of animals in the rich areas will result in an increase in the intensity of grazing there. This will have little effect on the actual concentration of plant cells present until the rate of removal exceeds the rate of increment by cell division. When this happens the whole relation-

ship will change rapidly. In the matter of a day or two, or even hours, depending upon the numbers of animals present, the plant cells will be grazed down to a negligible number.

In the meantime, the neighbouring areas of sea will, by the departure of the zooplankton, have acquired those conditions necessary for a fresh outburst of diatoms to take place. This will be more or less uninhibited and will quickly



Text-fig. 4. Scheme of grazing and migration cycle. A, initial state with inverse relationship B, start of migration with some grazing; C, completion of migration and heavy grazing; D, reversal of initial state and return to inverse relationship. Oblique hatching represents concentrations of phytoplankton.

reach high concentrations. The situation will thus be reversed and the animals will again be inversely distributed in relation to the plants but a new cycle of migration, grazing and growth will immediately be initiated. This is shown diagrammatically in Text-fig. 4.

The speed with which this cycle of events will take place must depend on several variables including the densities of animals and plants, the speeds of

migration, and the rate of division of the plants and of grazing by the animals. These are all factors to which definite values can be ascribed and it should eventually be possible to describe such a cycle mathematically. This is not however within the scope of the present work. The only necessary assumption is that the rates of migration must not be too high as compared with the rate of division of the phytoplankton, or otherwise a perfectly uniform sea would result and any increase in phytoplankton would be eaten down before developing noticeably. The small, when regarded absolutely, but relatively large difference in the proportions swimming up in the vertical tubes would seem in this connexion to be reasonable in extent; while an all or nothing reaction with a threshold value of a fairly high phytoplankton concentration would suffice as regards horizontal movements. Further, if there should be a lag in the movements of the animals then, starting from the slightest inequalities in distribution, these will oscillate with gradually increasing amplitude until the largest possible differences come into being.

The evidence at present available would seem to be more in accord with this hypothesis than with the others so far put forward. The inverse relationship in the sea is never absolute, often not clearly marked and sometimes completely reversed. This latter state, where high concentrations of both animals and plants occur in the same area, cannot be explained on the exclusion hypothesis, nor would it be expected on a simple theory of grazing. A dense concentration of animals and a dense concentration of plants are mutually incompatible entities. Their occurrence together must produce a situation of instability. The possibility of their having grown up together is surely much less likely than that of the animals having only recently come into the area, and being at that point in the cycle immediately prior to overcoming the rate of division of the plant cells. This state of distribution, the more common reverse one, and the intermediate ones can all be accounted for on a combination of migration and grazing, as can also the various experimental observations.

Hardy & Gunther (1935) found three species directly correlated with high concentrations of phytoplankton. Two of these (*Calanus simillimus* and *Drepanopus pectinatus*) were species which showed a most marked vertical migration, coming up to the surface before, and remaining there longer than, most other forms; and the third was a mysid (*Antarctomysis maxima*). It would now seem possible that this positive correlation occurred because these three forms are such active swimmers and have well-developed powers of navigation and migration. They can perhaps collect in regions of high phytoplankton density more readily than other forms and, their total numbers being insufficient to produce a marked grazing effect, they would therefore more often be found directly correlated with the plants than other forms.

Lucas's (1936) results, which might at first seem incompatible with the new hypothesis, can in fact be interpreted quite simply. He found a greater

number of animals visible in the unshaded portion of half-shaded tubes when no diatoms were present than when the water was rich in diatoms. This he ascribes to the production by the diatoms, in sunlight, of some substance distasteful to the animals and the consequent avoidance by the latter of this part of the tube. An equally reasonable explanation is that the mysids, generally negatively phototropic in captivity (Tattersall & Tattersall, 1951), swim away from the light in the enriched tubes where they are well fed, while in the unenriched tubes they are continuously making excursions out of the shaded part in search of food and consequently more are counted here.

Fleming (1939) shows mathematically that any large increase in grazing associated with an increase in the number of grazers will reduce the diatom population very rapidly and that similarly the development of dense diatom populations can only take place when the grazing is less than the production of new cells. He regards such increases and decreases generally as due to reproduction and death of the animals but remarks that similar results would be obtained by 'the intrusion of an outside population'.

When currents in a particular region are sweeping on undeflected for large distances, the theoretical effect of navigation as postulated by Hardy is to form the animals into long straight lines (Hardy & Gunther, 1935, p. 351). This aggregation he takes to occur in regions of poor phytoplankton, but the argument can as well apply to the formation of long patches or belts either within, or more probably at first, along the edges of regions rich in phytoplankton. These concentrations of animals will imprint, by grazing, a similar banded pattern upon the phytoplankton; both eating out bands and leaving bands of water free for fresh outbursts. Such a system, distorted and broken occasionally by cross-winds, could account for the prevalence in oceanic areas of long narrow patches of phytoplankton which have been observed so repeatedly.

The essential feature of this migration hypothesis is the dynamic and fluctuating property which it attributes to distributions in the sea. Harvey's hypothesis of grazing pictures the whole process as rather rigid and automatic with concentrations of zooplankton growing and breeding in one body of sea and naturally precluding any outburst of diatoms there; while this will occur unrestrictedly in other areas. Hardy's hypothesis pictures a more or less rigid pattern of phytoplankton distribution imposing, by its exclusive powers, an inverse pattern upon the animals. The migration hypothesis, on the other hand, pictures a constant fluctuation and movement of both animals and plants relative to each other; the former constantly searching for the latter in an endless cycle of horizontal and vertical migrations, and the latter constantly flowering up when the pressure of grazing is eased. Such a picture, although less precise, would seem more likely to be true of living organisms in an environment so unrestrictive to movement as the sea.

The attribution to some of the commonly termed 'planktonic' animals, of

considerable powers of horizontal migration need cause little concern. If a definition such as Bigelow's[1] is accepted, then it is clear that these animals should simply be referred to the already existing category of nekton.

The idea of a directive swimming by plankton animals is by no means new. No less an authority than Gurney says (1924, p. 39): 'It seems likely that the larvae of Decapods and Euphausids are not so much at the mercy of the currents as might be supposed. It is not very unusual to find swarms of the larvae of one species in different stages of development, which seems to indicate a power of keeping together from hatching onwards, or of collecting in a suitable locality'; and 'Some species seem to have decided preferences for certain regions, if one may judge from the behaviour of the British species of *Leander*. Quite early stages of the larvae are taken singly or in small numbers near Plymouth Sound, but later stages are exceedingly rare in in-shore waters, although the adults are abundant. But as the time for transformation approaches, the larvae seek the shore....Larvae are no doubt frequently carried by currents out of their normal habitat, and Foxon has given the examples of *Porcellana macrocheles* and *Calappa marmorata* which are sometimes so carried by the Gulf Stream to the shores of New England, but I believe that this is exceptional, and that the larvae for the most part have power to control their movements.'

Russell (1927) speaks of encountering a swarm of *Corystes* zoeas which was plainly visible from the ship; while 6 weeks later a swarm of *Corystes* megalopas was encountered in the same locality, and he considers the possibility of it being the same one. The megalopas, interestingly enough, were restricted to an area rich in *Rhizosolenia*. Macdonald (1927), in an account of the food and habits of *Meganyctiphanes norvegica*, speaks of congregations of both this form and *Thysanoessa raschii* in the Cumbrae Deep, on occasions when there was much detritus in the water, and also when *Calanus finmarchicus* was abundant. The euphausids were feeding upon these two, and the clear implication is that they migrated to and remained swarming in this place only while the food was available. Macdonald says he is not suggesting that the vegetable detritus or *Calanus* attracted the *Meganyctiphanes* to this particular locality, but the possibility of this having occurred is more plausible than that of their concurrence being fortuitous. He reports Bigelow as giving an account of a similar swarming of *Meganyctiphanes* in the neighbourhood of a sardine factory from which refuse was being dumped.

Euphausids can, especially in the Antarctic, be completely herbivorous. The likelihood of their migrating into areas rich in diatoms is surely high, and a similar activity on the part of other vigorously swimming forms also

[1] Bigelow (1924): 'By *plankton* we understand all such forms as float or swim freely in the water, but which, however active, are unable to carry out voluntary horizontal journeys of any extent, though certain of them perform considerable vertical migrations under the directive influence of sunlight or of some other physical stimulus.'

plausible. This, combined with the preferential vertical migration of the remaining organisms into areas rich in food, must automatically result in such a cycle as we have postulated; and the continued occurrence of this would give just such a degree of inverse relationship to catches of animal and plant plankton as is, in fact, found in nature.

Summary

An apparatus in which it is possible to observe the horizontal migrations of zooplankton in gradients of phytoplankton is described, and an account given of its use in experiments involving various organisms. A preliminary search for toxic organisms is also described.

The experiments comprise a demonstration of a migration by various animals into concentrations of the diatoms *Skeletonema*, *Thalassiosira*, *Biddulphia*, *Nitzschia* and various mixed cultures and the flagellates *Chlamydomonas*, *Peridinium*, *Dicrateria*, K, and *Oxyrrhis*. Cultures of *Rhodomonas* and *Gymnodinium* II are found to produce the opposite effect, and no reaction towards *Lauderia*, *Coscinodiscus*, *Eucampia*, *Syracosphaera* and *Exuviaella* could be shown. Results with bacteria cultures and various inorganic gradients are also discussed.

A second apparatus in which it is possible to observe the vertical migrations of zooplankton in the presence and absence of phytoplankton is also described, and an account given of experiments in it involving various organisms.

The experiments comprise a demonstration that greater numbers of animals swim up in the presence of mixed phytoplankton cultures, *Coscinodiscus*, *Skeletonema*, *Ditylum*, *Chlamydomonas*, *Gymnodinium* I and II and *Oxyrrhis* than in unenriched water. Flagellate One has no effect and *Chlorella* possibly depresses the number swimming up.

Under-water observations on the swimming movements of some of the animals used are described.

The possible application of the observations towards resolving the problem of the inverse distribution of phytoplankton and zooplankton in the sea is discussed, and the hypothesis is proposed that this may be accounted for by a combination of migration and grazing. It is considered that plankton animals must migrate both horizontally and vertically into patches of phytoplankton and, when present in sufficient numbers, graze these down very quickly. In the meantime fresh growths of phytoplankton will have occurred in neighbouring areas of sea now devoid of animals and the inverse relationship will be re-established. This changing cycle of distribution is thought to be continuous.

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Appendix I. List of Phytoplankton Organisms Used

Class	Order	Family	Name or number	Size (in μ)	Remark
Chrysophyceae	Chrysomonadales	Prymnesiaceae	Flagellate 3	5–6	Gold, 3 flagella
			Flagellate 4	6–12	Gold, 3 flagella
			Flagellate 10	5–7	Gold, 3 flagella
			Flagellate 25	8 × 5–10 × 6	Deep gold, 3 flagella
			Flagellate K	3–6 diam.	Deep gold, 3 flagella
			Flagellate Lancelot II, Clone 8	5–8	3 flagella
			Flagellate L 4 (IV), Clone 3	5–8	3 flagella
			Flagellate L 4 (IV), Clone 6	5–8	3 flagella
			Flagellate Lancelot I, Clone 4	3–7	Gold
			Flagellate L 4 (13/4) 2 L, Clone 1	3–6	—
			Flagellate L 4 (4/5), Clone 3	5–8	—
		Chromulinaceae	*Chromulina pleiades*	3·5–6	(Flagellate E)
			Chromulina pusilla	1·5–2	—
		Ochromonadaceae	*Pavlova gyrans*	3 × 4–6 × 10	—
		Coccolithophoridaceae	*Coccolithus huxleyi*	5–7	Milky coloured
			Syracosphaera carterae	12–14	Brown
		Isochrysidaceae	*Isochrysis galbana*	5 × 2–6 × 4	(Flagellate I)
			Dicrateria inornata	3–5·5	—
		Cyrtophoraceae	*Pseudopedinella* I	6 × 6–10 × 10	Pale gold
		Chrysocapsaceae	*Phaeocystis pouchetii*	1–2 mm.	Golden brown. Colonies of various shapes
		Unplaced	Flagellate 1	3–5	Pale gold
			Flagellate 5	3–5	Gold
			Flagellate 22	3–4	Deep gold
			Flagellate J	6–8 long	Gold
			Flagellate A	4–6	Gold
			Flagellate Lancelot I, Clone 1	2–4	Gold
			Flagellate Lancelot I, Clone 5	3–5	Gold
			Flagellate Lancelot I, Clone on 19/5	4–6 m.	Mixed with *Pyramimonas grossii*
			Flagellate Lancelot II, Clone 10	2·5–4	—
			Flagellate L 4 (13/4) 2 L, Clone 6	3–6	Mixed with a cryptophycean red 5–8 μ
Cryptophyceae	Cryptomonadales	Nephroselmidaceae	Flagellate 6	4 × 2·5–8 × 4	Red and bean-shaped
			Flagellate 6*a*	Slightly larger than Flagellate 6 but may be same species	
			Hemiselmis rufescens	4–8·5 diam.	—
		Cryptomonadaceae	Flagellate 23	15–25 long	Red
			Flagellate 23*a*	Slightly smaller than Flagellate 23 but may be same species	
			Flagellate 14	5–8	Red
			Flagellate 16	6–8	Red. Tailed
			Flagellate 19	6–10	Red. Tailed
			Flagellate 20	12 × 6–20 × 9	Red
			Flagellate 21	15–25 long	Red
			Flagellate 29	15–20	Red
			Flagellate Lancelot No. 16 (250 ml.), Clone 3	15–20	Red
			Flagellate L 4 (13/4) 2 L, Clone 6	5–8	Red. Mixed with 3–6 μ chrysophycean
			Flagellate 'Gross's μ'	—	—
			Rhodomonas sp.	—	Red. Mixed with *O. marina* and *N. closterium*
		Unplaced	Flagellate L 4 (13/4) 2 L, Clone 5	5–8	Red
Desmokontae	Desmomonadales	Prorocentraceae	*Exuviaella* I	10–18	—
			Exuviaella baltica	9–15 long	—
			Prorocentrum micans	30–40	Golden brown
			Prorocentrum triestinum	8–14	Golden brown
		Desmomonadaceae (?)	Flagellate 12	10–15	Golden brown. Probably a primitive form

Appendix I (*continued*)

Class	Order	Family	Name or number	Size (in μ)	Remarks
Dinophyceae	Gymnodiniales	Pronoctilucaceae	*Oxyrrhis marina*	10–30	Colourless. Mixed with *Rhodomonas* sp. and *N. closterium*
		Gymnodiniaceae	*Gymnodinium* I	6–20	Brown
			Gymnodinium II	6–22	Brown
			Massartia rotundata	12–14	Brown
	Peridiniales	Peridiniaceae	*Peridinium trochoidium*	20–30	Reddish brown
Chlorophyceae	Volvocales	Chlamydomonadaceae	*Chlamydomonas* III	6×4–12×8	Green
			Platymonas apiculata	7–10	Green
		Polyblepharidaceae	*Pyramimonas* sp.	6–8	Green
	Chlorococcales	Chlorellaceae	*Chlorella stigmatophora*	4–6	Green
	Ulotrichales	Ulotrichaceae	*Stichococcus* I	$3–5 \times 2$. Rods	Probably *S. cylindricus* Butcher
Bacillariophyceae	Centrales	Coscinodiscaceae	*Coscinodiscus concinnus*	150–450 diam.	
			Skeletonema costatum	7–16 diam.	
			Thalassiosira gravida	17–62 diam.	
			Thalassiosira nordenskiöldii	12–43 diam.	
			Lauderia borealis	34–38 diam.	
		Biddulphiaceae	*Biddulphia sinensis*	120–240 long	All the diatom cultures appear brown to a greater or lesser extent
			Ditylum brightwellii	25–60 diam.	
			Eucampia zoodiacus	25–73 broad	
		Chaetoceraceae	*Chaetoceros decipiens*	12–78 broad	
	Pennales	Fragilariaceae	*Licmophora lyngbyei*	50–75 long	
		Naviculaceae	Naviculoid	*c.* 100 long	
		Nitzschiaceae	*Nitzschia closterium*	20–90 long	
			Nitzschia closterium (forma *minutissima*)	25–35 long	Containing some of the 'tri-radiate' form
			Nitzschia seriata	100×6	

Appendix II. List of Zooplankton Organisms Used

Crustacea
- Branchiopoda
  - Anostraca
    - *Artemia salina.*
- Copepoda
  - Eucopepoda
    - Calanoida
      - *Calanus finmarchicus*, *Eurytemora hirundoides*, etc.
    - Harpacticoida
      - *Tigriopus fulvus.*
- Malacostraca
  - Mysidacea
    - *Hemimysis lamornae*, *Praunus neglectus*, *P. flexuosus*, *Neomysis integer*, *Mesopodopsis slabberi.*
  - Euphausiacea
    - *Thysanoessa inermis.*
  - Decapoda
    - Various larvae.

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OBSERVATIONS ON THE BREEDING AND SETTLEMENT OF *MYTILUS EDULIS* (L.) IN BRITISH WATERS

By P. N. J. Chipperfield

Liverpool University and I.C.I. Marine Paints Research Station, Brixham

(Text-figs. 1–8)

The literature on *Mytilus edulis* is particularly extensive. The most comprehensive account yet published is that of Field (1922), who gives excellent descriptions of the anatomy, physiology and embryology, as well as the bionomics, of this species. White (1939) adds little additional information upon the breeding and growth of *Mytilus*. Pelseneer (1935) gives an extensive bibliography covering both breeding and growth, whilst of the more recent work upon the breeding behaviour, the contributions of Battle (1932), Whedon (1936) and Young (1942) are most important. The occurrence of larvae in the plankton has been described in detail by Thorson (1946) and others; Visscher (1927), Orton (1933), Kändler (1926) and many others have given much information upon the spat-fall and settlement in several localities.

In spite of this wealth of published data there still remains considerable doubt over the time of onset and the duration of the breeding period of *M. edulis*, especially in relation to environmental factors. In particular, few recent investigations on *Mytilus* from beds in the sheltered and estuarine areas of the British coasts have been published, whilst the conclusions to be drawn from the older work (Herdman & Scott, 1895; Johnstone, 1898; Scott, 1900, etc.) are conflicting and lacking in precision. Finally, no attempt has previously been made to investigate the breeding, settlement and growth of this species simultaneously in a number of distinct geographical localities. This was the aim of the investigation, described below, which was commenced in 1946 as part of a general investigation of the breeding, settlement and growth of a number of common sessile littoral animals, and which is still in progress.

The author is indebted to Mr J. Baird of the Mussel Purification Station, Conway, to Mr J. Porteous of Portree, and to Mr R. Chestney of Brancaster for their invaluable assistance in supplying material regularly throughout the investigation. In particular, the author is indebted to the late Prof. J. H. Orton, F.R.S., for generously given advice and criticism, and to the Directors of Imperial Chemical Industries, Paints Division, under whose auspices the investigation was conducted.

Materials and Methods

The major part of the investigation was completed in 1946 and 1947. In the former year, samples of at least fifty specimens of adult *M. edulis* were obtained every two weeks from Liverpool (Prince's Landing Stage pontoons); Plymouth (Marine pier); the Blyth Estuary, Northumberland; Ramsey, Isle of Man, and also from one of the outdoor sea-water tanks at the Marine Biological Station, Port Erin, Isle of Man, where a supply of *Mytilus* taken originally from Ramsey was kept. In 1947, weekly (and sometimes more frequent) samples of at least 100 individuals were examined during the spring and early summer. Some of these were obtained from Liverpool, Port Erin and Ramsey as before, but the bulk of the material was obtained from Loch Portree, Isle of Skye; Conway, from the beds below the Benarth Road; and Brancaster Staithe (north Norfolk), from the Creek. Only one sample was received from Plymouth in 1947. Owing to the poor physiological condition of the *Mytilus* obtained from the Blyth Estuary during 1946, samples from this source were discontinued in the following year.

In subsequent years, the spawning of *Mytilus* was investigated at Brixham, South Devon, whilst the settlement and growth was followed closely at Liverpool in 1946 and 1947, and at Brixham in 1948, 1949 and 1950 under intertidal and continuously submerged conditions. Some observations on settlement were also made at Ramsey, Conway, Blyth, Portree, and Brancaster in 1946 and 1947, and at the River Dart in 1950.

Wherever possible, records of air and sea temperature were kept, and in a few localities salinity samples or records of salinities were also obtained.

The stages of gonad development were assessed from the colour and thickness of the mantle, and the degree of development of ova and sperm ascertained from the microscopic examination of smears. In some cases, sections of the mantle were prepared in order to establish the criteria of each of the five stages of development, described below.

Settlement of the spat and its subsequent growth were followed by observations upon the density and size of young *Mytilus* clustered upon the byssus threads of adults, or from the examination of non-toxic panels exposed for the purpose at Liverpool (1946–7) and Brixham (1948–50). Further information was obtained by the examination of submerged and intertidal substrata where and when opportunity arose.

Anatomy and Development of the Gonads

In *M. edulis* the sexes are separate, although in the early stages of development it is difficult to distinguish between them. In both sexes, the reproductive system consists of many branching ducts, which ramify throughout the mesosoma or 'abdomen' (see White, 1939, p. 33) and the mantle, and which, in a well-developed specimen, frequently spread into the connective tissue

between the other internal organs, and over the digestive gland. In the mantle, the gonads extend as far as the pallial muscle.

Outgrowths from the ducts terminate in follicles, in which ova and sperm are produced. In males, the follicles are more uniform in size than those in the female. In both they are extremely numerous.

There are five principal gonoducts in the mantle and these converge on each side below the pericardium. From this point, the main genital canal, on either side, runs to the corresponding genital papilla, which is situated immediately anterior to the posterior adductor muscle. Field (1922, pp. 182 *et seq.*) gives a full description of the morphology and histology of the gonads.

Stages of Gonad Development

The development of the gonads was referred to five arbitrary, but readily distinguished, stages, including a 'resting-spent' stage in which no trace of sexuality is apparent, as follows:

Stage O. Neuter or resting spent stage. No follicles in mantle tissue.
Stage I. Onset of gametogenesis and appearance of follicles in the mantle.
Stage II. Follicles well developed and filled with unripe ova and sperm.
Stage III. Ova ripe and capable of fertilization, sperm activated by sea water.
Recently spent stage. Most of follicles empty; a few relict ova and sperm present only.

Stage O. The mantle characteristics vary according to the amount of reserve food material stored therein. When 'fat' the mantle is thick, yellow-cream or buff in colour, and of a very smooth appearance. It contains abundant glycogen and considerable fat. No ova or sperm can be seen and the genital ducts are usually obliterated by the great growth of connective tissue containing the reserve food products.

In starved specimens, or those in poor 'fattening' areas, where little accumulation of glycogen occurs, the neuter mantles are thin and semi-transparent, and in these the onset of gametogenesis is more easily distinguished.

Stage I. This stage includes all specimens showing the first signs of gametogenesis. In this stage, the ovarian and testicular follicles can be distinguished in the mantle tissue. Regeneration of the follicles commences at the outer face of the mantle, forming a single layer.

In the male follicles, only sperm mother cells and spermatids are present, whilst in the female, the oocytes are small and few have budded from the germinal epithelium.

In this early stage of the development, the colour of the mantle in each sex

varies considerably, depending on the degree of development of the follicles and the amount and distribution of residual glycogen in the connective tissue. The female mantle is usually a red-brown or orange, and the male a light orange upon which background the small, but opaque follicles are readily apparent.

Stage II. In this stage well formed but unripe spermatozoa and ova are present in the follicles, which are well developed and prominent. Owing to wide variations in the nutritional condition of *Mytilus* from different localities, the thickness of the mantle (i.e. the number of layers of follicles) cannot be taken as a criterion of development. At this stage, however, the colours of the male and female mantles are distinct, owing to the mass of sperm or ova in the follicles. The male mantle has a brownish ground colour, which is almost obscured by the opaque white follicles. The female mantle is reddish orange or amber in colour, although the presence of ova bestows upon it a distinct apricot hue. Owing to the smaller size and more irregular distribution of the follicles, the female mantle appears to have a much smoother texture than the male mantle.

Stage III. Morphologically stage III is similar to stage II, except that the mass of the gonad is greater, and the connective tissue lying between the follicles in the mantle is even more occluded, although great variability occurs in material from the several localities, presumably owing to differences in nutrition. The general colour of the stage III female mantle is a very definite apricot, due entirely to the colour of the ova. The stage III male mantle is usually a full cream, or a yellowish cream in colour. The colour changes at maturity occur in individuals with mantles of any thickness. Thus within certain limits maturity appears to be independent of nutritional conditions, and ripe gametes can be obtained from poorly nourished individuals with thin mantles, whilst individuals with very thick mantle walls may yet contain unripe ova and sperm (see Young, 1945).

The ripeness of the ova and sperm in these mature individuals was determined from observations of their behaviour in sea water, and whether artificial fertilizations could be induced. The spermatozoa, when ripe, appear to be activated by sea water, whilst unripe sperm remains immotile. In no case has motile sperm been observed within the testicular follicles, and motility is induced solely by contact with sea water. Artificial fertilizations can be made with motile sperm only. Smears of the follicles of stage III males show few spermatids; thus it is clear that in *Mytilus*, as in *Pecten maximus* (Tang, 1941), a decline in spermatogenesis takes place as the gametes become mature. Occasionally individuals are found in which the gonoducts are filled with sperm; in smears of the follicles of these, no spermatids are found. The ripe sperm are arranged in lamellae converging towards the centre of the follicle.

In the stage III female, owing to the great development of the ovaries, the oocytes are of irregular shape. Within each oocyte is a large germinal vesicle containing a prominent nucleolus. The extreme growth of the follicles and the reduction in reserve food materials almost obliterates the interfollicular connective tissue. The ovarian egg possesses a distinct vitelline membrane about 1 μ in thickness, which readily bursts under slight pressure. The cytoplasm is very granular.

On being shed into sea water, the ripe oocytes rapidly assume a more or less spherical form (*c.* 0·068 mm. in diameter). During this process, maturation occurs, the germinal vesicle breaking down and the first polar body being budded off, the entire process occurring spontaneously in less than 20 min. at 12° C. Field (1922), however, states that fertilization is necessary before the first polar body can be budded off. Unripe oocytes do not round off in sea water, nor does the germinal vesicle break down.

The breakdown of the germinal vesicle in sea water was regarded as a criterion of the physiological maturity of the oocyte, and was used as such throughout this investigation.

Recently spent. Recently spawned individuals of both sexes are readily distinguished by the semi-transparent mantle and mesosoma (see Field, 1922), usually of a reddish brown colour, in which a few relict ova or sperm can usually be found. It is only the presence of these residual gametes which enables the sex of the spawned individual to be determined. When the gametes are absorbed—probably by phagocytosis—all trace of sexuality is lost, and in *Mytilus* from most localities glycogen is rapidly accumulated in the connective tissue. It is unusual for less completely spawned individuals to be found, except at such times when spawning is slow.

The process of spawning has been observed *in vitro*. In both sexes the gametes are passed between the valves of the shell to the exterior, via the exhalant aperture. Sperm is emitted in a continuous stream, whilst ova are aggregated into short rods, which break up as they reach the exterior. No flapping movements of the shell have been observed. Emission of the eggs and sperm normally continues for 30–60 min., after which time spawning is virtually complete, only a few residual gametes remaining in the follicles.

Spawning Behaviour in Relation to Environmental Conditions

Data obtained from samples examined during the spawning investigations carried out from 1946 to 1950 are given in Tables I–VII for Liverpool (1946, 1947), Plymouth (1946), Conway (1947), Portree (1947), Brancaster (1947), Ramsey (1947), and Brixham (1948, 1949), respectively. In order to assess clearly the state of sexual maturity in each sample, an 'index' or 'mean stage' of gonad development was calculated from the data, the number of individuals falling into each of the four developing stages (i.e. 'O' to 'III') being multiplied by a numerical factor equal to the arbitrary rating of the stage and the sum

of these products divided by the number of individuals in the sample. Thus, a weighted mean stage of development is obtained, which has a minimum

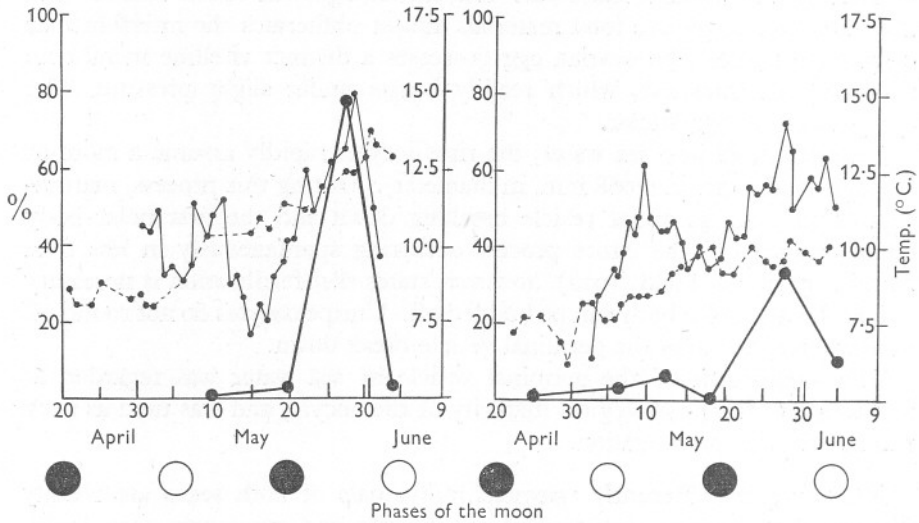

Fig. 1. Spawning of *M. edulis* at Conway in 1947, in relation to the lunar period and the sea and air temperatures, and the mean temperature to which the intertidal mussels were exposed during the spawning period.

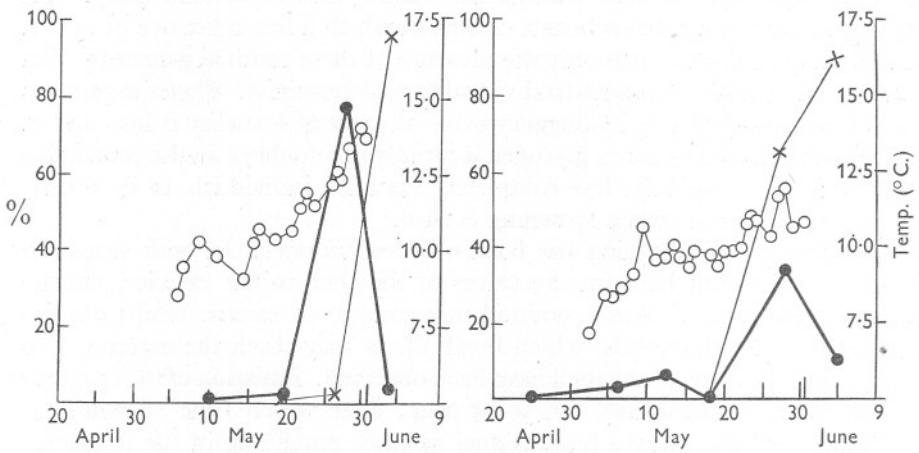

Fig. 2. Spawning of *M. edulis* from the intertidal zone at Portree, Isle of Skye, in 1947.

●——●, % in sample recently spawned; ×——×, % resting spent; •– – –•, sea temperature (daily mean); •——•, air temperature (daily mean to which mussels exposed); ○——○, mean of sea and air temperatures.

value of zero, and a maximum value of 3·0 when the entire sample is sexually mature and ready to spawn. The weighted mean stage of gonad development for each sample is given in the tables.

The proportions of spawned males and females and of 'resting spent' individuals in each sample are plotted against time in Figs. 1–4 for Conway, Portree, Liverpool, Brancaster, together with the lunar phases, and daily sea temperatures. Where intertidal material was employed, mean air temperatures are also plotted.

TABLE I. BREEDING OF *MYTILUS EDULIS* AT LIVERPOOL

Date of collection 1946	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % spawned	% Neuter	Total spawned and neuter	Index of gonad development
7. v.	75	48·0	25·3	1·3	49·3	26·7	2·7	4·0	2·7	6·7	2·39
17. v.	43	51·2	27·9	11·6	39·6	23·2	7·0	18·6	9·3	27·9	1·95
29. v.	50	52·0	6·0	46·0	22·0	4·0	18·0	64·0	26·0	90·0	0·30
13. vi.	45	2·2	0·0	2·2	4·5	0·0	4·5	6·7	93·3	100·0	0·0
27. vi.	31	0	0	0	0	0	0	0	100	100·0	0·0
16. vii.	43	0	0	0	2·3	0	0	0	97·7	97·7	0·023
28. viii.	29	0	0	0	0	0	0	0	100	100·0	0·0
16. ix.	33	16·1	0	0	21·4	0	0	0	63·6	63·6	0·36
22. x.	41	46·5	0	0	26·8	0	0	0	26·8	26·8	1·00
5. xi.	33	45·5	0	0	45·5	0	0	0	9·1	9·1	1·50
11. xii.	35	51·4	0	0	34·4	0	0	0	14·2	14·2	1·20
1947											
20. i.	19	36·8	0	0	63·2	0	0	0	0	0	1·31
27. ii.	25	40·0	0	0	48·0	0	0	0	12·0	12·0	1·08

TABLE II. BREEDING OF *MYTILUS EDULIS* AT PLYMOUTH

Date of collection 1946	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % spawned	% Neuter	Total spawned and neuter	Index of gonad development
16. v.	42	57·0	42·8	4·7	43·0	43·0	0	4·7	0	4·7	2·76
3. vi.	45	48·9	11·1	37·8	51·1	8·8	42·3	80·1	0	80·1	0·60
17. vii.	49	22·4	0	8·2	10·1	0	4·0	12·2	67·5	79·7	0·36
29. vii.	58	8·6	0	1·7	6·9	0	3·4	5·1	84·5	89·6	0·19
13. viii.	69	23·2	0	5·0	11·6	0	2·2	7·2	65·2	72·4	0·46
28. x.	54	9·3	0	0	9·3	0	0	0	81·4	81·4	0·26
1947											
7. v.	69	45·0	1·5	0	43·5	1·5	0	0	11·6	11·6	1·25

From Fig. 4 it will be noted that, for a short period before spawning commenced, material from Brancaster was obtained from two sources, viz. the Creek, and a mussel pond, filled at high water, in which mussels are stored for marketing. The *Mytilus* obtained from this latter source were collected from the Creek and placed in the pond when the gonads were beginning to mature, two weeks before spawning took place. Daily temperatures of the water in this pond were taken, and from Fig. 4 it is clear that a considerably more rapid increase occurred here than in the Creek.

TABLE III. BREEDING OF *MYTILUS EDULIS* AT CONWAY

Date of collection 1946	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
5. x.	99	36·3	0	0	51·5	0	0	0	12·1	12·1	0·95
23. xi.	102	52·8	0	0	46·1	0	0	0	1·2	1·2	1·16
10. xii.	48	45·9	0	0	43·8	0	0	0	10·3	10·3	1·83
1947											
27. i.	51	—	0	0	—	0	0	0	8·0	8·0	1·35
19. ii.	138	—	0	0	—	0	0	0	5·9	5·9	1·20
29. iii.	73	49·3	1·4	0	39·7	0	0	0	10·9	10·9	1·18
19. iv.	85	54·0	3·5	0	40·0	3·5	1·2	1·2	5·9	7·1	1·36
28. iv.	78	44·9	0	0	55·0	0	0	0	0	0	1·45
10. v.	107	51·4	5·6	0	48·6	1·9	0	0	0	0	1·59
20. v.	87	55·1	37·9	1·1	44·8	23·0	1·1	2·7	0	2·2	2·48
27. v.	84	41·7	0	33·3	55·9	0	46·4	79·9	2·4	82·1	0·35
3. vi.	110	0·9	0	0·9	1·8	0	1·8	3·6	97·2	99·9	0·0
16. vi.	97	0	0	0	0	0	0	0	100	100·0	0·0
1. vii.	165	0	0	0	0	0	0	0	100	100·0	0·0
15 vii.	83	0	0	0	0	0	0	0	100	100·0	0·0
28. vii.	57	—	0	0	—	0	0	0	91·5	91·5	0·9
14. viii.	40	12·5	0	0	7·5	0	0	0	80·0	80·0	0·20
24. ix.	57	26·3	0	0	22·8	0	0	0	51·0	51·0	0·65
1. x.	38	36·8	0	0	23·6	0	0	0	39·5	39·5	0·92
10 x.	50	38·0	0	0	48·0	0	0	0	14·0	14·0	1·13
31. x.	42	45·2	0	0	40·4	0	0	0	14·3	14·3	1·07
21. xi.	53	43·6	0	0	38·0	0	0	0	17·4	17·4	1·15

TABLE IV. BREEDING OF *MYTILUS EDULIS* AT PORTREE

Date of collection 1946	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
16. ix.	32	9·4	0	0	6·6	0	0	0	84·0	84·0	0·16
22. x.	54	13·0	0	0	16·7	0	0	0	70·3	70·3	0·36
27. xi.	46	50·0	0	0	47·8	0	0	0	2·2	2·2	1·23
1947											
8. ii.	21	52·4	0	0	38·1	0	0	0	9·5	9·5	1·19
19. iv.	66	42·4	27·3	0	57·5	30·3	0	0	0	0	2·57
25. iv.	121	49·6	31·4	0	49·6	38·0	0	0	0·8	0·8	2·58
7. v.	85	43·5	37·6	1·2	54·0	41·2	1·2	2·4	2·4	4·8	2·69
12. v.	109	48·6	27·5	4·6	51·4	25·7	1·8	6·4	0	6·4	2·46
19. v.	177	39·6	31·7	0	60·4	44·6	0	0	0·5	0·5	2·76
28. v.	96	17·7	2·2	15·6	17·7	1·1	16·7	32·3	64·5	96·8	0·94
4. vi.	90	5·5	0	5·5	5·5	0	5·5	11·0	89·0	100·0	0·0
25. vi.	201	0	0	0	0	0	0	0	100	100·0	0·0

Table V. Breeding of *Mytilus edulis* at Brancaster

Date of collection	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
28. iv.	144	53·5	12·5	0	43·0	6·25	0	0	3·5	3·5	1·70
5. v.	157	42·0	14·7	1·9	58·0	21·6	2·5	4·4	0	4·4	2·17
10. v.*	131	45·0	27·5	3·8	55·0	25·2	1·6	5·4	0	5·4	
14. v.*	119	53·0	5·0	44·7	39·5	6·7	30·9	75·6	7·5	83·1	
14. v.	128	51·8	28·1	0	46·8	26·6	0	0	1·6	1·6	2·55
19. v.	133	54·1	25·6	3·0	41·4	18·1	2·3	5·3	4·5	9·8	2·24
26. v.	124	39·6	8·2	31·4	43·6	6·5	37·1	68·5	17·0	85·5	0·28
4. vi.	216	42·6	0	42·6	41·6	0	41·6	84·2	15·7	99·9	0·0
14. vi.	210	16·4	0·9	15·5	8·6	0	8·6	24·1	75·3	99·4	0·03
30. vi.	150	0	0	0	0	0	0	0	100	100·0	0·0
15. vii.	119	1·5	0	0·5	3·0	0	1·5	2·0	95·5	97·5	0·04
6. viii.	43	14·0	0	0	9·3	0	0	0	76·7	76·7	0·23
26. ix.	127	19·7	0	0·8	15·0	0	0	0·8	65·4	66·2	0·34
28. x.	46	41·4	0	0	43·4	0	0	0	15·2	15·2	1·00
19. xi.	37	59·4	0	0	35·1	0	0	0	5·4	5·4	1·24

* From Mussel Pond.

Table VI. Breeding of *Mytilus edulis* at Ramsey (Isle of Man)

Date of collection 1946	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
18. vii.	40	15·0	0	15·0	12·5	0	12·5	17·5	72·5	100·0	0·0
30. viii.	49	0	0	0	0	0	0	0	100	100·0	0·0
12. ix.	100	14·0	0	0	7·0	0	0	0	79·0	79·0	0·26
6. xi.	48	6·2	0	0	20·8	0	0	0	73·0	—	0·33
2. xii.	40	15·0	0	0	15·0	0	0	0	70·0	—	0·53
1947											
3. v.	69	47·8	4·6	0	40·6	0	0	0	11·6	11·6	1·29
30. v.	48	20·8	0	20·8	12·5	0	12·5	33·3	66·6	99·9	0·0

Table VII. Breeding of *Mytilus edulis* at Brixham

Date 1948	No. in sample	% of sample: Female Total	Female Ripe	Female Spawned	Male Total	Male Ripe	Male Spawned	% Neuter	Total % spawned	% standard error of spawned individuals	Total % ripe
20. iv.	40	45	25	0	53	35	0	2	0	0	60
28. vi.	45	53	44	2	47	35	0	0	2	6·7	79
5. v.	58	47	35	4	50	41	5	3	7	3·3	76
12. v.	57	44	14	25	49	9	30	7	55	6·7	23
20. v.	58	36	9	22	41	5	29	22	51	6·6	14
1. vi.	42	14	2	10	17	2	10	69	20	6·2	4
16. vi.	40	25	0	0	5	0	0	93	0	0	0
1949											
7. iv.	25	44	28	0	56	44	0	0	0	0	84
14. iv.	51	47	39	0	53	41	0	0	0	0	80
21. iv.	36	56	39	3	44	30	3	0	6	4·0	69
29. iv.	75	43	27	11	52	33	16	5	26	5·1	50
3. v.	86	36	15	14	40	21	17	24	31	4·8	35
20. v.	41	29	2·5	22	37	5	24	34	46	7·8	7·5
25. v.	60	25	0	25	35	7	15	40	40	7·5	7·0
3. vi.	35	6	0	0	14	0	6	80	6	4·0	0

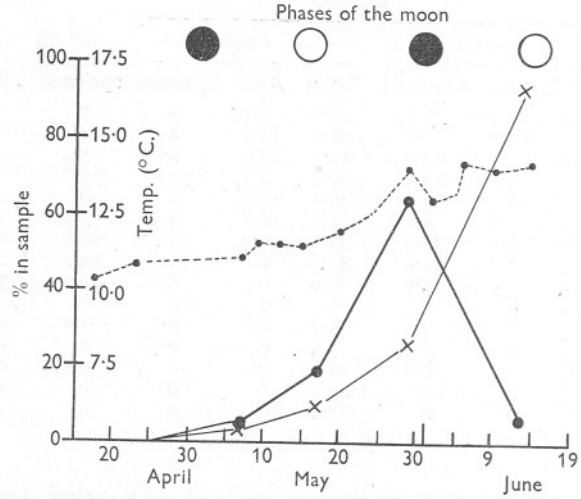

Fig. 3. Spawning of *M. edulis* at Liverpool in 1946. ●——●, % recently spawned; ×——×, % resting spent; ●– – –●, sea temperature.

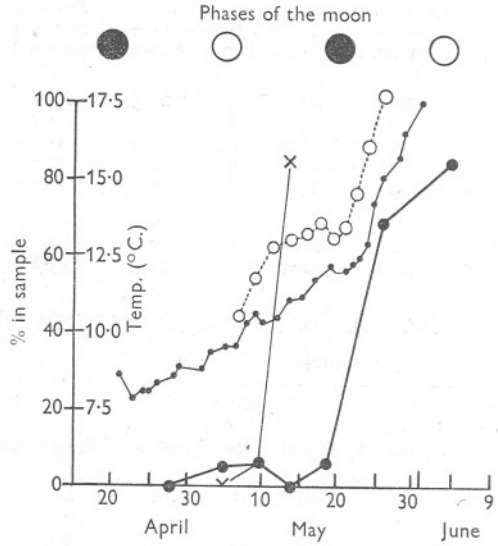

Fig. 4. Spawning of *M. edulis* at Brancaster Staithe, Norfolk, in 1947. ●——●, % recently spawned in creek; •——•, temperature in creek; ×——×, % recently spawned in pond; ○– – –○, temperature in pond.

The results for the material obtained from Blyth (1946) and for the material from the plaice tanks at Port Erin (1946) are not tabulated, as spawning had occurred before the first samples were obtained. In 1947 only two samples were obtained from Ramsey (Isle of Man), spawning having taken place

TABLE VIII. SUMMARY OF THE SPAWNING BEHAVIOUR OF *MYTILUS EDULIS*

Locality 1946	Date of commencement of spawning	Date of completion of spawning	Duration of spawning period (days)	Sea temp. range between spawning commencement dates (° C.)	Phase of moon at spawning and date	Approx. date of commencement of gonad ripening	Approx. temp. at commencement of ripening (° C.)
Liverpool	7. v.	13. vi	31	11·5–14·0	F.M. 17. v.	Mid April	8·5
Plymouth	16. v.–3. vi	3. vi	18	11·5–13·0	F.M. 17. v.		
Blyth Estuary	—	6. vi†					
Ramsay (I. of Man)	—	10. vi†					
1947							
Liverpool	19–27. v	6. vi	18	12·5–15·0	N.M. 20. v.	Early May	8·0
Ramsey	3–30. v	30. v	27	?	F.M. to N.M. 20. v.	Early May	
Brancaster (pond)	10–14. v	19. v	9	12·0–14·5	L.Q. 12. v.	Late April	8·0
Brancaster Creek	19–26. v	14. vi	26	12·0–15·5	N.M. 20. v.	Late April	8·0
Portree	19–28. v	4. vi	16	10·0–10·5 (9·5–12·0)*	N.M. 20. v.	Late March	6·5
Conway	10–14. v	19. v	9	11·5–13·0	N.M.	Late April	8·5
1948							
Brixham	5–12. v	1. vi	27		N.M.	March	9·0
1949							
Brixham	21–29. iv	3. vi	51	8·5–12·0	F.M. 13. iv. N.M. 28. iv.	March	6·5
1950							
Brixham	29. iv.–11. v	5. vi	37	10·5–13·0	F.M. 2. v.		
River Dart (Greenway)	28. iv.–18. v	18. v	21	10·5–13·5	F.M. 2. v.		

* Means of sea and air temperatures.
† Completed before this date.

between the dates of collection (see Table VI). Only small numbers of adult *Mytilus* could be found upon the pontoons of the floating landing stage at Liverpool in 1947, owing to severe storms during April, when the bulk of the population had been washed away. Observations were, however, made upon those which remained.

In Table VIII the spawning behaviour of *Mytilus* in the several localities is summarized, together with sea temperatures, etc.

In every case spawning commenced during the period late April–May and was completed within 3–4 weeks except at Brixham where a few (5%) ripe individuals were found a week or two later. Emission of ova and sperm during the first 7 days was always intensive, at least 70–80% of the population releasing its genital products (see Figs. 1–4). The remaining proportion of the population spawns during the ensuing fortnight or 3 weeks. No evidence of periodic spawning, nor of more than one discrete spawning period, was obtained in any locality during the 4 years of investigation.

Spawning, in each locality except Portree, commenced when the sea temperature had risen to 11·0–13·0° C. At Portree, however, the sea temperature (taken at high water) at the commencement of spawning was 10·0–10·5° C. If, however, the daily mean temperature to which the intertidal mussel bed was exposed during this period is computed from the maximum and minimum air temperatures, and the sea temperature, in relation to the times of high and low water, using a linear interpolation in estimating the air temperatures at the times of low water (where these do not correspond with the times of minimum and maximum air temperatures), it is shown that spawning commenced during a period when the daily mean temperature rose from 9·5 to 12·0° C. Similarly, spawning took place in the intertidal bed at Conway at the period when the daily mean temperature to which the bed was exposed rose from 10·5 to 13·0° C.

In the majority of the fourteen series of observations spawning was initiated during a period of spring tides, e.g. at Liverpool in 1946 and 1947, and Brixham in 1948 and 1949 after a new moon, and at Plymouth in 1946, and Brixham in 1949 and 1950, after a full moon. The mussels in the storage pond at Brancaster, however, spawned at a last quarter period, although those on the Creek beds commenced to spawn a week later, i.e. at new moon. Further, in each locality and in each year, spawning of *Mytilus* coincided with a period of predominantly bright sunny weather with little or no rainfall.

Period of Spawning

Of the many published accounts of the spawning period of *M. edulis*, two only refer to localities in which the present investigations were made, viz. Plymouth and the Isle of Man.

Matthews (1913) states that the spawning period of *M. edulis* at Plymouth in 1911 lasted from January until March, although samples which she took

between May and August suggested that spawning took place in early spring. However, Matthews found that artificial fertilizations could be made only in late May, which suggests that natural spawning, in that year, took place at this period. The results of the investigations in 1946 (see Table II) are in conformity with this suggestion, for few ripe individuals were observed later than May. Additional evidence of a spring spawning at Plymouth is given by Lebour (1938) who states that the veliger of *Mytilus* is a principal constituent of the plankton in late spring and early summer.

Bruce (1926) obtained mussels from Ramsey, Isle of Man, and kept them for experimental purposes in a shallow concrete tank of running sea water at Port Erin. He states that spawning occurred at the end of May, eggs and sperm 'running freely on May 27th, 1925'. The results for Port Erin tank material in 1946 and for Ramsey material in 1947 point to a May spawning (see Table VI).

The majority of the published records of the spawning of *M. edulis* in British waters are based upon observations of the presence of larvae in the plankton or of settlement, although a few are based upon observations of gonad development. These records, in the main, suggest April and May as the spawning months (cf. Table VIII). In the Barrow and Morecambe Bay area, Herdman & Scott (1895) found mature *Mytilus* in May. Working in the same area and employing microscopic examination of the gonads as well as observations upon the plankton and upon settlement, Johnstone (1898) describes rapid emission of genital products in July 1898, but suggests that spawning may begin in April, and concludes that spawning occurs throughout May, June, July and August. Scott (1900) reports spawning of females only in tanks at Piel, and upon the Barrow beds, in May 1899, the emission of ova continuing until 13 June, when the males also spawned. In more recent years, Daniel (1921) gives April and May as the spawning period in 1921, but as late March in the following year (a warm year). The heavy spat-fall in Morecambe Bay in June 1933, reported by Orton (1933), suggests a May spawning (cf. spawning at Liverpool in 1946 and 1947).

McIntosh (1891) gives March–May as the spawning period of *Mytilus* in the Tees, Esk and Humber, whilst Williamson (1907) concludes that March and April are the months during which maturity occurs on the Scottish beds. At Millport, Elmhirst (1923) describes the spawning period of *Mytilus* as April to June, although Pyefinch (see Harris, 1946) finds that the heaviest settlement takes place during August and September. In a more recent paper (Pyefinch, 1950), however, he modifies this view extensively, suggesting that the heaviest settlement occurs in June–July. It would thus seem that spawning at Millport normally takes place in late May or early June.

There are a number of references to the spawning of *Mytilus* upon the western sea-board of Europe. Spärck (1920) states that spawning occurs in the Limfjord in May and June, and Thorson (1946) describes the sudden

appearance of the veligers of *Mytilus* in late May or early June in the Øresund and the Isefjord. Kändler (1926), however, describes the occurrence of larvae of *Mytilus* at Heligoland over a very long period, although he states that a maximum occurs from April to June. Werner (1939), in reviewing the literature upon the spawning of North Sea lamellibranchs, suggests that *M. edulis* spawns from spring to autumn, and quotes in support the work of Havinga (1929) based upon the state of maturity of the gonads. On the other hand, Berner (1935), as the result of observations upon the gonads of *Mytilus* at Calais, states that the spawning period is February and March. The season of sexual maturity of *M. edulis* in the Gulf of Naples is stated by Lo Bianco (1899) to be March and April.

The spawning of *M. edulis* in the western hemisphere has been described by Stafford (1912), Field (1922), and Battle (1932). The former states that spawning takes place on the west coast of Canada in June, whilst Battle (1932) gives the spawning period as July to September in Passamoquoddy Bay. Field (1922) states that spawning commences on the Atlantic coast in April and continues until September. The work of Engle & Loosanoff (1944) suggests a principal spawning in late May in Milford Harbour, Long Island.

The past records of the period of spawning of *Mytilus* thus, in general, show considerable variation in the time of onset and duration, although a number agree with the observations described in this paper.

The Duration of the Spawning Period

In the present investigation the duration of the spawning period of *Mytilus* was followed on fourteen occasions. In eight of these, spawning was completed in 3–4 weeks (see Table VIII), in two in 9 days, whilst in two others (at Blyth and Ramsey in 1946) it was not possible to make an estimate of the duration of the spawning period. On the remaining occasions (at Brixham in 1949 and 1950) a few unspawned but ripe *Mytilus* (both males and females) were observed until 3 June in 1949, and 5 June in 1950, giving spawning periods of 51 and 37 days respectively. Observations at Brixham were too limited to enable the incidence of spawning to be followed during the spawning period in 1950, but in 1949 55% of the population had spawned during the first 14 days, whilst over 90% had completed spawning within 28 days from the commencement. It is possibly significant that, in both years, the sea temperature rose rapidly immediately prior to spawning, and then fell again, to rise slowly towards the maximum previously recorded. Further, the mean sea temperature at Liverpool in 1946 rose more slowly than in 1947, whilst the duration of spawning in the previous year was greater than in 1947 (31 days and 18 days respectively). The rapid spawning-out of the population at Conway and in the pond at Brancaster in 1947 may be associated with the rapid rise in temperature in those localities at the onset and throughout the period of spawning. It, therefore, seems possible that not only is the onset of

spawning determined directly or indirectly by a rapid rise in temperature, but that the rate of increase of temperature subsequently influences the rate of spawning of *M. edulis*, a rapid increase of temperature producing a higher spawning intensity.

Once spawning was completed, in no locality or year was any evidence obtained of rapid re-development of the gonads, leading to a later spawning. The later maturation of the gonads of smaller and younger individuals, described by Jensen & Spärck (1934) was not observed in the present investigation.

Thus, in the localities investigated, spawning is limited to a period of 3–4 weeks in the late spring, during which time the entire adult population releases its genital products. This appears to be true even of semi-starved individuals with thin poorly developed mantles (e.g. those from Blyth).

Many writers in the past have ascribed a longer breeding season to *M. edulis* than the above. Care must be taken in comparing their findings with the present ones as many of the former are based upon the results of systematic plankton observations (e.g. Stafford, 1912, on the west coast of Canada; Kändler, 1926, at Heligoland; Borisiak, 1909, in the Black Sea; Thorson, 1946, in the Øresund; and Lebour, 1938, at Plymouth) or upon observations of intensity of settlement (Harris, 1946). In these instances there is no assurance of a homogeneous larval population (i.e. larvae spawned in one hydrographical area), and the long season of larval abundance or settlement may be due entirely to the incursion of larvae from other localities or from deeper beds in which the local hydrographical conditions cause a later, or earlier spawning. In this connexion, it should be noted that Thorson (1946) observed the presence of the larvae of *M. edulis* in the plankton over a considerably longer period in Øresund than in the relatively enclosed body of shallow water in the Isefjord.

Field (1922), although making observations in many localities on the eastern sea board of America, does not give details of the variation in the spawning period, and in America only Battle (1932) investigated the length of the breeding period of *Mytilus* by gonad examinations as well as plankton observations; she concluded that, in Passamoquoddy Bay, the spawning period lasts for 3 months.

Bruce (1926) and Daniel (1921) ascribe a short breeding period to *Mytilus*. The former states that spawning in laboratory tanks at Port Erin, which commenced in 1925 on 27 May, was completed by 5 June, whilst the latter observed a short spawning period on the Morecambe beds in 1921 and 1922 which were both warm years. He quotes Mr F. Gardner, the bailiff, as saying spawning occurs in April, the actual date varying according to the weather, and that some beds ripen before others. Berner (1935), too, finds that *M. edulis* at Calais spawns for a brief period only. Similarly, the fishermen at Brancaster Staithe, Norfolk, give the spawning period of the mussels in the

creeks as April or May and state that the water becomes milky with milt suddenly, this condition lasting for a few days only, after which the mussels are thin and watery.

Thus, of those workers whose conclusions were based upon gonad examinations, only Battle (1932) and Havinga (1929, quoted by Werner, 1939), have found evidence of a lengthy period of spawning.

The intense initial spawning so strongly in evidence in most localities in 1947 has been noted by several workers, e.g. Field (1922), Daniel (1921), and Bruce (1926) and suggested by Thorson (1946), who observed a very sudden rapid increase in the number of larvae in the Limfjord, this high density lasting for a short time only.

It would thus appear that from observations upon the gonad condition, *M. edulis* may spawn during a period of 3–4 weeks, with the major release of ova and sperm occurring during the first 7 days, as was recorded in most localities in the present investigations, or that this species may spawn over a considerably longer period, the gonads possibly regenerating and discharging several times during the period (e.g. Battle, 1932; Havinga, 1929). At Plymouth and Brixham in 1946 and in 1949 and 1950 respectively the period during which a small number of ripe individuals could be found was greater than in other localities. Such observations suggest that where the initial rate of increase in temperature is small, the spawning period of *Mytilus* becomes protracted. In this respect it is significant that the rate of increase of sea temperature in the two localities in which Battle made her investigations (St Croix and Birch Cove, Passamoquoddy Bay) was low, the maximum temperature being slightly less than 14° C. in each instance, and the mean rate of increase 0·05° C./day during the spawning period. It appears probable that an upper limiting temperature to the regeneration of the gonads is achieved in those localities where spawning takes place during a short period, or that the temperature coefficient for laying down reserve materials is greater than that controlling the regeneration of the gonads; either or both would limit the spawning period in those localities where the sea temperature rises rapidly.

Ripening of the Gonads

In all localities, ripening of the ova and sperm commenced during a period of up to 6 weeks before the onset of spawning. This is shown clearly in Tables I–VII by the increase in the proportion of ripe individuals and the index of gonad development. Ripening of the gonads took place rapidly within 3 weeks of spawning at Conway and Brancaster in 1947, whilst at Portree in the same year a fair proportion of ripe individuals were found 5 weeks before the commencement of spawning (see Figs. 1, 2 and 4 for Conway, Portree and Brancaster respectively). Similarly, at Brixham in 1948 and 1949 some ripe individuals were present in samples taken during March,

5 or 6 weeks before the beginning of the spawning period. It is possibly significant that the minimum sea temperature at Brixham and Portree is considerably higher than at Conway and Brancaster (see Table IX), where the rate of increase in sea temperature from the annual minimum is greater, this greater rate of increase in temperature leading to a later but more rapid development of the gonads and a more rapid increase in the proportion of ripe individuals immediately before the occurrence of spawning.

TABLE IX. RATE OF INCREASE OF SEA TEMPERATURE

Locality	Seasonal minimum temperature (mean of 7 days)	Rate of increase in temperature from minimum to time of spawning
Portree (1947)	4·5° C.	0·066°/day
Conway (1947)	1·0° C.	0·151°/day
Brancaster (1947)	0° C.	0·142°/day
Brixham (1949)	7·5° C.	0·102°/day

A possible relation between sea temperature and rate of development of the gonads of *M. edulis* is further emphasized by the observation that the sea temperature at the commencement of the period of ripening is 7–8·5° C. in most localities where such estimates could be made (see Table VIII). It would thus seem that the gonads of *M. edulis* mature when the sea temperature is above *c.* 7·0° C., and that the rate of ripening is roughly proportional to the rate of increase in temperature.

There appears to be no direct relationship between nutrition and ripening of the gonads of *Mytilus*. Loosanoff (1942) has shown that *Mytilus* can ingest food at any time at temperatures above 0° C., whilst the observation that many *Mytilus* with very poorly developed mantles from Portree contained ripe ova and sperm, and spawned normally, suggests that maturity is not dependent upon good nutritional condition. Young (1942 and 1946) has also reached this conclusion in the case of *M. californianus*. In this connexion it is possibly significant that the glycogen content of *M. edulis*, as in *Echinus* and *Ostrea*, is reduced to a minimum value immediately prior to spawning (Daniel, 1921; Stott, 1931), suggesting that the development of the gonads occurs at the expense of reserve nutrient and is not dependent upon food ingested at this period, (see Fig. 8, p. 469).

During the period of ripening of the gonads in 1947, estimates were made of the proportion of mature ova obtained from the mantles of ripe individuals. As the spawning period approached, the proportion of ova rounding off in sea water, with the loss of the germinal vesicle, increased from a small value to over 90%. Artificial fertilizations made at these latter times were very successful, 80–90% of the fertilized ova segmenting normally. Further, as the gonads develop, an increasing proportion of the ova ripen at the same time in each individual.

Spawning Inducements

The rapidity of onset and the magnitude of the initial spawning phase of *Mytilus* strongly suggests that spawning is induced by a stimulus affecting the bulk of the population. That this stimulus is external is indicated by the variation in the length of the period during which a high proportion of mature individuals is found in the several localities before the onset of spawning.

The information derived from the present investigation strongly suggests that it is a rapid rise in the mean temperature to which the mussels are exposed to 11·5–13·0° C. which induces spawning (see Table VIII). It is also possible that the coincidence of the onset of spawning with a period of spring tides, in twelve of the fourteen instances summarized in Table VIII, is significant. Inshore temperatures, however, have been shown to rise in many localities during periods of spring tides in late spring and early summer, so that the apparent positive correlation between spawning and spring tides may in fact be due to the rise in sea temperature at new and full moon at this time of year.

Berner (1935) has described the spawning of *M. edulis* at Calais after a rapid change of sea temperature from 5 to 10° C., and a sudden decrease in salinity, whilst Nelson (1928) and Runnström (1929) give 10–12° C. and 14–16° C. respectively as the temperatures at which spawning takes place. Hutchins (1947) lends further support to the conclusion that *Mytilus* spawns at *c.* 11·5° C. in observing that the northern limit of distribution of the species is coincident with the 10° C. summer isotherm. Battle (1932), however, discovered no correlation between spawning and temperature in Passamaquoddy Bay. Whedon (1936), Coe & Fox (1942) and Young (1942, 1946) all state that there is no definite evidence that spawning in *M. californianus* is induced by temperature, or by temperature changes.

However, Battle (1932) found that the gonads of *M. edulis* at Passamoquoddy Bay matured at new moon during the summer months and that spawning followed immediately. Korringa (1947) implicitly suggests that the full moon spring tides were almost obliterated in this area in 1930, when the observations were made, and therefore ripening in normal years might occur at both new and full moon tides. Lunar periodicity in spawning is well known in many species (see Korringa, 1947, for an extensive bibliography), but the mechanism is extremely obscure. In *M. edulis* the effect of the greater variations in hydrostatic pressure at spring tides upon individuals with mature gonads may be of importance in inducing spawning. However, personal observations of spawning *in vitro*, and the observation of Bruce (1926) of rapid spawning in static tanks at Port Erin suggest that pressure is not a principal factor in stimulating mussels to spawn.

During 1947 attempts were made to induce *Mytilus* to spawn in the laboratory as the result of changes in environmental conditions. The results

were extremely conflicting. It was found that mechanical shock (by pulling the byssus threads, or agitating vigorously) almost invariably produced a positive response from a number of the ripe mussels employed in the experiments. Young (1945) suggests that mechanical shock is the stimulus inducing spawning in *M. californianus* under natural conditions. Similarly, Field (1922) states that rough handling induces spawning in *M. edulis*, whilst Orton (1924) has shown that dredging operations during the breeding season can cause oysters to spawn. On the other hand, instances of spawning of *M. edulis* have been observed frequently under completely static conditions *in vitro*.

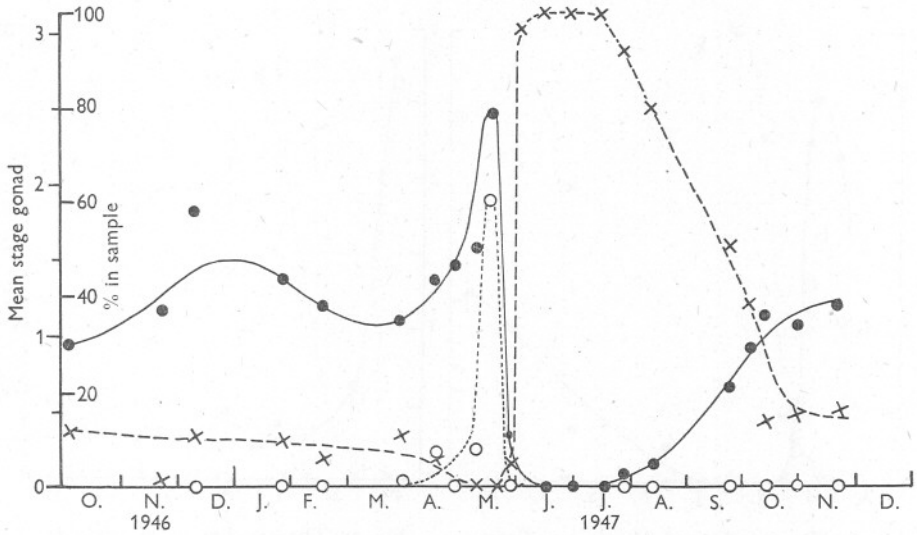

Fig. 5. *M. edulis*. Breeding at Conway, 1946–7. ●—— ●, mean stage of gonad development; × – – – ×, % of resting spent; ○·····○, % of ripe adults.

Alternations of high and low temperatures, variations in salinity, and a mixture of mature sperm and ova with the sea water in which the experimental *Mytilus* were immersed produced conflicting results. Few experiments were made, however, and the results therefore are of little significance. A more detailed investigation of spawning inducements is now in progress.

Post-Spawning Changes in the Gonads

Within 10–20 days after spawning, a great regression of the gonads occurs. The decrease in volume on spawning is compensated to some degree by the intake of water (see Daniel, 1921), the mantle tissues becoming soft, spongy and semi-transparent. The residual ova and sperm are probably absorbed by phagocytosis by the cells of the follicle walls, and all trace of sexuality is lost. These individuals are in the 'neuter' or 'resting spent'

stage. Growth of the connective tissue of the mantle and mesosoma also takes place immediately, and glycogen and some fat appear. During the latter part of June and throughout July, the amount of glycogen increases at a rate dependent upon the supply of food, until, by the end of July, *Mytilus* from good fattening beds (e.g. Brancaster and Conway), possess thick mantles filled with granules of glycogen. In general, there is no sign of the re-development of the gonads during this period, but at Plymouth in 1946 a small proportion of males and females could be distinguished in July, the proportion remaining constant until the final sample was taken in October 1946 (see Fig. 7).

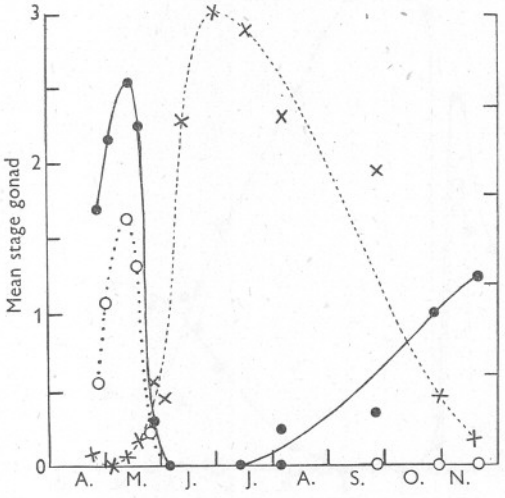

Fig. 6. The breeding of *M. edulis* at Brancaster Staithe, north Norfolk, in 1947.

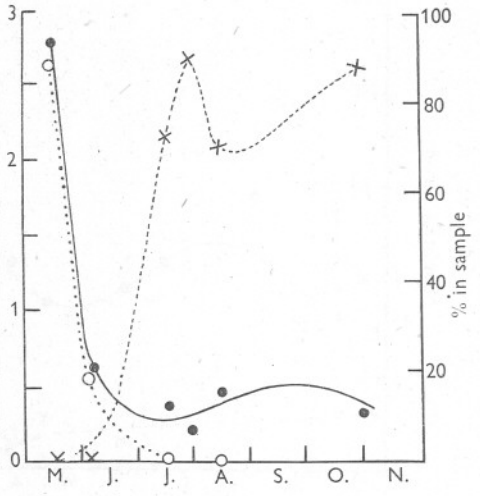

Fig. 7. The breeding of *M. edulis* at Plymouth, in 1946.

●—— ●, mean stage of gonad development; × – – – ×, % resting spent; ○·····○, % ripe adults.

In the other localities the first traces of returning sexuality were observed in late July or during August, the gonads beginning to invade the mantle lobes in some specimens (stage I) (Figs. 5, 6 and 8). This process continues throughout September and October, until, by mid-November, the proportion of 'neuter' individuals is small. The connective tissue becomes concentrated as the follicles develop and the glycogen and fat become wedged between them (see Daniel, 1921).

From December 1946 until the end of March 1947, no further development of the gonads took place, but during late March and April a rapid increase in the mass of the gonads occurred, culminating in maturation and spawning in May.

Thus, in *M. edulis*, a non-reproductive period, during which large amounts of reserve food products are accumulated, occurs between successive reproductive phases.

The seasonal variations in the glycogen and fat content of *Mytilus* have been studied by Daniel (1921), who employed material from the Mersey and from Morecambe Bay. If Daniel's figures for the proportion of glycogen and fat per unit weight of the ash-free dry substance are compared with the index of gonad development for the Liverpool material employed in the present investigation (see Fig. 8) it is significant that whilst gonad development was stationary from December to March 1946, the glycogen content of

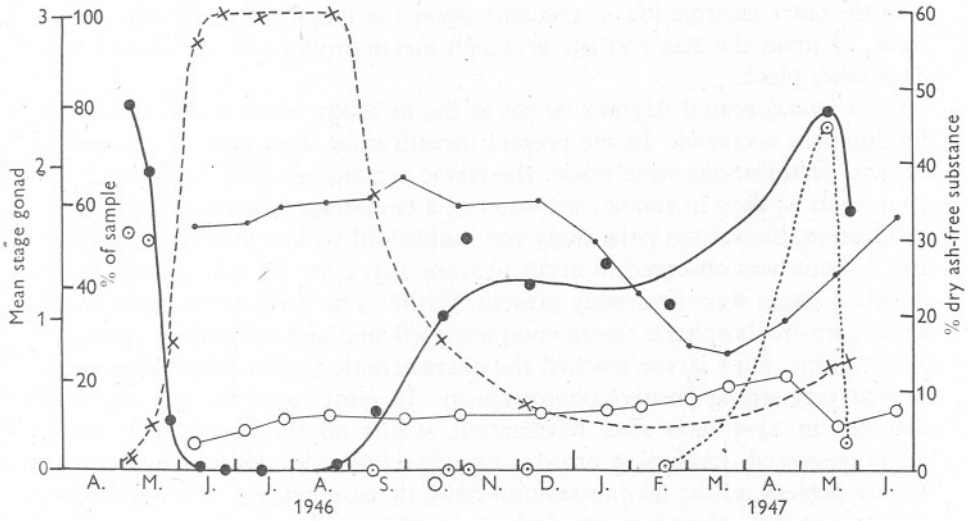

Fig. 8. *M. edulis*. Breeding at Liverpool, 1946–7. •——•, mean stage of gonad development. ×----×, % of resting spent. ●—— ●, % glycogen; ○——○, % fat; % composition by weight of ash-free substance, from Daniel (1921).

Mytilus declined markedly, suggesting that some was used in the ordinary metabolic processes during a period of low temperatures and possible scarcity of food. This is also suggested by the continuance of the high value of the respiratory quotient after the period of rapid accumulation of glycogen (Bruce, 1926); the decrease in the R.Q. in March and April suggests the conversion of carbohydrates to fats, for storage in the maturing ova. It appears probable that scarcity of food, or the inhibition of assimilation of food by low temperatures, is responsible for the decrease in glycogen content during the early months of the year, as although Dodgson (1928) has reported the opening of the shells and the maintenance of ciliary currents in *Mytilus* even at 0·0° C., he further states that assimilation does not occur at temperatures below 4° C.

Development of the Larvae

Berner (1935) suggests that, in *M. edulis*, the sperm is shed into the sea and drawn into the mantle cavity of the female mussel, where fertilization takes place, the developing embryos being ejected when ciliated. No evidence to substantiate this has been obtained in the course of the present investigation; of the many mussels examined during the spawning period, none has been observed to contain developing larvae in the mantle cavity. Further, sperm from ripe males retains its activity in sea water at a temperature of 15° C. for at least 3 hr., and the ova, once discharged into sea water, are fertilizable for at least the same period.

Little exact information is available upon the length of larval life of *M. edulis*, or upon the size and age at which metamorphosis to the dissoconch stage takes place.

Field (1922) reared *Mytilus* larvae to the fifth day, after which abnormal development occurred. In the present investigation, a number of successful artificial fertilizations were made, the larvae remaining viable for periods of from 10 to 14 days in aerated sea water at a temperature of 16–18° C. After fertilization, the second polar body was budded off within 30 min., whilst the first division was observed to occur in from 1 to 2 hr. In 5 hr., many 8- to 32-celled stages were invariably present. Within 9 hr. from fertilization most larvae (pre-trochospheres) were completely ciliated and swimming strongly. In 24–30 hr., most larvae reached the characteristic trochosphere stage, and showed very strong positive phototropism. In most cases, the gut was first observed in $2\frac{1}{2}$–3 days after fertilization, whilst on the fourth day, many larvae possessed developing pro-dissoconchs. Fully developed veligers were usually present on the fifth to seventh days, the size being *c.* 100–110μ long and 80μ broad. Owing to the lack of suitable food organisms no further development occurred, although living larvae persisted for a further 3–7 days. No dissoconch larvae were observed, and no significant increase in size was recorded.

Estimates of the duration of larval life in the sea, based upon observations of the onset of spawning and the commencement of settlement, are accurate only if the larval stock is homogeneous, and if larvae developing from the initial spawning do not suffer a heavy mortality. Thus, the estimates based upon the observations set out in Table X cannot be taken as strictly accurate, but only as an indication of the probable duration of larval life. As a result of these observations, it would seem that the duration of the period between initial spawning and the commencement of settlement is 3–4 weeks. However, the normal larva life may well be 4 or 5 weeks, for in most of the localities in Table X the maximum rate of settlement was attained 2 weeks after the first few spat were observed. Battle (1932) records the occurrence of larvae 310–360μ in length in 14–21 days, whilst Field's (1922) figures combined

with those of Matthews (1913), suggest a considerably lower rate of development, metamorphosis and settlement occurring in *c.* 10 weeks.

From the sizes of spat found upon panels exposed for periods of 7 days during the period of settlement of *M. edulis*, settlement can take place at any time or size after the dissoconch is formed, i.e. from a length of 400μ to 1 mm. Thorson (1946) comments upon the great variation in size at which metamorphosis occurs (250–400μ) and also the great differences in the rates of development, as shown by gill filament differentiation at different sizes.

Nelson (1928) records the ability of the advanced larvae to float near the surface by means of a gas bubble within the valves of the shell, even at a size of 900μ. Attachment is effected initially by the foot, after the larva has become stereotropic. Nelson (1928) states that many surfaces may be explored before attachment by the byssus takes place. The foot is frequently employed as a means of locomotion on the surface film of the water.

Settlement of the Larvae

In general, the period of settlement of *M. edulis* in British waters lasts from May to early July. The observed duration of settlement of the spat of this species in five localities (see Table X) is from 4 to 7 weeks. In each case it is

Table X. Estimated Duration of Larval Life and of the Period of Settlement of *Mytilus edulis*

Locality	Date of commencement of settlement	Estimated larval life (days)	Duration of period of settlement (weeks)
Liverpool	31. v. 46–6. vi. 46	18	6
	13. vi. 47–20. vi. 47	24	5
	8. vi. 48–15. vii. 48		6
Conway	3. vi. 47–16. vi. 47	21	4
Portree	4. vi. 47–25. vi. 47	21	6
Brancaster	4. vi. 47–14. vi. 47	25	5
Brixham	7. vi. 48–11. vi. 48	28	7
	29. v. 49–25. v. 49	26	6
	17. v. 50–31. v. 50	24	6

Mode (of 100) at 0·5 mm.

probable that the extended period is largely due to the incursion of larvae from later-spawning beds in the same general locality. This is exemplified by the occurrence of two distinct maxima separated by about 2 weeks in the number of spat settling at Brixham in 1948. The long periods of settlement described by several workers (e.g. Harris, 1946; Pyefinch, 1950) are possibly due to such an incursion of larvae. Thus a longer period of settlement would be more likely to occur in a deep-water locality, than in a shallow-water locality in which a greater degree of homogeneity in spawning might be expected.

Plankton observations at Brixham in 1949 suggest that although *Mytilus* larvae may be present during July and early August in small numbers, settlement is rarely observed later than mid-July (see also Engle & Loosanoff, 1944).

Mytilus settles most abundantly in areas offering some degree of shelter from water currents and wave action. Settlement is invariably small upon clean smooth non-toxic exposure panels (e.g. glass, bakelite), but can be heavy on panels providing some degree of shelter by reason of the fouling already upon them. Settlement is always heavier upon inset panels than upon panels the surfaces of which are raised 0·5–1·0 cm. above the substratum; in this latter instance, settlement is greater in the depressions between panels, even where the panels are known to be completely non-toxic. Settlement is also greater upon the upper edges of these panels. The heaviest settlements always occur upon hydroid colonies, the rough or frayed surfaces of ropes, and rough shells. In raft exposures, *Mytilus* settles more abundantly in the darker positions, possibly owing to the colonization of the lighter positions by algae, with the consequent reduction in area upon which the spat can attach and grow. Initial settlement can, however, be quite high upon filamentous algae, but the mussels rarely persist, even in small numbers.

When settlement occurs upon a non-toxic panel freely exposed in the sea at a constant depth, it is invariably heavier at the upper edge, becoming rapidly smaller as the distance from the upper edge of the panel increases. In years of good spat-fall an entire panel may be fouled by *Mytilus*, but initially the upper edge and the surfaces bordering it bear more and larger mussels. As settlement declines, greater growth is usually observed where the density of settlement is less. A projection across the centre of the panel will give rise to a heavier settlement of *Mytilus* immediately below it than immediately above. It would thus appear that *Mytilus* spat accumulates where the surface upon which it settles is interrupted by some discontinuity. It is probable that the spat, after initial settlement, migrates vertically upwards upon the surface by means of its ciliated foot until a discontinuity is reached, whereupon it fixes itself by means of its byssus, the discontinuity providing a stimulus for byssus formation. A similar tendency to accumulate at the upper parts of panels, possibly for a similar reason, has been noted in the settlement of *Balanus crenatus* and *B. improvisus* at Liverpool and Burnham-on-Crouch (Chipperfield, 1948).

The spat of *Mytilus edulis* attaches to floating structures most abundantly from just below the surface to a depth of about 2 ft.; below this the density of settlement declines. This is clearly shown by counts of the number of mussels settled on 3 in. long sections of tubular steel panel holders $1\frac{1}{4}$ in. diameter, suspended from a raft at Brixham in 1948 and 1949, made towards the end of the period of settlement. The counts are set out in Table XI. It is apparent that settlement will also occur in the region of the nominal water line, and in that zone above the water line which is subject to constant

immersion by wave-action, although the settlement falls off very rapidly in this region.

A similar distribution of settlement is seen in the case of fixed vertical substrata. In 1949 a chain covered with mussels and hanging freely to the bottom from a naval dolphin in Brixham Harbour, was denuded in parts for spawning investigations. Subsequently, the patches cleared of mussels were

TABLE XI. SETTLEMENT OF *MYTILUS EDULIS* IN RELATION TO DEPTH BELOW WATER LINE

Distance from water line (in.)	Numbers/3 in. length of 1¼ in. diam. vertical rod						
	1. vii. 48			16. vi. 49			
	1	2	Mean	1	2	3	Mean
+6 to +3	0	0	0	5	22	7	11
+3 to 0·0	8	15	11	35	28	58	40
0·0 to 3	33	43	38	101	85	158	115
−6 to −9	284	265	274	412	358	392	387
−12 to −15	221	294	257	329	400	360	363
−24 to −27	236	160	188	266	206	252	241
	Mean size: 4·5 mm.			Mean size: 3·0 mm.			

TABLE XII. INTERTIDAL SETTLEMENT OF *MYTILUS EDULIS* ON CHAIN, BRIXHAM, 14 JULY 1949

(Numbers (approx.) per link of chain. Mean size: 8·0 mm.)

Approx. level	No.	Approx. level	No.
H.W.N.T.	0	L.W.S.T.	50
M.T.L.	0	5 ft. below L.W.S.T.	180
M.T.L.–L.W.N.T.	0	15 ft. below L.W.S.T.	160
L.W.N.T.	8		

extended, and more made at known distances below approximate L.W.S.T. mark. Rough counts of the recently settled and surviving *Mytilus* in these areas were made on 14 July (see Table XII). Below L.W.S.T., settlement was not significantly greater at a depth of 15 ft. than at L.W.S.T. Above L.W.S.T., settlement was considerably smaller, no surviving settlement at all persisting at M.T.L. Similarly, at Liverpool in 1946, although a heavy surviving settlement occurred upon the low-tide floor of the jetty at the Prince's landing stage (see Corlett, 1948), that upon the mid-tide floor and upon the beams immediately beneath was extremely sparse.

Engle & Loosanoff (1944) explain similar results by suggesting that settlement of *M. edulis* larvae occurs only at low-water slack, or at late ebb. It is possible, however, that settlement by adhesion to the substratum by the foot may occur at any state of the tide, but that the time required for secure attachment by the byssus to be completed is of the order of 4½–6 hr. As the byssus is secreted only when the mussel is immersed, spat adhering in positions higher in the intertidal zone would not persist unless they happen to be in a

position in which they are constantly wetted, e.g. a small depression in a rock surface. Tidal currents and wave action are obviously complicating factors, but the restricted settlement of mussel spat between L.W.N.T. and M.T.L. on a rocky shore subject to some wave action such as Shoalstone Beach, Brixham, in narrow cracks and small hollows lends support to the suggestion detailed above. The effect of exposure to the air upon the physical and chemical properties of the liquid, or partly solidified, byssus material cannot be ignored in this context.

Summary

During 1946 and 1947, regular samples of *Mytilus edulis* from a number of localities on the British coasts, including Conway, Brancaster and Liverpool, were examined for gonad condition and spawning. For each sample, the mean stage of gonad development was computed. The criteria employed in distinguishing the stages of gonad development are described.

Ripening of the gonads takes place within a few weeks of the onset of spawning, in general commencing when the sea temperature has risen above 7·0° C. There appears to be no correlation between nutritional condition and ripening of the gonads, or subsequent spawning.

In all localities and in each year in which observations were made spawning occurred in late spring (mid-April to the end of May) and in most areas lasted for a short period only (2–4 weeks). At Brixham, in 1949 and 1950, the duration of the spawning period was longer (4–6 weeks). In most cases, 70–80% of the mature population spawned during the first 7–10 days of the breeding period. No evidence of periodic spawning was obtained.

In all cases, spawning commenced in a period during which the mean temperature to which the mussels were exposed was rising from *c.* 9·5° C. to 11–12·5° C. In most cases, the onset of spawning was coincident with a period of spring tides, and of predominantly bright sunny weather. The initial rate of spawning appears to be directly related to the rate of increase in mean temperature to which the mussels are exposed.

After spawning, mussels enter into a 'neuter' or 'resting spent' stage in which all traces of sexuality are lost. In good 'fattening' areas, the connective tissue of the mantle and 'mesosoma' rapidly becomes packed with glycogen and some fat. Re-development of the gonads usually commences in July or August, 2–3 months after spawning. A rapid increase in the mass of the gonads occurs during early spring.

The period of free swimming larval life is variable and is probably usually *c.* 4 weeks. Settlement can occur at any time after the formation of the dissoconch, corresponding to a length of from 0·4 to 1·0 mm.

Settlement is heaviest on floating structures in the region between the water line and a depth of 2 ft. Below this level the density of settlement declines somewhat. Similarly, upon intertidal structures and upon beds in

estuarine areas, little persistent settlement occurs above M.T.L. and the heaviest settlements take place at and below L.W.S.T. Settlement almost invariably occurs initially at the upper edge of submerged panels, etc.

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THE LARVAE OF THE SPATANGIDAE

By C. B. Rees, D.Sc.

The Scottish Marine Biological Association, Oceanographic Laboratory, Edinburgh

(Text-figs. 1–5)

Introduction

Mortensen (1927) has given an account of the distribution of the six species of Spatangidae which occur in British waters. Of these, four may be regarded as widespread and, in places, numerous: *Echinocardium cordatum* (Pennant), *E. flavescens* (O. Fr. Müller), *Spatangus purpureus* O. Fr. Müller, and *Brissopsis lyrifera* (Forbes). The fifth species, *Echinocardium pennatifidum* Norman, has been found on the south, west and north British coasts, and down to Durham on the east coast, though nowhere in large numbers. The sixth species, *Spatangus raschi* Lovén, is much more restricted, and is known only from fairly deep water off the west coast of Ireland and off the Shetland Islands.

Our knowledge of the larval forms of these species is not satisfactory. The larva of *Echinocardium cordatum* has been described by Mortensen (1898) and MacBride (1914). However, Thorson (1946) refers to the possibility that the larva of *E. flavescens* may be very similar to that of *E. cordatum*.

Chadwick (1914) described some young larvae which he assigned to *E. flavescens*, but Mortensen (1920) would not accept the certainty of this identification and gave *Brissopsis lyrifera* as a possible alternative. Despite the fact that Chadwick's drawings are poor (Mortensen, 1920, who saw the original material, pointed out that the post-oral arms are fenestrated, not simple as shown in the drawings), there is little doubt that Chadwick's identification was correct. It is a curious fact that Mortensen acknowledged seeing Chadwick's '*E. flavescens*' and gave *Brissopsis* as an alternative in the same paper (1920) in which he described the larva of *B. lyrifera*. As will be seen below, the larvae of the two species are, in fact, easily distinguishable even in very young stages. It has been accepted up to now that the larvae of *Echinocardium flavescens* are unknown (Mortensen, 1927; Thorson, 1946).

Young larvae described by Krohn (1853) were assigned by him to *Spatangus purpureus*. In this case, also, Mortensen (1913) questioned the identity. Mortensen (1913) has described the fully grown larva of *S. purpureus*, giving as its main characteristic the exceedingly long posterior process, but there is reason to believe (see below) that identification based on such a characteristic is unsatisfactory. The best account of *S. purpureus* larvae is given by Ohshima

(1921). Mortensen (1920) was unable to say how the younger larvae of *Spatangus* and *Brissopsis* may be distinguished.

The late larva of *B. lyrifera* was described by Mortensen (1920). He showed that it is easily distinguished from the late larvae of *Echinocardium cordatum* and *Spatangus* by the absence of postero-lateral arms. Mortensen (quoted by Thorson, 1946, p. 358) subsequently raised the possibility that there may be a still later stage than that described, in which the postero-lateral arms are more distinct. It may be said now that, even in larvae at the point of metamorphosis, no sign of a postero-lateral rod is present in the skeleton. The younger larvae have not been described.

Nothing is known of the development of *Echinocardium pennatifidum* and *Spatangus raschi*.

From the published descriptions, it is not possible to identify with any certainty most of the spatangid larvae which occur in plankton samples from British waters. A large proportion are usually in early stages of development which have not been adequately described. In most areas the larvae of *Echinocardium flavescens* and *E. pennatifidum* will present alternative possibilities even in the identification of later stages. *Spatangus raschi* is so much more restricted in its distribution that lack of knowledge of its larva is not so important.

The object of this report is to provide means of identifying the larvae of the four more common species from very young stages onwards. The first section is devoted to a more complete account of the general structure of the spatangid skeleton than appears to be available in the literature.

In view of the variation involved in the larvae it would be as well to specify the source and extent of the material on which the descriptions are based. Abundant larvae of *Echinocardium cordatum* and *E. flavescens* have been available in the samples collected by the Continuous Plankton Recorder in the plankton survey of the North Sea (Rae, 1952). Rather better preserved material has been obtained in Plankton Indicator samples from the north-east Scottish fishing grounds. *Spatangus purpureus* larvae have been much less abundant in both sets of samples; of the order of a hundred larvae have been identified. Approximately the same number of larvae of *Brissopsis lyrifera* have been identified, partly from samples taken from the Clyde (kindly provided by Dr D. T. Gauld) and partly from Recorder samples taken over the Continental Slope south-west of Ireland.

It is regretted that, for various reasons, so few measurements are given. Few as these are, however, they serve a more definite function than words such as long, short, longer, shorter, which are frequent in descriptions.

The descriptions are based entirely on the skeletal parts which may be seen clearly by immersing the larvae, if necessary, in hypochlorite solution; a 'domestic bleach' is very suitable for this purpose.

General Account of the Spatangid Skeleton

Fig. 1 gives in diagrammatic fashion the essential structure of the body skeleton of an early larva (A) and of a fully developed larva (B). Corresponding plans of the skeleton are given in the adjoining figures *a* and *b*. Characteristic of spatangid larvae is an unpaired posterior arm[1] arising from the posterior transverse rod.

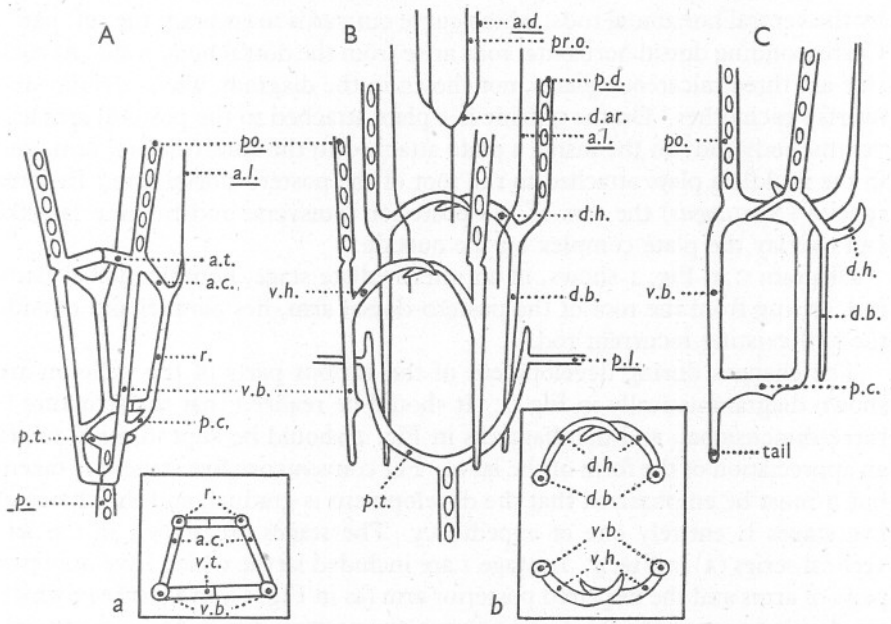

Fig. 1. Diagram of the general structure of the body skeleton of spatangid larvae. The young stage is shown in A and the late stage in B; corresponding plan views are shown in *a* and *b*. C shows part of the intermediate stage. Abbreviations applying to arm rods are underlined. All abbreviations, except for *po.*, postoral, and *pr.o.*, preoral, are composite: *a.*, anterior; *ar.*, arch; *b.*, body; *c.*, connexion; *d.*, dorsal; *h.*, horizontal; *l.*, lateral; *p.*, posterior; *r.*, recurrent; *t.*, transverse; *v.*, ventral. The term 'rod' should be added where appropriate.

In the young larva the body skeleton is box-like with the fenestrated postoral arms arising from the two anterior ventral corners and the simple antero-lateral arms from the two anterior dorsal corners. The box is made up of two side pieces, each piece consisting of a ventral body rod and a recurrent rod, joined by an anterior and a posterior connexion. The two side pieces are connected in the median plane by three projections, of which the anterior transverse rod is the most distinct and persistent. The connexion at the posterior end of the body rod is not firm and gives way early in development.

[1] Throughout this account the term 'arm' refers to the skeletal arm rods, as distinct from other rods (see Fig. 1).

When present it is convenient to recognize a 'tail' in the body rod, that part posterior to the posterior connexion.

The late larva is very much larger and the rigid box of the young larva becomes transformed to cope with the increase in size. The skeleton is still box-like, but the anterior and posterior connexions have disappeared and the recurrent rod is replaced by the dorsal body rod which is part of the root of the fenestrated postero-dorsal arm. The anterior transverse rods are replaced by the ventral horizontal rods, which curve outwards to embrace the soft parts. Corresponding dorsal horizontal rods arise from the dorsal body rods. At each side are three calcareous plates, not shown in the diagram, which overlay and overlap each other. On the outside is a plate attached to the postoral arm and ventral body rod, on the inside a plate attached to the antero-lateral arm, and in the middle a plate attached to the root of the postero-dorsal arm. In some species (*Spatangus*) the tips of the posterior transverse rod become fan-like and overlay the plate complex on the outside.

Diagram C in Fig. 1 shows, in an intermediate stage, how the dorsal body rod, arising from the root of the postero-dorsal arm, lies parallel and outside the still existing recurrent rod.

The changes during development of the various parts of the skeleton are shown diagrammatically in Fig. 2. It should be realized that the structure is three-dimensional, and the diagrams in Fig. 1 should be kept in mind to aid an appreciation of the form of the larva. For convenience five stages are taken, but it must be emphasized that the development is gradual, and the choice of five stages is entirely one of expediency. The stages are shown in the left vertical series (A) in Fig. 2. In stage 1 are included larvae which have only two pairs of arms and the unpaired posterior arm (as in Fig. 1), and larvae in which the development of the third pair of arms, the postero-dorsal, has commenced. Stages 2–5 are shown in the diagrams of Fig. 2 A only by the dorsal arch and its associated arms. The disposition of the dorsal arch in the complete larva is illustrated in Fig. 1 B. In stage 2 larvae the dorsal arch and the beginnings of the pre-oral arms are present. In stage 3 larvae there are the rudiments of the antero-dorsal arms. In stage 4 the antero-dorsal arms are half-developed and stage 5 is the final stage.

The root of the postero-dorsal arm in stage 1 is a three-rayed star (Fig. 2B). By stage 2 one of the star rays has increased in size and become the dorsal body rod with a short branch, the dorsal horizontal rod. In later stages the dorsal horizontal branch increases greatly in size. The remaining two rays of the star elongate slightly, curve and coalesce to form the circumference of a calcareous plate which is developed by stage 5.

The posterior transverse rod bearing the posterior arm is three-rayed in stage 1 (Fig. 2C). Two rays elongate by stage 2 and by stage 3 the elongated rays have the rudiments of the postero-lateral arms, which increase greatly through stage 4 to their full length in stage 5.

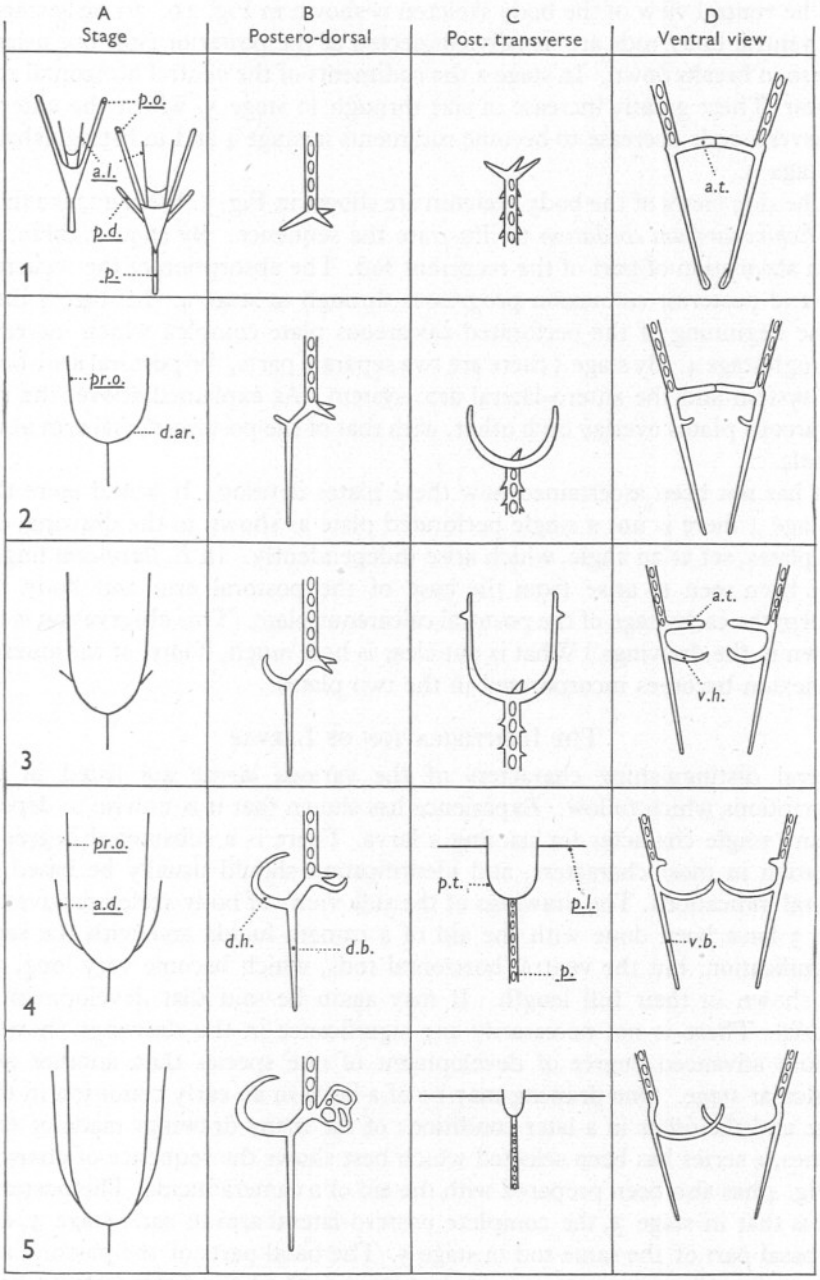

Fig. 2. Showing diagrammatically in vertical series the development, through five stages (A), of the postero-dorsal arm rod (B), the posterior transverse rod (C) and the body skeleton as seen in ventral view (D). Abbreviations as in Fig. 1.

The ventral view of the body skeleton is shown in Fig. 2D. In early stage 1 the ventral body rods are usually connected at the posterior end, but usually this soon breaks down. In stage 2 the rudiments of the ventral horizontal rods appear. These greatly increase in size through to stage 5, whilst the anterior transverse rods decrease to become rudiments in stage 4 and indistinguishable in stage 5.

The side views of the body skeleton are shown in Fig. 3. We may take those for *Echinocardium cordatum* to illustrate the sequence. By stage 2 there has been absorption of part of the recurrent rod. The absorption of the recurrent rod and posterior connexion progresses through to stage 4. In stage 3 there is the beginning of the perforated calcareous plate complex which increases through stage 4. By stage 5 there are two separate parts, the postoral arm-body rod system and the antero-lateral arm system. As explained above, the two calcareous plates overlay each other, with that of the postero-dorsal arm in the middle.

It has not been ascertained how these plates develop. It would seem that in stage 4 there is not a single perforated plate as shown in the drawing, but two plates, set at an angle, which arise independently. In *E. flavescens* fingers have been seen to arise from the base of the postoral arm and body rod to form the early stage of the postoral calcareous plate. (This observation is not shown in the drawings.) What is not clear is how much, if any, of the anterior connexion becomes incorporated in the two plates.

The Identification of Larvae

Several distinguishing characters of the various larvae are listed in the descriptions which follow. Experience has shown that it is unwise to depend on any single character for naming a larva. There is a substantial degree of variation in most characters, and identification should usually be based on several indications. The drawings of the side views of body skeletons given in Fig. 3 have been done with the aid of a camera lucida and with the same magnification, but the ventral horizontal rods, which become very long, are not shown in their full length. It may again be said that development is gradual. There is not necessarily any significance in the drawings showing a more advanced degree of development of one species than another at a particular stage. One drawing may be of a larva in an early condition in that stage and the other in a later condition; of the many drawings made of each species, a series has been selected which best shows the sequence of changes.

Fig. 4 has also been prepared with the aid of a camera lucida. The posterior arm is that in stage 3, the complete postero-lateral arm in early stage 4, and the basal part of the same rod in stage 5. The basal parts of the postoral and postero-dorsal arms are in stage 2, but the length of the unfenestrated part, which is the feature illustrated, remains more or less the same throughout the development of a larva and is independent of stage of development.

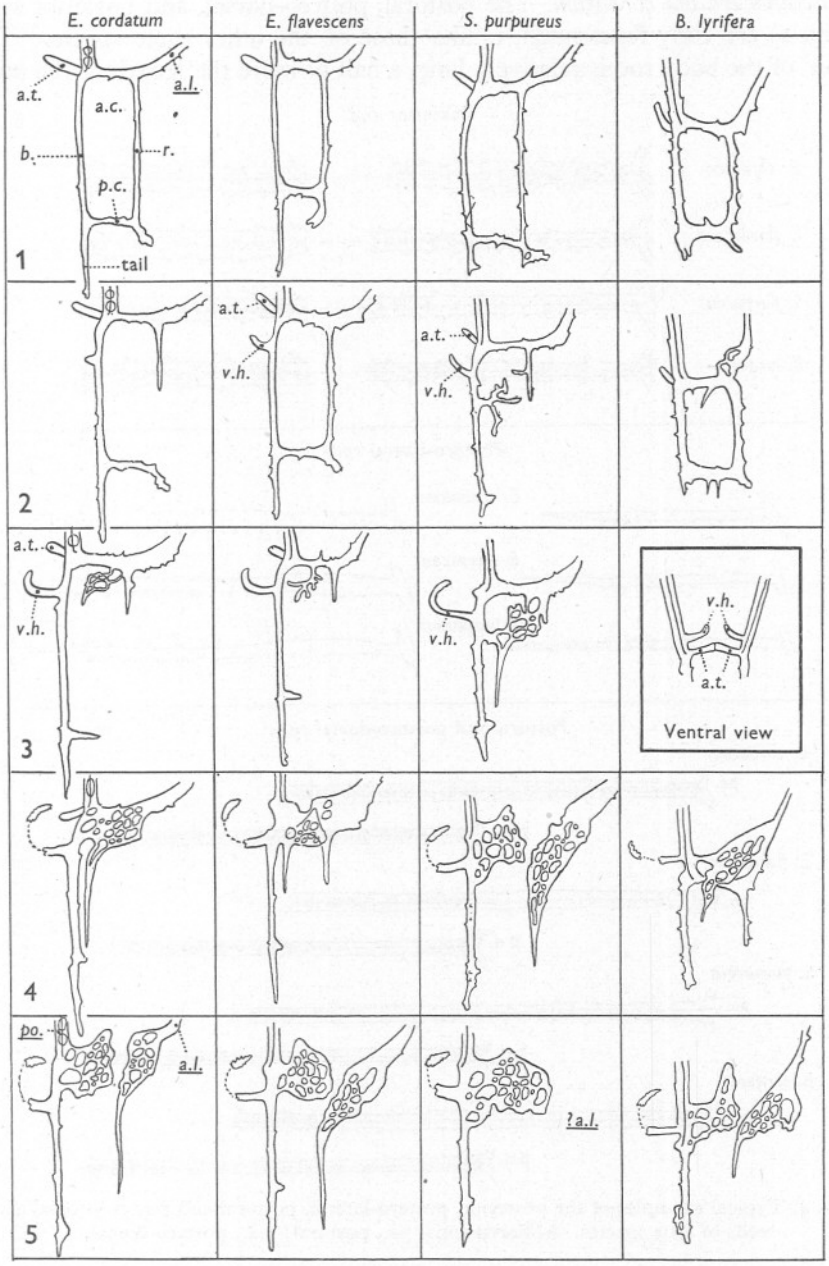

Fig. 3. The development, through five stages, of the body skeleton of four species, as seen in side view. A diagrammatic ventral view only is given for stage 3 of *B. lyrifera*. Abbreviations as in Fig. 1.

Echinocardium cordatum. The postoral, postero-dorsal, and posterior arms (Fig. 1) are fully fenestrated, unlike those of the other three species. The 'tail' of the body rod is relatively long, a half or more the length of the main

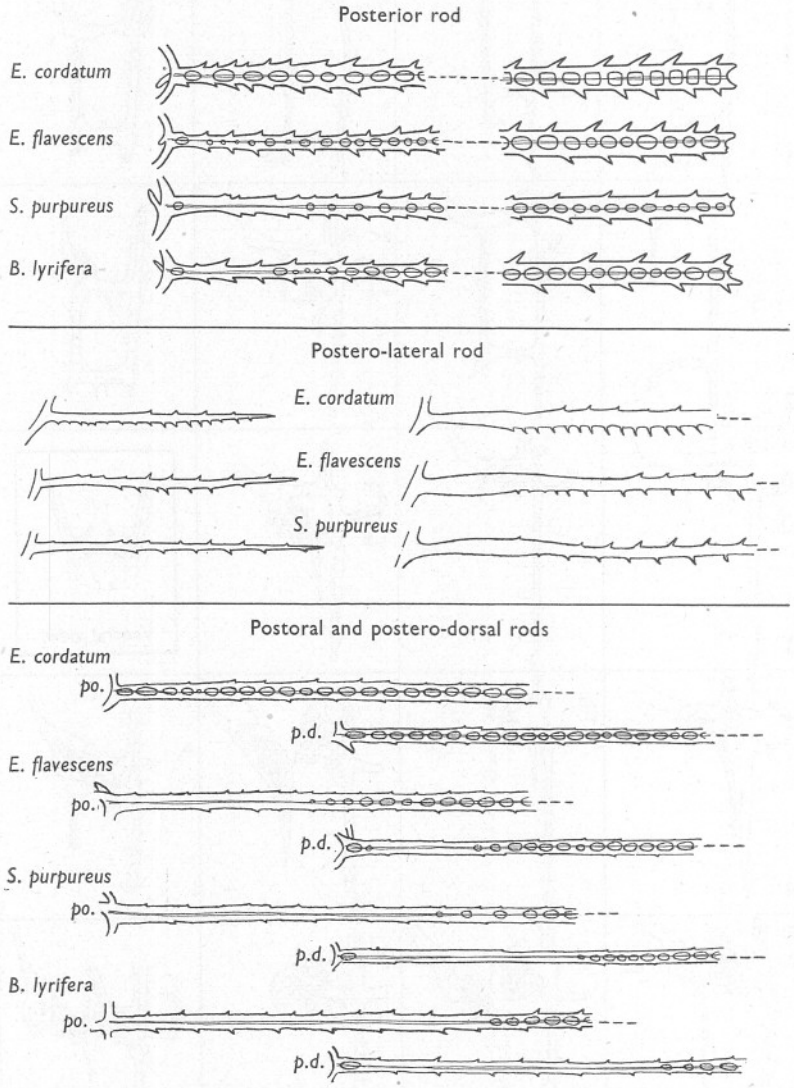

Fig. 4. Typical examples of the posterior, postero-lateral, postoral and postero-dorsal arm rods of four species. Abbreviations: *po.*, postoral; *p.d.*, postero-dorsal.

body rod. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated some distance posterior to the base of the postoral arm (and the anterior transverse rod). In the last

stage, the support for the posterior edge of the calcareous plate of the postoral arm is markedly anterior to the place of origin of the ventral horizontal rod. The postero-lateral arm is very spiny; there are approximately twice as many spines on the posterior edge as on the anterior.

Echinocardium flavescens. The posterior arm is, with rare exceptions, fully fenestrated. The fenestrations in the proximal part may be fairly large or very small; rarely a section may appear to be unfenestrated. The proximal part of the postoral arm is unfenestrated to a variable degree (Fig. 5), the great majority (forty-seven out of fifty measurements) to a length of 0·10–0·17 mm.

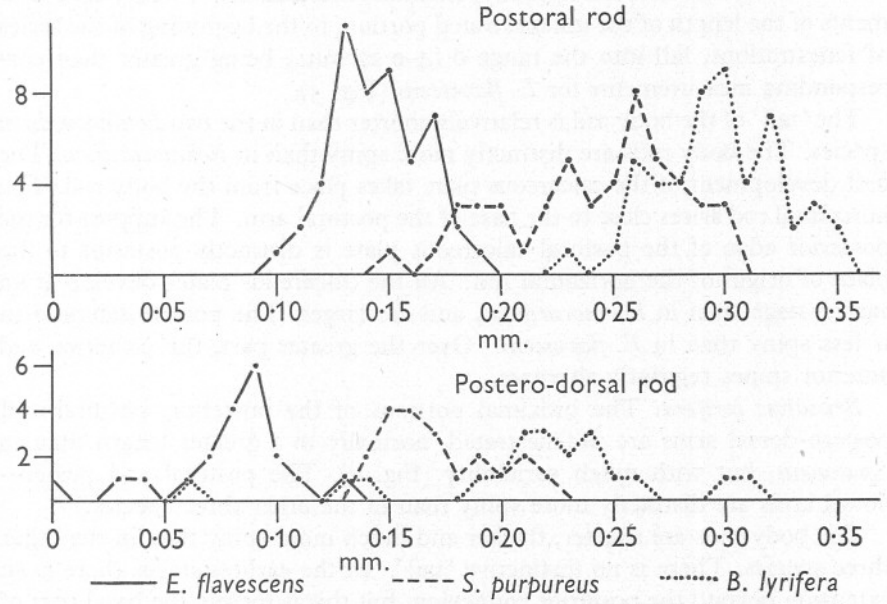

Fig. 5. Length-frequency distribution of the unfenestrated portion of the postoral arm (fifty measurements in each species) and the postero-dorsal arm (twenty measurements in each species) of *Echinocardium flavescens*, *Spatangus purpureus* and *Brissopsis lyrifera*.

inclusive. The proximal part of the postero-dorsal is unfenestrated, apart from a single basal fenestration. Along the unfenestrated part there may occasionally be an isolated fenestration. The length of the unfenestrated part, from the base of the arm to the beginning of the regular series of fenestrations, is variable (Fig. 5), twenty measurements falling into the range 0–0·13 mm.

The 'tail' of the body rod, relative to the full length of the body rod, is as long as that of *E. cordatum*. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated nearer to the base of the postoral arm than in *E. cordatum*, and the support for the posterior edge of the postoral calcareous plate is more or less at the same level as the ventral horizontal rod, or very slightly anterior. The postero-lateral arm

is not so spiny as in *E. cordatum*; over the greater part the spines on the posterior and anterior edges regularly alternate.

Spatangus purpureus. The proximal part of the posterior arm is unfenestrated to a variable length, but commonly about 0·1 mm., apart from a single basal fenestration. In occasional larvae the variation in degree of fenestration may overlap that of *Echinocardium flavescens*. The postoral arm is unfenestrated to a length substantially greater than in *E. flavescens*, forty-eight out of fifty measurements falling into the range 0·18–0·3 mm. inclusive (Fig. 5). The proximal part of the postero-dorsal arm is unfenestrated except for a single basal fenestration and, occasionally, isolated fenestrations. Twenty measurements of the length of the unfenestrated portion, to the beginning of the series of fenestrations, fall into the range 0·14–0·22 mm., being greater than corresponding measurements for *E. flavescens* (Fig. 5).

The 'tail' of the body rod is relatively shorter than in the two *Echinocardium* species. The body rods are distinctly more spiny than in *Echinocardium*. The first development of the calcareous plate takes place from the body rod. The horizontal rod arises close to the base of the postoral arm. The support for the posterior edge of the postoral calcareous plate is distinctly posterior to the place of origin of the horizontal rod. All the calcareous plates develop at an earlier stage than in *Echinocardium*, and are larger. The postero-lateral arm is less spiny than in *E. flavescens*. Over the greater part, the posterior and anterior spines regularly alternate.

Brissopsis lyrifera. The proximal portions of the posterior, postoral and postero-dorsal arms are unfenestrated, normally to a greater length than in *Spatangus*, but with much variability (Fig. 5). The postoral and postero-dorsal arms are distinctly more spiny than in the other three species.

The body rods are shorter, thicker and much more spiny than in the other three species. There is no distinctive 'tail'. In the earliest stages there is an extension beyond the posterior connexion, but this is merely the basal part of the connexion which meets its partner in the median line.

The earliest development of the calcareous plates takes place off the basal part of the antero-lateral arms. The ventral horizontal rod emerges off the postoral arm proper, and *anterior* to the base of the arm (and anterior transverse rod). There is no postero-lateral arm even in the last stage.

Discussion

To my knowledge, the most satisfactory account of a spatangoid skeleton is that by Ohshima (1921) of the larva of *Spatangus purpureus*. Some developmental stages were, however, not available to him, so his account is incomplete and significantly in error. He was unable to recognize that the anterior transverse rod is replaced during development by the ventral horizontal rod. Krohn (1853) gave a ventral view of a larva, corresponding to stage 3 in

Fig. 2, in which both transverse and horizontal rods are present, but did not comment on this. Ohshima (1921) judged that Krohn's drawing was either a misrepresentation or of an abnormal larva. It is, on the contrary, a normal stage of development.

I have not followed the development of the only clypeastroid known in British waters, *Echinocyamus pusillus* (O. Fr. Müller), in any detail, since there is no difficulty in identifying the larva. It is certain, however, that the same replacement of the transverse rod by the horizontal rod occurs, though this was not appreciated by Théel (1892), who described the larva in great detail. Onoda (1938) described the replacement as occurring in the larvae of *Echinarachnius brevis* Ikeda and *Astriclypeus manni* Verrill. Mortensen (1921, p. 231), on the other hand, refers only to the reduplication of the ventral transverse rod in clypeastroids. Later, he described a replacement as occurring in *Fibularia craniolis* (Leske) but called it 'a curious feature' (Mortensen, 1937). Despite this, it is probable that the replacement is a normal feature in clypeastroid larvae, as well as in spatangoid larvae.

Mortensen (1921, p. 202) pointed out that spatangoid larvae may be divided into two groups, one group containing species with postero-lateral arms and the other group those species without. This grouping has the disadvantage that young larvae (and of several spatangoid species only the young larvae have been described) cannot be assigned to a group, for the postero-lateral arm, if present in a species, is rather a late development. A review of the literature on spatangoid larvae suggests that the same grouping will result if the presence or absence of a 'tail' to the body rod is taken as the dividing character; this character would allow the division of very young larvae.

The larva of *Brissopsis lyrifera* is a representative of the group without postero-lateral arms and without a 'tail' to the body rod. A particularly striking characteristic of the larva is the development of the ventral horizontal rod anterior to the transverse rod. In the larvae of *Echinocardium* and *Spatangus*, which are representative of the other group, the ventral horizontal rod develops posterior to the transverse rod.

The larvae of *Echinocardium* and *Spatangus* are very similar; the only difference observed which is likely to be of generic value is the mode of formation of the calcareous plate complex. More information about this process is required than is now available. That there is a basic difference is indicated by the position where the early development occurs, in the region of the anterior connexion in *Echinocardium*, off the body rods in *Spatangus*. It is noteworthy that *Brissopsis* differs from both *Echinocardium* and *Spatangus* in this respect also.

It is unfortunate that the larva of *Echinocardium pennatifidum* remains unknown, for it may prohibit certain identification of *E. cordatum* or *E. flavescens* in the plankton from some places. Arguing that the characters which are common to the larvae of the two latter species are generic characters, it is likely

that the larva of *E. pennatifidum* has postero-lateral arms and a 'tail' to the body rod, the ventral horizontal rod is posterior to the anterior transverse rod, and that the earliest development of the plate complex is in the region of the anterior connexion.

It is not unlikely that the larva of *E. pennatifidum* was present in my material. One of two types of larvae observed may, in fact, be of this species. A single larva observed had all features indicating *E. flavescens* except that the arms were fully fenestrated. This larva was probably outside the range of variation of either *E. cordatum* or *E. flavescens*, but it may have been a hybrid form rather than the unknown larva of *E. pennatifidum*. Several larvae suspected of being *E. cordatum* had postero-lateral arms which were more spiny than usual in undoubted *E. cordatum* larvae. In the main part of the arm in *E. cordatum*, the ratio of posterior spines to anterior spines is approximately 2:1; but in these particular larvae the ratio was greater than 3:1, due to more crowded spines in the posterior edge. These larvae may have been within the range of variation of *E. cordatum* larvae.

It has been usual to indicate the length of the arms, particularly of the posterior process, in describing echinoplutei. Little attention has been given to this point in the present investigation, partly because the available material has not been very suitable, and partly because it has appeared that too much attention to this may be misleading. To be really useful it is required to know the length at each stage and the variation in length. There is good reason for believing the variation in length is considerable. Mortensen (1927) says of *E. cordatum* that the posterior process is 'exceedingly variable in length, sometimes a mere short stump, sometimes as long as the postoral arms'. The larva of *Brissopsis* is described as having a short posterior process (Mortensen, 1920). This is true for larvae which I have seen which had been taken from the Clyde, but some undoubted *Brissopsis* larvae from south-west of Ireland had processes 2 mm. long, that is, longer than that given in Mortensen's (1913) drawing of the larva of *Spatangus purpureus*, whose process is said to be 'exceedingly long'. It would seem at least possible that the variation in the *Spatangus* process is also considerable. On the whole, the posterior process of *E. flavescens* appears to be longer than that of *E. cordatum*.

There is a difficulty in describing echinoplutei, in that the variations are on the whole considerable. The length of the processes and the extent of development of calcareous plates would seem in part determined by the environment. Hörstadius (1940) concluded, following an investigation of echinoid hybrids, that the amount of skeleton produced is increased by higher temperatures. Tennent (1929) pointed out that slight changes in the environmental medium may induce considerable changes in the character of the echinoderm larval skeleton. The length of the unfenestrated portion of the fenestrated arms is also very variable (Fig. 5). The position of the postoral calcareous plate in relation to the ventral horizontal rod, and the region of

early development of the plate complex should, however, prove to be good specific and generic characters.

Summary

An account is given of the general structure of the body skeleton of the spatangid larva. A considerable change occurs in the skeleton as the larva increases in size, some parts being absorbed and new parts appearing. Of particular interest is the replacement of the anterior transverse rod by the ventral horizontal rod.

The skeletons of larvae, young stages to late stages, of *Echinocardium cordatum*, *E. flavescens*, *Spatangus purpureus* and *Brissopsis lyrifera* are described. The larvae of *Echinocardium* and *Spatangus* are, in essentials, very similar; that of *Brissopsis* differs greatly from them. A striking difference is that in *Brissopsis* the ventral horizontal rod arises from the postoral arm and anterior to the anterior transverse rod, in the other two genera it arises from the body rod posterior to the anterior transverse rod.

The replacement of the anterior transverse rod by a ventral horizontal rod is probably normal in clypeastroid larvae also.

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CELLULOLYTIC ACTIVITY IN THE LAMELLIBRANCH CRYSTALLINE STYLE

By B. S. Newell

East African Marine Fisheries Research Organisation, Zanzibar

Lavine (1946) gives the first report of a lamellibranch style cellulase. Whilst investigating the style amylases of *Mactra* and *Mya*, he dialysed aqueous solutions of crystalline style material through Visking casing (regenerated cellulose) and observed that the casing eroded away. Strips of Visking casing immersed in style solution were also dissolved, but filter paper was little affected. Up till then the existence of cellulolytic activity in the lamellibranch style had been denied by Yonge (1926) and Fox *et al.* (1936), for though Fox did obtain some very slight evidence of cellulose utilization, he dismissed it as negligible.

However, the lamellibranch diet seems to consist mainly of dinoflagellates (in fact the sea mussel *Mytilus californianus* is a selective feeder, rejecting diatoms and ingesting dinoflagellates, see Fox *et al.*, 1936), and since many of these latter organisms are reputed to possess a cellulose cell wall or cuticle, their assimilation would seem to require a cellulase. Cellulases have, of course, been reported in many other Mollusca, e.g. *Pterocera* (Yonge, 1932), *Xylophaga* (Purchon, 1941), *Teredo* (Potts, 1923), and the gastric cellulase of *Helix* has been known for many years.

It seemed, in view of Lavine's findings, that the absence of cellulolytic activity previously reported in the lamellibranchs might have been due to the use of an unsuitable substrate. Boswell (1941) quotes evidence that regenerated cellulose is much more easily utilized in the cellulolytic processes of micro-organisms. In view of the report by Yonge (1926) of the presence of active spirochaetes in the crystalline style of the oyster, *Ostrea edulis*, it seemed worth while to make use of this lamellibranch, and also of the mussel, *Mytilus edulis*, in an investigation of the possible presence of a style cellulase with an action upon regenerated cellulose.

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Methods

The cellulose substrate used was in the form of a finely divided suspension of filter-paper, prepared by the method of Scales as given by Stephenson (1939), The stability of the suspension was found to be improved by passage through

an 'Ormerod' emulsifier. In view of the method used (precipitation from 62·5%, v/v, sulphuric acid) the following tests for possible breakdown products of cellulose were carried out: (*a*) Reducing sugars. Qualitatively by Benedict's reagent and the picramate test. Quantitatively by Somogyi's method (1930). (*b*) Amyloids. Iodine coloration test.

No reducing activity was obtained either in the suspension itself or in filtrates from it, subjected to acid hydrolysis. There were no amyloids present, and no cellulose could be detected in filtrates of the suspension (chlor-zinc-iodine test). The suspension gave a green ring in the Molisch test, changing to the more usual violet coloration after about 15 min. Filtrates gave no Molisch reaction at all.

Utilization of cellulose was followed turbidimetrically in a Harvey absorptiometer (Harvey, 1948), and the units of 'turbidity' given in Tables I and II are those of the absorptiometer scale—which are logarithmic, being based on Beer's Law. The data show that the turbidity of the cellulose suspension alone remains constant over the periods of time used in the experiments, and the turbidity of the suspension was found to be proportional to the amount of cellulose present (cf. Stadi & Riggs, 1943).

The style solutions were made by dissolving crystalline style material from *Mytilus* and *Ostrea* in citrate-phosphate buffer at various pH values in the region of 5·5. Preliminary experiments showed that the optimum pH of the cellulolytic activity lay in this region, which approximates to the pH of the stomach fluid in *Ostrea* (Yonge, 1926). The cellulose in later experiments was also suspended in citrate-phosphate buffer. All experiments were conducted at laboratory temperature, which varied between 15 and 19° C.

Results

Boiling tubes were set up in series, each containing an equal volume of reaction mixture. A typical experiment was as follows, using style material from *O. edulis*.

Tubes A and B, 5 ml. buffer + 3 ml. cellulose suspension.

Tubes C, E and F, 5 ml. style solution + 3 ml. cellulose suspension.

Tubes B and C were boiled for 3 min. to act as controls. The turbidities of the five tubes were measured at intervals and are given in Table I as absorptiometer scale readings.

TABLE I

	Hours								
Tube	0	1	2	3	4	6	7	8	20
A	275	270	269	270	262	270	270	265	268
B	275	270	268	270	262	268	272	269	270
C	408	406	410	406	404	400	402	408	409
E	235	225	207	200	187	176	177	171	146
F	238	223	213	194	184	173	168	164	142

After this experiment, 5 ml. samples from tubes A, B, E and F were taken (shaking well to disperse the suspension as uniformly as possible). Each sample was centrifuged, decanted, and washed, three times, and the residual cellulose then dried to constant weight. The weights are shown below:

Tube	Residual cellulose (mg.)	Tube	Residual cellulose (mg.)
A (control)	14·9	E (active)	10·2
B (control)	15·0	F (active)	8·6

The activity is clearly thermo-labile.

In the case of experiments using material from *Mytilus edulis*, a similar decline in turbidity in tubes containing unboiled style solution and cellulose occurred, but activity was less than with style material from *Ostrea*. The styles from the two animals differ in appearance, and Berkeley (1935) has shown that qualitative differences in the glyco-proteins, at least, of various lamellibranch styles do occur. Since it might have been that the cellulose utilization was merely due to some of the numerous bacteria inhabiting the style, various preservative agents were used in later experiments. 'Merthiolate' (1 in 200,000) and chloroform (0·5–8 ml. of active preparation) did not appear to inhibit cellulose utilization. Xylene and toluene (also 0·5–8 ml. of active preparation) made turbidity measurements impracticable, but after one experiment, using *Mytilus* material, and toluene, the residual cellulose in each of three tubes after 20 hr. activity was collected, washed, dried and weighed. The weights are given below:

Tube	Residual cellulose (mg.)
Control	14·3
Preparation with toluene	8·9
Preparation without toluene	8·6

Thus toluene, at least, does not abolish cellulose utilization.

It was found that reducing substances appeared in active preparations as cellulose disappeared, and in some experiments the increase in such reducing activity, with time, was followed.

In one such experiment, five tubes were set up containing cellulose suspension in buffer and *Mytilus* style material, also in buffer. Relevant controls were set up as before, but this time included a tube with style solution only, to compensate for reducing activity in the style itself. This proved to be a necessary precaution.

The turbidities of tubes containing the whole system showed a regular decline, whilst those of the controls remained constant. After various periods of time, single tubes were withdrawn and activity stopped by the addition of sodium hydroxide solution to a pH above 8·5 (phenol red). The reaction mixtures were filtered and the filtrates retained. Estimation of reducing activity was effected by the Somogyi method (Somogyi, 1930), on 5 ml.

samples from two tubes before, and all five tubes after, acid hydrolysis. This hydrolysis was carried out by heating the samples for 10 hr. at 90° C., after adding five drops of concentrated sulphuric acid. The reducing activity is given below, corrected for reagent and style solution reducing substances, and expressed as milligrams of glucose per millilitre of reaction mixture.

Incubation time (hr.)	Reducing substances	
	Before acid hydrolysis	After acid hydrolysis
0	Nil	Nil
6½	0·051	0·066
19	—	0·116
25	—	0·144
43	0·072	0·166

It will be seen that substances able to reduce the Somogyi reagent are found in the *Mytilus* style preparation, but that the large increase in the reducing power of filtrates after acid hydrolysis suggests that the principal immediate products of cellulose breakdown are soluble compounds with little reducing power.

When active tubes were evacuated or tightly stoppered, the activity was slowed down or stopped. Using style material from *Mytilus*, five experimental tubes were set up (Table II). Control tube B showed no change with time. Boiling the control tube 1 increased the turbidity which then showed no further change with time. After 5 hr. the active tube 3 was tightly stoppered which prevented further reduction of turbidity. In the other two active tubes reduction continued until the end of the experiment.

TABLE II. TURBIDITY, AS LOGARITHMIC SCALE READINGS OF THE ABSORPTIOMETER

Tube	Hours								
	0	1	2	3	5	7	19	22	25
B (control)	363	364	366	365	370	367	367	370	370
1 (control)	662	662	662	663	662	661	662	662	662
3 (active)	432	402	390	384	375	377	370	376	374
4 (active)	417	392	374	371	358	352	327	322	312
5 (active)	412	382	367	362	352	350	314	314	314

From this investigation and from Berkeley's (1933 *a*, *b*) work on style oxidase, presence of glucosone (2-keto-glucose) was expected but neither glucosone nor glucose could be detected, no keto groups could be demonstrated by the Rothera or nitro-prusside tests.

On warming protein-free filtrates of reaction mixtures with phenyl hydrazine hydrochloride, in 2N-hydrochloric acid, a phenyl hydrazone of colourless pinnate crystals could be obtained. After acid hydrolysis, filtrates gave an ozazone resembling glucosazone, and having a decomposition point at 205° C. Further identification of the products of this cellulose utilizing system was not possible in the limited time available.

This investigation extends Lavine's findings of cellulase in *Mactra* and *Mya* to *Ostrea* and *Mytilus*. It has not established an actual relation between the cellulolytic action of the style and the presence of spirochaetes, and in *Mytilus* the bacterial population of the style is not yet known. In view, however, of the known ability of some groups of spirochaetes (see Walker & Warren, 1938) to hydrolyse cellulose to simpler carbohydrates, a connexion between style spirochaetes and cellulolytic action remains an interesting possibility. Morton (1952) discusses this point more fully.

Summary

A method of demonstrating cellulose utilization in biological systems by measurement of the turbidity of a cellulose suspension in a photo-electric absorptiometer is described. The crystalline styles of *Ostrea edulis* and *Mytilus edulis* appear to contain a cellulolytic factor as yet uncharacterized.

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A PRELIMINARY CHECK-LIST OF BRITISH MARINE ALGAE

By Mary Parke, D.Sc.
The Plymouth Laboratory

This preliminary list of some of the classes of the algae has been prepared, mainly for the use of British phycologists, to form a basis on which to work for the eventual compilation of a complete check and locality list of all species of British marine algae. The compiler would be grateful for all additions and corrections to this list, and for records of marine species from the algal classes not included in the present list.

The classes of algae treated in this list are the Chlorophyceae (53 gen., 156 spp.), Xanthophyceae (7 gen., 15 spp.), Chrysophyceae (15 gen., 22 spp.), Phaeophyceae (96 gen., 199 spp.), Rhodophyceae (126 gen., 324 spp.), and Cyanophyceae (39 gen., 125 spp.). The genera are listed alphabetically under the family and the species alphabetically under the genus. If a name has been changed from that given in Newton (1931), the name in Newton is added, in brackets, below. Names preceded by a question mark appear to need revision for nomenclatural or taxonomic reasons. Most of the so-called varieties listed are very probably merely habitat forms of the species, but further investigation is needed before new combinations are made for them. Notes are given at the end of each class, the number in brackets following a name in the list refers to these notes.

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CHLOROPHYCEAE

VOLVOCALES

Chlamydomonadaceae

BRACHIOMONAS Bohlin, 1897.
simplex Hazen
submarina Bohlin
? *westiana* Pascher

CARTERIA Diesing, 1866.
excavata Mass. ex Carter

CHLAMYDOMONAS Ehrenberg, 1833.
brachyura G. S. West
microplankton Rke.
quadrilobata Carter

PLATYMONAS G. S. West, 1916.
apiculata Butcher
contracta Carter
tetrathele G. S. West

Polyblepharidaceae

BIPEDINOMONAS Carter, 1937.
pyriformis Carter
rotunda Carter

DUNALIELLA Teodoresco, 1905.
salina (Dunal) Teodor.

HETEROMASTIX Korshikov, 1923.
minuta Carter

PYRAMIMONAS Schmarda, 1850. (3)
angulata Carter
grossii Parke
obovata Carter
octociliata Carter
olivacea Carter

Chlorodendraceae

PRASINOCLADUS Kuckuck, 1894.
lubricus Kuck.

CHLOROCOCCALES

Chlorococcaceae

CHARACIUM A. Braun, 1849.
? *marinum* Kjellm. (1)

CHLOROCHYTRIUM Cohn, 1872.
cohnii Wright
dermatocolax Rke.
facciolae (Borzi) Bristol
immersum Masse
inclusum Kjellm.
moorei Gardner
willei Printz

CODIOLUM A. Braun, 1855. (2)
petrocelidis Kuck.
? *pusillum* (Lyngb.) Kjellm.

SYKIDION Wright, 1881.
dyeri Wright

Chlorellaceae

CHLORELLA Beijerinck, 1890.
marina Butcher
ovalis Butcher
salina Butcher
stigmatophora Butcher

NANNOCHLORIS Naumann, 1919. (3)
atomus Butcher
maculatus Butcher

TREBOUXIA de Puymaly, 1924.
humicola (Treboux) Puymaly

ULOTRICHALES

Ulotrichaceae

STICHOCOCCUS Nägeli, 1849.
cylindricus Butcher

ULOTHRIX Kützing, 1833. (4)
consociata Wille
flacca (Dillw.) Thur.
pseudoflacca Wille
subflaccida Wille
(? *implexa* Kütz.)

Monostromaceae

MONOSTROMA Thuret, 1854. (5)
crepidinum Farl.
fuscum (Post. et Rupr.) Wittr.
(*blyttii* (Aresch.) Wittr.)
grevillei (Thur.) Wittr.
oxyspermum (Kütz.) Doty
(*laceratum* Thur.)
(*latissimum* (Kütz.) Wittr.)
(*orbiculatum* Thur.)
(*quaternarium* (Kütz.) Desm.)
(*wittrockii* Born.)
undulatum Wittr.

Ulvaceae

BLIDINGIA Kylin, 1947.
minima (Näg.) Kylin
(*Enteromorpha minima* Näg.)
(*Enteromorpha micrococca* Kütz.)

CAPSOSIPHON Gobi, 1879. (6)
fulvescens (Ag.) Setch. et Gardn.
(*C. aureolus* (Ag.) Gobi)

ENTEROMORPHA Link, 1820. (7)
ahlneriana Bliding
clathrata (Roth) Grev.
compressa (L.) Grev.
intestinalis (L.) Link
? *lingulata* J. Ag.
linza (L.) J. Ag.
? *marginata* J. Ag.
prolifera (Müll.) J. Ag.
ralfsii Harv.
ramulosa (Sm.) Hook.
torta (Mert.) Reinb.
? *usneoides* (Bonnem.) J. Ag.

PERCURSARIA Bory, 1823.
percursa (Ag.) Bory

ULVA Linnaeus, 1753.
? *lactuca* L.
var. ? *lactuca*
var. ? *latissima* (L.) D.C.
var. ? *rigida* (Ag.) Le Jol.

PRASIOLALES

Prasiolaceae

GAYELLA Kolderup Rosenvinge, 1893.
polyrhiza K. Rosenv.
PRASIOLA Agardh, 1822.
crispa (Lightf.) Menegh.
stipitata Suhr

CHAETOPHORALES

Chaetophoraceae

ACROCHAETE Pringsheim, 1862.
parasitica Oltm.
repens Pringsh.
BOLBOCOLEON Pringsheim, 1862.
piliferum Pringsh.
CHAETOBOLUS Kolderup Rosenvinge, 1893.
gibbus K. Rosenv. (8)
ECTOCHAETE (Huber) Wille, 1909. (9)
leptochaete (Huber) Wille
(*Endoderma leptochaete* Huber)
wittrockii (Wille) Kylin
(*Endoderma wittrockii* Wille)
ENTOCLADIA Reinke, 1879.
perforans (Huber) Levring
viridis Rke.
(*Endoderma viride* Lagerh.)
EPICLADIA Reinke, 1889.
flustrae Rke.
(*Endoderma flustrae* Batt.)
var. *flustrae*
var. ? *phillipsii* Batt.
OCHLOCHAETE Thwaites ex Harvey, 1849. (8)
ferox Huber
hystrix Thwaites
PHAEOPHILA Hauck, 1876.
dendroides (Crn.) Batt.
engleri Rke.
PILINIA Kützing, 1843.
maritima (Kjellm.) K. Rosenv.
rimosa Kütz.
PRINGSHEIMIELLA v. Hoehnel, 1920.
scutata (Rke.) Marchew.
(*Pringsheimia scutata* Rke.)
PROTODERMA Kützing emend. Borzi, 1895
marinum Rke.
PSEUDOPRINGSHEIMIA Wille, 1909.
confluens (K. Rosenv.) Wille
(*Ulvella confluens* K. Rosenv.)
fucicola (K. Rosenv.) Wille (10)
(*Ulvella fucicola* K. Rosenv.)
PSEUDULVELLA Wille, 1909.
applanata Setch. et Gardn.
ULVELLA Crouan, 1859.
lens Crn.

Trentepohliaceae

? GOMONTIA Bornet et Flahault, 1888. (11)
? *manxiana* Chodat
? *polyrhiza* (Lagerh.) Born. et Flah.
TELLAMIA Batters, 1895.
contorta Batt.
? *intricata* Batt.

CLADOPHORALES

Cladophoraceae (12)

ACROSIPHONIA J. Agardh; sensu Wille, 1909.
centralis (Lyngb.) Kjellm. (13)
(*Cladophora* (*Spongomorpha*) *arcta* (Dillw.) Kütz.)
CHAETOMORPHA Kützing, 1845.
? *aerea* (Dillw.) Kütz.
capillaris (Kütz.) Børg. (14)
crassa (Ag.) Kütz.
linum (Müll.) Kütz.
? *litorea* Cooke
melagonium (Web. et Mohr) Kütz.
CLADOPHORA Kützing, 1843.
albida (Huds.) Kütz.
var. *albida*
var. *refracta* (Harv.) Thur.
? *arctiuscula* Kütz.
? *balliana* Harv.
brownii (Dillw.) Harv.
cornea Kütz.
var. *verticillata* Kütz.
? *corymbifera* Kütz. (17)
corynarthra Kütz.
var. *spinescens* Batt.
expansa (Mert.) Kütz.
? *falcata* Harv.
? *flexuosa* (Griff.) Harv.
fracta (Müll.) Kütz. (15)
glaucescens (Griff.) Harv.
gracilis (Griff.) Kütz.
hirta Kütz.
hutchinsiae (Dillw.) Kütz.
var. *hutchinsiae*
var. *distans* (Ag.) Kütz. (16)
var. ? *divaricata* Harv.
macallana Harv.
magdalenae Harv.
pachyderma (Kjellm.) Brand
var. *tenuior* (Børg.) Brand
pellucida (Huds.) Kütz.
var. *pellucida*
var. ? *comosa* Kütz.
var. ? *cristata* Kütz.
var. ? *curvata* Kütz.
prolifera (Roth) Kütz.
ramosissima (Drap.) Kütz.
f. *humilis* (Kütz.) Hamel
(*C. neesiorum* Kütz. var. *humili* Batt.)
rectangularis (Griff.) Harv.
f. *rectangularis*
f. *hispida* Kütz.
f. *horrida* Kütz.
f. *subnuda* Kütz.
? *refracta* (Roth) Kütz. (17)
repens (J. Ag.) Harv.
rudolphiana (Ag.) Harv.

CLADOPHORA (*cont.*)
rupestris (L.) Kütz.
f. *rupestris*
f. ? *distorta* (Harv.)
f. *nuda* (Harv.) Hamel
? *sericea* (Huds.) Kütz. (18)
sonderi Kütz.
stolonifera (Kjellm.) Batt.
? *traillii* (Batt.) Batt.
? *trichocoma* Kütz.
utriculosa Kütz.
var. *utriculosa*
var. ? *diffusa* Hauck
RHIZOCLONIUM Kützing, 1843.
arenosum (Carm.) Kütz.
? *hieroglyphicum* (Ag.) Kütz.
implexum (Dillw.) Kütz.
? *kerneri* Stockm.
kochianum Kütz.
riparium (Roth) Harv.
tortuosum (Dillw.) Kütz. (19)
SPONGOMORPHA Kützing, 1843.
bombycina (Kjellm.) Wille
lanosa (Roth) Kütz.
var. *lanosa*
var. ? *uncialis* (Müll.)
pallida (Kjellm.) Kylin
UROSPORA Areschoug, 1866.
bangioides (Harv.) Holm. et Batt.
penicilliformis (Roth) Aresch. (2)
(*isogona* (Sm.) Batt.)
sporophyte *Codiolum gregarium* A.Br.
speciosa (Carm.) Leblond ex Hamel
(*Ulothrix speciosa* (Carm.) Kütz.)
wormskioldii (Mert.) K. Rosenv.
(*collabens* (Ag.) Holm. et Batt.)

? SIPHONALES

Caulerpaceae
BRYOPSIS Lamouroux, 1809.
hypnoides Lamour.
plumosa (Huds.) Ag.
var. *plumosa*
var. ? *nuda* Holm.
var. ? *subsimplex* Holm. et Batt.

Derbesiaceae
DERBESIA Solier, 1847. (20)
marina (Lyngb.) Kjellm.
gametophyte = *Halicystis ovalis* (Lyngb.) Aresch.
tenuissima (De Not.) Crn.

Codiaceae
CODIUM Stackhouse, 1797.
adhaerens (Cabr.) Ag.
? *amphibium* Moore
bursa (L.) Ag.
? *fragile* (Sur.) Hariot
f. *atlanticum* (Cotton) Levring
(*tomentosum* var. *atlanticum* Cotton)
? *tomentosum* Stackh.

Chaetosiphonaceae
BLASTOPHYSA Reinke, 1888.
rhizopus Rke.

Phyllosiphonaceae
OSTREOBIUM Bornet et Flahault, 1889.
quekettii Born. et Flah.

Notes on Chlorophyceae

(1) Not an independent species according to Levring (1937)—a *Cladophora* sporeling.
(2) *C. gregarium* A.Br., diploid generation of *Urospora penicilliformis*; *C. petrocelidis*, probably an independent form.
(3) Systematic position uncertain; see Chadefaud (1941) on *Pyramimonas* Schmarda.
(4) British species of *Ulothrix* require revision.
(5) British species in urgent need of revision: see Hamel (1931) for species included under *M. oxycoccum* (Kütz.) Thur. = *M. oxyspermum* (Kütz.) Doty.
(6) Chapman (1952) advocates the removal of the genus *Capsosiphon* from the Ulvaceae to separate family, Capsosiphonaceae.
(7) Until British material belonging to this genus has been fully investigated on the lines of the work of Bliding, the limits of the species occurring in Britain cannot be assessed.

Dr C. Bliding has checked the list of species personally, and has very kindly allowed me to publish his records of *E. ahlneriana* for the British coast. He reports:

'During an excursion to Anglesey, North Wales, in July 1953, I found *Enteromorpha Ahlneriana* Bliding in the intertidal zone at two different localities, Rhosneigr and Newborough.

'At Rhosneigr the main axes of the thalli were often very broad with few branches, as in Typus III (Bliding, 1944, p. 343, fig. 16). The material from Newborough, however, was more branched and the axes of the plants, as well as the branches, were more slender.

'*E. Ahlneriana* has so far been recorded only from the west coast of Sweden and from the Baltic Sea. In the Baltic this species is very abundant.' (Carl Bliding, Plymouth, 17 July 1953.)

(8) *Chaetobolus gibbus*, *Ochlochaete ferox* and *O. hystrix*—possibly forms of one species, see Waern (1952).
(9) *Endoderma-Ectochaete-Entocladia-Epicladia* complex—British material needs revision.
(10) *Ulvella fucicola* var. *globosa* Batt.; before making new combination under *Pseudopringsheimia* further investigation seems desirable.
(11) If Kylin (1935) is right *Gomontia* is a name of a taxon derived from two entirely discordant elements and should perhaps be rejected (Art. 76, Lanjouw, 1952).
(12) The British species of all genera in this family are in urgent need of revision.
(13) Part at least, if not all, material recorded for Britain under *Cl.* (*Spongomorpha*) *arcta* should be referred to *Acrosiphonia centralis* (Lyngb.) Kjellm.
(14) ='*Conferva tortuosa* J. Ag.', Harv., *Phyc. Brit.* 54A, *Chaetomorpha tortuosa* Kütz., in Newton, 1931, p. 91 (Chapman, 1939).
(15) British forms referred to this species are in need of revision.
(16) It is generally considered that the alga described and figured by Harvey, *Phyc. Brit.*, 130, as *Cladophora diffusa* is *C. hutchinsiae* Kütz. var. *distans* (Ag.) Kütz., which differs from *Conferva diffusa* Roth (=*Cladophora diffusa* (Roth) Harv.).
(17) Possibly forms of *Cladophora hamosa* Kütz.
(18) Should possibly be *Cladophora crystallina* (Roth) Kütz., see Levring (1937, 1940), Waern, 1952.
(19) =*Conferva tortuosa* Dillw., *Conferva implexa* Harv., *Phyc. Brit.*, 54B, *Lola implexa* (Harv.) Hamel (Chapman, 1939).
(20) HALICYSTIS Areschoug, 1850; gametophyte generation of *Derbesia marina* (Lyngb.) Kjellm. (*Halicystis ovalis* (Lyngb.) Aresch.) recorded for Britain but not the gametophyte generation (*H. parvula* Schmitz) of *Derbesia tenuissima* (De Not.) Crn.

XANTHOPHYCEAE

HETEROCHLORIDALES

Heterochloridaceae
CHLOROMESON Pascher, 1930.
parvum Carter
NEPHROCHLORIS Geitler et Gemisi, 1925.
? *salina* Carter (1)
OLISTHODISCUS Carter, 1937. (1)
luteus Carter
RHIZOCHLORIS Pascher, 1917.
arachnoides Carter

HETEROCOCCALES

Halosphaeraceae
HALOSPHAERA Schmitz, 1879.
viridis Schm.

HETEROTRICHALES

Tribonemataceae
TRIBONEMA Derbès et Solier, 1856.
bombycinum Derb. et Sol.
endozooticum (Wille) Magne
(*Rhizoclonium kerneri* Stockm.
f. *endozooticum* Wille)

HETEROSIPHONALES

Vaucheriaceae (2)
VAUCHERIA De Candolle, 1801. (3)
compacta (Collins) Collins s.lat.
(*sphaerospora* Nordst. var. *dioica*
K. Rosenv.)
coronata Nordst.
dichotoma (L.) Ag.
intermedia Nordst.
litorea Hofm. et Ag.
sphaerospora Nordst.
synandra Woron.
thuretii Woron.

Notes on Xanthophyceae

(1) Systematic position uncertain.
(2) Reasons for placing in Xanthophyceae—see Chadefaud (1945, 1951), Smith (1950).
(3) The list of species given has been checked by Mr T. Christensen, who also says that the *Vaucheria* sp. recorded by Carter as possibly *V. woroniniana* Heer. is definitely not this species. See also Christensen (1952) for information on distribution.

CHRYSOPHYCEAE

CHRYSOMONADALES (1)

Chromulinaceae
CHROMULINA Cienkowski, 1870.
lunaris Carter
pleiades Parke
pusilla Butcher

Cyrtophoraceae
PSEUDOPEDINELLA Carter, 1937.
pyriformis Carter

Isochrysidaceae
DICRATERIA Parke, 1949.
gilva Parke
inornata Parke
ISOCHRYSIS Parke, 1949.
galbana Parke

Ochromonadaceae
OCHROMONAS Wystozki, 1887.
oblonga Carter
PAVLOVA Butcher, 1952.
gyrans Butcher

Prymnesiaceae
PRYMNESIUM Massart, 1920.
minutum Carter
parvum Carter

RHIZOCHRYSIDALES

Platychrysidaceae
PLATYCHRYSIS Geitler, 1930.
pigra Geitler

CHRYSOCAPSALES

Chrysocapsaceae
CHRYSOTILA Anand, 1937. (2)
lamellosa Anand
stipitata Anand
GLOEOCHRYSIS Pascher, 1925.
litoralis Anand
maritima Anand
PHAEOCYSTIS Lagerheim, 1893.
globosa Scherff.
pouchetii (Hariot) Lagerh.

CHRYSOTRICHALES

Nematochrysidaceae
NEMATOCHRYSIS Pascher, 1925.
sessilis Pascher
var. *vectensis* Carter

Phaeothamnionaceae
APISTONEMA Pascher, 1925. (3)
carteri Anand (4)
CHRYSONEMA Anand, 1937.
litorale Anand

Thallochrysidaceae
THALLOCHRYSIS Conrad, 1920.
litoralis Anand

Notes on Chrysophyceae

(1) Coccolithophoridaceae not included in this preliminary list.
(2) Systematic position uncertain: should possibly be placed as the type of a new family.
(3) *Phaeococcus adnatus* G. S. West ex Cotton (1912), imperfectly described by Cooke and Rabenhorst under the name *Gloeocystis adnata* Näg., belongs, very possibly, to the genus *Apistonema* Pascher.
(4) See Waern (1952)—possibly a form of *A. pyrenigerum* Pascher.

PHAEOPHYCEAE

ECTOCARPALES

Ectocarpaceae (1)
ACINETOSPORA Bornet, 1891.
crinita (Carm.) Kornmann (2)
(*pusilla* (Griff.) Born.)
(*pusilla* var. *crinita* (Carm.) Batt.)
haploid generation =
? *Ectocarpus lebelii* Crn.
? *Ectocarpus padinae* Sauv.
CHILIONEMA Sauvageau, 1897. (3)
ocellatum (Kütz.) Kuck.
(*nathaliae* Sauv.)
(*ocellatum* (Rke.) Sauv.)
reptans (Crn.) Sauv.
COMPSONEMA Kuckuck, 1899. (3)
microspongium (Batt.) Kuck.
(*Ectocarpus microspongium* Batt.)
saxicola Kuck.
(*Myrionema saxicolum* Kuck.)

? DICHOSPORANGIUM Hauck, 1885.
? *chordariae* Wollny (4)
(*Streblonema chordariae* Cotton in Newton, p. 128, non *S. chordariae* (Farl.) De Toni)
ECTOCARPUS Lyngbye, 1819.
? *acanthophorus* Kütz.
confervoides (Roth) Le Jol. s. lat.
(*confervoides* s.str.)
(*amphibius* Harv.)
(*arctus* Kütz.)
(*dasycarpus* Kuck.)
(*hiemalis* Crn.)
(*penicillatus* (Ag.) Kjellm.)
(*siliculosus* (Dillw.) Lyngb.)
congestus Crn.
crouanii Thur.
? *distortus* Carm.
draparnaldioides (Crn.) Kjellm.
? *erectus* Kütz.
fasciculatus Harv.
? *fenestratus* Berk. ex Harv.
holmesii Batt.
? *landsburgii* Harv.
? *minimus* Näg. (5)
reinboldii Rke.
ENDODICTYON Gran, 1897.
infestans Gran (9)
(*Streblonema infestans* Batt.)
FELDMANNIA Hamel, 1939.
battersii (Born.) Hamel
(*Ectocarpus battersii* Born.)
globifera (Kütz.) Hamel
(*Ectocarpus globifer* Kütz.) (6)
? *irregularis* (Kütz.) Hamel
(*Ectocarpus irregularis* Kütz.)
simplex (Crn.) Hamel
(*Ectocarpus simplex* Crn.)
GIFFORDIA Batters emend. Hamel, 1939.
granulosa (Sm.) Hamel
(*Ectocarpus granulosus* (Sm.) Ag.)
hincksiae (Harv.) Hamel
(*Ectocarpus hincksiae* Harv.)
mitchellae (Harv.) Hamel
(*Ectocarpus mitchellae* Harv.)
ovata (Kjellm.) Kylin
(*Ectocarpus ovatus* Kjellm.)
var. *ovata*
var. *arachnoidea* (Rke.) Kylin
sandriana (Zanard.) Hamel
(*Ectocarpus sandrianus* Zanard.)
secunda (Kütz.) Batt.
(*Ectocarpus secundus* Kütz.)
HECATONEMA Sauvageau 1897. (3)
liechtensternii (Hauck) Batt.
maculans (Coll.) Sauv.
speciosum (Børg.) Cotton
HERPONEMA J. Agardh emend. Hamel, 1939.
luteolum (Sauv.) Hamel
(*Ectocarpus luteolus* Sauv.)
solitarium (Sauv.) Hamel
(*Ectocarpus solitarius* Sauv.)
valiantei (Born.) Hamel
(*Ectocarpus valiantei* Born.)
velutinum (Grev.) J. Ag.
(*Ectocarpus velutinus* (Grev.) Kütz.) (7)
LAMINARIOCOLAX Kylin, 1947.
tomentosoides (Farl.) Kylin
(*Ectocarpus tomentosoides* Farl.) (8)
MIKROSYPHAR Kuckuck, 1895. (9)
polysiphoniae Kuck.
porphyrae Kuck.
PHAEOSTROMA Kuckuck, 1895.
pustulosum Kuck. (10)
(*P. prostratum* Kuck.)
PYLAIELLA Bory, 1823.
littoralis (L.) Kjellm. (11)
SOROCARPUS Pringsheim, 1862.
micromorus (Bory) Silva
(*uvaeformis* (Lyngb.) Pringsh.)
SPONGONEMA Kützing, 1849.
tomentosum (Huds.) Kütz.
(*Ectocarpus tomentosus* (Huds.) Lyngb. *E. terminalis* Kutz.) (3)
STREBLONEMA Derbès et Solier, 1851. (12)
? *aequale* Oltm. (10)
breve (Sauv.) De Toni
(*Ectocarpus brevis* Sauv.)
effusum Kylin
fasciculatum Thur.
var. *fasciculatum*
var. ? *simplex* Batt.
? *helophorum* (K. Rosenv.) Batt.
intestinum (Reinsch) Holm. et Batt.
parasiticum (Sauv.) De Toni
(*Ectocarpus parasiticus* Sauv.)
sphaericum (Derb. et Sol.) Thur.
stilophorae (Crn.) De Toni
(*Ectocarpus stilophorae* Crn.) (13)
var. *stilophorae*
var. *caespitosum* (K. Rosenv.) De Toni
? *tenuissimum* Hauck (13)
volubile (Crn.) Thur.
zanardinii (Crn.) De Toni
WAERNIELLA Kylin, 1947.
lucifuga (Kuck.) Kylin

Lithodermataceae

LITHODERMA Areschoug, 1875.
extensum (Crn.) Hamel (14)
(*fatiscens* Aresch. emend. Kuck.)
PETRODERMA Kuckuck, 1897.
maculiforme (Wollny) Kuck.
SORAPION Kuckuck, 1894.
simulans Kuck.

Ralfsiaceae

RALFSIA Berkeley, 1831. (15)
clavata (Carm.) Farlow
disciformis Crn.
pusilla (Strömf.) Fosl.
spongiocarpa Batt. (16)
verrucosa (Aresch.) J. Ag.

Myrionemataceae
ASCOCYCLUS Magnus, 1874.
foecundus (Strömf.) Cotton
hispanicus Sauv.
magni Sauv.
(*orbicularis* Magn. in Newton, p. 159)
MICROSPONGIUM Reinke, 1888.
globosum Rke.
(*Hecatonema globosum* (Rke.) Batt.)
MYRIONEMA Greville, 1827.
aecidioides (K. Rosenv.) Sauv. (12)
corunnae Sauv.
papillosum Sauv.
polycladum Sauv.
strangulans Grev.
SYMPHYOCARPUS Kolderup Rosenvinge, 1894.
strangulans K. Rosenv.
? ULONEMA Foslie, 1894. (17)
? *rhizophorum* Fosl.

Elachistaceae
ELACHISTA Duby, 1830. (18)
flaccida (Dillw.) Aresch.
fucicola (Vell.) Aresch.
f. *fucicola*
f. *grevillei* (Arnott) Hamel
(*E. grevillei* Arnott)
scutulata (Sm.) Duby
HALOTHRIX Reinke, 1888.
lumbricalis (Kütz.) Rke.
LEPTONEMA Reinke, 1888.
fasciculatum Rke.
var. *fasciculatum*
var. ? *majus* Rke. (19)
var. ? *subcylindrica* K. Rosenv. (19)
var. ? *uncinatum* Rke. (19)
SYMPHORICOCCUS Reinke, 1888.
stellaris (Aresch.) Kuck. (20)
(*Elachista stellaris* Aresch.)

Leathesiaceae
CORYNOPHLAEA Kützing, 1843.
crispa (Harv.) Kuck.
(*Leathesia crispa* Harv.)
CYLINDROCARPUS Crouan, 1851.
(*Petrospongium* Näg.)
berkeleyi (Grev.) Crn.
(*Petrospongium berkeleyi* Näg.)
microscopicus Crn.
(*Ectocarpus microscopicus* Batt.)
LEATHESIA S. F. Gray, 1821.
difformis (L.) Aresch.
MICROCORYNE Strömfelt, 1888.
ocellata Strömf. (21)
MYRIACTULA Kuntze, 1898.
(*Myriactis* Kütz.)
areschougii (Crn.) Hamel
chordae (Aresch.) Levring
(*Elachista stellaris* var. *chordae* Aresch.)
clandestina (Crn.) J. Feldm.
(*Ectocarpus clandestinus* (Crn.) Sauv.)
haydenii (Gatty) Levring
rivulariae (Suhr) J. Feldm.
(*Myriactis pulvinata* Kütz.)
stellulata (Griff.) Levring

Mesogloiaceae
CHORDARIA Agardh emend. Lyngbye, 1819.
flagelliformis (Müll.) Ag.
var. *flagelliformis*
var. ? *firmus* Kjellm.
var. ? *minor* J. Ag.
CLADOSIPHON Kützing, 1843.
contortus (Thur.) Kylin
(*Castagnea contorta* Thur.)
zosterae (J. Ag.) Kylin
(*Castagnea zosterae* (J. Ag.) Thur.)
EUDESME J. Agardh, 1880.
virescens (Carm.) J. Ag.
(*Castagnea virescens* (Carm.) Thur.)
LIEBMANNIA J. Agardh, 1842.
leveillei J. Ag.
(*Mesogloia leveillei* Menegh.)
MESOGLOIA Agardh, 1817.
lanosa Crn.
? *neglecta* Batt. (22)
vermiculata (Sm.) Le Jol.
MYRIOCLADIA J. Agardh, 1841. (23)
tomentosa Crn.
SAUVAGEAUGLOIA Hamel, 1939.
griffithsiana (Grev.) Hamel
(*Mesogloia griffithsiana* Grev.)
SPHAEROTRICHIA Kylin, 1940.
divaricata (Ag.) Kylin
(*Chordaria divaricata* Ag.)
STREPSITHALIA Sauvageau, 1896.
buffhamiana (Batt.) Batt.

Acrothricaceae
ACROTHRIX Kylin, 1907.
gracilis Kylin

Spermatochnaceae
SPERMATOCHNUS Kutzing emend. Reinke, 1889.
paradoxus (Roth) Kütz.
STILOPHORA J. Agardh, 1841.
rhizodes (Ehrh.) J. Ag.
tuberculosa (Horn.) Rke.
STILOPSIS Kuckuck, 1929.
lejolisii (Thur.) Kuck.
(*Spermatochnus lejolisii* Rke.)

DICTYOSIPHONALES

Striariaceae
ISTHMOPLEA Kjellman, 1877.
sphaerophora (Carm.) Kjellm.
STICTYOSIPHON Kützing, 1843.
griffithsianus (Le Jol.) Holm. et Batt. (24)
(*Phloeospora brachiata* Born. in Newton, p. 187)
soriferus (Rke.) K. Rosenv. (25)
subarticulatus (Aresch.) Hauck (25)
tortilis (Rupr.) Rke. (25)
STRIARIA Greville, 1829.
attenuata Grev.
f. *attenuata*
f. *crinita* (Ruch.) Hauck

Myriotrichiaceae
BUFFHAMIA Batters, 1895. (26)
speciosa Batt.
LEBLONDIELLA Hamel, 1939.
densa (Batt.) Hamel
(*Myriotrichia densa* Batt.)
MYRIOTRICHIA Harvey, 1834.
clavaeformis Harv.
var. *clavaeformis*
var. ? *minima* Holm. et Batt.
? *filiformis* Harv. (27)
repens (Hauck) Karsakoff

Giraudiaceae
GIRAUDIA Derbès et Solier, 1851. (26)
sphacelarioides Derb. et Sol.

Punctariaceae
DESMOTRICHUM Kützing, 1845.
undulatum (J. Ag.) Rke.
(*balticum* Kütz.) (28)
(*Punctaria tenuissima* Grev.)
LITOSIPHON Harvey, 1849.
filiformis (Rke.) Batt.
var. *filiformis*
var. ? *gracilis* Batt.
laminariae (Lyngb.) Harv.
(*hibernicus* (Johns.) Batt.)
pusillus (Carm.) Harv.
PUNCTARIA Greville, 1830. (29)
crispata (Kütz.) Batt.
latifolia Grev.
var. *latifolia*
var. ? *laminarioides* Holm. et Batt.
var. ? *lanceolata* Batt.
plantaginea Grev.
var. *plantaginea*
var. ? *crouanii* Thur.
var. ? *rubescens* Batt.

Asperococcaceae
ASPEROCOCCUS Lamouroux, 1813.
bullosus Lamour.
compressus Griff. ex Hook. (30)
fistulosus (Huds.) Hook.
f. *fistulosus*
f. *vermicularis* (Griff.) Harv.
scaber Kuck.

Dictyosiphonaceae
DICTYOSIPHON Greville, 1830. (31)
chordaria Aresch.
(*mesogloia* Aresch.)
(*Gobia baltica* Rke.)
ekmanii Aresch.
foeniculaceus (Huds.) Grev.
f. *foeniculaceus*
f. *hippuroides* (Lyngb.) Levring
(*D. hippuroides* Kütz.)

SCYTOSIPHONALES (32)
Phaeosaccionaceae
PHAEOSACCION Farlow, 1882.
collinsii Farl.

Scytosiphonaceae
COLPOMENIA Derbès et Solier, 1856.
peregrina Sauv.
PETALONIA Derbès et Solier, 1850. (33)
fascia (Müll.) Kuntze (34)
(*debilis* (Ag.) Derb. et Sol.)
(*Phyllitis fascia* Kütz.)
? *filiformis* (Batt.)
zosterifolia (Rke.) Kuntze
(*Phyllitis zosterifolia* Rke.)
SCYTOSIPHON Agardh, 1811.
lomentaria (Lyngb.) Endl.
(*pygmaeus* Rke.)
var. *lomentaria*
var. ? *zostericola* Thur.

TILOPTERIDALES
Tilopteridaceae
HAPLOSPORA Kjellman, 1872.
globosa Kjellm.
TILOPTERIS Kützing, 1849.
mertensii (Sm.) Kütz.

CUTLERIALES
Cutleriaceae
CUTLERIA Greville, 1830.
multifida (Sm.) Grev.
sporophyte = *Aglaozonia parvula* (Grev.) Zanard. (*A. reptans* Crn.)
ZANARDINIA Nardo, 1841.
prototypus Nardo
(*collaris* Crn.)

SPOROCHNALES
Sporochnaceae
CARPOMITRA Kützing, 1843.
costata (Stackh.) Batt.
SPOROCHNUS Agardh, 1817.
pedunculatus (Huds.) Ag.

DESMARESTIALES
Arthrocladiaceae
ARTHROCLADIA Duby, 1832.
villosa (Huds.) Duby

Desmarestiaceae
DESMARESTIA Lamouroux, 1813.
aculeata (L.) Lamour.
dudresnayi Lamour.
ligulata (Lightf.) Lamour.
var. *ligulata*
var. ? *angustior* Batt.
var. ? *dilatata* Batt.
viridis (Müll.) Lamour.

LAMINARIALES

Chordaceae

CHORDA Stackhouse, 1797. (35)
filum (L.) Stackh.
tomentosa Lyngb.

Laminariaceae

LAMINARIA Lamouroux, 1813.
digitata (Huds.) Lamour.
hyperborea (Gunn.) Fosl.
(*cloustonii* Edm.)
ochroleuca La Pylaie
saccharina (L.) Lamour.
(*hieroglyphica* J. Ag.)
SACCORHIZA La Pylaie, 1829.
polyschides (Lightf.) Batt.
(*bulbosa* (Huds.) La Pylaie)

Alariaceae

ALARIA Greville, 1830.
esculenta (L.) Grev.

SPHACELARIALES

Choristocarpaceae

CHORISTOCARPUS Zanardini, 1860.
tenellus (Kütz.) Zanard.

Sphacelariaceae

BATTERSIA Reinke, 1890.
mirabilis Rke.
CHAETOPTERIS Kützing, 1843.
plumosa (Lyngb.) Kütz.
SPHACELARIA Lyngbye, 1819. (36)
bipinnata (Kütz.) Sauv.
britannica Sauv.
caespitula Lyngb.
cirrosa (Roth) Ag.
fusca (Huds.) Ag.
plumigera Holm.
plumula Zanard.
racemosa Grev.
radicans (Dillw.) Ag.
? *saxatilis* (Kuck.) Sauv.
tribuloides Menegh.
SPHACELLA Reinke, 1890.
subtilissima Rke.

Stypocaulaceae

HALOPTERIS Kützing emend. Sauvageau, 1903.
filicina (Grat.) Kütz.
var. *filicina*
var. *patentissima* Sauv.
(*sertularia* Batt.)
scoparia (L.) Sauv.
var. *scoparia*
var. *patentissima* Sauv.
(*scoparioides* Holm. et Batt.)

Cladostephaceae

CLADOSTEPHUS Agardh, 1817.
spongiosus (Huds.) Ag.
verticillatus (Lightf.) Ag.

DICTYOTALES

Dictyotaceae

DICTYOPTERIS Lamouroux, 1809.
membranacea (Stackh.) Batt.
DICTYOTA Lamouroux, 1809.
dichotoma (Huds.) Lamour.
var. *dichotoma*
var. *intricata* (Ag.) Grev.
var. ? *latifrons* Holm. et Batt.
ILOPHUS J. Agardh, 1880.
spiralis (Mont.) Hamel
(*Dictyota ligulata* Kütz.)
PADINA Adanson, 1763.
pavonia (L.) Lamour.
TAONIA J. Agardh, 1848.
atomaria (Woodw.) J. Ag.
var. *atomaria*
var. ? *divaricata* Holm. et Batt.

FUCALES

Fucaceae

ASCOPHYLLUM Stackhouse, 1809.
nodosum (L.) Le Jol.
f. *nodosum*
f. *mackaii* (Turn.)
(*A. mackaii* (Turn.) Holm. et Batt.)
f. *scorpioides* (Hornem.) Hauck
FUCUS Linnaeus, 1753. (37)
ceranoides L.
inflatus L.
(*anceps* Harv. et Ward.)
serratus L.
spiralis L.
vesiculosus L.
PELVETIA Decaisne et Thuret, 1845.
canaliculata (L.) Dcne. et Thur.

Himanthaliaceae

HIMANTHALIA Lyngbye, 1819.
elongata (L.) S. F. Gray
(*lorea* (L.) Lyngb.)

Cystoseiraceae

BIFURCARIA Stackhouse, 1809.
rotunda (Huds.) Papenf.
(*tuberculata* (Huds.) Stackh.)
CYSTOSEIRA Agardh, 1820.
fibrosa (Huds.) Ag.
foeniculacea (L.) Grev. emend. Sauv.
(*discors* Ag.)
granulata Ag.
tamariscifolia (Huds.) Papenf.
(*ericoides* (L.) Ag.)
HALIDRYS Lyngbye, 1819.
siliquosa (L.) Lyngb.

Notes on Phaeophyceae

(1) British material belonging to genera in this family is in urgent need of revision.

(2) See Kornmann (1953): Dr Kornmann very kindly sent me proofs of his paper and also of that of Kuckuck (1953), so that the information contained in these papers could be included here.

(3) See Kuckuck (1953).

(4) The genus *Dichosporangium* is included here for the present to cover *Streblonema chordariae* Cotton in Newton (1931). If remaining in *Streblonema* it would need a new name because of earlier *S. chordariae* (Farl.) De Toni.

(5) Requires investigation. Hamel (1939), suggests it should be placed in *Herponema* J. Ag. emend. Hamel.

(6) New combination not made for var. *rupestris* Batt., investigation needed.

(7) New combination not made for var. *laterifructus* Batt., investigation needed.

(8) New combination not made for var. *punctiformis* Batt., investigation needed.

(9) See Levring (1945) and Waern (1952).

(10) Jaasund (1951) considers genus should be placed in the Chordariaceae; also considers that *Streblonema aequale* Oltm. is possibly a form of *Phaestroma pustulosum*. According to Cotton (1912) British material differs slightly from Kuckuck's plant and may possibly be a distinct species.

(11) Concerning validity of name and also *Pylaiella rupincola* (Aresch.) Kylin—see information given in Waern (1952).

(12) *Streblonema* s.lat.; British material belonging to this complex in urgent need of investigation and revision, see Hamel (1939), Kylin (1947), Waern (1952). British species placed by Hamel (1939) in *Entonema* Reinsch sensu Hamel (*Streblonema parasiticum* (Sauv.) De Toni, *S. breve* (Sauv.) De Toni, *Ectocarpus clandestinus* (Crn.) Sauv.) and by Kylin (1947) in *Entonema* Reinsch sensu Kylin (*Streblonema effusum* Kylin, *S. aequale* Oltm., *Myrionema aecidioides* (K. Rosenv.) Sauv.) have, for the time being, been left, either in the genus *Streblonema* s.lat., or in other genera in which they have been placed.

(13) *Ectocarpus stilophorae* Crn. var. *cervicornis* Batt. recorded, investigation needed. Is *Streblonema tenuissimum* Hauck a form of *S. stilophorae* (Crn.) De Toni?

(14) Almost certainly more than one species recorded for British coast under name *L. fatiscens* Aresch., see Waern (1952). British material of whole family in need of investigation.

(15) Genus in need of revision, see Hamel (1939), Hollenberg (1941), Kylin (1947).

(16) According to Batters = *R. clavata* Rke. *non* Farlow; see Kylin (1947) who gives new name *R. tenuis* for Reinke's *R. clavata*; requires investigation.

(17) See Cotton (1912), Levring (1937), Kylin (1947), Jaasund (1951).

(18) Conserved as *Elachista*, original spelling *Elachistea*, which is etymologically incorrect according to Fritsch (1945).

(19) Habitat forms of species?

(20) = *Areschougia stellaris* (Aresch.) Menegh. See Silva (1952), where *Areschougia* Harv. is proposed for conservation against *Areschougia* Menegh.

(21) See Hamel (1939).

(22) See Batters (1906), and Cotton (1912, p. 124, under *Acrothrix*.)

(23) According to Kylin (1933) the plant recorded on the British coast under the name *Myriocladia lovenii* is not the true *M. lovenii* J. Ag.; it may be an undescribed species.

(24) The following notes are from Mr R. Ross and Miss L. Newton concerning the confusion that has been found in the synonomy dealing with this plant. The type specimen of *Conferva brachiata* Sm. in Engl. Bot. has been examined and is thought to be a *Pylaiella*. This specimen is also the nomenclatural type of *Ectocarpus brachiatus* (Sm.) Harv. *ex* Hook. *non* C. Ag., *Phloeospora brachiata* (Sm.) Bornet, *Stictyosiphon brachiatus* (Sm.) Hygen et Jorde. The plant found by Mrs Griffiths and described and figured under the above names by all authors is quite different. Its earliest legitimate name is *Ectocarpus griffithsianus* Le Jolis in *Trans. bot. Soc. Edinb.*, vol. 7, p. 37 (1863), and a combination based on this name in the appropriate genus is its correct name. The plant has been left for the time being in the genus *Stictyosiphon*, but further study is needed.

(25) British material in urgent need of investigation; kept as three separate species for the present, but see Kolderup Rosenvinge (1935), Levring (1937, 1940), Kolderup Rosenvinge & Lund (1947), Waern (1952).

(26) Systematic position uncertain.

(27) British material needs investigation; should be perhaps *M. clavaeformis* f. *filiformis* (Harv.) Kjellm., see Kolderup Rosenvinge & Lund (1947), Kylin (1947).

(28) See Kylin (1947), Kolderup Rosenvinge & Lund (1947).

(29) British material needs study, see Hamel (1939), Kolderup Rosenvinge & Lund (1947).

(30) See Hamel (1939), who places this species in genus *Haloglossum* Kütz.

(31) British material of genus in need of investigation; see Du Rietz (1940), Levring (1940), Kolderup Rosenvinge & Lund (1947), Sinclair (1949).

(32) See J. Feldmann (1949).

(33) See Silva (1952) concerning name of genus.

(34) See Setchell & Gardner (1925) under *Ilea* Fries; field observations needed before British forms of species can be revised and the limits of the species assessed.

(35) Recorded varieties of both species require investigation before being listed. Kolderup Rosenvinge & Lund (1947) state that the var. *subtomentosa* of *Chorda filum* is not to be maintained.

(36) British material of genus in need of revision, see Waern (1945, 1952), Kylin (1947), Lund (1950).

(37) No varieties or forms given in list; awaiting the results of Dr E. Burrows and Dr S. Lodge's work on the form-range of the *Fucus* species (except *F. inflatus*) on the British coast. Mr H. Powell is investigating *F. inflatus* L.

RHODOPHYCEAE

BANGIOPHYCIDAE

GONIOTRICHALES

Goniotrichaceae

ASTEROCYTIS Gobi, 1879.
ramosa (Thwaites) Gobi (1)
GONIOTRICHUM Kützing, 1843.
? *elegans* (Chauv.) Le Jol.
cornu-cervi (Reinsch) Hauck
NEEVEA Batters, 1900.
repens Batt.

BANGIALES

Erythropeltidaceae

ERYTHROCLADIA Kolderup Rosenvinge, 1909.
irregularis K. Rosenv.
subintegra K. Rosenv.
ERYTHROPELTIS Schmitz, 1896. (2)
? *discigera* Schm.
var. ? *discigera*
var. ? *flustrae* Batt.
ERYTHROTRICHIA Areschoug, 1850. (3)
bertholdii Batt.
boryana (Mont.) Berth.
var. *boryana*
var. ? *crispa* Batt.
carnea (Dillw.) J. Ag.
ciliaris (Carm.) Batt.
investiens (Zanard.) Born.
reflexa (Crn.) Thur.
welwitschii (Rupr.) Batt.
PORPHYROPSIS Kolderup Rosenvinge, 1909.
coccinea (J. Ag.) K. Rosenv.

Bangiaceae

BANGIA Lyngbye, 1819.
fuscopurpurea (Dillw.) Lyngb. (4)
CONCHOCELIS Batters, 1892. (5)
? *rosea* Batt.
PORPHYRA Agardh, 1824.
amethystea Kütz.
leucosticta Thur.
linearis Grev.
miniata (Lyngb.) Ag.
f. *miniata*
f. *abyssicola* (Kjellm.) K. Rosenv.
f. *amplissima* (Kjellm.) K. Rosenv.
f. *tenuissima* (Strömf.) K. Rosenv.
umbilicalis (L.) Kütz.
f. *umbilicalis*
f. *laciniata* (Lightf.) J. Ag.

FLORIDEOPHYCIDAE

NEMALIONALES

Acrochaetiaceae

ACROCHAETIUM Nägeli, 1861. (6)
alariae (Jonss.) Born.
battersianum Hamel
bonnemaisoniae (Batt.) J. et G. Feldm.
(*Colaconema bonnemaisoniae* Batt.)
bornetii Papenf.
(*corymbiferum* (Thur.) Batt.)
brebneri (Batt.) Hamel
(*Rhodochorton brebneri* Batt.)
caespitosum (J. Ag.) Näg.
? (*lorrain-smithiae* (Lyle) Newton)
chylocladiae (Batt.) Batt.
f. *chylocladiae*
f. *pulchrum* Batt.
daviesii (Dillw.) Näg.

ACROCHAETIUM (*cont.*)
emergens (K. Rosenv.) Web.-v. Bosse
endophyticum (Batt.) Batt.
endozoicum (Darb.) Batt.
microscopicum (Näg.) Näg.
minutum (Suhr) Hamel
(*Rhodochorton minutum* (Suhr) Rke.)
mirabile Näg.
nemalionalis (De Not.) Born.
pallens (Zanard.) Näg.
(*Rhodochorton pallens* (Zanard.) Hauck)
parvulum (Kylin) Hoyt
reticulatum (Batt.) Papenf.
(*Colaconema reticulatum* Batt.)
sanctae-mariae (Darb.) Hamel
scapae (Lyle) Papenf.
secundatum (Lyngb.) Näg.
seiriolanum (Harvey-Gibs.) K. Rosenv.
sparsum (Harv.) Näg.
thuretii (Born.) Collins et Hervey
trifilum (Buff.) Batt.
virgatulum (Harv.) J. Ag.
f. *virgatulum*
f. *luxurians* (J. Ag.)
f. *tetrica* (K. Rosenv.)
? *griffithsianum* Näg.
? *irregulare* Reinsch
? *lanuginosum* (Dillw.) Näg.
? *pulvereum* Näg.
AUDOUINELLA Bory emend. Papenfuss, 1945.
efflorescens (J. Ag.) Papenf.
(*Acrochaetium efflorescens* Näg.)
membranacea (Magn.) Papenf.
(*Rhodochorton membranaceum* Magn.)
RHODOCHORTON Nägeli, 1861. (7)

Helminthocladiaceae
HELMINTHOCLADIA J. Agardh, 1851.
calvadosii (Lamour.) Setch.
(*purpurea* J. Ag.)
? *hudsonii* J. Ag.
HELMINTHORA J. Agardh, 1851.
divaricata (Ag.) J. Ag.
NEMALION Duby, 1830.
elminthoides (Vell.) Batt.
? *multifidum* (Web. et Mohr) J. Ag.

Chaetangiaceae
SCINAIA Bivona, 1822.
furcellata (Turn.) Bivona
subcostata (J. Ag.) Chemin ex Hamel
(*furcellata* var. *subcostata* J. Ag.

GELIDIALES

Gelidiaceae
GELIDIUM Lamouroux, 1813. (8)
PTEROCLADIA J. Agardh, 1852.
pinnata (Huds.) Papenf.
(*capillacea* (Gmel.) Born. et Thur.)

GIGARTINALES

Cruoriaceae
CRUORIA Fries, 1835.
pellita (Lyngb.) Fries
rosea Crn.
var. *rosea*
var. *purpurea* (Crn.) Batt.
CRUORIOPSIS Dufour, 1864.
gracilis (Kuck.) Batt.
? *hauckii* Batt. (9)
PETROCELIS J. Agardh, 1852.
cruenta J. Ag.
hennedyi (Harv.) Batt.

Calosiphoniaceae
CALOSIPHONIA Crouan, 1852.
vermicularis (J. Ag.) Schm.

Nemastomaceae (10)
PLATOMA Schmitz, 1889.
bairdii (Farl.) Kuck.
marginifera J. Ag.
SCHIZYMENIA J. Agardh, 1851.
dubyi (Chauv.) J. Ag.

Furcellariaceae
FURCELLARIA Lamouroux, 1813.
fastigiata (L.) Lamour. (11)
HALARACHNION Kützing, 1843.
ligulatum (Woodw.) Kütz.

Rhabdoniaceae
CATENELLA Greville, 1830.
repens (Lightf.) Batt.

Rhodophyllidaceae
CALLIBLEPHARIS Kützing, 1843.
ciliata (Huds.) Kütz.
var. *ciliata*
var. ? *angusta* Holm. et Batt.
lanceolata (Stackh.) Batt.
CYSTOCLONIUM Kützing, 1843.
purpureum (Huds.) Batt.
RHODOPHYLLIS Kützing, 1847.
? *appendiculata* J. Ag.
divaricata (Stackh.) Papenf. (12)
(*bifida* Kütz.)

Plocamiaceae
PLOCAMIUM Lamouroux, 1813.
coccineum (Huds.) Lyngb.
f. *coccineum*
f. *uncinatum* (Ag.) Levring

Sphaerococcaceae
SPHAEROCOCCUS Stackhouse, 1797.
coronopifolius Stackh.

Gracilariaceae
CORDYLECLADIA J. Agardh, 1852.
erecta (Grev.) J. Ag.
GRACILARIA Greville, 1830.
bursa-pastoris (Gmel.) Silva
(*compressa* (Ag.) Grev.)
foliifera (Forssk.) Børg.
(*multipartita* J. Ag.)
verrucosa (Huds.) Papenf.
(*confervoides* (L.) Grev.)
f. *verrucosa*
f. *gracilis* (Stackh.)
f. *procerrima* (Turn.)

Phyllophoraceae
AHNFELTIA Fries, 1835.
plicata (Huds.) Fries
GYMNOGONGRUS Martius, 1833.
griffithsiae (Turn.) Mart.
norvegicus (Gunn.) J. Ag.
patens J. Ag.
? PETROGLOSSUM Hollenberg, 1943.
nicaeense (Duby) Schotter (13)
(*Rhodymenia palmetta* var. *elisiae* Chauv.)
PHYLLOPHORA Greville, 1830. (14)
brodiaei (Turn.) J. Ag.
membranifolia (Good. et Woodw.) J. Ag.
palmettoides J. Ag.
rubens (L.) Grev.
(*epiphylla* (Müll.) Batt.)
traillii Holm. et Batt.
STENOGRAMME Harvey, 1841.
interrupta (Ag.) Mont.

Gigartinaceae
CHONDRUS Stackhouse, 1797.
crispus (L.) Stackh. (15)
GIGARTINA Stackhouse, 1809.
acicularis (Wulf.) Lamour.
pistillata (Gmel.) Stackh.
stellata (Stackh.) Batt. (15)
teedii Lamour.

CRYPTONEMIALES

Gloiosiphoniaceae
GLOIOSIPHONIA Carmichael ex Berkeley, 1833.
capillaris (Huds.) Carm.

Dumontiaceae
DILSEA Stackhouse, 1809.
carnosa (Schmidel) Kuntze
(*edulis* (Stackh.) Stackh.)
DUDRESNAYA Crouan frat., 1835.
verticillata (With.) Le Jol.
DUMONTIA Lamouroux, 1813.
incrassata (Müll.) Lamour.

Polyideaceae
POLYIDES Agardh, 1822.
caprinus (Gunn.) Papenf.
(*rotundus* (Gmel.) Grev.)

Squamariaceae (16)
ERYTHRODERMIS Batters, 1900. (17)
allenii Batt.
HAEMATOCELIS J. Agardh, 1852. (17)
rubens J. Ag.
PEYSSONELIA Decaisne, 1841.
atropurpurea Crn.
dubyi Crn.
(*Cruoriella dubyi* (Crn.) Schm.)
harveyana Crn.
rosenvingii Schm.
rubra (Grev.) J. Ag.
rupestris Crn.
PORPHYRODISCUS Batters, 1897. (17)
simulans Batt.
RHODODERMIS Crouan, 1852.
elegans Crn.
var. *elegans*
var. *polystromatica* Batt. (18)
georgii (Batt.) Collins
(*Rhodophysema georgii* Batt.)
? *parasitica* Batt. (19)
RHODODISCUS Crouan, 1859.
pulcherrimus Crn.

Hildenbrandiaceae
HILDENBRANDIA Nardo, 1834.
crouanii J. Ag.
prototypus Nardo

Corallinaceae (20)
CHOREONEMA Schmitz, 1889.
thuretii (Born.) Schm.
CORALLINA Linnaeus emend. Lamouroux, 1812.
? *elongata* Johnst. (21)
granifera Ellis et Soland.
(*virgata* Zanard.)
officinalis L.
f. *officinalis*
f. *compacta* (Crn.)
squamata Ellis
EPILITHON Heydrich, 1897. (22)
? *membranaceum* (Esper) Heydr.
(*Lithothamnion membranaceum* Fosl.)
JANIA Lamouroux, 1812.
corniculata (L.) Lamour.
(*Corallina rubens* var. *corniculata* Hauck)
rubens (L.) Lamour.
(*Corallina rubens* Ellis et Sol.)
LITHOPHYLLUM Philippi, 1837.
adplicitum (Fosl.) Newton
corallinae (Crn.) Heydr.
(*Melobesia corallinae* Crn.)
crouanii Fosl.
fasciculatum Fosl.
f. *complanata* Fosl.
f. *divergens* Fosl.
f. *incrassata* Fosl.
hapalidioides (Crn.) Heydr.
f. *hapalidioides*
f. *confinis* (Crn.) Fosl.
incrustans Phil.
f. *incrustans*
f. *depressa* (Crn.) Fosl.
f. *harveyi* Fosl.
f. *subdichotomum* Heydr.
orbiculatum Fosl. (23)
pustulatum (Lamour.) Fosl.
f. *pustulatum*
f. *laminariae* (Crn.) Fosl.
LITHOTHAMNION Philippi, 1837.
bornetii Fosl.
calcareum (Pall.) Aresch.
f. *calcareum*
f. *compressa* (McCalla) Fosl.
f. *crassa* Lem.
f. *squarrulosa* Fosl.

LITHOTHAMNION (*cont.*)
calcareum (*cont.*)
f. *subsimplex* (Batt.) Fosl.
f. ? *subvalida* Fosl.
? *colliculosum* Fosl. (24)
f. *colliculosum*
f. *rosea* Batt.
compactum Kjellm.
? *fruticulosum* (Kütz.) Fosl.
glaciale Kjellm.
granii Fosl. (24)
f. *reducta* Fosl. (*battersii* Fosl.)
laeve (Strömf.) Fosl.
(*stroemfeltii* Fosl.)
laevigatum Fosl.
lenormandii (Aresch.) Fosl.
f. *lenormandii*
f. *squamulosa* Fosl.
f. *sublaevis* Fosl.
norvegicum (Aresch.) Kjellm.
(*calcareum* var. *norvegicum* Fosl.)
polymorphum (L.) Aresch.
sonderi Hauck
f. *sonderi*
f. *sublaevigata* Fosl.
MELOBESIA Lamouroux, 1812. (22)
? *farinosa* Lamour.
var. *farinosa*
var. *borealis* Lemoine
var. *solmsiana* Fkbg.
? *lejolisii* Rosan.
? *minutula* Fosl.
? *zonalis* (Crn.) Fosl.
MESOPHYLLUM Lemoine, 1928.
lichenoides (Ellis) Lemoine
(*Lithothamnion lichenoides* Fosl.)
f. *lichenoides*
f. *agariciformis* (Johnst. ?)
f. *depressa* (Fosl.)
SCHMITZIELLA Bornet et Batters, 1892.
endophlaea Born. et Batt.

Grateloupiaceae
GRATELOUPIA Agardh, 1822.
dichotoma J. Ag.
filicina (Wulf.) Ag.
var. *filicina*
var. *intermedia* Holm. et Batt.
minima Crn.
HALYMENIA Agardh, 1817.
latifolia Crn.

Kallymeniaceae
CALLOCOLAX Schmitz, 1895.
neglectus Schm.
CALLOPHYLLIS Kützing, 1843.
flabellata Crn.
laciniata (Huds.) Kütz.
EUTHORA J. Agardh, 1847.
cristata (L.) J. Ag.
KALLYMENIA J. Agardh, 1842. (25)
? *larterae* Holm.
reniformis (Turn.) J. Ag.
var. *reniformis*
var. *ferrarii* J. Ag.
var. *undulata* J. Ag.
MEREDITHIA J. Agardh, 1892.
microphylla J. Ag.
(*Kallymenia microphylla* J. Ag.)

Choreocolaceae
CHOREOCOLAX Reinsch, 1875.
polysiphoniae Reinsch
HARVEYELLA Schmitz et Reinke, 1889.
mirabilis (Reinsch) Schm. et Rke.
HOLMSELLA Sturch, 1926.
pachyderma (Reinsch) Sturch

BONNEMAISONIALES (26)

Naccariaceae
ATRACTOPHORA Crouan, 1849.
hypnoides Crn.
NACCARIA Endlicher, 1836.
wiggii (Turn.) Endl.

Bonnemaisoniaceae
ASPARAGOPSIS Montagne, 1840.
armata Harv.
tetrasporophyte = *Falkenbergia rufolanosa* (Harv.) Schm.
BONNEMAISONIA Agardh, 1822.
asparagoides (Woodw.) Ag.
tetrasporophyte = *Hymenoclonium serpens* (Crn.) Batt.
clavata (Schousb.) Hamel
hamifera Hariot
tetrasporophyte = *Trailliella intricata* Batt.

RHODYMENIALES

Champiaceae
CHAMPIA Desvaux, 1808.
parvula (Ag.) Harv.
CHYLOCLADIA Greville, 1833.
reflexa Lenorm.
squarrosa (Kütz.) Le Jol.
(*kaliformis* var. *squarrosa* Harv.)
verticillata (Lightf.) Bliding
(*kaliformis* (Good. et Woodw.) Grev.)
var. *verticillata*
var. *patens* (Kütz.)
GASTROCLONIUM Kützing, 1843.
ovatum (Huds.) Papenf.
(*Chylocladia ovata* Batt.)
var. *ovatum*
var. *subarticulatum* (Kütz.)
LOMENTARIA Lyngbye, 1819.
articulata (Huds.) Lyngb.
clavellosa (Turn.) Gaill.
var. *clavellosa*
var. *sedifolia* Harv.
orcadensis (Harv.) Collins
(*rosea* (Harv.) Thur.)

Rhodymeniaceae
RHODYMENIA Greville, 1830.
ardissonei J. Feldm.
(*corallicola* Ardiss.)
palmata (L.) Grev. (27)
pseudopalmata (Lamour.) Silva (27)
(*palmetta* Grev.)

CERAMIALES

Ceramiaceae

AGLAOTHAMNION G. Feldmann-Mazoyer, 1940.
brodiaei (Harv.) G. Feldm.
(*Callithamnion brodiaei* Harv.)
polyspermum (Ag.) Parke
(*Callithamnion polyspermum* Ag.)
f. *polyspermum*
f. *scopulorum* (Ag.) Parke
(*Aglaothamnion scopulorum* (Ag.) G. Feldm.)
tenuissimum (Bonnem.) G. Feldm.
(*Callithamnion tenuissimum* (Bonnem.) Kütz.)
tripinnatum (Grat.) G. Feldm.
(*Callithamnion tripinnatum* (Grat.) Ag.)

ANTITHAMNION Nägeli, 1847.
boreale (Gobi) Kjellm.
cruciatum (Ag.) Näg.
var. *cruciatum*
var. *pumilum* (Harv.) Batt.
floccosum (Müll.) Kleen
plumula (Ellis) Thur.
var. *plumula*
var. *crispum* (Ducluz.) Hauck
var. *spinescens* Strömf.
sarniense (Lyle) G. Feldm. (28)
(*Antithamnionella sarniensis* Lyle)
spirographidis Schiffner

BORNETIA Thuret, 1855.
secundiflora (J. Ag.) Thur.

CALLITHAMNION Lyngbye emend. G. Feldmann-Mazoyer, 1940. (29)
arbuscula (Dillw.) Lyngb.
byssoides Arnott
corymbosum (Sm.) Lyngb.
dudresnayi Crn.
? *fruticulosum* J. Ag. (30)
granulatum (Ducluz.) Ag. (31)
hookeri (Dillw.) Ag.
rabenhorstii Crn.
? *roseum* Harv. (32)
? *spongiosum* Harv. (31)
tetragonum (Wither.) Ag. (30)
f. *tetragonum*
f. *brachiatum* (Bonnem.) K. Rosenv.
tetricum Ag.

CERAMIUM Roth, 1797. (33)
acanthonotum Carm. ex Harv.
arborescens J. Ag.
areschougii Kylin
atlanticum Peterson
boergesenii Peterson
ciliatum (Ellis) Ducluz.
circinatum (Kütz.) J. Ag.
? *crouanianum* J. Ag.
derbesii Solier
deslongchampsii Chauv.
diaphanum (Lightf.) Roth
echionotum J. Ag.
var. *echionotum*
var. *transcurrens* (Kütz.) Batt.
fastigiatum Harv.
flabelligerum J. Ag.
fruticulosum (Kütz.) J. Ag.
gracillimum Griff. et Harv.
pedicellatum (Duby) J. Ag.
pennatum Crn.
rubrum (Huds.) Ag.
secundatum J. Ag.
strictum Harv.
tenue J. Ag.
tenuissimum (Lyngb.) J. Ag.
vimineum J. Ag.

COMPSOTHAMNION Nägeli, 1861.
gracillimum (Harv.) Näg.
thuyoides (Sm.) Näg.

CORYNOSPORA J. Agardh emend. J. Agardh, 1876.
pedicellata (Sm.) J. Ag.
(*Monospora pedicellata* (Sm.) Solier)
f. *pedicellata*
f. *comosa* (Holm. et Batt.)

CROUANIA J. Agardh, 1842.
attenuata (Bonnem.) J. Ag.

GRIFFITHSIA Agardh, 1817.
barbata (Sm.) Ag.
corallinoides (L.) Batt.
devoniensis Harv. (34)
flosculosa (Ellis) Batt.

HALURUS Kützing, 1849.
equisetifolius (Ag.) Kütz.
var. *equisetifolius*
var. *simplicifilum* J. Ag.

MICROCLADIA Greville, 1830.
glandulosa (Soland.) Grev.

PLEONOSPORIUM Nägeli emend. Nägeli ex Hauck, 1883.
borreri (Sm.) Näg. ex Hauck
var. *borreri*
var. *fasciculatum* (Harv.) Holm. et Batt.

? PLUMARIA Schmitz, 1896. (35)
? *elegans* (Bonnem.) Schm.

? PTILOTA Agardh, 1817. (35)
? *plumosa* (Huds.) Ag.

PTILOTHAMNION Thuret, 1863.
? *lucifugum* Cotton (36)
pluma (Dillw.) Thur.

SEIROSPORA Harvey, 1846. (37)
griffithsiana Harv.
hormocarpa (Holm.) Batt.
interrupta (Sm.) Schm.

SPERMOTHAMNION Areschoug, 1877. (38)
barbatum (Ag.) Näg.
irregulare (J. Ag.) Ardiss.
repens (Dillw.) K. Rosenv.
(*roseolum* Pringsh.)
(*turneri* (Mert.) Aresch.)
strictum (Ag.) Ardiss.

SPHONDYLOTHAMNION Nägeli, 1861.
multifidum (Huds.) Näg.
var. *multifidum*
var. *piliferum* (Ag.) Batt.

SPYRIDIA Harvey, 1833.
filamentosa (Wulf.) Harv.

Delesseriaceae
ACROSORIUM Zanardini, 1869.
reptans (Crn.) Kylin (39)
uncinatum (J. Ag.) Kylin (39)
APOGLOSSUM J. Agardh, 1898.
ruscifolium (Turn.) J. Ag.
CRYPTOPLEURA Kützing, 1843.
ramosa (Huds.) Kylin
f. *ramosa*
f. *ciliifera* (Kütz.)
f. *lobata* (Kütz.)
f. *uncinata* (Grev.)
DELESSERIA Lamouroux, 1813.
sanguinea (Huds.) Lamour.
ERYTHROGLOSSUM J. Agardh, 1898.
sandrianum (Zanard.) Kylin
GONIMOPHYLLUM Batters, 1892.
buffhamii Batt.
HYPOGLOSSUM Kützing, 1843.
woodwardii Kütz. (40)
MEMBRANOPTERA Stackhouse, 1809.
alata (Huds.) Stackh.
NITOPHYLLUM Greville, 1830. (41)
? *bonnemaisonii* Grev.
(*Myriogramme bonnemaisonii* Kylin)
var. *bonnemaisonii*
var. *crassinerve* Batt.
punctatum (Stackh.) Grev.
? *versicolor* Harv.
(*Myriogramme versicolor* Kylin)
PANTONEURA Kylin, 1919.
angustissima (Turn.) Kylin
PHYCODRYS Kützing, 1843.
rubens (Huds.) Batt.
POLYNEURA Kylin, 1924.
gmelinii (Grev.) Kylin
hilliae (Grev.) Kylin
litterata (J. Ag.) Kylin
RHIZOGLOSSUM Kylin, 1924.
thysanorhizans (Holm.) Kylin

Dasyaceae
DASYA Agardh, 1824.
arbuscula (Dillw.) Ag.
var. *arbuscula*
var. ? *caespitosa* J. Ag.
corymbifera J. Ag.
ocellata (Grat.) Harv.
punicea Menegh.
HETEROSIPHONIA Montagne, 1842.
plumosa (Ellis) Batt.
f. *plumosa*
f. *patens* (Grev.)
f. *tenuior* (Dillw.)

Rhodomelaceae (42)
BOSTRYCHIA Montagne, 1838.
scorpioides (Huds.) Mont.
BRONGNIARTELLA Bory, 1822.
byssoides (Good. et Woodw.) Schm.
CHONDRIA Agardh, 1817.
caerulescens J. Ag.
dasyphylla (Woodw.) Ag.
tenuissima (Good. et Woodw.) Ag.
HALOPITYS Kützing, 1843.
incurvus (Huds.) Batt.
LAURENCIA Lamouroux, 1813. (43)
hybrida (D.C.) Lenorm. ex Duby
(*caespitosa* Lamour.)
obtusa (Huds.) Lamour.
pinnatifida (Huds.) Lamour.
LOPHOSIPHONIA Falkenberg, 1897.
obscura (Ag.) Fkbg.
(*Polysiphonia obscura* J. Ag.)
ODONTHALIA Lyngbye, 1819.
dentata (L.) Lyngb.
POLYSIPHONIA Greville, 1824. (44)
brodiaei (Dillw.) Grev.
denudata (Dillw.) Kütz.
(*variegata* (Ag.) Zanard.)
elongata (Huds.) Harv.
elongella Harv.
fibrata (Dillw.) Harv.
fibrillosa (Dillw.) Grev. (45)
foetidissima Cocks
fruticulosa (Wulf.) Spreng.
(*Pterosiphonia fruticulosa* (Wulf.) Batten)
furcellata (Ag.) Harv.
var. *furcellata*
var. *forcipata* J. Ag.
insidiosa Crn.
lanosa (L.) Tandy (*fastigiata* Grev.)
macrocarpa Harv.
nigra (Huds.) Batt.
nigrescens (Sm.) Grev.
f. *nigrescens*
f. *affinis* (Moore) J. Ag.
f. *fucoides* (Huds.) J. Ag.
f. *protensa* J. Ag.
f. *senticosa* J. Ag.
opaca (Ag.) Zanard.
rhunensis Thur.
richardsonii Hook.
? *sanguinea* (Ag.) Zanard.
simulans Harv.
spinulosa Grev.
var. *spinulosa*
var. *major* J. Ag.
spiralis Batten
subulifera (Ag.) Harv.
var. *subulifera*
var. *templetonii* Harv.
urceolata (Dillw.) Grev.
f. *urceolata*
f. *comosa* (Ag.) J. Ag.
f. *formosa* (Suhr) J. Ag.
f. *patens* (Dillw.) J. Ag.
violacea (Roth) Grev. (46)
PTEROSIPHONIA Falkenberg, 1889.
complanata (Clem.) Fkbg.
parasitica (Huds.) Fkbg.
pennata (Roth) Fkbg.
thuyoides (Harv.) Schm.
RHODOMELA Agardh, 1822. (47)
confervoides (Huds.) Silva
(*subfusca* (Woodw.) Ag.)
lycopodioides (L.) Ag.

Notes on Rhodophyceae

(1) See Waern (1952).

(2) See Hamel (1924, p. 283).

(3) British material of genus in need of revision.

(4) According to Koster (1952) should be *B. atropurpurea* (Roth) Ag. f. *fuscopurpurea* (Dillw.) Ag. as there are no differences to be found between the fresh-water and marine material; see also Hamel (1924), and Kylin (1944). Kept the marine form distinct for the present awaiting the results of Dr K. M. Baker's investigations.

(5) See Drew (1949).

(6) *Acrochaetium* Nägeli, sens. lat.

(7) Dr K. M. Baker is revising the British material of this genus.

(8) Awaiting the results of the investigation of this genus carried out by Dr M. de Valèra and Mr P. Dixon.

(9) Requires investigation.

(10) *Nemastoma* J. Agardh, 1842; *N. dichotoma* J. Ag., recorded by Lyle (1920) for Guernsey. J. Feldmann (1941) thinks that several species may have been confused under the above name, so Lyle's specimen should be examined.

(11) See Papenfuss (1950), Waern (1952), Silva (1952).

(12) *Rhodophyllis bifida* var. *incrassata* Harv., requires investigation before any new combination is made under *R. divaricata*. *R. appendiculata* may be a form of *R. divaricata* (J. Feldmann, 1941).

(13) See J. Feldmann (1941, p. 86) and G. Schotter (1952).

(14) The form-range of the species on the British coast require investigation.

(15) See Marshall, Newton & Orr (1949).

(16) British material belonging to this family requires monographic treatment.

(17) Systematic position uncertain.

(18) See Cotton (1912).

(19) See Kylin (1944).

(20) In need of monographic treatment.

(21) Needs investigation—should be possibly *C. mediterranea* Aresch. or *C. officinalis* L. var. *mediterranea* (Aresch.) Hauck.

(22) The genus *Fosliella* Howe not adopted for the present—appears to require further investigation. If adopted the British species would be *F. farinosa* (Lamour.) Howe, *F. lejolisii* (Rosan.) Howe, *F. minuta* (Fosl.), *F. zonalis* (Crn.) J. Feldm., and *Epilithon membranaceum* would again be known as *Melobesia membranacea* (Esper.) Lamour.

(23) Should possibly be placed in genus *Pseudolithothamnion* Lemoine, 1913; see Lemoine (1928).

(24) Regarded by some workers as a form of *L. glaciale*.

(25) *K. larterae* Holm. requires investigation—may be a form of *K. reniformis* (Turn.) J. Ag.

(26) See J. & G. Feldmann (1942, 1952) and J. Feldmann (1952).

(27) The form-range of the species requires investigation and revision.

(28) Possibly a form of *A. spirographidis*, see J. Feldmann (1942).

(29) British species require investigation as no doubt some of the species listed here under *Callithamnion* should be placed under *Aglaothamnion*. The range of form of species of this genus on the British coast is in urgent need of attention.

(30) Kolderup Rosenvinge (1924) and Kylin (1944) consider *C. tetragonum*, *C. spiniferum* Kylin (Dutch coast) and *C. fruticulosum* forms of one species. Kolderup Rosenvinge (1924) unites them under *C. tetragonum* but Kylin (1944) prefers to keep them separate and to use the name *C. tetragonum* for plants on the British coast and *C. fruticulosum* for the plants on the Swedish coast. The form-range of the series on the British coast needs investigation and comparison with material from Sweden, Holland and Denmark.

(31) Cotton (1912) thinks that *C. spongiosum* and *C. granulatum* may be two distinct species, having found no intermediate forms between them. They have been listed separately awaiting monographic treatment of British material.

(32) Left as *C. roseum* Harv. until it is certain that Harvey's plant is the same as *C. roseum* (Roth) Lyngb.

(33) British material in urgent need of revision, see work of Petersen, Kylin, Sjösted), Waern (references to literature in Waern, 1952), Kolderup Rosenvinge (1924), G. Feldmann-Mazoyer (1940), Lucas (1950). The different workers' interpretations of many of the species and of the form-range within any one species vary so much that no attempt has been made to link up the 'species' recorded for the British coast with these treatments. This must await monographic treatment of British material.

(34) Possibly an ecological form of *G. corallinoides*, Koster (1952).

(35) *Plumaria* Schm. and *Ptilota* Ag. used awaiting decision concerning conservation of *Plumaria* Schm. over *Plumaria* Stackh.—see Silva (1952).

(36) See Cotton (1912), G. Feldmann-Mazoyer (1940); *P. lucifugum* Cotton may be a form of *P. pluma* since G. Feldmann considers that *P. micropterum* (Mont.) Born. is a reduced form of *P. pluma*. Fertile material of Cotton's plant needs investigation.

(37) British material in need of revision.

(38) British material requires investigation and revision. Kolderup Rosenvinge places *S. turneri* (Mert.) Aresch. as var. *turneri* (Mert.) K. Rosenv. and *S. roseolum* (Ag.) Pringsh. as var. *roseolum* (Ag.) K. Rosenv. under *S. repens* (Dillw.) K. Rosenv. If *Conferva repens* Dillw. is the same plant as that described by Mertens as *Ceramium turneri*, which Kolderup Rosenvinge believed it was, then var. *turneri* should be placed as a synonym under *S. repens* (Dillw.) K. Rosenv. var. *repens*. See J. Feldmann (1942, p. 59) concerning *S. strictum*.

(39) Doubtful if these two species are distinct.

(40) Forms of this species occurring on British coast in need of revision.

(41) Kylin (1934) considered that *N. bonnemaisonii* and *N. versicolor* are closer to the genus *Nitophyllum* than to the genus *Myriogramme* Kylin and so should remain in the genus *Nitophyllum* for the present; he considered, however, that they are sufficiently different from the type species *N. punctatum* to warrant their being placed in a new genus.

(42) *Ctenosiphonia* Falkenberg, 1897; *C. hypnoides* (Welw.) Fkbg. recorded for Guernsey by Lyle (1920, 1923).

(43) Form-range of species on British coast needs revision.

(44) Form-range of species on British coast requires investigation.

(45) Considered by some workers a form of *P. violacea*; British material needs investigation.

(46) The forms of this species on the British coast need investigation, see Kolderup Rosenvinge (1924), Kylin (1944), Veldkamp (1950). *P. subulata* (Ducluz.) J. Ag. considered a distinct species by some workers and not a variety of *P. violacea*.

(47) Forms of *R. confervoides* require revision before any new combinations are made. *R. lycopodioides* should possibly be included as a variety under *R. confervoides*.

CYANOPHYCEAE (1)

Chroococcaceae (2)
- ANACYSTIS Meneghini, 1837.
  - *marina* (Hansg.) Dr. et Daily (*Aphanocapsa marina* Hansg.)
  - ? *parasitica* Kütz.
- ? APHANOCAPSA Nägeli, 1849.
  - ? *litoralis* Hansg.
- ? APHANOTHECE Nägeli, 1849.
  - ? *pallida* (Kütz.) Rabenh.
- ? CHROOCOCCUS Nägeli, 1849.
  - ? *calcicola* Anand
  - ? *turgidus* (Kütz.) Näg.
- ? GLOEOCAPSA Kützing, 1843.
  - ? *crepidinum* Thur.
- ? GLOEOTHECE Nägeli, 1849.
  - ? *palea* (Kütz.) Rabenh.
- ? HOLOPEDIA Lagerheim, 1883.
  - ? *sabulicola* (Lagerh.) Kirchn.
- MERISMOPEDIA Meyen, 1839.
  - ? *convoluta* Bréb. ex Kütz.
  - ? *glauca* (Ehrb.) Näg.
    - f. *glauca*
    - f. ? *mediterranea* (Näg.) Collins
  - ? *revoluta* Ask.
- ? SYNECHOCOCCUS Nägeli, 1849.
  - ? *bacillaris* Butcher

Chamaesiphonaceae (2, 3)
- ? CHAMAESIPHON A. Braun. et Grunow, 1865.
  - ? *marinus* Wille (4)
- ? CHLOROGLOEA Wille, 1900.
  - ? *tuberculosa* (Hansg.) Wille
- ? DERMOCARPA Crouan, 1858.
  - ? *enteromorphae* Anand
  - ? *leibleiniae* (Reinsch) Born. et Thur.
  - ? *olivacea* (Reinsch) Tilden
  - ? *incrustans* (Reinsch) Batt.
  - ? *rosea* (Reinsch) Batt.
  - ? *sphaerica* Setch. et Gardn. ex Gardn.

? DERMOCARPA (*cont.*)
? *violacea* Crn.
ENTOPHYSALIS Kützing, 1843.
conferta (Kütz.) Dr. et Daily
(*Dermocarpa prasina* (Reinsch) Born. et Thur.)
(*Xenococcus schousboei* Thur.)
crustacea (Ag.) Dr. et Daily
(*granulosa* Kütz.)
(*Hyella caespitosa* Born. et Flah.)
(*Pleurocapsa fuliginosa* Hauck)
? ONCOBYRSA Agardh, 1827.
? *marina* (Grun.) Rabenh.
? PLEUROCAPSA Thuret, 1885.
? *amethystea* K. Rosenv.
? *crepidinum* Collins
? *entophysaloides* Setch. et Gardn. ex Gardn.
? *kerneri* (Hansg.) Dr.
? XENOCOCCUS Thuret ex Bornet et Thuret, 1880.
? *gilkeyae* Setch. et Gardn. ex Gardn. (forma)
? *pyriformis* Setch. et Gardn. ex Gardn.
? *violacea* Anand

Stigonemataceae

BRACHYTRICHIA Zanardini; Bornet et Flahault, 1886.
balani (Lloyd) B. et F.
MASTIGOCOLEUS Lagerheim; Bornet et Flahault, 1887.
testarum Lagerh. ex. B. et F.

Nostocaceae

ANABAENA Bory; Bornet et Flahault, 1888.
? *berkeleyana* (Thwaites) Batt.
? *broomei* (Thwaites) Batt.
torulosa (Carm.) Lagerh. ex B. et F.
variabilis Kütz. ex B. et F.
NODULARIA Mertens; Bornet et Flahault, 1888.
harveyana (Thwaites) Thur. ex. B. et F.
spumigena Mert. ex. B. et F.
var. *litorea* (Kütz.) B. et F.
NOSTOC Vaucher; Bornet et Flahault, 1888.
commune Vauch. ex B. et F.
entophytum B. et F.
linckia (Roth) Born. ex B. et F.
PSEUDANABAENA Lauterborn, 1916.
brevis Carter

Rivulariaceae

AMPHITHRIX Kützing; Bornet et Flahault, 1886.
violacea (Kütz.) B. et F.
CALOTHRIX Agardh; Bornet et Flahault, 1886.
aeruginea (Kütz.) Thur. ex B. et F.
confervicola Ag. ex B. et F.
var. *confervicola*
var. *purpurea* B. et F.
? *consociata* (Kütz.) B. et F. (5)
? *contarenii* B. et F. (6)
cottonii nov. nom. (7)
(*endophytica* Cotton)
crustacea Thur. ex B. et F.
fusca (Kütz.) B. et F.
litoralis Anand
parasitica (Chauv.) Thur. ex. B. et F.
pulvinata (Ag.) B. et F.
var. *pulvinata*
var. *prostrata* Anand
scopulorum (Web. et Mohr) Ag. ex B. et F.
(*fasciculata* Ag. ex B. et F.) (8)
vivipara Harv. ex B. et F.
DICHOTHRIX Zanardini; Bornet et Flahault, 1886.
gypsophila (Kütz.) B. et F.
ISACTIS Thuret; Bornet et Flahault, 1886.
plana Thur. ex B. et F.
var. *plana*
var. *fissurata* B. et F.
RIVULARIA Roth; Bornet et Flahault, 1886.
atra Roth ex B. et F. (9)
australis Harv. ex B. et F.
biasolettiana Menegh. ex B. et F.
bullata (Poir.) Berk. ex B. et F.
mesenterica Thur. ex B. et F.
nitida Ag. ex B. et F. (9)

Scytonemataceae

? DIPLOCOLON Nägeli; Bornet et Flahault, 1887. (10)
? *codii* Batt. (10)
FREMYELLA J. De Toni, 1936.
(MICROCHAETE Thuret, 1875)
aeruginea (Batt.) nov.comb.
(*Microchaete aeruginea* Batt.)
grisea (Thur. ex B. et F.) J. De Toni
(*Microchaete grisea* Thur.)
PLECTONEMA Thuret; Gomont, 1893.
battersii Gom.
utorale Anand
norvegicum Gom.
nostocorum Born. ex Gom.
terebrans Born. et Flah. ex Gom.
TOLYPOTHRIX Kützing; Bornet et Flahault, 1887.
tenuis Kütz. ex B. et F.

Oscillatoriaceae

HYDROCOLEUM Kützing; Gomont, 1892.
glutinosum (Ag.) Gom.
lyngbyaceum Kütz. ex Gom.
var. *lyngbyaceum*
var. *rupestre* Kütz. ex Gom.
LYNGBYA Agardh; Gomont, 1893.
aestuarii Liebm. ex Gom. (11)
f. *aestuarii*
f. *aeruginosa* Gom.
f. *ferruginea* Gom.
f. *limicola* Gom.
f. *natans* Gom.

LYNGBYA (*cont.*)
aestuarii (*cont.*)
f. *spectabilis* Gom.
f. *symplocoidea* Gom.
agardhii Gom.
confervoides Ag. ex. Gom.
gracilis Rabenh. ex Gom.
var. *maritima* Anand
lutea Gom. ex Gom.
majuscula Harv. ex Gom.
meneghiniana Gom. ex Gom.
rivulariarum Gom.
semiplena J. Ag. ex Gom.
MICROCOLEUS Desmazières; Gomont, 1892.
acutirostris Gom.
chthonoplastes Thur. ex Gom.
tenerrimus Gom.
OSCILLATORIA Vaucher; Gomont, 1893. (12)
amphibia Ag. ex Gom.
bonnemaisonii Crn. ex Gom.
brevis Kütz. ex Gom.
var. *neapolitana* Gom.
corallinae Gom. ex Gom.
formosa Bory ex Gom.
laetevirens Crn. ex Gom.
limosa Ag. ex Gom.
margaritifera Kütz. ex Gom.
nigro-viridis Thwaites ex Gom.
sancta Kütz. ex Gom.
PHORMIDIUM Kützing; Gomont, 1893.
ambiguum Gom.
angustissimum W. et G. S. West
autumnale Gom.
corium Gom.
ectocarpi Gom.
foveolarum Gom.
f. *foveolarum*
f. *minus* Anand
f. *moniliforme* Anand
fragile Gom.
monile Setch. et Gardn. (forma)
papyraceum Gom. ex Gom.
persicinum Gom.
submembranaceum Gom.
var. *minor* Anand
subuliforme Gom.
tenue Gom.
uncinatum Gom. ex Gom.
valderianum Gom.
SCHIZOTHRIX Kützing; Gomont, 1892.
cresswellii Harv. ex Gom.
fritschii Anand
lardacea Gom.
vaginata Gom.
SPIRULINA Turpin; Gomont, 1893.
major Kütz. ex Gom.
subsalsa Oerst. ex Gom.
f. *subsalsa*
f. *oceanica* Gom.
subtilissima Kütz. ex Gom.
versicolor Cohn ex Gom. (13)
SYMPLOCA Kützing; Gomont, 1893.
atlantica Gom.
var. *atlantica*
var. *purpurea* Batt.
dubia Gom.
hydnoides Kütz. ex Gom.
var. *hydnoides*
var. *fasciculata* Gom.

Notes on Cyanophyceae

(1) For various systems of classification see Geitler (1932), Frémy (1934), Fritsch (1945), Smith (1950); families only given in list.

(2) See Drouet (1951) and Drouet & Daily (1952) who are also preparing for publication a detailed revision of the coccoid Cyanophyceae. Dr F. Drouet has very kindly given the following information for inclusion here. In the revision of the Chroococcaceae the genus *Anacystis* Menegh. will include *Aphanocapsa*, *Chroococcus* and *Gloeocapsa* of this list and the genus *Coccochloris* Spreng. will include *Aphanothece*, *Gloeothece*, and *Synechococcus* of this list. In the revison of the Chamaesiphonaceae the genus *Entophysalis* Kütz. will include all the genera given under this family in this list including *Pleurocapsa* Thur. as to type, but not as to many of the species which largely are forms of *Anacystis*.

(3) See also Nadson (1932).

(4) Probably not a member of the Cyanophyceae.

(5) = *C. confervoides* Ag.?

(6) Possibly a form of *C. scopulorum* Ag.

(7) = *Calothrix endophytica* Cotton, *Proc. Roy. Irish Acad.*, Vol. 31, p. 104, 1912 [non *Calothrix endophytica* Lemmerman, *Forsch. Ber. d. Biolog. Stat. zu Plön*, p. 184, 1896 = *Homoeothrix endophytica* (Lemm.) Lemm., *Krypt. Flor. Mark Brandenburg*, Bd. 3, p. 240, 1910.]

(8) Both Lindstedt (1943) and Du Rietz (1947) consider that *C. fasciculata* Ag. is a luxuriant growth form of *C. scopulorum*. The plant figured and described as *C. fasciculata* by Harvey in Phyc. Brit., may be, according to some authorities, a species quite distinct from the true *C. fasciculata* Ag.

(9) Geitler (1932) suggests that *R. atra* is possibly the young stage of *R. nitida*.

(10) The value of this genus appears to be debatable since *D. heppii* Näg., on which the genus was founded, is, according to some authorities, only a form of *Scytonema crustaceum* Ag. There also seems to be doubt concerning Batters' plant (Frémy, 1934) since his diagrams do not show false branching but true branching—this requires investigation.

(11) Intermediates occur between all the described forms (Frémy, 1934).

(12) Two species omitted from list as they are in need of investigation. (*a*) *O. rosea* (Crn.) Batt. [non *O. rosea* Utermöhl, 1925, = *O. utermoehlii* J. De Toni, 1939] given as a sp. inquir. in Gomont. (*b*) *O. subuliformis* Thwaites ex Harv., Phyc. Brit.; does Thwaites' plant = *O. subuliformis* Kütz.?

(13) Probably a form of *S. subsalsa*—no essential difference between the two species (Geitler, 1932).

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

RECEPTOR ELEMENTS IN THE THORACIC MUSCLES OF *HOMARUS VULGARIS* AND *PALINURUS VULGARIS*

By J. S. Alexandrowicz

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 315–46

In the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris* the presence of receptor elements of various kinds has been recorded. Muscle receptor organs belonging to the same category as described previously in the abdomen have been found in the two posterior (7th and 8th) thoracic segments. Like those in the six abdominal segments they are linked with the system of the extensor muscles. The topography and the structure of these organs have been described.

Nerve cells regarded as receptors of a different category have been found in some of the muscles inserting in the median surface of the epimeral plate. These cells, termed '*N*-cells', are smaller elements than those of the first category and have not a special muscle of their own, but end with long processes between the fibres of the ordinary muscles. In each of the species investigated five such elements have been found. It is suggested that the *N*-cells may represent more primitive forms of muscle receptors, and the receptor organs of the extensor muscles in the thorax and abdomen are more highly evolved forms. J.S.A.

NOTES ON THE NERVOUS SYSTEM IN THE STOMATOPODA.

II. THE SYSTEM OF DORSAL TRUNKS.
III. SMALL NERVE CELLS IN MOTOR NERVES

By J. S. Alexandrowicz

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 29–45

On the ventral surface of the extensor muscles in *Squilla mantis* a pair of nerve trunks has been found which run longitudinally through the thoracic and abdominal segments. In each of the first five abdominal segments there are four ganglion cells in each trunk. In the same segments five paired nerves arise from the trunks and give off branches ending in two neuropile-like networks situated in the pericardial cavity: the first in its lateral wall and the second in a lamella spanned between the dorsal muscles of the opposite sides. It is suggested that these nervous elements have a neurosecretory function.

In the motor nerves running on the ventral surface of the extensor muscles in *Squilla mantis* nerve cells of small size have been found. They have long processes running alongside the motor fibres. There is no evidence of any relations of these cells with the system of dorsal trunks. J.S.A.

Resistenz der Meeresalgen gegen sichtbares Licht und gegen kurzwellige UV-Strahlen

By R. Biebl

Protoplasma, Bd. 41, 1952, pp. 353–77

The tissues of marine algae show a typical 'ecological resistance' to direct sunlight. After 2 hr. exposure to sunlight or 3–5 days' exposure to two 350 W. electric-light bulbs all the cells of sublittoral algae were killed, while those of intertidal algae remained intact.

The degree of resistance to ultra-violet light is characteristic for algae within each ecological zone (intertidal, low-water, or sublittoral): it is constitutional, and not conditioned by the environment. Resistances to sunlight and to ultra-violet light are different characteristics of the tissues.

Photographs of dead cells show that those killed by visible light are in general rapidly bleached, in contrast to those killed by ultra-violet light or other deleterious agents. R.B.

Lichttransmissionsänderungen an Meeresalgen, im besonderen an *Porphyra umbilicalis* f. *laciniata*

By R. Biebl

Oesterr. botan. Zeitschr., Bd. 100, 1953, pp. 179–202

The light-transmission between 400 and 800 mμ was measured with an Unicam Photoelectric Quartz Spectrophotometer on small pieces of the red alga *Porphyra umbilicalis* (L.) Kütz. f. *laciniata* (Lightf.) J.Ag. in order to compare the values obtained before and after various treatments.

If *Porphyra*, after being kept for 17 hr. in hypotonic (10% normal) or hypertonic (300% normal) sea water, is desiccated for 3 hr. or exposed for 2 hr. to direct sunlight, it exhibits a remarkable raising of light-transmission, which is fully reversible if the alga is brought back to normal conditions for about 20 hr. If the pieces are put in darkness after 2 hr. exposure to sunlight, the light transmission continues to rise for about 5 hr., but afterwards returns

gradually to the original values. Heat (34° C.) also causes raising of light-transmission. Cold (−2° C.) does not.

There is a certain contrast between the extinction curves obtained with algae treated with distilled water or 10% sea water on the one hand, and those treated with concentrated sea water, or desiccated, on the other. For descriptive purposes, the terms 'swelling type' (Quellungstyp) and 'shrinking type' (Entquellungstyp) have been adopted.

It was not possible to study the cause of changes in light transmission in the time available. Alterations in the form of the big plastids as well as changes in the colloidal structure of a reversible nature in the plastids ought to be taken into consideration. It is unlikely that the pigments could be decomposed and resynthesised during the short duration of the experiments.

R.B.

Some structural Proteins of *Mytilus edulis*

By **C. H. Brown**

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 487–502

The byssus threads, periostracum, hinge and ground substance of the shell and the supporting material of the gills of *Mytilus edulis* were examined by physical, chemical and histochemical means. The byssus threads, periostracum and hinge were shown to consist of a quinone-tanned protein. The byssus threads are formed in the posterior groove of the foot from the secretions of two glands, the 'white' gland which supplies the bulk of the protein of the thread and the 'purple' gland which supplies the aromatic material responsible for the tanning. The periostracum is secreted by gland cells in the epithelium of the outer lobe of the mantle edge. The supporting material of the gills is a fibrous protein without quinone-tanning. This protein is in some respects similar to the untanned protein of the byssus. C.H.B.

Presenza di spicole in *Diplosoma listerianum* (Milne Edwards). Contributo alla sistematica degli Ascidiacea, Didemnidae (The presence of Spicules in *Diplosoma listerianum* [Milne Edwards]. A contribution to the Systematics of the Ascidiacea, Didemnidae)

By **D. B. Carlisle**

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 62–8

The presence of calcareous spicules is reported in the test of some colonies of this species collected at Naples and in a much less proportion of colonies collected at Plymouth. They are much smaller than is usual in the Didemnidae and are rapidly destroyed by the trace of formic acid present in formalin so

that they are normally absent from preserved material. They are invisible except with phase contrast when a piece of test is mounted in canada balsam. The nomenclature of the species is discussed and it is suggested that the report of these spicules destroys the last remaining difference between the genera *Lissoclinum* and *Diplosoma*, so that they should now be merged under the older name—*Diplosoma* Macdonald. It is possible that *Lissoclinum pseudoleptoclinum* (Drasche) may be a subspecies of *Diplosoma listerianum*.

D.B.C.

Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental Evidence for a Growth- and Moult-Accelerating Factor obtainable from Eyestalks

By **D. B. Carlisle** and **P. F. R. Dohrn**

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 69–83

A solution made by extracting the eyestalks of fast-moulting (summer) female *Lysmata* or *Palaemon* spp. with distilled water acidified to pH 3·5–3·8 with hydrochloric acid was active in accelerating the rate of moulting when injected intramuscularly into *Lysmata*. There was no significant activity in male eyestalk extracts or in extracts made from eyestalks taken from females in winter when the moult rate is very low. The active material was destroyed by boiling. The moult rate was also accelerated by vertebrate pituitary extracts and crude human chorianic gonadotrophin. It is suggested that the active material in the eyestalk extracts is a hormone whose site of origin is probably neurosecretory cells in the central nervous system of the eyestalk and other ganglia. It is concluded that the initiation of the moulting process may be under the control of the moult-inhibiting hormone, but the process of the premoult, once begun, is controlled by the moult-accelerating hormone.

D.B.C.

Plankton of the Benguela Current

By **T. J. Hart**

Nature, Vol. 171, 1953, pp. 631–4

Preliminary results from net-caught phytoplankton samples showed good agreement with the hydrological results described in a previous article by Currie, and some analogy with conditions off California. Many cosmopolitan species seemed especially abundant in both areas. In the Benguela, chaetocerids predominated in the richest coastal area both in autumn and in spring. *Planktoniella* formed a good indicator of the much poorer offshore current at both seasons, and followed intrusions of this water into the area of the

upwelling current very closely, as Gunther had found off Peru. *Goniaulax spinifera* was dominant in a localized area where lateral mixing between the two main types of surface waters seemed most probable, but the total plankton there was not rich. Many individual species of diatoms showed distributional patterns conforming to the disposition of the water masses. The population of the rich inshore area tended to be greater in autumn than in spring, and this is thought to be due to the lessened upwelling intensity in autumn, some time-lag favouring maximum development of the succession in the previously upwelled water.

Samples of discoloured surface-water near Walvis Bay at the time of a moderate fish mortality showed *Peridinium triquetrum* dominant in reddish areas (3–6 million cells/l.). Khaki areas were less rich (some half-million cells/l.) and contained many diatoms (*Asterionella*) and *Prorocentrum* in addition to the *P. triquetrum*. Water bloom of several different types is now known to occur in the region, though blooms due to *Gymnodinium* or *Goniaulax*, such as are known to be lethal elsewhere, have not yet been identified. It is suggested hypothetically that the fish mortalities may result from multiple causes, with the emphasis on lack of oxygen in the subsurface layers, rather than from the action of noxious dinoflagellates alone.

Some outstanding features of the zooplankton distribution are also briefly mentioned. T.J.H.

Contraction and Relaxation in the Adductor Muscles of *Mytilus edulis*

By J. Lowy

Journ. Physiol., Vol. 120, 1953, pp. 129–40

The spontaneous electrical and mechanical activity in the intact posterior adductor muscle of *Mytilus edulis* has been recorded simultaneously and continuously for long periods of time with the animal in water or exposed to air. It was found that both phasic and tonic contractions of the adductor are accompanied by muscle action potentials. Tonic contraction is associated with intermittent excitatory volleys as well as with electrical activity in the muscle during the intervals between volleys.

Experiments with preparations of the posterior adductor muscle show that nerve elements in the visceral ganglia control the duration and termination of a state of contraction. On the present evidence no explanation can as yet be given about the mechanism involved in the nervous control of relaxation.

When placed in oxygenated sea water the denervated posterior adductor muscle relaxes within a few hours. Strips of this muscle can also be isolated in a relaxed state. Such preparations remain excitable to electrical stimuli and exert a maximum tension for up to two days at 14° C. J.L.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1952–53

Patron

The Council has pleasure in reporting that His Royal Highness The Duke of Edinburgh, K.G., has graciously consented to become Patron of the Marine Biological Association of the United Kingdom.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was seventeen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Council has to record with regret the deaths of two Vice-Presidents, Viscount Astor and Dr W. T. Calman, C.B., F.R.S.; of Dr Th. Mortensen, an Honorary Member of the Association; and of Prof. J. H. Orton, F.R.S., sometime member of Council and for many years a member of the Staff of the Plymouth laboratory.

The Plymouth Laboratory

Following the completion last year of the building of the laboratory for research with radioactive substances, the opportunity has been taken of paving the area between this laboratory and the main building with concrete.

The facilities of the main workshop have been improved by the purchase of a milling machine.

The Council is grateful to the Development Commissioners for the supply of a new Morris 10 cwt. truck.

The Aquarium

Throughout the summer months the aquarium was crowded with visitors, and their appreciation and interest is reflected in the sale of large numbers of the aquarium guide book. Tanks have been stocked to capacity, perhaps the most notable exhibits being the wreck-fish (*Polyprion americanus*), mentioned in last year's report and now in fine condition, and about twenty boar-fish (*Capros aper*), which make an especially colourful display. Some of the sea-water mains which had corroded have been renewed and three new panes of glass have been inserted on the south side. Dr D. P. Wilson has published in Volume XXXI, No. 1, of the *Journal* an account of the aquarium and circulation system in use at Plymouth, with suggestions for improvement and some suggestions of application to all large marine aquaria.

Research Ships

During the year the piston and liner of one of the cylinders of the *Sabella* have been renewed, and it has been necessary to fit a new tail shaft and rudder spindle to the *Sula*. Both ships, and the *Gammarus*, are in good condition and have been working regularly throughout the year.

New Research Ship

The keel of the new research ship now being built in the yard of Messrs Philip and Son Ltd., at Dartmouth, was laid in August 1952, and work is proceeding according to schedule. Dr G. A. Steven and Captain C. A. Hoodless have given a considerable amount of time to consideration of the many details involved, and Mr F. J. Warren has given much assistance in the planning of the electrical installations.

The Staff

Dr J. A. C. Nicol has been promoted to the grade of Principal Scientific Officer.

By arrangement with University College, London, Mr B. C. Abbott joined the staff of the Plymouth Laboratory on 1 September 1952 on a special temporary appointment.

Dr H. W. Harvey, F.R.S., has been awarded the Agassiz Medal for 1952 by the National Academy of Sciences, Washington.

Mr F. S. Russell attended the forty-first meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1952; and he and Dr Mary Parke attended the International Seaweed Symposium held in Edinburgh in July 1952.

Mr G. M. Spooner was awarded a grant by the Institut Océanographique to spend the month of June 1952 in the Musée Océanographique at Monaco.

Mr D. B. Carlisle was awarded a grant to spend the month of May 1952 at the Stazione Zoologica, Naples. At the end of August he went to the Stazione to assist in the Physiological Department for four months.

Occupation of Tables

The following one hundred and eight workers have occupied tables at the Plymouth laboratory during the year:

B. C. ABBOTT, London (Physiology of muscle).
E. ADAMS, Plymouth (Library).
J. A. ALLEN, Glasgow (Ciliary mechanism of Lucinacea).
Dr DAPHNE ATKINS, London (Biology of *Pinnotheres*).
K. H. BAIN, Gold Coast (Fisheries).
R. BAINBRIDGE, Oxford (Interrelationships of plankton organisms).
R. H. BAIRD, Conway (Oyster culture).
Miss D. BALLANTINE, Development Commission (Taxonomy and culture of flagellates).

I. D. BEALL, Plymouth (Library).
Dr ANNA M. BIDDER, Cambridge (Cephalopods).
Dr H. BLASCHKO, Oxford (Enzymes in *Loligo*).
B. BOCZAROW, Plymouth (Library).
A. D. BONEY, Plymouth (Algae).
E. R. BRAITHWAITE, Plymouth (Library).
Dr ELEANOR M. BROWN, London (Parasites of copepods).
Prof. & Mme L. BRULL, Liège (Physiology of kidney in *Lophius*).
G. O. BURGESS, Hull (Epidermal secretions of fish).
Dr J. N. CARRUTHERS, London (Bottom sampling).
P. CHILDS, Plymouth (Comparative anatomy of biliary tract).
Dr P. N. J. CHIPPERFIELD, Brixham (Library).
R. B. CLARK, Glasgow (Behaviour of polychaetes).
Dr H. A. COLE, Conway (Oyster culture).
Miss M. COLLIS, London (Anatomy and physiology of *Sabellaria*).
C. A. COSWAY, Torquay (Library).
C. B. COWEY, Oxford (Biochemistry).
R. I. CURRIE, National Institute of Oceanography (Oceanography).
L. CUYPERS, Liège (Physiology of kidney in *Lophius*).
Dr R. PHILLIPS DALES, London (Behaviour of *Amphitrite*).
Dr A. K. DAS, India (Biochemistry of fish).
Dr D. DAVENPORT, Santa Barbara, Cal., U.S.A. (Physiology of commensalism).
Prof. J. H. DAY, Cape Town (General).
K. ERIKSEN, Oslo (General).
Dr MARIA FELIŃSKA, Cheltenham (Ciliates).
L. R. FISHER, Reading (Vitamin A in cephalopods).
Miss V. E. FORD, Brighton (Algae).
Cdr R. H. C. F. FRAMPTON, R.N. (Rtd.), Plymouth (Library).
Dr VERA FRETTER, London (Osmoregulation in *Nereis diversicolor*).
B. L. GINSBORG, London (General).
Dr C. P. GNANAMUTHU, Madras (General).
Dr HELEN GOODRICH, Oxford (*Phascolosoma*).
J. N. R. GRAINGER, Hull (Respiration in crustaceans).
Miss S. G. GREENING, Birmingham (*Arenicola*).
Dr ANTOINETTE GUELIN, Paris (Bacteriophages in fishes).
D. N. F. HALL, Colonial Fisheries (Biology of decapods).
Miss M. B. HARLEY, London (Feeding habits of *Nereis*).
Dr T. J. HART, National Institute of Oceanography (Plankton).
F. A. B. HAWES, London (Fouling organisms; settlement in *Tubularia*).
R. H. HEDLEY, Newcastle (Tube formation in serpulids).
Dr C. F. HICKLING, Colonial Office (General).
Miss J. M. HIMMS, Oxford (Enzymes in *Loligo*).
Prof. A. L. HODGKIN, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).
E. P. HODGKIN, Western Australia (General).
Dr L. B. HÖGLUND, Uppsala (*Spirorbis*).
G. HOYLE, Glasgow (Spontaneous activity of tunicates).
O. D. HUNT, Newton Ferrers (Library).
R. F. HUTTON, Miami, Fla., U.S.A (Fulbright Scholar) (Trematode parasites).
L. A. J. JACKMAN, Paignton (Library).
Miss M. W. JARVIS, London (*Hemimysis* and *Macromysis*).
F. J. JEFFERY, Plymouth (Library).

L. W. G. JONES, Plymouth (Library).
Dr W. C. JONES, Cambridge (*Leucosolenia*).
K. A. & Mrs JOYSEY, London (Biometrics of *Echinus esculentus*).
Dr G. Y. KENNEDY, Sheffield (Porphyrins in marine invertebrates).
G. KERKUT, Cambridge (*Astropecten*; behaviour of gastropods).
Dr R. D. KEYNES, Cambridge (Giant nerve fibres in *Loligo*; electric organs in rays).
Miss L. LAKE, London (Ecology of buoys in Plymouth Sound).
Dr MARIE V. LEBOUR, Cawsand (Decapod crustaceans).
Prof. O. E. LOWENSTEIN, Birmingham (Electrophysiology of elasmobranch labyrinth).
Dr A. G. LOWNDES, Peterborough (Density of marine organisms; Entomostraca).
D. C. H. MCDONALD, London (Architecture of marine laboratories).
D. A. MCGILL, Bucknell, Pa., U.S.A. (Fulbright Scholar) (Iodine metabolism in *Ciona*).
Prof. S. A. MATTHEWS, Williamstown, Mass., U.S.A. (Endocrinology of fish).
Dr A. J. MATTY, Nottingham (Elasmobranch endocrinology).
R. B. MAYNE, Plymouth (Library).
Dr J. E. MORTON, London (Tunicate digestion; pteropod morphology).
Miss M. P. MOSS, Oxford (General).
I. S. R. MUNRO, C.S.I.R.O. Australia (General).
Dr E. NIZET, Liège (Physiology of kidney in *Lophius*).
Miss M. OLIVER, Oxford (General).
A. H. PAPWORTH, Northampton (Plankton).
Miss J. PELLY, Oxford (General).
E. J. PERKINS, London (pH of seashore soils).
D. G. POLLARD, Plymouth (Library).
Dr H. H. POOLE, Dublin (Photoelectric measurement of light).
S. RANASINGHE, Reading (Fauna of cast seaweed).
Dr E. RASMUSSEN, Copenhagen (*Polydora hoplura*).
Dr W. J. REES, British Museum (Natural History) (Library).
Miss B. RICKARD, Plymouth (Library).
Dr J. P. RILEY, Liverpool (Chemistry of sea water).
G. S. SAETERSDAL, Bergen (General).
Dr C. P. SPENCER, Bangor (Physiology of diatoms).
Miss F. A. STANBURY, Plymouth (*Cladophora*).
J. H. STEELE, Aberdeen (Chemistry of Sea Water).
F. C. STOTT, Kingston (Library).
Dr MURIEL F. SUTTON, London (Regeneration in *Ciona*; feeding in *Terebella*).
H. WILSON THOMAS, Plymouth (Library).
Prof. E. B. VERNEY, F.R.S., Cambridge (Physiology of kidney in *Lophius*).
Miss M. WAGSTAFF, Horsham (Movements of *Patella*).
Dr G. P. WELLS, London (*Arenicola*).
Dr MARY WHITEAR, London (Histology of fish skin).
Miss E. WHITEHEAD, London (Biology of *Lasaea rubra*).
J. H. WICKSTEAD, Colonial Fisheries (General plankton).
Dr D. R. WILKIE, London (Dynamic constants of *Raia* muscle).
Miss L. WILSENS, Liège (Physiology of kidney in *Lophius*).
R. F. WRIGHTON, Birmingham (General).
H. V. WYATT, London (Life history of *Calyptraea*).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members

of the scientific staff, the following have come from overseas: Dr L. R. Donaldson, Seattle; Dr A. Stephanides, Athens; Dr F. C. Withler, Nanaimo; Dr E. Dahl, Oslo; Dr Y. R. Desai, India; Prof. Irving M. Klotz, Woods Hole; Dr Anton Fr. Bruun, Dr H. Lemche, Torben L. Wolff and F. Jensenius Madsen, Copenhagen; A. E. Hefford, New Zealand; Dr Lois E. TeWinkel, U.S.A.; Dr Olaf Elofson, Uppsala; D. L. Belding, Woods Hole; Dr E. C. T. Holsinger, Ceylon; R. Knapp-Fisher, Newfoundland; A. Richards, La Jolla; Dr A. von Brandt, Hamburg; Dr H. Mann, Hamburg; Dr H. Mertens, Bonn; Dr H. Wiehr, Hamburg; F. W. Moorhouse, South Australia; Prof. S. Stankovic, Belgrade; D. W. le Mare, Penang; Prof. H. Etcheverry and Prof. Regina Cubillos, Valparaiso.

The Royal Danish Research Ship *Galathea* arrived at Plymouth on 20 June 1952, being the last port of call before returning to Copenhagen after her voyage round the world under the scientific leadership of Dr Anton Fr. Bruun.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-four students from the following Universities and University Colleges: Oxford, Cambridge, Glasgow, Edinburgh, London, Leeds, Sheffield, Nottingham, Newcastle, Southampton, Aberystwyth, Cardiff, Exeter, and from Plymouth Technical College.

Also during the Easter Vacation Mrs F. B. J. Sewell brought a party of five girls from Southampton Grammar School and Eltham High School, Mr I. F. Thomas four boys from Oundle, and Mr R. J. Jones twelve boys from Whitgift School.

Scientific Work of the Plymouth Laboratory Staff

During the year the scope of the work of a number of members of the staff has been much extended by opportunities to take part in the research cruises of R.R.S. *Discovery II*. The Council wish to record their thanks to the Director of the National Institute of Oceanography and his staff for this valuable co-operation, and for the assistance they have given in the work at sea.

In a number of ways also there has been collaboration and co-operation with the Fisheries Laboratories of the Ministry of Agriculture and Fisheries at Lowestoft and of the Scottish Home Department at Aberdeen.

Physics and Chemistry of Sea Water

In conjunction with Dr H. H. Poole, who again visited the laboratory after a long interval, Dr W. R. G. Atkins has worked at a modification of the 'balance by depth' method of measuring transparency of sea water which appears to have certain advantages, though the apparatus is more expensive. A copy of the original apparatus was supplied, from London, to a French laboratory.

Now that their joint work on the scattering of light in sea water has been published in the *Proceedings of the Royal Society* (B, Vol. 140), they are working up further early post-war measurements with the cube photometer to compare the clarity of the water with that obtaining before the war and to study the angular distribution of the light. Dr Atkins has made such a comparison of the phosphate and silicate contents of the water at Station E 1, comparing the years 1923, 1925 and 1948, using precisely the same methods of analysis. The winter phosphate maximum in 1948 was 25% below that for 1923, but owing to better utilization the minimum value for the production (minimum because of the possibility of some phosphate being used more than once) was only 16% below 1923. Very curiously the winter silicate maximum in 1948 was also almost exactly 25% below that of 1923.

The seasonal variation in the copper content of sea water was also studied, mainly for 1948. A winter maximum of about 25 mg./m.^{3} fell to an autumn minimum of under 2 mg./m.^{3} for surface water. In April a surface 8 mg./m.^{3} was accompanied by 18 mg. at 50 m., a reduction comparable to that for phosphate, and, like it, due to utilization by the phytoplankton. Papers on the phosphate, silicate and copper have been published in Volume XXXI, No. 3, of the *Journal*.

Dr L. H. N. Cooper has continued to seek an understanding of the richness of the English Channel in plant nutrients and the associated rich production of animals which occurred during the 1920's. In the Celtic Sea in 1950 productive waters containing *Sagitta elegans* and associated biological indicators and reasonably rich in nutrients were found. It is likely that these waters owe their enrichment, at least in part, to regeneration from muds and oozes on the sea bed. Nowhere was there water as rich as that found in the English Channel a quarter of a century ago by Dr Atkins. Large areas of the Celtic Sea were definitely poor in nutrients and plankton.

Since it seemed that the answer was not to be found in some vagary of the circulation of the Celtic Sea, attention was switched to the continental slope. Ways in which deeper richer water may be raised to the surface were examined (see Volume XXX, No. 3, of the *Journal*). Again, no explanation is to be seen in terms of events confined to the continental slope and neighbouring waters. To-day sufficiently rich water in the deeper Atlantic is not within reach of the forces which could bring it up to the surface.

Further evidence accrued from a chance investigation. At the end of October 1951, large numbers of boar-fish, *Capros aper*, were taken by the Laboratory research vessels for the first time for many years. It has been suggested (see Volume XXXI, No. 2, of the *Journal*) that these were thrown up from a canyon by a 'submarine eagre' on 25 September 1951 and then carried towards the Eddystone by currents.

It would now seem that the known facts can be explained only if in the 1920's much of the water in the eastern North Atlantic between 400 m. depth

and the bottom was thrust up bodily by 100 m. or more, so raising the level of nutrients accessible to upwelling and capsizing processes. A hypothetical means of achieving this has been developed which requires that the oxygen content of the bottom water of the Atlantic should have decreased steadily since 1921. A station in the Bay of Biscay worked by the Danish Research Vessel *Dana* in 1922 and 1930 has been twice repeated by R.R.S. *Discovery II* in 1952. Below 3000 m. the oxygen content has fallen from 5·8 ml. in 1922 to 5·4 ml. in 1930 and 4·9 ml. in 1952. The distribution of silicate (see Volume xxx, No. 3, of the *Journal*) in the Atlantic bottom water and in the Norwegian and Greenland Seas, if it can be rightly interpreted, holds promise of clarifying the problem.

Dr Cooper and Mr F. A. J. Armstrong spent three weeks on R.R.S. *Discovery II* in the North Atlantic in June and Mr Armstrong a week in September. Observations were made of the distribution of temperature, salinity, inorganic and total phosphorus, silicate and aluminium at four stations, one down to 6000 m. in a deep basin. Mr Armstrong also determined inorganic suspended matter at several depths. He has, in addition, been able to analyse for silicate samples collected by R.V. *Ernest Holt* from the waters around Bear Island and Greenland. These samples were obtained through the courtesy of Mr A. J. Lee of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft.

Monthly cruises to Station E 1, and routine determinations of phosphate, total phosphorus and silicate, have been continued by Mr Armstrong, who has also determined and partially analysed the inorganic matter in suspension at various depths at this station throughout a year. The amount found at the surface has been consistently larger than that in deeper water, and there was a decrease at most depths during the summer months.

A spectrophotometric method of determining aluminium in solution in sea water has been studied, to help in estimating that part of the silicate in sea water which may be derived from solution of aluminosilicates.

In January 1952 Mr Armstrong took another series of samples in the Teign Estuary and analysed them for the late Prof. J. H. Orton. Technical and chemical assistance has been given by him to Dr D. P. Wilson for his investigations into the biological differences between sea waters.

Plankton

Dr W. R. G. Atkins and Miss P. G. Jenkins have followed the phytoplankton through a complete year by spectrophotometric measurements of chlorophyll and by identification of the organisms each month. The water column, 0–70 m. at Station E 1, had over 1·0 g./m.^{2} of plankton, wet weight, in March and September, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}. A comparison with the production obtained from phosphate analyses suggests that the phytoplankton crop each month is

rapidly devoured. Identifications were effected by adding nutrient salts to samples of the water and allowing the plants to multiply in diffuse light. By this means the common diatoms were found, as well as seven Chlorophyceae not recorded in previous plankton studies of the region. Chrysophyceae and Dinophyceae were also found, and, of the Cryptophyceae, *Hemiselmis rufescens* Parke appeared in almost pure growth in a 50 m. sample taken in January. The results of this work have been published in Volume XXXI, No. 3, of the *Journal*.

Dr H. W. Harvey has continued investigation of the uptake of inorganic nitrogen compounds by the diatom *Nitzschia closterium*, and the synthesis of pigments in the dark by nitrogen-deficient cells when supplied with a nitrogen source. The uptake of organic phosphorus compounds—inositol hexaphosphate and glycerophosphoric acid—by phosphate-deficient cells in the dark has also been examined. Accounts of these investigations are being published in Volume XXXI, No. 3, of the *Journal*. An account of the technique used for microdetermination of organic phosphorus is in press in the *Analyst*.

A technique is being sought to examine the behaviour of a marine *Chlamydomonas* which is attracted by and swims into a concentration gradient of a number of compounds. It is hoped that such observations may throw light upon the nutritional requirements of this alga, if it proves possible to disentangle the effects of different compounds. Observations at present indicate that these flagellates swim into very low concentrations of ammonium if they are growing in nitrate (when growing in a mixture of ammonium and nitrate they absorb and utilize ammonium in preference to nitrate); into concentrations of manganese when growing in nitrate, but not when supplied with ammonium (a part played by manganese in plant metabolism is stated to lie in the process of utilization of nitrate); into low concentrations of iron; and into a higher partial pressure of carbon dioxide.

The collection of species-pure cultures of marine plant organisms has been maintained throughout the year by Dr Mary Parke, and a number of plant forms not already in the collection have been isolated either from sea-water samples or from mixed cultures. In addition to plant forms, a ciliate, *Uronema schewiakoff*, has been isolated in species-pure culture and has, so far, been kept successfully. The study of new members of the Chrysophyceae with three flagella has been continued.

Owing to the illness of her technical assistant, the maintenance of this collection of marine forms has taken up a great deal of Dr Parke's time this year.

During the year cultures for research purposes have been sent abroad and also to institutions in this country. A great number have also been produced for the use of research workers in the laboratory.

Work has also continued on the compilation of the preliminary Check List of the British Marine Algae.

Miss D. Ballantine, holding a Development Commission Fisheries Research Training Grant, has continued the comparison of methods for the estimation of numbers of nanoplankton organisms other than diatoms in sea water, and this investigation is now approaching completion. It is evident that, of the methods employed, centrifuging and filtering through a collodion membrane give strictly comparable results. The serial dilution technique gives very variable and usually lower results than the above two methods of counting.

Miss Ballantine has carried out some preliminary experiments on the use of penicillin and streptomycin (C. P. Spencer's technique) to isolate pure cultures of naked flagellates, but to date no success has been achieved.

Mr F. S. Russell has given much time to the correction of the proofs of the monograph on the Medusae of the British Isles. In this he has received critical assistance from Dr P. L. Kramp, of the Zoological Museum of the University of Copenhagen, and from Dr W. J. Rees, of the British Museum (Natural History). Dr J. S. Alexandrowicz has given much help in checking the bibliography. The final proofs have now been returned to the printers.

During 1952, Mr P. G. Corbin has observed no change from the prevailing low level of macroplankton production off Plymouth as sampled by half-hour oblique hauls with the 2 m. stramin ring-trawl.

Dr D. P. Wilson, in collaboration with Mr F. A. J. Armstrong, has made some further experiments on biological differences between natural sea waters. As it was not possible this year to obtain water from the Celtic Sea, water from the Firth of Clyde was substituted. This was possible by the kind co-operation of the Scottish Marine Biological Association's staff at Millport. Water collected in the Clyde was sent to Plymouth by rail, and in one experiment yielded better cultures of *Echinus esculentus* larvae than did water from the English Channel. In a mixture of equal volumes of the two waters the larvae did almost as well as in the Clyde water alone. It is interesting to note that both the Celtic Sea and the Firth of Clyde are characterized by a *Sagitta elegans* plankton, the English Channel at the present time being characterized by a *S. setosa* community. In another experiment it was shown that hydrogen-ion concentrations within the range likely for natural sea water have little or no effect on the growth and form of early *Echinus* larvae. Both Clyde and Channel waters, after passing through active carbon, gave poor, abnormal cultures of the larvae. From the carbon through which the waters had been passed weighable amounts of unidentified materials were extracted with acetone; these materials, added to waters of both kinds, resulted in the early death of developing *Echinus* eggs, whereas the control blank extracts from the carbon did not. These results have been published in Volume XXXI, No. 2, of the *Journal*.

Fauna and Flora of the Sea Floor

Dr D. P. Wilson has continued his work on the settlement of *Ophelia bicornis* larvae and considerable progress has been made. The memoir

mentioned last year has been published in the *Annales de l'Institut Océanographique*, and since then another paper based on experiments during the summer of 1951 has been prepared for publication in Volume XXXI, No. 3, of the *Journal*. In this paper it is shown that sands may be classed as attractive, neutral or repellent, and that there is considerable variation in the extent to which they are attractive or repellent. Further experiments during the summer of 1952 gave confirmatory evidence in support of this classification, and, in addition, an attractive factor was transferred to acid-cleaned sands, which are normally neutral, and to a purely artificial sand of fused alumina. Similar transferences of a repellent factor were also obtained, though less markedly than for the attractive factor. It was shown that the grade of a sand does influence settlement and metamorphosis to some extent. The results of the latest experiments indicate that organic material, living or dead, on the sand grains plays an important role in inducing or discouraging settlement.

With a view to bringing up to date our knowledge of the Isopoda and Tanaidacea of the Plymouth area, Mr G. M. Spooner has re-examined all available material, preserved and freshly collected. The identity of a number of species has been cleared up. Among additions to the Plymouth fauna is *Anilocra physodes* (L.), first observed in 1951, a warm-water species apparently spreading northward. *Limnoria* is represented by two species, not one only, of which *L. quadripunctata* Holthuis is distinctly more widely spread and abundant than the true *L. lignorum* (Rathke). Observations have been made on the relative distribution of the various *Idotea* species, of which *I. metallica*, an open-water form, is new to the area.

During a month spent at Monaco in June Mr Spooner was able to investigate all obtainable Amphipoda and Isopoda, for a comparison of Mediterranean species with those of Britain. Special attention was given to the genera *Gammarus* and *Jassa*. The dominant marine *Gammarus* of Mediterranean waters, hitherto assumed to be *G. locusta* (L.), is not this but a related species already known from southern England and Belgium which is yet to be described.

Continued work on *Jassa* has largely confirmed the view that more than one distinct species has been confused under the name of *J. falcata* (Mont.), contrary to the views of Sexton & Reid. There are at least three in Britain, and evidently others in the colder antarctic waters. *J. falcata* (Mont.) *sens. str.* is known only from Europe, and may be the only form in more northern parts. It is especially associated with growths of *Tubularia*, though not confined to that habitat. *Jassa marmorata* Holmes is abundant amongst weeds and ascidians on buoys and rafts, and appears to be the characteristic American species, from which area it may have originally spread. *Jassa valida* Dana has been newly recognized from Brixham and Monaco Harbours. Its home apparently is the temperate zone of the southern hemisphere. It is supposed that these three species have arisen under geographical isolation and have

been brought together (as in Britain) prematurely by transportation on ships.

Dr H. G. Vevers has published a second paper on the photography of the sea floor in Volume XXXI, No. 2, of the *Journal*. It has now been found that the large aggregations of the brittle-star *Ophiothrix fragilis* previously photographed have been remarkably constant in distribution and density over a period of more than two years. An additional area with a very dense population of the same brittle-star has been photographed near the Eddystone Reef and has been shown to coincide with an area from which Dr E. J. Allen dredged enormous hauls of brittle-stars more than 50 years ago.

A study of variation in the sex ratio of *Asterias rubens* populations has been published in Volume XXXI, No. 1, of the *Journal*, and, in collaboration with Mr James Fisher, a paper on the present population of the North Atlantic gannet (*Sula bassana*) has been published in the *Proceedings of the Xth International Ornithological Congress at Uppsala, 1950*.

Dr Vevers has also continued his work on the carotenoid pigmentation of echinoderms and crustaceans, and, in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, on the isolation and estimation of protoporphyrin in the integument of *Asterias rubens*.

Mr N. A. Holme has nearly completed a paper on the biomass of the bottom-fauna at Plymouth. This will provide a basis for following changes in the productivity and faunistic composition of the benthos in the area.

In continuation of a study of the distribution of certain lamellibranchs, particularly *Ensis*, in relation to grade of the substratum, he visited Tresco in the Scilly Isles in March 1952. The beaches are mostly of a coarse sand derived from the granite rocks of which the islands are formed, and characteristic species of the *Spatangus-Venus fasciata* community were found. The fauna is quite rich and varied, and some species seemed to attain a greater size than in the English Channel.

In September Mr Holme spent a week shore-collecting from the Marine Biological Station at Roscoff, the main object being to trace the southward distribution of *Ensis siliqua*. This species is rare or absent in Jersey, where it is replaced by *Solen marginatus* on open sandy beaches. At Roscoff, *Ensis siliqua* is not very common, and there is reason to believe that it is here close to the southern end of its range. While in France he made a short visit to the Marine Laboratory at Dinard.

In July and August 1952 Mr Holme spent three weeks at sea on R.R.S. *Discovery II* in the North Atlantic, and worked fourteen stations with the bottom-sampler in depths down to 70 fathoms, mainly in the Celtic Sea area. These preliminary samples of the bottom fauna will be of value when it becomes possible to sample in the area with the Association's new ship.

Mr G. R. Forster has designed and developed a dredge which can collect some of the deeper-burrowing animals, such as *Upogebia* and *Callianassa*.

The first model, made in the laboratory from scrap material, has been worked satisfactorily from the *Gammarus* and the *Sula*. An account of this dredge is being prepared for publication, and plans have been drawn up of a stronger and slightly larger model.

With the aid of a Royal Society grant, a Siebe-Gorman compressed-air self-contained breathing apparatus has been purchased for Mr Forster. A considerable amount of diving has been undertaken by him, but he has not yet been able to explore below the *Laminaria* belt outside the Sound, as it extends to depths of 9 or 10 fathoms. With further practice there should be little difficulty in reaching the 'sub-*Laminaria*' zone. A few underwater observations have been made on different types of dredge to see how deeply they dig into the sea bed.

Observations on the migratory behaviour of *Leander* have been continued.

Mr Forster has also made a study of membranes in the gut of decapods, especially *Leander* and *Hippolyte*. These membranes, which are chitinous, were first noticed on the faecal pellets. They may not be true peritrophic membranes as they are probably secreted largely in the posterior end of the pyloric filter press, which is part of the fore-gut.

Comparative studies on marine furcocercous cercariae are being made by Mr R. F. Hutton, working on a Fulbright Research Scholarship. To date, four new species have been isolated from the Plymouth area. The description of one of these species has been published in Volume XXXI, No. 2, of the *Journal*, and another in Volume XXXI, No. 3.

Mr D. B. Carlisle has continued his studies on the Plymouth tunicates, and two papers on the larva of *Polyclinum* and on the British species of *Tridemnum* have been published in Volume XXXI, Nos. 2 and 3, of the *Journal*.

Mr P. G. Corbin's re-examination of the sessile Scyphomedusae (Lucernariidae) has revealed the presence of five species in the Plymouth area. *Haliclystus auricula* is common from time to time; the remaining four species are uncommon. They are *Lucernaria campanulata*, to which *L. discoidea* of Eales is undoubtedly referable; a *Lucernaria* sp., formerly frequently identified as *L. campanulata* but which appears to be undescribed; *Craterolophus tethys*, previously unrecorded; and *Depastrum cyathiforme*, recorded from the area but not recently taken.

Physiology of Marine Animals

Investigations of the physiology of luminescence in marine animals have been continued by Dr J. A. C. Nicol. With the aid of photoelectric recording further information has been obtained about the luminescent responses of *Chaetopterus*. Interest has centred on nervous control of the luminescent cells. It has been found that a single electric stimulus is effective, but an increase in the intensity of the light emitted can be secured by using bursts of

stimuli, or by raising the frequency of stimulation. These effects are ascribed to a process of summation in the contractile elements of the glandular cells responsible for discharging the luminescent secretion, and the theoretical implications are discussed in an account which has appeared in Volume XXXI, No. 1, of the *Journal*.

In contrast to *Chaetopterus*, the polynoid worms show intracellular luminescence, and this condition has facilitated a more exact analysis of the processes of nervous regulation. There are six or seven polynoid worms in the Plymouth fauna that emit luminous flashes from their elytra when disturbed, and oscilloscope recordings have been obtained of the luminescent flashes of these animals. It has been found that the normal response is a series of flashes to each stimulus. By regulation of the experimental conditions it has been possible to investigate the interplay of summation, facilitation, and fatigue in the luminescent responses. Part of this work is ready for publication, and the studies are being continued.

Dr Nicol has also been able to extend his investigations to luminescent pelagic species on board R.R.S. *Discovery II* during her third cruise in 1952. Interesting records were obtained of luminescence in *Beroë*, *Pelagia* and *Pyrosoma*, and much material collected for later examination.

Dr J. S. Alexandrowicz has completed his study of the muscle receptors in the thorax of *Homarus vulgaris* and *Palinurus vulgaris*. A paper recording the results of this research has been published in the *Quarterly Journal of Microscopical Science*. Further work on the muscle receptors in crustaceans has revealed that hermit crabs have receptor organs in the abdominal segments situated in the layer of the extensor muscles. These results were published in Volume XXXI, No. 2, of the *Journal*.

The study of the innervation of the heart in *Ligia oceanica* has resulted in establishing the presence of nerve elements arranged on the same lines as in the Decapoda and Stomatopoda. It has also been found that the pericardium is reinforced by alary muscles which have an innervation independent of the heart nerves. The results were published in Volume XXXI, No. 1, of the *Journal*.

Two further parts of the 'Notes on the nervous system of the Stomatopoda' have been prepared for publication. The first of these deals with the system of nervous trunks which run through the thoracic and abdominal segments on the ventral side of the extensor muscles and contain twenty ganglion cells on each side. The nerves arising from these trunks end in the five abdominal segments in neuropile-like networks situated in the pericardial cavity. It is suggested that these nerves have a neurosecretory function. The second contains some observations on small nerve cells with long processes situated in the trunks of the motor nerves. The results have been published in Volume XXIV of *Pubblicazioni della Stazione Zoologica di Napoli*.

Extending these investigations of the nerve elements in the pericardium to decapod crustaceans, Dr Alexandrowicz has found that in all the species

investigated (several crabs, *Eupagurus bernhardus*, *Leander serratus*, *Homarus vulgaris*) there are nervous trunks suspended in the pericardial cavity which contain, apart from thicker nerve fibres, a sheath of fine neuropile-like terminations. In some species similar terminations spread also on the pericardium wall. The term 'pericardial organs' has been proposed for these structures, and it is assumed that they are neurosecretory in nature. A paper recording the results of this work has been published in Volume XXXI, No. 3, of the *Journal*.

Dr Alexandrowicz and Mr D. B. Carlisle have collaborated in an investigation of the function of these pericardial organs in crustaceans. It has been shown that extracts from the organs are active in the control of heart beat, and that there are probably two hormones present, one of which gives the fluorescence test for adrenaline.

Mr Carlisle has also investigated the endocrinology of moulting in prawns and crabs. The moult-accelerating hormone, previously found in collaboration with Dr P. Dohrn, in the Mediterranean shrimp *Lysmata* (*Pubb. Zool. Stat. Napoli*, Vol. XXIV), has been found also in *Leander*, but not so far in *Carcinus*. The hormone has been shown to be present in extracts of the cerebral and thoracic ganglia and of the eye-stalks, but not in extracts of muscle or legs. An attempt has been made to isolate the hormone which appears, on the evidence available so far, to be a polypeptide. For the purpose a paper electrophoresis apparatus has been constructed. Further work on this hormone in *Lysmata* was performed in Naples during May 1952.

Dr J. Lowy has completed his work on contraction and relaxation in the adductor muscles of *Mytilus edulis*. It was found that nerve elements located in the visceral ganglia control the speed of relaxation as well as the maintenance of tonic contraction. During normal tonus electrical activity in the muscles consists of intermittent excitatory volleys; muscle potentials also occur in the intervals between volleys. Prolonged tonus is accompanied by a decrease in frequency and amplitude of the excitatory volleys. In connexion with this work it was necessary to develop a more sensitive recording technique, and this was accomplished by adapting a two-channel ink-writing oscillograph. An electrode system was devised which allows the recording of electrical activity of muscle in sea water without artifacts.

In experiments with preparations of the posterior adductor muscle it has been shown that a state of tonus can be abolished by stimulating the visceral ganglia (or the cerebral ganglia, or the sense organs situated along the mantle edge) at an intensity greater than that required for excitation. This causes inhibition of the electrical response in the muscle, followed by relaxation. However, in the intact animal there is often an increase in the frequency of muscle potentials during relaxation after prolonged tonic contraction. This observation is difficult to explain and more experimental work with isolated preparations is needed before a satisfactory hypothesis of the mechanism of

relaxation can be formulated. An account of this work will appear in the *Journal of Physiology*.

In continuation of work on the energetics of isolated muscle begun at University College, London, Mr B. C. Abbott is investigating the mechanical, electrical and thermal properties of *Mytilus* retractor muscles in collaboration with Dr Lowy.

Mr Abbott has also started investigations on the uptake of radioactive isotopes of certain elements by unicellular algae.

Ion exchanges in the byssus retractor muscles of *Mytilus* are also being studied.

Fish and Fisheries

Dr G. A. Steven has continued his observations on the Newlyn (Cornwall) spring mackerel fishery with a view to forecasting fishing prospects. A moderately successful season was predicted for this year's fishery. In spite of a very poor start which worried the trade for a time, the total yield was finally in reasonable agreement with expectation. The indications are that next season's fishery will also be a moderately successful one; but, as pointed out in last year's report, the Newlyn mackerel fishery is so greatly influenced by marketing conditions that the fishing yield may bear little relation to actual fish stocks. Occasional divergences from prediction must, therefore, be expected even if the predictions are basically sound.

The trawling survey of grounds in the vicinity of Plymouth for comparison with similar surveys made in 1913–14 and in the early 1920's has been continued. The preliminary trends reported last year continue, i.e. that present catches are on the whole poorer than those of the 1920's but better than those of the 1913–14 period. Much work yet remains to be done, however, before really conclusive results can be presented.

In connexion with the development of the large mid-water net, Mr Steven has been experimenting with a simple type of depressor which can be used and stored as simply as a trawl board. Promising results are being obtained.

Mr P. G. Corbin has obtained further collections of sand-eels (Ammodytidae) from off-shore during trawling with a small-mesh cover on the cod-end of the trawl.

This year Mr Corbin had an opportunity of carrying out open-ocean trials aboard R.R.S. *Discovery II* of the 30 ft. diameter mid-water net referred to in last year's Report of the Council. Several oblique hauls were made with the net; it operated satisfactorily and can be shot and hauled inboard easily and quickly without requiring special tackle or extensive deck space, and when not in use it stows compactly ($1\frac{1}{2} \times 2\frac{1}{2}$ yards). The strain exerted by a net of this size on a fairly long warp (1000–1500 m.) is considerable and presents the major obstacle to towing at speeds fast enough to catch rapidly moving larger creatures. Further information on this problem is being sought.

The preservation of fishing nets, trawl twines and ropes was further studied by Dr W. R. G. Atkins and Mr F. J. Warren, attention being paid to the water absorption. The preservatives tried, save tar on coir, add little weight to the wet ropes because less water is taken up. Care was taken to dry the treated specimens to constant weight before immersion. For use in the sea a longer immersion period than that in the British Standards Institution specification seems desirable. On thin sisal ropes six copper preparations are still under test. These results were published in Volume XXXI, No. 3, of the *Journal*.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

Dr Mavis Gunther has generously presented to the Library a collection of reprints relating to marine biology and oceanography which belonged to the late Dr E. R. Gunther.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume XXXI, No. 1, of the *Journal* was published in June 1952, No. 2 in October 1952, and No. 3 in February 1953.

The following papers, the outcome of work done at the Plymouth Laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C. & AUBERT, X. M., 1952. The force exerted by active striated muscle during and after change of length. *Journ. Physiol.*, Vol. 117, pp. 77–86.

ALEXANDROWICZ, J. S., 1952. Notes on the nervous system in the Stomatopoda. I. The system of median connectives. *Pubbl. Staz. Zool. Napoli*, Vol. 23, pp. 201–14.

ALEXANDROWICZ, J. S., 1952. Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris*. *Quart. Journ. Micr. Sci.*, Vol. 93, pp. 315–46.

ALEXANDROWICZ, J. S., 1953. Notes on the nervous system in the Stomatopoda. II. The system of dorsal trunks. III. Small nerve cells in motor nerves. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 29–45.

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BIEBL, RICHARD, 1952. Ultraviolettabsorption der Meeresalgen. *Ber. Deutsch. Bot. Gesellschaft*, Vol. 65, pp. 37–41.

BIEBL, RICHARD, 1952. Resistenz der Meeresalgen gegen sichtbares Licht und gegen Kurzwellige UV-Strahlen. *Protoplasma*, Vol. 41, pp. 353–377.

BIEBL, RICHARD, 1953. Lichttransmissionsänderungen an Meeresalgen, im besonderen an *Porphyra umbilicalis* f. *laciniata*. *Österreich. Bot. Zeits.*, Vol. 100, pp. 179–202.

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COOPER, L. H. N., 1952. Water movements over the Continental Slope in relation to fisheries hydrography. *Rapp. Proc.-Verb., Cons. Int. Explor. Mer*, Vol. 131, pp. 44–50.

COOPER, L. H. N., 1952. Utilization of total phosphorus determinations in physical oceanography. *Proc.-Verb.: Assoc. d'Océanographie Physique*, No. 5, pp. 199–200 (Misc. Papers G. 24).

COWEY, J. B., 1952. The structure and function of the basement membrane muscle system in *Amphiporus lactifloreus* (Nemertea). *Quart. Journ. Micro. Sci.*, Vol. 93, pp. 1–15.

CURRIE, RONALD, 1953. Upwelling in the Benguela current. *Nature*, Vol. 171, pp. 497–500.

FISHER, JAMES & VEVERS, H. G., 1951. The present population of the North Atlantic gannet (*Sula bassana*). *Proc. Xth Internat. Ornithological Congress, Uppsala*, June 1950, pp. 463–7.

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HARVEY, H. W., 1953. Micro-determination of phosphorus in biological material. *The Analyst*, Vol. 78, pp. 110–114.

HODGKIN, A. L. & HUXLEY, A. F., 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *Journ. Physiol.*, Vol. 116, pp. 449–72.

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HODGKIN, A. L., HUXLEY, A. F. & KATZ, B., 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *Journ. Physiol.*, Vol. 116, pp. 424–48.

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KEYNES, R. D. & LEWIS, P. R., 1951. The sodium and potassium content of Cephalopod nerve fibres. *Journ. Physiol.*, Vol. 114, pp. 151–82.

LEBOUR, MARIE V., 1952. A larval hoplophorid (Crustacea) from Bermuda. *Proc. Zool. Soc. London*, Vol. 121, pp. 753–57.

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MOORE, JANET, 1952. The induction of regeneration in the hydroid *Cordylophora lacustris*. *Journ. Exp. Biol.*, Vol. 29, pp. 72–93.

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SILÉN, LARS, 1952. Researches on Phoronidea of the Gullmar Fiord area. (West coast of Sweden.) *Ark. f. Zool.*, Ser. 2, Bd. 4, pp. 95–140.

SPOONER, G. M., 1952. A new subterranean gammarid (Crustacea) from Britain. *Proc. Zool. Soc. London*, Vol. 121, pp. 851–59.

SUTTON, MURIEL F., 1951. On the food, feeding mechanism and alimentary canal of Corixidae (Hemiptera, Heteroptera). *Proc. Zool. Soc. London*, Vol. 121, pp. 465–99.

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Membership of the Association

The total number of members on 31 March 1953 was 709, being 50 more than on 31 March 1952; of these the number of life members was 89 and of annual members 620. The number of Associate members is six, Dr W. T. Calman, F.R.S., having died during the year, and Mr W. H. Searle having been elected.

Gift

The Council wishes to record its thanks to Dr Anna M. Bidder for the gift of two elm seats to be placed in the garden of the Plymouth Laboratory.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledges the following generous grants for the year:

From the Fishmongers' Company (£400), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20),

Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Nottingham (£10. 10*s*.), Southampton (£15. 15*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), Hull (£10. 10*s*.), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1953–54:

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MORLEY H. NEALE, C.B.E.
The Rt. Hon. Major Sir THOMAS L. DUGDALE, Bt., M.P.
The Earl of VERULAM

COUNCIL

To retire in 1954

J. S. COLMAN
H. CARY GILSON
Prof. ALASTAIR GRAHAM, D.Sc.
N. A. MACKINTOSH, C.B.E., D.Sc.
Prof. J. Z. YOUNG, F.R.S.

To retire in 1955

Prof. H. GRAHAM CANNON, Sc.D., F.R.S.
O. D. HUNT
Prof. O. E. LOWENSTEIN, D.Sc.
G. P. WELLS, Sc.D.
R. S. WIMPENNY

To retire in 1956

J. N. CARRUTHERS, D.Sc.
Prof. H. MUNRO FOX, F.R.S.
Prof. A. L. HODGKIN, F.R.S.
Prof. J. E. SMITH, Sc.D.
Prof. V. C. WYNNE-EDWARDS

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2

Secretary

F. S. RUSSELL, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

G. P. BIDDER, Sc.D.
R. G. R. WALL (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
S. SMITH, Ph.D. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
H. W. PARKER, D.Sc. (Zoological Society)
Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S. (Royal Society)

BALANCE SHEET 1952–53

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31ST MARCH 1953

	£ s. d.	£ s. d.
CAPITAL RESERVE ACCOUNT:		
As at 31st March 1952	38,903 17 6	
Add: Grants received and receivable during the year from the Development Fund in respect of cost to date of the New Research Vessel	72,194 18 4	
		111,098 15 10
SURPLUS ACCOUNT:		
As at 31st March 1952	8,234 6 10	
Add: Excess of Income over Expenditure for the year ...	1,546 17 7	
		9,781 4 5
		120,880 0 3
AQUARIUM SINKING FUND:		
As at 31st March 1952	251 19 10	
Add: Donations for Rebuilding Aquarium Tanks	27 4 8	
		279 4 6
E. T. BROWNE BEQUEST FUNDS:	£ s. d.	
Library Fund as at 31st March 1952	1,224 17 1	
Add: Interest on Investments ...	35 14 3	
	1,260 11 4	
Special Apparatus Fund as at 31st March 1952	2,633 2 6	
Add: Interest on Investments ...	80 14 10	
	2,713 17 4	
Scientific Publications Fund as at 31st March 1952	2,229 17 5	
Add: Interest on Investments ...	60 11 9	
	2,290 9 2	
		6,264 17 10
ROYAL SOCIETY GRANT—PUBLICATION OF MONOGRAPH ON MEDUSAE OF THE BRITISH ISLES:		
As at 31st March 1952	752 15 8	
Add: Bank Deposit Interest credited during year	13 5 10	
		766 1 6
COMPOSITION FEES FUND:		
As at 31st March 1952	919 18 2	
Add: Fees Received during the year	94 10 0	
		1,014 8 2

	£ s. d.	£ s. d.
FIXED ASSETS:		
At valuations as estimated by the Director at 31st March 1953:		
Boats and Equipment:		
F.V. 'Sula'	11,000 0 0	
Motor Boat 'Gammarus'	200 0 0	
Nets, Gear and General Equipment	100 0 0	
	11,300 0 0	
Laboratory apparatus, equipment and machinery	9,907 19 1	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost	19,487 0 0	
	40,694 19 1	
New Research Vessel (under construction). Cost to 31st March 1953	78,410 7 8	
		119,105 6 9
GENERAL FUND INVESTMENT, at Book Value £352. 2*s.* 3*d.* 2½% Treasury Stock ... ... (Market value £208; last year £204)		232 7 10
E. T. BROWNE BEQUEST FUND INVESTMENTS, at cost £5,901. 8*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ... ... (Market value £4,750; last year £4,544)		5,795 3 6
COMPOSITION FEES FUND INVESTMENTS, at cost:		
£18. 8*s.* 6*d.* 2½% Treasury Stock	15 15 0	
£1,072. 12*s.* 3*d.* British Transport 3% Guaranteed Stock 1978/88 ... ... (Market value £874; last year £729)	998 13 2	
		1,014 8 2
CURRENT ASSETS:		
STOCKS ON HAND, as valued by the Director:	£ s. d.	
Specimens	600 0 0	
Chemicals	250 0 0	
Journals	400 0 0	
	1,250 0 0	

BUILDINGS RECONSTRUCTION FUND:			
As at 31st March 1952		808 7 1	
Add: War Damage Compensation received during the year	1,433 10 5		
Less: Receivable at 31st March 1952	139 14 4		
		1,293 16 1	
		2,102 3 2	
Less: Expenditure during the year		803 8 2	
			1,298 15 0
RADIOACTIVE SUBSTANCES LABORATORY BUILDING FUND:			
Grants Received from the Development Fund		559 15 2	
Expenditure during the year...		743 4 10	
Balance *per contra*		183 9 8	
RADIOACTIVE SUBSTANCES RESEARCH FUND:			
Grants Received from the Development Fund		876 0 0	
Less: Expenditure during the year		291 16 8	
			584 3 4
RESEARCH FUND—MISS D. BALLANTINE:			
Grants Received from the Development Fund		389 7 6	
Less: Expenditure during the year		302 9 3	
			86 18 3
CURRENT LIABILITIES:			
Accrued Expenses, etc.		1,113 19 5	
Subscriptions and Grants Received in advance		167 1 10	
Instalments due under contract for construction of New Research Vessel		—	
			1,281 1 3
Note: The Association had a capital commitment at 31st March 1953 in respect of the balance of the cost of the New Research Vessel of £60,670 which will be recoverable under a Development Fund Grant.			
			£132,455 10 1

SUNDRY DEBTORS:			
Sales of Specimens, Journals, Nets and Hydrographical Gear	926 3 1		
Recoverable Expenditure	436 7 5		
		1,362 10 6	
PREPAYMENTS		81 7 4	
RADIOACTIVE SUBSTANCES LABORATORY BUILDING FUND:			
Balance *per contra*		183 9 8	
BALANCE AT BANK AND CASH IN HAND:			
Coutts & Co., London	£ s. d		
Current Account...	1,903 15 8		
Deposit Account...	766 1 6		
	2,669 17 2		
Lloyds Bank Ltd., Plymouth ...	699 19 0		
Cash in Hand	61 0 2		
		3,430 16 4	
			6,308 3 10
			£132,455 10 1

JOHN E. HARRIS
O. D. HUNT } *Members of the Council*

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1953, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

Norwich Union House
2, St Andrew's Cross
Plymouth
13th May 1953.

PRICE WATERHOUSE & CO.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1953

	£ s. d.	£ s. d.
SALARIES, including Pay Additions, and Association's contribution to Superannuation Scheme and National Insurance		22,877 16 4
LABORATORY AND BOATS' CREWS' WAGES, including Pay Additions, National Insurance, contributions to Superannuation Scheme, War Bonus and Employers' Liability Insurance		17,991 18 7
UPKEEP OF LIBRARY		854 19 0
SCIENTIFIC PUBLICATIONS, *less* sales		1,700 9 10
UPKEEP OF LABORATORIES AND AQUARIUM:		
Buildings and Machinery	568 19 11	
Electricity, Gas, Coal and Water	870 5 2	
Chemicals and Apparatus	936 6 9	
Insurances, Ground Rent and Rent of Stores	163 10 0	
Travelling Expenses	610 0 6	
Audit Fee	63 0 0	
Stationery, Postages, Telephone, Carriage and Sundries	778 15 5	
Specimens	197 2 8	
		4,188 0 5
MAINTENANCE AND HIRE OF BOATS:		
Petrol, Oil, Paraffin, etc.	679 4 9	
Maintenance of and Repairs to Nets, Gear and Apparatus	2,467 11 3	
Boat Hire, Collecting Expenses and Upkeep of Truck	221 11 9	
Insurances	631 13 4	
Hire of R.V. 'Sabella'	1,380 0 0	
		5,380 1 1
ENTERTAINMENT EXPENSES		59 8 3
BALANCE, being excess of Income over Expenditure for the year		1,546 17 7
		£54,599 11 1

	£ s. d.	£ s. d.
GRANTS AND TABLE RENTS:		
Ministry of Agriculture and Fisheries grant from Development Fund	47,834 0 0	
Fishmongers' Company	400 0 0	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of: London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Sheffield £10. 10*s*., Imperial College £10, and Ministry of Works £104)	1,176 2 1	
		49,410 2 1
SUBSCRIPTIONS (excluding those received in advance)		587 4 0
FEES FOR TESTS OF MATERIALS		—
SALES:		
Specimens	2,432 12 0	
Fish	452 15 2	
Nets, Gear and Hydrographical Apparatus	£ s. d. 870 7 7	
Less: Cost of Materials	585 14 11	
	284 12 8	
		3,169 19 10
INTEREST ON INVESTMENTS		37 4 10
INTEREST ON BANK DEPOSITS LESS CHARGES		42 12 7
SALE OF DR M. V. LEBOUR'S BOOK		6 17 6
SALE OF 'PLYMOUTH MARINE FAUNA'		1 7 6
AQUARIUM:		
Admission Fees	1,739 4 10	
Sale of Guides	128 18 6	
	1,868 3 4	
Less: Maintenance of Building	101 17 11	
Printing Guides and Tickets	169 11 8	
Advertising	38 17 0	
Food	107 8 0	
Wages	106 6 0	
	524 0 7	
		1,344 2 9
		£54,599 11 1

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. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £25,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; 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. XXVII (p. 761) and Vol. XXXI (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.

TERMS OF MEMBERSHIP

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers 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.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

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