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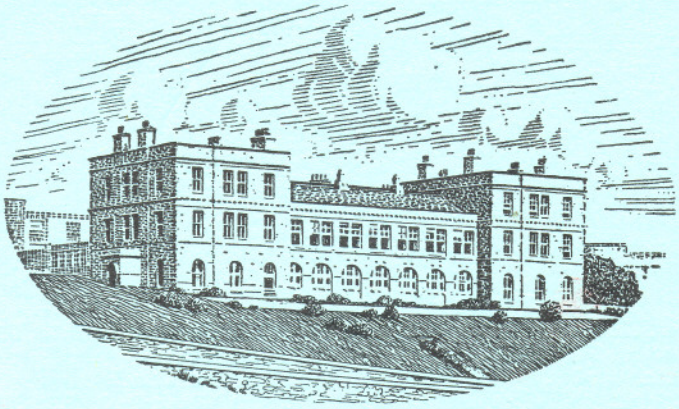

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BIOLOGICALLY ACTIVE COMPOUNDS IN THE SEA

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Introduction

It is becoming recognized that metabolites released into aquatic and other environments may have vital significance for associated communities. For the particular habitat of the sea, Lucas (1938) indicated tentatively some of the possibilities and later (1947, 1949) reviewed some of the extensive evidence which provided support for a new concept of 'non-predatory' inter-relationships. External metabolites ('ectocrines') formed by some organisms were envisaged as favouring or hindering the growth of other members of the community, their effects varying according to their concentrations. Although a few might extend their effects in relatively large concentrations, many (perhaps the most important) were regarded as effective at extreme dilution. Metabolites could conceivably neutralize the growth-inhibiting or growth-promoting effects of other substances, presumably including other metabolites. The production and persistence of metabolites in a body of sea water imposed a 'biological history' on the water and provided a basis for ecological 'succession'. The general evidence of 'succession', like that implying 'animal exclusion' (Hardy, 1935), almost demanded such a concept in support.

The concept of 'non-predatory' relationships drew support from a wide variety of biological observations but, until recently, little biochemical work has been carried out to detect or identify metabolites. The early experiments (e.g. Allen & Nelson, 1910), showing the importance of organic matter for establishing successful cultures, led logically to Harvey's demonstration (1933 and later) of the necessity for accessory factors in the growth of some marine diatoms, while other workers showed the significance of pro- and antibiotic substances in the culture of algae and other micro-organisms (e.g. Lefevre *et al.*, 1952). A few workers (e.g. Harvey, 1939; Matsudiara, 1939) had demonstrated differences in sea waters as culture media, and Hutchinson (1943), for example, produced evidence of a free vitamin (thiamin) in lake water. Recently a number of prominent contributions have been made by the workers at the Haskins Laboratories (New York) who have investigated with great precision the nutrition of a few marine and freshwater micro-organisms. Their work is demonstrating interdependencies such as: '(1) the interchange of growth factors; (2) the lowering of inhibitory concentrations of several major mineral

nutrients, especially phosphate; and (3) the preferential utilization of minerals, including trace metals, [which] may condition waters, bringing their concentrations into the optimal zones for succeeding forms'. They consider that the practical aim, 'to predict algal successions and blooms—may be achieved through a comprehensive knowledge of vitamin cycles as well as mineral cycles. An immediate problem is to trace the thiamin and cobalamin cycles' (Provasoli & Pintner, 1953, p. 849).

Meanwhile, Wilson (1932 and later) had observed that some polychaete larvae would only settle on limited types of sand which were almost certainly associated with other and perhaps specific forms of life. In another series of experiments (Wilson, 1951; Wilson & Armstrong, 1952, 1954) he demonstrated that the eggs and larvae of the sea urchin (and two species of marine worms studied less intensively) would grow more satisfactorily in water from the Celtic Sea than in English Channel water. It is significant that these waters can be distinguished by the characteristic species of *Sagitta* so that Wilson's experiments link directly with Russell's demonstration (1935) of the importance of 'western' water for good productivity in the English Channel. The general idea of sea-water characterization by chaetognath indicator species was developed for more extensive sea areas by Fraser (1937, 1939) and some postulates were made with regard to possible operative factors (Fraser, 1952).

Although in a broad sense closely related to their hydrographic environment, it is now familiar knowledge that planktonic communities may sometimes be sharply distinguished even within a water mass which is homogeneous with regard to all the usual hydrographic characteristics, including nutrients. Not only may hydrographic and nutrient contents of natural waters be significant, and inorganic micro-constituents at a more subtle level, but also the organic compounds present as the result of past and present metabolic processes. Indeed, in so far as final productivity depends on the success of photosynthetic micro-organisms, any demonstration of the dependence of these organisms on external metabolites points indirectly, if not directly, to the significance of such metabolites for the rest of the community.

It may even be legitimate to speculate further on the possible value of such work. The sensitivity of some micro-organisms to certain metabolites has been shown to be extremely high, their productivity being entirely dependent on the availability of these metabolites, whatever the grosser nutrients available (see, for example, Hamilton, Hutner & Provasoli, 1952). The possibility arises, therefore, of initiating a chain reaction of biological events ('succession') by adding to natural waters metabolites likely to encourage a desirable sequence and to suppress undesirable types. Since no additional nutrients are involved, and since the quantities of the metabolites may be relatively very small, this possibility may lead to more practical methods of controlling some aspects of aquatic life than have so far been suggested.

Thus the study of biologically active organic substances[1] in the sea is at least indirectly relevant to fishery problems. In time, some compounds may be shown to be directly relevant—perhaps by favouring or hindering the development of fish eggs or larvae, or even affecting the adult life of shellfish and true fish. One hint may be found in the observations of Collier, Ray, Magnitzky & Bell (1953) that the active feeding of oysters is related to the concentration in the sea water of certain organic substances, which respond to the *N*-ethyl carbazole test for carbohydrates. Perhaps another lies in the results of fish-behaviour studies. It has been suggested that salmon may be able to distinguish the products of one organism from another even at great dilutions and perhaps thereby the waters of one stream from others (see, for example, Lissmann's review, 1954). The requirements which must be fulfilled to satisfy the theory that fish discriminate 'home' waters by perception of special characteristic chemical constituents have been described by Hasler & Wisby (1951). They have tracked down the characteristic odour in one instance to a volatile aromatic substance.

Previous Organic Analyses of Sea Water

Much of the early work on the analysis of organic matter in the sea relied on combustion or powerful oxidizing agents (Bond, 1933; Krogh & Keys, 1934) to give an estimate of the total carbon present, and gave no clue regarding the nature of the compounds involved. A most extensive review of analyses of marine organisms, including plankton, from the earliest records to 1944 has been prepared by Vinogradov (1953). The analyses are chiefly concerned with moisture content, ash and assays of C, H, N, P and many other elements. Recently, the particulate matter (organic and inorganic) in sea water has been studied and partly analysed by Armstrong & Atkins (1950) and also by Fox, Oppenheimer & Kittredge (1953). The inanimate organic matter in the sea, according to the most recent estimate (Fox *et al.*, 1953), occurs to the extent of approximately 60% as particulate matter and about 40% as dissolved substances.

The separation and analysis of dissolved organic matter isolated from sea water has seldom been reported. Wangersky (1952) isolated by dialysis what was considered to be a rhamnose-containing polysaccharide which affected the feeding of oysters (Collier *et al.*, 1950).

It is perhaps reasonable to consider as potentially soluble some of the organic compounds found internally in marine plants and animals. An up to date and comprehensive review and bibliography has been prepared by Black (1954) of the organic chemical analysis of many of the constituents of brown, red and green algae.

[1] When the organic matter is isolated from a sample of sea water whose biological history will normally be unknown, it is more accurate to describe any active compounds simply as biologically active (to organism 'X') than to describe them as 'ectocrines' which may imply knowledge of the original sources which is not yet available.

Work at Aberdeen

With such possibilities in mind, work was begun at Aberdeen to see whether natural waters could be distinguished by their organic content and, specifically, whether the organic content could be shown to have variable significance for representative marine micro-organisms. This paper gives a brief outline of the first results. It will have served its purpose if it provokes discussion, perhaps some pooling of ideas, and stimulates further work on the biological and biochemical sides, for assuredly the two must go together.

Possible lines of advance in the study of organic matter in the sea may lie in (*a*) direct organic analysis of extracts from large amounts of sea water, (*b*) intensive assay of natural and enriched sea waters with specific micro-organisms, and (*c*) isolation of metabolites from pure mass cultures. Each of these possibilities might make an important contribution to marine science but the total work required would be very large. A more incisive attack, which might enable important features to be detected relatively quickly, demands a speedy method of isolating organic matter from relatively small samples of filtered sea water, followed by analysis of the extract and biological testing of some of the components. Final details of the method to be adopted are not yet settled, but the following paragraphs describe its evolution and the results to date.

Methods of Isolation

The earliest experiments were straightforward applications of normal methods of isolating organic compounds to the separation of dissolved organic matter from sea water. In each experiment the sea-water sample was first passed through a 1 μ filter soon after collection to remove detritus, plankton and, as far as possible, all the nanoplankton. Various methods of isolation were tried and the results obtained are summarized below.

(1) *Evaporation and extraction.* Sea water was evaporated at 30–40° C. under reduced pressure and portions of the salt residue extracted with various organic liquids. Most common solvents, e.g. alcohol, acetone, ether and, to a lesser extent, chloroform, dissolved troublesome amounts of salts in addition to any organic matter. Benzene gave least salt contamination but only a very small amount of extract. Handling of the original hygroscopic mass of salts was difficult and complete extraction was laborious.

(2) *Solvent extraction.* Extraction with organic liquids immiscible with sea water was a convenient and rapid method of isolating some of the fatty, waxy and highly coloured substances. Only solvents which did not dissolve appreciable amounts of salts were suitable, such as benzene.

(3) *Dialysis.* Sea water was dialysed against tap water to yield on evaporation organic residues of high molecular weight. The method demands the handling of fairly large volumes of liquid in order to obtain a reasonable yield but is perhaps one of the most useful, although only applicable to a restricted range of organic compounds.

(4) *Ion-exchange treatment.* Direct ion-exchange treatment of sea water proved quite unsatisfactory owing to the high salt concentration. The process can be useful, however, for removing final traces of salts from any type of extract.

(5) *Adsorption.* Many substances can be used to adsorb some part of the organic (and at the same time some inorganic) matter from sea water. Carbon, alumina, cotton-wool and cellulose powder were tried. The method is simple, fairly rapid, and not very selective in the types of organic compounds removed. Some results for carbon extracts are described later.

(6) *Electrodialysis.* The most recent separations were by this method, which in theory should yield the total organic matter more or less unchanged and free from salts. Supplies are becoming available of ion-exchange membranes which are impermeable to organic substances except perhaps the most mobile fatty acid ions, e.g. formate, acetate. Objections to the use of membranes such as parchment or cellophane, which permit the passage out of the solution of, for example, amino-acids and neutral organic compounds of low molecular weight are thus overcome. The method is convenient but slow, and further development of a suitable type of apparatus is required. Multi-membrane types have the particular advantage of minimizing temperature breakdown and electrolytic reduction or oxidation of sensitive organic materials.

Qualitative Analysis of Organic Extracts

Many methods were attempted. In general, direct organic analysis, spot tests, micro-reactions and paper chromatograms sprayed with selective reagents yielded relatively indecisive results on the gross extracts, and also after attempted fractionation, perhaps because separations were not complete. However, several clues were obtained.

Among the most common components of the carbon extracts were (1) an insoluble white substance similar to that described by Wilson & Armstrong (1952, p. 338), (2) coloured material, possibly carotenoid, soluble in benzene, petroleum ethers, etc., (3) a fairly large proportion of brownish waxy or fatty matter, and (4) yellowish orange matter with a bright blue fluorescence in ultra-violet light (probably related to the 'gelbstoff' of Kalle, 1949). A number of other components were detected by paper chromatography and examination in ultra-violet light but could not be satisfactorily separated by preliminary attempts using column chromatography.

Empirical Organic Analysis of Sea Water Samples

Salt-free carbon extracts from filtered samples of sea water are readily compared by preparing paper chromatograms of portions of the extracts (see, for example, Balston & Talbot, 1952, for general technical details).

Sea water (2 l.) is shaken with activated carbon (*c.* 10 g) and allowed to stand. Most of the liquid can then be sucked or siphoned off, the carbon recovered on a filter and washed with distilled water until chloride-free. A cold extraction process, using alcohol followed by an alcohol-benzene mixture, has been used to recover the organic matter from the carbon.

Because of variations in the adsorptive powers of various carbons and in their extractable impurities, it is essential to standardize the technique by

using only one type of carbon and by including carbon blanks. These blanks consist of carbon, shaken with sample volumes of 3·5% saline solution, and washed and extracted in the same way as the sea-water samples.

When chromatograms of the extracts and the carbon blanks were compared by ultra-violet light, different patterns and numbers of spots were seen. Provided interfering inorganic matter has been removed, these spots represent different organic components of the sea-water samples. Unknown proportions of only those compounds which are adsorbed by carbon from sea water are represented in the extract, however, and not necessarily all the soluble organic matter present.

Any suitable combination of adsorbent, solvent or paper chromatogram technique can be tried. The method yields comparative organic chemical analyses of extracts from small sea-water samples with minimum effort. For known constituents it could be developed to give approximate quantitative analysis by colorimetric methods or for bio-assay by plating or culture techniques. Especially in the case of microbiological assay it would be necessary to ensure adequate control measures to eliminate spurious effects arising from impurities in the activated carbon and in the solvents used.

Biological Effects of Some Organic Isolates

Since the preliminary qualitative organic analysis of such extracts had proved unrewarding, it was important to decide whether further attempts were worth while. The immediate value of the study of organic matter must be dependent largely on some components proving to be of biological interest, possessing, for example, growth-promoting or growth-inhibiting properties. It was not possible to test biological activity in a wide range of ways at a number of different concentrations because of the small quantities of each fraction then available, but it was felt that the responses of some common marine phytoplankton species might provide indications of biological activity of possible significance for fisheries.

An artificial basal medium was prepared according to formulae devised by Provasoli & Pintner (1953, p. 842). The plankton cultures were kindly supplied by Dr Mary Parke of the Laboratory at Plymouth. Growth was assessed by measuring change in optical density of small cultures in closed tubes. A list of the eleven types which were grown in sixteen different media is given in Table I. The various additions to the basal inorganic medium are listed in Table II. Usually the organic extract was obtained from sea water by means of adsorption on carbon. In assessing the subsequent growth of cultures, differences in optical density of $\geqslant 8$ units are significant at the 90% level; $\geqslant 10$ units at the 95% level.

The algae reacted to several of the fractions, exhibiting both increased and decreased growth, as compared with controls. Further (and perhaps different) instances of these effects would probably have been observed

TABLE I

Culture used	Medium no. (see Table II) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Number of experiments with values ≥ 10 +	≥ 10 −	Others
Skeletonema costatum (Grev.) Cleve	−12	5	−5	0	7	0	−13	6	3	9	−10	5	−7	−8	7	3	0	3	13
Mixed culture (Aberdeen)*	14	29	1	8	35	−3	6	27	−5	12	−9	21	2	−5	−3	9	6	0	10
Thalassiosira gravida Cleve	3	−3	−11	9	10	12	−1	32	6	10	−6	18	5	2	4	5	5	1	10
Chromulina pusilla Butcher	3	−4	5	3	−5	−22	−13	39	−7	7	6	−2	2	3	−1	15	2	2	12
Chaetoceros decipiens Cleve	5	3	2	2	−3	−5	12	−1	10	−6	−9	−3	9	6	−2	−1	2	0	14
Thalassiothrix frauenfeldii Grun. in Cleve & Grun.	−5	2	12	−4	10	17	−2	30	−8	3	13	11	−8	6	−6	3	6	0	10
Prorocentrum micans Ehrenberg	4	5	−4	1	8	−9	18	24	−10	−10	1	1	−1	7	2	−1	2	2	12
Phaeodactylum tricornutum Bohlin	4	21	10	19	26	7	−9	35	22	10	−16	19	16	−1	30	23	11	1	4
Plymouth No. 103 *Gymnodinium* sp.	3	2	−4	5	11	6	8	36	13	2	38	6	10	−16	−3	5	5	1	10
Plymouth No. 81 *Dunaliella* sp.	51	61	93	96	47	73	57	254	130	57	50	176	65	59	116	68	16	0	0
Isochrysis galbana Parke	0	40	9	−12	13	−4	5	39	−4	15	17	2	4	10	−11	4	6	2	8
																	61	12	103
Successful cultures	2	4	3	2	7	3	3	9	4	5	4	5	3	2	2	3	34·6 %	6·8 %	58·6 %
Unsuccessful cultures	1	0	1	1	0	1	2	0	1	1	2	0	0	1	1	0			
Little change†	8	7	7	8	4	7	6	2	6	5	5	6	8	8	8	8			

Values in the table represent changes in optical density ($\log I_0/I \times 10^3$) between 1st (10. viii. 53) and 18th day (28. viii. 53), for 5 ml. cultures in 1 cm diameter tubes. Light filter for Spekker, Ilford 601. Temperature range 15·4–15.7° C. Light intensity *c.* 2200 lux, continuous. Media adjusted to initial pH 8·0.

* *Chaetoceros ceratosporus* (or *gracilis*) and a *Chlorococcus* sp.

† Includes unsuccessful cultures of colourless species.

by testing a range of concentrations. One particular fraction was found to promote greater growth in nine of the eleven species tested. Other fractions affected smaller numbers of species favourably or unfavourably. The algal cultures used, although species-pure, were not bacteria-free, so that the observed changes in optical density of the cultures may not refer directly to the original medium supplied but to a medium modified by bacterial metabolism. Large-scale bacterial proliferation could also directly alter the optical density of the cultures. To what extent such effects complicate the assessment

TABLE II

Medium no.	Supplement to basal medium (5 ml.)
1	0·2 ml. of a stable alkaline saline earth extract
2	0·2 ml. distilled water
3	0·18 ml. D.W. + 0·02 ml. of the 5 % alcohol extract of carbon adsorbate
4	0·18 ml. D.W. + 0·02 ml. of the 20 % alcohol extract of carbon adsorbate
5	0·18 ml. D.W. + 0·02 ml. of Fraction 3, cellulose powder column chromatogram of carbon adsorbate
6	0·18 ml. D.W. + 0·02 ml. of Fraction 8, cellulose powder column chromatogram of carbon adsorbate
7	0·18 ml. D.W. + 0·02 ml. of Fraction 19, cellulose powder column chromatogram of carbon adsorbate
8	0·18 ml. D.W. + 0·02 ml. of Fraction 23, cellulose powder column chromatogram of carbon adsorbate
9	0·18 ml. D.W. + 0·02 ml. of Fraction 37, cellulose powder column chromatogram of carbon adsorbate
10	0·18 ml. D.W. + 0·02 ml. of Fraction 48, cellulose powder column chromatogram of carbon adsorbate
11	0·18 ml. D.W. + 0·02 ml. of Fraction 52, cellulose powder column chromatogram of carbon adsorbate
12	0·18 ml. D.W. + 0·02 ml. of the aqueous extract of Whatman No. 1 filter paper
13	0·18 ml. D.W. + 0·02 ml. of the second carbon extract of water from 30 m, 58° 35′ N., 00° 00′ April 1953
14	0·18 ml. D.W. + 0·02 ml. of the whole carbon extract, water B 21c, February 1953
15	0·18 ml. D.W. + 0·02 ml. of 18/44 Sutcliffe Speakman and Co., Quality 110, carbon blank
16	0·18 ml. D.W. + 0·02 ml. of the 50 % alcohol extract of carbon adsorbate

of the biological activity on the algae is not known at present, although examination of the cultures at the end of the experiment showed no obvious signs of bacterial colonies. Many of the cultures persisted for several months after the completion of the experiments. In any event, although of fundamental interest, the distinction is of less immediate importance, since most, if not all, algae normally grow in association with bacteria, from which they may well acquire some accessory nutrients.

It is difficult to relate the concentrations of organic isolates in the culture media to those of the appropriate fractions in the original sea-water samples since so many losses inevitably occur during isolation and fractionation. The *in vitro* concentrations used probably ranged between 2 and 40 times those occurring naturally, but it should be stressed that this is a very approximate reckoning, and these estimates may be too high.

Future Lines of Research

This preliminary work suggests that a method based on (*a*) isolation of organic matter by electrodialysis using ion-exchange membranes, (*b*) separation by paper chromatography, and (*c*) biological testing using a number of organisms, should meet the requirements of sensitivity and selectivity. An examination of the complete organic matter in solution is still reasonably economic in labour.

It is hoped that, in this way, it may be possible to 'label' water samples (and consequently the water masses from which they derive) somewhat grossly according to (1) the number and chromatographic position of some organic compounds, and (2) the nature of some of their biological effects—even before it is possible to indicate the chemical nature of the organic differences concerned. Such results should be of immediate help in the interpretation of the ecological situation, whilst the observed biological differences should give pointers to the sections of the extracts most deserving chemical analyses. It may conceivably be possible to apply the results to fish behaviour and/or development.

Although the main chemical effort will be directed to this end, it is not intended to omit analyses of the dissolved organic matter. Preparations are in hand for continuous de-salting of sea water in fairly large quantities by ion-exchange electrodialysis. Work already done can be utilized to construct a more effective course of preliminary separation, and automatic fractionation apparatus has recently been obtained which will facilitate chromatography. Individual fractions can then be tested for biological activity and facilities are available for ultra-violet and infra-red spectroscopic analysis of interesting components. It may be possible, using this knowledge and experience, to obtain by a shorter method sufficient of the interesting fractions to permit fuller qualitative organic micro-analysis.

Summary

The concept of external metabolites in the sea, which has received support from biological investigations, invites chemical corroboration which, until recently, has not been forthcoming. Separations of organic matter from filtered sea water using a variety of methods are reported and a preliminary assessment made. Organic residues obtained from different open sea sources by adsorption on carbon give rise to different paper chromatogram patterns. Certain organic components of a carbon adsorbate contain substances which affect the growth of a number of marine phytoplankton organisms. Further research is planned employing methods of isolation and chromatography of the dissolved organic matter from different sea waters and the assessment of biological activity of certain fractions by means of common marine organisms. Some aspects of the significance of biologically active compounds in the sea are mentioned.

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UNDERWATER OBSERVATIONS ON ROCKS OFF STOKE POINT AND DARTMOUTH

By G. R. Forster
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The Lower Limit of *Laminaria* and other Algae

In the vicinity of the Stoke Point rocks which lie ¼ mile offshore, plants of *Laminaria—L. hyperborea* (Gunn.) Fosl.—disappear below approximately 17 m, from low-water spring tide level. This limit, described in the preliminary note (Forster, 1954, this *Journal*, Vol. 33, pp. 341–4) has been confirmed during five dives in the same area in August 1954. The lower limit of *Dictyopteris membranacea* (Stackh.) Bate, which largely replaces *Laminaria*, has not yet been fully determined, but in fairly broad gulleys it fades out at 25 m and is followed by purely animal growths. Twenty-five metres is, therefore, probably close to its lower limit for this particular area.

In contrast, at the East Blackstone which is ½ mile offshore near Dartmouth, the *Laminaria* only penetrated to 5–6 m. It was followed, down to 8 m, by scattered plants of *Dictyota dichotoma* (Huds.) Lamour., and very sparse red algae chiefly *Heterosiphonia plumosa* (Ellis) Batt. Closer inshore, and in Torbay, *Saccorhiza polyschides* (Lightf.) Batt. apparently replaced the *Laminaria hyperborea*. The lower limit of *Saccorhiza* was not observed, but at two separate positions it became very sparse by 6 m. These observations were made during nine dives in September 1954.

The Sessile Fauna

The results obtained in 1953 from Stoke Point rocks have been confirmed during 1954, and further dives made to slightly greater depths. From the end of the *Laminaria* zone at 17 m down to 25 m, the rocks are often fairly flat with a series of widely separate ridges about 2 m high, running roughly north and south. The sea bottom, viewed from 3 or 4 m away, appeared as a dark brown field of *Dictyopteris* relieved by large yellow clumps of *Cliona celata* Grant (non-boring form) and pink sea-fans, *Eunicella verrucosa* (Pallas). There were roughly two *Cliona* and three *Eunicella* colonies per 10 sq.m.

Beyond 25 m the rocks were sometimes irregular, forming gulleys and pockets with a bottom of coarse sand. The gulley walls from 25 to 28 m, though not steeply sloping, were devoid of algae. Here the fauna was composed chiefly of Coelenterata and Bryozoa. *Corynactis viridis* Allman, though still common, was not as abundant as it was on vertical gulley walls at 18–20 m, when the algae were also excluded. Both *Alcyonium glomeratum* (Hassall) and

Epizoanthus wrightii (H. and S.) also occurred frequently, the latter with numerous bright orange and yellow polyps forming patches of up to approximately 1 sq.m in area. A few colonies of *Sertularella gayi* (Lamouroux) appeared for the first time, this species being common on the Eddystone trawling grounds in 45–65 m. The chief Bryozoa were erect forms, *Cellaria fistulosa* (L.), *Flustra papyracea* (Ellis & Solander), with scattered colonies of *Lepralia foliacea* (E. & S.).

The sessile rock fauna at the E. Blackstone was considerably different from that of Stoke Point rocks. From 4·5 to 6 m, very approximately, on vertical or overhanging rock faces small mussels (*Mytilus* sp.) were abundant. The small white anemone *Actinothoë sphyrodeta* (Gosse) occurred commonly both with the *Mytilus* and in patches on its own.

From 6 m down to 18 m, which was the lower limit reached, the following species were very common: *Nemertesia* (*Antennularia*) *antennina* (L.), *Tubularia indivisa* L., *Sertularella gayi* (hydroids); *Scrupocellaria scruposa* (E. & S.), *Cellaria fistulosa*, *Flustra papyracea* (Bryozoa); and *Antedon bifida* (Pennant). No zonation of these species with depths was observed; the rock surface being covered over considerable areas either by the hydroids, the Bryozoa, or *Antedon* or combinations of these groups especially hydroids and Bryozoa. There were also small patches of *Actinothoë*, groups of *Metridium senile* (L.), numerous *Caryophyllia—C. smithi* Stokes, cup coral—and the ubiquitous *Corynactis*, though only scattered polyps. Only two *Eunicella* and one *Axinella—A. polyploides* (Schmidt)—were seen and may, therefore, be taken as rare.

One dive was made at the E. Cod Rock near Berry Head. In contrast to the igneous rock of E. Blackstone, this rock was composed of limestone. At a depth of 6–7 m the rock surface was honeycombed by the boring mollusc *Hiatella arctica* (L.). Besides *Antedon*, *Alcyonium digitatum* L., and *Mytilus* sp., which were common, there were also many anemones usually inhabiting *Hiatella* burrows. The anemones were *Tealia felina* (L.) and *Cereus pedunculatus* (Pennant). *Metridium* was present in smaller numbers and growing normally on the rock.

Discussion

During the diving the water was found to be much more turbid between Dartmouth and Torbay than near Stoke Point, where during a calm spell of weather the bottom can be seen clearly at a depth of 11 m, whereas in Torbay even during a prolonged period of offshore winds the comparable limit was only 2–3 m. The shallow penetration of the algae at the E. Blackstone and nearby is clearly the result of growing in more continually turbid water. With the fauna, however, only *Sertularella*, *Flustra* and *Cellaria* were common to both areas. The contrast in the fauna between the two areas was not simply an alteration in the life zones of various species, but the species themselves

were mostly different. Much of the E. Blackstone fauna was not unfamiliar, since *Nemertesia* (Antennularia), *Actinothoë*, *Mytilus* sp. and *Antedon* are rather common species in Plymouth Sound. Both the Sound and the Dartmouth–Torbay area are sheltered from the prevailing west and south-west winds, and thus from the more violent wave action; but they also differ from the Stoke Point area in the greater turbidity of the water. Thus the change in the sessile fauna may not be solely related to wave action.

It was surprising to find the sea so much more turbid in the sheltered Dartmouth–Torbay area than in the exposed Stoke Point area, as the reverse had been expected. Although this situation might possibly be attributed merely to the sea-bed being largely mud in Torbay but sand beyond the rocks at Stoke Point, it seems more reasonable to assume that the turbidity does not arise *in situ* but results from a continual replacement of water containing suspended material. For Torbay itself has extremely weak tidal streams and is very well sheltered from wave action except from the east. In September 1954, when the observations were made, there had been no strong east wind for at least two months; so unless the water was being replaced one would expect it to have been much clearer than at Stoke Point where any south-westerly gale turns the sea cloudy for several days. Dr L. H. N. Cooper suggests that the turbidity may originate in the violent water mixing which takes place off Portland Bill, 40 miles to the eastward.

Acknowledgement

I am much indebted to Mr R. U. Gooding for his help and assistance both under and above water; to Messrs Siebe Gorman and Co. who were kind enough to loan Mr Gooding apparatus; and to the Royal Society for a grant with which much of my own apparatus was obtained.

Summary

A description is given of the commonest sublittoral algae and sessile animals found on rock at Stoke Point Reef and at the E. Blackstone rock near Dartmouth to a maximum depth of 28 m. The E. Blackstone fauna resembled that of Plymouth Sound. The marked change which has been found is ascribed to the greater turbidity of the water near Dartmouth and the increased shelter from wave action.

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OBSERVATIONS ON THE CILIARY CURRENTS OF THE JELLY-FISH *AURELIA AURITA* L.

By A. J. Southward

From the Plymouth Laboratory

(Text-figs. 1–7)

The common jelly-fish *Aurelia aurita* has been a popular species for the study of medusan physiology, for its abundance, transparency and docility make it easy to observe. Unfortunately, studies of the animal as a whole are rare, many investigators having confined themselves to isolated parts or functions, and it is sometimes difficult to combine the accounts in the literature. Such a difficulty arose on attempting to assess the role of ciliary currents in feeding. Widmark (1913) described only the internal currents, Orton (1922) the external currents, Gemmill (1920) the currents of the ephyra stage, while Henschel (1935) investigated the reactions of the oral arms alone. A study of the external and internal currents of the whole animal cleared up certain apparently conflicting details in these accounts, and disclosed several new features (Southward, 1949). It is now possible to give an account of a more complete investigation of the ciliary currents, both in the adult and in the larval stages of *Aurelia*, and to correlate some points of morphology with the currents.

In accordance with established use, the term 'ciliary currents' has been used to describe what are strictly flagellar currents. There is no ciliated epithelium as the term is understood in the higher metazoa; instead, as in most medusae (Hyman, 1940; Krasinska, 1914), a proportion of the ectodermal cells bear single flagella. In formalin-preserved material the flagella vary from 9 to 18 μ in length, somewhat longer than the cells themselves.

Observations on the living *Aurelia* were made at Liverpool in 1947 and 1948 on specimens from the Mersey estuary; at Port Erin in 1947–51 on specimens from the west coast of the Isle of Man; and at Plymouth in 1954 on specimens from the Tamar estuary. The external currents were investigated by adding suspensions of carmine or graphite particles to the water in which the specimens were kept, or by feeding with diatom cultures, mixed plankton or various proteins. The finer details of the currents were observed on specimens restricted in small dishes, but without narcosis, and were sometimes checked on isolated parts. Similar procedures often sufficed to show the internal currents, but it was sometimes necessary to inject suspensions directly into the canals and pouches.

Material was fixed in calcium-formalin, and much structure could be made out by simple sagittal sections. For more microscopic detail, standard paraffin

embedding proved unsatisfactory due to shrinkage of both cells and mesogloea, and sections were cut by the gelatin-freezing method (Baker, 1944) and the glycol wax procedure (Miles & Linder, 1952). The gelatin-freezing method produced least shrinkage of the mesogloea, and was found satisfactory for grosser detail, while the glycol wax procedure was preferable for cytological detail. All material was bulk-stained with Mayer's haemalum, but sections were sometimes reinforced with iron haematoxylin and eosin.

The figures are based on camera-lucida drawings of the fixed or sectioned material, to which the details of currents were added later from rough sketches of the living material.

The External Ciliation of the Adult

The Umbrellar Surfaces

The cilia of both the ex-umbrellar and sub-umbrellar surfaces beat centrifugally, towards the edge of the disc. Particles and food material are trapped in mucus, which accumulates in rolls and travels gradually to the umbrella margin.

The Umbrella Margin

The margin of the umbrella, except in the region of the eight sense organs (rhopalia), bears an outer row of lappets, an intermediate row of tentacles, and an inner fold of ectoderm (Fig. 2 A); the latter, from its superficial resemblance to the velum of the hydromedusae, is called the velarium (Figs 1 and 2 A, *V*). The cilia on the tentacles beat distally, towards the tip, but the direction of travel of the material depends on the position of the tentacle. If the tentacle is relaxed and hanging free vertically the material is rejected, but if the tentacle is contracted, for example by stimulus of food, the material is caught up by the other structures of the margin. Each of the lappets has a peripherally directed current on the outer side and a centripetal current on the inner side: the result is that material collected by the lappets, and that reaching them from the ex-umbrellar surface, is passed between the lappets into the marginal groove (Fig. 2 A, *Mg*). Material collected on the sub-umbral surface is passed smoothly over the velarium and also accumulates in the marginal groove. In this groove the currents move away from the rhopalia and towards the regions of the adradial canals (Fig. 1, right half) and material accumulates beneath the adradial canals in slight widenings of the velarium, termed food pouches by Orton (1920). The marginal groove, the food pouches and the side of the velarium directed towards the marginal groove are all richly ciliated, and in section show tall columnar cells similar to those of the inner, endodermal surfaces of the oral arms.

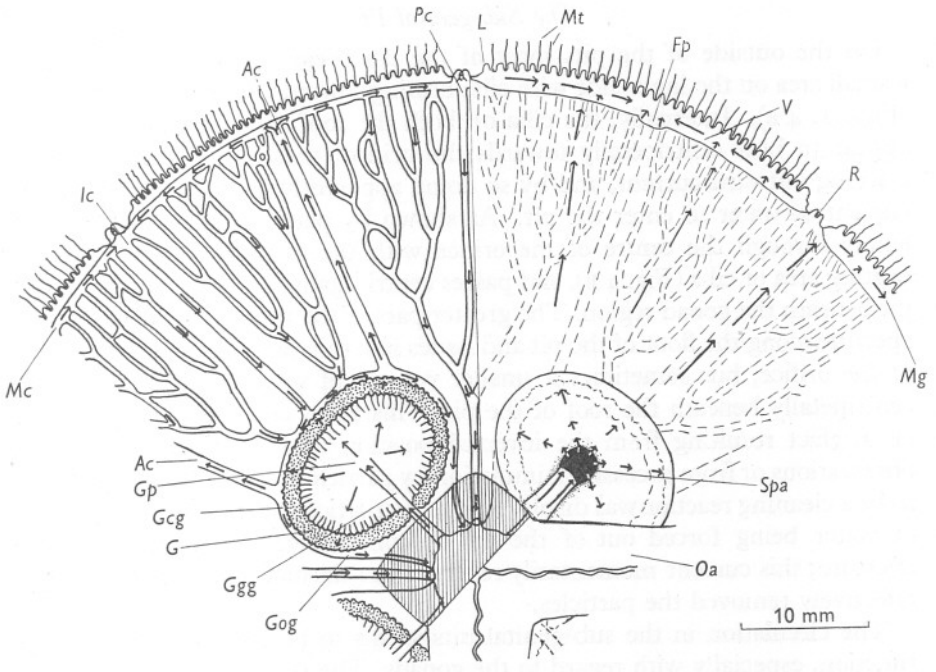


Fig. 1. *Aurelia*: part of the disc seen from the sub-umbrellar surface, with two of the oral arms removed (the cut bases hatched). The right side shows the currents on the sub-umbrellar surface and at the margin, while the left side shows the major currents in the canals and in one of the gastric pouches. *Ac*, adradial canal; *Fp*, food pouch; *G*, gonad; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral arm groove; *Gp*, gastric pouch; *Ic*, interradial canal; *L*, lappet; *Mc*, marginal canal; *Mg*, marginal groove; *Mt*, marginal tentacle; *Oa*, oral arm; *Pc*, perradial canal; *R*, rhopalium; *Spa*, aperture of sub-genital pit; *V*, velarium.

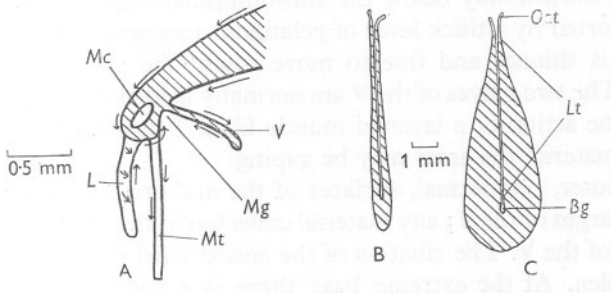


Fig. 2. *Aurelia:* diagrammatic sections of: (A) the umbrella margin; (B) the distal, and (C), the proximal regions of an oral arm. *Bg*, basal groove tract; *L*, lappet; *Lt*, lateral tract; *Mc*, marginal canal; *Mg*, marginal groove; *Mt*, marginal tentacle; *Oat* oral arm tentacle; *V*, velarium.

The Sub-genital Pits

On the outside of the pits most of the cilia beat outwards, but there is a small area on the inner side with cilia beating towards the aperture of the pit (Figs. 1, 4 B). Internally, the cilia on both the roof and the floor of the pit appear to beat centripetally towards the orifice; those on the floor set up a strong outward current, and by so doing apparently cause a compensatory current of water to enter the pit. As shown by particles in it, this current enters through the centre of the orifice with the assistance of the inward beating area of cilia (Fig. 4 B), and passes centrifugally to the periphery of the pit beneath the gonad region. The greater part of the water passes back to the aperture along the floor of the pit and issues in a thin layer around the margin of the orifice, but sometimes a smaller volume of water was seen to travel centripetally beneath the roof of the pit. This latter current may have been an artefact resulting from the inverted position of the animal during the observations or from excessive stimulation by particles. In fact, what appeared to be a cleaning reaction was displayed if many particles were present, a current of water being forced out of the pit by contraction of the sphincter-like aperture; this current momentarily reversed the normal ingoing current and effectively removed the particles.

The circulation in the sub-genital pits seems to be largely respiratory in function, especially with regard to the gonads. The latter are separated from the pits only by a single layer of thin ectoderm and the merest trace of genital epithelium.

The Internal Currents of the Adult

The Oral Arms

The oral arms of the adult jelly-fish are formed by elongation of the four corners of the simple mouth of the ephyra stage. They are V-shaped in section, and extend horizontally below the sub-umbrellar surface, the proximal part being supported by a thick layer of gelatinous mesogloea (Fig. 2 C), while the distal half is thinner and free to move round the margin of the umbrella (Fig. 2 B). The two halves of the V are normally held close together, apparently partly by the action of a layer of muscle fibres beneath the ectoderm, for in preserved material the arms may be gaping.

On the outer, ectodermal, surfaces of the oral arms the cilia beat towards the open margin of the V; any material collected in the mucus passes over into the groove of the V. The ciliation of the endodermal surfaces of the groove is more complex. At the extreme base there is a continuous distally-moving current, the basal groove current, which is largely excretory, for the particles carried by it have usually passed through the gastric pouches and canals (Fig. 3, *Bg*). This tract of cilia seems distinct from the remaining cilia of the groove which can act in two ways. Food material, including the mucous

masses picked up from the marginal food pouches, travel proximally along the arm in a tract formed by the apposition of the two sides of the groove (Fig. 3, *Lt*), and is passed on to the gastric pouches. The width of the lateral tract involved varies with the amount of food in it; in some cases the whole of the groove, with the exception of the basal tract, may be seen moving food towards the gastric pouches. These two opposing tracts have never been seen to mix in a healthy jelly-fish, yet they are apparently separated only by the close apposition of the two halves of the V, which must nevertheless leave room for the cilia to work.

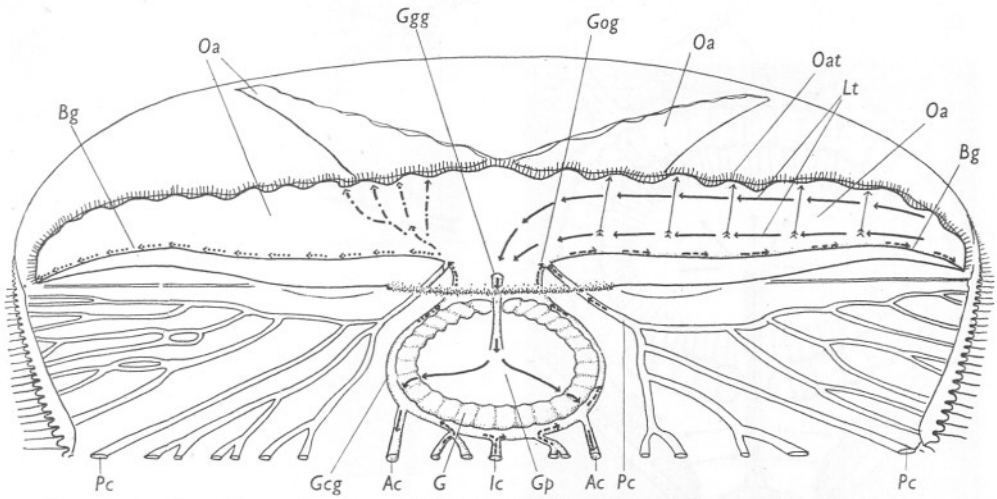


Fig. 3. *Aurelia:* oblique view of part of the disc from the sub-umbrellar surface, showing some of the currents in two of the four oral arms and in one of the four gastric pouches. The right side shows the path of food (solid arrows) in the lateral tract, and of excretory matter (broken arrows) in the basal groove, while the left side illustrates the main paths of the gametes at spawning (path of sperm in male shown by dotted arrows, path of eggs in female shown by dotted and dashed arrows). The feathered arrows represent the rejection reaction in the lateral tract. *Ac*, adradial canal; *Bg*, basal groove tract; *G*, gonad; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral groove; *Gp*, gastric pouch; *Ic*, interradial canal; *Lt*, lateral tract; *Oa*, oral arm; *Oat*, oral arm tentacle; *Pc*, perradial canal.

In the absence of food the lateral tract rejects nearly all inert particles placed in it. More rarely inert material may be picked up and passed a short distance proximally, but sooner or later there comes a marked change in the current, and the inert material is carried laterally to the margins of the V and rejected (Fig. 3, feathered arrows).

The Gastric Pouches

The currents in the gastric pouches are more complex than suggested by Widmark (1913), for selection mechanisms are present as in the oral arms. Food from the lateral tracts of the oral arms passes into the gastric pouches by

the gastro-genital grooves (Figs. 3, 4, *Ggg*). These grooves, first noted by Goodey (1908, 1909), have two tracts of cilia beating in different directions. In the upper part of the groove, between the roof of the gastric pouch and the sides of the groove, there is an ingoing food-bearing current continuous with the lateral tracts of the oral arms (Fig. 4 B, D, *Gggu*). In the lower part of the groove, the cilia beat outwards towards the mouth, but this is not noticed

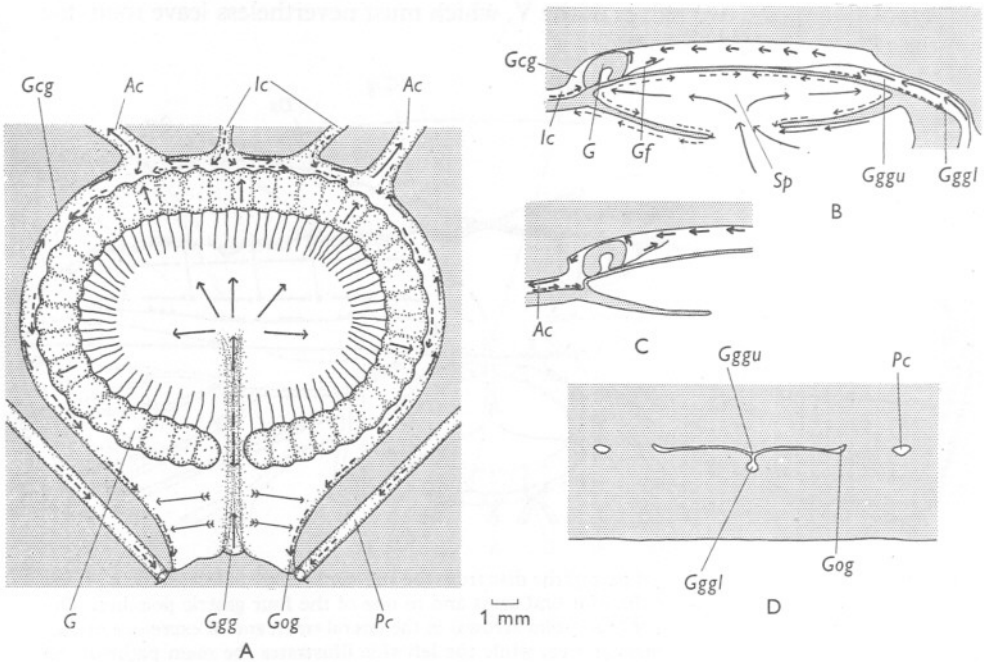


Fig. 4. *Aurelia:* A, diagrammatic view of a gastric pouch from above, with the ex-umbral surface and upper mesogloea removed; B, a radial section across the same pouch and the sub-genital pit below; C, a part section of the same pouch at the origin of an adradial canal; D, a section across the gastro-genital groove inward of the gonad. The solid arrows show ingoing currents, the broken arrows outgoing currents, the feathered arrows rejection reactions, and the thin arrows in B represent the water circulation in the sub-genital pit. *Ac*, adradial canal; *G*, gonad; *Gf*, gastric filament; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gggl*, lower, and *Gggu*, upper, parts of the gastro-genital groove; *Gog*, gastro-oral groove; *Ic*, interradial canal; *Pc*, perradial canal; *Sp*, sub-genital pit.

superficially unless inert material is injected into the groove, when a rejection reaction occurs and material in the upper part of the groove may pass through to the lower part and be carried outwards (Fig. 4 B, D, *Gggl*). If much inert material is present in the groove a further rejection reaction may take place, the material being passed laterally to the origin of the oral arm basal tract, apparently by withdrawal of the sides of the groove from apposition with the roof (Fig. 4 A, feathered arrows).

Food material entering the gastric pouch by the gastro-genital groove is swept centrifugally by cilia on the roof of the pouch, and comes to rest against the gastric filaments. The cilia of the filaments beat distally, and function mechanically as a means of breaking up food masses. The separated particles pass up and over the gonads into the gastro-circular groove running round the gonads (Fig. 4 A, B, *Gcg*). Food materials travel along the roof of this groove, to the openings of the adradial canals, into which they pass. The floor of the groove is largely excretory, for indigestible matter from the interradial canals travels round it to the gastro-oral grooves (Fig. 4 A, *Gog*) along which it passes to the basal grooves of the oral arms (Fig. 3, right half). Thus the gastro-circular groove forms a further selection mechanism. Selection can also occur in the main body of the pouch, where the centripetally beating cilia on the floor can carry material to the lower part of the gastro-genital groove.

The Canals

It must be noted that the details of currents given in this paper may not apply to all specimens of *Aurelia*, since structural variation is not uncommon (Browne, 1901). This is especially true of the canals, and slight differences in the method of branching of the perradial and interradial canals can be found in practically every specimen; in some cases there may exist anastamoses between the adradials and the side branches of the other canals, which may by-pass the normal circulation.

The cilia on the roof and sides of the adradial canals beat centrifugally, and convey food from the gastric pouches to the marginal canals (Fig. 1, left half). The cilia lining the floor of the adradial canals beat in the opposite direction, and indigestible particles that have penetrated this far may be carried back to the gastro-circular groove (Fig. 4 A). Water and particles entering the marginal canal from the adradial canals pass round on either side of the adradials to the side and main branches of the perradial and interradial canals along which they travel towards the centre of the disc (Fig. 1, left half). The interradial canals open into the gastro-circular grooves, and particles are transferred to the floor tract of the latter, while the perradial canals communicate directly with the bases of the oral arm grooves (Fig. 3, right half).

So far, only one current has been detected in the interradial and perradial canals. These canals are flattened horizontally, while the adradials, which carry two currents, one above the other, have their greatest dimension vertically. In all canals the circulation seems to be assisted by the muscular pulsations of the umbrella, the walls of the canals expanding and contracting with the coronal muscles.

The Ciliary Currents of the Larval Stages

The direction of beat of the cilia remains fairly constant through the successive larval stages to the adult. For example, the locomotion of the planula is effected by cilia which beat orally (Fig. 5 A); after settlement on the aboral end, the same direction of beat now serves to accumulate food particles near the mouth (Fig. 5 B). A similar direction is maintained on the developing tentacles, which can, however, vary the ultimate direction of the particles collected by changing their posture. After strobilation the orally directed current of the scyphistoma becomes the centrifugal current of the exumbral surface of the ephyra, while the orally directed current near the mouth of the scyphistoma is represented by similarly directed currents on and near the manubrium of the ephyra (cf. Gemmill, 1920). The other external currents of the ephyra are centrifugal.

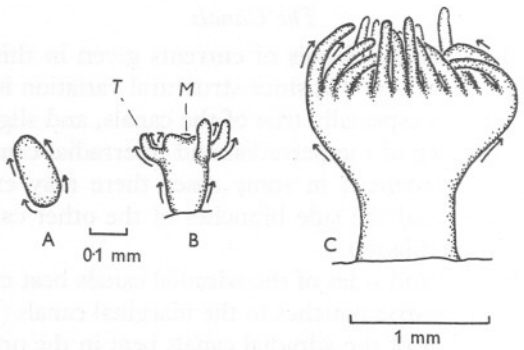
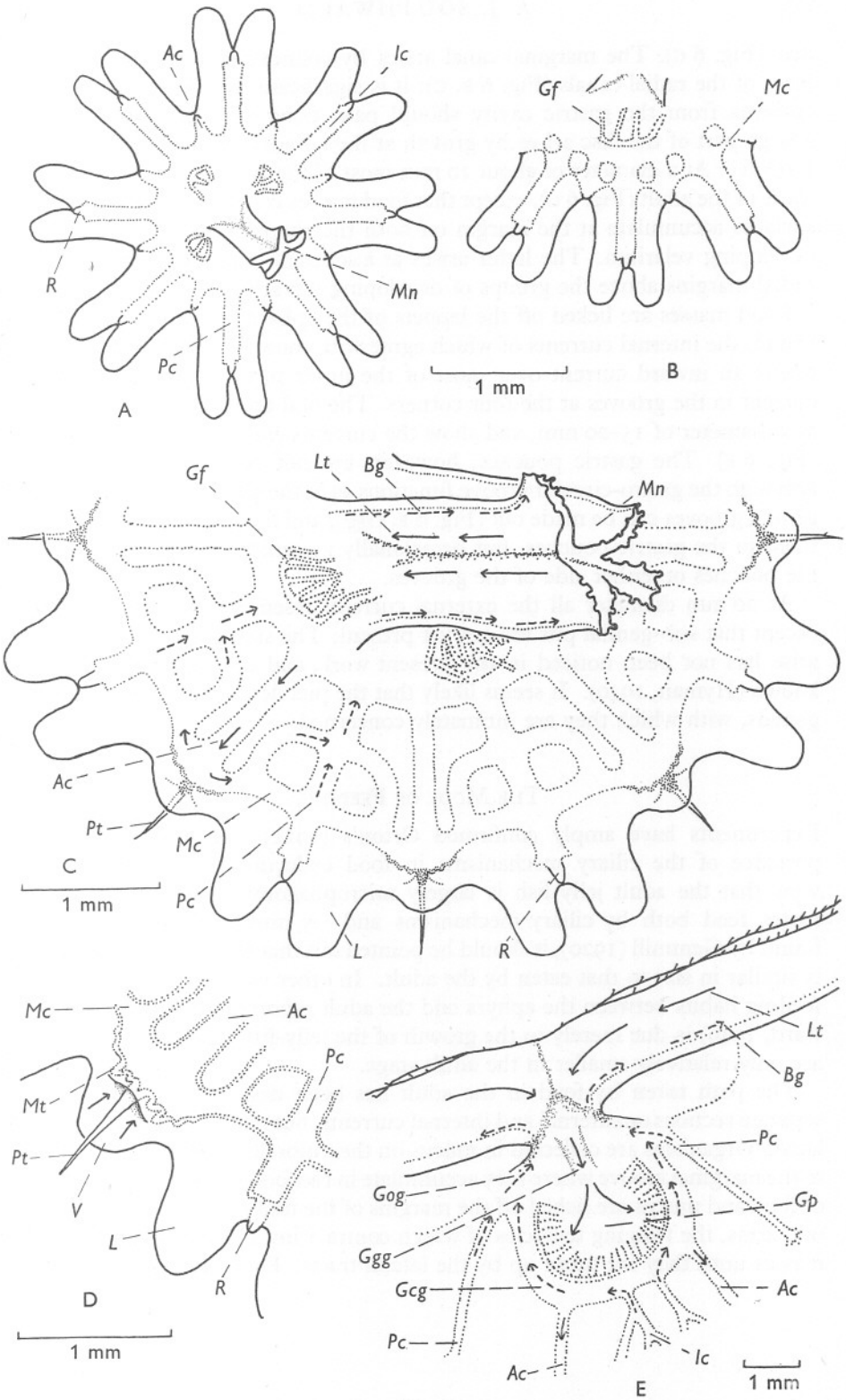


Fig. 5. *Aurelia:* A, planula, B, early scyphistoma, and C, later scyphistoma stages, showing external currents. *M*, mouth; *T*, tentacle.

The internal currents of the early ephyra are simple and adapted to the wide open gastric cavity and absence of a marginal canal (Fig. 6 A). The cilia on the roof of the gastric cavity beat centrifugally, and those on the floor centripetally, but much swirling is caused by the gastric filaments and by the muscular contractions of the animal. The more complicated canal system and circulation of the later stages develops with the expansion of the area of the

Legend to Fig. 6.

Fig. 6. *Aurelia:* A, an early ephyra, the currents of which were reported by Gemmill (1920); B, a slightly older ephyra than A, showing the origin of the marginal canal; C, 10 mm. stage, with marginal tentacle and velarium developing; D, a slightly larger view of an adradius of the specimen in C, but from the ex-umbral aspect; E, 15–20 mm stage, with oral arms and gastric pouch. All except D from the sub-umbrellar surface. *Ac*, adradial canal; *Bg*, basal tract; *Gf*, gastric filament; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral groove; *Gp*, gastric pouch; *Gprg*, gastric pouch ring groove; *Ic*, interradial canal; *L*, lappet; *Lt*, lateral tract; *Mc*, marginal canal; *Mn*, manubrium; *Mt*, marginal tentacle; *Pc*, perradial canal; *Pt*, primary tentacle; *R*, rhopalium; *V*, velarium.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Pc
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Gp
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Gcg
Ac
Pc
Ic
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1 mm

Fig. 6.

disc (Fig. 6 C). The marginal canal arises by connexion between the outer parts of the radial canals (Fig. 6 B, C): it is significant that the main outward currents from the gastric cavity should pass along the adradial canals, as a large part of the disc arises by growth at the adradial margins, between the rhopalia. At a diameter of about 10 mm most of the ciliary currents resemble those of the adult (Fig. 6 C), except that food masses collected by the umbrellar surfaces accumulate at the margin on both the rhopalial lappets and on the developing velarium. The latter arises as a series of small lappets at the adradial margins above the groups of developing tentacles (Fig. 6 D).

Food masses are licked off the lappets of the ephyra by the mobile manubrium, the internal currents of which agree with those of the oral arms of the adult: an inward current over most of the inner surfaces, and an outward current in the grooves at the four corners. The oral arms are fully developed at a diameter of 15–20 mm, and show the currents and reactions of the adult (Fig. 6 E). The gastric pouches, however, are not complete at this stage, although the gastro-circular groove functions as in the adult. Incipient gastro-genital grooves can be made out (Fig. 6 E, *Ggg*), and food usually passes down them to the gastric pouches, but occasionally material may be seen to enter the pouches on either side of the grooves.

At 20 mm diameter all the external currents resemble those of the adult, except that sub-genital pits are not yet present. The stage at which these pits arise has not been noticed in the present work, and does not appear to be known (Hyman, 1940). It seems likely that the pits develop together with the gonads, with which they are intimately concerned.

The Mode of Feeding

Experiments have amply confirmed Orton's (1922) evidence of the importance of the ciliary mechanisms in food collection and reinforced the view that the adult jelly-fish is largely microphagous. Although the larval stages feed both by ciliary mechanisms and by muscular macrophagy as found by Gemmill (1920), it should be pointed out that the food they consume is similar in size to that eaten by the adult. In other words, the difference in feeding habits between the ephyra and the adult reported previously (Southward, 1949) is due merely to the growth of the jelly-fish: the food organisms are only relatively smaller in the adult stage.

The path taken by food in the adult has been described already in the separate sections on external and internal currents, but may be briefly recapitulated. Organisms are collected in mucus on the umbrellar surfaces and passed to the marginal groove where they accumulate in the food-pouches. The masses of food and mucus are licked off the margins of the umbrella by the tips of the oral arms, the fringing tentacles of which contract inwards and hold the food masses until they are taken up by the lateral tracts. The food is passed along

the lateral tracts to the gastro-genital grooves, by way of which it enters the gastric pouches. After being separated by the gastric filaments the food particles are distributed along the canals. Digestion has not yet been studied in *Aurelia*, but it seems possible that, as in *Cassiopeia* (Smith, 1936), the gastric filaments can produce some extracellular enzymes.

The collection of food can be observed directly by placing *Aurelia* in a suspension of plankton organisms: indirectly, the type of food being collected can be determined by examination of the mucous masses in the food pouches. The ex-umbrellar surface plays least part in the process of food collection, since large copepods and other organisms are swept off during pulsation of the bell. The main collecting takes place on the sub-umbrellar surfaces, on the oral arms and at the margin of the umbrella, the action of swimming causing a current of water to pass over these parts.

A specimen 10 cm in diameter cleared all the coarse plankton from 700 ml. of water in less than an hour, but much larger volumes of sea water should be passed over the external surfaces in nature. *Aurelia* easily picks up small copepods (e.g. *Tigriopus*) on the external surfaces, and at 15° C these can reach the food pouches within 10 min, the oral arms in 12 min and the gastric filaments in 50 min. Material picked up by the oral arms directly reaches the gastric pouches more quickly, sometimes within 10 min.

Examination of the mucous masses in the food pouches of freshly captured specimens usually shows plankton typical of the place of capture. Thus, the food pouches of *Aurelia* from Port Erin Bay in early June showed principally copepods, which were then present in great abundance in the sea. Specimens from the River Mersey had large amounts of detritus (probably silt and sewage), in addition to diatoms, ciliates, flagellates and some crustacean eggs, in the food pouches. The pouches of further specimens from a tide pool near the mouth of the Mersey estuary contained, in addition to large amounts of detritus, many *Noctiluca* and some balanoid cyprids, both common in the local plankton at the time. Other specimens kept in tanks at Port Erin had collected plankton typical of the local sea-water supply, namely diatoms, algal fragments, polychaete larvae and harpacticid copepods. *Aurelia* placed in pure cultures of the diatom *Nitzschia* or the copepod *Tigriopus* rapidly accumulated these organisms in the food pouches, and later, in the gastric pouches.

The evidence for plankton feeding, and microphagy in general, is thus very strong. Attempts to show macrophagy in the adult have always failed, as food particles above 5 mm diameter are always dropped or rejected from the margin or the oral arm grooves. A further limitation of the size of food masses is imposed by the narrowness of the gastro-genital grooves. Although, theoretically, the mouth communicates with a gastric pouch by the whole width of the tract between the two gastro-oral grooves (Fig. 4 A), in practice food enters a pouch only along the gastro-genital grooves.

Selection mechanisms play an important part in the feeding processes of

microphagous animals, and *Aurelia* shows such mechanisms at several stages in the food path. The tentacles may accept or reject according to their position relative to the marginal groove, and their degree of contraction, but the oral arms constitute the major selective device outside the gastric pouches. As Henschel (1935) showed, the arms have a well-marked chemical sense, and respond to proteins and nitrogenous substances, but not to carbohydrates or inorganic particles. The reaction to food masses, plankton, or mussel extract is seen first as a contraction of the fringe of capitate tentacles (which bear batteries of nematocysts), and the material is then taken up by the lateral tract. Materials such as carmine, graphite or starch grains cause little or no contraction of these tentacles and are not usually taken up by the lateral tract. If such indigestible particles are taken up by the lateral tract (possibly as a result of the presence of unseen food organisms in the water) they are later rejected before reaching the gastric pouches, by a lateral movement in the tract (Fig. 3, right half). This rejection reaction can often be halted, and the material caused to be taken up again, by stimulation with proteins (e.g. egg albumen), and if the stimulus is sufficiently strong the indigestible material may pass to the gastric pouches. On the other hand, further addition of much indigestible material to the lateral tract may cause the arm to reject the whole contents of the tract, including any food masses that may be present.

In the absence of obvious structural features, such as the grooves in the throat of the anemone *Metridium* (Elmhirst, 1925; but cf. Parker & Marks, 1928), it might be thought that the rejection reaction shown by the oral arms of *Aurelia* was due to a change in direction of beat of the cilia. Unfortunately it has not been possible to see the direction of beat of a cilium directly *in situ* in the arm, and it is difficult to expose a single inner surface without damaging the cilia. However, it is possible that the 'reversal' is only apparent. Thus, the normal ingoing current of the lateral tract creates its own 'canals' by apposition of the sides of the groove around the food or other particles; relaxation of the muscle fibres partly responsible for this apposition would allow these canals to communicate with the exterior, and the rejection might merely result from the material taking the easier path to the exterior. Further investigation of the rejection reaction is necessary before this theory can be accepted.

The other selection mechanisms occur in the gastric pouches and in the adradial canals. That described for the gastro-genital groove (p. 206) is probably of chemical nature like the oral arm reaction, but the remainder may be mechanical, serving to sort out the heavier, and therefore presumably inorganic particles from the food.

Although scattered batteries of nematocysts are present on the ex-umbrellar surface of *Aurelia*, they appear to play little part in food capture in the adult; their penetrating powers, assessed on human epidermis, are much poorer than those of other semaeostome medusae. In the ephyra, however, the

nematocysts help to hold captured organisms on the lappets (Gemmill, 1921) until they can be licked off by the manubrium. The smallest ephyra stage (Fig. 6 A) has been seen to hold and later ingest in this way as many as six nauplii of *Balanus balanoides*, almost simultaneously: at the same time, masses of smaller organisms collected by the ciliary currents were also eaten. Ephyrae of up to 10 mm diameter were found feeding on the newly hatched plaice larvae in the fish-hatchery at Port Erin, and one specimen accepted up to three larvae, one after the other, in the same way that the earlier stage dealt with nauplii. In these stages the manubrium showed marked selection mechanisms, and, like the oral arms of the adult, rejected indigestible particles.

The Currents and Reproduction

As is well known (Agassiz, 1860; Hargitt & Hargitt, 1910), the gametes of *Aurelia* issue from the gonads and gastric pouches along the oral arms; the eggs, which are already fertilized, become enclosed in pockets of the inner, endodermal, surfaces of the oral arms, near the margins of the grooves, where they develop to the planula stage. Goodey (1908, 1909) believed that the eggs and sperm left the gastric pouches by way of the gastro-genital grooves ('gonadial grooves'), and was able to show their presence in this channel in preserved and sectioned material. However, in the course of the present work it was frequently observed that mature female *Aurelia*, after handling, passed eggs and planulae round the canals, and their presence in any part of the system is not remarkable. Widmark (1913) observed the passage of gametes along the gastro-oral arm grooves ('eck-canalen'). During the present study only four specimens have been seen to spawn, two males and two females, but in all the gametes likewise issued along the gastro-oral arm grooves (Fig. 3, left half). In the males the bulk of the sperm was passed to the basal grooves of the oral arms, in which it travelled to the tips of the arms, where it was ejected in mucous masses. The females differed in that the eggs travelled only a short distance in the basal grooves, and were quickly passed to the lateral tracts, in which they moved laterally, quite like rejected material, to the margins of the grooves.

Internal fertilization is presumably effected by the females picking up the sperm masses ejected by the males, and passing them to the gastric pouches in the normal feeding currents. Sperm masses were, in fact, found in the marginal food pouches of a female kept in the same tank as a spawning male. The process is probably assisted by the shoaling that frequently occurs during the breeding period (Agassiz, 1860; Southward, 1954).

Comparison with Other Jelly-Fish

In its restriction to a largely microphagous habit, and in its reliance upon ciliary mechanisms for food transport and internal circulation, *Aurelia* resembles the rhizostome medusa *Cassiopeia* (Smith, 1936). However, studies

of other semaeostome and rhizostome mudusae, not completed, show that the two groups have many common features of physiology. Thus, the centrifugal currents of the ex-umbrellar and sub-umbrellar surfaces of *Aurelia* are present, slightly modified, in *Cassiopeia* and apparently in the semaeostomes *Chrysaora* and *Cyanea*, but in these species the currents play little part, if any, in food collection. In all these species, and in *Rhizostoma* as well, the inner surfaces of the oral arms show similar currents and functions: an outgoing current in the base of the groove (in rhizostomes that part of the oral arm canal nearest the sub-umbrellar surface) and an ingoing current, often capable of rejection, on the remaining surfaces.

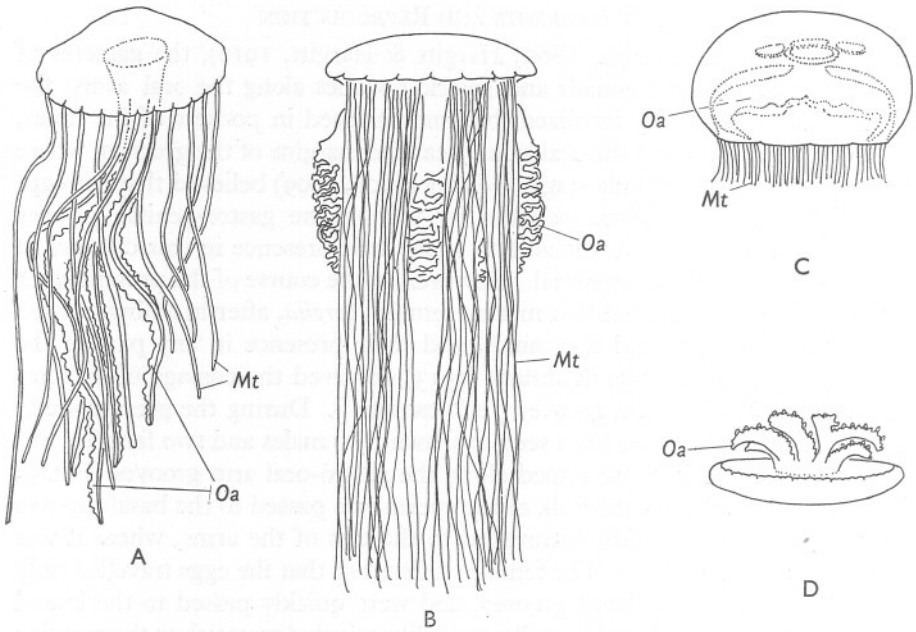

Fig. 7. Diagrammatic sketches of A, *Chrysaora*, B, *Cyanea*, C, *Aurelia* (in swimming position), and D, *Cassiopeia* (in attached posture), all in side view to show the relative proportions of the marginal tentacles (*Mt*) and the oral arms (*Oa*). A, C and D to the same scale, about $\frac{1}{3}$ natural size; B, approximately one-half the scale of the rest.

Internally the species so far studied have a centrifugal current on the roof of the gastric pouches or gastric cavities, and a centripetal current on the floor. *Cyanea* and *Chrysaora*, however, differ from the rest in their lack of a marginal canal or plexus, and hence show no regular circulation in the radial canals (Widmark, 1911): in this they resemble the ephyra of *Aurelia*, in which the circulation is also partly ciliary and partly muscular. These two species differ from the adult *Aurelia* and the rhizostomes in possessing a wide central gastric cavity communicating freely with the oral arms, and might be said to be less

specialized. However, they diverge widely from the possibly ancestral ephyral type in another direction, for the oral arms and marginal tentacles are greatly elongated—specializations apparently directed towards a drift-net habit of feeding (Fig. 7).

If *Chrysaora* (Fig. 7 A) is the least specialized of these species (cf. Agassiz, 1860), *Aurelia* can be regarded as an intermediate stage in the direction of the rhizostomes, by reduction of the marginal tentacles and by the greater reliance placed on ciliary currents for food collection: the rhizostomes themselves have no tentacles, and the oral arms alone gather food. The final development of this trend may perhaps be found in *Cassiopeia*, which is sessile and employs the pulsations of the bell to pass water over the oral arms (Fig. 7 D). On the other hand, *Cyanea* shows greater adaptation than *Chrysaora* for the drift-net type of feeding; the enlarged frills of the oral arms (Fig. 7 B) must be necessary to cope with the increased number and catching area of the tentacles, to ensure that organisms captured are transferred to the arms.

This paper owes much to the late Prof. J. H. Orton, F.R.S., who suggested the investigation to me, and who gave freely of advice and encouragement. Mr F. S. Russell, F.R.S., kindly advised me on several matters, and Dr D. J. Crisp and Dr D. Atkins helpfully criticized the typescript. I am indebted to Captain Nicholson, the Lancashire and Western Sea Fisheries Committee bailiff for Liverpool, Mr A. Cregeen, the Port Erin Marine Biological Station boatman, and Mr A. Briggs, the Marine Biological Association collector, for the supply of living material. Mr T. R. Tozer kindly supplied well-preserved specimens of all stages.

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Summary

The jelly-fish *Aurelia aurita* possesses external and internal ciliary currents that play a large part in food collection and in the transport of food, reproductive products and excretory matter.

Adults feed on relatively small organisms, which are collected in mucus on all external surfaces and eventually passed to the inner surfaces of the oral arms.

The inner surfaces of the oral arms bear two ciliated tracts which operate simultaneously in opposite directions. The lateral tract carries food materials proximally towards the gastric pouches, but is capable of rejecting inedible matter. The basal tract carries excretory matter distally, away from the gastric pouches and canals to the exterior.

Rejection reactions are also found in the gastric pouches and radial canals, parts of which have currents moving in opposite directions on the roof and on the floor. These opposing currents appear to be derived from the system in the ephyra stage, where the circulation in the wide gastric cavity and blind-ending

canals is maintained partly by centripetal currents on the floor and centrifugal currents on the roof.

The directions of the main currents remain constant throughout the larval stages to the adult, although slight variations are introduced by morphological changes. The currents also remain the same during spawning, when the eggs and sperm leave the gastric pouches by the normal excretory path.

Many of the ciliary currents found in *Aurelia* are present in other semaeostome and rhizostome medusae, but only in *Aurelia* do the umbrella surfaces and currents play a large part in food collection. Some of the major morphological differences in the Scyphomedusae can be related to the different feeding habits of the various species.

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

III. ACTION OF CO_2, CYANIDE AND FLUORIDE

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(Text-figs. 1 and 2)

Proceeding with our studies on urine secretion of the aglomerular kidney of *Lophius piscatorius* L., in the Plymouth Laboratory (Brull & Nizet, 1953; Brull, Nizet & Verney, 1953; Brull & Cuypers, 1954*a*, 1954*b*) and using the method of blood perfusion we described previously, we attempted to study the influence of CO_2, and of cyanide and fluoride on water secretion and on Mg concentration, which seems to be the most characteristic function of that kidney.

Methods

Two experiments were carried out with CO_2, three other experiments with cyanide, and two attempts were made with fluoride, the first one on a preparation used for cyanide, after recovery from that intoxication (Expt. G).

A sufficiently large kidney was perfused with heparinized blood from a pool obtained from several *Lophius*, a pool which was large enough to wash the preparation after removal of the modified or toxic blood, and to replace it by a fresh supply.

The drops of urine were registered, and after a constant or an increased urine flow was noticed, either blood in equilibrium with a different gas mixture replaced the previous one, or the toxic substance was added, dissolved in a small amount of Ringer and neutralized.

Whenever blood was removed, the circulation was stopped for a very short time, which, with practice, could be reduced to 15 sec. As soon as the effect of the toxic substance or of the modified blood was clearly established, the preparation was washed out with fresh blood, and then new blood from the same pool was put in.

Rough determinations of magnesium were made on one drop of plasma or diluted urine, with the 'Tüpfelmethode' of Feigl (1931), using titane yellow as reactive. The margin of error in these determinations is $\pm 20\%$.

Effect of CO_2

In a previous publication (Brull & Cuypers, 1954*a*), we have shown that the blood of *Lophius piscatorius* contains between 1·87 and 2·35 g of haemoglobin. Because of this low content, and of the fact that ferricyanide coagulates this kind of blood, determinations of the O_2 content are inaccurate in arterial blood, and practically impossible in venous blood, from which no measurable volume of oxygen is liberated in the Van Slyke apparatus.

Yet there remains no doubt that the kidneys of *Lophius*, receiving venous blood only, live on a very low oxygen tension. If the blood is oxygenated when perfusing these kidneys, there is no change in the order of magnitude of urinary volume or concentration (Brull, *et al.* 1953). We concluded therefore that the oxygen requirement of the *Lophius* kidney is very light.

Owing to the fact that in our previous perfusion experiments the artificial blood circulation was in contact with air or with oxygen, there resulted a progressive loss of CO_2 and a rise of pH. These alterations were not accompanied, even in the long run, by a parallel change in the water output or in the concentration capacity for Mg, except for a slow decrease of urine volume when the preparation becomes too old. Thus, the secretion is not affected by enriching the blood with O_2 and impoverishing it in CO_2.

It seemed interesting to find out whether *an increase* in CO_2 would have an influence, especially as it is well known that the Bohr effect is of high magnitude in the blood of marine teleosteans (Florkin, 1944, p. 86). This was soon confirmed by the aspect of the blood when equilibrated with a mixture of 5% CO_2 and 95% O_2; it was much darker than any venous blood we had previously seen from *Lophius*.

Fig. 1 (Expt. H and I) illustrates the results of two experiments during which kidneys were successively perfused (1) with blood in equilibrium with air; (2) with blood in equilibrium with 5% CO_2 and 95% O_2; (3) with the same as in the first.

The perfusion pressure was constantly kept at 200 mm water in the first perfusion, and at 300 mm in the second, as we know from our previous work (Brull & Cuypers, 1954*b*) that these figures are above the optimum required to produce maximum urine output.

It is obvious that high CO_2 tension produces two effects: (1) an important reduction in the flow of urine, which is totally reversible, and (2) a vasoconstriction which is not reversible after 1 hr.

Since the urine flow recovers after removal of the CO_2-enriched blood, while the blood flow remains low, one may conclude that the two phenomena are independent of each other. This is not surprising, when considering that the blood flows, even reduced by the CO_2, are still above the optimum flows, according to our previous experiments.

Now, which is the factor responsible for the reduction in the urine

output: the drop of available oxygen, the CO_2 tension itself, or the drop of pH? The question remains open.

The following experiments may shed some light on the problem.

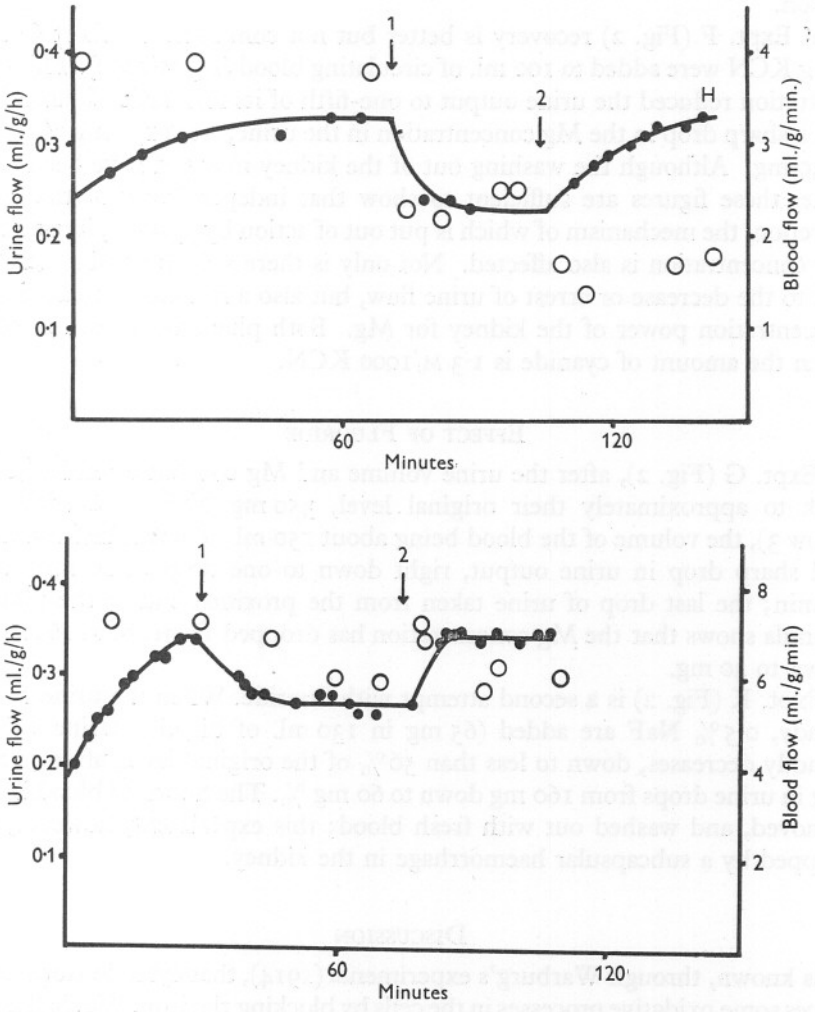

Fig. 1. Upper curve. Expt. H: a kidney of 7 g is perfused with blood oxygenated with air. At $\downarrow^1$ blood in equilibrium with carbogen replaces the air-oxygenated blood. At $\downarrow^2$ back to air-oxygenated blood. Perfusion pressure constant at 200 mm water. Lower curve. Expt.I: a kidney of 11 g is perfused with blood oxygenated with air. At $\downarrow^1$ blood replaced by blood in equilibrium with 5 % CO_2–95 % O_2. At $\downarrow^2$ back to previous blood. Perfusion pressure constant at 300 mm water.

Effect of Cyanide

In the first experiment (Fig. 2, Expt. E), the amount of cyanide given is apparently too large, there being practically no recovery after removal of the poison.

In Expt. F (Fig. 2) recovery is better but not complete. In Expt G only 8 mg KCN were added to 100 ml. of circulating blood (1·3 M/1000). This concentration reduced the urine output to one-fifth of its former level, and there was a sharp drop in the Mg concentration in the urine, from 150 mg %, down to 30 mg. Although the washing out of the kidney may not have been complete, these figures are sufficient to show that independently of the water secretion, the mechanism of which is put out of action by cyanide, its power of Mg concentration is also affected. Not only is there a drop of Mg excretion due to the decrease or arrest of urine flow, but also a marked decrease in the concentration power of the kidney for Mg. Both phenomena are reversible when the amount of cyanide is 1·3 M/1000 KCN.

Effect of Fluoride

In Expt. G (Fig. 2), after the urine volume and Mg concentration had come back to approximately their original level, 350 mg NaF were added (at arrow 3), the volume of the blood being about 150 ml. There is an immediate and sharp drop in urine output, right down to one drop in 10 min, after 16 min; the last drop of urine taken from the proximal end of the ureteral cannula shows that the Mg concentration has dropped from about 160 mg % down to 40 mg.

Expt. K (Fig. 2) is a second attempt with fluoride. When the urine flow is steady, 0·5% NaF are added (65 mg in 130 ml. of blood). Urine volume steadily decreases, down to less than 50% of the original level, after 40 min. Mg in urine drops from 160 mg down to 60 mg %. The fluorized blood is then removed, and washed out with fresh blood; this experiment, however, was stopped by a subcapsular haemorrhage in the kidney.

Discussion

It is known, through Warburg's experiments (1914), that cyanide stops or reduces some oxidative processes in the cells by blocking the iron. We shall not go into the extensive literature on the mechanism of action of HCN and of fluoride. Both chemicals reduce aerobic respiration, and fluorides especially affect the carbohydrate cycle, namely by an inhibition at the level of pyruvic acid (Peters, Rydin & Thompson, 1935; Melrose & Terner, 1953). Their effects on water secretion and Mg concentration add new evidence in favour of the concept that these kidney activities are really active processes involving energy derived from the carbohydrate cycle and oxygen consumption. Although

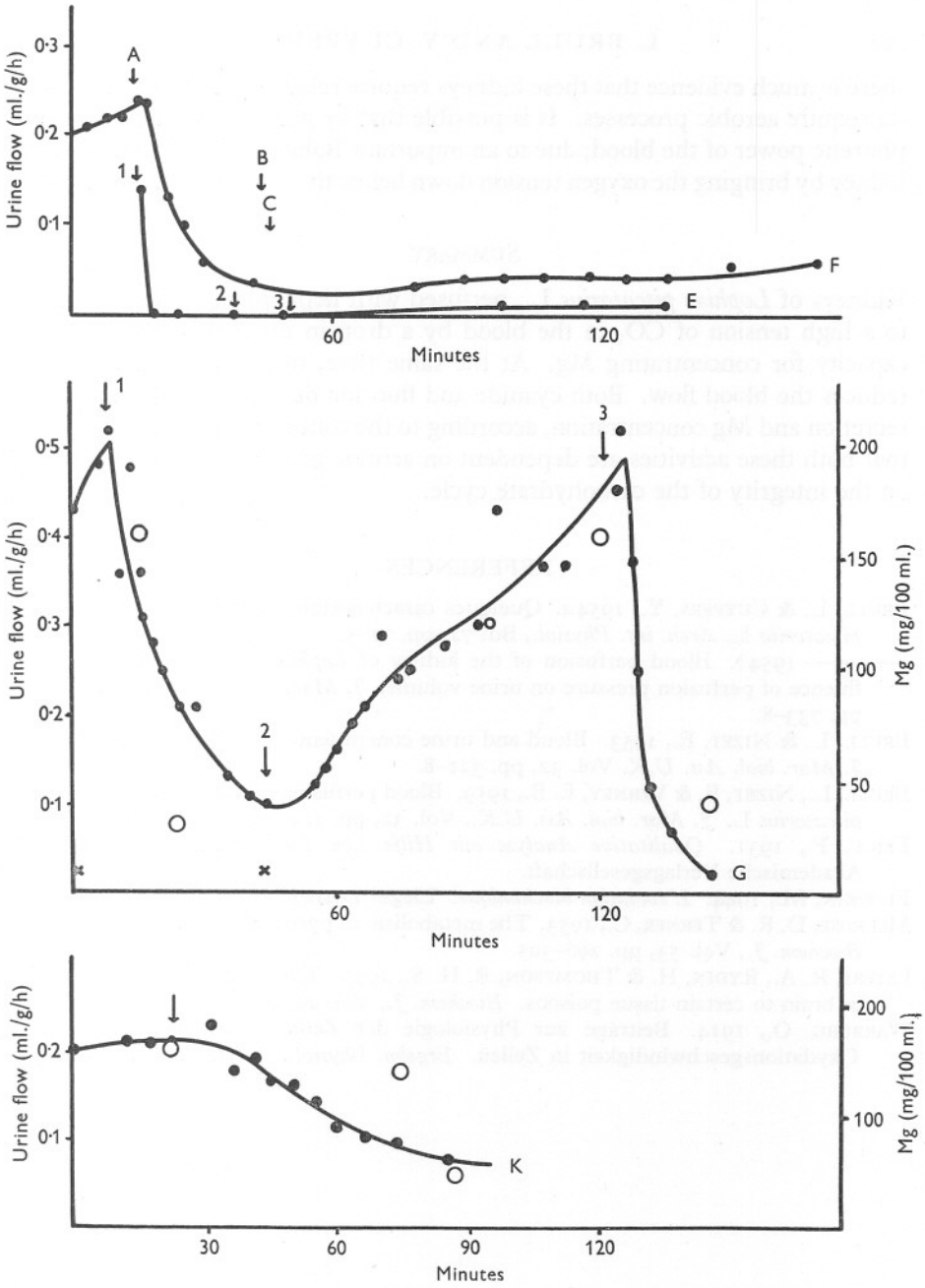

Fig. 2. Expts. E, F, G, K. Expt. E (lower curve): 160 mg KCN are added to 175 ml. of blood at 15 min (arrow 1). At 27 min the cyanided blood is removed; the preparation is washed with 100 ml. fresh blood (arrow 2). At 49 min this blood is removed and 150 ml. new blood put in (arrow 3). Expt. F (upper curve): at 14 min (arrow *A*) 20 mg KCN added to 130 ml. circulating blood. At 43 min (arrow *B*) the cyanided blood is removed. At 45 min circulation removed with 210 ml. fresh blood from which the fifty first ml. from the renal outflow are discarded. Expt. G: at the first arrow 8 mg KCN are added to 100 ml. circulating blood. At arrow 2, cyanided blood removed; the preparation is washed with 50 ml. fresh blood and new blood from the same pool is put in. At arrow 3, 350 mg NaF are put in. Expt. K: at the arrow, 65 mg NaF are added to 130 ml. circulating blood.

there is much evidence that these kidneys require relatively little oxygen, they do require aerobic processes. It is possible that by greatly lowering the oxyphoretic power of the blood, due to an important Bohr effect, CO_2 acts on the kidney by bringing the oxygen tension down below the minimum requirement.

Summary

Kidneys of *Lophius piscatorius* L., perfused with heparinized blood, respond to a high tension of CO_2 in the blood by a drop in urine flow and in their capacity for concentrating Mg. At the same time, but independently, CO_2 reduces the blood flow. Both cyanide and fluoride may stop or reduce water secretion and Mg concentration, according to the concentration used, showing that both these activities are dependent on aerobic processes and presumably on the integrity of the carbohydrate cycle.

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PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING 1954

By F. A. J. Armstrong
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(Text-figs. 1–4)

Analyses of water collected during 1954 at the International Hydrographic Station E 1 (lat. 50° 02′ N., long. 4° 22′ W.) are here reported in the same form as in a previous report (Armstrong, 1954). The methods of collection and analysis of samples were unchanged.

There are no observations below 30 m. at the end of the year because of a mishap with the sampling gear in November, and because bad weather curtailed work at sea in December.

Temperature and Salinity

The vertical distribution of temperature during the year is shown in Fig. 1. The water column showed vertical uniformity until May. The minimum surface temperature recorded was 9·0 on 12 March. The mean salinity increased between 19 January and 16 February from 35·31 to 35·37. The significance of this small change is discussed below. On 10 May, after rough weather there was only 0·5° C. difference between the top and bottom of the water column, but on 19 May the difference was 2·3° C and there was a marked thermocline at 30 m. This thermocline remained at 25 to 30 m during the summer, but had broken down by 11 October. Summer temperatures in the upper layers were unusually low, the surface maximum of 14·92° C being recorded on 14 September. The observations for 11 August and 14 September are given in full in Table I, from which it may be seen that marked temperature increases took place. Those in the deeper water are larger than in the upper layers and cannot be put down to vertical mixing. The sharpness of the thermocline remains unimpaired. A small but significant increase in salinity occurred. The weather records from Mount Batten Station show that mean air temperatures (at Plymouth) in the interval lay between the sea surface temperatures at E 1 observed on 11 August and 14 September, and that hours of sunshine were a little less than average. Although direct absorption of solar radiation causes some warming of the sea surface irrespective of the temperature of the air, it is improbable that the observed rises in temperature throughout the water column could have occurred locally. The change therefore must have been caused by a displacement of water masses on a considerable scale.

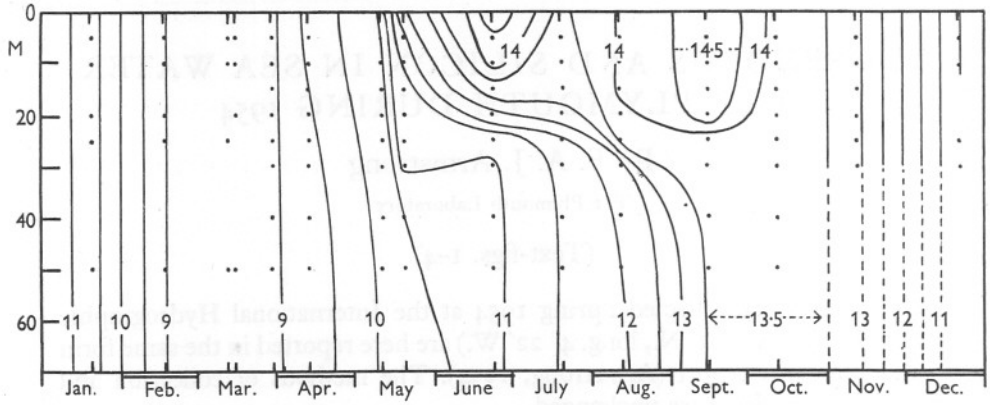

Fig. 1. Vertical temperature distribution at International Hydrographic Station E 1, 1954. Contour lines at 0·5° C intervals.

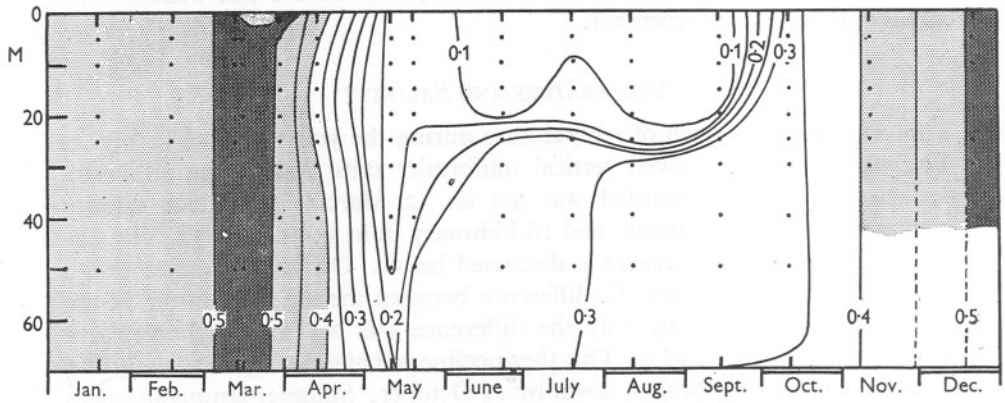

Fig. 2. Vertical distribution of phosphate as μg atom P/l. at International Hydrographic Station E 1, 1954. Contour lines at 0·05 μg atom P/l. intervals.

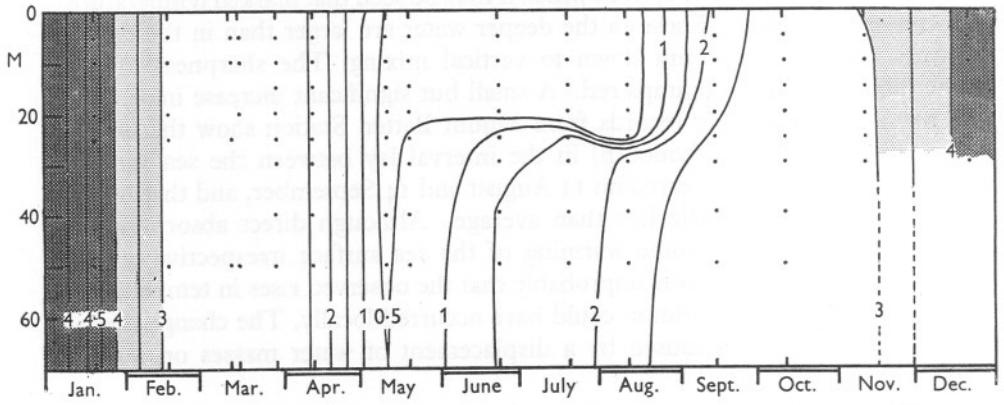

Fig. 3. Vertical distribution of silicate as μg atom Si/l. at International Hydrographic Station E 1, 1954. Contour lines at 0·5 μg atom Si/l. intervals.

During the whole of this period of 34 days a weak south-westerly air stream flowed over the area, becoming a strong south-westerly breeze (18–26 knots) for 2 days on 9–10 September. The weather remained cool and remarkably equable. The mean temperatures with standard deviations were

Max. day temp.	$17{\cdot}3 \pm 1{\cdot}3°$ C
Min. night temp.	$12{\cdot}0 \pm 2{\cdot}0°$ C
Overall mean	$14{\cdot}7°$ C

Mean hours of sunshine were 5·46 h/day. The average for this period is estimated as about 5·9.

After the breakdown of the thermocline, temperatures decreased as is normal. On 21 December the upper 10 m. was colder and less saline than at 25 and 30 m (e.g. 5 m 10·48° C, 34·76‰ S; 25 m 11·07° C, 35·10‰ S) which suggests the presence of coastal water after the heavy rainfall of late November and early December.

TABLE I. TEMPERATURE AND SALINITY AT INTERNATIONAL HYDROGRAPHIC STATION E1, 11 AUGUST AND 14 SEPTEMBER 1954

Depth (m)	Temperature (° C)		Salinity (‰)	
	11 Aug.	14 Sept.	11 Aug.	14 Sept.
0·5	14·05	14·92	35·24	35·25
5	14·05	14·87	35·21	35·25
10	14·05	14·87	35·24	35·27
15	14·03	14·87	35·24	35·27
20	13·99	14·77	35·25	35·27
25	13·98	13·70	35·25	35·30
30	12·38	13·59	35·29	35·35
40	12·22	13·57	35·30	35·35
50	12·00	13·54	35·29	35·35
70	11·91	13·53	35·33	35·33

PHOSPHATE

The vertical distribution of phosphate is shown in Fig. 2, and values at 10 and 50 m in Fig. 4. The water column was vertically almost homogeneous until early May. There was no significant change between 19 January and 16 February. During April phosphate concentrations fell sharply, and on 10 May were low throughout the water column. It is reasonable to assume that the vertical mixing possible before the establishment of the thermocline had carried part of the phytoplankton crop below the photosynthetic zone. After 19 May when the thermocline restricted vertical movement of water, this part of the phytoplankton would be trapped at depths where it could not long survive for lack of light. The consequence is interesting. Above the thermocline phosphate remained low (minimum 0·07μg atom P/l. on 11 August), but at deeper levels it increased, presumably by regeneration from the dying phytoplankton.

By 11 October, when the water had become isothermal, phosphate also became uniform, and increased until the end of the year.

Silicate

The vertical distribution of silicate is shown in Fig. 3 and values at 10 and 50 m in Fig. 4.

On 19 January silicate was 4·7 μg atom Si/l., a little higher than in December 1953, and the highest value recorded here since the present series of observations began in 1950. On 16 February the concentration was 2·8 μg atom Si/l.

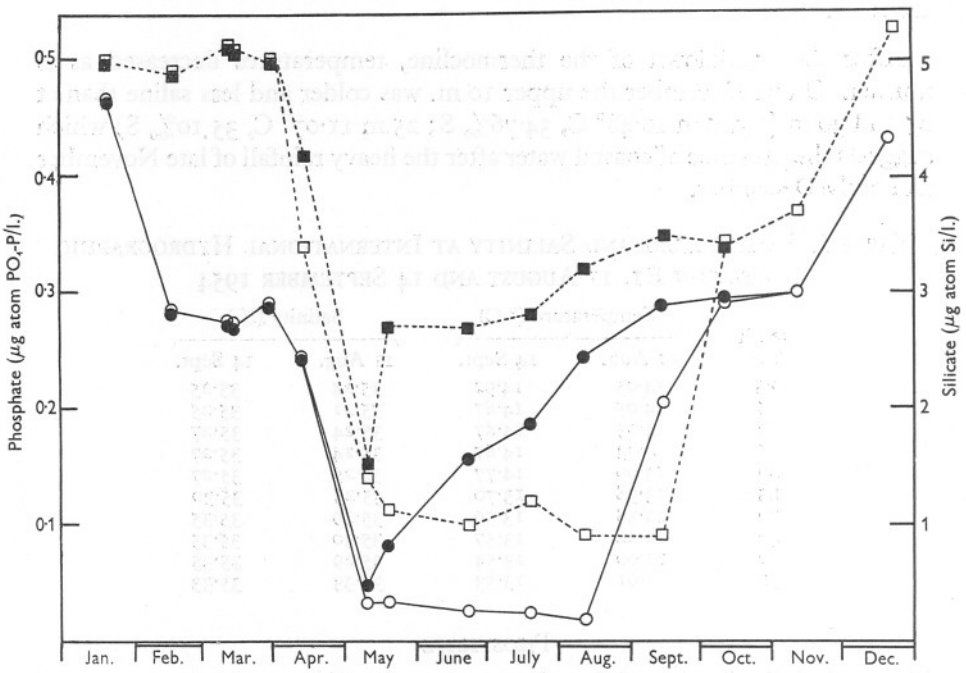

Fig. 4. Phosphate (μg atom PO_4-P/l.) and silicate (μg atom Si/l.) at 10 and 50 m at International Hydrographic Station E1 during 1954. Phosphate at 10 m, □; at 50 m, ■. Silicate at 10 m, ○; at 50 m, ●.

This very large decrease cannot have been caused by growth of diatoms. Such a growth would have been unprecedented at this time of the year, but is ruled out of account since there was no corresponding decrease in phosphate. The change in silicate must be ascribed to an influx of another body of water. There was, as noted above, a small change in salinity.

Silicate values fluctuated somewhat until 12 April, when the mean value was 2·5. On 10 May this had fallen to 0·4 when phosphate had also diminished throughout the water column. After the establishment of the thermocline, silicate remained low in the upper layers (minimum value 0·17 μg atom Si/l. on 11 August) though not so low as in 1953. Below the thermocline there was a steady increase, more gradual than that for phosphate. The values at 50 m in

Fig. 4 show an almost linear increase with time, which suggests that silica was dissolving from the frustules of diatoms trapped and dying below the euphotic zone.

On 14 September silicate had risen to 2·1 μg atom Si/l. in the upper layers, although phosphate remained low. The possibility that diatoms may have been supplanted by non-siliceous algae cannot be excluded, though without more knowledge of the flora at this station this is speculative. It seems certain, considering the marked temperature change and the appreciable alteration in salinity, that there was a change in the water mass. Silicate concentrations increased further in November and December and high values around 4·3 μg atom Si/l. were found in the top 10 m layer on 21 December.

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

Date	Phosphate-P (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)
19. i. 54	0·50	0·57	4·70
16. ii. 54	0·49	0·61	2·83
12. iii. 54	0·51	0·61	2·74
15. iii. 54	0·51	0·62	2·62
29. iii. 54	0·49	0·62	2·88
12. iv. 54	0·41	0·61	2·47
10. v. 54	0·14	0·40	0·39
19. v. 54	0·19	0·36	0·63
22. vi. 54	0·21	0·37	1·10
19. vii. 54	0·23	0·41	1·32
11. viii. 54	0·24	0·45	1·54
14. ix. 54	0·26	0·44	2·61
11. x. 54	0·34	0·46	2·95
11. xi. 54	0·41*	0·55*	2·94*
21. xii. 54	0·53*	0·64†	3·71†

* Upper 30 m only. † Upper 25 m only.

INTEGRAL MEAN CONCENTRATIONS

Computed figures are shown in Table II, and need little comment. The decreases representing consumption of nutrients in the spring outburst of plants were: phosphate 0·37 μg atom P/l., total phosphorus 0·26 μg atom P/l., silicate 2·49 μg atom Si/l.

SUMMARY

The results of analyses of water from the International Hydrographic Station E1 during 1954 are discussed. The seasonal variation is shown, in which it appears that consumption of nutrients in the spring outburst of plants was: phosphate 0·37 μg atom P/l., total phosphorus 0·26 μg atom P/l., silicate 2·49 μg atom Si/l., these figures being means for the whole water column. Unusual changes in silicate concentrations between January and February,

and between August and September, are ascribed to changes in the water mass at the station. This conclusion is supported by the temperature, salinity and phosphate observations.

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A PELAGIC MARINE DIATOM REQUIRING COBALAMIN

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The Marine Station, Millport

A bacteria-free culture of the important centric diatom *Skeletonema costatum* has recently been established at Millport, and some exploratory nutritional experiments have been carried out with it. *S. costatum* is a photo-autotroph whose autotrophy is, apparently, limited to cobalamin (vitamin B_{12}).

Details concerning isolation and maintenance of *S. costatum* differ from those already published (Droop, 1954*a*, 1955) only in the matter of pH control, a vital factor in the successful culture of this species whose range of tolerance is narrow (pH 7·5–8·5).

A synthetic solution (*S 36*) was developed for and used in the experiments to be described:

S 36

KNO_3	100 mg	Cobalamin	100 mμg
K_2HPO_4	10 mg	'S.W. 1'*	250 ml.
$Na_2SiO_3 . 9 H_2O$	100 mg	'S.W. 2'*	5 ml.
Tris-(hydroxymethyl)-aminomethane	500 mg	'T.M. 2'*	10 ml.
Thiamin	1 mg	Glass distilled water	735 ml.

S. costatum has been carried through more than ten transfers in this medium and growth has been as good as in any containing soil and liver extracts and natural sea water. The medium was adjusted to pH 8·1 before being decanted into test-tubes and autoclaved for a few moments at 15 lb., inoculations being carried out 18 hr later from exponentially growing cultures.

The sensitivity of *S. costatum* to pH was such as to require the use of replications in all vitamin experiments. This rendered conventional dose-response layouts very unwieldy. Consequently, the requirement for cobalamin was demonstrated qualitatively by the use of a serial transfer technique in which cultures receiving a full dose of the vitamin were compared with those receiving none. At each stage inoculations were taken from a vitamin-free culture of the previous stage to one batch of medium containing the vitamin and to one vitamin-free, the two batches having been prepared and adjusted as one, then divided for the addition of the vitamin, and autoclaved simultaneously.

The results of these experiments are shown in Table I. The magnitude of the errors occasioned by the difficulty of adequately controlling pH is not sufficient to obscure the fact that significantly poorer yields were obtained in the vitamin-free controls in each trial after the first. Inocula for the latter

* For composition of these metal solutions, see Droop (1955).

were from stock, hence carry-over of cobalamin in this instance would not be negligible. The results show that cyanocobalamin, the vitamin proper, can be spared by the variants, pseudo-vitamin B_{12}, factor A and factor B; but the experiments were not such as to be able to determine the relative activities of these variants, nor indeed to determine whether the requirement for cobalamin is absolute.

TABLE I. EFFECT OF COBALAMIN AT A CONCENTRATION OF 100 mμg/l. ON GROWTH OF *Skeletonema costatum* ON SERIAL TRANSFER IN MEDIUM *S 36* LESS COBALAMIN

Inocula were taken from controls of previous transfer (dilution factor: 100). Final yields measured optically (per cent absorption).

	Mean percentage absorption	Standard error	Replications
First transfer			
With cyanocobalamin	17	1·6	5
Control	16	0·81	5
Second transfer			
With cyanocobalamin	26	1·1	5
Control	7	0·84	5
Third transfer			
With cyanocobalamin	15	3·8	7
With factor A	13	2·2	7
With factor B	10	2·2	7
With pseudo-B_{12}	8	5·0	7
Control	2	0·62	7
Fourth transfer			
With cyanocobalamin	39	7·5	7
With factor A	44	4·6	7
With factor B	33	9·0	7
With pseudo-B_{12}	37	5·9	7
Control	7	1·9	7

S. costatum resembles *Bacterium coli* in being able to utilize factor B as the sole source of the vitamin, factor B being that part of the molecule, common to the various forms, which remains after removal of the respective nucleotides (Ford & Porter, 1952). The requirement for cobalamin in this instance, therefore, is likely to be occasioned by a synthetic disability concerning this non-nucleotide portion, as in the case of *B. coli* (Ford, Holdsworth & Kon, 1955). It cannot, however, yet be concluded that *S. costatum* is autotrophic with respect to the nucleotide, because the experiments were undertaken with a culture medium containing thiamin; but this may be the case.

A similar series of experiments with thiamin (in the presence of cobalamin) failed to demonstrate a significant requirement for this vitamin, but the use of cotton-wool plugs may account for this.

The culture of *S. costatum* in a state of bacteriological purity has allowed the first insight into the nutritional requirements of an important pelagic

organism. It also provides a measure of hope that success will attend efforts to culture other pelagic species so necessary to an understanding of the biochemical ecology of the sea. Indeed, the concept of non-predatory relationships, though more than fifty years old (cf. Lucas, 1938), has, for want of proper experimental material, yet received no detailed support in the field of phytoplankton studies. The need for accessory substances for growth of marine diatoms, first postulated by Allen (1914) and later by Harvey (1939) and others, is paralleled by nutritional deficiencies in investigated euryhaline diatoms and flagellates (Hutner & Provasoli, 1953; Provasoli & Pintner, 1953; Lewin, 1954; Droop, 1954*b*; Sweeney, 1954). Latterly, each investigation has tended to make more probable the general importance of cobalamins in the economy of the sea, though the species investigated have mostly been supralittoral or estuarine. The requirement in *S. costatum* is, therefore, of interest, and it is noteworthy that in this species, which possibly resembles other pelagic diatoms, there is a lack of specificity in the form of the vitamin preferred.

Grateful acknowledgement is made to Dr L. Provasoli (Haskins Laboratories, New York) for an introduction to the useful pH buffer, tris-(hydroxymethyl)-aminomethane, and to Dr J. E. Ford (N.I.R.D., Shinfield) for samples of factors A and B and pseudo-vitamin B_{12}.

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SOME NEW SUPRA-LITTORAL PROTISTA

By M. R. Droop

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(Text-figs. 1–19)

This communication is devoted to furnishing diagnoses of six organisms recently isolated from supra-littoral rock pools on the island of Cumbrae. Much of their interest derives from their being available in bacteria-free cultures; on the other hand, with one exception, all are frequent and typical members of the brackish supra-littoral.

Accounts of habitats in many ways similar to those on Cumbrae are to be found in Bohlin (1897), Levander (1900), Carter (1937), Conrad (1939) and Droop (1953). According to Bohlin's classification the pools may be divided broadly into three categories: (1) 'brackish', rock pools in which the influence of sea splash is dominant; (2) 'rain', rock pools beyond the normal reach of splash but not influenced by land drainage; (3) 'peat', pools with coloured water receiving drainage from surrounding peaty soil. On Cumbrae pools are found both on the friable Devonian rock and the hard basalt dykes. 'Rain' pools are not plentiful, probably from absence of any agency keeping the rocks beyond the immediate range of sea splash free from phanerogamic vegetation. Where they do occur they usually contain *Haematococcus pluvialis*. The 'brackish' pools are mostly under 10 l. in capacity and are subject to extreme fluctuations in salinity and alkalinity and dry up frequently. Typical dominants other than the species to be described are *Oxyrrhis marina*, *Monochrysis lutheri*, *Chlamydomonas pulsatilla* and *Platymonas* spp. The 'peat' pools, though mostly fresh, are subject to occasional inundations by the sea. Their reaction is normally neutral, as they do not support heavy populations.

Cultivation

A modification of Pringsheim's (1946) technique was employed for making isolations (Droop, 1954*a*). Cultures were at first maintained in a complex medium such as medium I below, but later it became possible in a number of cases to substitute for this completely 'synthetic' solutions, of which *S 20* and *S 22* are examples.

Medium I

Soil extract	20 mg[1]	K_2HPO_4	10 mg
Liver extract ('Oxoid')	100 mg	Natural sea water diluted to appropriate strength with glass-distilled water and autoclaved separately from the nutrients	1000 ml.
Tryptone peptone ('Difco')	100 mg		
KNO_3	100 mg		

S 20

KNO_3	100 mg
K_2HPO_4	10 mg
'S.W. 1'	250 ml.
'S.W. 2'	5 ml.
'T.M. 2'	10 ml.
Glass-distilled water	730 ml.

S 22

Glycine	40 mg
Guanine	40 mg
Uric acid	4 mg
Thiamin	1 mg
Cobalamin	100 mμg
Medium *S 20*	1000 ml.

'S.W. 1'

NaCl	60 g
$MgCl_2.6H_2O$	10 g
KCl	1·5 g
$CaSO_4$	2 g
Glass-distilled water to	1000 ml.

'S.W. 2'

$SrCl_2$	1000 mg
KBr	3000 mg
$Al_2(SO_4)_3$	50 mg
$LiCl.H_2O$	10 mg
RbCl	10 mg
Glass-distilled water	1000 ml.

'T.M. 2'

EDTA[2]	2000 mg	$NaMoO_4$	40 mg
Fe-EDTA	70 mg	$CoSO_4$	1·7 mg
$ZnSO_4$	560 mg	$CuSO_4$	0·3 mg
$MnSO_4$	200 mg	Glass-distilled water	1000 ml.

[1] 4 ml. of an extract of soil which yielded 5 g dry wt. of humic substance per l. on acidification.
[2] Disodium ethylene diamine tetra-acetate.

S 20 is a 'synthetic' substitute for enriched half strength sea water (cf. Hutner & Provasoli, 1951; Provasoli & Pintner, 1953). Although possibly needlessly complex, it may be quickly prepared from the stock solutions. It has formed the core of several successful solutions for 'marine' organisms. *S 22* is suitable for *Prymnesium parvum* and *Monochrysis lutheri* and the *Syracosphaera* and *Hemiselmis* described below. The function of the organic ingredients glycine, guanine and uric acid is not at present clear; all appear to be necessary. It is possible that they serve to increase the buffer capacity of the medium in the alkaline region. The growth factors represented may be superfluous in some instances, but a quantitative dose-response relation has been established for the four species mentioned with cobalamin (Droop, 1954*b*), and this

vitamin is probably needed by other Chrysophyceae. pH of the medium should be adjusted with Na_2CO_3 to about 8 for most supra-littoral species. Media below pH 7 are toxic and above pH 9 they tend to precipitate unless autoclaving is reduced to an absolute minimum.

CHLOROPHYCEAE

CHLOROCOCCALES

Nannochloris oculata n.sp. (Figs. 1–6) (Strain no. 66)[1]

Cellulae globosae 2–4 μ diam., aut solitariae aut aggregatae; chromatophoro singulo, pallido viridi, parietino per parte peripheriam cellulae occupanti; stigmate pallido rubro, rotundo, ad chromatophorum locato; sine pyrenoide, sed 1–3 granis magnis amylaceis parietinis praeditae; tegumento delicatissimo persistanti, sed circum cellulas juvenes inviso et vix maturas apparenti. Propagatur per fissionem pariens cellulas filias duas (non autosporas). Cellulae filiae aut liberes aut ad liminem tegumenti materni dirupti manentes.

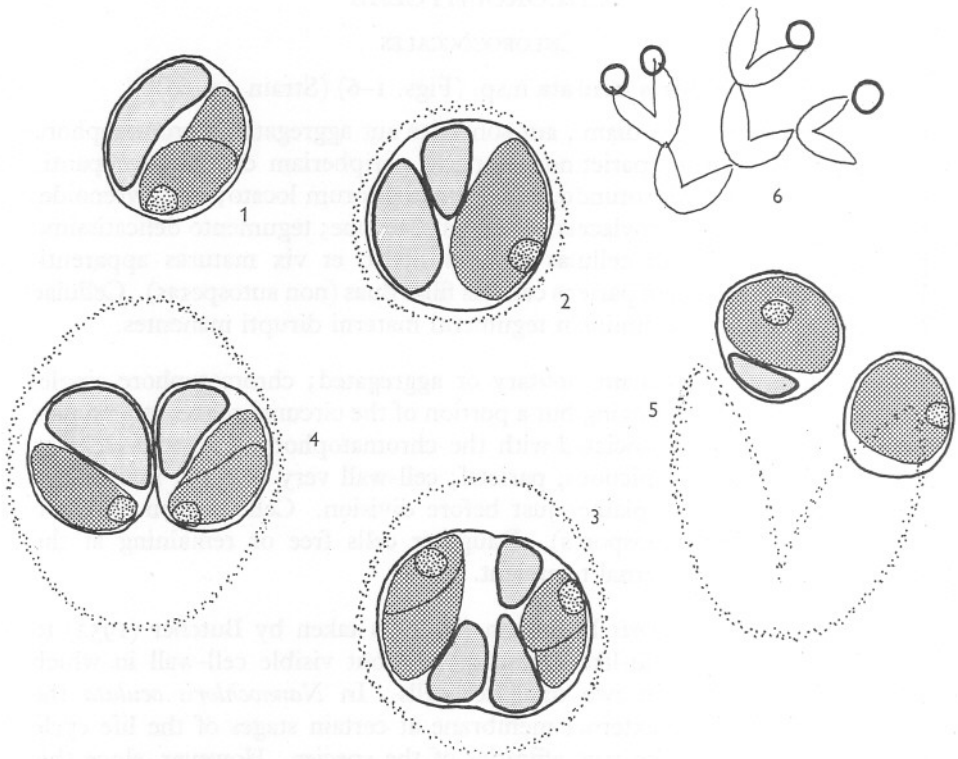
Cells globose 2–4 μ diam. solitary or aggregated; chromatophore single, pale green, parietal occupying but a portion of the circumference; stigma pale orange-red, circular, associated with the chromatophore; pyrenoid lacking; starch grains 1–3, conspicuous, parietal; cell-wall very delicate, invisible in young cells, becoming plainer just before division. Cell division into two daughter cells (not autospores). Daughter cells free or remaining at the mouth of the split maternal tegument.

The genus *Nannochloris* Naumann (1931) is taken by Butcher (1952) to include minute *Chlorella*-like organisms without visible cell-wall in which cell-division resulted in two daughter cells. In *Nannochloris oculata* the presence of a delicate external membrane at certain stages of the life cycle casts some doubt on the true affinities of the species. However, since this character varies in its expression, being quite undetectable under some conditions, and at no time sufficiently firm to modify the mode of cell-division characteristic of *Nannochloris*, it was thought most expedient to place the species there. *N. oculata* differs from other described species also in the possession of a pale circular stigma.

The cell-wall is never visible in young cells, which always appear naked. It is seen first in mature cells just before division, when it has the appearance of a faintly granular investment. As division proceeds this investment enlarges so that space is visible between it and the cell. After division, daughter cells are freed by splitting of the investment, but one or both may remain attached to it, leading in extreme cases to a loose subdendroid colony reminiscent of

[1] Strain numbers refer to Millport cultures.

Dichotomococcus capitatus Korschikof (1928). The investment stains well with 1% cresyl blue, when the granulation is very marked. At no time does it appear gelatinous. The environmental conditions leading to its greatest expression are not yet clear to me but I have the impression that they are concomitant with optimum conditions for growth. Colony formation is encountered in the field as well as in cultures.



Figs. 1–6. *Nannochloris oculata* n.sp. Figs. 1–5, stages in cell division, × 7,500. Fig. 6, 'Colony' formation, × 1,750.

The protoplast of *Nannochloris oculata* is clear and structureless and has no visible inclusions other than those mentioned. The chromatophore is pale at all times, but in actively growing cultures is dark enough to make the stigma hard to observe. In nitrogen-deficient cultures, however, it becomes very pale indeed and the stigma is then quite plain. The change in the relative intensity of stigma and chromatophore is reflected in the naked-eye aspect of cultures which turn from yellow-green to brown as if there were carotenoids accumulating.

N. oculata is a frequent inhabitant of brackish rock pools on Cumbrae, particularly those of low salinity on the basalt. Ten out of 115 pools examined

in the summer of 1953 contained *N. oculata* as a dominant. Table I shows salinity analysis of these records, including data of the eight species most frequently encountered.

A pure clone (no. 66) of *N. oculata* was obtained by plating a suitable dilution of a sample on 1% agar with 50 mg/l. soil extract prepared with 50% natural sea water. Growth was quite good in soil-extract agar but liquid media

TABLE I. SALINITY DISTRIBUTION OF SOME FIELD RECORDS

(Entries refer to number of records.)

Species	Salinity (‰) 0 to 0·4	0·4 to 1·6	1·6 to 6·4	6·4 to 25	Above 25	Total species records
Haematococcus pluvialis	7	1	.	.	.	8
Chlamydomonas pulsatilla	1	3	3	.	.	7
Nannochloris oculata	1	3	3	2	1	10
Euglena proxima	2	2	4	3	.	11
Hemiselmis virescens	.	2	5	4	1	12
Brachiomonas submarina	5	7	15	5	3	35
Platymonas spp.	1	2	1	8	4	16
Syracosphaera elongata	.	1	1	2	2	6
Monochrysis lutheri	.	3	5	7	4	19
Oxyrrhis marina	.	.	7	14	10	31
Total salinity records	12	22	32	23	17	—

TABLE II. UTILIZATION OF SOME N COMPOUNDS IN THE PRESENCE OF SOIL EXTRACT

(+, efficient; ?, doubtful; o, not utilized; −, compound harmful.)

	Isoleucine	Glutamate	Histidine	Methionine	Tryptophane	Lysine	Leucine	Phenylalanine	Serine	Valine	Glycine	Arginine	Aspartate	Urate	Urea	Ammonium	Nitrate
Haematococcus pluvialis	o	?	?	?	+	?	o	o	o	o	?	+	?	+	+	+	+
Nannochloris oculata	o	?	o	o	+	o	o	o	o	o	o	o	o	+	+	+	+
Monochrysis lutheri	o	o	o	o	−	o	o	o	o	o	+	o	o	+	+	+	+
Syracosphaera elongata	?	o	o	?	−	?	?	?	o	?	?	?	?	+	?	o	+
Prymnesium parvum	+	o	?	?	−	+	?	+	?	+	?	+	+	?	o	?	+

were improved by casein digest or any of a number of carboxylic acids, purines, and nucleotides, particularly if pH of the medium was on the low side. Medium *S 20*, pH at least 7·5 is satisfactory, but it is more reliable with the addition of 40 mg/l. glutamic acid. *N. oculata* is an obligate phototroph and is non-auxotrophic. It is able to use as N sources nitrate, ammonium, urea, uric acid, and the amino-acid tryptophane. Glutamic acid is also utilized, but very inefficiently (Table II). Tolerance of a wide salinity range in the field indicated in Table I is matched by its performance in cultures in which relative growth rate was found to be maximal (a daily twofold increase with 200 ft candles warm white fluorescent light for 16 h each day) in salinities between 4 and 36‰, and cultures could be maintained in salinities between 2 and 54‰.

VOLVOCALES

Brachiomonas submarina var. **pulsifera** n.var. (Strains nos. 44 and 45)

Monada speciei *Brachiomonas submarina* Bohlin em. Droop, antice quatuor vacuolis contractilibus praedita.

Cells with the characters of *Brachiomonas submarina* Bohlin em. Droop, but provided with four anterior contractile vacuoles.

B. submarina is a sexual organism with, apparently, two mating types (Droop, 1953); var. *pulsifera* is no exception. Originally twenty-five clones of individuals possessing four contractile vacuoles were isolated, nine of which were of one mating type and 16 of the other. One of each type was retained in culture (strains nos. 44 and 45), and proved to be compatible with one or other of the existing strains of *B. submarina* var. *submarina* (strains nos. 42 and 43). Table III shows the pattern of compatibility.

TABLE III. COMPATIBILITY IN *BRACHIOMONAS*

(Z, compatible; O, incompatible.)

		Strain			
	Strain	42	43	44	45
Var. *submarina*	42	O	Z	Z	O
	43	Z	O	O	Z
Var. *pulsifera*	44	Z	O	O	Z
	45	O	Z	Z	O

Although there has not been undertaken a zygote analysis, which would determine the underlying genetical mechanism, the fact that cells possessing vacuoles can mate with those not possessing them suggests that the difference between the two forms is less than specific. The status Variety seemed appropriate.

Var. *pulsifera* is widespread in brackish pools on Cumbrae. Curiously, the type variety appears to be completely absent.

CRYPTOPHYCEAE

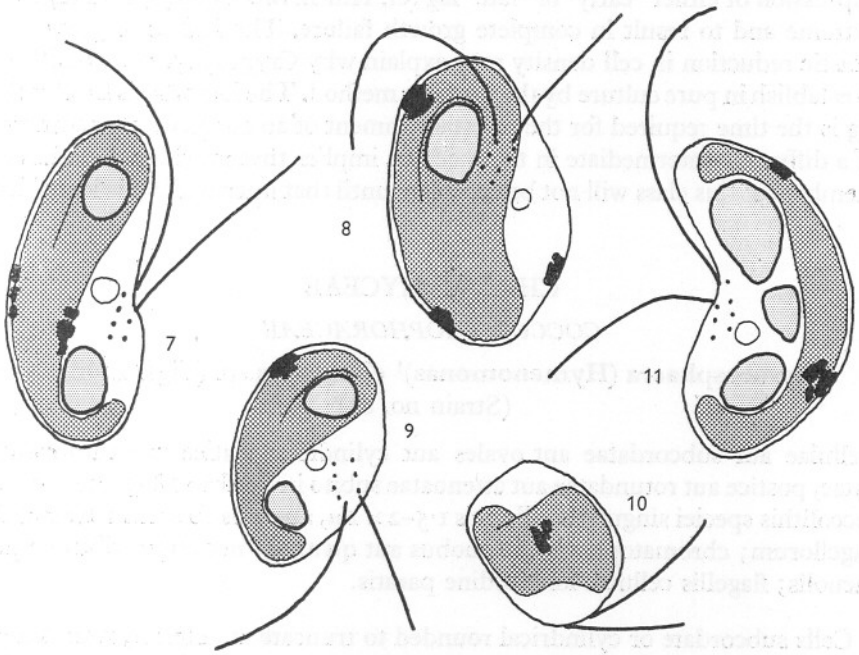
NEPHROSELMIDACEAE

Hemiselmis virescens n.sp. (Figs. 7–11) (Strain no. 64)

Monada generis *Hemiselmis* Parke chromatophore laete viride.

Having examined a culture of *H. rufescens* ('Flagellate D' (Parke, 1949)) kindly supplied by Dr Parke, I have been unable to detect any difference between the present species and *H. rufescens* other than colour of the chromatophore. In healthy cultures of *H. virescens* the latter is an almost brilliant

green, so that to the naked eye cultures appear pale turquoise to bottle-green according to their density. Figs. 7–11, however, differ from Dr Parke's of *H. rufescens* in the following points: length and stiffness of the flagella, flagellar insertion, number and position of stigmata, and orientation of the two rows of trichocysts. The number and position of the stigmata vary in both species and should in all probability be related to the health of the material. The other differences are, in my opinion, differences in interpretation of small structures. Cells of *H. virescens* are mostly between 5 and 7 μ in length.



Figs. 7–11. *Hemiselmis virescens* n.sp., × 5,000.

H. virescens is widely distributed in supra-littoral pools on Cumbrae; its appearance is rather spasmodic, but on occasions it is to be found in enormous numbers (*c.* 30 million per ml.). Pools of low salinity are favoured but it has a great range (Table I). It is also recorded from Öregrund, Sweden.

The type strain (no. 64) was isolated in July 1953 from a very small pool on Skate Point. The difficulties encountered in isolation of this strain are matched by those of its maintenance. Paradoxically, it has proved easier to maintain in the synthetic medium *S22* than in any variant of the original isolation medium containing soil and liver extracts. Nutritional requirements are not fully worked out; that room exists for improvement in the medium is shown both by the high yields in nature which cannot yet be achieved in the laboratory and by the improvement frequently obtained in two-member

cultures, e.g. cultivated with another flagellate such as *Monochrysis lutheri*. In that case, incidentally, growth of *M. lutheri* is largely suppressed.

The only vitamin requirement of *Hemiselmis virescens* thus far definitely identified is one for cobalamin.

Successful maintenance of this species depends both upon sufficient material (0·2 ml. per 5 ml.) being transferred on subculturing and on cultures not being allowed to age. Subculturing should be carried out fortnightly. Apparently enzymatic changes occur so quickly in cells not actively multiplying that any expression of either 'early' or 'late' lag (cf. Hinshelwood, 1946) is likely to be extreme and to result in complete growth failure. The failure to grow after drastic reduction in cell density may explain why Cryptophyceae are difficult to establish in pure culture by the washing method. The hypothesis that 'early' lag is the time required for the re-establishment of an adequate concentration of a diffusible intermediate in the medium implies that nutritional studies on members of this class will not be complete until that intermediate is identified.

CHRYSOPHYCEAE

COCCOLITHOPHORACEAE

Syracosphaera (Hymenomonas)[1] **elongata** n.sp. (Figs. 12–14)
(Strain no. 62)

Cellulae aut subcordatae aut ovales aut cylindricae antice truncato-rotundatae, postice aut rotundatae aut attenuatae subito in caudam 18–30 × 12–15 μ; coccolithis speciei singularis ellipticis 1·5–2 × 1 μ, absentis ab area instructantis flagellorum; chromatophoris aut duobus aut quatuor; nec stigmatibus neque vacuolis; flagellis cellulae longitudine paratis.

Cells subcordate or cylindrical rounded to truncate at anterior, rounded or suddenly attenuated to a short point at posterior 18–30 × 12–15 μ; coccoliths of a single type, oval, 1·5–2 × 1 μ, absent from area surrounding flagellar insertion; chromatophores 2 or 4; neither stigma nor vacuoles; flagella as long as the cell, not longer. Euryhaline.

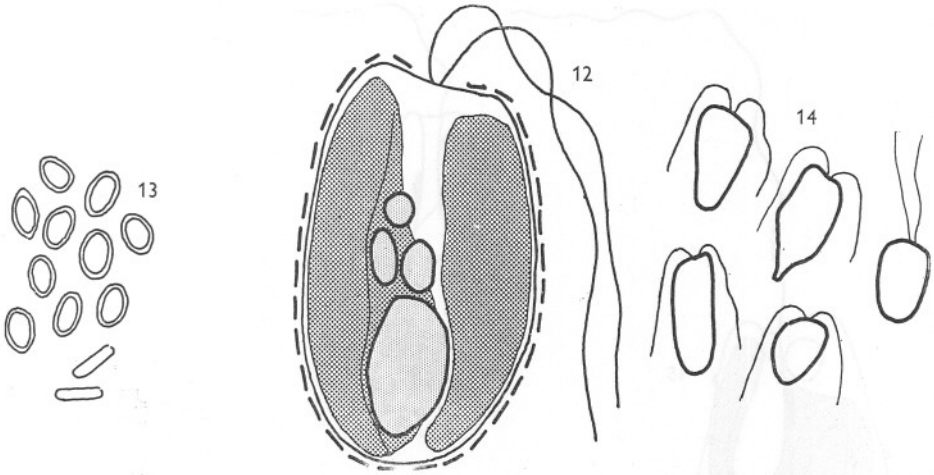
S. elongata most nearly resembles *S. carterae*[2] Braarud & Fagerland (1946) particularly as to size, shape and structure of coccoliths. I am indebted to Prof. T. Braarud for electron-microscope observations on the coccoliths of my strain.

The points of difference between *S. elongata* and *S. carterae* which warrant the creation of a new species are size and shape of the cell and length of flagella. *S. elongata* is 1½ times the size of *S. carterae* and the cell length/breadth ratio tends on the whole to be much greater. A ratio of 2–4 is usual. The

[1] Nomenclature under review (T. Braarud, personal communication.)
[2] Living material of both the Blindern and Plymouth strains has been examined.

flagella, on the other hand, are relatively shorter than those of *S. carterae*, being rarely longer and frequently shorter than the cell length. On account of this, *S. elongata* moves noticeably more sluggishly than does *S. carterae*. There is no doubt, however, that the two species are closely related.

S. elongata is a moderately frequent inhabitant of brackish pools of rather high salinity on Cumbrae. Sometimes it occurs in great numbers, though more usually few individuals are encountered.



Figs. 12–14. *Syracosphaera elongata* n.sp. Fig. 12, typical cell in culture, × 2,500. Fig. 13, coccoliths, × 3,500. Fig. 14, cells from wild material, × 500.

A clone of this species has been maintained for a year in medium *S22*, but has also been cultured in media with salinity as low as 8 and as high as 90‰. There is an absolute requirement for cobalamin and, in certain circumstances, thiamin has a stimulating effect; no other growth requirements have been identified. It appears to be an obligate phototroph, as none of a large range of organic carbon compounds support growth in the dark. Nitrate and uric acid are good sources of nitrogen for this species while ammonium, urea and amino acids are utilized not at all or with difficulty (Table II).

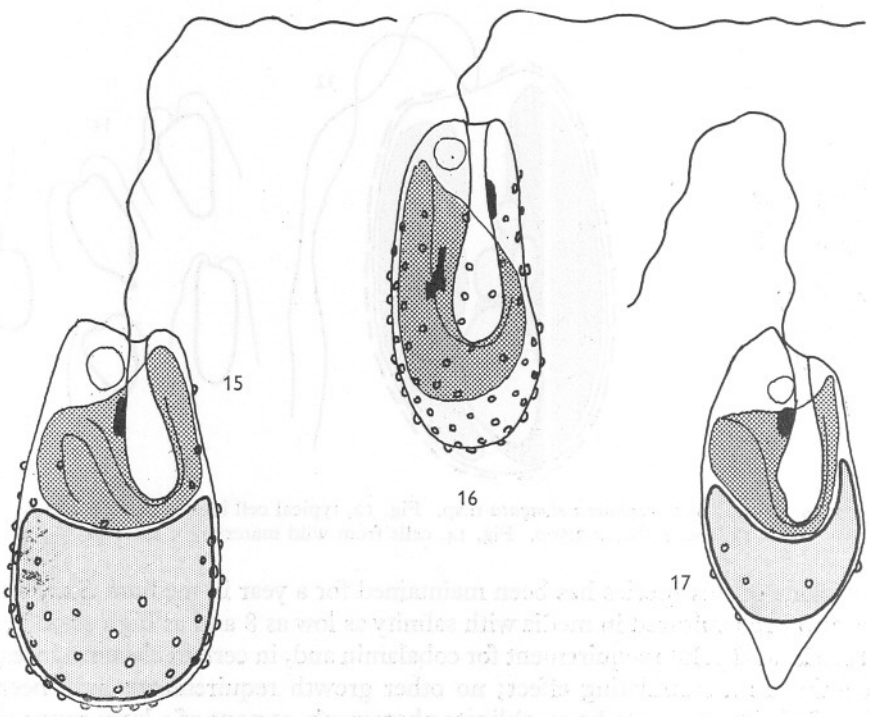
MALLOMONADACEAE

Microglena arenicola n.sp. (Figs. 15–17) (Strain no. 72)

Monada submarina generis *Microglena* Ehrenberg, cylindricato-ovata, paulum asymmetrica et compressa, antice oblique truncato-rotundata depressione vadosa instructa ad oram ampullae, postice aut rotundata aut attenuata subito in caudam, 12–16 μ longae, 8–11 μ crassae; periplasto delicatissimo, antice paucis, postice pluris, siliculis minutis praedito; flagello ad oram ampullae inserto, longiore paulum longitudine cellulae, clinato antice; vacuolo con-

tractili singulo, in ampullam fundenti; chromatophoro singulo aut poculiformi aut sinuato, ventum ampullae circumdato; stigmate magno, laete rubro, ad collum ampullae locato; nucleo postice, sed ante leucosini guttam locato.

Cells cylindrical to ovate slightly compressed and asymmetrical, anteriorly obliquely truncate to rounded with a small shallow depression leading to the reservoir mouth, posteriorly rounded or attenuated suddenly to a point;



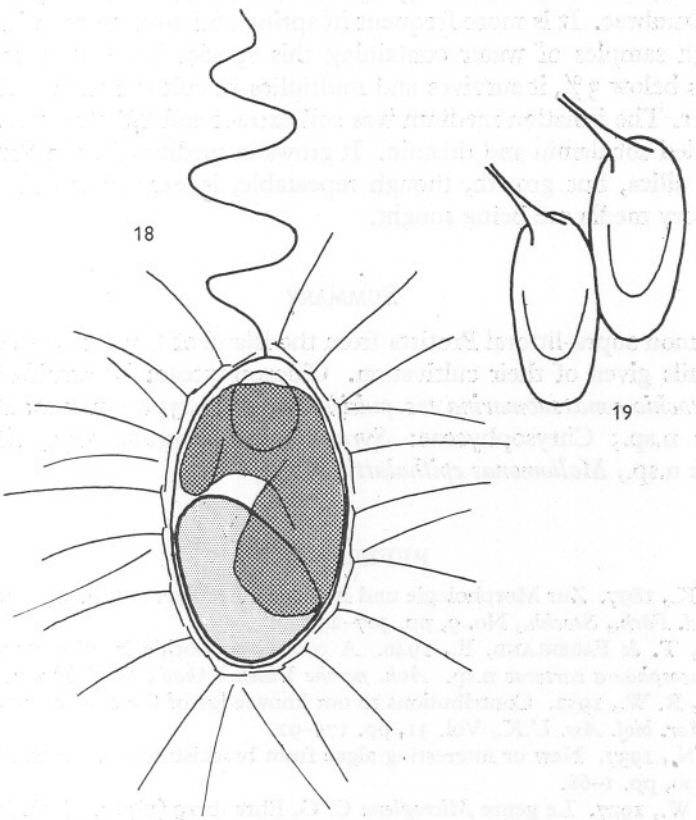
Figs. 15–17. *Microglena arenicola* n.sp., ×5,000.

12–16 × 8–11 μ; periplast thin with small silicious punctae, mostly at rear; flagellum arising at reservoir mouth, slightly longer than cell, anterior tip inclined sharply during forward movement; contractile vacuole single, emptying into the reservoir; chromatophore single, embracing reservoir belly, cup shaped or folded to an S; stigma large, bright red, at reservoir neck; nucleus lying between chromatophore and posterior leucosin body. Euryhaline.

M. arenicola is similar in shape to *M. punctifera* Ehr., particularly as regards the anterior indention but it is much smaller and relatively narrower anteriorly. Figs. 15 and 16 show typical specimens, while Fig. 17 shows one with the posterior point, possibly associated with exudation of leucosin. The vacuolar apparatus differs from that of other species (cf. Conrad, 1927) in that there is but a single contractile vacuole.

During March and April 1954 high seas had left on the sand of Kames Bay, Millport, a large quantity of brown weed which had become partly buried and rotten. The result was a fine bloom of flagellates and algae on the sand where there was freshwater seepage. Dark green patches were mostly *Euglena proxima*; light green or yellow patches, *Microglena arenicola*. Salinity of the water containing the flagellates was less than 4‰.

No difficulty was encountered in isolating *M. arenicola*, but satisfactory maintenance media await development. At present it is maintained in *S 22* with or without the addition of silica. It is extremely phototactic in cultures.



Figs. 18, 19. *Mallomonas epithalattia* n.sp. Fig. 18, typical cell, × 1,500. Fig. 19, two plates, × 8,000

Mallomonas epithalattia n.sp. (Figs. 18, 19) (Strain no. 71)

Cellulae ovatae vel ellipsoideae 20–30 × 10–15 μ; scutis ellipticis 4 × 1·5 μ, setis simplicibus aequaliter dispersis et radiantibus 10–15 μ longis; chromatophoro singulo parietino; nucleo antice.

Cells ovoid to ellipsoid 20–30 × 10–15 μ with elliptical plates 4 × 1·5 μ and setae evenly distributed and radially divergent, 10–15 μ long, entire; with single parietal chromatophore and anterior nucleus.

Neither reservoir, contractile vacuoles nor cysts have been observed in *M. epithalattia*. This species differs from others with oval plates in having but a single chromatophore (Conrad, 1933). Except for the shape of the plates, there is a strong resemblance to *M. fresenii* Kent. The plates of *M. epithalattia* are very delicate and are nearly invisible unstained, but they take up aqueous cresyl blue satisfactorily.

M. epithalattia is found in slightly brackish peat pools mainly on Farland Point, Cumbrae. It is more frequent in spring and autumn than in summer. Although samples of water containing this species have been confined to salinities below 3‰ it survives and multiplies in cultures with half strength sea water. The isolation medium was soil extract and 1/8 strength sea water with added cobalamin and thiamin. It grows in medium I or in *S 22*, with or without silica, but growth, though repeatable, is exceedingly light. More satisfactory media are being sought.

Summary

Six common supra-littoral Protista from the island of Cumbrae are described and details given of their cultivation. Chlorophyceae: *Nannochloris oculata* n.sp., *Brachiomonas submarina* var. *pulsifera* n.var.; Cryptophyceae: *Hemiselmis virescens* n.sp.; Chrysophyceae: *Syracosphaera elongata* n.sp., *Microglena arenicola* n.sp., *Mallomonas epithalattia* n.sp.

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THE SALP *RITTERIELLA* OFF THE ENGLISH COAST—A CORRECTION

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In a recent short note in this *Journal* (Fraser, 1954) the occurrence was announced, off the entrance to the English Channel, of the solitary form of the salp *Ritteriella picteti* (Apstein), and seven aggregate forms of *R. amboinensis* (Apstein), taken by H.M.S. *Challenger* in 1953. The identification of the aggregate forms as *R. amboinensis* and not as *R. picteti* was based, as then stated, on Thompson's (1948) description from a very young specimen of *R. picteti* which emphasized the similarity of its muscle arrangements to those of *Salpa cylindrica* and not to *Ritteriella amboinensis*.

Since this was written, Berner (1954) has described the aggregate form of *R. picteti* from good specimens found off the west coast of U.S.A., and he has shown that the musculature was in fact not like that of *Salpa cylindrica* but very similar to *Ritteriella amboinensis*. Differences were noted in the number of fibres constituting the various body muscles and in the number and arrangement of the oral muscles. A still more recent paper by Yount (1954) includes comparisons of the two forms, based on Berner's characters but also including differences in the length of the endostyle and in body shape (length of anterior and posterior processes, and degree of extension of the gut outside the rest of the body). Yount also goes into greater detail in describing the oral musculature in the two species.

A re-examination of the specimens taken by H.M.S. *Challenger* shows them to be in fairly close agreement with both Berner's and Yount's descriptions of *R. picteti*, but with one or two small differences. The actual number of fibres in the muscles is rather smaller than given by Berner for *R. picteti*, though the precise figure is difficult to ascertain, and there is no median interruption of the first sphincter muscle in the upper lip which Berner believed to be characteristic, and which Yount accepted. I am particularly grateful to Mr Berner for sending me some of his material for comparison and, although I certainly found this interruption in his specimens I do not regard its absence from the Atlantic specimens, or their slightly fewer fibres, as significant differences. Such variations in the musculature are not unexpected, and Sewell (1926), for example, gives a warning (p. 67) against regarding interruptions of muscles, their branching or joining, as diagnostic characters.

Again, the differences in the length of the endostyle and body processes appear to be variable with size, and there is in the Atlantic specimens a range

from short blunt processes with the endostyle extending only to M. 5 in the smallest specimen to long filiform processes and extended endostyle in the largest specimens. It thus seems unlikely that these characters can be used diagnostically as Yount suggests.

However, the Atlantic specimens have the four ventral oral muscles as described in detail by Yount and the number of muscle fibres, though less than given by Berner, is considerably greater than that for *R. amboinensis*. In a personal communication Dr H. Thompson tells me that he accepts Berner's description of the aggregate form of *R. picteti* which was based on much more extensive material, and he agrees that his statement (1948) that it has muscle arrangements resembling that of *Salpa cylindrica* can now be ignored. I therefore conclude that the seven aggregate specimens taken by H.M.S. *Challenger* are indeed *Ritteriella picteti* and I wish to correct the original statement. This satisfactorily disposes of the apparent but somewhat surprising coincidence of two most unusual species of salp.

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Contribution No. 1 from the University of Florida Field Station.

CERCARIA TURRITELLAE N.SP., A 'HUGE-TAILED' MONOSTOME LARVA FROM *TURRITELLA COMMUNIS* RISSO

By Robert F. Hutton

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(Text-figs. 1 and 2)

Rothschild (1935) reported that Dr M. V. Lebour drew her attention to a 'huge-tailed' monostome which had occurred during 1931 in specimens of *Turritella communis* collected from the Rame Mud, Plymouth. From 1932 to 1934 Rothschild (1935) examined 541 specimens of this gastropod from that locality without finding this larva. During the summer of 1952, the present author examined a number of specimens from the same locality and was also unsuccessful in finding such a larval trematode. However, in November 1952, the monostome described below was found in two of 110 *Turritella* collected from Cawsand Bay, Plymouth Sound.

The author wishes to express his indebtedness to the Director and Staff of the Plymouth Laboratory of the Marine Biological Association for their friendly co-operation and assistance in all phases of this work. The work was carried out while the author was holding a Fulbright Scholarship.

Methods

Fully developed cercariae were obtained by isolating infected specimens of *T. communis* in jars of sea water and allowing the cercariae to emerge. Rediae and immature cercariae were obtained by crushing the shells of the hosts. Living specimens as well as permanent preparations were studied. Whole mounts were fixed in sublimate, stained with borax-carmine, and mounted in Canada balsam, or fixed in sublimate or formalin, unstained, and mounted in Faure's medium or in Cristalite (E. Gurr). Serial sections (5μ) of several cercariae were stained with Ehrlich's haematoxylin and eosin. The orientation of the objects in the desired position at imbedding was achieved by the method of Péterfi (after Romeis, 1948, p. 99). The incidence of infection was determined by crushing the gastropods and examining the digestive glands and gonads under the dissecting microscope.

Cercaria turritellae n.sp.

Specific diagnosis. The body of this non-swimming, non-oculate, monostome larva is elongate. Cuticular spines were not observed on the body. The digestive system (Fig. 2*f*) consists of the following structures: a mouth, located in the centre of a subterminal sucker; a short prepharynx; a small pharynx (?); and a narrow oesophagus leading into two long intestinal caeca that terminate near the posterior end of the body. The excretory vesicle is of moderate size with two lateral branches, containing large refringent inclusions, extending forward and joining in the mid-line of the body well in back of the oral sucker. Underneath the cuticle of the body wall in fully formed cercariae, numerous cystogenous glands, containing small refringent rods, obscure much of the internal structure. The tail is extremely large, wider and somewhat longer than the body; characteristically, it curves ventrally. On the dorsal side of this structure, two finely spined fin-like ridges (Figs. 1*d* and 2*c*, *d*) extend over more than half of its length. Annulations which appear to be circular muscle fibres uniformly cover the tail. Internally, the tail is composed mainly of large hyaline cells, each of which contains a nucleus (Fig. 1*d*). Measurements of fifteen living specimens in various stages of extension and contraction and slightly compressed between a cover-glass and glass slide are (in microns): body length 108–226 (av. 167); body width 46–124 (85); oral sucker width 24–32 (28); tail length 164–260 (212); tail width 62–128 (95). The redia (Fig. 2*e*) is elongate, without collar or ambulacral processes, and provided with a short intestine and a birth pore which is near the pharynx. Lateral to the pharynx is an undetermined number of cephalic glands. The largest rediae contain fully formed cercariae as well as cercarial embryos; no daughter parthenitae were observed. Measurements of three living specimens in various stages of extension and contraction and slightly compressed between a cover-glass and glass slide are (in microns): body length 596–1190 (av. 893); body width 154–306 (230); pharynx width 26–32 (29). The excretory formula of the redia is 2 (1+2).

Host. *Turritella communis* Risso.

Habitat. Digestive gland and gonad.

Locality. Cawsand Bay, Plymouth Sound.

Incidence of infection. 0·57% (two out of 350 specimens examined).

Remarks. The large tail of these cercariae appears to be an organ used for flotation because there is never any indication that the animals are able to swim, and, as they are usually suspended in the water with the body hanging below the tail, it seems likely that the hyaline cells of which the tail is mainly composed aid the buoyancy of the cercariae. In addition to the large cells, the tail contains near its base, and also bordering the two dorsally placed fin-like ridges, a number of smaller cells (Fig. 1*d*). These two finely spined ridges begin near the base of the tail and extend posteriorly over slightly more than

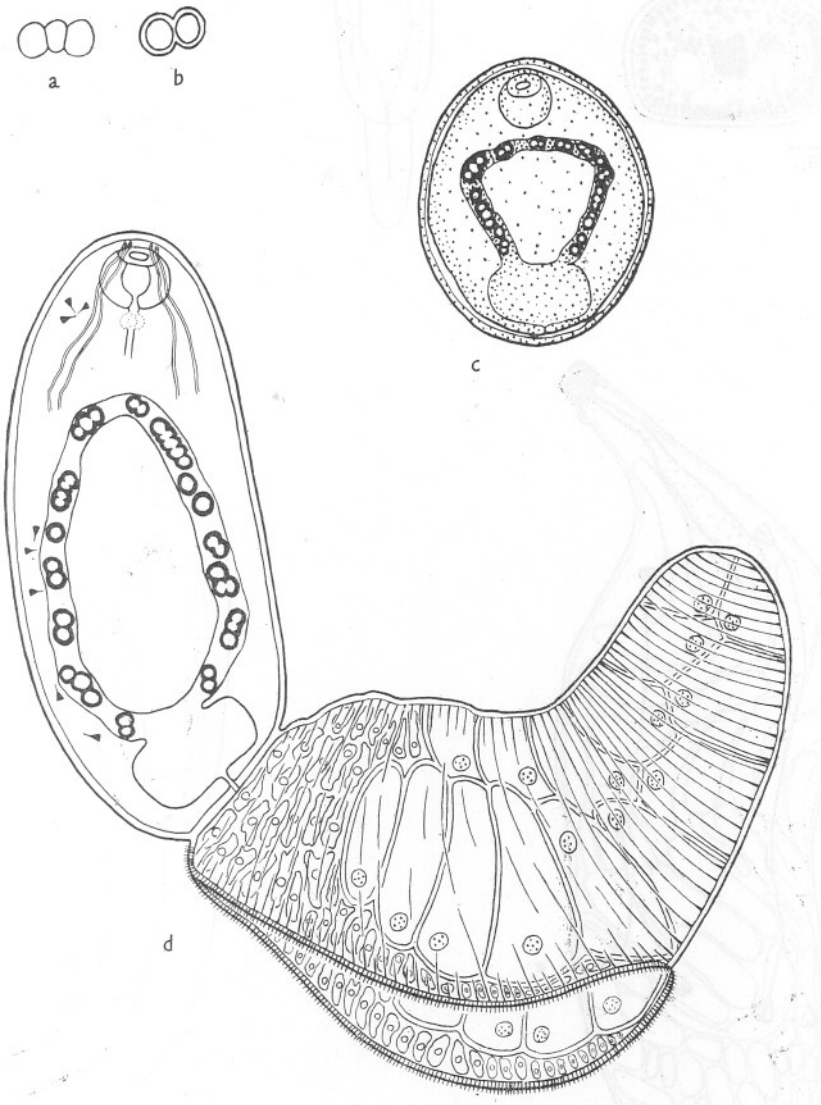

Fig. 1. *Cercaria turritellae*. *a*, thin-walled inclusions in the branches of the excretory vesicle; *b*, thick-walled inclusions in the branches of the excretory vesicle; *c*, encysted cercaria as it appears after sudden pressure from a cover-glass; *d*, fully developed cercaria as it appears under slight pressure from a cover-glass.

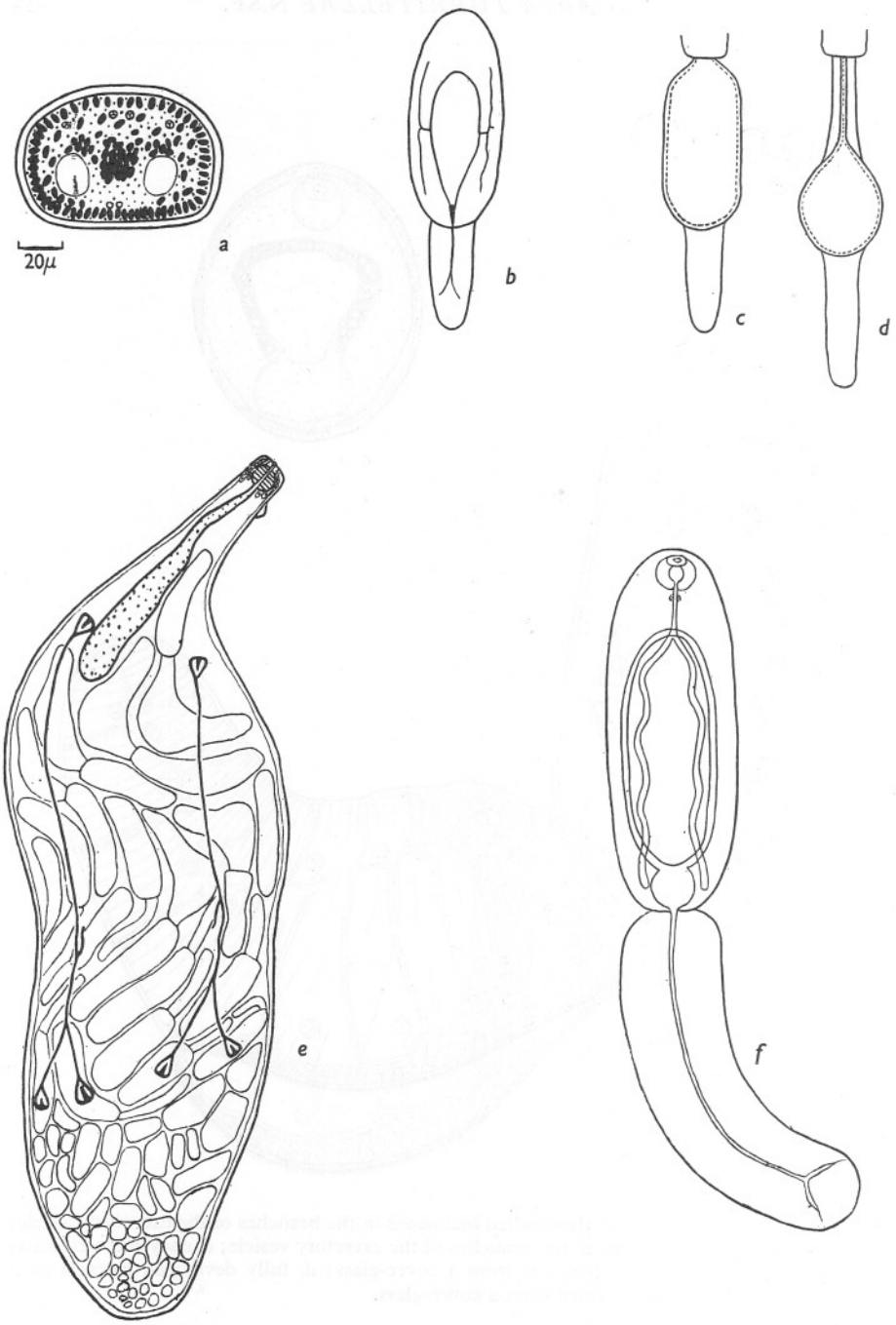

Fig. 2. *Cercaria turritellae*. *a*, transverse section (5 μ) through the body of a fully developed cercaria at a level slightly anterior to the excretory vesicle; *b*, view from ventral side of an immature cercaria showing the type branching of the excretory system in the body and in the tail; *c*, dorsal view of tail showing fin-like ridges as they appear when the tail is contracted; *d*, dorsal view of tail showing fin-like ridges as they appear when the tail is extended; *e*, mature redia showing germ balls, developing cercariae and the excretory system: *f*, immature cercaria showing the digestive tract and part of the excretory system.

half of its length. At that point they curve medially and join together at the mid-dorsal line of the body (Fig. 1*d*). While the cercariae are suspended in the water, the tails are slowly extended and contracted intermittently. This causes a change in the appearance of the fin-like ridges. Fig. 2*c* shows the shape of the ridges in a specimen with a contracted tail, while Fig. 2*d* is a specimen with an extended tail; both figures are views from the dorsal side.

In laboratory containers, immediately after being released from the molluscan host, members of this species rise in the water and float just below the surface film. Later, if the water remains quiet, the cercariae gradually settle to the bottom of the containers. Slight agitation of the water causes the larvae to rise and float for some time after the agitation stops.

A short time after being released, the cercariae begin to secrete a substance which forms large cocoon-like cysts enclosing the larvae completely. These cysts are composed of a thin substance that is translucent except for numerous scattered wart-like knobs which are composed of small rods similar to, but smaller than, those present within the cystogenous glands of the fully-formed cercaria. While within these cysts, the bodies and attached tails continue to extend and contract intermittently.

Sudden pressure of a cover-glass on unencysted cercariae just released from their molluscan host causes decaudation and encystment. These cysts (Fig. 1*c*) are approximately five to six times smaller than the cocoon-like structures previously mentioned. Cysts of ten living specimens produced in this manner measure 118–136 μ in length and 94–108 μ in width.

As was stated previously, there are underneath the cuticle of the body numerous cystogenous glands containing a number of refringent rods. The largest of these rods measure 12 μ in length and almost 2 μ in width. They are evidently cystogenous granules similar to those reported by Sinitsin (1911) and Sewell (1922), as well as by other writers. The cuticle of the body is pierced by numerous small openings, each of which appears to lead into a cystogenous gland.

In living fully formed cercariae well extended under pressure from a cover-glass, a structure evidently representing a small pharynx (Fig. 1*d*) is visible. Only in immature larvae (Fig. 2*f*), in which the cystogenous glands are not so well developed, are the digestive caeca distinguishable. They extend to the posterior end of the body and terminate lateral to the excretory vesicle.

On each side of the oesophagus, a pair of small ducts run forward and open to the exterior in front of the subterminal sucker. Although in serial sections the penetration glands could not be discerned, it seems likely that because of their position these ducts are penetration gland canals. Serial sections (5 μ) of several specimens stained with Ehrlich's haematoxylin and eosin reveal six ducts in addition to those of the lateral branches of the excretory vesicle; however, I was unable to follow their ultimate courses.

Large rounded inclusions within the branches of the excretory vesicle are

very conspicuous structures of mature cercariae. They do not occur in the vesicle itself and in the immature larvae are altogether absent. Usually they exhibit characteristic thick walls (Fig. 1*b*), but thin walls sometimes occur (Fig. 1*a*). Figs. 1*c*, *d* show the thick-walled inclusions as they appear under the dissecting microscope using incident light.

A special effort was made to study the details of the excretory system, but the refringent rods underneath the cuticle of the body obscured the pattern of the excretory system of the mature cercaria; however, there is a possibility that the flame cells occur in groups of three since such a group was observed near the oral sucker (Fig. 1*d*). Immature cercariae revealed the type-branching of the excretory system as shown in Fig. 2*b*. From each lateral branch of the vesicle, a single collecting tubule extends for a short distance before bifurcating into anterior and posterior branches. From the posterior end of the vesicle, a single median tubule passes through the tail and bifurcates as shown in Fig. 2*b*, *f*.

Of the two gastropods infected with *Cercaria turritellae* one was a male 29 mm. long while the other was a female 35·5 mm. long.

Discussion

Sinitsin (1911) described a binoculate monostome, *C. equitator*, with a huge tail. This species was described from *Cerithiolum exille* collected from the Black Sea. Miller (1925*a*) described a similar binoculate monostome, *Cercaria purpuracauda*. It was found in the digestive gland of *Bittium eschrichtii* collected from Puget Sound; later (1925*b* and 1929) he described very briefly four additional species (*Cercaria* F, *Cercaria* T, *Cercaria* U, *Cercaria* W) with eye-spots collected from the Dry Tortugas area. Cable (1952)[1] also reported a magnacercous monostome cercaria with eye-spots from *Turritella exoleata* collected from the marine waters of Puerto Rico. This cercaria, like those reported by Sinitsin and Miller, is an active swimmer. Cable believes this larva, from morphological and ecological observations, is a heterophid trematode belonging to the subfamily Galactosominae.

Cercaria turritellae n.sp. differs conspicuously from the above species by lacking eye-spots, by not being an active swimmer, by having a smaller proportion of tail length to body length (with the possible exception of *Cercaria* W since Miller did not give any measurements for this species) and by having fin-like ridges on the proximal part of the tail. Also, this cercaria differs from *C. equitator*, *C. purpuracauda* and the species reported by Cable by not having spines at the anterior end of the body. Large rounded inclusions which occur in the branches of the excretory vesicle of *C. turritellae* are very conspicuous in the fully formed larva; they have not been reported for other marine 'huge-tailed' monostome larvae.

[1] And personal communication.

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THE FUNCTIONAL MORPHOLOGY OF THE ROCK-BORING LAMELLIBRANCH *PETRICOLA PHOLADIFORMIS* LAMARCK

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(Text-figs. 1–16)

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Introduction

While the writer was engaged in a study of the British species of Pholadidae, an opportunity arose also to study *Petricola pholadiformis* Lamarck (see Winckworth, 1932), a rock-boring lamellibranch belonging to the Petricolidae. This species is of special interest as it is considered to exhibit convergent evolution with respect to *Barnea candida*, one of the British species of Pholadidae.

The specimens were obtained from Burnham-on-Crouch, Essex, where they were collected near low-water mark by Mr G. D. Waugh, to whom the writer wishes to express his gratitude. Living and preserved specimens of *Petricola pholadiformis* were studied in 1950 at the Department of Zoology and Comparative Anatomy, University College of South Wales and Monmouthshire, Cardiff.

The most important paper on *Petricola* is that of Lamy (1923), who reviewed the genus, describing *P. pholadiformis* Lamarck, and giving its synonyms. He described the distribution of this species on the Atlantic Coast of North America, and on the West Coast of Africa. He recorded its arrival in European waters, where it was observed on the coast of Kent in 1893. The genus is briefly mentioned by Sikes (1910), Calman (1919), Schlesch (1932), Calman & Crawford (1936), and Coe (1943). Other direct references to *Petricola* will be referred to in the appropriate places; wider references to literature are made in a paper on the British Pholadidae (Purchon, 1955).

The Siphons

The basal half of the siphonal process is formed by fusion of the inhalant and exhalant siphons (Fig. 4). The siphons are separate for their distal halves, the inhalant being slightly longer than the exhalant siphon (Morse, 1919). When fully extended, the siphons are a little longer than the shell valves. The siphons are formed solely from the inner lobes of the mantle margin, and are unprotected by periostracum. The middle and outer lobes of the mantle form a crescentic ridge passing round the base of the siphonal process, parallel to the edge of the shell (Figs. 4, 9, *Olm*), and giving rise to a thin transparent sheet of periostracum which passes to the margin of the shell valves. Thus the siphons are of type 'A' as defined by Yonge (1948).

The siphons of *P. pholadiformis* are smooth and the flesh is of an opaque creamy colour with irregular patches of light brown pigment distally.

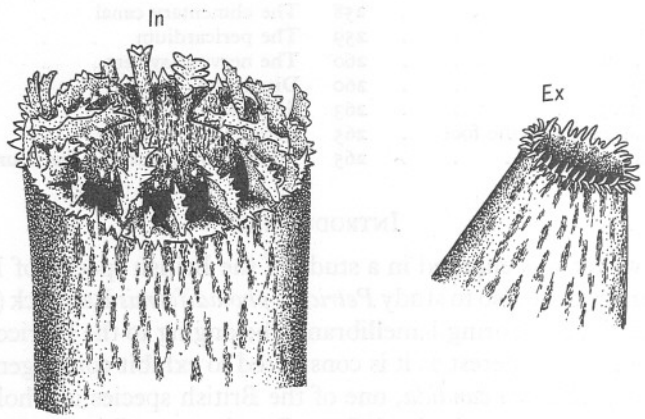

Fig. 1. *P. pholadiformis*, free-hand drawing of the tips of the siphons. Shell length 1·5 in. ×7·5.

The inhalant aperture is surrounded by a series of irregularly pinnate tentacles of various sizes (Fig. 1, *In*). These may be roughly classified as primary, secondary, tertiary and even quaternary according to size, the quaternary tentacles lying closest to the outer margin and being smallest and simplest. There was much variation from specimen to specimen in the sample studied, but there tended to be about six primary tentacles which normally projected across the inhalant aperture. There were about as many smaller secondary tentacles and, external to these, a larger number of still smaller and simpler tertiary tentacles. At the periphery, external to the twenty-four or more tertiary tentacles, there was a ring of about forty-eight small marginal papillae.

The primary, secondary and tertiary pinnate tentacles are very similar in form and pigmentation to the pinnate tentacles of *Zirphaea crispata* (see

Purchon, 1955). In addition, however, in *Petricola pholadiformis* there are occasional opaque white patches on the sides of the tentacles and on the inner surface of the inhalant siphon.

Unlike the inhalant siphon, the exhalant siphon tapers slightly towards its extremity which is similarly ornamented by irregular brown patches (Fig. 1, *Ex*). The exhalant aperture is surrounded by a variable number of small conical tentacles which tend to be arranged in two circles, the inner consisting of about twenty-four tentacles, some of which may be united at their bases in pairs. The outer ring consists of a larger number of smaller tentacles.

When observed in aquaria, the siphons were seldom extended far, the base of the siphonal process generally being partly introverted between the posterior ends of the shell valves. They undergo slight contractions and expansions which are possibly more pronounced under natural conditions.

The Shell Valves

The shell of *P. pholadiformis* superficially resembles that of *Barnea candida* (see Purchon, 1955) and the two species may be found burrowing side by side. *Petricola* may be readily distinguished, however, by the absence of accessory shell plates and by the presence of a conspicuous external ligament. Internally the differences between *Petricola* and *Barnea* are equally pronounced.

The hinge teeth are well developed (Figs. 2, 3, *Ht*) and these, together with the elongated external ligament (Fig. 2, *L*) will undoubtedly prevent any rocking of the shell valves about a vertical axis, as has evolved in the Adesmacea. The adductor scars are in the typical positions ventral to the hinge line, the anterior adductor scar being only a little less in area than that of the posterior adductor muscle.

In the shell drawn, the pallial sinus penetrated to 60% of the total shell length, a value of the same order as has been found in the Pholadidae (Purchon, 1955); *Petricola pholadiformis* is certainly well adapted for burrowing, if not for boring. There is no 'accessory ventral adductor' in *P. pholadiformis* (as is present in the Adesmacea), but there is an 'accessory dorsal adductor muscle', dorsal to the rectum (Fig. 14, *Apa*).

The habit of the specimens here studied has been described by Mr G. D. Waugh (personal communication) as boring in lumps of chalk which lie on the mud at low-water mark. In some cases specimens had bored through the chalk and their anterior ends had entered the underlying mud. *Petricola* is apparently only able to bore into very soft rocks, perforation being presumably by opening and closing of the shell valves on the hinge line as an axis. This view was held by Otter (1937) for *P. lapicida*, the burrow of which he described as oval in section.

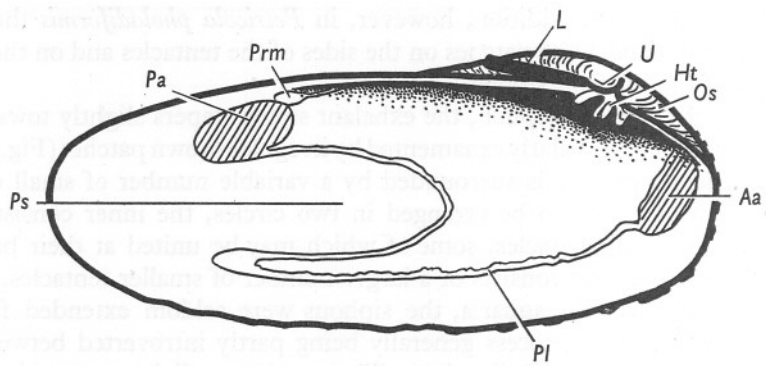

Fig. 2. *P. pholadiformis*, interior of the left shell valve. Shell length 2·3 in. × 1·5. For lettering see pp. 277–8.

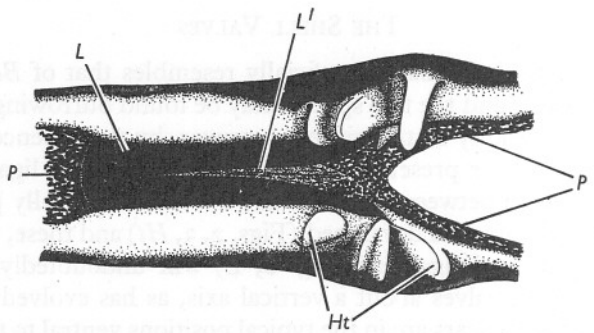

Fig. 3. *P. pholadiformis*. Ventral view of a portion of the hinge line of two shell valves, the valves being widely parted. The anterior end is to the right. Shell length 1·7 in. × 5·3. For lettering see pp. 277–8.

The Mantle Cavity

The disposition of the organs in the mantle cavity is shown in Fig. 4. Detailed description is not necessary. The most important features of the various organs will be outlined in the following sections.

The pedal gape is long, and amounts to about half of the length of the shell (Fig. 4). There is an annular backwardly directed valve in the bases of both the inhalant and the exhalant siphons (Fig. 4, *V*). According to Haas (1929–40) such valves occur in many families. Similar valves are present in *Pholadidea loscombiana* (see Purchon, 1955).

The Ctenida

The outer demibranch (Fig. 4, *Alod*) does not reach as far forwards as the inner demibranch, much of which is therefore exposed to the action of the sorting areas of the outer labial palp (*Rolp*). Particles carried forwards either

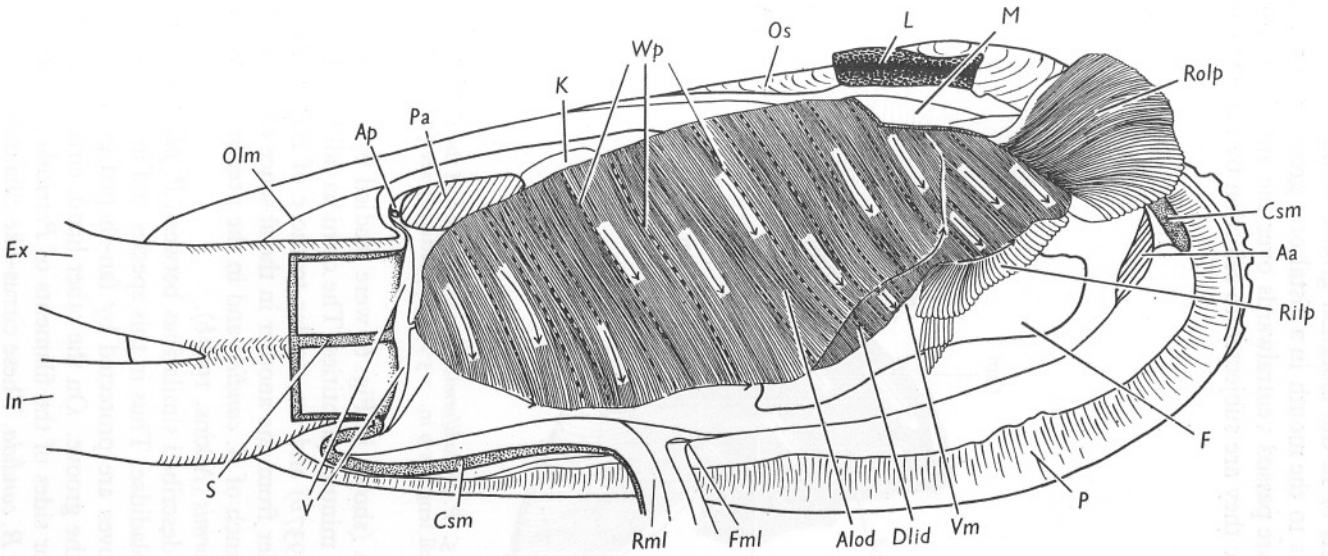

Fig. 4. *P. pholadiformis*. Organs in the mantle cavity. The right shell valve and most of the right mantle lobe have been removed. Shell length 1·9 in. ×3. For lettering see pp. 277–8.

in the ctenidial axis or in the marginal groove of the outer demibranch do not travel forwards to the mouth in a distal oral groove anterior to the inner demibranch, but are passed ventralwards over the anterior part of the inner demibranch where they are subjected to selection by the outer labial palp.

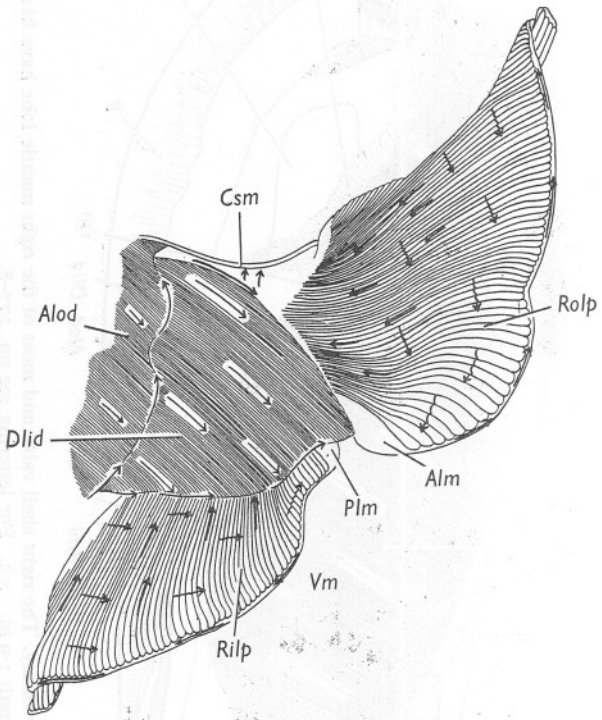

Fig. 5. *P. pholadiformis*. The labial palps of the right side. Shell length 1·9 in. × 5·25. For lettering see pp. 277–8.

Ciliary currents (shown in Fig. 6) were studied by the application of carborundum *F 3* in minute quantities. The ctenidia fall into Atkins's Category C (1*c*) (Atkins, 1937*b*) and are similar to those of *Barnea candida* (Atkins, 1937*a*). They differ from one another in the absence of a marginal groove in the outer demibranch of *B. candida* and in the presence of weak plication in *Petricola pholadiformis* (Atkins, 1937*b*).

Atkins (1937*a*) described similarities between *P. pholadiformis* and various species of the Pholadidae. Thus in this species and in *Pholadidea loscombiana* the marginal grooves are protected by fan-shaped groups of guarding cilia which arch over the groove. On the other hand, cirrus-like cilia are present along the posterior sides of the filaments of *Petricola pholadiformis* and both *Barnea parva* and *B. candida*. These cirrus-like cilia may aid in the removal of large particles of sand or rock which fall on the ctenidia.

There are a number of points in which *Petricola pholadiformis* resembles *Barnea candida*, the most remarkable of which is the presence of opposing ciliary currents on the filaments of the descending lamella of the outer demibranch (Fig. 6). The two species live side by side occasionally, e.g. at Burnham-on-Crouch, and the similarity between their ctenidia may reasonably be regarded as parallel adaptation to similar environmental conditions. This has been discussed by Purchon (1955).

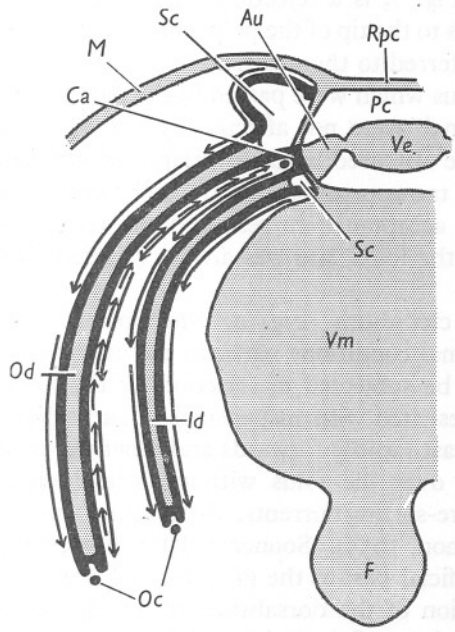

Fig. 6.

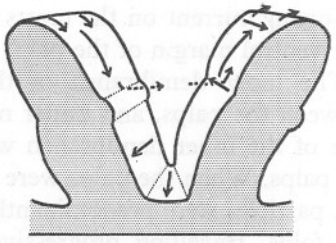

Fig. 7.

Fig. 6. *P. pholadiformis*, a thick hand-cut transverse section through the body in the region of the pericardium. × 10.

Fig. 7. *P. pholadiformis*. Diagrammatic representation of the ciliary pattern on the folds of the labial palps. Anterior side to the right.

For lettering see pp. 277–8.

The Labial Palps

The labial palps are extremely active, and are continually coiling and uncoiling as is indicated in Fig. 4, *Rilp*. The ridges on the opposed surfaces of the palps may be thrown violently into zigzags on the slightest mechanical stimulation of the palps.

The detailed ciliary pattern on the folds of the palps is shown in Fig. 7. The following ciliary currents may be recognized:

(1) *A rejection current* on the floor and sides of the groove between adjacent folds on the palp. Particles are driven to the free ventral border of the palp.

(2) *An acceptance current* operating on the superficial slopes and on the crests of the folds, carrying particles forwards from fold to fold, towards the mouth.

(3) *A ventralward re-sorting current* on the crest of each fold, carrying particles towards the ventral border of the palp.

(4) *A dorsalward re-sorting current* carrying particles in the superficial part of each groove, towards the base of the palp and the lateral oral groove.

In addition, and not shown in Fig. 7, is a rejection tract which conveys material from the grooves backwards to the tip of the palp along its free ventral border. This material is then transferred to the mantle.

Skeins of particles bound in mucus which were passed to the palp from the anterior end of the inner demibranch were not affected by the dorsalward re-sorting currents, since these were obscured by the overlapping of adjacent folds. Such material was rapidly transported anteriorly and ventralwards under the combined action of the acceptance current and the ventralward re-sorting current on the crests of the folds, and was always rejected via the free ventral margin of the palp.

The inner demibranch of the ctenidium (Fig. 4, *Dlid*) passes deeply between the palps, and under normal conditions particles moving over that part of the inner demibranch will be subjected to selection by the folds of the palps. When the palps were presented with suspensions of carborundum *F 3*, particles were predominantly cast rapidly forwards and downwards over the folds, travelling progressively over the folds without being deflected dorsalwards or ventralwards by the re-sorting currents. Such action was never to be seen in the Pholadidae (Purchon, 1955). Sooner or later, however, the particles were trapped in the superficial part of the grooves, and were transported dorsalwards due to the action of the dorsalward re-sorting current. These particles accumulated at the base of the palp, and together with any particles brought thither by the ctenidial axis, passed into the lateral oral groove and thence rapidly to the mouth where they were ingested. Very little entered the deepest part of the grooves to be rejected via the free ventral border of the palp.

In the specimens studied, there was no suggestion of intermittent feeding, as was noted in the Pholadidae (Purchon, 1955), and it was concluded that the palps were far less selective than those of the Pholadidae.

It appears that the palps accept fine and medium-sized particles and convey them to the mouth, but reject mucus-bound material such as is likely to be presented to the palps when the water contains much suspended material.

On the outer smooth surfaces of the palps the cilia beat dorsalwards, and particles were carried over the postero-dorsal free margin of the palps and on to the ridged inner surfaces.

The Visceral Mass and the Foot

The foot is plough-shaped, and lies along the whole ventral border of the visceral mass (Fig. 4, *F*). A broad, apparently unciliated area on the side of the visceral mass (Fig. 8, *Ia*) corresponds in shape and position with that of the inner labial palp, while no ciliary activity could be detected on the sides of the foot. Over the postero-dorsal half of the visceral mass, the ciliary currents carried particles ventralwards, and into posteriorward rejection tracts which carried all material on the sides of the visceral mass to its posterior end. Particles falling on the sides of the visceral mass will presumably be cleared by the labial palps.

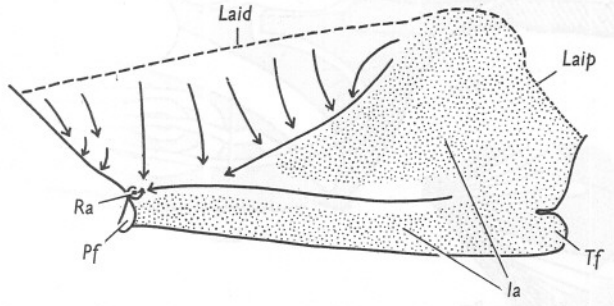

Fig. 8. *P. pholadiformis*. Ciliary currents on the right side of the visceral mass. Shell length 2·3 in. × 2·7. For lettering see pp. 277–8.

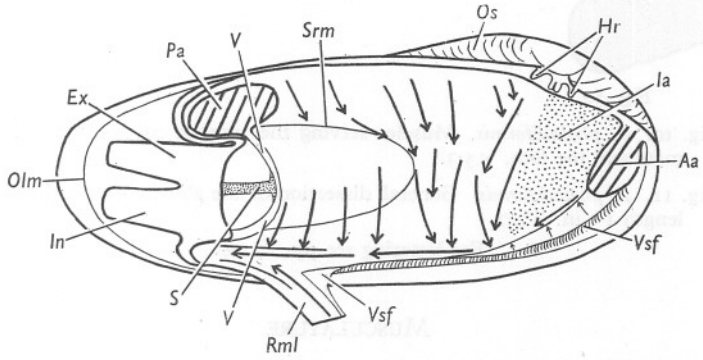

Fig. 9. *P. pholadiformis*. Ciliary currents on the inner surface of the left lobe of the mantle. Shell length 1·9 in. × 1·35. For lettering see pp. 277–8.

The Mantle

In order to study the ciliation of the inner surface of the mantle, the visceral mass and ctenidia were removed and the preparation was left for some hours to allow the muscles to relax and the siphonal process to expand somewhat. There is a broad triangular area anteriorly, which is apparently unciliated and which corresponds in shape and position with that of the outer labial palp (Fig. 9, *Ia*).

Over the greater part of the mantle the ciliary action is ventralwards, carrying particles towards the margin of the mantle. There is a powerful rejection tract near the mantle margin. This arises near the mouth, passes backwards on the side of the pedal gape, and joins with its partner from the other side of the body posterior to the pedal gape. Under experimental conditions, waste material collects in a small pocket at the base of the siphonal process; it is not certain whether this would or would not occur under natural conditions.

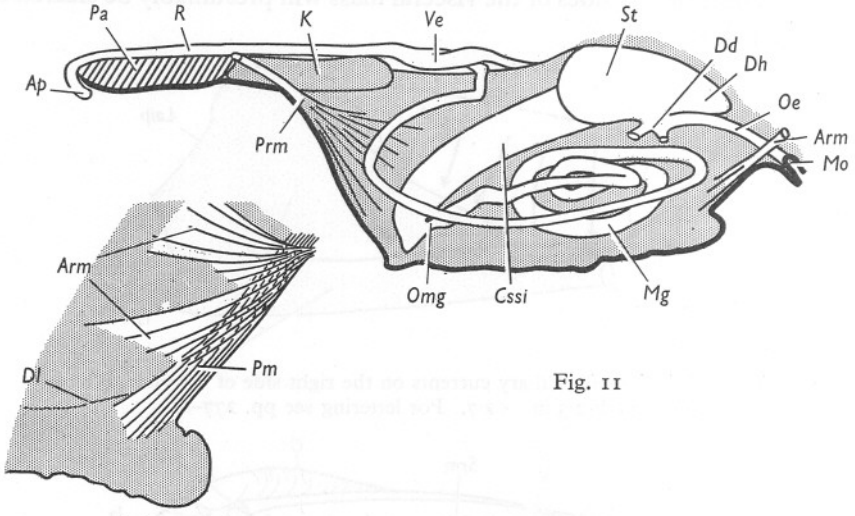

Fig. 11

Fig. 10

Fig. 10. *P. pholadiformis*. Muscles serving the anterior part of the visceral mass and the foot. × 5·3.

Fig. 11. *P. pholadiformis*. General dissection of the alimentary canal. Shell length 1·9 in. × 2·7.

For lettering see pp. 277–8.

Musculature

Unlike the Pholadidae (Purchon, 1955), the anterior adductor scar of *Petricola pholadiformis* is smaller in area than that of the posterior adductor, being 70% of the area in the shell drawn (Fig. 2). Another difference is the position of the anterior adductor scar ventral to the hinge line. There is an 'Accessory Posterior Adductor Muscle', which lies anterior to the posterior adductor and *dorsal* to the rectum (Fig. 14, *Apa*).

The anterior retractor muscle of the visceral mass. This arises as a flattened sheet a little posterior to the anterior adductor (Fig. 10, *Arm*). Its fibres spread out immediately below the epidermis on the antero-dorsal surface of the visceral mass.

The posterior retractor muscle of the visceral mass. This arises anterior to the posterior adductor (Figs. 2, 11, *Prm*), and it is more powerfully developed than that of the Pholadidae. At the postero-dorsal angle of the visceral mass the left and right muscles meet and a proportion of fibres cross over from side to side.

Pedal retractor muscle. This arises immediately behind the anterior adductor, and internal to the base of the anterior retractor of the visceral mass; it is a stout bundle, elliptical in section (Fig. 10, *Pm*). The muscle passes downwards along the anterior face of the visceral mass and as it approaches the muscular tissues of the foot it spreads out posteriorly, and there is a mingling of fibres from left and right sides as the two muscles enter the foot.

Transverse muscle fibres in the visceral mass. Here and there, transverse muscle fibres arise in the epithelium of the visceral mass and pass inwards either to the walls of the alimentary canal or transversely across the visceral mass to the opposite side. No such muscle bundles were found in the Pholadidae, though a few muscle fibres were found in *Zirphaea crispata* inserted into the sides of the oesophagus and the anterior face of the stomach (Purchon, 1955).

The Alimentary Canal

A general dissection of the alimentary canal was carried out on specimens that had been relaxed with menthol and magnesium sulphate, and had then been preserved in alcohol (Fig. 11). The oesophagus enters the ventral wall of the stomach and its base is overlapped by an outgrowth of the stomach, the dorsal hood (Fig. 11, *Dh*). The apertures from the stomach into the digestive diverticula (*Dd*) lie on the ventral surface of the stomach. The combined style sac and mid-gut, the cavities of which are incompletely separated by a typhlosole, arise on the postero-ventral surface of the stomach and pass backwards and downwards towards the heel of the foot. The mid-gut passes from the distal end of this wider tube, coils on the ventral side of the stomach and then passes backwards and upwards to the pericardium. The hind-gut penetrates the ventricle, passes ventral to the accessory posterior adductor (Fig. 14, *Apa*), as noted in the previous section, and terminates in an anal papilla in the typical manner.

The internal structure of the stomach was studied on living specimens. The epithelium covering the visceral mass was removed and the stomach exposed by scraping away gonadial tissues, and by loosening the mass of digestive diverticula. A mid-dorsal incision was made in the stomach wall, and the right side of the stomach was drawn downwards. The dorsal hood was opened, giving a view comparable to that shown in Fig. 12, in which the oesophageal aperture is hidden from view by the anterior part of the gastric shield (*Gs*).

The dorsal hood arches forwards above the oesophagus and opens into the stomach anteriorly on its right side (*Odh*). Its ventral wall is covered with a finely ridged sorting area (Fig. 12, *Sa*). Here cilia beat transversely over the

crests of the folds towards the apex of the hood on the left-hand side (*Dh'*), whereas in the grooves the cilia beat backwards and finally reject material via the mouth of the hood (*Odh*) into the intestinal groove (*Gi*).

The roof of the dorsal hood is smooth, and ciliary currents on it pass material backwards, over the right side of the stomach and into the mid-gut. The posterior wall of the dorsal hood bears a minor sorting area (*Sa'*) which passes out of the mouth of the dorsal hood, and across the anterior face of the

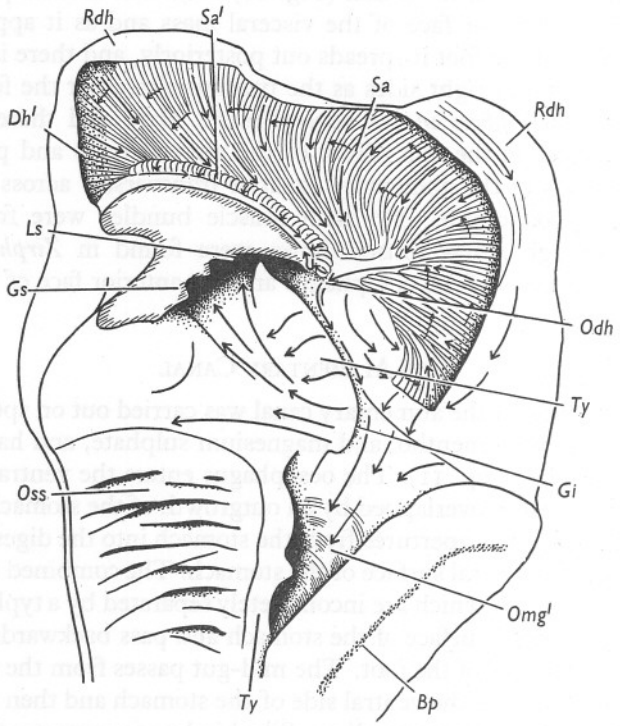

Fig. 12. *P. pholadiformis*. Interior of the stomach. The stomach has been opened by a mid-dorsal incision through the roof of the stomach and style sac, and through the roof of the dorsal hood. Shell length 1·9 in. ×9. For lettering see pp. 277–8.

stomach below the oesophageal aperture (Fig. 13, *Sa'*, *Sa''*). It probably serves to carry particles from the anterior face of the stomach into the dorsal hood.

The left pouch of *Petricola pholadiformis* can be exposed by cutting transversely to the left through the gastric shield (Fig. 12, *Gs*). The sides and floor of the left pouch can now be seen, as also the aperture of the oesophagus into the stomach (Fig. 13). Reference to this figure shows that there is a slender belt of transverse ridges on the roof of the left pouch, there is an extensive sorting area (*Sa'''*) on the floor of the left pouch, and there are also three ducts

leading from the left pouch into the digestive diverticula (*Ddd*). It is important to note that the ciliary currents detected in the left pouch do not assist particles to pass into the ducts of the digestive diverticula—on the contrary, the reverse is true.

The major typhlosole (Fig. 13, *Ty'*) arises in the left caecum (*Ddl*), crosses the anterior face of the stomach ventral to the oesophageal aperture and enters the mouth of the right caecum (*Ddr*). It emerges from the right caecum and travels posteriorly along the right side of the floor of the stomach (*Ty*), accompanied all the way by the intestinal groove (*Gi*) and passes into the mid-gut (Fig. 12, *Omg'*).

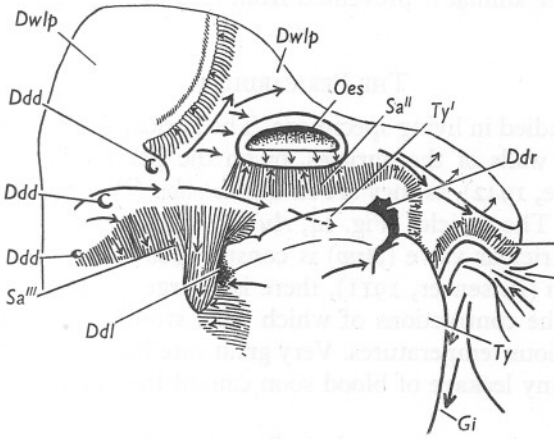

Fig. 13. *P. pholadiformis*. A dorsal view of the anterior part of the stomach, the roof of the left pouch having been cut and turned forwards. × 8. For lettering see pp. 277–8.

The sorting areas described above, in the left pouch, on the anterior face of the stomach, and in the dorsal hood, appear to be expressly designed to hinder the passage of particles from the stomach into the ducts of the digestive diverticula, and to reject any particles captured into the intestinal groove. This applies as much to the ducts of the digestive diverticula which open via the left caecum and the right caecum, as to the ducts that open into the stomach via the left pouch.

Within the mouths of the left and right caeca, the cilia beat inwards, and will carry particles into the ducts of the digestive diverticula. There is no definite ciliary route carrying particles to the left caecum, but particles rejected from the left caecum via the intestinal groove are carried into the right caecum. The intestinal groove finally removes particles rejected by the right caecum, and passes them into the mid-gut.

It appears probable therefore that, as previously noted for the Pholadidae, food material cannot be passed from the stomach into the digestive diverticula solely by ciliary activity. Probably muscular activity is also involved. Changes

in the relative volumes of the stomach and the various parts of the digestive diverticula, their ducts and the left and right caeca, could cause stomach contents to be sucked or injected into the digestive diverticula.

Such volumetric changes might be caused by action of the adductor muscles, and by movements of the foot by the pedal muscles. The walls of the stomach may also contribute to movement of the stomach contents. Considerable muscular activity was noted in the wall of the living stomach of *P. pholadiformis*.

It has not been possible to study the action of the crystalline style in the stomach of *P. pholadiformis*. Where the style sac is not completely separated from the lumen of the mid-gut, as in this species, the crystalline style readily dissolves when the animal is prevented from feeding (Yonge, 1926).

The Pericardium

The heart was studied in living specimens. The pericardial glands, which may either lie on the walls of the auricles, or on the pericardial walls, are here pericardial (White, 1942), as they are in *Choristodon* (*Petricola*) *lapicidum* (see Pelseneer, 1911). The auricles (Fig. 14, *Au*) are triangular with thin walls and the auriculo-ventricular valve (*Avv*) is conspicuous by its opacity and size. As in *Choristodon* (Pelseneer, 1911), there is a large aortic bulb (*Ab*) on the posterior aorta, the contractions of which were studied on freshly captured specimens, at various temperatures. Very great care had to be exercised in the dissection since any leakage of blood soon caused the aortic bulb to collapse permanently.

In dissected specimens the aortic bulb remained inactive as long as the siphons remained withdrawn, but a short time after the work of dissection was completed the siphons were usually protruded slightly and the aortic bulb then became active. After some time, due probably to the gradual loss of blood, the activity of the aortic bulb decreased, and it diminished in size. In Fig. 14 the ventricle is drawn in a contracted state, and the aortic bulb in a moderately expanded state.

By subjecting animals to variations in water temperature, from about 6° C to about 30° C, the rate of beat of the ventricle was found to increase with increasing temperature, but the rate of beat of the aortic bulb was unrelated to temperature. Each beat of the aortic bulb was related to movements of the siphons, which were not related to change in temperature.

The aortic bulb evidently acts both as a safety valve, protecting the ventricle from the effects of a sudden flow of blood from the siphons along the posterior aorta, and also as a reservoir which can quickly supply blood for the subsequent expansion of the siphons. Siphonal contraction and expansion involve considerable movements of blood, and the value of an aortic bulb to a bivalve with very active siphons is obvious.

Specimens of *Scrobicularia plana* were dissected for comparison. This

species, which feeds on bottom deposits by means of its inhalant siphon, has very long, separate, and active siphons (Yonge, 1949). As was expected, a large and active aortic bulb was found on the posterior aorta in this species. An animal such as *Petricola pholadiformis*, living in a burrow, and possessing siphons that are united at their bases, may not have to execute very extensive contractions of the siphons in order to bring them within the protection of the burrow. The retention of an active aortic bulb, therefore, may perhaps be taken to indicate that this form has recently taken to a rock-boring mode of life.

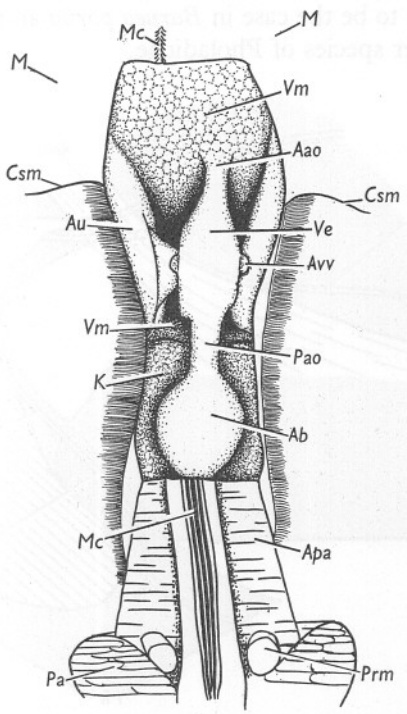

Fig. 14. *P. pholadiformis*. Dorsal view of the contents of the pericardium. Shell length 1·9 in. ×4·5. For lettering see pp. 277–8.

In the Pholadidae, on the other hand, the slightest contraction of the tips of the siphons carries them into the protection of the burrow. Frequent and extensive contractions of the siphons are less likely to occur, and it is not surprising to find that an aortic bulb, if present, is not developed to the same extent as in the case of *P. pholadiformis*. The Pholadidae may be regarded as being more highly adapted to a rock-boring mode of life.

Pelseneer (1906) noted that such highly developed aortic bulbs on the posterior aorta are commonly found in siphonate lamellibranchs. Large aortic bulbs occur in the Veneridae, Petricolidae and Mactridae, for example,

and also in the Tridacnidae. Although these last are not siphonate, they probably have similar problems in controlling the movement of blood to and from the mantle whenever the shell valves contract or the mantle margins are withdrawn.

The excretory organ of *P. pholadiformis* (Fig. 14, *K*) lies anterior to the posterior adductor muscle, being overlaid by the aortic bulb which presses slightly into its dorsal surface. It resembles that of the Pholadidae (Purchon, 1955). There is an anterior oval aperture in the median line, causing the cavities of the distal limbs of the left and right excretory organs to communicate. This was found to be the case in *Barnea parva* and in *Zirphaea crispata*, but not in three other species of Pholadidae.

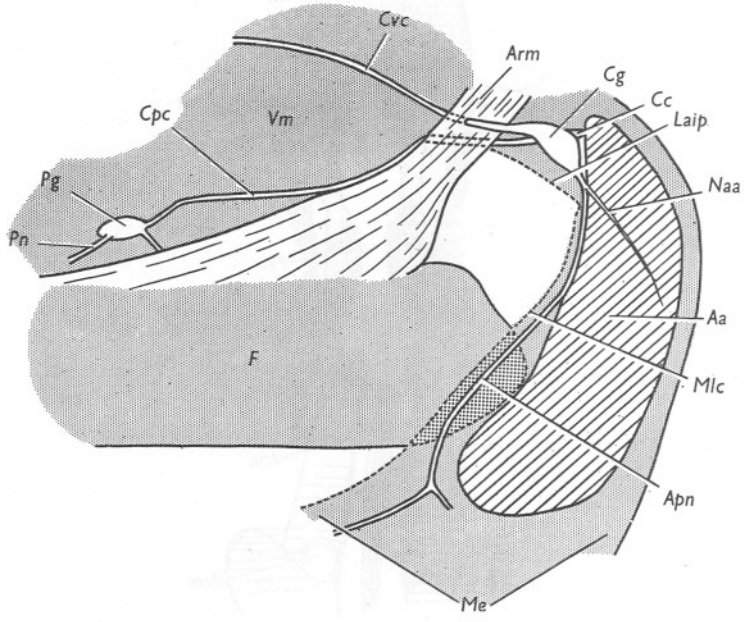

Fig. 15. *P. pholadiformis*. General dissection of the anterior part of the nervous system. Seen from the right side. Shell length 1·8 in. ×9. For lettering see pp. 277–8.

The Nervous System

The cerebral ganglia are of simple fusiform shape and they lie embedded in a tough fibrous matrix, anterior to the anterior retractor muscle of the visceral mass (Fig. 15, *Cg*). The cerebral commissure (*Cc*) passes transversely, close to the postero-dorsal margin of the anterior adductor muscle. The cerebro-pedal connective (*Cpc*) passes backwards internal to the base of the anterior retractor muscle of the visceral mass (*Arm*) and enters the anterior face of the pedal ganglia. The cerebro-visceral connective penetrates the base of the

anterior retractor muscle and then passes backwards within the substance of the visceral mass, not close to the epithelium.

The pedal ganglia are large and rounded, and can easily be dissected free from the surrounding tissues. Two large pedal nerves pass out ventrally into the muscles of the foot.

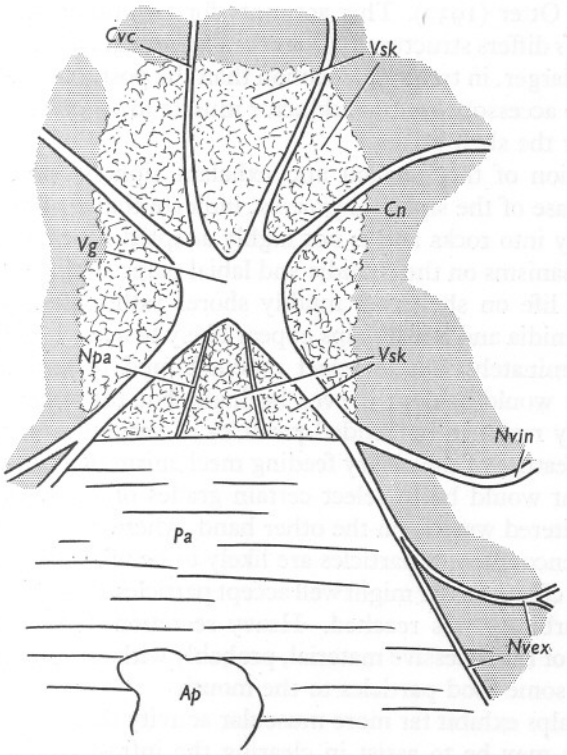

Fig. 16. *P. pholadiformis*. Ventral view of the posterior part of the nervous system. Shell length 1·9 in. ×9. For lettering see pp. 277–8.

The visceral ganglia lie, not on the ventral surface of the posterior adductor muscle, but a little further forward on the ventral surface of the excretory organ (Fig. 16, *Vg*). The yellowish ganglia are easily cleared from surrounding tissues. There is no transverse connective between the cerebro-visceral connectives anterior to the visceral ganglia, as is found in the rock-boring Pholadidae (Purchon, 1955). The following nerves leave the visceral ganglia: a ctenidial nerve (*Cn*), a nerve to the posterior adductor muscle (*Npa*) and a nerve to the siphonal process. The last-named nerve divides into two branches, one serving the inhalant siphon (*Nvin*) and one the exhalant siphon (*Nvex*). Each of these subdivides further in the vicinity of the annular valve in the base of the siphon which it serves. There is no macroscopic indication of a siphonal ganglion on the course of the siphonal nerve.

Discussion

The presence of a large external ligament and of well-developed hinge teeth in *Petricola pholadiformis* indicates that the only motion of the shell valves can be by normal opening and closing about the hinge line as an axis, as shown for *P. lapicida* by Otter (1937). This accounts for two other features in which *P. pholadiformis* differs structurally from the Pholadidae: the anterior adductor is smaller, not larger, in transverse section than the posterior adductor muscle, and there is no accessory ventral adductor muscle to secure a ventral point of articulation for the shell valves.

The separation of the inhalant and exhalant siphons, and the ability to introvert the base of the siphonal process, indicate that *P. pholadiformis* does not bore deeply into rocks and is not highly adapted for rock-boring.

Ciliary mechanisms on the ctenidia and labial palps confirm that the species is adapted for life on sheltered, muddy shores rather than on open rocky coasts. The ctenidia and labial palps appear likely to accept particulate matter rather indiscriminately, but to reject mucus-bound material. On an open coast turbidity would seldom if ever be high, but turbulence of the water would probably result in suspended particles of various sizes being present. The essential feature of the ciliary feeding mechanism of an organism in such an environment would be to select certain grades of particles and to reject others. In sheltered waters, on the other hand, where extreme turbidity may be frequently encountered, particles are likely to be of small size. In such an environment, a ciliary feeder might well accept particles almost indiscriminately until excess turbidity was reached. Heavy secretion of mucus would then cause rejection of the excessive material, probably without completely stopping the passage of some food particles to the mouth.

The labial palps exhibit far more muscular activity than those of the Pholadidae, and this may be to assist in clearing the infra-branchial chamber of excessive quantities of mucus-bound material.

The stomach of *P. pholadiformis*, though oriented differently, is of the same fundamental plan as that of the Pholadidae, and it functions in the same manner. The only significant anatomical differences are the anterior displacement of the dorsal hood, and the conjoined style sac and mid-gut in *P. pholadiformis*. As noted elsewhere for the Pholadidae (Purchon, 1955), ciliary sorting mechanisms within the stomach appear to collect particles for passage into the mid-gut; they would seem to hinder passage of food particles into the ducts of the digestive diverticula. This may be brought about by a muscular pumping or suction action, by relative changes in the volumes of the stomach and of the digestive diverticula.

The bulbus on the posterior aorta appeared at first sight to be directly related to the ventricle in its mode of action, but this was later found not to be the case. Frequency of ventricular contraction was found to be directly related to the temperature, whereas that of the bulbus was related, not to the

water temperature, but to the movements of the siphons. It seems probable that the bulbus acts as a safety valve, protecting the ventricle from sudden return of blood from the contracting siphons. It also probably acts as a reservoir from which blood may flow into the siphons when these are extended again.

P. pholadiformis, as its name implies, has been considered to exhibit some degree of convergent evolution with reference to the Pholadidae, and especially with regard to *Barnea candida* (L.) which it resembles superficially, and with which it may occur side by side on the same beach.

During the present study over thirty anatomical points were noted in which *Petricola pholadiformis* differs from all of the British Pholadidae. There can be no doubt that the Pholadidae and the Petricolidae are unrelated. Nevertheless, there are points of resemblance, no less than fourteen of these being noted in which *P. pholadiformis* resembles one or more species of the Pholadidae. But from the point of view of illustrating convergent evolution through adaptation to a rock-boring mode of life only three of these need to be considered, namely:

(*a*) Fan-shaped groups of guarding cilia arch over and protect the marginal grooves of the ctenidium (compare *Pholadidea loscombiana*).

(*b*) Cirrus-like cilia are present along the posterior sides of the ctenidial filaments (compare *Barnea parva*, *B. candida*).

(*c*) The ciliary currents on the ctenidia are of Atkins's type C (1*c*) (Atkins, 1937*b*) (compare *Barnea candida*). An opinion has been expressed elsewhere (Purchon, 1955) that this ciliary pattern on the ctenidia of *B. candida* and *Petricola pholadiformis* has been achieved by simplification of the ciliary mechanisms on three of the ctenidial lamellae and not by complication of the ciliary mechanisms on the fourth lamella.

It seems probable that there must have been some measure of adaptive radiation in the Pholadidae, and also, doubtless, a certain amount of evolutionary 'drift' in each species. Due to variation in structure from species to species within the Pholadidae, it seems inevitable that there should be fortuitous similarities in structure between *P. pholadiformis* and individual members of the Pholadidae. Though the superficial resemblance is to *Barnea candida*, similarities also exist between *Petricola pholadiformis* and other members of the Pholadidae, but these similarities appear to be fortuitous.

Summary

Living specimens of *Petricola pholadiformis* Lamarck were obtained from Burnham-on-Crouch, Essex.

Studies of the principal organ systems were made on the living animal, particular attention being paid to the ciliary feeding and cleansing mechanisms in the mantle cavity, and the ciliary sorting mechanisms within the stomach. The action of the bulbus on the posterior aorta was also studied.

A detailed comparison was made between *P. pholadiformis* and all the British species of Pholadidae. Thirty anatomical features were found in which *P. pholadiformis* differed from *all* of the Pholadidae, and fourteen features in which *P. pholadiformis* resembled one or more species of Pholadidae. It was concluded that these resemblances were purely fortuitous.

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Abbreviations used in the Figures

Aa	Anterior adductor (muscle, or scar)
Aao	Anterior aorta
Ab	Aortic bulb
Alm	Anterior lip of the mouth
Alod	Ascending lamella of the outer demibranch
Ant	Anterior
Ap	Anal papilla
Apa	Accessory posterior adductor muscle
Apn	Anterior pallial nerve
Arm	Anterior retractor muscle of the visceral mass
Au	Auricle
Avv	Auriculo-ventricular valve
Bp	Brown pigment on the wall of the style sac
Ca	Ctenidial axis
Cc	Cerebral commissure
Cg	Cerebral ganglion
Cn	Ctenidial nerve
Cpc	Cerebro-pedal connective
Csm	Cut surface of the mantle
Cssi	Combined style sac and mid-gut
Cvc	Cerebro-visceral connective
Dd	Openings into the digestive diverticula of the right side
Ddd	Ducts of the digestive diverticula
Ddl	The left caecum, into which open the majority of the ducts of the digestive diverticula on the left side of the body
Ddr	The right caecum, into which open all of the ducts of the digestive diverticula on the right side of the body
Dh	Dorsal hood
Dh′	Distal end of the dorsal hood
Dl	Dividing line between the musculature of the foot and the glandular visceral mass
Dlid	Descending lamella of the inner demibranch
Dwlp	Dorsal wall of the left pouch
Ex	Exhalant aperture
F	Foot
Fml	Anterior limit to the fusion of the mantle lobes
Gi	Intestinal groove
Gs	Gastric shield
Ht	Hinge teeth
Ia	Areas of ciliary inactivity
Id	Inner demibranch
In	Inhalant aperture
K	Excretory organ
L	Ligament
L′	Split in the ligament
Laid	Line of attachment of the inner demibranch
Laip	Line of attachment of the right inner labial palp
Ls	The left pouch of the stomach
M	Mantle
Mc	Median mantle crest
Me	Thickened mantle edge
Mg	Mid-gut
Mlc	Line along which the right mantle lobe has been cut away
Mo	Mouth
Naa	Nerve to the anterior adductor muscle
Npa	Nerve to the posterior adductor muscle
Nvin	Nerve to the muscles and valve of the inhalant siphon
Nvex	Nerve to the muscles and valve of the exhalant siphon
Oc	Oralward current
Od	Outer demibranch
Odh	Opening of the dorsal hood into the stomach
Oe	Oesophagus
Oes	Opening of the oesophagus into the stomach
Omg	Origin of the mid-gut at the base of the style sac
Omg′	Origin of the mid-gut from the stomach
Olm	Outer and middle lobes of the mantle edge
Os	Outer surface of shell
Oss	Opening of the style sac into the stomach
P	Periostracum
Pa	Posterior adductor (muscle, or scar)
Pao	Posterior aorta
Pc	Pericardium
Pf	Posterior end of the foot
Pg	Pedal ganglia
Pl	Pallial line
Plm	Posterior lip of the mouth
Pm	Pedal retractor muscle
Pn	Pedal nerves
Post	Posterior
Prm	Posterior retractor muscle of the visceral mass (muscle, or scar)
Ps	Pallial sinus
R	Rectum
Ra	Region where small particles accumulate

Rilp Right inner labial palp
Rml Portion of right mantle lobe, turned downwards
Rdh Roof of the dorsal hood of the stomach, turned outwards
Rolp Right outer labial palp
Rpc Roof of the pericardium
S Septum in the siphonal process
Sa Sorting area which extends from the interior of the dorsal hood to the intestinal groove
Sa′ Additional sorting area within the dorsal hood, which passes out towards the oesophagus
Sa″ Extension of *Sa′* which lies ventral to the opening of the oesophagus into the stomach
Sa‴ Sorting area within the left pouch of the stomach
Sc Supra-branchial chamber
Srm Line of attachment of the siphonal retractor muscles to the shell
St Stomach
Tf Tip of the foot, greatly contracted
Ty Major intestinal typhlosole
Ty′ The part of the major intestinal typhlosole which passes transversely across the stomach from the aperture of the left caecum to that of the right caecum
U Umbo
V Valves in the base of the exhalant and inhalant siphons
Ve Ventricle
Vg Visceral ganglia
Vm Visceral mass
Vsf Velum surrounding the foot
Vsk Ventral surface of the excretory organ
Wp Weak plication of the ctenidia

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CAROTENOID PIGMENTS IN THE OPTIC CUSHION OF *MARTHASTERIAS GLACIALIS* (L.)

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(Text-figs. 1–3)

Knowledge of the pigments of photosensitive structures in invertebrates is scanty; this is especially so in echinoderms. In other groups of animals and in plants, carotenoids have frequently been associated with light-sensitive processes (Wald, 1945), though their precise function is still uncertain.

Observations on the occurrence and distribution of carotenoids in various starfishes have been made by several authors whose work is reviewed by Fox (1953). Those in the integument of *Asterias* have recently been described by Vevers (1952), but, apart from the work of Lönnberg & Hellström (1931), and Lönnberg (1933), there have been no recent studies on the carotenoids of *Marthasterias*.

A study of the carotenoids in the light sensitive areas of echinoderms is therefore highly desirable, and the prominent and accessible 'eye spots' of *M. glacialis* (L.) form an obvious choice for such studies, although their functioning as photoreceptors has not yet been demonstrated experimentally in this species.

Several workers, especially Smith (1937), who reviewed the earlier work, have studied the structure of the 'eye spots' in this animal, but no special attention has been given to their pigments. In view of the possible importance of these pigments in visual physiology, it was decided to examine them in living material.

Distribution of Pigment

Individuals were obtained from trawl hauls taken in 20–30 fathoms off Plymouth and their optic cushions excised and examined under the microscope in sea water either directly, or in the form of frozen sections, in order to determine the distribution of pigment. It is essential to avoid the use of fixatives or processes causing shrinkage (as shown by Fox & Millott, 1954), or denaturation, and of solvents which leach out the pigment. Paraffin sections could not, therefore, be used.

The appearance of the excised optic cushion with its pigments is shown in Fig. 1. The main anatomical and histological features of the organ (as they

appear in fixed material), have already been described by Smith (1937) and will be mentioned here only in so far as they differ from previous accounts and are essential to understanding pigment distribution. In general form the optic cushion resembles a saddle astride the base of the azygos tentacle, measuring 1·5 mm in length in an average-sized specimen. It bears about 150 deeply pigmented cups (*o.c.*) disposed regularly in rows. Each cup is orientated with its long axis normal to the surface of the cushion, and most measured some 150 μ in length.

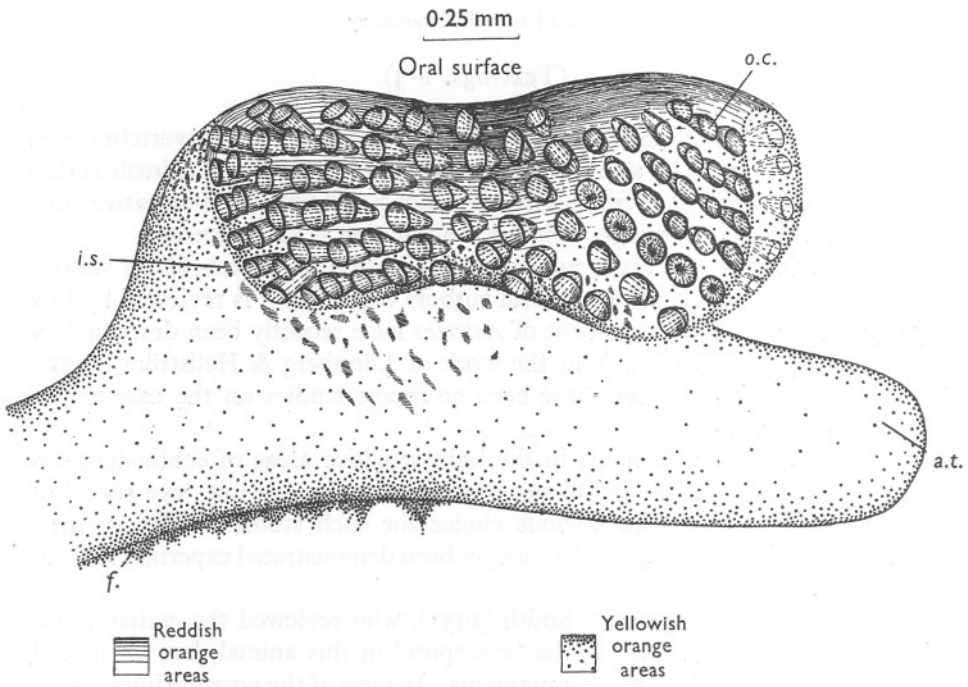

Fig. 1. The optic cushion and azygos tentacle of *Marthasterias glacialis* as it appears when dissected from the arm tip. Semi-diagrammatic, side view, looking obliquely from the tip of the arm. *a.t.*, azygos tentacle; *f.*, fringe marking region where the cushion has been dissected away from the ambulacrum; *i.s.*, one of the scattered, isolated spots of reddish orange pigment; *o.c.*, optic cup.

The cups were called 'optic cups' by Smith, and though their functioning as photoreceptors has not been demonstrated directly, their structure and experimental evidence of the function of similar organs in other starfish justify at least tentative retention of the term.

The cushion and azygos tentacle is yellowish orange, whilst the cups are very conspicuous owing to their deep reddish orange colour. The latter pigment also occurs on the cushion in the form of irregular scattered spots (*i.s.*).

In each cup, as shown in Fig. 2, the pigment is confined to certain cells (*p.c.*) in the wall, which as a result appears brilliant reddish orange in life; the cells at the bottom and around the rim of the cup appear to contain the most pigment. Between the reddish orange cells the wall of the cup appears pale yellow, and in these areas no cellular structure was discernible. It is essential to be cautious in interpreting this appearance, for it might be an artifact, the yellow areas being due to gaps between the deeply pigmented cells,

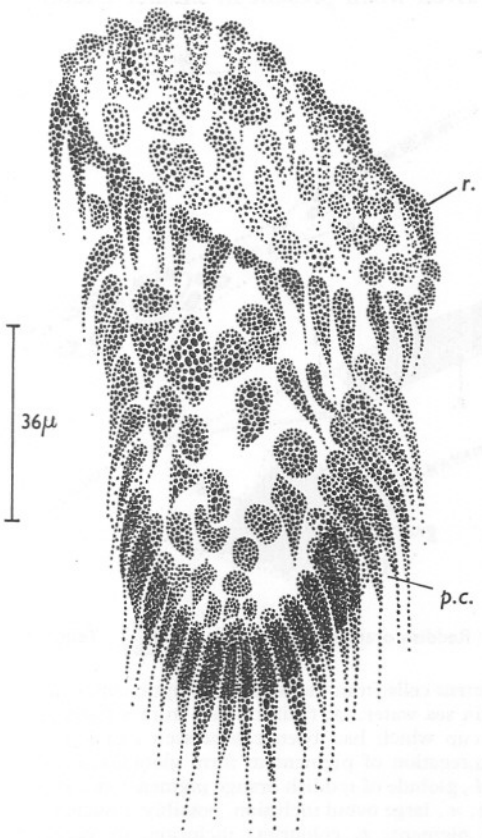

Fig. 2. The distribution of pigment cells in a single optic cup. Drawn from a fresh, unstained optic cushion mounted in sea water. No attempt has been made to show the precise character of cell contents. *r.*, rim of cup; *p.c.*, pigment cell.

caused by compression of the optic cup by the coverslip during examination. That this is not so is shown by the fact that such areas were observed in fresh cups examined by a water immersion objective and which were not, therefore, subjected to pressure.

Fig. 3 shows the form of the cells bearing the reddish orange pigment. Most are long and narrow with one end drawn out (Fig. 3A); some are irregular in

form (Fig. 3 C, D) though still attenuated. Within the cell the precise disposition of pigment is difficult to determine. Undoubtedly some is carried in discrete red, or brownish, vacuoles or granules, measuring 0·5–2·0 μ (*gv.*), but more often the pigment is so dense as to prevent accurate observation. Though this can be made out in fresh material, it is more evident in cups which have been left beneath a coverslip for some hours, with the result that some of the pigment disappears (possibly due to oxidation, see p. 283), and observation becomes easier. Even when present in smaller quantity, however, we could

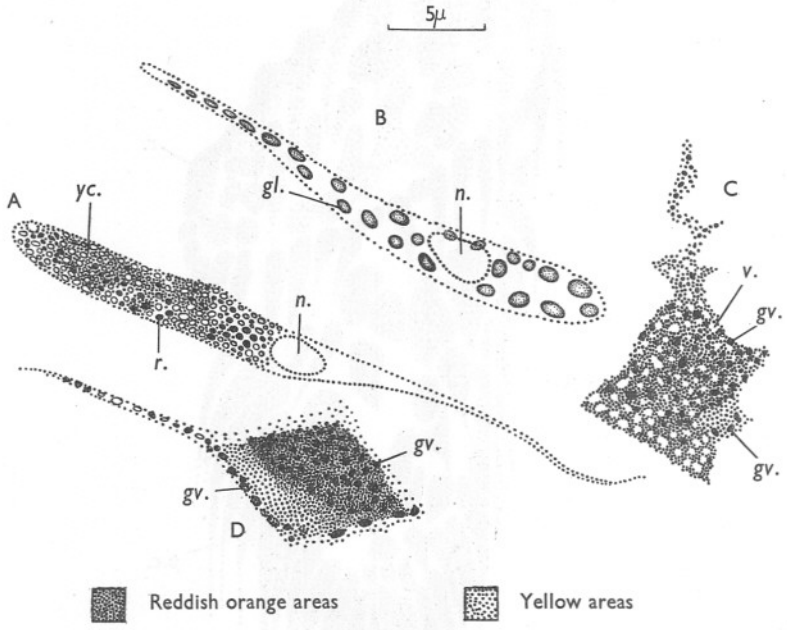

Fig. 3. Isolated pigment cells from the wall of the optic cup, drawn from unstained preparations mounted in sea water. A, fusiform cell from a fresh preparation; B, fusiform cell from an optic cup which had been compressed and left under a coverslip for 36 h, showing the aggregation of pigment to form globules; C, D, irregular cells from fresh preparations. *gl.*, globule of reddish orange pigment; *gv.*, granule (or vacuole) of reddish orange pigment; *n.*, large ovoid inclusion, possibly a nucleus; *r.*, granule (or vacuole) of reddish orange pigment; *v*, colourless inclusion, or vacuole; *yc.*, area of cytoplasm bearing yellowish pigment. The stipple key is applicable only to C and D.

not determine the precise way in which some pigment was carried, for it coloured the cytoplasm uniformly yellow or reddish orange, without any discernible association with discrete cellular inclusions (Fig. 3 A, C and D).

A condition that may possibly be significant was observed in preparations which had been squashed or kept beneath a coverslip for 36 h. In the resultant disorganization, the reddish orange pigment had aggregated into ovoid bodies (Fig. 3 B), suggesting that originally the pigment may have been disposed in the form of liquid, or semi-liquid, droplets.

Identification of the Pigments

Previous investigations into the pigmentation of asteroids have shown the importance of carotenoids in the general body colour (e.g. Fox, 1953; Vevers, 1952). In *M. glacialis* previous work has suggested the presence of carotenes and xanthophylls (Lönnberg, 1933; Lönnberg & Hellstrom, 1931) and whole extracts of the integument have yielded no evidence of porphyrins, despite the occurrence of protoporphyrin in the integument of the related *Asterias rubens* (Kennedy & Vevers, 1953).

Extracts of the optic cushions of *Marthasterias* were prepared as follows. The optic cushions and azygos tentacles of individuals from the hauls mentioned above were excised intact and freed from adhering integument. Some 200 of these were transferred to refined absolute methanol maintained in the dark in an atmosphere of nitrogen. Extraction was continued for about 12 h to several days at room temperature, and produced an orange solution, leaving behind colourless tissue. It proved essential to conduct the extraction in nitrogen as the solution became colourless in a few hours if left in air.

The crude extract was examined in a Unicam S.P. 500 spectrophotometer for its absorption in the range 320–620 mμ. It showed a broad peak in the region of 472 mμ, suggesting that the colour is due to a mixture of carotenoids.

The pigments were further characterized by two methods.

Method A

Sufficient water was added to the extract to make a concentration of approximately 90% methanol and this was shaken with an equal volume of 'Analar' petroleum ether (B.P. 40–60° C). Nearly all of the pigment passed into the petroleum ether epiphase, leaving only traces of yellow colour in the hypophase of which there was never sufficient for definite characterization. This pale yellow hypophase probably represented small amounts of xanthophylls. The epiphase was slowly evaporated to dryness on a warm bath, then dissolved in a small quantity of ethanol and saponified by warming the solution with $2\frac{1}{2}$ times its volume of KOH (concentration of 160 g KOH in 106 ml water). The resulting saponification mixture was cooled by adding distilled water and then extracted with di-ethyl ether. About two-thirds of the pigment passed immediately into the ether which was collected and evaporated to dryness. This formed fraction *a* 1.

The hypophase, which was in aqueous ethanolic KOH, remained in the lower layer when shaken with more ether, and only passed into the epiphase on acidification with glacial acetic acid. This pigment was collected as fraction *a* 2.

The two fractions were examined spectrophotometrically and showed the absorption maxima (in mμ) given below:

	Carbon disulphide	*n*-hexane
a 1	484	450
a 2	499–500	—

Method B

An extract similar to that used in method A was made and taken by dilution into 'Analar' petroleum ether. This was passed down a column of alumina (B.D.H. for chromatography) and elution was continued with petroleum ether. Two bands formed, one (the lower) being deep orange, the other (upper) pale orange. Development of the chromatogram was continued using 0·5% methanol in petroleum ether, until the two bands were well separated. They were then collected separately, the lower forming fraction *b*1, the upper, fraction *b*2. Both were taken into appropriate solvents for spectrophotometric determination of their absorption maxima which are reported (in mμ) below:

	Carbon disulphide	*n*-Hexane
*b*1	485	449
*b*2	503	—

The phase partition behaviour of fraction *a*1 and the behaviour on the chromatogram of fraction *b*1, together with their absorption maxima, suggest that both are β-carotene.

The absorption maximum of fraction *b*2 and its behaviour when chromatographed on alumina, suggest that it is esterified astaxanthin.

Fraction *a*2 was epiphasic before saponification, but after this it became hypophasic and remained so when in alkaline solution. The absorption maximum of this portion after saponification suggested astacene, and this, together with the original phase partition behaviour, indicates derivation from esterified astaxanthin.

The Effect of Exposure to Light

A methanol extract of optic cushions in an atmosphere of nitrogen, contained in a tube of ordinary glass, was irradiated by a 'Hanovia' ultra-violet lamp. Care was taken to place the solution far enough from the lamp to avoid any significant rise in temperature. The optical density of the extract was determined in the region of its maximum absorption in the visible range. Before irradiation log *E* was found to be 413 at 476 mμ. After 1 h irradiation, it had fallen to 404 at 476 mμ and after a further 2 h, to 276 at the same wavelength. At least one of the pigments is therefore unstable in light.

Discussion

The discovery of β-carotene and astaxanthin in the optic cushions of *Marthasterias* is not surprising in view of their occurrence in the integument of *Asterias* (Vevers, 1952), and since β-carotene has been reported as the predominant carotene in asteroids (Fox, 1953).

It would appear from considerations of molecular size, that the yellowish orange colour is due to β-carotene, while the darker reddish orange is due to

esters of astaxanthin. If this is so, from appearances under the microscope, β-carotene is widely distributed in the cushion while astaxanthin appears confined to the optic cup and pigment cells scattered irregularly over the surface of the cushion. Both pigments appear to exist in some of the pigmented cells of the optic cups, although it is not yet clear whether they occur free, or combined, for example, with protein. As regards astaxanthin, combination with protein appears unlikely, since the pigment does not appear in the living optic cushion with the dark hues commonly associated with such a combination, and correlated with this is the fact that the colour of the optic cushion is not markedly changed by a denaturant such as methanol. Some of the reddish orange colour could, however, be due to molecular associations involving β-carotene.

Our findings concerning the appearance of the pigment-bearing cells of the optic cup differ somewhat from those of Smith (1937), who described them as prolonged into tails which are bent so as to lie parallel to the long axis of the optic cup, with their pigment in granular form, confined to the outer third of the cell.

As will be evident from Fig. 2, the form and disposition of the pigment cells leaves no doubt that they correspond to the cells similarly distinguished by Smith, but we find that their abundant pigment is present throughout the cytoplasm. It is also noteworthy that they are about twice the size of those figured by Smith. As previously noted, some of the cells are irregular, though still attenuated.

In general, these differences can be reconciled with the different methods used, for the preparation of paraffin sections by Smith's methods would not only leach out and destroy the carotenoid pigments, but would also cause shrinkage. What the granular pigment mentioned by Smith may be, we cannot suggest, for we saw nothing in the living optic cups that might correspond with it.

As noted by Smith, who reviewed the earlier work, the histology of the optic cup is difficult, but he concluded that it is lined by cells that bear pigment and those that are sensory. A study of the histology of the cup is beyond the scope and methods of this investigation, but we feel it necessary to urge caution in discriminating between sensory and pigmented cells on histological grounds alone. For if the lining of the optic cups is truly photosensory (see below), then since the very nature of the photoreceptive process renders a light absorber necessary, the possession of an orange or yellow pigment absorbing at the high-energy end of the spectrum would seem to constitute an admirable adaptation for this. It is thus possible that the pigment cells are also sensory.

There is general agreement that asteroids are light sensitive, but since the function of the optic cushions in *Marthasterias* has not yet been demonstrated, the role of the carotenoid pigments must remain uncertain. Nevertheless, it must be admitted that in so far as their structure is concerned (see Smith,

1937), the optic cups present evidence of participation in some form of light perception, though due caution must be exercised in using such evidence alone. Furthermore, in the related *Asterias*, the 'eye spot' has been shown unquestionably to be photosensitive by Hartline, Wagner & MacNichol (1952), who recorded its action potential in response to stimulation by light. In addition, however, evidence has accumulated showing that photosensitivity in starfish is not confined to the eye spot and may extend to the tube feet, and, as in other echinoderms, to the general body surface (Cowles, 1910, 1911; v. Frisch, 1909; McCurdy, 1912; Mangold, 1909; Millott, 1955; van Weel, 1935).

The discovery of the same carotenoids in the optic cushion of *Marthasterias* as have been found in the skin of other asteroids, may be significant in possibly providing a clue to the common basis of photosensitivity in these areas, especially since carotenoids have been found in a wide variety of photosensitive structures (Wald, 1945).

If such an indication proves to be well founded, then the optic cushion might well represent a localized concentration of such skin pigments and constitute a stage in the evolution of the condition where photosensitivity is largely confined to highly specialized eyes.

It must be borne in mind, however, that these pigments may not be the only ones occurring in the optic cushion, for the methods of observation and extraction used here would not reveal any fugitive photosensitive pigments of the rhodopsin type, and neither time nor the amount of material available permitted us to determine whether there were other indications of such pigments in the form of vitamin A. There is also the possibility that the pigments may play only a secondary part in light perception by acting as filters, etc. Astaxanthin appears to play such a role in some specialized eyes (Wald, 1945).

Regrettably the experiments made to determine whether the pigments were stable in light could not be completed. Though decomposition occurred, it appears too slow to be significant in vision, but as the pigments were dissolved in methanol they were far removed from their normal chemical environment. Such stability, however, would not in itself prevent the pigments from functioning in photoreception, since their primary function of absorbing light energy would be discharged, whether they remained intact, or were decomposed and reformed.

We wish to thank Dr G. A. Steven and the crews of the Plymouth research vessels for their efforts in obtaining material for us during inclement weather. Our thanks are also due to Mr F. A. J. Armstrong for help and suggestions, and to Prof. J. E. Smith who has kindly read the manuscript. One of us (N.M.) wishes to acknowledge the generosity of the Director and Staff of the Plymouth Laboratory in providing facilities for research.

SUMMARY

The optic cushion of *Marthasterias glacialis* is seen to contain yellowish orange and reddish orange pigments. The former occurs throughout the cushion, the latter is confined to the wall of the optic cups and a few scattered, irregular patches. Both pigments occur in the cytoplasm of long and narrow or irregular cells, some in the form of granules or vacuoles.

Optic cushions yield an orange extract in methanol which decomposes in light.

The behaviour of methanolic extracts after partitioning, saponification, chromatographic separation and spectrophotometric examination indicates the presence of β-carotene and esterified astaxanthin.

The significance of the results in relation to previous findings and their possible bearing on photoreception is discussed.

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A NOTE ON THE RELATIONS OF CERTAIN PARAMETERS FOLLOWING A LOGARITHMIC TRANSFORMATION

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(Text-fig. 1)

The logarithmic transformation has been used in the statistical analysis of certain marine biological data. Parameters calculated from the transformed distributions have been used in the description of the observations, but it appears that some of the mathematical procedures adopted have not been fully understood.

Winsor & Clarke (1940) have analysed data from plankton hauls. The problem considered by them was the determination of the variability in numbers of animals caught by repeated hauls through the same body of water. The raw data were characterized by a constant coefficient of variation, i.e. the variability in catch was proportional to the size of the catch. By transformation from the actual numbers caught to their logarithmic values, it was possible to equalize the variances and to apply the method of analysis of variance to estimate the variability due to the different sources. Finally, an estimate of the coefficient of variation was obtained from the logarithmic values. Their method of estimation was quoted by Snedecor (1946, p. 451) and was employed by Barnes & Bagenal (1951) in their study of repeated trawl hauls. Barnes & Bagenal also calculated confidence limits for comparison of observations following the method of Silliman (1946) in his work on pilchard eggs. These methods seem to be based on a misunderstanding of the nature of a transformation. The formal relation between the variance of such transformed data and the coefficient of variation of the untransformed data follows from the moments of the 'log-normal' distribution, given first by Wicksell (1917). From these moments it will be shown that the method given by Winsor & Clarke is mathematically unsound. However, the discrepancies between their values and those now obtained are comparatively small (ranging from about 2% for a coefficient of variation of 20% to about 15% for one of 60%). In all cases the method given in this paper results in a smaller estimate.

For comparison of catches Silliman gave confidence limits which were expressed as percentages of the mean catch. The mean used, although not explicitly stated, was the geometric mean of the catches.

The Logarithmic Transformation

For untransformed data with a positively skew frequency distribution a transformation from the values of x, to the logarithmic values $y = \log_{10} x$, may result in a distribution of the normal form. The relation between the two distributions is such that the proportion of observations falling within a given range of

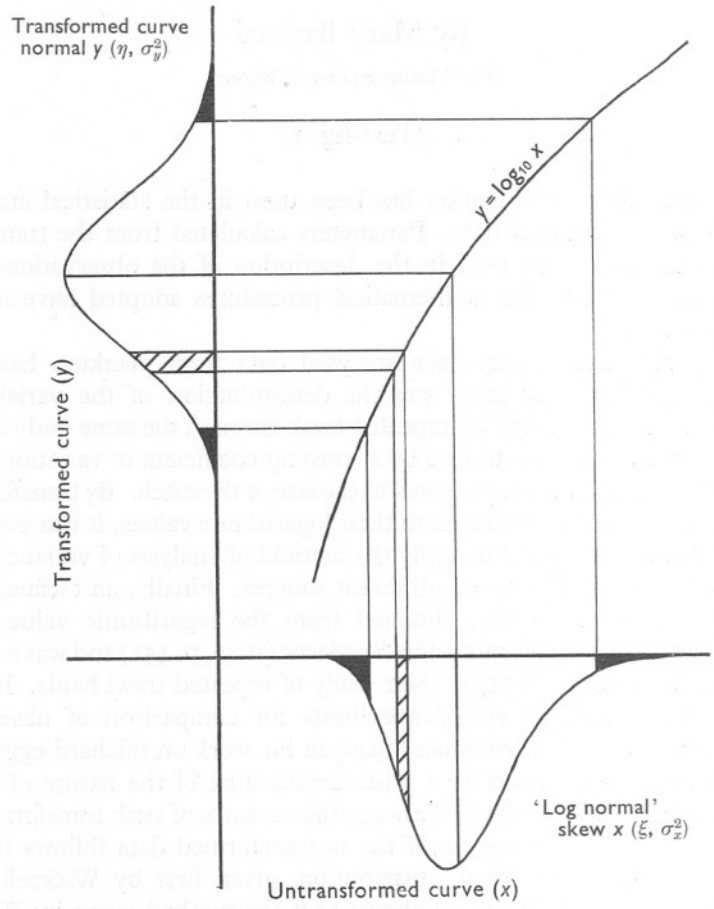

Fig. 1. Diagram to show the relation between the 'log-normal' and the normal distributions. For explanation see text.

the x values will equal the proportion of observations falling within the corresponding range of y values. This is so since there is a corresponding value of y to every value of x.

Such a transformation is shown in Fig. 1 (after N. L. Johnson, 1949).

An untransformed frequency distribution, which is noticeably skew, is represented on the lower margin. Let this be the distribution of x with mean

ξ and variance σ_x^2. In the centre of the figure the relation $y = \log_{10} x$ is represented graphically by the smooth curve. On the left-hand margin is the transformed frequency distribution, projected from the original distribution by means of the curve $y = \log_{10} x$. This distribution of y, with mean η and variance σ_y^2, is normal.

The cross-hatched columns representing frequencies over corresponding ranges of the two variates are equal in area. By consideration of this property of correspondence of areas certain relations between parameters of the two distributions become apparent. The mean, median and mode of the transformed distribution, which is normal, coincide and divide the area under the curve equally. The corresponding value in the untransformed distribution also divides the area under the curve equally and is the median, but because the curve is skew will not coincide with the mean and mode.

The normal curve has been thoroughly investigated and tabulated and use has been made of many of its properties. The 95% confidence limits are those values of the variate between which 95% of the total observations lie, and for the normal curve are approximately given by the mean plus and minus two standard deviations (or for means of small samples t standard deviations). In the figure these are at $\eta \pm 2\sigma_y$ for the transformed distribution, and the corresponding values in the untransformed distribution will also represent 95% confidence limits since the shaded 'tail areas', are equal.

The mean and variance of a frequency distribution do not depend simply on area and therefore the values for one distribution cannot be obtained simply by transformation of the appropriate values for the transformed distribution; but must be obtained from the mathematical relations between them.

Wicksell (1917) gives for the transformation $y = \log_e x$, where y is normally distributed with mean η and variance σ_y^2, the relation:

$$r\text{th moment about origin of } x = e^{r\eta + \frac{1}{2}r^2\sigma_y^2}, \tag{1}$$

whence,
$$\text{mean } x = e^{\eta + \frac{1}{2}\sigma_y^2}, \tag{2}$$

and
$$\text{variance } x = e^{2\eta + \sigma_y^2}(e^{\sigma_y^2} - 1). \tag{3}$$

For the transformation $y = \log_{10} x$, rearrangement gives the relations

$$\text{mean } y = \eta = \log_{10}\left[\frac{\xi}{(1 + \sigma_x^2/\xi^2)^{\frac{1}{2}}}\right], \tag{4}$$

$$\text{variance } y = \sigma_y^2 = \log_{10} e \log_{10}(1 + \sigma_x^2/\xi^2). \tag{5}$$

Now $\sigma_x/\xi = V_x$ is the ratio of the standard deviation of x to its mean value and is the coefficient of variation of the untransformed data. Rewriting (5) we have

$$\sigma_y^2 = 0{\cdot}43428 \log_{10}(1 + V_x^2),$$

from which it is seen that if the coefficient of variation of x is constant the variance of the transformed variate y will be constant and the methods of the 'analysis of variance' may be used. From equation (4) it is seen that the relation between the means of the transformed and untransformed distributions is not simple. For a sample the mean of the transformed values equals the logarithm of the *geometric mean* of the actual numbers.

Since $$y = \log_{10} x,$$

and $$\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i = \frac{1}{n}\sum_{i=1}^{n} \log_{10} x_i = \log_{10} \sqrt[n]{(x_1 \times x_2 \times \ \dots \ \times x_n)},$$

i.e. $$\text{mean } y = \log_{10} \ (\text{geometric mean } x).$$

The mean of a sample drawn from a normal population is itself distributed normally with a variance equal to that of the parent population divided by the number in the sample, and therefore appropriate confidence limits may be calculated; since the *geometric mean* of a sample of n observations is related to the arithmetic mean of the transformed values in the same way as a single observation of x is to the transformed value $y = \log_{10} x$, for all values of n. If the limits for use with the arithmetic mean of the untransformed data are required, estimates of the population mean will have to be made from the actual numbers observed. Finney (1941) has shown that for data of this type the usual estimate of the sample mean, i.e. $\frac{1}{n}\sum_{i=1}^{n} x_i$ decreases in efficiency as an estimator of the population mean as the coefficient of variation increases. He has given corrections which can be used, but it is probably better to perform all comparisons on the transformed values, and thus also to avoid confusion between the geometric and arithmetic means.

The Method of Winsor & Clarke

The data considered by Winsor & Clarke consisted of catches from plankton nets hauled vertically, obliquely and horizontally. In each case similar methods of analysis were used. The observations were given in the form of estimated total catches of each of given groups of animals (defined by species, age and sex) calculated from laboratory samples, which represented numbers caught by nets hauled consecutively, and therefore effectively through the same body of water. The subsequent analysis of variance divided the total variance observed into its component parts.

In order that such an analysis of variance may be performed the data should satisfy several conditions, one of which is that the variances within different groups and due to different factors must be estimated on comparable scales, or 'equalized', and the resultant residual variations should be normal with zero mean. A transformation from the raw data may result in variates which satisfy these conditions.

Winsor & Clarke applied the logarithmic transformation and subsequent analysis of variance, which they used to estimate the variances and appropriate coefficients of variation. To illustrate this latter step it is best to quote from their paper.

From the estimates 0·00781 and 0·00600 for the within haul and haul to haul variances, we obtain 0·0884 and 0·0775 as the estimated standard deviations. These are obtained from the logarithms of the catches. To interpret these figures in terms of variability of the actual catches we proceed as follows:

$$0{\cdot}0884 = \log 1{\cdot}226,$$

$$0{\cdot}0775 = \log 1{\cdot}195.$$

Now a deviation of 0·0884 in the logarithm of the catch means that the catch has been multiplied (or divided) by 1·226. Hence we may say that one standard deviation in the logarithm corresponds to a percentage standard deviation, a coefficient of variation, of 22·6 % in the catch. Similarly, a logarithmic standard deviation of 0·0775 corresponds to a coefficient of variation of 19·5 %.

This implies that the relation between σ_y and V_x is

$$\sigma_y = \log_{10}(1 + V_x), \tag{6}$$

but from equation (5) we see that this is not so. Equation (6) will always overestimate the value of V for a given σ_y, since it gives

$$V_x = 10^{\sigma_y} - 1, \tag{7}$$

whereas from (5)
$$V_x = \sqrt{(10^{\sigma_y^2/0{\cdot}43429} - 1)}. \tag{8}$$

and the magnitude of the bias may be calculated. For the case quoted by Winsor & Clarke, calculation from equation (5) gives

$$\sigma_x^2 = 0{\cdot}00781 = 0{\cdot}43429\,(1 + V_x^2),$$

$$1 + V_x^2 = \text{antilog}_{10}\frac{(0{\cdot}00781)}{(0{\cdot}43429)} = 1{\cdot}0422,$$

and
$$V_x = 0{\cdot}205 \quad \text{or} \quad 20{\cdot}5\,\%$$

for which Winsor & Clarke obtained 22·6 %.
For $\sigma_y^2 = 0{\cdot}0400$ eqn. (5) gives 48·6 % and the method of Winsor & Clarke 58·5 %; and $\sigma_y^2 = 0{\cdot}0900$ eqn. (5) gives 78·2 % and the method of Winsor & Clarke 99·5 %.

The bias due to the method of Winsor & Clarke increases with increase in σ_y^2.

The Method of Silliman

Silliman's data consisted of observations on the number of pilchard eggs in hauls made with two identical nets, each hauled once at a number of different stations. The data was not given as estimated catch, but as the numbers

counted in laboratory samples. Two laboratory samples were taken from each haul and both counts were given. This data, like that of Winsor & Clarke, has a constant coefficient of variation, and a logarithmic transformation was used. The analysis of variance showed a highly significant variation between stations; estimates of the 'within station between haul' and the 'between samples' variances were obtained.

Winsor & Clarke suggested the use of confidence limits in order to compare catches, and if these are calculated from the numbers obtained in several hauls from a given station, or at a given time, they provide a test of significance for a further observation from, say, a different station, or at a different time.

From equations (4) and (5) it is seen that the 95 % limits for the untransformed distribution correspond to the antilogarithms of the values for the 95 % limits for the transformed (normal) distribution; and are therefore $\text{antilog}_{10}(\eta - t\sigma_y)$ and $\text{antilog}_{10}(\eta + t\sigma_y)$. If these are to be expressed as proportions of the mean of the untransformed distributions, we have

$$\frac{\text{antilog}_{10}(\eta - t\sigma_y)}{\xi} \quad \text{and} \quad \frac{\text{antilog}_{10}(\eta + t\sigma_y)}{\xi}$$

or
$$\log_{10}\frac{(\text{lower limit})}{\text{mean}} = -t\sqrt{[0{\cdot}43429\log_{10}(1+V_x^2)]} - \tfrac{1}{2}\log_{10}(1+V_x^2), \quad (9)$$

$$\log_{10}\frac{(\text{upper limit})}{\text{mean}} = +t\sqrt{[0{\cdot}43429\log_{10}(1+V_x^2)]} - \tfrac{1}{2}\log_{10}(1+V_x^2), \quad (10)$$

in which form they are expressed in terms of the coefficient of variation only. Silliman, recognizing this property, has argued as follows:

> Solution of these equations gives $\sigma_H^2 = 0{\cdot}024$ as the variance of hauls and $\sigma_S^2 = 0{\cdot}007$ as the variance of samples. Therefore $\sigma_H = \sqrt{0{\cdot}024} = 0{\cdot}155$ and $\sigma_S = \sqrt{0{\cdot}007} = 0{\cdot}084$. The 95 % fiducial limits are of interest and may be calculated from these estimates of σ. For hauls $2 \times 0{\cdot}155 = 0{\cdot}310$ (2σ limits include 95 % of the distribution). Since these values are logarithmic, the antilogs are used to convert to ratios. The antilog of 0·310 is 2·04 giving fiducial limits of 49 % ($100 \times 1/2{\cdot}04$) to 204 % ($100 \times 2{\cdot}04$). Thus the egg number from one haul may not be considered significantly different from the egg number at another, unless it is less than one half, or more than double, that of the other. Similarly, the 95 % fiducial limits for samples are 68 % to 147 %, and may be interpreted in a like manner.

This method implies that the ratios are

$$\frac{\text{antilog}_{10}(\eta - 2\sigma_y)}{\text{antilog}_{10}\,\eta} \quad \text{and} \quad \frac{\text{antilog}_{10}(\eta + 2\sigma_y)}{\text{antilog}_{10}\,\eta},$$

i.e. the mean employed is the antilogarithm of the mean of the transformed distribution, the geometric mean of the untransformed data. Similarly, estimates of the confidence limits for the mean of a sample are expressed as percentages of the geometric mean of the sample. Throughout Silliman has used the word mean, which is generally taken to apply only to the arithmetic mean.

If the arithmetic mean is used in this way, where the method is strictly only applicable to the geometric mean, anomalous results may be obtained. A numerical example taken from Silliman's data may illustrate the different results obtained with the use of the geometric and arithmetic means.

The laboratory counts of pilchard eggs for two samples drawn from each of two hauls taken at twenty-four different stations were given. Do the mean values of the first samples differ significantly between the two hauls? The means of the first samples are:

	Haul A	Haul B
Mean of actual numbers	70·5	85·6
Mean of logarithmic values	1·6499	1·7031
Geometric mean catch	44·66	50·48

The variance to be associated with a single haul, obtained from the analysis of variance, and based on 69 degrees of freedom, is 0·031.

The variance of the mean of 24 hauls is therefore 0·031/24
and the standard deviation 0·0359
2 × standard deviations* 0·0718

and the confidence limits are antilog 1·6499 + 0·0718 = 52·69
antilog 1·6499 − 0·0718 = 37·85.

From which it is seen that the geometric mean of the samples for the B hauls falls within the confidence limits derived from the A hauls. If the limits are calculated following Silliman and applied to the arithmetic mean of the untransformed observations, they are:

Lower limit 84·7% of 70·5 = 59·11,
Upper limit 117% of 70·5 = 83·19,

and the observed value of 85·6 for the B hauls appears to be significant as it falls outside the confidence limits derived from the A hauls. This result differs from that obtained above.

For confidence limits for a single observation the limits in actual numbers given by the two methods are equal as there is no difference between the estimate of the arithmetic mean and the geometric mean for a sample of one.

Discussion

Although the estimates of the coefficient of variation as given by Winsor & Clarke have been shown to have been incorrectly obtained, the correct coefficient of variation does give a useful indication of the variability to be expected with any particular method and gear. The estimates given in this paper are lower than those given by them, but for their data are nevertheless high. For critical comparisons of catches, using the same gear and method confidence

* In this case $t = 2$ for 95% probability level and 69 degrees of freedom.

limits are more useful. These may be calculated from the transformed data and expressed as actual numbers for a single observation, or as limits for the geometric mean of a number of catches calculated as percentages of the geometric mean of the observed data. This is equivalent to performing the comparisons on the logarithmic values.

It must be emphasized that the derivation of estimates, both of the coefficient of variation and of confidence limits, given in this paper, relies on the assumption that the logarithmic transformation employed has normalized the observed distribution. In practice this will not be completely realized, but the estimates given will be better than those obtained by the methods of Winsor & Clarke and Silliman, which take little account of the mathematical relations involved.

For some observed data, a logarithmic transformation of the form $y = \log_{10}(x+a)$ is more appropriate and, from the equations given above, estimates of the coefficient of variation can be obtained. For such a transformation, the variance of the untransformed data remains σ_x^2, but the mean becomes $(\xi + a)$. From Fig. 1 it will be seen that confidence limits for a single observation depend only on area, and may therefore be obtained for any *normalizing* transformation, even when the mathematical relations between the parameters of the two distributions are not readily available. Comparisons of sample means may be carried out on the transformed values.

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THE GROWTH RATE OF THE LONG ROUGH DAB *HIPPOGLOSSOIDES PLATESSOIDES* (FABR.)

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(Plate I and Text-figs. 1–6)

The Long Rough Dab, *Hippoglossoides platessoides* (Fabr.), is a common fish in the Clyde Sea Area, being indeed the most abundant flatfish. However, despite its abundance and its wide geographic range (it is found from the British coasts as far north as Spitzbergen and the Murman coast, Iceland, Greenland and south to Cape Cod in America), very little work has been done on its general biology. This is no doubt owing to its having little or no economic value. Concerning its growth rate, which is the subject of this paper, the European subspecies *limandoides* (Bloch) (see Norman, 1934) has been investigated to some extent in the Barents Sea (Essipow & Slastnikow, 1932; and Milinsky, 1944), and has been mentioned in more general papers by Saemundsson (1925) and Krüger (1942) for Icelandic and Baltic populations respectively. The American population which constitutes a separate subspecies *platessoides*, has been discussed by Huntsman (1918), though he does not give much numerical data on the growth rate.

In the work described below the ages of 1561 *H. platessoides limandoides* caught in the Clyde area have been determined and their growth rate calculated.

Gear

The gear used in obtaining the samples on which this work is based was a small-mesh cotton V.D. trawl of the following dimensions: headline, 49 ft.; foot rope, 78 ft.; bridles, 15 fathoms; lower wings and belly mesh size, $2\frac{1}{2}$ in. bar; all other meshes, 1 in. bar. This gear took an adequate sample of the population, except that the mesh-size selection gave a noticeably biased sample of the younger age-groups. This point will be discussed in a later section (p. 305). In order to illustrate the adequacy of the gear in obtaining representative samples two tests were carried out on a pair of hauls taken over the same ground on 8 July 1954. In the first test (see Table 1) the mean lengths of the age-groups present were compared and no significant differences were found. In the second test the numbers of fish of each age-group were compared, and again no significant differences were found.

The Material and Methods

The samples were obtained monthly, and for the most part the fish were caught in an area at about 40 m. depth off Mountstuart House on the east side of the Isle of Bute. Before this area was finally chosen some of the earlier hauls were taken on various grounds round the Isle of Cumbrae, and prior to the spawning time hauls were also taken in the deeper water between Bute and Cumbrae. Owing to the depth segregation of spawning and immature fish, and so also of the relative proportions of different sizes and ages, these latter hauls led to the samples being more representative of the population as a whole.

Table I. Comparison of two Replicate Catches of Long Rough Dabs

	Haul I		Haul II	
Age-group	Number	Mean length (cm)	Number	Mean length (cm)
2	15	13·2	12	13·4
3	33	18·8	26	18·3
4	22	23·1	24	22·7
5	9	23·1	5	24·0

The dates on which the samples were obtained are given in Table II.

The examination of the fish after capture was carried out in the laboratory, and for the purposes of this investigation, the sex and length of each fish was noted and the otoliths were extracted and stored in separate packets. The lengths were measured from the tip of the lower jaw to the end of the longest caudal fin ray to the nearest 0·5 cm. These measurements however were later grouped into centimetre groups, and the resulting groups of from $x-0{\cdot}25$ cm to $x+0{\cdot}75$ cm were all classed as x cm rather than $x.25$ cm for ease in computation.

Age Determination

The ages of the fish were determined from the otoliths (Pl. I). These were examined as soon as possible after extraction, in a strongly illuminated black dish. They were found to be very clear and easy to read. The number of translucent rings that alternate with opaque bands was noted and taken to represent the age of the fish in years.

In order to test the accuracy of the otolith readings some scales were examined from a few fish, but they proved to be very obscure and difficult to read and the attempt was abandoned. However, the edges of the otoliths were examined and the percentage that were translucent was calculated. The data are given in Table III and it can be seen that the assumption that the rings are annual structures is confirmed.

From the age analysis each fish was allocated to a population *a–f* dependent on when it was supposed to have been spawned. This obviates the difficulty

during the winter and spring when fish that were spawned in a given year may have a different number of translucent rings dependent on the condition of the outer edge.

TABLE II. DETAILS OF LONG ROUGH DAB CATCHES

Population ...	*a*		*b*		*c*		*d*		*e*		*f*		Totals		Grand totals
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1953															
23 Oct.	—	—	—	4	1	13	—	22	1	21	5	5	7	65	72
19 Nov.	—	—	—	—	3	9	2	15	3	18	2	9	10	51	61
15 Dec.	—	—	—	2	1	5	1	17	1	18	2	6	5	48	53
1954															
21 Jan. 26 Jan.	—	—	—	6	1	21	1	28	2	22	1	3	5	80	85
15 Feb.	—	—	—	3	—	12	—	30	—	4	—	—	—	49	49
2 Mar.	—	—	—	7	2	26	2	78	4	149	1	8	9	268	277
30 Mar.	—	4	—	3	—	18	—	36	—	44	—	—	—	105	105
8 Apr.	—	1	—	18	—	27	—	52	—	48	—	1	—	147	147
3 May	—	—	—	8	1	15	2	30	2	59	—	5	5	117	122
2 June	—	1	—	2	4	16	12	54	10	98	3	6	29	177	206
8 July	—	—	—	1	1	14	7	46	5	59	3	15	16	135	151
9 Aug.	—	—	—	1	4	10	13	36	15	46	5	23	37	116	153
7 Sept.	—	—	—	2	1	5	8	16	—	35	3	10	12	68	80
Total	—	6	—	57	19	191	48	460	43	621	25	91	135	1426	1561

TABLE III. PERCENTAGE OF OTOLITHS WITH TRANSLUCENT EDGES

Oct.	36·5	Apr.	50·2
Nov.	46·1	May	10·7
Dec.	75·2	June	18·0
Jan.	74·2	July	7·4
Feb.	83·7	Aug.	10·6
Mar.	74·3	Sept.	15·2

GROWTH RATE

In the analysis of the growth rate the two sexes were treated separately. For each population sample in each month the mean length was calculated, and its 95% fiducial limits obtained for all samples where sufficient numbers were caught. The data for the females are given in Table IV and for the males in Table V, though for the latter the numbers are unfortunately too small to make calculation of fiducial limits worth while. It can immediately be seen that the males are not only of a smaller size than females of a corresponding age, but also that they have a shorter life span (see also Fig. 3).

The data in Table IV are shown in Fig. 1. From this it can be seen that the most rapid growth takes place in the younger fish and falls off in later years. The seasonal differences in growth rate can also be seen, the faster growth taking place between April and November. It will be noticed that this period of faster growth is at a time when the edge of the majority of otoliths is opaque

TABLE IV. MEAN LENGTHS (AND THEIR FIDUCIAL LIMITS) OF FEMALE LONG ROUGH DABS FROM POPULATIONS *a* TO *f*

Population ...	*a*	*b*			*c*			*d*			*e*			*f*		
	Mean	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit
Oct.	—	—	25·5	—	22·0	23·4	24·7	21·2	21·9	22·7	16·3	17·0	17·8	—	10·0	—
Nov.	—	—	—	—	20·8	22·7	24·4	20·4	21·6	22·8	16·6	17·6	18·5	11·1	12·3	13·6
Dec.	—	—	17·5	—	17·7	21·0	24·3	19·8	21·1	22·3	17·1	18·0	18·9	11·0	12·7	14·3
Jan.	—	24·2	26·3	28·5	23·1	24·1	25·2	21·1	21·7	22·3	16·9	17·8	18·7	—	11·7	—
Feb.	—	—	27·7	—	23·1	24·5	25·9	22·3	22·8	23·3	17·2	18·5	19·9	—	—	—
Mar.	—	24·7	26·4	28·2	21·7	22·7	23·6	21·3	21·5	21·7	17·1	17·4	17·7	—	9·8	—
Mar.	26	—	25·3	—	21·5	22·6	23·8	21·3	22·0	22·6	17·9	18·5	19·1	—	—	—
Apr.	22	23·4	24·4	25·5	22·1	23·1	23·9	21·7	22·1	22·6	18·0	18·6	19·1	—	11	—
May	—	23·1	25·3	27·4	22·1	23·7	25·3	21·3	22·2	23·1	17·6	18·2	18·7	—	10·8	—
June	26	—	25·0	—	23·2	24·5	25·8	21·8	22·5	23·1	17·8	18·2	18·6	10·7	12·0	13·3
July	—	—	21	—	22·5	23·4	24·4	22·3	22·9	23·5	18·0	18·5	19·0	12·4	13·2	14·0
Aug.	—	—	23	—	22·1	24·1	26·1	21·5	22·1	22·8	19·4	19·9	20·4	13·0	13·6	14·2
Sept.	—	—	24·5	—	21·7	23·8	25·9	21·5	22·8	24·0	19·5	20·3	21·0	13·4	14·6	15·8
Mean for the year	25·3	24·7	25·3	26·0	23·1	23·4	23·7	21·9	22·1	22·3	18·1	18·3	18·4	12·2	12·6	13·0

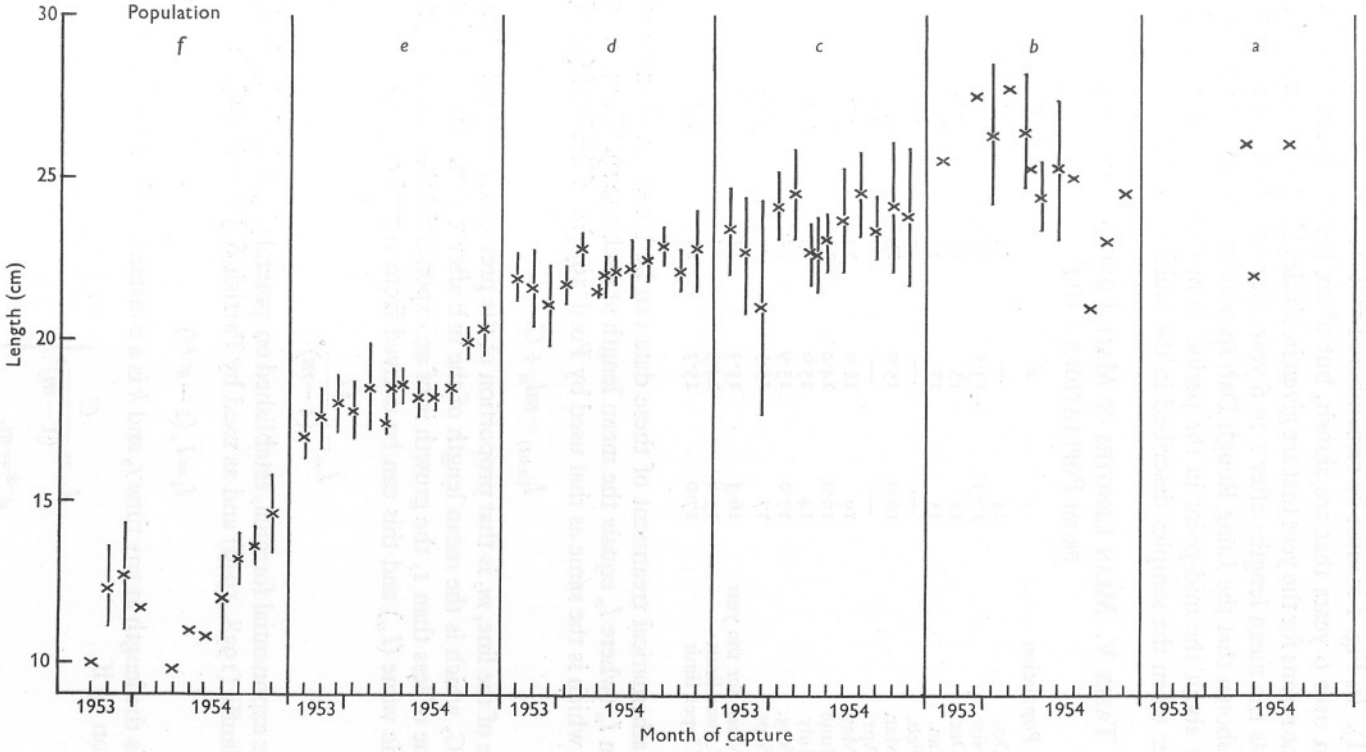


Fig. 1. The means (×) and their 95% fiducial limits (vertical lines) of female Long Rough Dabs of six populations, *a–f*, from samples taken from October 1953 to September 1954

(Table III). In Fig. 1 it must be remembered that it is not samples from one generation over 6 years that are shown, but of six age-groups sampled over 1 year. The means for the year that are given in Tables IV and V can reasonably be taken as the mean lengths after 1 to 6 years (for populations *f* to *a*), since it can be shown that the Long Rough Dab spawns at the beginning of April, that is, at about the mid-point in the period from October to the following September when the samples described in the tables were taken.

TABLE V. MEAN LENGTHS OF MALE LONG ROUGH DABS FROM POPULATIONS *c* TO *f*

Population ...	*c*	*d*	*e*	*f*
Oct.	14	—	11	8·8
Nov.	17·3	13·5	12·0	10·5
Dec.	14	15	14	10·5
Jan.	15	17	13·0	10
Feb.	—	—	—	—
Mar.	16·0	15·0	13·0	13
Apr.	—	—	—	—
May	19	16·0	13·5	—
June	16·0	14·4	13·0	11·0
July	14	15·9	13·8	11·3
Aug.	17·0	15·9	14·3	10·8
Sept.	17	15·5	—	10·7
Mean for the year	16·3	15·3	13·5	10·5
Lower limit	15·6	14·9	13·0	9·9
Upper limit	17·0	15·7	14·0	11·0

The mathematical treatment of these data can be based on the regression of $l_{(n+1)}$ on l_n (where l_n equals the mean length at each age n). The regression equation, which is the same as that used by Ford (1933) and Walford (1946), is

$$l_{(n+1)} = ml_n + C. \tag{i}$$

The slope of the line, m, is that proportion of the previous length added to the constant C, which is the mean length of the fish after 1 year. Since m always has a value of less than 1, the growth is of an exponential form, tending to an asymptotic value (l_∞) and this can be derived from equation (i) as

$$l_\infty = \frac{C}{(1-m)}. \tag{ii}$$

A suitable exponential formula, established on general physiological principles by Bertalanffy (1938, 1949) and as used by Parrish & Jones (1953), is

$$l_t = l_\infty(1-e^{-kt}), \tag{iii}$$

where l_t is the length at any time t, and k is a constant. This will be equivalent to equation (i) if

$$\left.\begin{aligned} l_\infty &= \frac{C}{(1-m)}, \\ e^{-k} &= m, \end{aligned}\right\} \tag{iv}$$

and the constant (C) is the length at 1 year old. This last condition is necessary because the exponential equation passes through the origin, whereas the regression allows a value for l_0 (which may perhaps represent the length of the fish when the otolith formation commences). However, if a comparison of the regression equation and the data suggests that a value for l_0 should be calculated, this can be obtained from equation (i), and if one wishes to fit the exponential equation (iii) so that l_0 has this value, the origin of t must be changed by an amount x, such that the lengths are calculated for values of $t=n+x$. Equation (iii) now becomes:

$$l_n = l_\infty (1 - m^{n+x}), \tag{v}$$

and writing $n=0$ we get

$$x = \frac{\log (l_\infty - l_0) - \log l_\infty}{\log m}. \tag{vi}$$

In the analysis of the data by these methods, the regression equations (i) were calculated from the mean lengths given in Tables IV and V, care being taken to weight the points in proportion to the number of fish contributing to each mean. The equations found were

$$\left.\begin{aligned} &\text{for the females: } l_{n+1} = 0{\cdot}574\, l_n + 11{\cdot}262, \\ &\text{for the males: } \quad l_{n+1} = 0{\cdot}585\, l_n + 7{\cdot}368; \end{aligned}\right\} \tag{vii}$$

and the l_∞ values therefore were given by

$$\left.\begin{aligned} &\text{for the females: } l_\infty = \frac{11{\cdot}262}{(1 - 0{\cdot}574)} = 26{\cdot}437 \text{ cm}, \\ &\text{for the males: } \quad l_\infty = \frac{7{\cdot}368}{(1 - 0{\cdot}585)} = 17{\cdot}754 \text{ cm}. \end{aligned}\right\} \tag{viii}$$

These values in the exponential equation (3) give

$$\left.\begin{aligned} &\text{for the females: } l_t = 26{\cdot}437\ (1 - 0{\cdot}574^t), \\ &\text{for the males: } \quad l_t = 17{\cdot}754\ (1 - 0{\cdot}585^t). \end{aligned}\right\} \tag{ix}$$

Successive values of l_t are given in Table VI and Fig. 2. It will be noticed that the constant (C) in equation (vii) is not equal to the observed l_1 so that a value for l_0 can be obtained. If this is calculated from l_1

$$\left.\begin{aligned} &\text{for the females: } l_0 = 2{\cdot}33 \text{ cm}, \\ &\text{for the males: } \quad l_0 = 5{\cdot}35 \text{ cm}. \end{aligned}\right\} \tag{x}$$

The accuracy of these values is dependent on the accuracy of l_1, and a better estimate can be obtained from the data as a whole by finding

a weighted l_0 from all the age groups (using equation (vii)). This method gives

$$\left.\begin{aligned} &\text{for the females: } l_0 = 2{\cdot}130 \text{ cm,} \\ &\text{for the males: } \quad l_0 = 5{\cdot}335 \text{ cm.} \end{aligned}\right\} \tag{xi}$$

Using these values in equation (vi) we get

$$\left.\begin{aligned} &\text{for the females: } x = 0{\cdot}151 \text{ years,} \\ &\text{for the males: } \quad x = 0{\cdot}667 \text{ years;} \end{aligned}\right\} \tag{xii}$$

TABLE VI. SUCCESSIVE VALUES OF l_t, FROM EQUATION (ix)

	Females		Males	
t	Observed	Calculated	Observed	Calculated
1	12·6	11·3	10·5	7·4
2	18·3	17·7	13·5	11·7
3	22·1	21·4	15·3	14·2
4	23·4	23·6	16·3	15·7
5	25·3	24·8	—	—
6	25·3	25·5	—	—

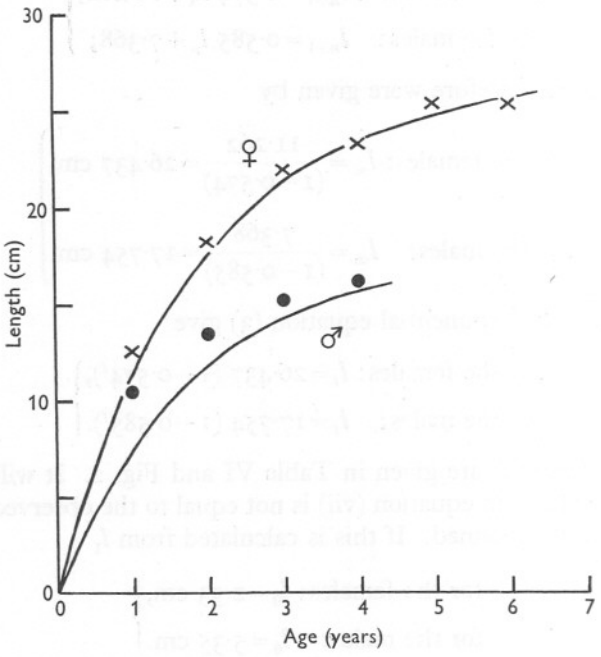


Fig. 2. Observed means and generalized age/length relation of Long Rough Dabs.
Females (×), with curve $l_t = 26{\cdot}437\ (1 - 0{\cdot}574^t)$.
Males (●), with curve $l_t = 17{\cdot}754\ (1 - 0{\cdot}585^t)$.

and so equation (v) becomes

$$\left.\begin{aligned}\text{for the females: } l_n &= 26{\cdot}437\ (1-0{\cdot}574^{n+0{\cdot}151}),\\ \text{for the males: } \quad l_n &= 17{\cdot}754\ (1-0{\cdot}585^{n+0{\cdot}667}).\end{aligned}\right\} \qquad \text{(xiii)}$$

Successive values of l_n are given in Table VII.

TABLE VII. SUCCESSIVE VALUES OF l_n, FROM EQUATION (xiii)

Males				Females			
Observed		Calculated		Observed		Calculated	
$n=1$	10·5	$n=1{\cdot}667$	10·49	$n=1$	12·6	$n=1{\cdot}151$	12·48
2	13·5	2·667	13·51	2	18·3	2·151	18·44
3	15·3	3·667	15·27	3	22·1	3·151	21·84
4	16·3	4·667	16·30	4	23·4	4·151	23·80
	—		—	5	25·3	5·151	24·92
	—		—	6	25·3	6·151	25·57

If the lengths given by equation (xiii), which fit the data very well, are the best mathematical approximations to the population, we must examine the meaning of the change x in the time scale; and various hypotheses can be suggested.

First, the values of l_0 may represent the length of the fish when the otolith formation commenced—or the otolith formation does not start until the fish are x years old—but if this were true it is difficult to see why the values are so different for the males and females. A second interpretation would be to suppose that the value of x may represent the difference between the true anniversary of spawning and that implied in the observed data. This can only mean that the males and females are produced at quite different times, which is absurd.

A third interpretation supposes the sampling contains an inherent bias. It has so far been assumed that the observed mean lengths ($\bar{x}$) are the best estimates of the population means (ξ). If, however, we allow that the sample means may be biased and do not necessarily approximate to the population values, it is suggested that the apparently good fit given in Table VII is a spurious one. The type of factor which would lead up to the kind of bias we are considering might be net mesh-size selection. With such a factor the observed mean lengths would all be too large (cf. Table VI), but if the bias for l_n and $l_{(n+1)}$ were even approximately the same, the biased mean lengths of the sample would give similar values of m and C in equation (i) as would the correct population means, since the regression is based on the ratio of l_{n+1} to l_n and not on their absolute values. This can be seen from the equation,

$$m=\frac{l_{n+1}-l_n}{l_n-l_{n-1}},$$

since if the mean lengths are similarly biased, the amounts of bias will cancel each other. If this interpretation is correct the calculated mean lengths given

in Table VI are more likely to represent those of the population than are the observed sample means, and the difference is produced by a factor such as net selection.

TABLE VIII

	Females						Males			
Age	1	2	3	4	5	6	1	2	3	4
$\bar{x}$	12·6	18·3	22·1	23·4	25·3	25·3	10·5	13·5	15·3	16·3
Approximate upper range limit	19	26	30	31	31	?	14·5	18	19	20
ξ	11·3	17·7	21·4	23·6	24·8	25·5	7·4	11·7	14·2	15·7
3σ	7·7	8·3	8·6	7·4	6·2	—	7·0	6·3	4·8	4·4
$\xi+2\sigma$	16·5	23·3	27·2	28·6	29·0	—	12·0	15·9	17·4	18·6
$\xi-2\sigma$	6·1	12·1	15·6	18·6	20·6	—	2·8	7·5	11·0	12·6

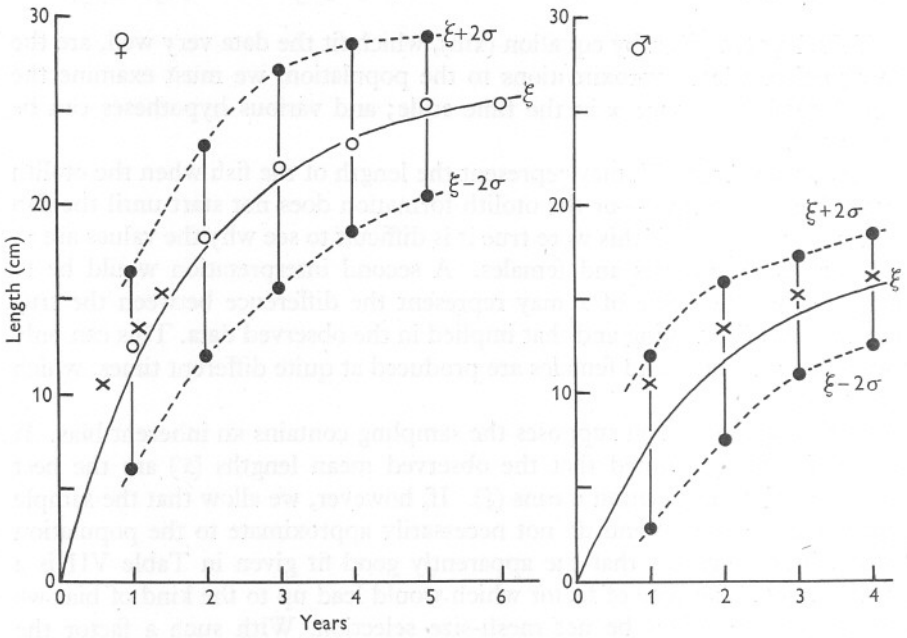


Fig. 3. Diagrams to illustrate the relation of theoretical population means (ξ) and the sample means ($\bar{x}$): ○·····○ for females; ×·····× for males. (In the left-hand diagram × represents $\bar{x}$ for males of comparable theoretical size, having been simply transferred from the right-hand diagram.)

Since net-selection bias operates mostly on the smaller fish, we may take the largest observed fish of any age-group to indicate the upper limit of the population range, and if we assume that the length frequency is normally distributed we may take this to represent three standard deviations and thus we may deduce the 2σ limits about the mean which should include 95% of the

population. These values have been calculated for the males and females and are given in Table VIII and Fig. 3. It will be noticed that the observed lengths are within the 2σ limits. It can also be seen from Fig. 3 that the discrepancy between the males and females is very small; the observed mean lengths for the first three age-groups of the males have been transposed to the appropriate position on the graph for the females, and it can be seen that the net selection bias operates very similarly for comparable lengths of both sexes.

The hypothesis of net-selection bias being the reason for the discrepancy between the observed and calculated lengths for each age is not put forward to the exclusion of the other factors given above. It is very unlikely that the observed lengths are those appropriate for whole numbers of years after spawning or the time of otolith formation; nevertheless, it is believed that a hypothesis concerning net selection, or some similar factor, is necessary for a true understanding of the facts. If, because of the time of sampling, it is necessary to change the value of t, it must be remembered that the otolith formation will probably commence at the same length in the males and females, so that the lengths at l_0 should be the same, but that this will be reached sooner by the faster growing sex, which, in Long Rough Dabs of this size, are the females.

TABLE IX. CALCULATED VALUES OF l_t, FROM EQUATION (iii), WHERE $m = 0{\cdot}4897$

Age	Observed	Calculated
1	10·5	9·1
2	13·5	13·5
3	15·3	15·7
4	16·3	16·7

The mathematical treatment of the data in this paper has been based on the regression line of $l_{(n+1)}$ on l_n, which is the 'best' straight line obtained by the method of least squares, weighted according to the number contributing to the mean length for each age-group. The values of the growth constants obtained in this way are more satisfactory and objective than any that could be obtained by fitting the curve of equation (iii) to the data by trial and error; or by purely graphical methods. The real advantage of the method used in this paper will be appreciated if we consider the growth of the males. By substituting the observed lengths into equation (iv) a weighted mean value for k can be obtained and has been found to be 0·714, corresponding to $m = 0{\cdot}4897$. The calculated values of l_t using this figure in equation (iii) are given in Table IX and give what is apparently a good fit, but there are numerous objections to the method.

It is always easier and more accurate to fit a straight line than a curve; and since the line is based on ratios, if there is bias in the mean lengths which is even approximately similar, better results will be obtained than by using the

biased observed values separately. Secondly, the slope of the line is not 0·4897, and it can hardly be fortuitous that the values of m in equation (i) are so similar for both sexes. Furthermore, Bertalanffy (1938) showed that a

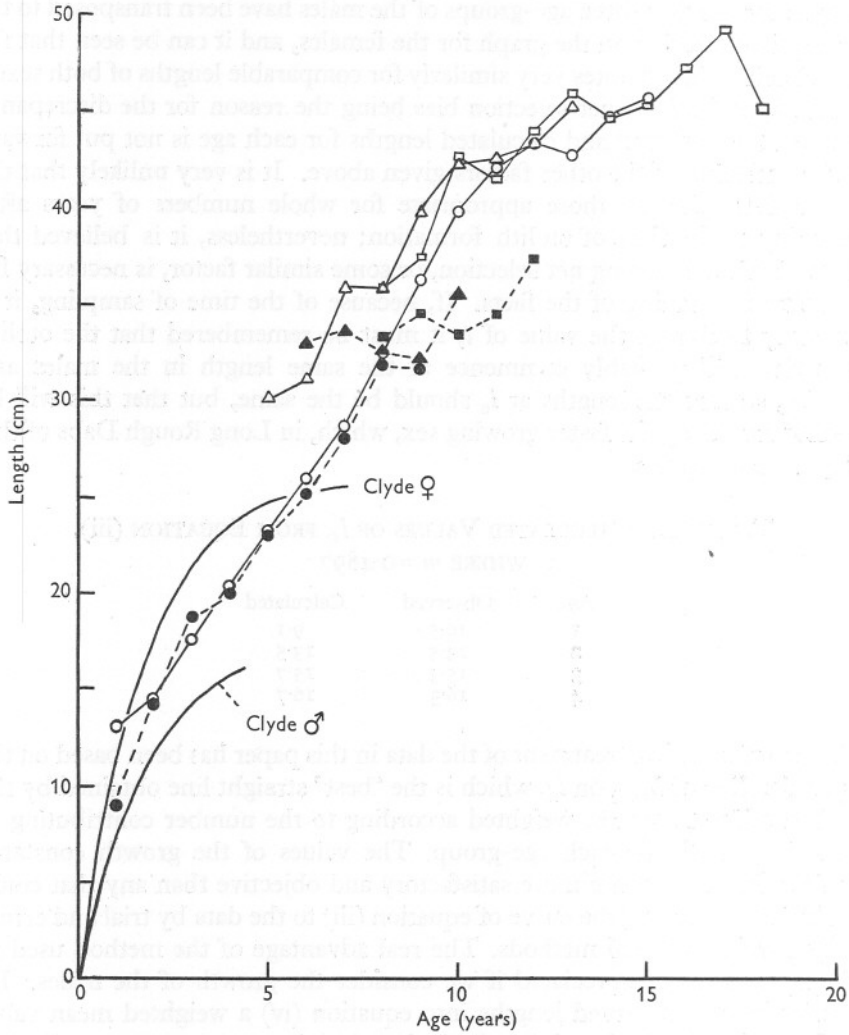


Fig. 4. Comparison of Clyde Long Rough Dab growth rate with that given by Milinsky (○ females, ● males, from Table II; □ females, ■ males from Table III) and by Essipow & Slastnikow (△, females, ▲ males) for the Barents Sea.

curve of the form in equation (iii) could be established on physiological principles. If his reasoning is correct the curve should fit the observed data, and any discrepancy will most probably be due to some factor such as biased

sampling or faulty age determination. The discrepancies found in Table VI can be explained very satisfactorily in this way, but it is hard to see why in Table IX the observed values for both $t=3$ and $t=4$ are too low. It is very important in work of this kind to bear in mind the limitations of the sampling device and to anticipate what differences these may produce on the parameters of the sample as compared with those of the population.

Comparison with Previous Studies

The most striking point that emerges from a comparison of the age and growth of Clyde-caught Long Rough Dabs and those from elsewhere is the difference in longevity. The ages recorded for Barents Sea specimens given by Essipow & Slastnikow (1932) range from 5 to 13, and Milinsky (1944) records females of up to 19 years old. Similarly, in Icelandic populations, Saemundsson (1925) found Long Rough Dabs of 17 years old, and Huntsman (1918), discussing the American subspecies *H. p. platessoides*, states that they can certainly live as long as 24 years, and probably 30 years should be assigned as the upper limit. In the Clyde no specimens have been found that have survived their sixth year.

Similar differences are found in the maximum sizes to which the fish grow. Specimens of 48·0 cm (Essipow & Slastnikow, 1932) and of over 50 cm (Milinsky, 1944) have been recorded from the Barents Sea, and of 2 ft. (61 cm) from Canada (Huntsman, 1918). Saemundsson (1925) found Long Rough Dabs of up to 42 cm at Iceland, and the longest recorded by Krüger (1942) for the Baltic was 34 cm. The largest specimen in the 1561 Clyde-caught Long Rough Dabs was 30·5 cm long.

With the rate of growth, however, no such very great differences are found, particularly in the younger age-groups. The data extracted from the Russian papers are shown in Fig. 4, together with the curves for the Clyde populations. It can be seen that the initial rates of growth are quite comparable, though later differing greatly, and the sexual differences are not apparent in the Russian data. Huntsman does not give any numerical data except as a generalized graph to illustrate that the growth rate varies markedly from place to place, and this is attributed to water-temperature differences. Saemundsson, however, gives sufficient data from Iceland for a comparison to be made between the Clyde and Icelandic populations. This is shown graphically in Fig. 6, which illustrates the data for 11–25 June 1924, chosen because it is sufficiently large and quite representative of the other data given.

Krüger's growth rate data from the Baltic (the nearest recorded to the Clyde) is shown in Fig. 6, where it can be seen that the rates are very similar, except for the older females, though the males are almost identical.

Summary

The ages of 1426 female, and 135 male Long Rough Dabs have been determined from otolith readings. The females were found to live into their seventh year and reach 30·5 cm, compared with males that live to their fifth year and reach 19·0 cm in length. However, the mean increase in length per year for a given length, has been found to be the same. The growth of the females has been shown to fit the equation $l_t = 26{\cdot}437\ (1 - 0{\cdot}574^t)$ and of the males

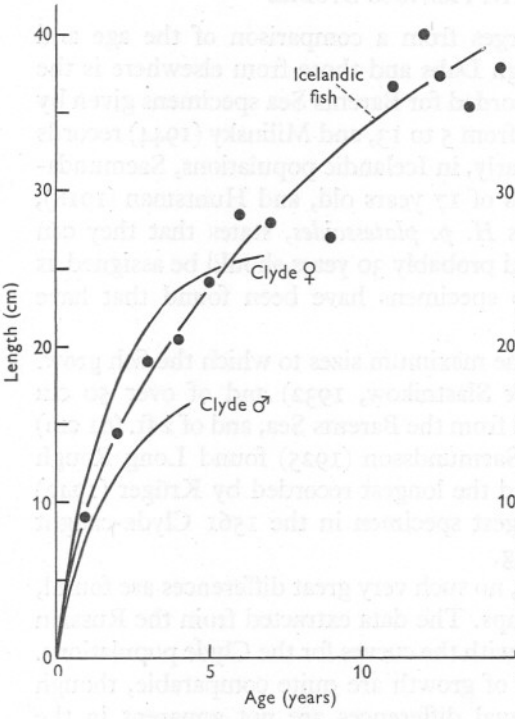


Fig. 5. Growth rate comparison of Clyde and Icelandic Long Rough Dabs.

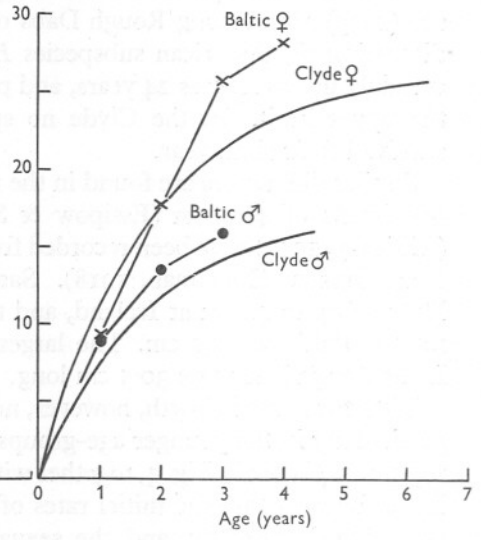
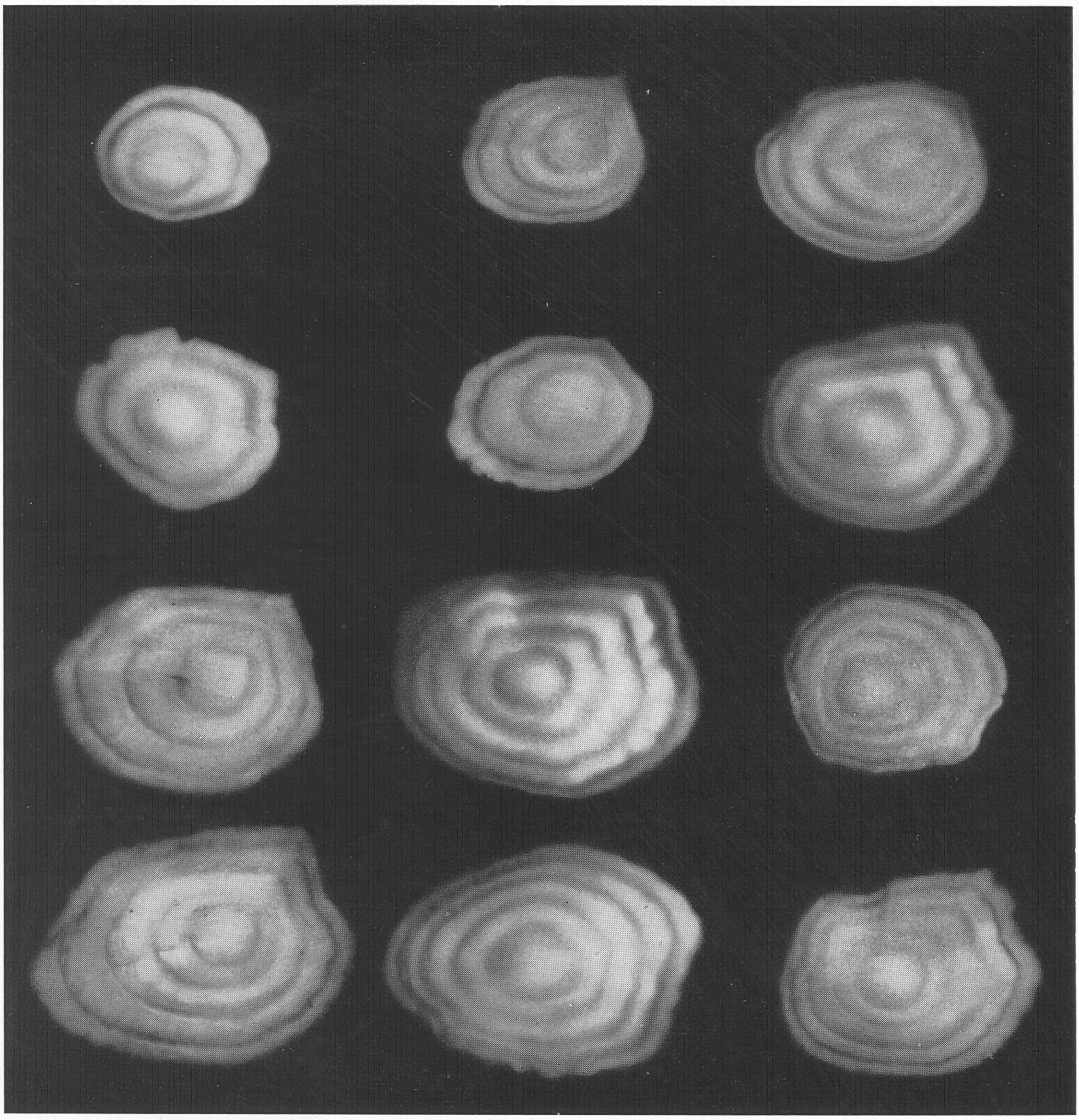


Fig. 6. Growth rate comparison of Clyde and Baltic Long Rough Dabs.

$l_t = 17{\cdot}754\ (1 - 0{\cdot}585^t)$. In the case of the females the observed and calculated values agree quite closely, but with the males there is a greater difference, and it is believed that the discrepancy is due to net selection. The suggestion is made that the calculated mean lengths for each age group are better estimates of those of the population than are the observed sample means. The two standard deviations limits can be estimated about the population means and the sample values are found to be within these limits.

Comparison with previous studies shows that the Clyde fish are smaller and shorter-lived than more northern specimens, though broadly speaking the rate of growth for comparable ages is not so dissimilar.



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EXPLANATION OF PLATE I

Otoliths of Long Rough Dabs. Top row: males left to right: 2 rings, 12·5 cm; 3 rings, 14·0 cm; 4 rings, 19·0 cm. Upper middle row: females, left to right: 2 rings, 17·5 cm; 2 rings, 16·5 cm; 3 rings, 22·0 cm. Lower middle row: females, left to right: 3 rings, 22·5 cm; 4 rings, 23·0 cm; 4 rings, 18·5 cm. Bottom row: females, left to right: 4 rings, 25·0 cm; 4 rings, 23·0 cm; 4 rings, 22·0 cm.

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THE FEEDING BEHAVIOUR OF STARFISH ON ESSEX OYSTER BEDS

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(Plate I and Text-figs. 1–3)

Starfish on Oyster Beds

Two species of starfish are found in large numbers in Essex rivers. They are *Asterias rubens* L. and the common sunstar *Solaster papposus* (L.). Of these, *Asterias rubens* has been regarded for many years as a voracious predator of oysters. Forbes (1841) recorded that it preyed on all kinds of Mollusca, and was commonly found with *Natica* in its stomach. He quotes Bishop Sprat's *History of the Royal Society*, in which the Admiralty Court laid penalties on those engaged in the oyster fishery 'who do not tread under their feet, or throw upon the shore, a fish which they call Five-finger, resembling a spur-rowel, because that fish gets into the oysters when they gape and sucks them out'. Of *Solaster papposus* he said: 'It is very ravenous, devouring shellfish. It frequents oyster and scallop banks, often in great numbers, and sometimes colonizes the sides of harbours frequented by oyster dredgers, in company with *Uraster rubens*.'

It has been readily confirmed since Forbes's time that *Asterias rubens* will feed on oysters, and it has been said to do so with such frequency as to cause very serious depredations. Cuénot (1887) recorded that a natural oyster bed of 10–12 km length had been completely destroyed by starfish, introduced incidentally by fishermen in their nets.

In Canada and the United States starfish present the oystermen with a severe problem. Needler (1941) regarded the starfish (*A. vulgaris*) as the worst enemy of oysters in Canadian Atlantic waters and one of the more serious obstacles to successful oyster farming. Galtsoff & Loosanoff (1939) stated that since the beginning of oyster culture in the United States in 1845, the starfish (*A. forbesi*) has been regarded as one of the most destructive enemies of shellfish on the Atlantic coast, and that by far the greater part of the loss caused is borne by the oyster growers. One estimate is that since 1921 no less than 500,000 bushels of oysters have been destroyed every year, representing an annual loss of 500,000 dollars. The decline in the scallop industry in Buzzards Bay was also attributed to the depredations of the starfish (Galtsoff & Loosanoff, 1939).

Korringa (1951) found that although *A. rubens* occurs on the Dutch oyster beds, the number of adult oysters devoured gives no cause for serious anxiety,

because the starfish apparently prefer mussels, or encounter less difficulty in opening them. Serious damage may, however, be caused by young starfishes among the tiny oyster spat on the tile-collectors.

In this country the starfish *A. rubens* has always been regarded by fishermen as the traditional enemy of mussels and oysters. Laver (1916) listed the starfish or 'Five-finger' as the most injurious of the direct enemies of the oyster in the Colchester Oyster Fishery. Collard (1902) referred to the starfish as a deadly foe of the Whitstable oyster.

Large numbers of *A. rubens* appear from time to time on oyster grounds in the River Crouch. They are numerous outside the mouth of the river, and particularly off the coast of Whitstable, where for many years the collection of starfish for fertilizer has been a substantial fishery.

Oystermen in the United States undertake costly control measures, usually with boats equipped with starfish mops. Lee (1951) estimated that the direct cost of control efforts amounts to more than 500,000 dollars annually. Scientists in the United States and Canada have enjoyed considerable success with the use of quicklime as a means of control (Galtsoff & Loosanoff, 1939; Loosanoff & Engle, 1942; Lee, 1951). Particles of quicklime spread over the oyster beds quickly sink to the bottom, and on to the surface membrane of the starfish. The caustic action of slaking lime causes lesions which penetrate and spread, eventually causing the death of the starfish. Its effects on oysters and other commercial species are considered to be negligible.

In Holland, the use of a spiked roller for catching starfish has been tried, but abandoned (Lambert, 1951), because of damage to the oysters. Copper sulphate and lime were found to be effective experimentally as methods of chemical control, but dredging is still the only means of destruction generally employed.

In this country, starfish are removed from the dredges during routine oyster cultivation and killed by drying out.

The work forming the subject of this paper was undertaken to test the efficiency of measures of chemical control of native starfish, and to decide whether more intensive control measures could be justified.

The Food of Starfish

Hunt (1925) examined the stomach contents of *A. rubens* from the Plymouth fishing grounds but found that only a small percentage contained recognizable remains. He rightly concluded that this was a result of their feeding method. The mouth of the starfish is small, and digestion of larger animals is largely external. He however recorded the feeding of *Asterias* on small specimens of the molluscs *Venus*, *Dosinia*, *Mactra*, *Pecten*, *Corbula*, *Cultellus*, *Lutraria*, *Syndosmya* and *Turritella*, and found the remains of the crustaceans *Portunus*, *Diastylis* and *Balanus* and the polychaete *Flabelligera*. In *Solaster papposus* he

found occasional remains of *Asterias rubens* and a small *Pecten*, but was unable to induce *Solaster* to feed under laboratory conditions. Blegvad (1914) had previously recorded that *Asterias* is devoured by larger individuals of its own kind and by *Solaster*. According to Mortensen (1927) the food of *Asterias* consists principally of molluscs, but it also eats crustaceans (especially barnacles), worms, echinoderms, even specimens of its own species, indeed almost everything eatable, living or dead. He said that it does considerable damage to oyster cultures, though it would appear that it cannot, by itself, open the large, undamaged oysters. It is itself eaten only by other sea-stars (*Solaster*, *Luidia ciliaris*). Vevers (1949) stated that while still in a young stage *Asterias rubens* eats barnacles and small lamellibranchs, but as an adult it feeds chiefly on worms, crustaceans, other echinoderms and many lamellibranchs, in particular *Chlamys opercularis* (L) and *Mytilus edulis*. In fact it would appear to be omnivorous, eating any living or dead material on which it can get a firm hold. Bull (1934) made some aquarium observations and found that *Solaster papposus*, *S. endeca* and the northern stone crab, *Lithodes maia*, fed in captivity mainly on *Asterias rubens*. *Solaster* also ate numbers of *Metridium senile* but would feed on molluscs or mollusc flesh only under conditions of extreme starvation.

The smallness of the mouth aperture in both *Asterias* and *Solaster* limits the size of prey which can be taken into the stomach in a recognizable form. The mouth aperture of *Solaster* is, however, larger than that of *Asterias*, a *Solaster* of 85 mm maximum radius having an aperture of 17 mm diameter, compared with only 4 mm for the mouth of an *Asterias* of similar size. For this reason recognizable stomach contents are more likely to be found in *Solaster*, but they still represent only the smaller items of its diet. Although the stomach contents of many starfish trawled in the Irish Sea were examined by the author, no *Asterias* and only two *Solaster* contained recognizable remains. Of these, one, of 75 mm maximum radius, contained a small *Chlamys* valve, a *Turritella* shell and several pieces of *Asterias* arm. The second, of 80 mm radius, contained a number of spines which were identical with those of *Psammechinus* taken in the same trawl.

In order to determine the larger food eaten by starfish they must be taken in the field when actually feeding, or observed feeding in aquarium tanks. During the course of dredging in the River Crouch, Essex, it has been established that the largest numbers of *Asterias rubens* are found on and in the neighbourhood of the Southward Laying, 1 mile below Burnham-on-Crouch (Text-fig. 2). Closely associated with it, but in less abundance, is *Solaster papposus*, and almost always in the same dredge are found several stone crabs, *Hyas araneus*. Subsequent dredging in the Rivers Crouch and Colne have confirmed that these three species are usually found together. *Asterias* taken from the dredges are frequently found in a characteristic arched feeding position but usually the prey has been released. Occasionally the prey is

retained and has been recorded. *Asterias* from the River Crouch have been observed feeding on oysters, slipper limpets (*Crepidula fornicata*), barnacles, *Spisula* and *Littorina*. *Solaster* has frequently been taken with the arms of *Asterias* in its stomach, and also quite often with pieces of *Alcyonidium gelatinosum*, which is abundant in this river. One small *Gibbula* and a small individual of *Crepidula* have also been found in the stomach of *Solaster* in the field.

The bottom community from which the *Asterias* is taken gives a good guide to the type of food which is being eaten. *Asterias* have frequently been recorded in large numbers on mussel beds off the Kent coast, and outside the mouth of the River Crouch, with obvious evidence of their depredations. Korringa (1951) records that Dutch oystermen occasionally see great numbers of the starfish *Asterias rubens* floating on the surface by means of gas bubbles. They believe that this is a method of migrating to other feeding grounds. In Britain this belief is also held locally, particularly at Whitstable, where the phenomenon is said to be associated with the destruction of one mussel bed and the search for another. It is agreed with Korringa that there is no scientific evidence to support this observation: it may be of abnormal occurrence, or due to pathological conditions, but the reports of local fishermen are seldom without foundation. In the River Crouch, particularly on the Southward Laying, where enormous numbers of *Crepidula* occur farther offshore than the main concentrations of oysters, the *Asterias* were found to be associated with the *Crepidula*, and were found in the dredges to be feeding on it. During 2 weeks in February and March, more than 10,000 *Asterias* were removed by the Ministry's two vessels from the offshore part of the Southward Laying, where the fauna consists almost entirely of *Crepidula* with some barnacles. In the mouth of the River Colne recent changes in conditions have led to the almost complete disappearance of oysters. *Crepidula* are abundant there in places, and also *Asterias*, which are often taken in the dredges feeding on *Crepidula*. Mr Francis, foreman of the Colne Fishery Board, was so convinced that the *Asterias* were substantially reducing the population of *Crepidula*, that, contrary to normal practice the starfish were returned to the ground to continue their good work.

Confirmation of the feeding behaviour suggested by field observations was sought in laboratory experiments. First, it was demonstrated that quicklime, sprinkled over the surface of the water in porcelain sinks containing *Asterias*, caused severe burns and lesions on the starfish, followed by their disintegration and death within about 3 days. Attention was then directed towards the advisability of using such drastic control measures on the oyster beds. It was decided that a series of laboratory experiments would help to assess the magnitude of the predations of starfish on the oysters.

Laboratory Observations

During the course of the experiments a large stock tank containing various shellfish and starfish was maintained, and provided a series of interesting observations. The tank contained a population of mixed sizes of *Asterias rubens* with several *Solaster papposus* and *Hyas araneus*, and an abundance of oysters and oyster spat, barnacles, *Urosalpinx* and *Crepidula*. During the period 11–27 January 1954, although many *Crepidula* and some *Urosalpinx* and barnacles had been devoured, only one small oyster and no oyster spat had been eaten. Between 17 March and 21 April 1954, a total of 262 *Crepidula*, and 28 *Urosalpinx* were devoured compared with only two brood oysters and no oyster spat. No count was kept of barnacles eaten. On one occasion, the introduction of a quantity of fresh *Crepidula* chains into the corner of the stock tank was followed almost immediately by the migration of thirteen out of twenty of the *Asterias* to that corner and extensive feeding.

Other shellfish devoured by *Asterias* in this stock tank included several *Pecten maximus* and *Mya arenaria*. One *Asterias* was observed attacking and devouring a smaller individual of the same species.

During the course of these observations *Solaster* was once observed feeding on a brood oyster, and very occasionally on a *Crepidula* chain. It was frequently observed to attack *Asterias*, individual arms of which were often taken from the stomach of *Solaster*.

Asterias, but rarely *Solaster*, was frequently attacked by *Hyas* (see 'Method of feeding' p. 321), which injures the arms by constricting them with its chelae, and biting the tips with its mandibles. A check on the *Asterias* in the tank showed that six *Solaster* and six *Hyas* had between them accounted for several complete *Asterias*, between one and three arms from each of nine others, and the tips or ends of arms from eight others during a period of 2 weeks in March. The *Solaster* had only rarely been damaged.

Several shore crabs, *Carcinas maenas*, which are found in the dredges with *Hyas*, were introduced into the tank, but on no occasion were they suspected of attacking starfish.

Further experiments were designed to discover the type of food on which *Asterias* and *Solaster* will feed. These were followed by offering *Asterias* various foods in equal quantity, in order to determine those which are the most important in its diet.

Experiment 1

This was designed to estimate the rate of destruction of brood oysters in the absence of other food.

Four large *Asterias* (maximum radius 140 mm) were placed in a large tank containing sixteen adult oysters. As each oyster was consumed it was removed, and replaced by another of approximately the same size. It was found that

the *Asterias* assumed a feeding position in less than 2 days, and within 3 days of commencing the experiment two oysters had been consumed. During the period 7 January–21 April 1954, the four starfish consumed an average of 4·8 oysters per week. The largest number of oysters eaten in 1 week was eight. The oysters used were between 44 and 76 mm average diameter. One *Asterias* (♂) spawned on 11 May at a temperature of 15·3° C. Two others spawned on 29 May. Feeding stopped a few days before and after spawning and did not resume its former intensity throughout the summer. Generally an oyster was opened and consumed in less than 2 days. One starfish was observed to complete the process in 22 h during March, and a period of less than 24 h was recorded on several occasions. Evidently the larger starfish can and will eat oysters consistently in the absence of other food, and their rate of feeding under such conditions would constitute a serious menace to oyster growers.

Experiment 2

This was designed to discover on which other animals the starfish will feed, in the presence of oysters. Four *Asterias* ranging from 50 to 120 mm maximum radius of arm, were confined with abundant food including oysters, oyster spat, *Mytilus*, *Cardium*, *Paphia*, *Macoma*, *Buccinum*, *Crepidula*, *Nassarius*, *Nucella*, *Urosalpinx*, *Littorina*, *Gibbula* and barnacles. It was not possible to keep equal numbers of each food throughout the experiment or to replace each one immediately it was consumed, but a stock of 6 oysters and 6 shells bearing oyster spat (17) was maintained, and at no time did the total of any other species exceed this figure. From 4 January to 24 March 1954, the 4 *Asterias* consumed 45 *Mytilus*, 12 *Macoma*, 10 *Cardium*, 4 *Urosalpinx*, 4 *Nucella*, 3 *Paphia*, 3 *Crepidula*, 1 *Nassarius*, 1 *Littorina* and 1 *Gibbula*. It is significant that during this time no oysters or oyster spat were consumed. It was concluded that barnacles provided little attraction because many of the food animals devoured were coated with undamaged barnacles, mainly *Elminius*.

Experiment 3

Four *Solaster* (55–70 mm maximum radius) were confined with abundant food supply including oysters, oyster spat, *Mytilus*, *Cardium*, *Littorina*, *Urosalpinx*, *Crepidula*, *Macoma*, *Metridium* and 1 *Psammechinus*, but no *Asterias*. From 5 January to 11 March, only 3 *Cardium*, 2 *Macoma*, 1 *Psammechinus*, 1 oyster spat and 1 *Metridium* were devoured. It has been found that the feeding rate of starfish decreases before and during spawning. The *Solaster* spawned in the sink on 25 February, but this low rate of feeding could not entirely be associated with this fact, but was almost certainly due to an unsuitable food supply. When, on 12 March, an *Asterias* was added it was rapidly devoured, the remains being found in the stomachs of 3 *Solaster*.

When further *Asterias* were added they were attacked almost at once by the *Solaster*. Evidently *Solaster* can eat other food, but its natural food certainly includes *Asterias*, confirming the observations made in the field.

Experiment 4

The preliminary experiments seemed to indicate that *Mytilus* provides the greatest attraction to *Asterias*, but the number of mussels occurring at the Southward Laying (Mistakidis, 1951, and own observations), and generally in the River Crouch, is insignificant compared with other organisms. For this reason, only the more important animals related to oyster cultivation were included as food in this experiment. Four *Asterias* (maximum radius 60–120 mm) were confined with 6 brood oysters, 6 chains of *Crepidula*, 6 oyster shells each bearing several oyster spat, and 6 *Urosalpinx*. Between 25 March and 22 June, 1 brood oyster, 8 oyster spat, 27 *Crepidula* and 10 *Urosalpinx* were devoured.

Experiment 5

During the field observations mentioned previously, it was found that *Asterias* is frequently found feeding on *Crepidula*. In order to determine to what extent *Asterias* will feed on *Crepidula* in the presence of oysters, 10 *Asterias* (maximum radius 70–108 mm) were confined with 20 *Crepidula* chains and 20 small oysters, the largest oyster measuring 54 mm average diameter. These were distributed at random in a large tank with running sea water. After 5 days (20–25 March 1954) it was found that every *Crepidula* chain had been attacked to some extent, with as many as five individuals eaten from one chain. A total of 53 individuals of *Crepidula*, most of them adult, were eaten. This must be compared with the consumption of only 3 oysters. In a subsequent experiment the same 10 *Asterias* were used and 20 adult individuals of *Crepidula* were distributed at random with 20 oysters. During a period of 14 days (25 March–8 April) 9 oysters and 13 *Crepidula* were eaten. The possible explanations of the lower proportion of *Crepidula* eaten are believed to be first, that a chain of individuals exerts a greater attraction than a single one, or, secondly, that, attraction being discounted, the random movements of the starfish were interrupted by the presence in one place of a series of readily accessible animals provided by a chain. It must be remembered, however, that in the first part of this experiment all 20 of the chains had been attacked by *Asterias*, and that under natural conditions there is an ample supply of *Crepidula* which are more likely than not to be concentrated in chains. In a subsequent experiment 20 oysters were placed in one corner of the tank (area 160 cm square) and 20 chains of *Crepidula* in the opposite corner, with 10 *Asterias* in the centre of the tank. In 6 days (8–14 April), 4 oysters and 33 *Crepidula* were eaten. The experiment was repeated, reversing the positions of *Crepidula* and oysters. In the subsequent 6 days, 4 oysters and 25 *Crepidula*

were consumed. This behaviour confirms the suggestion that *Asterias* prefers *Crepidula*, possibly because it is less inaccessible than the oyster.

Experiment 6

It became evident from observations made in the stock tank and subsequent experiments that there was a difference in feeding behaviour according to the size of the starfish. A population of *Asterias* was divided arbitrarily into size-groups by reference to a histogram composed from measurements of *Asterias* dredged from the River Crouch (Text-fig. 1). The groups selected were—group I: <30 mm; group II: 40–70 mm; group III: 70–100 mm; group IV: >100 mm maximum radius.

Group I. 23 *Asterias* of maximum radius 30 mm were confined with shells and stones bearing oyster spat, *Crepidula* spat and barnacles, and 6 *Urosalpinx*. The tanks were examined every day or two and the food eaten was replaced. During the period 22 April–10 May 1954, a total of 15 oyster spat, 13 *Crepidula* spat and 6000 barnacles, mainly *Elminius*, had been devoured. These results were consistent throughout the experiment and it was significant that on several occasions an oyster shell was cleared of barnacles leaving several undamaged oyster spat. Further, it was found that the number of oyster spat devoured was always greatest when virtually all the barnacles had been devoured.

Group II. 10 *Asterias* of maximum radius 40–70 mm were confined with 6 young oysters, 6 shells bearing oyster and *Crepidula* spat, 6 chains of *Crepidula*, 6 *Urosalpinx* and 2 stones covered by barnacles. During the period 22 April–10 May 1954, a total of 1 oyster spat, 2 *Crepidula* spat and 3500 barnacles were consumed. Although no selection was made of barnacles used in the experiments, those eaten by group II were mainly of a larger size than those eaten by group I, and it was found that the larger barnacles were eaten before the smaller sizes were attacked.

Group III. 7 *Asterias* of maximum radius 70–100 mm were confined with 6 young oysters, 6 shells bearing oyster and *Crepidula* spat and barnacles, 6 chains of *Crepidula*, 6 *Urosalpinx* and 2 stones covered by barnacles. Between 22 April and 10 May 1954, a total of 3 oyster spat, 13 *Crepidula* spat, 2 oysters, 47 adult *Crepidula*, 1 *Urosalpinx* and 425 barnacles were devoured.

Group IV. 5 *Asterias* of maximum radius 100–145 mm were confined with unlimited quantities of oysters and oyster spat, *Crepidula* and its spat, *Urosalpinx* and barnacle covered shells and stones. During the period 22 April to 14 May 1954, a total of 1 oyster, 29 adult *Crepidula* and 20 barnacles together with 1 *Pecten maximus*, which was being stored in the same tank, were eaten. This confirms the previous result (Exp. 2) that the barnacles provide little attraction for the larger starfish.

In Table I, a comparison is made of the average numbers of different foods eaten by one *Asterias* from each group in 18 days. It can be seen that the

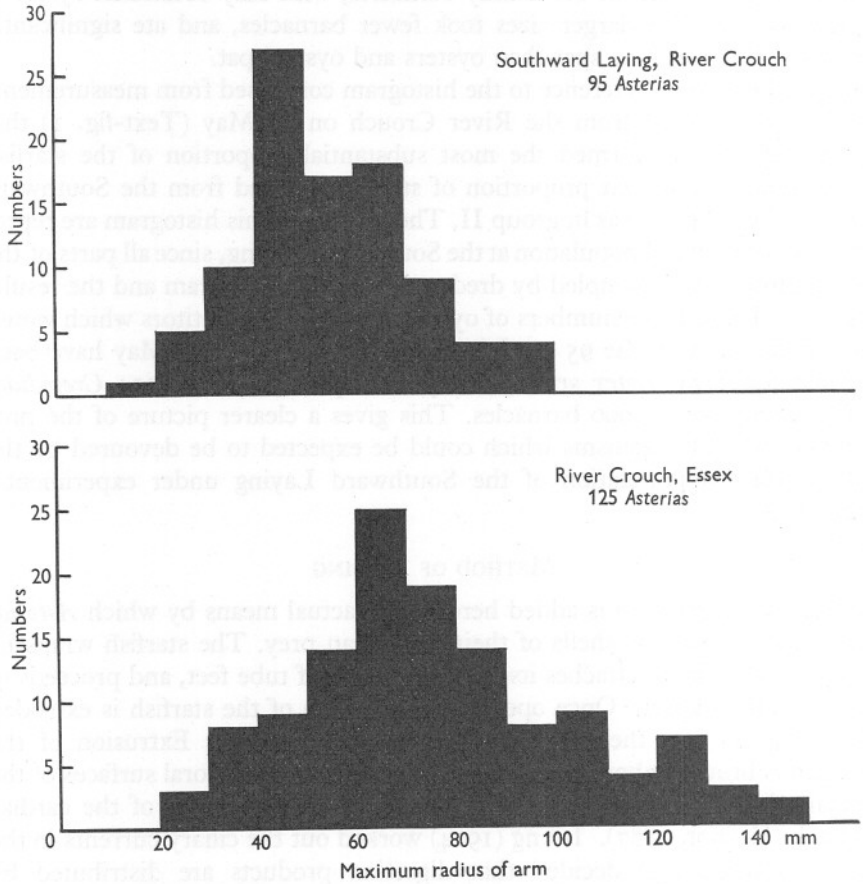
smaller sizes of *Asterias* ate mainly barnacles, with only occasional oyster or *Crepidula* spat. The larger sizes took fewer barnacles, and ate significantly more *Crepidula* and its spat than oysters and oyster spat.

It can be seen by reference to the histogram composed from measurements of *Asterias* dredged from the River Crouch on 10 May (Text-fig. 1) that groups II and III formed the most substantial proportion of the starfish population. The largest proportion of starfish dredged from the Southward Laying (Text-fig. 1) was in group II. The starfish in this histogram are representative of the total population at the Southward Laying, since all parts of the oyster ground were sampled by dredge. Using the histogram and the results shown in Table I, the numbers of oysters and their competitors which would have been eaten by the 95 starfish during 18 days in April–May have been calculated as: 24 oyster spat, 54 *Crepidula* spat, 5 oysters, 114 *Crepidula*, 2 *Urosalpinx* and 26,900 barnacles. This gives a clearer picture of the proportions of food organisms which could be expected to be devoured by the natural starfish population of the Southward Laying under experimental conditions.

Method of Feeding

Little new information is added here on the actual means by which *Asterias* and *Solaster* open the shells of their molluscan prey. The starfish wraps its arms round its prey, attaches itself by the series of tube feet, and proceeds to pull the valves apart. Once opened, the stomach of the starfish is extruded (Pl. I, fig. 1) into the prey and digestion commences. Extrusion of the stomach is brought about by contraction of the oral and aboral surfaces of the animal which forces coelomic fluid into loose separate folds of the cardiac stomach (Cuénot, 1887). Irving (1924) worked out the ciliary currents in the starfish *Patiria*, and decided that digestion products are distributed by ciliary action, the Tiedemann's diverticulum being a region specialized for distribution.

The mechanics of the process by which the prey is opened have provided a subject for controversy for many years. Schiemenz (1895) explored most possibilities and concluded that a starfish can develop sufficient force to open a bivalve unaided by any poisonous secretion. Van der Heyde (1922) established the presence of a toxic substance in the stomach extract of the starfish *Asterias forbesi*, using the heart of *Pecten* and the gastrocnemius muscle of a frog. Sawano & Mitsugi (1932) found that the stomach extract of *Asterias rollestoni* caused a tetanic contraction in the heart of the oyster. Cahn (1950) has described the feeding process in the Japanese starfish (*A. amurensis*), which begins its attack on the oyster by pouring a poison secreted by its stomach into the water in front of the oyster's inhalant siphon. The poison in the inhalant current of water stimulates the oyster to close its shell. The adductor muscle loses tonus and relaxes, and the valves are pulled apart by



Text-fig. 1. Population histograms of *Asterias rubens* from a dredge survey on 10 May 1954.

TABLE I. SHELLFISH DEVOURED BY *ASTERIAS* DURING THE INCLUSIVE PERIOD 23 APRIL–10 MAY 1954

Figures reduced to 1 *Asterias* in each size group

Size group ...	I	II	III	IV
Max. radius (mm) ...	15–30	40–70	70–100	100–145
Oyster spat	0·7	0·1	0·4	0
Crepidula spat	0·6	0·2	1·9	0
Oysters	0	0	0·3	0·2
Crepidula	0	0	6·7	4·7
Urosalpinx	0	0	0·1	0
Barnacles	260	350	61	3

the tube feet of the starfish. The time required to destroy an oyster is about 2 h from the first injection of the poison into the water to the complete digestion of the body. Experimentally, this paralysis remains effective for many hours, if it does not result in death.

Even if such an effect could be produced by the stomach extract of *A. rubens*, this explanation is still open to Schiemenz's criticism that a bivalve which has been opened but not digested will quickly recover. With oysters, mussels and *Pecten*, the author has observed on several occasions that the disengaging of the arms of the starfish has resulted in such rapid closure by the bivalve that the stomach of the starfish was trapped and torn. Evidently if *A. rubens* is able to produce a poison, it can only have a temporary effect and appears not to be lethal.

It is interesting that the outer margin of the oyster shell is invariably broken away during an attack by *Asterias*, but in other bivalves and *Crepidula* the shell margin remains intact. This could be said to result from a deliberate chipping away of the edge, which could allow the entry of a narcotic, but is more likely to be due to a strong pull by the starfish which causes the weakest part of the shell to break away first. This conclusion was strengthened when an *Asterias* attacked an oyster which had been suffering from a severe attack by *Polydora*, the shell-boring mudworm. Here the edge of the shell remained unbroken, while one valve was broken across where it had been weakened by the excavation of *Polydora* in the region of attachment of the adductor muscle.

A chain of *Crepidula* may be attacked from either end or at some intermediate point in the chain. Once an individual has been detached or the chain parted the diverticula of the stomach are everted around the now exposed body of its prey.

The barnacle is the favourite food of the smaller sizes of *Asterias*. *Elminius modestus*, the dominant barnacle in the bottom community, is eaten most frequently, but also *Balanus* where it and *Elminius* occur together. The method of feeding appears to depend on the strength of the starfish. The group I starfish usually ate all the barnacles which they were capable of detaching, by sucking the contents through the underside. The larger barnacles were then devoured by sucking the contents through the operculum. The group II starfish could feed on all sizes of barnacles by detaching them, but the smaller barnacles were not usually attacked until the larger ones had been eaten.

Solaster seldom overcomes a complete *Asterias* at once. An *Asterias* in captivity is actively pursued by *Solaster* which attaches itself to one or more arms of the *Asterias*. An arm is soon autotomized by the *Asterias*, and devoured by the *Solaster* (Pl. I, fig. 1). After digestion, the spicules of *Asterias* are rejected.

Hyas presents an interesting study in behaviour. When an *Asterias* is feeding on a mollusc, the *Hyas* appears to be waiting for scraps, and when the shell

of the prey is discarded it claws out what few remains the *Asterias* has left. In many instances, however, the *Hyas* becomes impatient and attacks the *Asterias* itself, which it grips between its two chelipeds then proceeding to use its mandibles to chew off the tip of the arm of the *Asterias* (Pl. I, fig. 2). Since the sensitive eye spot is located here such an action must cause serious damage to the starfish, but in many cases it is followed by complete autotomization of the arm, which is then devoured. In some instances the constriction of the arm by the chelipeds of *Hyas* is so severe that the distal portion of the arm becomes functionless and withers. In one of the specimens in Pl. I, fig. 2, a new arm tip appeared at the point of constriction after several weeks. It is a strange fact that although *Hyas* frequently attacks and feeds on *Asterias* it seldom attacks *Solaster*. This may be coupled with an interesting local tradition that *Solaster* may be used as bait to poison marauding garden cats, but *Asterias* is not mentioned in this capacity. It is possible that *Hyas* can detect some toxic substance in the flesh of *Solaster* and it was decided to investigate this by simple experiment. Ten small pieces, from each of a freshly dead *Asterias* and *Solaster*, were placed in a glass trough with two *Hyas*. After several hours it was found that all the pieces of *Asterias* had been consumed, while all ten of the pieces of *Solaster* remained. After another hour, no further feeding had taken place, and ten more pieces of *Asterias* were added with the same result. Evidently there is some substance in *Solaster* which renders it undesirable to *Hyas*. I have, however, observed one *Solaster* eat a smaller one of the same species, but it was almost certainly already dead.

Rate of Feeding

Experiments in Canada (Needler, 1941) showed that one starfish may kill three oysters of about half its own length in one week, and much larger numbers of spat. A starfish was found by experiment to require a diameter of about $1\frac{1}{2}$ times the length of an oyster to attack it successfully. Since most of the starfish on Canadian beds are between 2 and 3 in. in diameter, the greatest danger is to spat or small oysters.

In America Mead (1901) noticed that a single small *Asterias forbesi* devoured over 50 clams (*Mulinia lateralis*) in 6 days. Galtsoff & Loosanoff (1939) showed in the laboratory, at Milford, Conn., that one medium-sized starfish consumed thirteen 1-year-old oysters in 4 days. One small starfish of 1·7 cm diameter destroyed 25 oyster spat of less than 1 cm diameter in 3 days. They found that in experiments conducted in outdoor tanks during the pre-spawning period from May to July, the starfish fed only rarely, but after completion of spawning they became exceedingly voracious until the onset of low temperatures in winter and early spring.

In Exp. 1 (above) the starfish were confined in indoor tanks protected from the influence of lowest temperatures. Uninterrupted feeding took place from

the beginning of January to the beginning of February, although temperatures dropped at times to a minimum of 3·0° C. Feeding ceased completely between 3 and 12 February when minimum temperatures of 2·0° C. were recorded, and was resumed as temperatures began to rise again. One male *Asterias* spawned on 11 May and 2 others on 29 May, but feeding was interrupted only for a few days before and after spawning, although the *Asterias* were greatly swollen with gonads. In this instance the behaviour of *A. rubens* seems to differ slightly from *A. forbesi*, which is inhibited from feeding by temperatures between 0 and 6° C. The rate of feeding during the summer months was less than that during the first 4–5 months of the year. Although an oyster was devoured in less than 24 h, the maximum number eaten by four starfish in one week during the period under observation was eight, representing an average of two per starfish. These oysters were at least 3 years of age and between 44 and 76 mm average diameter.

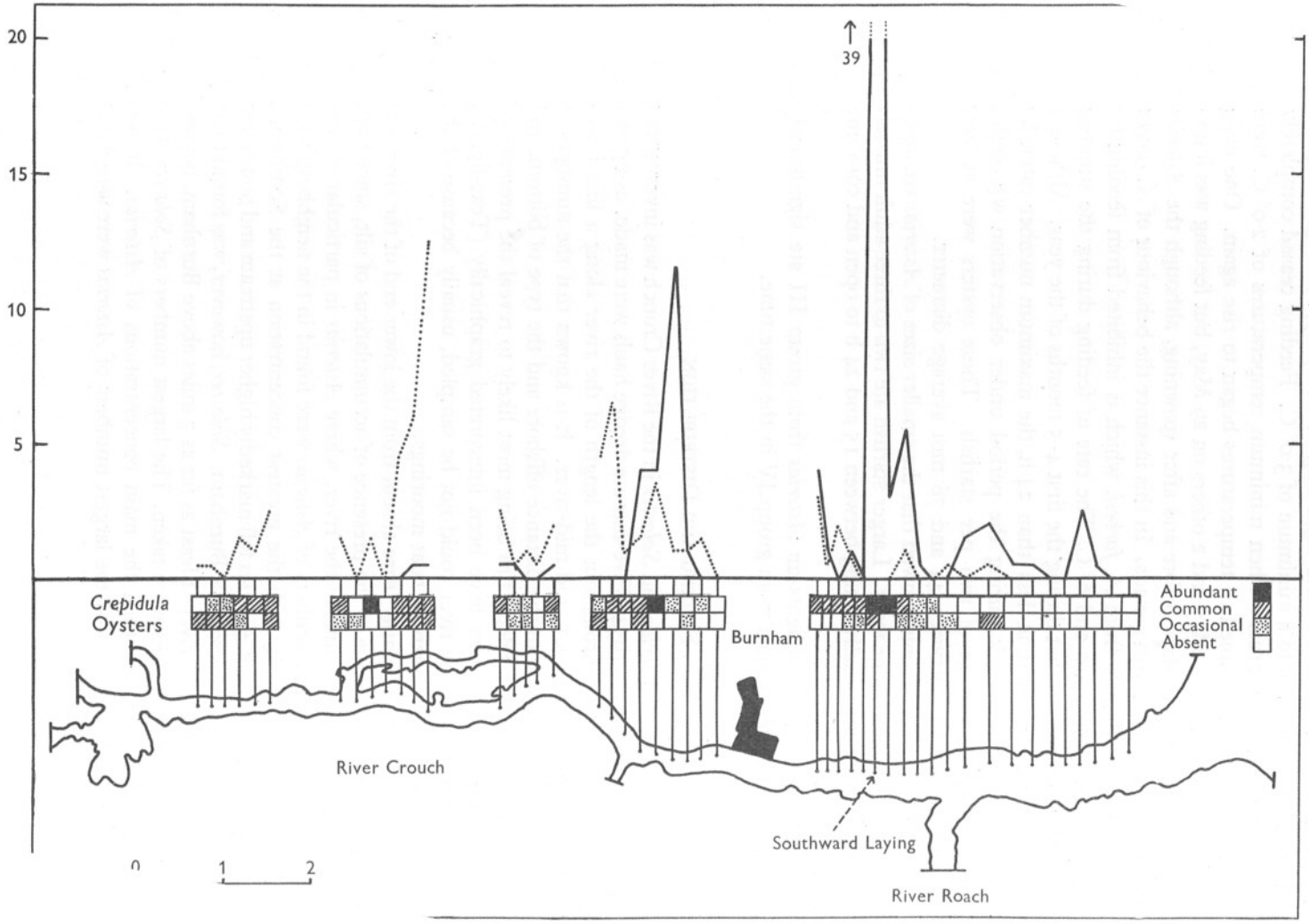
Subsequent experiments showed that the smaller sizes of *Asterias* ate up to 200 barnacles each in one week. Larger starfish ate two to three adult mussels per week, each one usually taking between 15 and 24 h to open and consume one mussel.

In the feeding experiments an *Asterias* from group III ate significantly more than a larger starfish from group IV in the same time.

Feeding and Distribution

The distribution of *Asterias* and *Solaster* in the River Crouch was investigated by a survey on 10 May 1954. Five-minute dredge hauls were made, using two 4 ft. power dredges, throughout the length of the river along a line intermediate between L.W.O.S.T. and mid-river. It is known that the numbers of starfish vary in relation to the distance offshore and the type of bottom, and this arbitrary line was selected as being most likely to reveal the presence of both species. The results have been interpreted graphically (Text-fig. 2). Certain stretches of the river could not be sampled, usually because of the presence of large numbers of boat moorings.

Both *Asterias* and *Solaster* were absent from the lower end of the river and this is believed to be due to the presence of accumulations of silt, since both species are found just outside the river, where *Asterias* in particular is very abundant. The largest numbers of *Asterias* were found in the neighbourhood of Burnham-on-Crouch, with the greatest concentration at the Southward Laying. The numbers of *Asterias* fell markedly higher upstream and none were found more than $4\frac{1}{2}$ miles above Burnham. *Solaster*, however, was found to be present throughout the river at least as far as 7 miles above Burnham, beyond which no further samples were taken. The largest numbers of *Solaster* were found higher upstream than the main concentrations of *Asterias*. It was evident from the results that the largest numbers of *Asterias* were associated



the River Crouch, Essex, and the density of *Crepidula* and oysters.

Solaster; ———, *Asterias*.

with derelict or semi-cultivated oyster ground with a bottom fauna characterized by a high proportion of *Crepidula*. *Asterias*, and less frequently *Solaster*, are sometimes found on the shore above L.W.O.S.T.

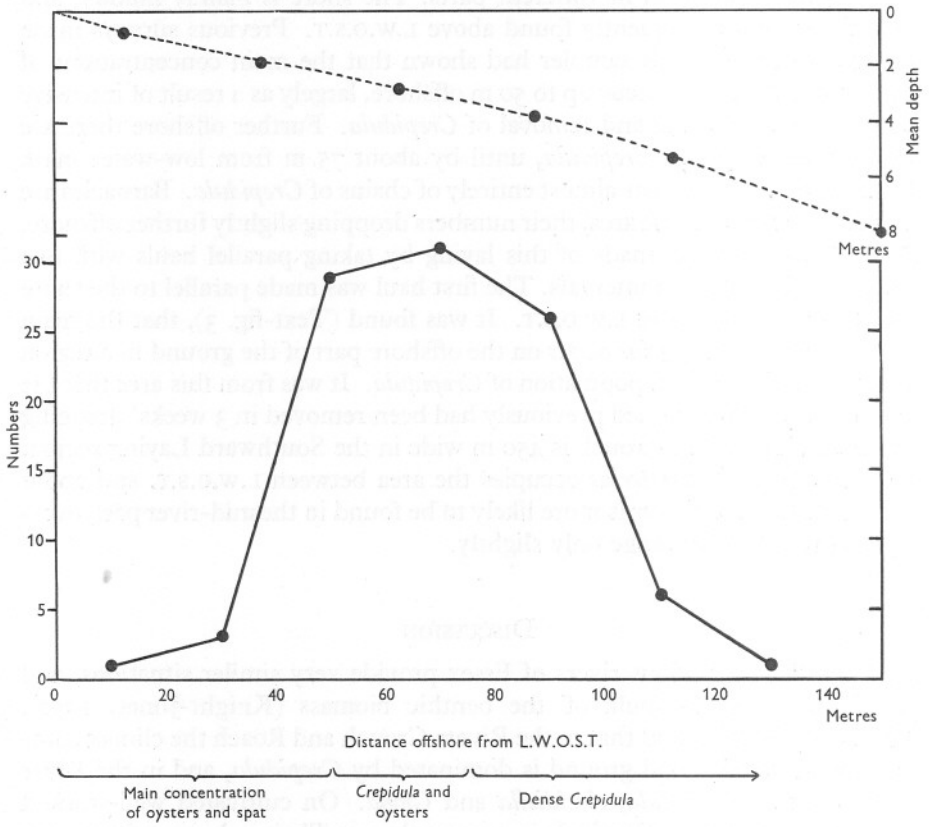
A more intensive survey was made of the distribution of starfish over the Southward Laying, where the highest concentration of *Asterias* was recorded. This is an oyster ground which has the characteristics of both cultivated and uncultivated ground in its different parts. The shore is mainly muddy, and *Asterias* is only infrequently found above L.W.O.S.T. Previous surveys made using a van Veen grab sampler had shown that the main concentrations of oysters and their spat occur up to 50 m offshore, largely as a result of intensive cultivation of this area and removal of *Crepidula*. Further offshore there are proportionately more *Crepidula*, until by about 75 m from low-water mark the bottom fauna consists almost entirely of chains of *Crepidula*. Barnacles are profuse over most of the area, their numbers dropping slightly further offshore. A dredge survey was made of this laying by taking parallel hauls with two oyster dredges at 20 m intervals. The first haul was made parallel to the shore and 10 m offshore from L.W.O.S.T. It was found (Text-fig. 3), that the main concentrations of *Asterias* occur on the offshore part of the ground in a region characterized by a high population of *Crepidula*. It was from this area that the 10,000 *Asterias* mentioned previously had been removed in 3 weeks' dredging in March. The River Crouch is 450 m wide in the Southward Laying region. It can be seen that *Asterias* occupies the area between L.W.O.S.T. and about 150 m offshore. *Solaster* is more likely to be found in the mid-river part, overlapping the *Asterias* range only slightly.

Discussion

Ecologically, the various rivers of Essex provide very similar situations, and *Crepidula* forms the bulk of the benthic biomass (Knight-Jones, 1952). Mistakidis (1951) found that in the Rivers Crouch and Roach the climax community on uncultivated ground is dominated by *Crepidula*, and in the River Blackwater by *Crepidula*, *Ascidiella* and *Ciona*. On cultivated well-stocked oyster grounds where *Crepidula* is present the equilibrium is an artificial one and has to be maintained by frequent dredging and harrowing. On such oyster beds the bottom community in Essex may be described as characterized by *Ostrea* with *Elminius* as epifauna. *Urosalpinx* occurs in varying numbers in the Essex rivers and is well suited by the conditions on cultivated grounds.

It is evident that there are ample quantities of food for starfish in the rivers, where cultivated and uncultivated grounds occur side by side. Although *Mytilus* is not abundant in the River Crouch, it appears to provide the favourite food of *Asterias*. *Mytilus* is usually discouraged on oyster beds because of its habit of binding oysters and shells bearing spat together with byssus threads and because its presence increases the deposit of silt.

During the laboratory experiments it was noted that when oyster spat was devoured by *Asterias* the two valves were only rarely detached from each other. At the end of 1953, a survey had been made of the Southward Laying, Essex, using dredges and a van Veen grab sampler. From the results of this survey it was calculated that there had been a total mortality of 73% of the 1953 settlement of oyster spat before the end of December 1953. It was estimated



Text-fig. 3. Distribution of *Asterias rubens* on the Southward Laying, River Crouch.

that 58% of the spat had been destroyed by *Urosalpinx*, leaving a mortality of only 15% attributable to other causes (Hancock, 1954). One of these causes is likely to be starfish, although smothering by silt and attacks by *Carcinus* are considered to be prominent amongst the dangers to which oyster spat are exposed (Knight-Jones, 1952). It can be concluded that *Asterias* is by no means the most important cause of mortality amongst oyster spat in these rivers, although it may account for some of it.

From the viewpoint of oyster culture, the problem is to what extent *Asterias* behaves as a predator on oysters and their spat. There is evidence

from observations in the field that the largest numbers of *Asterias* are to be found amongst the most dense concentrations of *Crepidula*. Laboratory experiments have shown that when oysters and *Crepidula* are present side by side, larger numbers of *Crepidula* are likely to be consumed. The smaller sizes of *Asterias* show a marked preference for barnacles over oyster spat. *Crepidula* and barnacles occur in enormous numbers in the River Crouch and compete with the oyster and its spat for space and food supply. *Asterias* often feeds on *Urosalpinx*, which has been shown to eat large numbers of oyster spat. It would not be possible to estimate the negative value of pests eaten by starfish in terms of oysters, but *Asterias* has been found to feed significantly more on animals which are important predators and competitors than on the oysters themselves. The figures from the feeding experiments show that *Asterias* must play a large part in reducing their numbers. They also show, however, that the larger starfish can and will eat many oysters in the absence of other food. In Essex, oyster grounds are seldom free from large numbers of barnacles or remote from quantities of *Crepidula*. It must be concluded that *Asterias* provides some measure of control of the pests of oyster grounds and as such can be regarded as beneficial to oyster culture. There is no doubt that a certain number of oysters and spat fall prey to *Asterias*, but it is felt that severe damage is only likely to occur when starfish are present on highly cultivated grounds with large concentrations of oysters and their spat. In such circumstances, removal and destruction of the starfish is recommended. When, however, a derelict ground is being cleaned by dredging and any *Asterias* taken can only be feeding on *Crepidula* and barnacles, to destroy the starfish would be a misguided policy. Starfish are known to be capable of migrating, but there has been no evidence that under the conditions in these rivers a cultivated ground provides a greater attraction than an uncultivated one. It is possible that under controlled conditions starfish could be transplanted to the more isolated of the derelict grounds and used as a means of reducing the numbers of pests. Before relaying oysters to such a ground, chemical control to remove the starfish might have some application, but it is felt not to be justified under normal conditions.

Solaster and *Hyas* have been found to be natural enemies of *Asterias* and must have some effect in limiting the size of its population. The two species of starfish differ slightly in distribution, and this fact may lessen the effects of feeding by *Solaster* on *Asterias*.

Summary

Laboratory and field observations were made on the food and feeding of the starfish in relation to its role as a predator on oyster beds in the rivers of Essex, particularly in the river Crouch.

Asterias rubens was found most likely to be associated with large numbers of *Crepidula*, the most serious competitor of the oyster.

Laboratory experiments showed that although *Asterias* occasionally ate spat and adult oysters, the greater part of its food was made up of organisms which are competitors of the oyster. The smaller sizes of *Asterias* ate large numbers of barnacles, with occasional spat of oysters and *Crepidula*. The larger occasionally ate oysters and oyster spat, but almost always exhibited a preference for mussels and, in the absence of these, for *Crepidula*, and sometimes even for *Urosalpinx*.

Some observations were made on the method and rate of feeding and distribution of *Asterias* and *Solaster papposus*, and certain aspects of the feeding behaviour of the stone crab, *Hyas araneus*.

It was concluded that *Asterias* is not such a serious enemy of the oyster as was previously supposed, and that under certain conditions, its presence may be beneficial to oyster culture.

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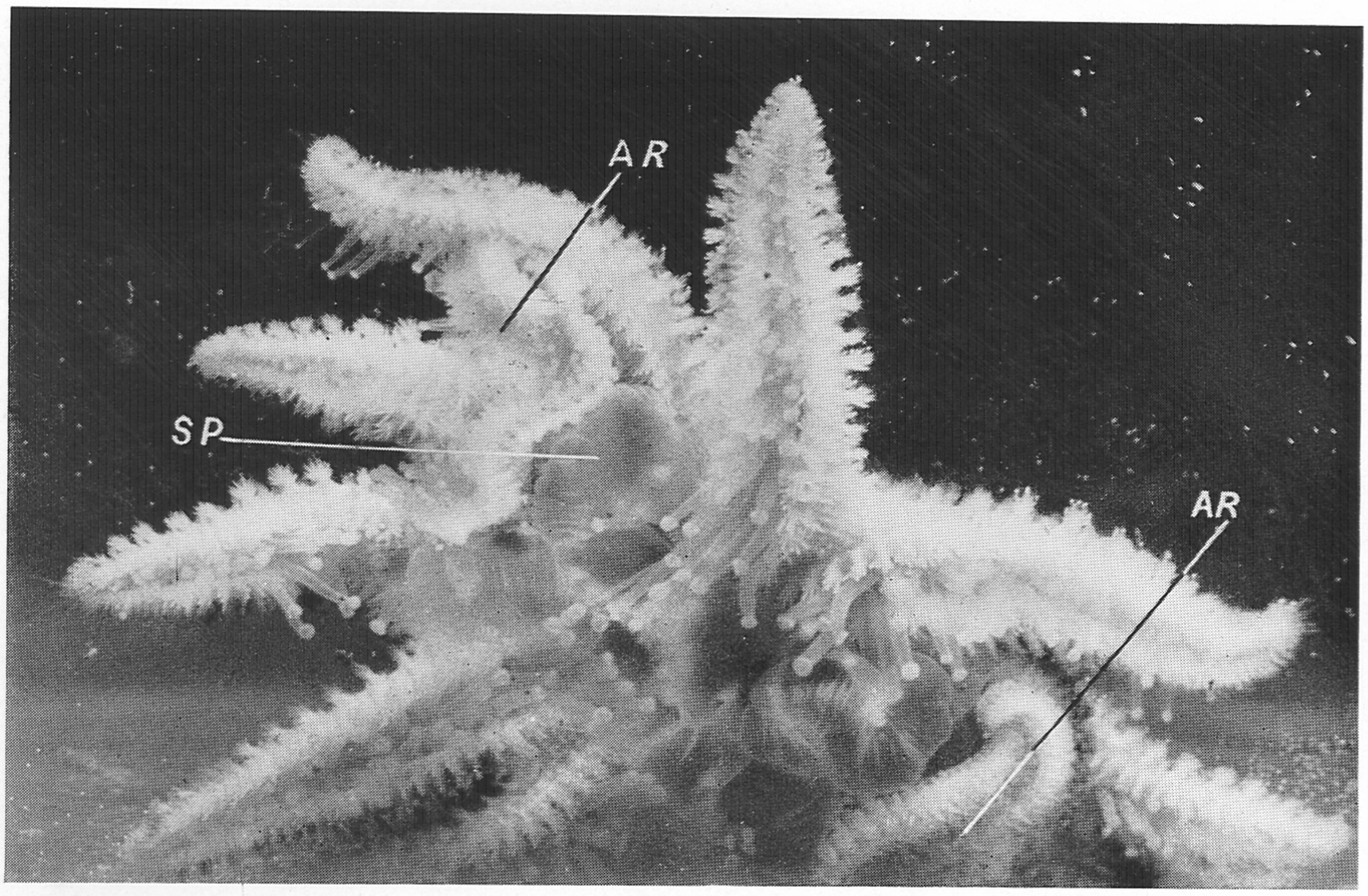

Fig. 1

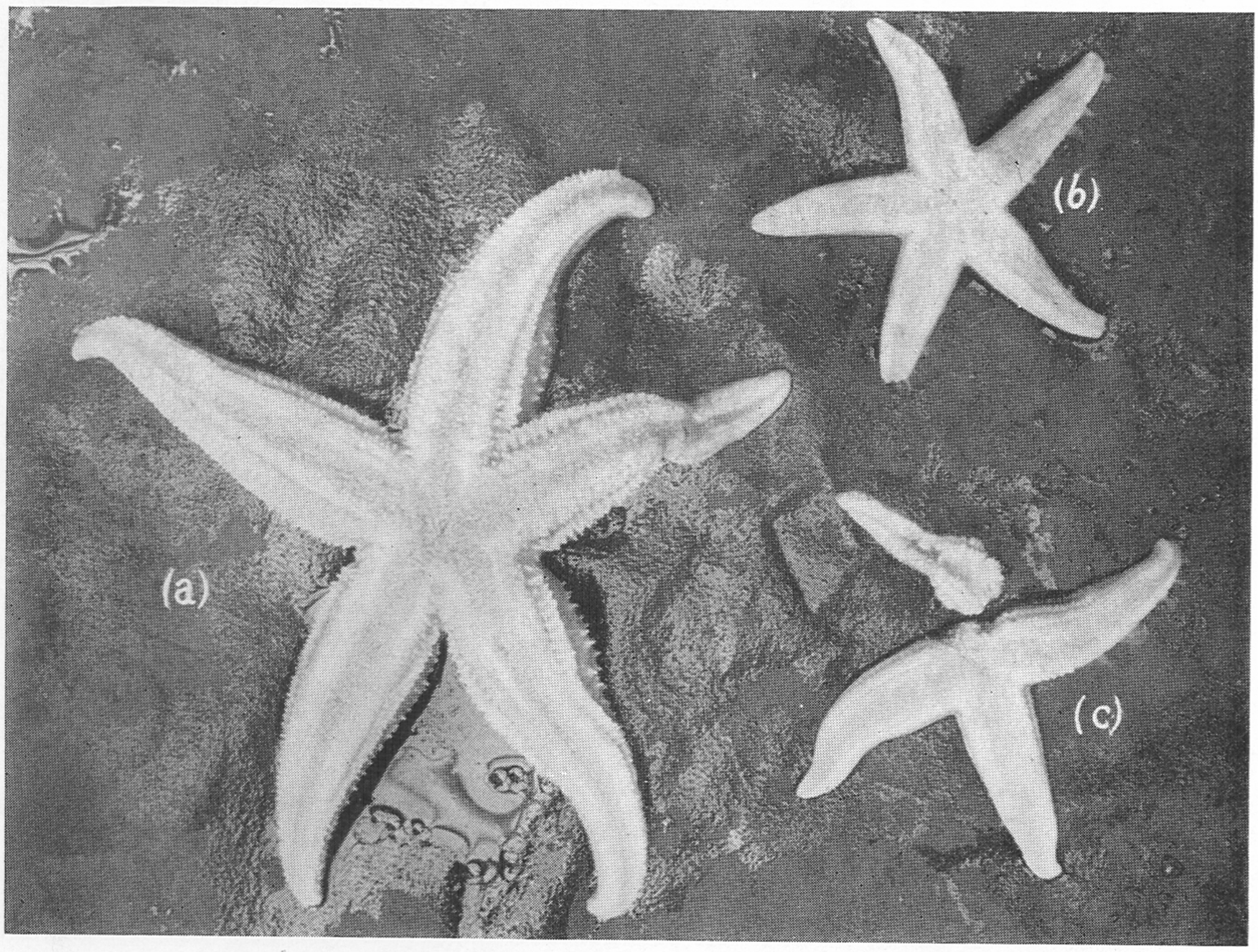

Fig. 2

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EXPLANATION OF PLATE I

Fig. 1. *Solaster papposus* attacking *Asterias rubens*, which has autotomized an arm. *AR.*, arms of *Asterias*; *SP.*, everted stomach pouches of *Solaster*. Photo: P. J. Warren.

Fig. 2. *Asterias rubens* attacked by *Hyas araneus*, showing (*a*) constriction of one arm, (*b*) removal of three arm tips, and (*c*) autotomization of two arms. Photo: D. Key.

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NOTES ON THE VARIATION OCCURRING IN *TUBULARIA LARYNX* ELLIS & SOLANDER

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(Text-figs. 1–5)

In the course of an investigation into the settlement of actinula larva of *Tubularia larynx* Ellis & Solander, the variations in the material collected suggested that more than one species of *Tubularia* might be present at Plymouth. Pyefinch & Downing (1949) had noted variations in the species occurring at Millport, but had decided that it could most satisfactorily be included in *T. larynx*.

Brink (1925*a*) drew attention to Fenchel (1905), who showed that twenty-two forms of *Tubularia* described as new species were *T. larynx*. Amongst these Fenchel included Hincks's (1868) *T. coronata* and Allman's (1871) species *spectabilis*, *pacifica*, *attenuata*, *polycarpa*, *tenella*, *bellis* and *humilis*. These had been given status as species because insufficient notice had been given to the variability of the specific characters.

Errors have also been made because immature individuals have been described. Brink (1925*b*) concluded that the species *Vorticlava* (Alder) and *Acharadria* (Wright) (see Hincks, pp. 131–4) are immature forms of *Tubularia larynx*.

A search through the literature has shown that many authors, including Stechow (1925*a*), Pérez (1925) and Vervoort (1946), have mentioned the instability of the 'specific' characters of *T. larynx*. Sometimes new species are described which are almost certainly *T. larynx*, such as *T. sphaerogona* Hargitt 1927, *T. radiata* Uchida 1936, and *T. venusta* Yamada 1950. Stechow (1925*b*) describes *T. australis* as synonymous with *T. gracilis* Harvey and *T. gracilis* Lendenfeld, but Fenchel considered both these two species to be synonymous with *larynx*. The descriptions given by other authors are often insufficient to distinguish the material from *T. crocea* or immature *T. indivisa*.

The aim in this paper is to illustrate the variations that occur in the specific characters of *T. larynx* (based on Allman's description) and, where possible, to explain them.

The work was undertaken in Plymouth in 1952. I am grateful for the advice and help I received from the Director and Staff of the Laboratory. I wish to thank Dr W. J. Rees for the interest he has shown. I am further indebted to the Chairman and the Members of the R.O.S.C.M. Ltd. for permission to publish this work.

Method

The material examined was collected from buoys in the Sound or taken from a raft moored near the Breakwater. On being brought into the laboratory, the clumps were washed to remove the tubes of *Jassa falcata*. They were placed in glass bowls and the water was changed 3 or 4 times a day.

All attempts at culturing were unsuccessful.

The measurements of the heights of the stems are approximate—there is no morphological difference between the stem and the stolon. Measurements and counts were made with the aid of a dissecting microscope.

A variety of terms is used in describing Coelenterata. As far as possible, the terms used by Allman are retained. A tubularian colony is formed by several hydranths with a common coelenteron. A clump or cluster of hydranths is made up of several colonies often too densely entangled to permit separation. To standardize, the hydranths are described as mature or immature. An individual is considered mature when the gonophores contain spermatozoa or developing ova.

Specific Characters

Allman's description of *T. larynx* can be summarized as follows.

Trophosome

Hydrocaulus, numerous branching stems with more or less distinctly marked transverse annulations, 3·5–5·0 cm or more in length.

Coenosarc forming a collar-like expansion below the hydranth. In describing *T. polycarpa* Allman states that it has a fluted collar, as found in *T. larynx*.

Hydranth, 14–20 distal (oral) tentacles in two closely approximate alternate series, with about 20 proximal (aboral) tentacles each about 1 cm long. The hydranth is 0·5 cm wide across the widest part of the body.

Gonosome

Gonophores in long, pendulous clusters; in the male long simple racemes, longer than the aboral tentacles. In the female they are shorter and the peduncle (blastotyle) is branched to form a panicle or compound raceme. The male gonophore is elongated, the female globular oval in form.

Apical processes, four conical ones, larger in the female than in the male.

Radiating canals: no gastro-vascular canals.

Actinula

No oral tentacles at the time of liberation.

Observations

As will be shown, few of these characters are stable. Many of them have been found to vary according to the size of the hydranth, and this depends on age and stage of development.

For recognition purposes it has been found necessary to concentrate on 'mature' characters, ignoring 'immature'. Though *Tubularia larynx* forms a colony of individual hydranths with a common coelenteron, these are never all the same age. The age differences are accentuated by the phenomena of autotomy and regeneration. In most colonies, however, some of the hydranths will be mature.

Trophosome

Hydrocaulus. Branching has been found to be of universal occurrence and is frequent during early stages of colony formation when the stolon of the newly settled actinula grows along the substrate, forming the hydrorhiza. False branches are formed by actinulae growing on the parent stems. These are very prevalent in some colonies and the false are difficult to distinguish from true branches.

The length of the stem is very variable and has no bearing on the maturity of the hydranth, because of autotomy and regeneration. Colonies have been examined with stems varying from 1·2 to 17·0 cm in length. Environmental factors such as salinity and current force may affect growth in length. D'Arcy Thompson (1942) mentions experiments by Loeb (1906), which showed that stems regenerating in hypotonic sea water were larger than those in hypertonic or normal; the maximum increase was in 2·2 % sea water. It was noticed that the longest colonies were taken from a raft which swung in response to the tidal currents: these colonies were unable to support the hydranths when placed in a jar, so weak were the stems. The weakness may be due to a thin perisarc secreted during a period of a fast growth brought about by factors other than current.

The factors affecting growth in length will also influence the occurrence of annulations in the perisarc. Berrill (1952*b*) has suggested that these represent surges in growth in *T. crocea* similar to those in other gymnoblastic hydroids described by him (Berrill, 1949*a*, *b*; 1952*a*) and by Hauschka (1944).

However, the rhythmic growth pulsations they record have never been seen in *T. larynx*, so this is not an adequate explanation for this species.

Growth in *T. larynx* occurs both at the stolon tip and in the stem below the hydranth, and it is in these areas that the annulations are formed. These areas are linked by the hydroplasmic tension in the coelenteron. Hydroplasmic pressure is defined by Hauschka (1944) as 'pressure over and above internal resistance of static form to compression from without'. The existence of such a pressure can be shown by cutting the stem below the growth region. The

diameter of the growth region will be immediately reduced, showing that it has been under a pressure from within and that the thin perisarc is still flexible and unable to withstand compression from without; the stolon apex is similar in this respect.

The annulations are formed in these zones whilst the chitin is still malleable. Observations suggest that they are formed mechanically through the agency of the hydroplasmic tension. This force is stable throughout the length of the coelenteron, so an increase of pressure at any point will be transmitted throughout the coelenteron. Pressure is increased by the nutritive movements of the hydranth.

These movements appear co-ordinated and have been analysed as three cycles. First, the aboral tentacles are raised vertically, the hypostome contracts and its base swells, dilating the peristome and manubria of the gonophores. This circulates the fluids within the hydranth; the manubria expand and contract once or twice independently. The hypostome relaxes and lengthens, peristaltic waves move up and down its length, circulating nutritive fluids. Finally, the whole hydranth is pulled down on the stem by contraction of the growing region, apparently by longitudinal muscles. This last movement is occasionally preceded by the opening of the gland dividing the stomach from the coelenteron, resulting in the exchange of fluids between them. The movement itself will result in increased hydroplasmic pressure at the stolon, distending it. This could be called a 'growth surge', but it is not produced by regular pulsations of the coenosarc as are the pulsations described by Berrill and Hauschka. Furthermore, when there are several hydranths with a common coelenteron their movements being unco-ordinated will produce varying pressures at the stolon apex. After the autotomy of a hydranth of a colony the hydroplasmic pressure induced by the others will be applied to the area of regeneration, possibly resulting in a reduction of stolonic growth.

Each distension at the stolon apex is limited in circumference by the chitin secreted by the coenosarcal ectodermis. The hardening of the chitin posterior to the apex results in the formation of annulations, marking each successive distension or 'growth surge' of the apex. The annulations are less regular and are often incomplete in the growth region below the hydranth where the downward movement of the hydranth results in a concertina-like in-folding of the coenosarc (Fig. 1). A series of contractions will result in the formation of an annula by the hardening of the perisarc. Further annulae will be formed as growth takes place; their irregularity being due to variations in growth rate as well as to environmental factors which may influence the angle of growth. The length of the region of growth, where the perisarc is still pliable, will be limited mechanically by the ability of the coenosarc aided by hydroplasmic tension to support the hydranth erect—these are constant factors—as well as by any environmental factors which aid or hinder growth. The inter-relationship of these influences will be secondarily reflected in the frequency and size

of the annulae. A slow-growing colony might then be expected to have a thickened well-annulated perisarc, whereas a fast-growing one would have a thinner and so less clearly annulated perisarc.

No colony examined during the period of this study has been entirely devoid of annulations.

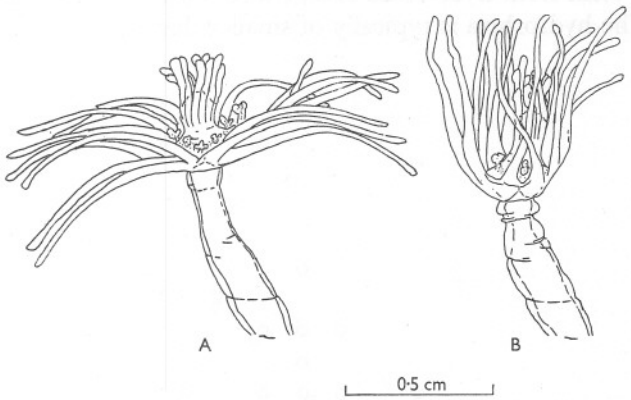

Fig. 1. A, immature hydranth with aboral tentacles extended horizontally. B, same, on completion of the third cycle of the nutritive movements—aboral tentacles vertical, peristome dilated and the growth region contracted folding the perisarc into annulae. (Drawn by camera lucida.)

Coenosarc

The coenosarc is said by Allman to form a collar below the hydranth similar to *T. polycarpa*.

This collar expands and contracts independently of the nutritive movements described previously. Beutler (1926) described these contractions as connected with the circulation of nutritive fluids within the coelenteron. Her diagrams are reproduced in Borradaile, Potts, Eastham & Saunders (1948). These contractions have been seen more frequently in the mature ageing polyp which has ceased to grow in length; the perisarc is stiff right to the base of the hydranth which is thus restricted in its movements. Such individuals are thought to be ageing because the clusters of gonophores on the peristome appear to prevent the aboral tentacles from rising to the vertical. The collar itself increases in circumference with the growth of the hydranth and there is little doubt that this increase is reflected in the increasing circumference of the perisarc of the stem, as compared with that of the hydrorhizal area.

It is difficult to decide whether this collar is itself a specialized organ or merely a dilation differentiated mechanically by the nutritive movements and the stomach contractions. The latter is the more probable explanation. It is recognized first some days after the settled actinula has started to feed and it increases in conformity with the width of the hydranth.

The fluting is formed by chitin secreted by the ectoderm and furrowed by the muscle movements of the collar.

Hydrorhiza

Allman gives no specific characters for the hydrorhiza. It is difficult to differentiate stem from hydrorhiza except where the latter is attached to the substrate. The hydrorhiza is typically of smaller diameter than the stem.

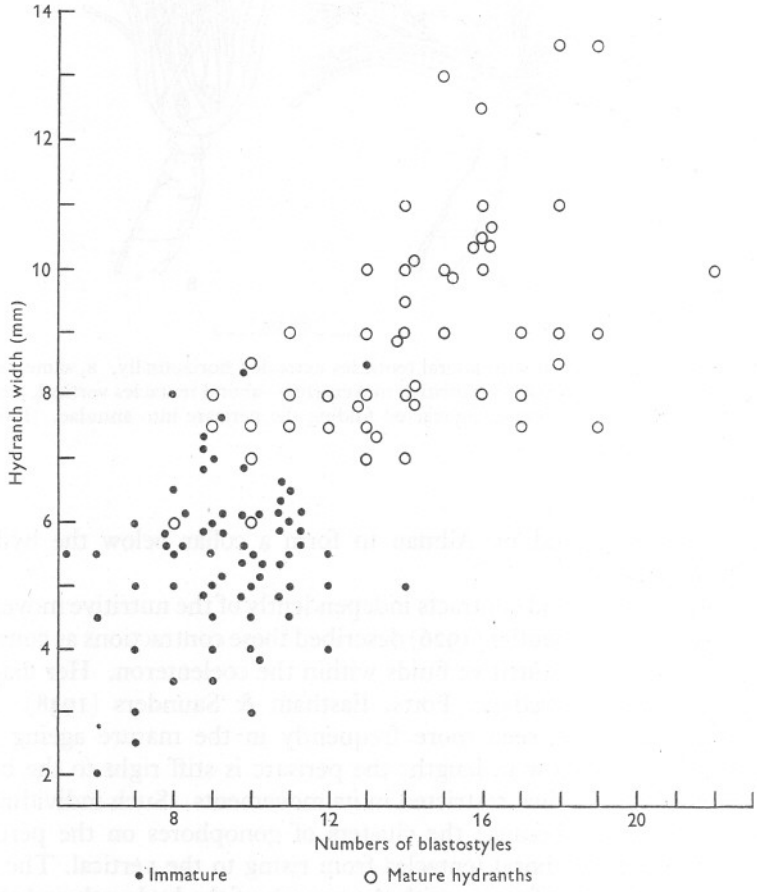

Fig. 2. Scatter diagram illustrating the correlation between the numbers of blastostyles and the widths of the hydranths. (Data in Appendix A.)

Hydranth

In describing the hydranth Allman describes his species as being of definite widths or having definite numbers of tentacles. The reliance given by other workers to these figures has resulted in the erection of invalid species.

Examination of many clusters showed these characters to be very variable.

A clump of 120 hydranths was then examined in detail. All individuals with gonophores, whether mature or not, were examined and the numbers of tentacles and gonophores, the sex and the widths of the hydranths were recorded (Appendix A). There is an intercorrelation between width, numbers of blastotyles and numbers of aboral tentacles. Figs. 2 and 3 show this, demonstrating that an increase in hydranth size is accompanied by increases in numbers of tentacles and blastostyles, which is to be expected. Any identification based on such characters must allow for this correlated increase.

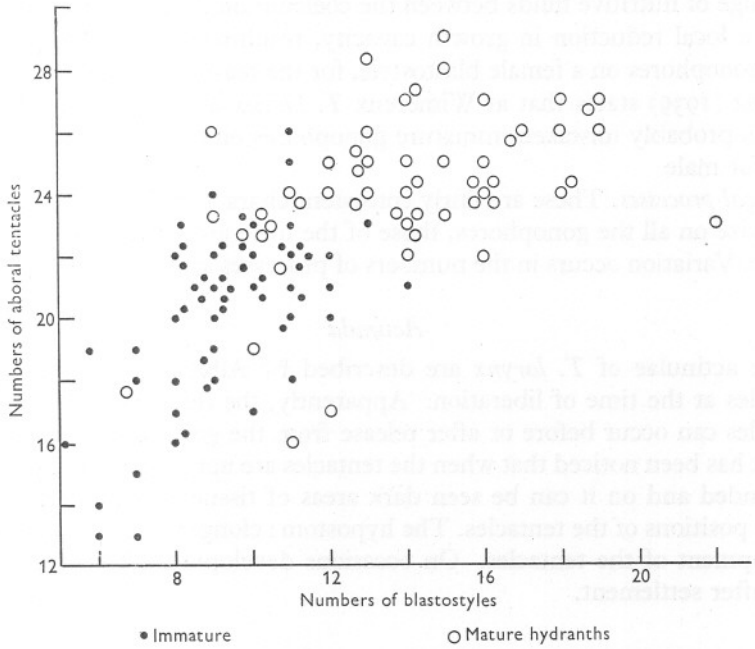

Fig. 3. Scatter diagram showing the correlation between the numbers of blastostyles and of aboral tentacles. (Data in Appendix A.)

Gonosome

Gonophores. In general, the mature male gonophores are smaller than the mature female; they are oval whereas the female are round. Moreover, the developing ovum displaces the manubrium within the gonophore. A male hydranth appears whiter because of the dense white spermatozoa and spermatocytes within the gonophore, mature and immature being distinguished from each other by size. Immature males and females are frequently indistinguishable.

Hermaphrodites have been found. The apical gonophores of a mature and ageing female hydranth are frequently male.

Liu & Berrill (1948) and Berrill (1952*b*) have recorded hermaphrodites in *T. crocea*. Berrill also terms such colonies as ageing. He suggests that male

gonophores are budded on a female blastostyle as the result of a reduction in growth capacity; a maximum growth rate being necessary for the initiation of the large female gonad. When this is not attained male gonophores develop in place of female.

In support of this suggestion is the observation made earlier that such individuals are considered aged because the nutritive movements of the hydranth are restricted by the numbers of blastostyles and gonophores on the peristome. The hardening of the perisarc below the hydranth reduces the exchange of nutritive fluids between the coelenteron and hydranth. This may cause a local reduction in growth capacity, resulting in the development of male gonophores on a female blastostyle, for the reasons suggested by Berrill.

Pérez (1939) states that at Wimereux *T. larynx* is always hermaphrodite. He has probably mistaken immature gonophores on a mature female blastostyle for male.

Apical processes. These are fairly consistent characters. They are not of the same size on all the gonophores, those of the male are often smaller than the female. Variation occurs in the numbers of processes as well as in their length.

Actinula

The actinulae of *T. larynx* are described by Allman as not having oral tentacles at the time of liberation. Apparently, the development of the oral tentacles can occur before or after release from the gonophore. At the same time it has been noticed that when the tentacles are not formed the hypostome is rounded and on it can be seen dark areas of tissue approximating to the future positions of the tentacles. The hypostome elongates at the same time as development of the tentacles. On occasions development has been delayed until after settlement.

Material Collected in Plymouth Sound

The material to be described can be divided into that collected from a raft near the Breakwater, over a period of several weeks, and that collected from various buoys, etc., in other parts of the Sound. Mature specimens were found at all times between June and late September, though they became scarce in August, when clumps without hydranths were frequently found.

Trophosome

Hydrocaulus. Length 1·5–17·5 cm. It was noticed that the perisarc of the longer stems—10 cm and more—was soft and thin in contrast with that of smaller individuals. The longest stems were those of a clump growing attached to a piece of twine trailing from the raft. In general, the material from the raft was of a greater length than elsewhere.

Regular annulations were common and more pronounced in the shorter stems, in which branching was more frequent. It is suggested that in the longer colonies collected from the raft where the current was persistent the actinulae were released and swept away from the parent hydranth. In colonies where the current direction varied, some actinulae are trapped amongst the parent stems to which they become attached, increasing the amount of apparent or false branching.

Ceonosarc. A collar occurs beneath the hydranth and it increases in size with age. It appears to be fluted in ageing individuals for reasons given earlier.

Hydrorhiza. Branching and annulations are frequent. This zone is smaller in circumference than the stem.

Hydranth. The numbers of tentacles show great variation; an increase in circumference of the oral cone and the peristome is accompanied by an increase in the numbers of tentacles. Examples of the amount of variation that occurs in mature individuals is recorded in Appendix B.

Gonosome

Gonophores. Blastostyles occur in one or two rows on the peristome; the number of rows increases according to the size of the hydranth. In the sample (Appendix A) the blastostyles vary from 5 to 22. The gonophores are occasionally pendulous; up to 35 have been counted on a single blastostyle.

Apical processes. Usually four in both sexes—occasionally less—easily distinguished in the female but seldom of equal size.

Actinula

Oral tentacles are usually developed prior to liberation from the gonophore. Numbers of aboral tentacles have varied between 7 and 11, and oral between 3 and 5.

Discussion

Brink (1925*a*) remarked that '. . . the weak point of hydroid systematics is due to the fact that the study of the variability of external features has not kept pace with the discovery of new species'. He drew attention to Fenchel's (1905) examination of the literature, which demonstrated this point. Many new species have been described since.

There is no doubt that the material described here constitutes a single species. Sufficient variation can be found within a large clump to cover many of the species described by Allman. For instance, *T. bellis* differs from *larynx* only in the height of the colony described. Similarly, in *T. humilis* he describes the 'male'; the small number of gonophores suggests that Allman was describing an immature, recently regenerated hydranth. A similar error could have been made in the species *T. attenuata*. *T. polycarpa* owes its creation to having been found on the bottom of a ship in Coquimbo harbour.

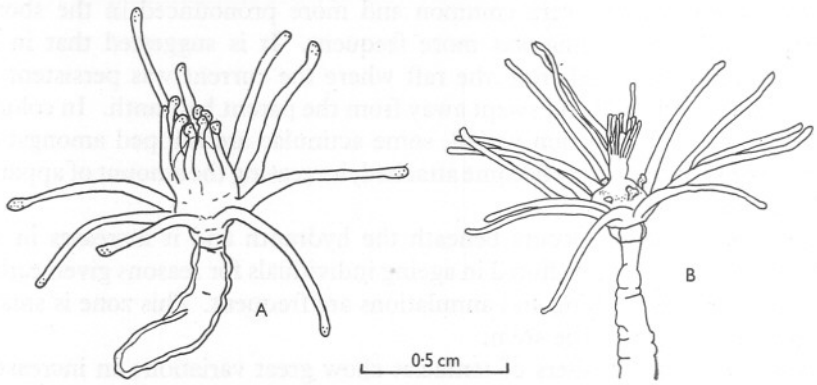

Fig. 4. A, a recently settled actinula drawn by camera lucida. Compare with Hincks, *Vorticlava*, Vol. 1, p. 132, and Vol. 2, pl. XXIII. B, an immature regenerated hydranth. Compare with Hincks, Vol. 2, pl. XXIII. (Drawn by camera lucida.)

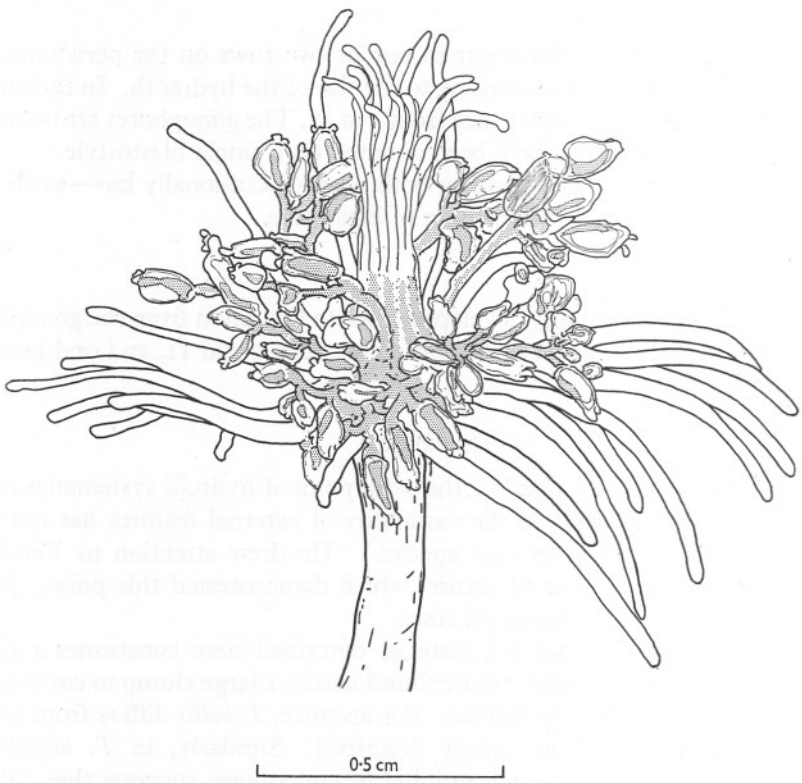

Fig. 5. Mature female hydranth showing the displacement of the manubria, within the gonophores, by the developing ova and actinula. (Drawn by camera lucida.)

Brink (1925*b*) considered the species of *Vorticlava* and *Acharadria* to be stages in the development of *T. larynx*. From Alder's description *Vorticlava* is certainly a newly settled actinula, as suspected by Hincks. Wright's drawing of *Acharadria*, however, resembles more closely an immature, regenerating hydranth, rather than a stage in the growth of a colony, because of the large number of tentacles and simultaneous absence of gonophores.

It is not possible to describe accurately the geographic distribution of *T. larynx*. It is certainly found in all oceans but it is, as yet, recorded under many different specific names.

It must be emphasized that Allman's specific characters are valid when full allowance is made for the diversity that occurs in the species. This is reduced when several mature individuals are used in identification.

Summary

As certain previous authors have observed, *T. larynx* is a species subject to much variation. Disregard of this has led to the erection of invalid species.

The descriptive characters used by Allman are discussed. In a comparison with material collected in Plymouth Sound these characters are shown to be so variable that used singly they are valueless. In particular, disregard of the stage of development and over-emphasis on size will be very misleading.

The causes of some of this variability are suggested.

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APPENDIX A

DATA OBTAINED FROM EXAMINING A CLUMP OF *TUBULARIA LARYNX* COLLECTED ON A BUOY NEAR REMAINS OF THE PIER

(The graphs are drawn from these data. M = mature; I = immature.)

Sex		Hydranth width (mm)	Length aboral tentacles (mm)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
♂+♂+♂+♀+♀+♂+♂+♂+♂+♂+♂+♂+♂	M	7·5	—	22	27	19	2
	M	10·5	—	20	24	16	2
	M	10·0	—	21	27	16	2
	M	8·0	—	17	22	14	1
	M	10·0	—	21	26	13	2
	M	10·0	—	22	23	22	2
	M	10·0	—	20	29	15	2
	M	11·0	—	22	24	18	2
	M	12·5	—	23	24	16	2
	M	8·0	—	19	28	13	2
	M	10·5	—	22	24	16	2
	M	9·0	—	20	26	19	2
	M	7·0	—	16	23	10	1
	M	6·0	—	18	22	8	1

APPENDIX A (*continued*)

Sex		Hydranth width (mm)	Length aboral tentacles (mm.)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
♀	M	7·5	—	21	26	17	1
♀	M	8·0	—	20	26	9	1
♀	M	8·5	—	23	26	18	2
♀	M	8·0	—	21	24	12	1
♀	M	8·5	—	20	23	10	1
♀	M	11·0	—	20	24	16	2
♀	M	13·0	5·0	19	25	15	2
♀	M	8·0	3·0	20	17	12	1
♀	M	11·0	—	23	25	14	2
♀	M	9·5	3·0	18	27	14	2
♀	M	8·0	—	22	27	14	2
♀	M	8·0	3·5	21	25	13	2
♀	M	7·5	3·0	16	23	9	1
♀	M	7·5	2·5	19	25	12	1
♀	M	9·0	—	19	27	18	2
♀	M	7·0	3·0	19	21	9	1
♀	M	10·0	3·7	23	23	14	2
♀	M	6·0	2·5	18	23	10	1
♀	M	10·0	3·7	19	23	14	2
♀	M	8·0	3·0	21	22	16	2
♀	M	9·0	3·0	18	22	11	1
♀	M	13·5	5·0	24	24	18	2
♀	M	6·0	2·0	19	22	9	1
♀	M	8·0	3·5	22	24	11	2
♀	M	13·5	5·0	19	27	19	2
♀	M	10·0	3·5	23	28	15	1
♀	M	9·0	3·0	24	25	13	—
♀	M	9·0	3·5	18	23	14	2
♀	M	10·5	3·7	21	25	16	2
♀	M	7·5	3·0	21	23	10	1
♀	M	9·0	3·5	25	26	17	2
♀	M	10·5	4·5	22	24	16	2
♀	M	9·0	3·5	22	24	14	1
♀	M	7·0	3·0	22	24	13	1
♀	M	7·5	3·0	19	25	13	1
♀	M	8·0	3·0	—	24	14	1
♀	M	7·0	3·0	23	23	14	1
♀	M	9·0	3·5	20	23	15	1
♀	M	5·0	2·0	12	16	11	1
♀	M	7·5	3·0	18	24	11	1
♀	I	5·5	—	20	20	12	1
♀	I	7·0	—	26	23	10	1
♀	I	8·5	3·0	23	23	13	1
♀	I	8·5	3·5	18	22	10	1
♀	I	6·0	—	14	22	11	1
♀	I	7·5	2·5	18	24	9	1
♂	I	6·0	2·5	18	23	10	1
♂	I	6·0	2·5	20	22	11	1
♀	I	6·0	2·2	14	21	9	1
♀	I	7·5	3·2	16	21	9	1
—	I	7·0	2·5	18	21	9	1
—	I	6·0	2·0	19	22	9	1
—	I	6·0	2·5	22	18	7	1
—	I	8·0	3·5	19	23	8	1
—	I	4·0	1·5	19	21	10	1
—	I	7·0	3·0	15	19	9	1
—	I	5·5	2·2	19	22	10	1
—	I	5·5	2·0	15	16	5	1
—	I	2·5	0·75	—	15	7	1
—	I	2·0	0·75	16	13	6	1
—	I	6·0	2·5	17	16	8	1
—	I	3·0	1·2	14	17	10	1
—	I	5·0	—	20	21	10	1

Appendix A (*continued*)

Sex		Hydranth width (mm)	Length aboral tentacles (mm.)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
—	I	5·0	—	19	21	11	1
—	I	5·0	—	16	21	9	1
—	I	5·5	—	19	20	11	1
—	I	3·5	—	10	16	8	1
—	I	5·0	—	20	22	12	1
♂	I	6·0	2·5	21	21	9	1
♂	I	6·0	2·5	17	22	10	1
♂	I	4·5	1·7	14	20	10	1
♀	I	6·5	2·7	21	22	11	1
♀	I	5·5	2·2	16	20	8	1
♀	I	5·5	2·5	18	14	6	1
♀	I	4·0	1·5	18	19	7	1
♀	I	5·5	2·2	19	20	10	1
♀	I	6·5	2·7	16	22	8	1
♀	I	5·5	1·5	15	20	11	1
—	I	5·0	2·0	22	19	10	1
—	I	5·5	2·2	15	21	10	1
—	I	5·0	2·0	18	22	8	1
—	I	6·0	2·5	17	22	9	1
—	I	5·5	2·2	18	22	11	1
—	I	3·5	1·2	16	18	9	1
—	I	5·5	2·2	19	20	8	1
—	I	5·5	2·2	22	19	10	1
—	I	6·0	2·5	15	22	11	1
—	I	4·0	1·5	14	18	10	1
—	I	6·0	2·5	18	21	11	1
—	I	4·0	1·5	17	21	12	1
—	I	6·5	2·7	20	23	11	1
—	I	5·5	2·2	21	17	8	1
—	I	5·0	2·0	19	21	14	—
—	I	6·0	2·5	19	26	11	—
—	I	4·0	1·5	15	19	9	1
—	I	5·0	2·0	18	19	10	1
—	I	5·0	2·0	18	18	9	1
—	I	6·0	2·2	16	18	8	1
—	I	4·5	1·7	17	21	9	1
—	I	6·5	2·7	21	25	11	1
—	I	4·5	1·7	14	19	6	1
—	I	4·5	1·7	17	18	11	1
—	I	3·0	1·2	15	13	7	1
—	I	5·0	2·0	14	18	7	1
—	I	5·0	—	20	21	10	1
—	I	5·0	—	19	21	11	1

Appendix B

Records of Clump Heights and Tentacle Numbers of Mature Individuals in Clumps Collected in Various Parts of Plymouth Sound

Locality	Clump height (cm.)	Variation in tentacle nos.		No. examined
		Oral	Aboral	
Buoy chain, breakwater	1·5–4·0	17–26	18–25	12
Buoy chain, Barn pool	1·5–5·0	14–37	13–35	29
Buoy chain, Mount Batten	1·5–5·0	17–22	18–25	30
Raft, breakwater	1·5–5·0	16–22	20–26	90
Raft, breakwater	5·0–7·5	21–31	22–29	
Raft, breakwater	7·5–12·5	17–32	19–32	
Pier, buoy	1·5–7·0	12–24	16–28	54
Total range of variation	1·5–12·5	12–37	13–35	215

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THE DIET AND FEEDING MECHANISM OF *IDOTEA*

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(Text-figs. 1 and 2)

CONTENTS

Introduction

Few published data are available on the feeding mechanism of isopods; Nicholls (1931) describes the feeding of *Ligia oceanica* (L.), but he deals mainly with the foregut and the digestive system. The feeding mechanism of the tanaid *Apseudes talpa* (Montagu) is described by Dennell (1937) but, unlike an isopod, this species still retains a fairly elaborate maxillary filter mechanism. A description of the mode of action of the mouthparts of an isopod such as *Idotea* is therefore of value for comparison with the general scheme of evolution of peracaridan-feeding mechanisms (Dennell, 1937). In addition, such an account forms a corollary to morphological descriptions of the mouthparts (Naylor, 1955*a*), and notes on the diet of *Idotea* supplement observations on the ecology of the various species (Naylor, 1955*b*).

Diet

According to Roux (1829) and Collinge (1917), idoteids feed mainly on animal remains, but Bate & Westwood (1868) maintained that *Idotea tricuspidata* (now *I. baltica* (Pallas) and other species) fed on algae. As for the habits of individual species, Dollfus (1895) states that *I. pelagica* Leach is commensal with, or parasitic on, barnacles on the shore, Kjennerud (1952) describes how *I. neglecta* G. O. Sars eats fish and fish waste in the fish-market harbour at Bergen, and Howes (1939) regards *I. viridis* (Slabber) from a saline lagoon in Essex as a carnivore feeding chiefly on coelenterates, *Membranipora* or dead organisms.

Present observations on the feeding habits of *Idotea*, when compared with details of the ecological distribution of the species (Naylor, 1955*b*), show how each species eats the organisms with which it is associated. Examination of the gut contents shows that most of the food is algal, but some animal remains have been observed, particularly in *I. pelagica*, which often has barnacle appendages in its gut. In addition, laboratory observations show that an idoteid will eat its own cast skin and other dead *Idotea*; cannibalism, particularly of moulting individuals, sometimes occurs.

To determine whether plant or animal food is preferred *Idotea* were kept in aquaria and fed experimentally. *I. emarginata* (Fabricius) and *I. neglecta* ate *Laminaria* and scallop muscle if both were provided as food, and *I. emarginata* attacked and ate living *Arenicola*, whether seaweed was present or not. Dead fish such as whiting, herring, pollack and coalfish were eaten till nothing but the skeleton remained, even when *Laminaria* had been the food for several days.

Thus, though seaweeds seem to provide the bulk of available food for these species in their particular habitat, animal remains, and even some living animals, are taken when available.

Probably most *Idotea* are omnivorous, but some feeding differences occur depending on the habitat and locality. *I. pelagica* appears to have similar habits in many localities (Dollfus, 1894–5; Sars, 1899; Elmhirst, 1946; Kjennerud, 1952; Naylor, 1955*b*). The species is found amongst barnacles on exposed shores, but rather than being an ectoparasite of barnacles (Dollfus, 1894–5), it seems as though it is a scavenger in its habitat, feeding on cast skins of previously moulted barnacles, as well as on algae. Local differences in diet occur in at least two species. *I. neglecta* appears to feed largely on decaying weed in Port Erin Bay, yet the species appears to be solely carnivorous in a Norwegian locality (Kjennerud, 1952), and *I. viridis* in the Isle of Man feeds chiefly on algae and not on animal matter as described by Howes (1939). Finally there may also be different food preferences by *Idotea* of different size; small *I. granulosa* Rathke, for instance, eat the *Cladophora* with which they are associated, whilst large specimens eat the fucoids which harbour them (Naylor, 1955*b*).

The Feeding Mechanism

Methods

In favourable conditions, without excess light or heat, *Idotea* will feed on algae in a Petri dish, and may be examined under a low-power dissecting microscope. In this way, part of the feeding process, particularly the biting and scraping of the weed surface, was observed directly. The remainder of the process of feeding is inferred from the structure and musculature of the mouthparts, and by observing the movements of the mouthparts when the ventral maxillipedes, which cover the other mouthparts, had been removed.

The removal of the maxillipedes might upset the phase of movement of the mouthparts, but it did not seem to affect the direction of movement of each appendage.

The structure of the mouthparts

The structure of the mouthparts of *Idotea* has been described in detail elsewhere (Naylor, 1955*a*), but it is necessary to give a brief summary of that description here so that the mode of action of the mouthparts may be fully understood.

Idoteids have four pairs of oral appendages, namely, mandibles, maxillules, maxillae and maxillipedes, the last of these originating from the thoracic segment incorporated into the cephalon. In addition to these there are the labrum, forming the anterior wall of the oral cavity, and the bilobed paragnath, forming the posterior wall. The mandibles themselves form the lateral walls of the oral cavity. All the paired appendages originate behind the paragnath; they project forwards beneath the head with their tips coming to lie below or just behind the oral cavity (Fig. 1).

The *labrum* is a heavily chitinized structure which is slightly spiny at its lower edge (Fig. 1); it abuts against the food mass during feeding.

The *mandibles* are well developed and are the largest of the oral appendages. Each consists of a ventral incisor process, a lacinia mobilis, a row of toothed spines, and a dorsal molar process (Fig. 1), and when the mandibles are closely apposed the structures of the right appendage lie above the corresponding ones of the left side (Fig. 2). In relation to this the two mandibles are structurally asymmetrical; whereas the left lacinia mobilis bears three broad teeth, that of the right mandible has one large spine and several smaller ones and all are less chitinized than those of the left lacinia mobilis. In addition, the molar process of the left mandible is inclined upwards, whilst that of the right side faces somewhat downwards.

The *paragnath* is bilobed and bears upwardly-directed spines in the mid-ventral groove between the lobes.

Each *maxillule* has an inner endite bearing three long, and one short, plumose bristles which project between the lobes of the paragnath (Fig. 1), and an outer endite bearing a number of stout spines. Each *maxilla* has a bilobed outer endite bearing comb setae, and an inner endite bearing plumose bristles like those of the maxillule.

The *maxillipedes* are plate-like and they protect the other mouthparts. They are joined medially by a coupling hook (Fig. 1) on each appendage and, unlike the other appendages, the maxillipedes bear palps. The palps are capable of independent lateral movement, and the spines on their inner borders aid in keeping the other mouthparts free of debris.

The *first pair of legs* on the thorax aid in feeding by holding the food mass and by combing the mouthparts. Unlike the other legs, the dactylopodite is palmate and bears a number of toothed, comb-like spines on its upper surface.

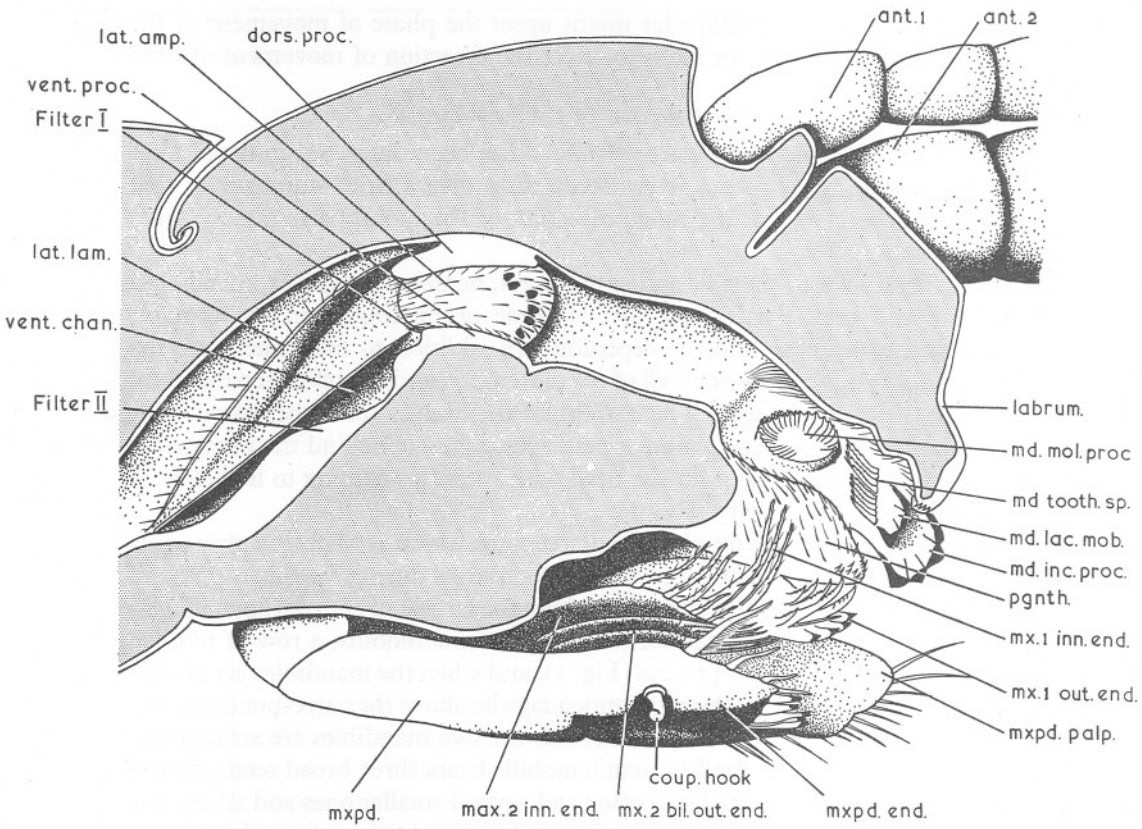

Fig. 1. Median view of the left half of the head of *I. emarginata*, showing the mouthparts and the foregut. *Ant.* 1, first antenna; *ant.* 2, second antenna; *coup. hook*, coupling hook on maxillipede; *dors. proc.*, dorsal process; *lat. amp.*, lateral ampulla; *lat. lam.*, lateral lamella; *md. inc. proc.*, incisor process of mandible; *md. lac. mob.*, lacinia mobilis of mandible; *md. mol. proc.*, molar process of mandible; *md. tooth. sp.*, toothed spines of mandible; *mx. 1 inn. end.*, inner endite of maxillule; *mx. 1 out. end.*, outer endite of maxillule; *mx. 2 bil. out. end.*, bilobed outer endite of maxilla; *mx. 2 inn. end.*, inner endite of maxilla; *mxpd.*, base of maxillipede; *mxpd. end.*, endite of maxillipede; *mxpd. palp*, palp of maxillipede; *pgnth.*, paragnath; *vent. chan.*, ventral channel; *vent. proc.*, ventral process.

The Mouthparts in action

General Movements

During feeding the mandibles bite sideways. The maxillules move obliquely forwards till they meet in the mid-line and then they may or may not move forwards together. It is important to note that the mandibles and maxillules alternate in their movements.

The maxillae move in a manner similar to that of the maxillules, converging anteriorly to about the same level as the posterior limit of the tips of the maxillules. The maxillipedes, being coupled together medially, move forwards and backwards, with no lateral movement except in the palps.

All these movements may vary according to whether the animal is actually biting the food material or only clearing the mouthparts of debris collected there.

Biting and Scraping

From a functional point of view all the mouthparts but the maxillae are built on a similar plan. Ventrally placed on each appendage are heavily chitinized structures for biting or scraping the food material. There are broad, scleratized teeth on the incisor process of each mandible, and on the lacinia mobilis of the left appendage. The outer endite of the maxillule has four, heavily chitinized, chisel-shaped spines ventral to the other spines, and the maxillipedes have five or six scraping spines situated ventrally on their inner parts.

When the animal is feeding, the maxillules come together as the mandibles separate, and in doing so they abrade the edge of the weed and facilitate the work of the mandibles when these next bite. To a certain extent the spines on the maxillipedes probably also help in abrading the weed, but this pair of appendages, like the maxillules, do not bite the food material.

The maxillae take no part in abrading or biting the weed; they are hardly scleratized at all.

Pushing

Above the biting and scraping parts of the appendages are structures concerned with the removal of food from the biting parts and with its transfer to the molar processes for chewing. Serving to transfer food upwards along the mandibles are the row of toothed spines and the lacinia mobilis of each side (Fig. 2). By their very arrangement these should push food upwards every time the mandibles come together. Cannon & Manton (1927, p. 235), in describing the asymmetrical arrangement of the mandibles in *Hemimysis*, state that it provides 'a mechanism by which simple lateral movements of the mandibles must transfer food from the ventral incisor processes to the dorsal molar processes'.

In addition to those on the mandibles there are toothed spines on the maxillules. These lie dorsal to the four scraping spines (Fig. 1) and they point upwards and inwards between the paragnaths; they must aid in pushing the bitten food mass upwards. Here again it is important that the mandibles and maxillules alternate in their movements. At the time when the food bitten by the mandibles is likely to fall between them as they retract, the maxillules are coming together; in doing so they will push inwards any food which is likely to fall, and the toothed nature of the spines should make them more efficient in this respect.

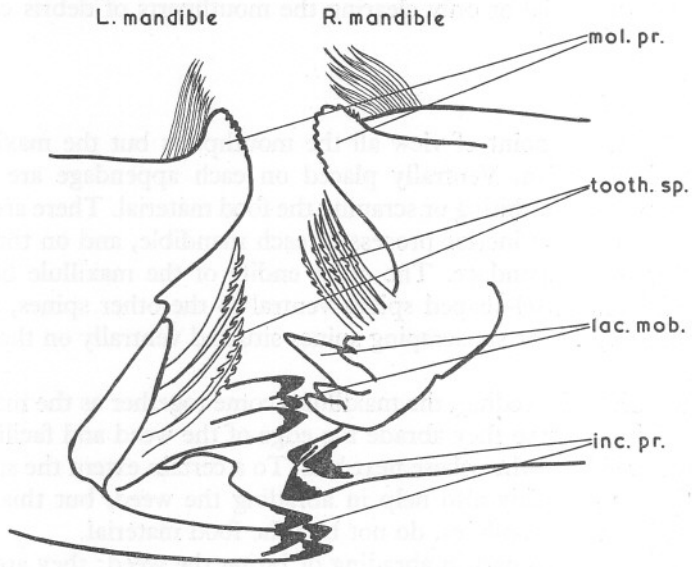

Fig. 2. The apposed mandibles, seen from behind (*mol.pr.*, molar process; *tooth.sp.*, toothed spines; *lac.mob.*, lacinia mobilis; *inc.pr.*, incisor process).

Brushing

Escaping particles of food are probably dealt with by the plumose, or brush setae, on the maxillules, maxillae and maxillipedes. It is difficult to observe these setae in action but, from the general movement of the appendages, some idea of their function may be inferred.

The plumose setae move mainly forwards and backwards, since they are on the inner parts of the appendages, and the setae of each member of the pair of appendages meet in the mid-ventral line. The three pairs of appendages normally move out of phase with each other, and particles of food are probably brushed forwards from one to the other until they are combed off the setae of the maxillules by the spines on the paragnath.

From there the food particles will be pushed between the mandibles by the toothed spines on the outer endite of the maxillule, and by the brush setae on the inner endite of that appendage. Once between the mandibles, as described earlier, food passes up to the molar processes by virtue of lateral movements of the mandibles themselves.

Combing

It is necessary in such a method of feeding, where small particles of food will tend to cling to various appendages, for there to be combing mechanisms to remove such debris. Comb spines on the first pair of legs and the long

smooth spines on the inner border of the maxillipedal palp, have already been described as aiding in keeping the mouthparts free of debris. In addition, short smooth spines on the paragnath have been mentioned as combing food particles from the brush setae of the inner endites of the last three pairs of mouthparts. Other structures which probably aid in combing the mouthparts are the spines on the outer endites of the maxillae (Fig. 1), and the two flexible, blade-like structures, fringed with stiff bristles, which are situated ventrally on the tips of the maxillules (Naylor, 1955*a*).

Trituration

All the movements of the mouthparts serve to push the food forwards and upwards to the molar processes of the mandibles. These have blade-like ridges on their apposing surfaces, which slide across each other to crush the food. From there the triturated food passes to the fore-gut.

The Foregut

The foregut of *Idotea* is very similar to that described for *Asellus* (Rehorst, 1914) and for *Ligia* (Nicholls, 1931). The latter author reviews much literature on the foregut of isopods, and the terminology adopted by him is used here.

Anteriorly, on each side-wall of the foregut, arise the paired, lateral ampullae. These appear to crush the food material so that fluid is sifted from the solid matter through paired, ventral bristle-plates, which open into a mid-ventral channel (filter I). The channel is formed by the anterior ventral lamellae, and it is divided medially, at its posterior end, by a second filter apparatus (filter II). On either side of this structure, running parallel with its upper edge, lies a groove covered with strong bristles. The grooves are blind anteriorly, but posteriorly they open into the three digestive caeca of each side; fluid passes between the setae and into the caeca. A backward projection of the second filter apparatus acts as a valve over the opening from the caeca into the intestine.

Solid food is prevented from being regurgitated by a series of membranous flaps in the foregut, namely, the paired lateral lamellae, the dorsal lamella, the paired, posterior ventral lamellae, and the ventral valve. All except the lateral lamellae arise at the posterior end of the foregut and project into the intestine.

Discussion

From what has been said of the diet and action of the mouthparts, it is clear that *Idotea* must feed on large food masses; it cannot filter food from suspension, and in this respect it is a typical 'higher' peracaridan (Dennell, 1937). The general method of feeding of *Idotea*, whereby the animal browses on its

food, scraping and biting pieces from it, is similar to that described for *Ligia* (Nicholls, 1931).

The 'higher' Peracarida, which feed on large food masses, are thought to have been derived from an entirely filter-feeding ancestor (Cannon & Manton, 1927; Manton, 1928; Dennell, 1937), and the first adoption of the bottom-living habit by a peracaridan with an elaborate maxillary filter may have been the result of seeking larger food particles from the bottom (Manton, 1928). The first stage in obtaining larger food appears to have been the direct intake of fairly large particles by the mandibles, the outer endites of the maxillules, and possibly other appendages as well; this has been observed in *Hemimysis* (Cannon & Manton, 1927), and in *Apseudes* (Dennell, 1937). It is then an easy step towards the elaboration of the mandibles and the outer endites of the maxillules, so as to bite into, and break pieces from, more solid food.

As the filter-feeding mechanism has become of less importance in the 'higher' Peracarida, the inner endite of the maxillule, and the whole of the maxilla, have become reduced, and the mouthparts have become covered ventrally by the plate-like maxillipedes. All these changes appear to have taken place with the loss of the ventral food current, and they have occurred in the evolution of the Isopoda.

In *Idotea* the equivalent of the filtering mechanism, namely the inner parts of the hinder three pairs of mouthparts, apparently serves to brush into the mouth particles of food which escape from the biting parts of the appendages. The mouthparts are very compact, but the feeding mechanism is still elaborate, particularly when the division of labour between the various parts of each appendage is considered.

With the adoption of a bottom-living habit and a raptatory mode of feeding, characteristic of the 'higher' Peracarida, and with its generalized food requirements (p. 348), *Idotea* is well suited to occupy a wide range of habitat.

Summary

Idotea feeds on large food masses. It is potentially an omnivorous scavenger, but each species may have a characteristic diet depending on the availability of food in its particular habitat.

The structure, topography and action of the mouthparts are described: spines on the maxillules and maxillipedes scrape the food material whilst the mandibles actually bite the food; toothed spines on the maxillules and mandibles push the food upwards to the molar processes of the mandibles; debris is brushed forwards between the lobes of the paragnath by setae on the inner endites of the maxillules, maxillae and maxillipedes; and spines on the maxillipedal palp, and on the first leg, comb the mouthparts.

The structure of the foregut resembles that of *Asellus* and *Ligia*.

The relation of the feeding mechanism to the functional evolution of the mouthparts of Peracarida as a whole is discussed.

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

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(Text-figs. 1–7)

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

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

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

* Working at I.C.I. Ltd., Paints Division, Marine Research Station, Brixham, at the time the experimental work described in this paper was carried out.

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

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

Methods of Investigation

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

Although a small regular settlement of *E. modestus*, of the order of 0·01 per sq.cm during the season, is now (1952) maintained at Brixham, this was insufficient for our work. The initial settlements were therefore obtained by exposing plates in the River Crouch, Essex, where *Elminius* is exceedingly abundant, settlements of 50–100 spat per sq.cm having sometimes been obtained during only a week's exposure in June and July. The plates, each bearing an identification number, were taken to Brixham harbour (where all the observations were carried out), and immersed as soon as possible. When the spat had attained a diameter of 2–3 mm about fifty to sixty of the more healthy and larger individuals were selected, and the remainder prised off the panel, but no individual was allowed to be separated by a distance of more than 2 cm from its fellows. The panel was then photographed at intervals from the underside, the individuals in the photographs being numbered for reference.

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

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

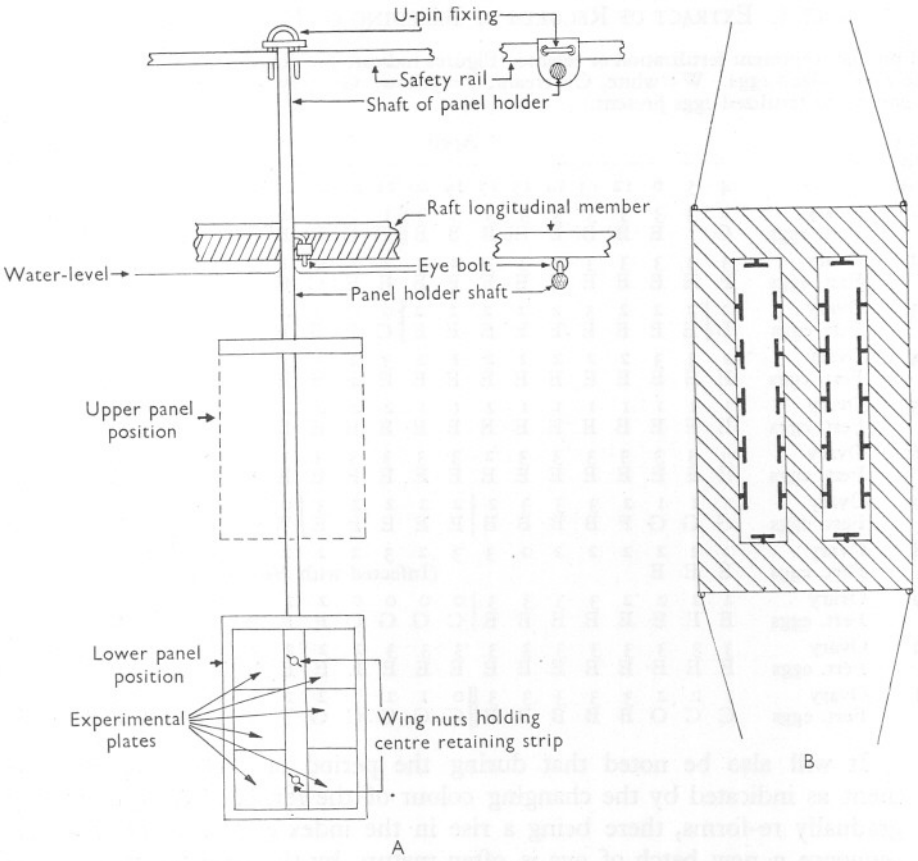

Fig. 1. Diagram of method of exposure of glass plates. A, Panel holder and fixing arrangements. B, Plan of raft showing wells for panel holders. The wells are darkened by wooden covers when the panel holders are in position.

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

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

the sudden appearance of white or cream egg masses, the ovarian index figure drops sharply, usually from 3 to 0 or to 1 (e.g. individual 2, 22–25 April). This indicates the discharge of the greater part of the contents of the ovary, for the ovarian tubules at the time the individual is fertilized are almost entirely filled with mature eggs.

TABLE I. EXTRACT OF RECORDS OF BREEDING OF *ELMINIUS MODESTUS*

(Full lines represent fertilization or release. Figures indicate the development of the ovary, letters the state of fertilized eggs. W = white, C = cream, Y = yellow, G = grey, O = ochre, F = fawn, B = brown, E = empty, no fertilized eggs present.)

No. of individual		April																May					
		4	5	6	12	13	14	15	17	19	20	21	22	25	26	27	28	29	2	3	5	8	10	12
1	Ovary	2	3	3	3	3	3	3	3	3	3	1	2	2	2	3	3	3	3	3	3	3	1	0
	Fert. eggs	O	?	E	B?	B?	B	E?	B	B	B	C	O	B	B	B	B	B	B	B	B	E	C	G
2	Ovary	3	3	3	3	3	3	3	3	2	3	3	3	0	1	1	1	1	2	2	3	3	3	3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	C	C	C	G	O	O	O	B	B	B	B
3	Ovary	2	2	2	2	3	2	2	2	2	2	0	0	2	2	2	1	2	3	2	3	3	0	0
	Fert. eggs	B	E	E	E	E	E	E	E	E	E	C	F	F	F	B	G	B	B	B	B	B	O	F
4	Ovary	2	3	3	2	2	2	2	2	2	2	3	2	3	3	0	1	2	3	2	3	2	2	2
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	C	G	F	F	F	B	B	B	B
5	Ovary	1	1	1	1	1	1	1	2	1	1	2	2	2	2	2	2	2	3	0	1	1	2	3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	C	C	O	F	E
6	Ovary	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	(Later found infected)					
7	Ovary	1	1	1	2	3	3	3	2	2	2	2	2	3	0	0	1	1	1	2	2	3	3	3
	Fert. eggs	G	G	G	F	B	B	B	B	E	E	E	E	E	W	C	C	G	G	G	G	F	E	E
8	Ovary	2	2	2	2	2	2	2	3	3	2	3	2	2	2	2	2	2	2	1	2	2	2	2
	Fert. eggs	E	E	E							(Infected with *Hemioniscus*)													
9	Ovary	2	2	2	2	3	3	3	3	0	0	0	0	2	2	2	2	2	3	3	3	3	0	0
	Fert. eggs	E	E	E	E	E	E	E	E	C	G	G	O	F	F	F	F	F	B	B	B	E	C	C
10	Ovary	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
11	Ovary	1	2	2	3	3	3	3	3	0	1	1	1	2	2	2	2	2	3	3	3	0	1	1
	Fert. eggs	C	C	O	B	B	B	B	B	C	C	C	C	G	Y	Y	Y	O	B	B	B	C	C	C

It will also be noted that during the period of embryonic development as indicated by the changing colour of the fertilized eggs, the ovary gradually re-forms, there being a rise in the index of the ovary. In consequence a new batch of ova is often mature by the time the nauplii are being liberated, and another fertilization can immediately supervene, as in individual 1 between 20 and 21 April. When this takes place dark brown egg masses are replaced by new pale ones within the interval of time between successive observations, and double lines are inserted in the table. The embryos are normally retained for an appreciable period after reaching what appears to be a completely developed condition, for they are then fully pigmented, and hatch immediately if removed from the parent and placed in sea water.

The most difficult observation to make with certainty was the date of liberation. In healthy individuals which were feeding rapidly the growth of

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

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

Definition of Terms

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

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

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

$$\theta \simeq \frac{\overline{T}_F}{\overline{T}}. \tag{1}$$

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

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

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

$$\phi_F = \phi_L.$$

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

$$\phi \simeq \frac{1}{\overline{T}}. \tag{2}$$

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

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

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

$$\alpha(1-\theta) = \phi_F. \tag{3}$$

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

$$\beta\theta = \phi_L. \tag{4}$$

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

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

$$\frac{d\theta}{dt} = \phi_F - \phi_L. \tag{5}$$

Hence
$$\frac{d\theta}{dt} = \alpha(1-\theta) - \beta\theta. \tag{6}$$

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

$$\beta \simeq 1/\overline{T}_F, \tag{7}$$

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

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

$$\alpha \simeq 1/(\overline{T} - \overline{T}_F).$$

Hence clearly
$$\alpha \simeq 1/\overline{T}_E, \tag{8}$$

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

Seasonal Changes in Reproductive Activity

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

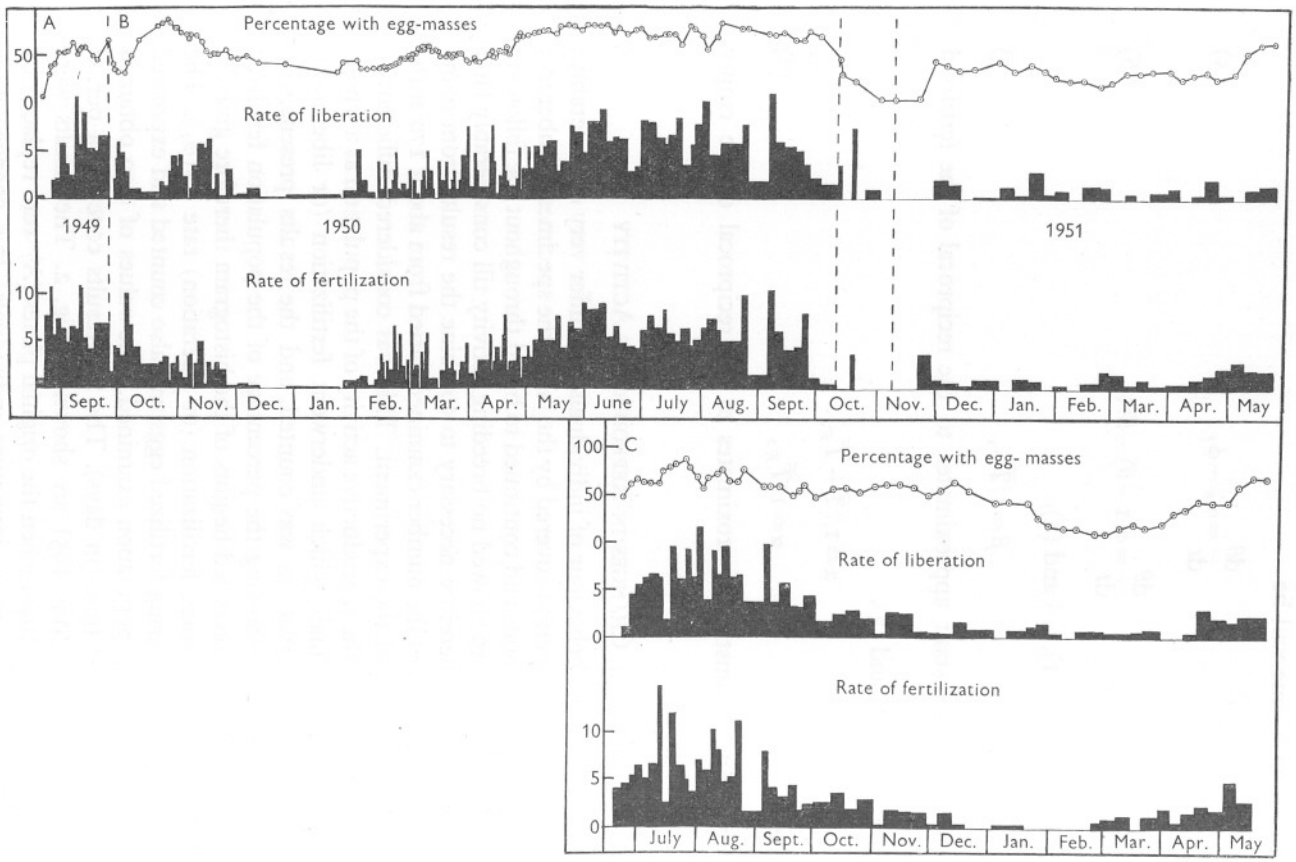

Fig. 2. Histograms showing frequency of liberation (upper) and fertilization (lower) for three series of barnacles, as percentage of population per day. Series A covers the period from August to September 1949, series B from September 1949 to May 1951, and series C, shown in the lower part of the figure,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

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

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

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

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

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

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

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

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

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

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

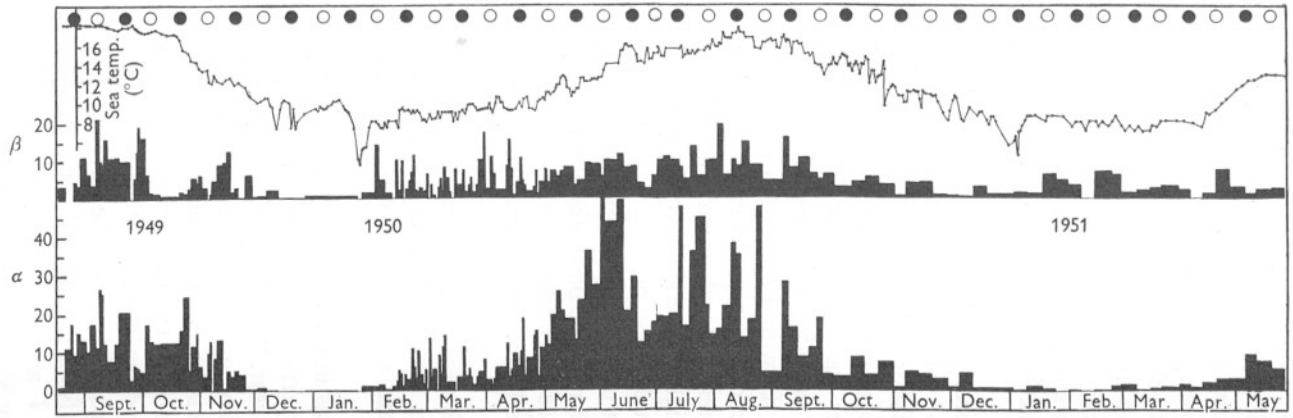

Fig. 3. Histograms showing the fluctuations in the values of the fertilization constant α and the liberation constant β over the period August 1949 to May 1951 for all series of barnacles. The sea temperatures are graphed above the histograms, and the times of maximum tides corresponding to full and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

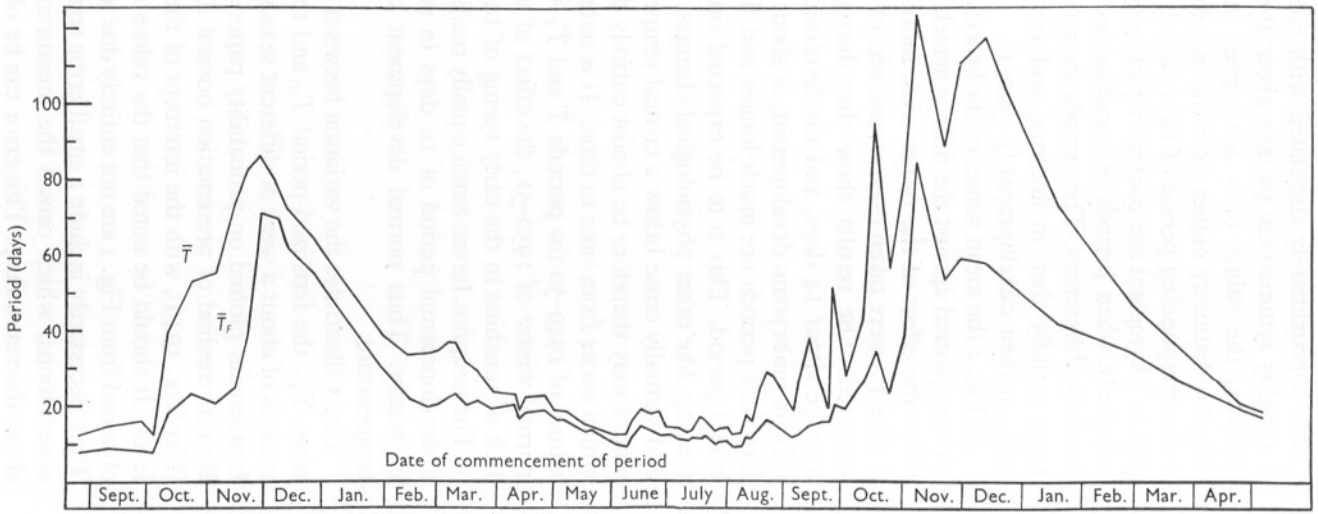

Fig. 4. Mean values of brood period $\overline{T}$ (upper), and fertilized period $\overline{T}_F$ (lower), from August 1949 to May 1951. The values of $\overline{T}$ and $\overline{T}_F$ are plotted against the date of commencement of the brood and fertilized periods respectively.

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

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

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

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

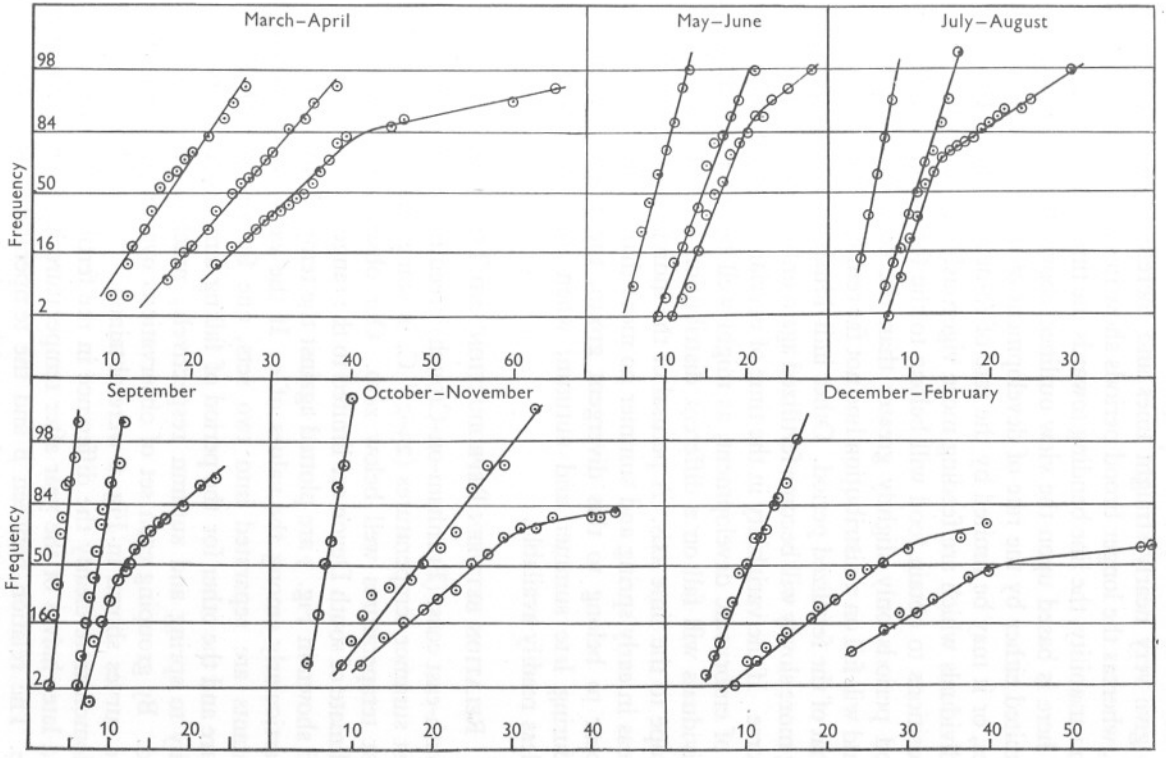

Fig. 5. Cumulative frequency curves for time of development T_D (left), the fertilized period T_F (centre) and the brood period T (right) at various seasons. The frequency axis is drawn on a probability scale, so that normal distributions are presented as straight lines. Horizontal axis gives the time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

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

Relation between Reproduction and Temperature

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

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

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

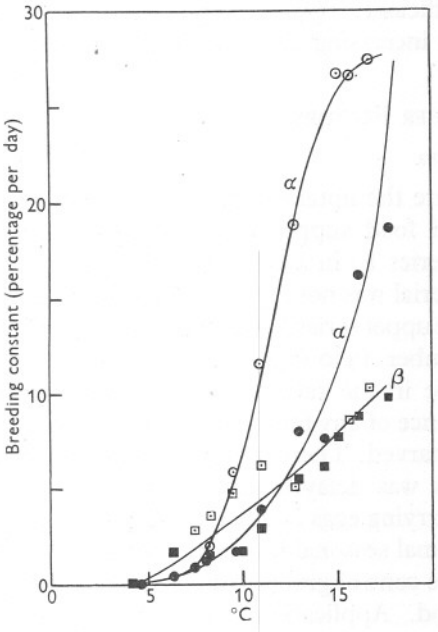

Fig. 6

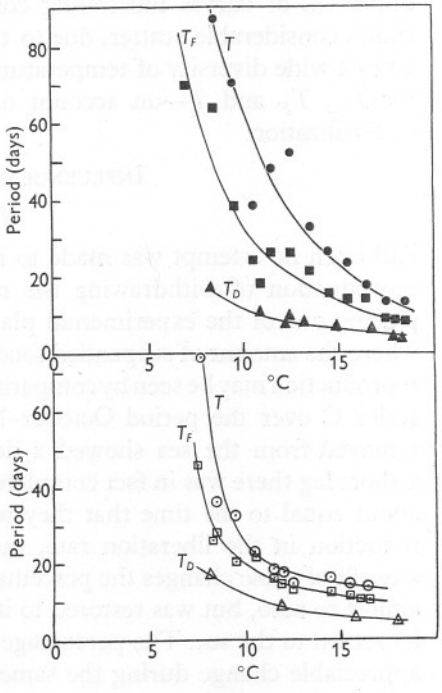

Fig. 7

Fig. 6. Temperature dependence of the fertilization constant α and the liberation constant β. ⊙ α, rising temperatures; ● α, falling temperatures; ⊡ β, rising temperatures; ■ β, falling temperatures.

Fig. 7. Temperature dependence of development, fertilized, and brood periods. Upper figure on falling, lower figure on rising temperature gradient. ○, brood period T; □, fertilized period T_F; △, time of development T_D.

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

part of figure) exhibit a relation to temperature closely similar to that of the rate of embryonic development. The values for Q_{10} based on the more reliable sections of the curves, namely those between 9 and 16° C., are 3·6, 3·3, and 3·6 for T_D, T_F and T respectively. These values are of a reasonable order for temperature coefficients of developmental processes (see Barnes, 1937). Their identity points to a common rate-governing process—that of the development of the embryos, which when completed leads to their release from the mantle cavity and then allows another fertilization to take place. The upper set of curves illustrating conditions in autumn, on the other hand, shows considerable scatter, due to the less regular individual behaviour, and gives a wide diversity of temperature coefficients—Q_{10} being 3·6, 5·4, and 5·8 for T_D, T_F and T—on account of the increasing delay in liberation and re-fertilization.

Influence of Other Factors

Nutrition

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

Water Movement

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

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

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

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

		Mean squares		
		A	B	C
Source of variation	Degrees of freedom	Fertilized and fully developed ovary	Fertilized	Fully developed embryos
Dates and position	4	1695·6	532·1	314·8
Sizes	2	1407·9	850·7	367·5
Access to water	1	3333·1	6579·3	1146·7
First-order interaction	14	130·6	98·5	120·5
Replicates and second-order interaction (residual)	38	67·2	60·2	55·3

Two samples each of ten to twenty individuals belonging to each sub-group (i.e. of a given size range, position, and relation to water access) were examined on each date and the number of individuals falling into classes A, B, or C recorded as a percentage. Percentages were transformed to angular values, and the variation about the mean (mean square) was calculated in respect of each of the sources of variation. The combined mean square due to replication and second-order interaction was used to determine the significance of each source of variation in turn, $P=0{\cdot}05$ as the limit of significance. All factors except first-order interactions had a significant effect.

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

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

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

Source of variation	Details		A	B	C
Dates of settlement and examination	Settled Sept. 1948	Ex. 19. i. 49	32·7*	18·4	14·4*
		Ex. 10. iii. 49	46·9*	18·3*	9·05
		Ex. 16. iii. 49	60·9*	24·8*	5·14*
		Ex. 30. iii. 49†	83·1	72·7	17·3
Size ranges	3–5 mm		48·2**	14·5*	4·4**
	5–6 mm		53·4*	20·7*	6·9*
	>6 mm		62·7	27·5	12·8
Position†	Fronts of panels		64·3	34·2	11·8*
	Backs of panels		62·9	43·0	18·8
Access to water	Centre of panel		47·8*	10·4*	3·6*
	Outside		61·8	31·4	12·3

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

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

* Indicates significant difference from entry one line below ($P < 0{\cdot}05$).

** Indicates significant difference from entry two lines below ($P < 0{\cdot}05$).

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

Age of individuals

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

individuals. To test this possibility a series of panels bearing younger individuals (series C, p. 365) was observed over the same period as the older individuals of series B. Both sets were of mature age and therefore not markedly different in average size. A comparison of the reproduction constants α and β was then made, excluding the short period when the two sets of panels were receiving different treatments. The difference between the constants α for the older and younger series (B–C) based on forty-three pairs of parallel observations was $+0{\cdot}407\%$ per day, and the standard error of the difference was $1{\cdot}30\%$ per day. The observed difference of means for β was $-0{\cdot}667\%$ per day and the corresponding standard error 0·481. In neither therefore was the difference significant.

Tidal periodicity

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

$\Delta\phi_F$ mean difference $= -0{\cdot}0028$, standard error of difference $= 0{\cdot}211$;
$\Delta\phi_L$ mean difference $= -0{\cdot}167$, standard error of difference $= 0{\cdot}246$.

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

Breeding in *Elminius* in Relation to Season

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

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

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

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

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

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

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

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

Fecundity of *Elminius* in Relation to its Ecology

The breeding behaviour of *Elminius* is well suited to its environment, namely sheltered coasts and estuaries. The remarkable eurythermy of its reproduction is clearly adapted to the wide fluctuations of temperature which occur in such habitats. Moreover, land-washed nutrients normally allow a high level of production to be maintained in enclosed waters throughout all but the coldest months. *Elminius* therefore has available abundant suspended food material for purposes of reproduction throughout most of the year. The continuous succession of broods necessitates intensive feeding, and it is noteworthy that *Elminius* has a faster rate of beat of the cirri than is found in most of the other British species, and far more rapid than that of *Balanus balanoides*. The advantage of feeding with access to unfiltered water, not only to growth but also to reproductive rate, has been shown. This effect is very pronounced in individuals exposed to a fast-flowing stream, as for example those attached to piles standing in a narrow part of a tidal estuary. Such individuals grow rapidly to a large size (1·0–1·5 cm) and a high percentage are fertile in the warmer months. The influence of water current on growth rate renders size

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

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

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

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

Summary

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

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

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

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

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

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

There is no lunar periodicity in the breeding cycle.

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

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

STUDIES ON *LYSMATA SETICAUDATA* RISSO (CRUSTACEA DECAPODA). VIII. THE LACK OF INFLUENCE OF EYESTALK ABLATION AND OF INJECTION OF EYESTALK EXTRACTS ON TESTICULAR WEIGHT AND DEGREE OF DEVELOPMENT OF THE MALE GENITAL DUCTS

By **D. B. Carlisle**

Pubbl. Staz. zool. Napoli, Vol. 25, 1954, pp. 241–5

Eyestalk ablation and an injection of eyestalk extracts has no effect on testicular weight or on degree of development of the male genital ducts in *Lysmata seticaudata*. This result is quite opposite to that found by Démeusy in the crab *Carcinides*.

D. B. C.

THE EARLY DEVELOPMENT STAGES OF THE BASS, *MORONE LABRAX* (L.)

By **L. A. J. Jackman**

Proc. Zool. Soc. Lond., B, Vol. 124, pp. 531–4

On 21 May 1952 some of the bass in the Plymouth Aquarium spawned, and a number of these eggs were collected and their development observed.

The eggs of the bass in British waters are larger than those in the Mediterranean, and hatching occurs on the fourth day. The mean length of newly hatched larvae is 3·83 mm.

During the first 24 hr these post-larval stages rest for long periods at the surface of the water, are insensitive to touch and no evasive action is taken.

On the third day the larvae rest at an angle of forty-five degrees to the surface. By the sixth day they commence to swim, and make rapid darts through the water easily avoiding the approach of a pipette.

By the tenth day the oil globule is partially absorbed.

Throughout these observations the mean temperature of the tanks was 15° C.

From the observed rate of growth of these reared larval stages, it is likely that those taken in the plankton hauls off the Eddystone during the period 1925–33 (Russell, 1935) were from 3–15 days old.

L. A. J. J.

The Effect of Galvanic Polarization on the Impulse Discharge from Sense Endings in the Isolated Labyrinth of the Thornback Ray (*Raja clavata*)

By O. Lowenstein

J. Physiol. Vol. 127, 1955, pp. 104–17

The effects of galvanic polarizing currents on the impulse discharges from the crista of the horizontal ampulla and from other labyrinthine end-organs show that galvanic polarization produces impulse responses similar to those occurring on natural rotatory stimulation. The responses from single sensory units of the horizontal semicircular canal to galvanic stimulation by ascending and descending currents sum with the responses to ipsilateral and contralateral accelerations. This result is held to provide circumstantial evidence for the existence of a so-called generator potential intervening between the mechanical deformation of the sense organ and the propagated impulse discharge in the sensory nerve. O. L.

The Planktonic decapod Crustacea and Stomatopoda of the Benguela Current. Part I. First Survey, R.R.S. *William Scoresby*, March 1950

By Marie V. Lebour, D.Sc.

Discovery Reports, Vol. 27, 1954, pp. 219–34

Most of the specimens are larvae. Among the few adults were several specimens of the Pasiphaeid *Pasiphaea semispina* recently described by Holthuis (1951–52). The present records show that it occurs much farther north than those found previously in the South Atlantic. Other interesting finds are a late larva of a *Periclimenes* with a long antennular flagellum and a peculiar pagurid larva of a type unknown before. Phyllosoma larvae of *Jasus lalandii* are common and at times a *Callianassa* larva is abundant which does not appear to belong to any known adult (the adults, always difficult to find, are very little known from their regions). Brachyura zoeae are abundant at times but not attributable to known species, the only Stomatopod present is a *Squilla* larva, almost certainly *Squilla armata* as it occurs in the same locality as the adult and agrees with it in most essential features. M. V. L.

The Pelagic Mollusca of the Benguela current. Part I. First Survey, R.R.S. *William Scoresby*, March 1950. With an Account of the Reproductive System and Sexual Succession of *Limacina bulimoides*

By J. E. Morton

Discovery Reports, Vol. 27, 1954, pp. 163–200

This paper records the occurrence and distribution in the Atlantic Ocean off south-west Africa of the following species of molluscs: *Ianthina ianthina*, *I. globosa*, *Atlanta peroni*, *Limacina inflata*, *L. bulimoides*, *Diacria trispinosa*, *Euclio pyramidata*, *Cavolinia inflexa*, *Cymbulia peroni*, *Thliptodon diaphanus*, *Pneumodermopsis paucidens*, as well as two species of larval lamellibranchs, one prosobranch larva, and several larval cephalopods. The ecology of *Limacina bulimoides* is discussed and information presented on its diurnal depth migrations. A size-depth division of the population appears to take place at night.

The reproductive system and sexual succession of *Limacina bulimoides* is described in detail. The genital ducts are typical of the lower level of both the groups Opisthobranchiata and Pulmonata, and in the condition of the gonad, as well as the development of penis and prostate, *Limacina* shows protandrous hermaphroditism. Six sexual stages are distinguished, ranging from small sexually undifferentiated individuals, through various stages of male development, to the females, which are the largest. A short discussion is added, dealing with the problem of the evolution of sexual succession in gastropods as a whole.

J. E. M.

Chemical Evidence on the Abundance and Botanical Composition of the Marine Phytoplankton

By W. R. G. Atkins

Proc. 7th Int. Bot. Congr. Stockh., 1950, p. 262 (published 1953)

The sea twenty miles off Plymouth is representative of a large area subjected to uniform meteorological conditions and not normally affected by water movements from areas under other conditions. Figures for the phytoplankton crop, calculated for a depth of 70 m. were concordant when based upon the changes in phosphate, carbon dioxide and nitrate, but with silica the values were over 10 times too small. The diatom tests do not dissolve or do so slowly, but suspended clay amounts to about one part per million, and this goes into solution in measurable amounts as shown by storing in polythene bottles for three months.

Productivity may also be followed by chlorophyll estimation after filtration through collodion discs or using fine paper after addition of aluminium sulphate. In the gel thus produced small flagellates can be seen under the microscope, in slow motion. Dr M. Parke's pure cultures afforded counts, which, excluding large diatoms, ran from 50 to over 3000 million cells per milligram of chlorophyll.

W. R. G. A.

Mechanical Stimulation in the Sea-anemone *Calliactis parasitica*

By L. M. Passano and C. F. A. Pantin

Proc. roy. Soc. B, 1955, Vol. 143, pp. 226–38

A method of administering measured local mechanical stimuli is described. Experiments were done upon the anemone *Calliactis parasitica.*

Mechanical stimuli show rapid apparent adaptation—partly due to simple mechanical causes such as contracture and passive deformation of the tissues.

When conditions are standardized a mechanical stimulus of sufficient intensity on the column gives a nervous impulse. These mechanical stimuli can be used in the same way as electric shocks to give facilitated responses.

Increasing the mechanical intensity shows (*a*) a threshold below which no impulse is generated, and (*b*) with further increase of strength, trains of increasing numbers of impulses.

There exists a graded mechanically excitable system. Gradation is to be observed below the threshold since there can be summation of subliminal impulses.

The excitable system appears to be orientated tangentially and responds to strains rather than pressure. The excitable system is purely endodermal. The ectoderm and mesogloea act only as an integument.

On histological grounds there are grave difficulties in supposing that impulses arise simply and directly in the numerous simple sense organs. The possibility is noted that the graded excitation of the mechanically sensitive system causes the nerve-net to fire off impulses.

The sensitivity of different parts of the animal varies greatly. In *Calliactis* the oral disc is at least 4000 times as sensitive as the column. The combination of a simple mechanically excitable system with the gross morphological features of the bodily organization permits purposive and varied responses. Thus strong stimulation of the column leads to the closure reflex, whilst weak stimulation of the disc by contact or by water movements leads to appropriate responses connected with feeding or rejection.

C. F. A. P.

Recherches préliminaires relatives à la separation et à la comparaison des Substances chromactives des Crustacés et des Insectes (Preliminary studies on the separation and comparison of the chromactive Substances of Crustacea and Insects)

By David Carlisle, Mme. Marie Dupont-Raabe and Sir Francis Knowles

C.R. Acad. Sci. Paris, T. 240, 1955, pp. 665–7

The chromactive substances of the sinus gland and post-commissural organs of crustacea and of the brain and *corpora cardiaca* of insects were separated by paper electrophoresis. The most striking results were the individuation and characterization of various chromactive substances. The first, designated substance A, was found in all these organs except the insect brain. It provoked concentration of all the red and yellow pigments of *Leander*. It was stable to boiling and did not pass freely through dialysis membranes. The second substance, substance B, was found only in the post-commissural organs. It concentrated the large red chromatophores of *Leander*, but expanded the small red chromatophores of the body and tail. These two substances were antagonistic. The brain of the insect contained a different chromactive substance, designated substance C. This substance caused darkening of *Carausius*, but had no effect on the pigments of *Leander*. It did, however, cause concentration of the chromatophores of *Crangon*. Other more mobile substances were found in the various extracts; they all pass freely through dialysis membranes and each acts only on a single pigmentary effector type. These substances appear to be the definitive hormones while substances A and B are probably the precursors.

Extracts of the X-organ of *Leander* have no effect when fresh, but after boiling, or after alcohol treatment, they attain an activity comparable to that of the sinus gland. It is probable that the site of production of the precursors of the hormone of the sinus gland is in the X-organ. D. B. C.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. 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

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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.

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