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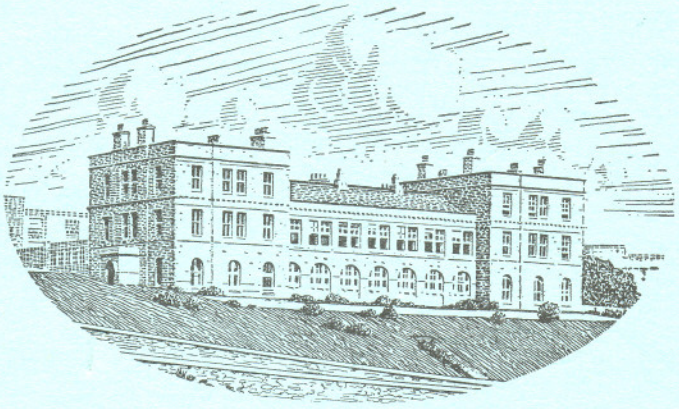

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THE DISTRIBUTION AND SIGNIFICANCE OF ORGANICALLY BOUND IODINE IN THE ASCIDIAN *CIONA INTESTINALIS* LINNAEUS

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(Plate I and Text-fig. 1)

INTRODUCTION AND PREVIOUS WORK

Attention has already been drawn (Barrington & Franchi, 1956*a*) to the presence of organically bound iodine in the endostyle of *Ciona*.[1] The purpose of the present work is to provide a fuller account of the distribution of this iodine, to examine how far the binding process is inhibited by the action of the goitrogen thiouracil, and to provide some cytological data for comparison with conditions in the endostyle of the ammocoete larva of the lamprey (Barrington & Franchi, 1956*b*). The importance of this latter aspect arises from recent demonstrations that significant amounts of thyroxin may be formed in various invertebrates (see, for example, Gorbman, Clements & O'Brien, 1954), possibly as the result of the iodination of skeletal scleroproteins. It has been plausibly suggested (Gorbman, 1955) that thyroid hormone may initially have arisen in this way as a biological accident; the thyroidal biosynthesis which is found in vertebrates, and which depends upon the secretion of thyroglobulin (Roche & Michel, 1955), would thus be a later evolutionary development consequent upon the iodination product having become metabolically important in the vertebrate line. From this point of view such protochordates as the ascidians occupy a position of key importance, and the crucial question to be answered is whether their iodine binding also is to be regarded, like that of invertebrates, as a chance by-product, or whether it shows any signs of being, like that of vertebrates, organized as a biochemical specialization.

MATERIAL AND METHODS

The animals were sent to Nottingham from the Laboratory of the Marine Biological Association, Plymouth. Some were fixed on arrival, while others were first immersed for 2 days in sea water containing 200 μc. of ^{131}I per litre. For investigation of the action of thiouracil the animals were immersed for

[1] Some evidence for this has also been obtained in unpublished work of B. C. Abbott and D. A. McGill (see Report of the Council of the Marine Biological Association for 1953–4, this Journal, Vol. 33, p. 775).

1 day in sea water containing 0·03 g. of the goitrogen per litre, after which the radio-iodine was added and they were left for a further 2 days. Constant aeration was provided, and temperatures ranged from 9 to 15° C.

Material was fixed in Susa, mercuric-formol, Champy's fluid in sea water, Bouin's fluid in sea water, and Hollande's modification of Bouin's fluid without acetic acid. For staining and histo-chemistry use was made of the Azan technique, mucicarmine, Kull's technique, the periodic-acid-Schiff (PAS) procedure, the pyronin-methyl-green test for ribonucleic acid (RNA), the ferric ferricyanide test (Fisher, 1953), and fluorescence microscopy; some further reference to these methods will be found in Barrington & Franchi (1956*b*). Autoradiographs were prepared by the stripping-film technique of Pelc, as outlined by Pearse (1954), and were counterstained in Harris's haematoxylin and eosin.

OBSERVATIONS

THE ENDOSTYLE

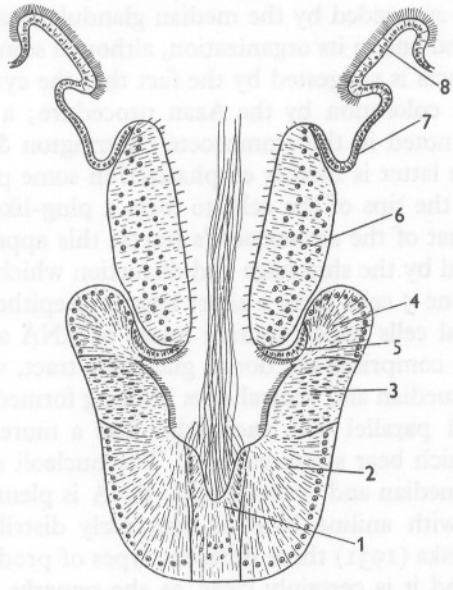
A comparative survey of the endostyle in seven species of ascidians has been reported by Sokólska (1931; see also Hûus, 1937), but the present study has disclosed some new points, both of fact and interpretation, which are of importance for the analysis of the functions and homology of the organ. In *Ciona* (Millar, 1953) it has the form of a deep groove, the epithelial wall of which is composed of a number of distinctive cell types; these will be referred to here for convenience as numbered zones (Text-fig. 1).

Zones 1–6

In the mid-ventral line, at the base of the groove, there is a group of cells (zone 1) which bear exceptionally long cilia. Near their nuclei there are occasional large vacuoles containing material which may be brownish in colour; the significance of these is obscure, but distally there is clear evidence of secretory function, for the apical part of the cell body contains material (diffuse or granular according to the mode of fixation) which stains intensely with aniline blue and with the PAS procedure, and which is the only product in the endostylar epithelium to react positively with mucicarmine. These cells differ, however, from the more typical secretory cells of the three pairs of glandular tracts (see below) in showing neither a large nucleolus nor a significant amount of RNA. Their cell bodies are slender and elongated, and their nuclei are crowded into two rather irregular layers, but the zone extends on each side as a layer of much shallower cells in which both the secretory contents and the ciliation are progressively reduced, the tips of these extensions becoming continuous with the ventral glandular tracts.

The latter are formed of elongated columnar cells (zone 2); each of these has a large spherical nucleus, a very conspicuous nucleolus, and cytoplasm rich in RNA, but it is difficult to judge whether they bear any cilia. In

Sokólska's figures these cells are shown lying more or less parallel with each other, and covered over (and therefore separated from the endostylar lumen) by an entirely separate layer of cubical cells. It is difficult to understand what could be the significance of such an arrangement, which would result in the separation of the glandular cells from the lumen into which they discharge, and in *Ciona* the actual situation proves to be a little different from this. The cells of the glandular tract are in fact arranged in a fan-shaped pattern (Text-fig. 1), with their apices crowded together in a narrow zone at which the discharge of their secretion must take place, and it is the lateral extension of the zone 1 cells over the main body of the fan which gives the misleading impression that the secretory cells are completely cut off from the lumen.



Text-fig. 1. Transverse section of the endostyle of *Ciona* (diagrammatic). The eight zones of the epithelium are indicated by corresponding numbers; for further explanation see text.

This detail is of some importance, not only because it makes the organization of the ventral tract functionally intelligible, but even more because it emphasizes the close resemblance between the endostyle of *Ciona* and that of the ammocoete larva. The cells of the glandular tracts in the latter animal are very like these zone 2 cells, and are arranged in a similar fan-shaped pattern, with discharge taking place through a narrow plug formed by the fusion of the tips of the cells.

The secretory functions of the endostyle of *Ciona* appear to be complex, and an analysis of the activity of the glandular tracts is outside the scope of the present work. It must suffice to say that a characteristic feature of the cells of

the ventral and median tracts is the accumulation above the nucleus of a material, presumably a secretory product, which stains pale grey-blue with the Azan technique, gives a weak PAS-positive reaction, and is negative to mucicarmine. This is sometimes seen as a compact mass lying within a large vacuole, but its actual form is probably much influenced by artifact, and in some preparations, notably after Champy or Bouin-Hollande (without acetic) fixation, it is not sharply demarcated from the surrounding cytoplasm.

The ventral glandular tract is abruptly succeeded by zone 3, composed of slender ciliated cells which are closely crowded and which have their granular nuclei irregularly arranged in a number of layers. RNA is negligible in these cells, but the presence of granular inclusions, PAS-positive after fixation in Bouin-Hollande (without acetic), suggests that they may have some secretory function. They are succeeded by the median glandular tract (zone 4), which resembles the ventral one in its organization, although some differentiation in the secretory products is suggested by the fact that the cytoplasm of the two tracts differs in its coloration by the Azan procedure; a somewhat similar situation has been noted in the ammocoete (Barrington & Franchi, 1956*b*). Resemblance to the latter is further emphasized in some preparations by the apparent fusion of the tips of the cells to form a plug-like structure exactly comparable with that of the ammocoete's tracts; this appearance may, however, be exaggerated by the shrinkage and distortion which is clearly induced by fixation. The zone 5 cells form a short length of epithelium composed of more or less cubical cells which contain traces of RNA and a few granular inclusions. Zone 6 comprises the dorsal glandular tract, which differs in its structure from the median and ventral ones in being formed of elongated cells which are arranged parallel with each other like a more normal columnar epithelium, and which bear scattered cilia. The nucleoli are large, although less so than in the median and ventral tracts, RNA is plentiful, and secretory material (staining with aniline blue) is irregularly distributed in vacuoles. According to Sokólska (1931) three different types of product are discharged from these cells, and it is certainly clear, as she remarks, that the so-called mucous secretions of the ascidian endostyle must be a product of considerable complexity.

In a recent account of the ammocoete's endostyle (Barrington & Franchi, 1956*b*) a distinction has been made between alimentary and thyroidal functions. From this point of view, which seems also applicable to *Ciona*, the portions of the endostyle so far described, constituting the main bulk of the organ, must be regarded as primarily alimentary; there is probably more to be learned as to the full function of its secretions, but they are well known in general terms to be concerned with the trapping of food particles (Orton, 1913). Over the whole of this region (zones 1–6) bound iodine is inconspicuous in autoradiographs and is possibly absent altogether except in association with the long cilia of zone 1, where a small amount of iodine is commonly to be

found; this, however, has a separate significance which will be discussed below (p. 8). In some areas of the epithelium of some specimens, however, the density of the autograph seems to be slightly greater than that of the background, particularly in zone 1 and in the upper part of zone 6, and it is just possible that this is indicative of a slight capacity for iodine binding; it is certain, however, that this would attract little attention if it were not for the highly significant images obtained from the remainder of the organ (zones 7 and 8).

Zone 7

Dealing first with zone 7 (Text-fig. 1; Pl. I, fig. 5), iodine is here found distributed more or less evenly over the cell bodies throughout the whole extent of this epithelium; the autoradiographic image (Pl. I, figs. 6, 11) is not dense, but it is a very definite and constant feature of this zone, and it seems evident that iodine binding must be occurring in the cells. Its clear definition in this particular zone shows that this binding cannot be merely a chance by-product of the general secretory activity of the endostyle, and a further examination of the properties of zone 7 provides good evidence that a specialized secretory process is, in fact, involved, for its cells contain numerous granules of variable size, and may sometimes show a slight indication of RNA. Often there appears to be one large granule per cell (Pl. I, fig. 12), but in some preparations additional smaller ones can be seen, and it is probable that a fusion of a group of these into larger ones may occur as an artifact of fixation. Mucus-like material, not dissimilar in shape although with less sharply defined contours, is plentiful elsewhere in the pharyngeal epithelium, so that the properties of the zone 7 granules, in comparison with the inclusions of other cells, need careful examination; they may be summarized as follows:

(*a*) Colour. They are pale yellow-brown in colour, a property not found anywhere else in the endostylar or pharyngeal epithelium except in the material seen in vacuoles in some of the zone 1 cells (see above, p. 2). The pigment, both in the latter and in the zone 7 granules, is bleached after 24–48 h treatment with 10% hydrogen peroxide, and is thus possibly melanic, although the initial colour is sometimes so weak that this result may not be of much significance.

(*b*) Fluorescence. After fixation in Susa, mercuric formol, or Bouin in sea water the granules exhibit a yellow-brown fluorescence which becomes very pale yellow after bleaching with hydrogen peroxide. Although fluorescence is a conspicuous feature of cells in the blood sinuses, it is found nowhere else in the endostylar or pharyngeal epithelium with the fixatives mentioned; in particular, the brown material in zone 1 is not fluorescent. After fixation in Bouin-Hollande (without acetic) some finely particulate whitish fluorescence appears in zone 6 and in the mucous region of zone 1, but the fluorescence of zone 7, although less striking than with the other fixatives, remains distinctive.

(*c*) Azan staining. The zone 7 granules commonly stain a characteristic

bright orange; the inclusions elsewhere show a varying degree of affinity for aniline blue, the apical secretion of zone 1 and the secretion of the pharyngeal epithelium staining deeply with this.

(*d*) PAS reaction. The granules give a moderately strong positive response. A noticeably stronger response is given by the secretion of zone 1 and of the pharyngeal epithelium, while the response of the remaining secretory products of the endostyle, together with the brown material in zone 1, is weak and indefinite.

(*e*) Mucicarmine. The granules are negative to mucicarmine, as also are all of the products of the endostyle, with the exception of the zone 1 secretion (see above). An occasional indefinite response may be given by the pharyngeal epithelium, but this also is commonly negative.

(*f*) Ferric ferricyanide test. The granules give a moderate but quite definite positive response. The secretion of the pharyngeal epithelium is also positive, but more strongly so, giving a deep blue colour. A strong response is also obtained from some of the contents of zone 1; the response of the remainder of the endostyle is indefinite, although the cytoplasm of the glandular cells may respond to a moderate extent. After fixation in Bouin-Hollande (without acetic) the response of the zone 7 granules is relatively more intense.

The above tests are not, of course, specific, a positive ferric ferricyanide response, for example, indicating no more than the presence of reducing groups (Adams, 1956), and the results are clearly influenced to some extent by the nature of the fixative. It can, however, be concluded that there is present in zone 7 a particular type of secretory inclusion which displays characteristics not found in the same combination elsewhere in the endostylar or pharyngeal epithelium. No useful inferences can be drawn at this stage as to the distinctive chemical nature of this secretion, but it can be said that the granules are associated with iodine binding, and it is significant that their properties, as outlined above, are virtually identical with those of the secretion of the thyroidal epithelium of the endostyle of the ammocoete (Barrington & Franchi, 1956*b*). It seems reasonable, therefore, to conclude that they are homologous with the latter, and, through them, with thyroid colloid, and that they thus constitute evidence for the existence in *Ciona* of a specialized secretory activity providing the molecular basis for the iodination process. On this interpretation the latter is not, therefore, a chance by-product of endostylar secretion, but seems very likely to represent a truly thyroidal biosynthesis. For this reason the zone 7 granules will be referred to as *thyroidal granules*, the name already applied to the corresponding secretion of the ammocoete.

Zone 8

In certain sections one or more of the thyroidal granules of zone 7 can be seen lying in the endostylar lumen, or else at the tip of a cell as though extrusion were about to take place. Such appearances could, however, very well be

an artifact of fixation or of sectioning, and they are too infrequent to be accepted as reliable evidence of discharge. On the other hand, there is reason for believing that in the ammocoete the iodinated product may be extruded into the endostylar lumen in a more fluid and non-staining form, and it is possible that the same may apply to *Ciona*. The importance of this matter lies in its relevance to the interpretation of the condition of the zone 8 epithelium (Text-fig. 1). This is somewhat thicker than zone 7, and is composed of strongly ciliated cells which lack RNA and which show less sign of secretory activity than any other part of the endostylar epithelium. These cells are known (Orton, 1913) to be responsible for driving the secretion of the endostyle laterally out of the groove and on to the pharyngeal wall, up which it is then swept by the cilia of the latter (see below, p. 8). It follows that any iodinated product which may be discharged from zone 7 would also be swept over these zone 8 cilia and some might well be adsorbed to them; thus would arise autoradiographic images which would, however, indicate a transmission of bound iodine across the cells rather than the existence of it within them.

This appears to be the most likely explanation of the fact that zone 8 actually gives a much more intense image than does zone 7 (Pl. I, figs. 5, 6), for close inspection of the distribution of the iodine shows a marked difference between the two zones, the weaker image of zone 7 being almost uniformly distributed over the cells, while that of zone 8 shows an intense concentration over the cilia and cell borders. Pl. I, figs. 6 and 11, illustrate this feature, although it is much less clearly defined in the photomicrographs than in the original preparations. The iodine image extends also over the cell bodies, but this extension is much less intense than that of the surface concentrations, and could quite well be a result of random scatter from them. There is, too, the possibility that if an iodinated secretion does accumulate over the surface of the epithelium there might be some diffusion from it into the cytoplasm. However, it is clearly impossible to be sure that no binding of iodine takes place within the zone 8 cells, but the absence of any obvious secretory basis for it suggests that its occurrence is highly improbable. Moreover, the view that the iodine image over zone 8 is a consequence of the movement of iodinated secretion by the cilia is strongly supported by consideration of the distribution of bound iodine elsewhere in the pharynx, as will now be explained.

THE PHARYNGEAL CONTENTS

First, there is clear evidence that an iodinated product is mixed with the visible secretion of the endostyle and pharynx, for the food cords in the lumen are found to be strongly iodinated (Pl. I, figs. 1, 2). Here, however, a complication is admittedly introduced by the capacity of marine organisms to accumulate bound iodine (see above, p. 1), for any such organisms included in the food would be expected to show this same property. This was, in fact, strikingly exemplified by one specimen in which a large unidentified arthropod

within the pharynx showed a substantial concentration of bound iodine within its exoskeleton (Pl. I, figs. 1, 2). This property could hardly, however, account for all of the iodination of the food cords, the exogenous contents of which are very variable in amount and miscellaneous in nature, including *Ciona* sperm, diatoms and unidentifiable cellular material and detritus. It is perhaps impossible to establish with absolute certainty that an iodinated secretion is added to the food cords from the endostyle, but the strong probability that this does happen is indicated by the fact that radioactive secretion, with very little exogenous material in it, can be observed in the mouth of the organ (Pl. I, figs. 5, 6). Equally significant is the almost invariable association of a weak but definite iodine image with the long cilia which arise from zone 1, for these are thought to have no function in the transporting of food particles, but are believed to assist in the deflecting of the endostylar secretion on to the cilia of zone 8 (Orton, 1913).

THE PHARYNGEAL EPITHELIUM

A second significant aspect of iodine distribution within the pharynx arises from the complicated structure of the wall of this organ. A current of water is maintained by the cilia of the gill openings or stigmata, but internally to the latter there is a system of longitudinal bars from which papillae project into the pharyngeal cavity (Roule, 1884; Berrill, 1950). Now the long threads of secretion which trap the food particles are swept up the walls mainly by the cilia of these papillae, aided in part by a waving movement of the bars themselves (Orton, 1913), with the ciliated dorsal languets becoming involved in the backward movement of the main food cord. If, therefore, there is any tendency for the iodinated component of the endostylar secretion to accumulate over the surfaces of the ciliated epithelia which propel the latter, as has been suggested above for zone 8, an autoradiographic image should be conspicuous on the ciliated regions of the papillae and languets. This is, in fact, readily seen to be so (Pl. I, figs. 1, 2, 9 and 10), the image, as with that of zone 8, showing a concentration over the surface rather than a uniform distribution over the cell bodies. In sharp contrast to this, the ciliated epithelia of the stigmata, which, by virtue of their position, are largely removed from contact with the endostylar secretion, yield very much lighter and often quite negligible images.

THE INTESTINAL CONTENTS

Within the intestine the food cords are distinguishable into two components, one being material which has entered from the pharynx and which is recognizable by the variety of its contents, and the other a clear mucus-like substance, positive to mucicarmine, which has no food material mixed with it, and which is a secretion of the mucus cells of the intestinal epithelium. Auto-

radiographs demonstrate an important difference between these two components (Pl. I, figs. 7, 8), for the former gives an iodine image while the latter does not; here, then, there is no evidence for any association of iodine binding with the normal process of alimentary secretion.

THE TEST

The presence of iodine in the test of ascidians has long been known from the work of Cameron (1914, 1915), who, without reporting on its chemical form, noted that it was especially abundant in the surface layer. The test is now known to be composed of two parts, the tunic proper and the superficial cuticle. The former is made up of the cellulose-like tunicin, with which is associated some glycoprotein, while the cuticle consists of pure protein and is thus chemically different from the remainder (Pérès, 1948). This difference is emphasized by the mode of development, for while the epidermis is mainly responsible for secreting the basic substance of the test, the cuticle arises from 'Tropfenzelle'; the latter initially contain a glycoprotein complex, but after migrating into the test they appear to give up the carbohydrate component to the tunicin and the remainder of their content is subsequently incorporated into the cuticle.

In view of these facts it is of interest that autoradiographs clearly demonstrate (Pl. I, figs. 3, 4) an accumulation of bound iodine in the cuticle, while none appears to be present in the remainder of the test. Mention has been made above (p. 1) of the way in which bound iodine becomes incorporated into the scleroproteins of the skeletal structures of invertebrates; the nature of the proteins of the test clearly demands further investigation from this point of view, for if the tough outer cuticle could be shown to contain scleroprotein the presence in it of bound iodine would be readily explained as a similar by-product of skeletal secretion. The facts at present available certainly suggest that iodination in the test is a process quite distinct from that in the endostyle, although it is tempting to speculate that the existence of the former might have been the starting point for the evolution in ascidians of a biochemically useful iodinated product.

In the earlier stages of this work there appeared to be indications of the association of bound iodine with the mantle epithelium (Barrington & Franchi, 1956*a*), but further studies have not substantiated this, and at the present time significant amounts of bound iodine have only been found consistently in the regions mentioned above. The iodination of the test merits further investigation, however, for, as has been suggested here, it raises an issue of some evolutionary interest, and it is hoped to report on it further in relation to the mode of secretion and regeneration of this tissue. Moreover, the existence of bound iodine in the stolonic septum of *Perophora* (Gorbman, 1941) shows that the present description does not exhaust the possibilities of iodine binding in ascidians.

THE ACTION OF THIOURACIL

In considering the effects of thiouracil it is necessary to bear in mind its supposed mode of action. Thyroidal biosynthesis is thought to depend upon the iodination and subsequent condensation of the tyrosine residues in thyroglobulin, the specific protein of the thyroid gland (Roche & Michel, 1955), but the means by which chemical goitrogens produce their interference with this process are not fully understood. It seems likely, however, that thiouracil, acting perhaps as a reducing agent, prevents the liberation from iodide of free iodine, the presence of the latter being essential if the iodination of tyrosine is to take place. This requirement has been mainly studied in relation to thyroidal biosynthesis, but it appears to be a necessary condition for any protein iodination, including *in vitro* reactions (Rawson, Rall & Sonenberg, 1955). It would seem to follow from this that the iodination of skeletal scleroproteins by radioactive iodide must depend upon the release of free iodine, and that this in its turn might well depend, at least in part, upon an appropriate oxidizing enzyme system which might, therefore, be inhibited by thiouracil. This means that a demonstration of such inhibitory action in any tissue cannot be held as a proof that it is carrying on thyroidal function in the strict sense of the iodination of a specific thyroid protein.

The effect of thiouracil treatment upon *Ciona* is to eliminate almost completely all traces of bound iodine from the sites mentioned above. None at all can be detected in the endostylar epithelium or on the pharyngeal cilia. It is, however, sometimes possible to detect a very slight but significant amount in the food cords, both in the pharynx and in the intestine, but not, of course, in the purely intestinal mucus in the latter (see above, p. 9). It is not clear whether this iodine is associated solely with the exogenous food material, or whether it results from a small amount of bound iodine continuing to be discharged from the endostyle without the prior storage which would be needed to enable that organ to produce a significant autoradiograph, but it is of interest that a similar situation appears to exist in *Amphioxus* (Thomas, 1956), the association of iodine with food particles being demonstrable after thiouracil treatment has eliminated it from the endostyle of that animal. The amount involved in *Ciona*, however, is very much less than in control specimens and could easily be overlooked if it were not being specially sought, and it is clear that the goitrogen substantially arrests the normal iodination process. This applies also to the cuticle of the tunic, for in thiouracil-treated specimens the bound iodine is either completely lacking or is present in a greatly reduced amount. It is impossible to judge the full significance of these results until there has been further clarification of the biochemical basis of the iodination process in the endostyle and cuticle, and until the mode of action of thiouracil is better understood. For the reasons already stated, however, the behaviour of the cuticle cannot be taken as evidence that this region is concerned with specifically thyroidal biosynthesis.

DISCUSSION

The observations recorded here show a close resemblance between the endostyle of *Ciona* and that of the ammocoete, not only in the organization of certain of the glandular tracts, but more especially in the ability of parts of the epithelium to bind iodine, and in the association of the latter property with a characteristic secretory product, here called the 'thyroidal granule'. So close is this resemblance that there can now be little doubt as to the correctness of the classical view that the protochordate endostyle is homologous with that of the ammocoete and, through the latter organ, with the thyroid gland of the vertebrates. It is necessary to consider further, however, the justification for regarding iodine binding in the ascidian endostyle as a truly thyroidal biosynthesis.

Spaul (1928) was unable to accelerate metamorphosis in frog tadpoles by feeding them with *Ciona* endostyle, but in view of the relative weakness of the autoradiograph of the latter organ as compared with that obtained from mammalian thyroid it may be doubted whether the amount of tissue used by him would have been adequate for securing a positive result. As against this negative finding, Sembrat (1953) was able to bring about metamorphic changes in axolotls by inserting into them as many as sixty-five dried endostyles of *Amphioxus*, in which organ iodine binding is known to occur (Thomas, 1956), and in view of this, and of the known occurrence of thyroxin in the ammocoete endostyle (Leloup & Berg, 1954), there is clearly no *a priori* reason why thyroid hormone should not be present in the endostyle of *Ciona*. It is hoped to report later on the chemical form of the bound iodine of this organ and of the test, but in the meantime it must be said that, for reasons indicated above (p. 1), this particular issue now seems less crucial than the question as to whether or not the iodine binding is organized as a biochemical specialization.

It is for future work to show whether, as seems quite likely, the iodination of the cuticle in *Ciona* is a chance result of the presence there of scleroproteins. The situation in the endostyle, however, seems much better defined, for the present work has shown that iodination in that organ takes place within a narrowly limited zone which is cytologically specialized in a manner directly comparable with the thyroidally active regions of the ammocoete's endostyle. The inference is that the product of iodination is a thyroid hormone which has become metabolically important for the ascidian, and that a particular epithelium is set apart for secreting, as thyroidal granules, the necessary molecular basis for the iodination process. It is not possible as yet to suggest what might be the functional importance of this hormone, but it is clearly essential that this inference should now be tested by examining whether thyroxin can be shown to exert any specific effects upon ascidians. At this stage also it is not clear whether any significance is to be attached to the slight traces of bound iodine in other parts of the endostyle. Such traces might represent a generalized capacity from which the special property of zone 7

evolved, but study of a wider range of species is necessary before any useful opinion can be expressed on this.

The autoradiographs do not disclose the route by which the iodine reaches these cells; presumably iodide might be absorbed by them direct from the water, or it might reach them in the body fluid after absorption elsewhere (by, for example, the pharyngeal wall). In this connexion it seems worth noting that the zone 7 cells are separated from the underlying body fluid by a rather thick layer of connective tissue; no doubt this would not preclude absorption through the latter, but, having regard also to the situation of this zone at the upper edge of the endostylar groove where it must be bathed by moving sea water, circumstances would seem to favour the possibility of direct absorption of iodide from the pharyngeal lumen. This would, of course, partly explain the use of endostylar epithelium for thyroidal biosynthesis, and other advantages are its well-developed secretory capacity, which would provide a basis for the evolution of the thyroidal granules, and the ease with which the hormonal product can be mixed with the remainder of the endostylar secretions and so be conveyed into the intestine. From there it is presumably taken up into the body tissues (although there is at present no proof of this), and it is the development of this particular route of absorption which, as Thomas (1956) has also pointed out in connexion with his study of *Amphioxus*, explains why thyroid hormone can be successfully administered by mouth throughout the vertebrates.

Comparison with the ammocoete is complicated by the very different organization of the pharynx in the vertebrates, but it is clear that the division of the endostyle in the latter animal into two halves, amounting to its virtual duplication, and its posterior elaboration into a spiral coil, provide a very large surface area within the limited space available. The proportion of this area devoted to iodine binding is, moreover, very substantially greater than in the ascidian, while many of the thyroidally active cells are columnar in form and, with their large complement of thyroidal granules, seem much more specialized than the corresponding cells of zone 7 (Barrington & Franchi, 1956*b*). These facts suggest a more intensive development of iodine binding in the ammocoete, and this must surely be correlated with the diminished availability of iodine which would have been an inevitable consequence of the migration into fresh water which marked the evolution of vertebrates from the protochordates. It may be suggested also that the form of the endostyle in the ammocoete, where it appears as a sac with only a narrow opening, is not as well adapted as is an open groove for the uptake of iodide from the water circulating through the pharynx; since, therefore, the endostylar epithelium in this animal has close contact with well-developed blood capillaries it seems probable that uptake from the blood stream may have become established with this advance in specialization, although here again the autoradiographs offer no clear evidence.

It is of great interest that iodine binding has also been shown to occur in the

endostyle of *Amphioxus* (Thomas, 1956), for this establishes the phenomenon as a common property of the organ in all three of the groups (Tunicata, Cephalochordata and Vertebrata) in which the latter is present. The endostyle of *Amphioxus* has only two pairs of glandular tracts, and the iodine binding appears to be located in or near the lateral pair, a fact which has led Thomas to regard the endostylar mucus as the direct evolutionary forerunner of thyroid colloid. The present work shows, however, that there are specialized iodine binding cells in the ascidian endostyle which are distinct from the glandular tracts and which would seem to resemble the thyroidally active cells of the ammocoete much more closely than do the mucus cells of *Amphioxus*, despite the fact that the latter animal, with its well-developed metamerism, is in some respects much closer to the vertebrates than are the ascidians. This situation, at first sight somewhat paradoxical, clearly needs further investigation. Its explanation may, however, be found in some recent arguments of Berrill (1955), who has developed with great cogency the view that *Ciona* represents a truly primitive level of chordate organization from which the vertebrates could have been derived by the process of neoteny on which Garstang (1929) placed such emphasis. Berrill suggests that *Amphioxus* is a specialized offshoot of the main chordate stem, and represents a secondary return to a fully marine existence from a stage at which the early chordates were already ascending estuaries and becoming established in fresh water. He ascribes to this the secondary degeneration of their anterior sensory equipment, and it would seem logical to expect that a comparable degeneration in the iodine-binding mechanism would result from such a return to an iodine-rich habitat. For the present this suggestion must remain a matter for speculation, but in the meantime some support for such an interpretation is possibly to be drawn from Thomas's interesting observation that *Amphioxus* does not take up radio-iodine when it is first removed from its natural habitat, but begins to do so when it is kept in the laboratory in circulating water in which the iodine content is probably lower than in the outside sea water.

I am indebted to Mr T. Berbank for preparing the photomicrographs and to Miss J. M. Plumtree for technical assistance. Comparisons with the ammocoete larva have been rendered possible by work carried out on that animal in collaboration with me by Mr L. L. Franchi.

SUMMARY

Some features of the organization of the endostyle of *Ciona intestinalis* are described. Autoradiographs show that bound iodine is present in a limited area of the epithelium (zone 7), where it is associated with characteristic secretory inclusions which are found only in this zone. These are regarded as providing the molecular basis for the iodination process, and are termed 'thyroidal granules', since they resemble in certain properties the similarly

named granules of the endostyle of the ammocoete; it is concluded that they are homologous with these and, through them, with thyroid colloid. Their existence provides evidence that the iodination process is a product of biochemical specialization, and implies that it results in the formation of an hormonal secretion. It is concluded from the distribution of bound iodine over the pharyngeal epithelium and in the food cords that this iodinated secretion is carried in the latter into the intestine. Bound iodine is also present in the cuticle of the test; it is suggested that this may differ from the situation in the endostyle in being a consequence of the iodination of a skeletal protein secretion such as is known to occur in many invertebrates. Prior immersion of the animals in thiouracil solution largely eliminates bound radio-iodine from all of the sites mentioned, although a little may sometimes be detectable in the food cords and cuticle. The results are discussed in the light of recent work on *Amphioxus* and the ammocoete larva, and attention is drawn to their bearing on current views on the relationship between vertebrates and protochordates.

REFERENCES

ADAMS, C. W. M., 1956. A stricter interpretation of the ferric ferricyanide reaction. *J. Histochem. Cytochem.*, Vol. 4, pp. 23–35.

BARRINGTON, E. J. W. & FRANCHI, L. L., 1956*a*. Organic binding of iodine in the endostyle of *Ciona intestinalis*. *Nature, Lond.*, Vol. 177, p. 432

—— —— 1956*b*. Some cytological characteristics of thyroidal function in the endostyle of the ammocoete larva. *Quart. J. micr. Sci.*, Vol. 97, pp. 393–410.

BERRILL, N. J., 1950. *The Tunicata*. London: Ray Society.

—— 1955. *The Origin of Vertebrates*. Oxford: The Clarendon Press.

CAMERON, A. T., 1914. The distribution of iodine in plant and animal tissue. *J. biol. Chem.*, Vol. 18, 335–80.

—— 1915. The distribution of iodine in plant and animal tissue. *J. biol. Chem.*, Vol. 23, pp. 1–39.

FISHER, E. R., 1953. Observations on thyroid colloid. *Arch. Path.*, Vol. 56, pp. 275–85.

GARSTANG, W., 1929. The morphology of the Tunicata, and its bearing on the phylogeny of the Chordata. *Quart. J. micr. Sci.*, Vol. 72, pp. 51–187.

GORBMAN, A., 1941. Identity of an iodine-storing tissue in an ascidian. *Science*, Vol. 94, p. 192.

—— 1955. Some aspects of the comparative biochemistry of iodine utilization and the evolution of thyroidal function. *Physiol. Rev.*, Vol. 35, pp. 336–46.

GORBMAN, A., CLEMENTS, M. & O'BRIEN, R., 1954. Utilization of radio-iodine by invertebrates, with special study of several annelids and mollusca. *J. exp. Zool.*, Vol. 127, pp. 75–92.

HŬUS, J., 1937. Tunicata: Ascidiaceae. *Handb. Zool. Berl.*, Bd. 5, Hlf. 2, pp. 545–672.

LELOUP, J. & BERG, O., 1954. Sur la présence d'acides amino-iodes dans l'endostyle de l'Ammocoete. *C.R. Acad. Sci., Paris*, T. 238, p. 1069.

MILLAR, R. H., 1953. *Ciona*. L.M.B.C. Memoir No. 35. (Liverpool: University Press).

ORTON, J. H., 1913. The ciliary mechanisms of the gill and the mode of feeding in *Amphioxus*, ascidians and *Solenomya togata*. *J. mar. biol. Ass. U.K.*, Vol. 10, pp. 19–49.

PEARSE, A. G. E., 1954. *Histochemistry, theoretical and applied*. London: Churchill.

PÉRÈS, J. M., 1948. Recherches sur la genèse et la régénération de la tunique chez *Clavelina lepadiformis* Müller. *Arch. Anat. micr. Morph. exp.*, T. 37, pp. 230–60.

RAWSON, R. W., RALL, J. E. & SONENBERG, M., 1955. The chemistry and physiology of the thyroid. *The Hormones* (ed. Pincus & Thimann), Vol. 3, pp. 434–519.

ROCHE, J. & MICHEL, R., 1955. Nature, biosynthesis and metabolism of thyroid hormones. *Physiol. Rev.*, Vol. 35, pp. 583–610.

ROULE, L., 1884. Recherches sur les ascidies simples des côtes de Provence. *Ann. Mus. Hist. nat. Marseille*, T. 2, pp. 1–270.

SEMBRAT, K., 1953. Effect of the endostyle of the lancelet (*Branchiostoma lanceolatum* Pall.) on the metamorphosis of axolotl. *Zool. Polon.*, Vol. 6, pp. 3–19.

SOKÓLSKA, J., 1931. Contribution à l'histologie de l'endostyle des ascides. *Folia Morph., Warsz.* T. 3, pp. 25–34.

SPAUL, E. A., 1928. Comparative studies of accelerated amphibian metamorphosis. *J. exp. Biol.*, Vol. 5, pp. 212–32.

THOMAS, I. M., 1956. The accumulation of radioactive iodine by *Amphioxus*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 203–10.

EXPLANATION OF PLATE I

Fig. 1. Scene in the pharynx of *Ciona*, showing, in transverse section, part of an unidentified arthropod to the left, a food cord to the right, and dorsal languets above. Azan.

Fig. 2. Autoradiograph of an adjacent section. Iodine is present in the exoskeleton of the arthropod, in the food cord, and over the ciliated lateral borders of the dorsal languets (see also figs. 9 and 10).

Fig. 3. Section through part of a colony of *Botryllus* which is growing upon the surface of the test of a *Ciona*. The cuticle of the former is at the top left and that of the latter at the bottom right. PAS.

Fig. 4. Autoradiograph of an adjacent section. Iodine is conspicuously concentrated in the cuticle of *Ciona* (extending across the bottom of the photograph) and is also present to a less extent in that of *Botryllus* (top left).

Fig. 5. Upper part of the right side of the endostyle of *Ciona* (compare Text-fig. 1). The upper part of zone 6 is at the bottom right, zone 7 is above it, and zone 8 forms a crescent to the left; the pharyngeal epithelium extends from the latter upwards and to the right. A small mass of secretion lies in the mouth of the endostyle towards the bottom left corner. PAS.

Fig. 6. Autoradiograph of an adjacent section. Iodine is present over zones 7 and 8, and in the small mass of secretion at the bottom left-hand corner. At the top of the crescent formed by zone 8 there is some indication of the concentration of iodine at the surface of this epithelium (see text).

Fig. 7. Food cord in the intestine of *Ciona*. The dense pharyngeal component, with exogenous material, extends to the left, and is sharply distinguished from the clear intestinal secretion which lies to the right. Azan.

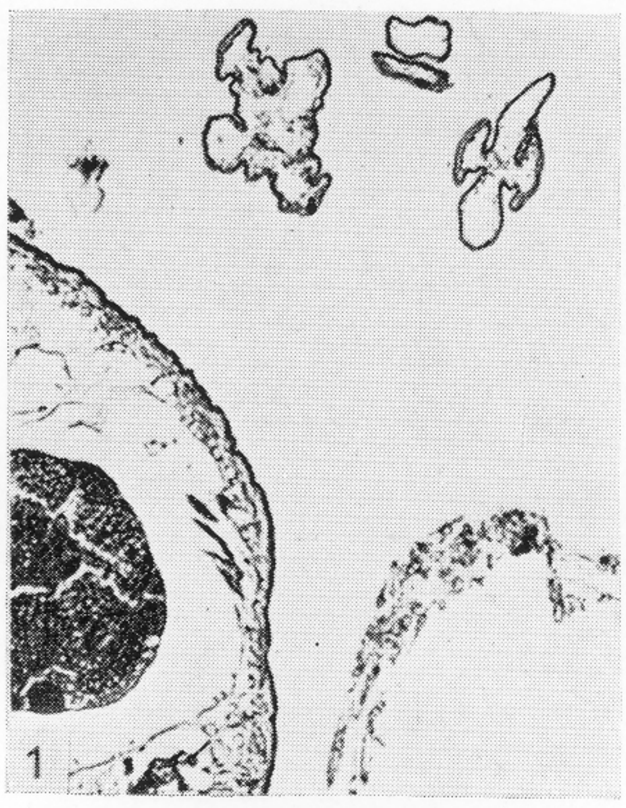
Fig. 8. Autoradiograph of an adjacent section. Iodine is conspicuous in the pharyngeal component and is absent from the intestinal secretion.

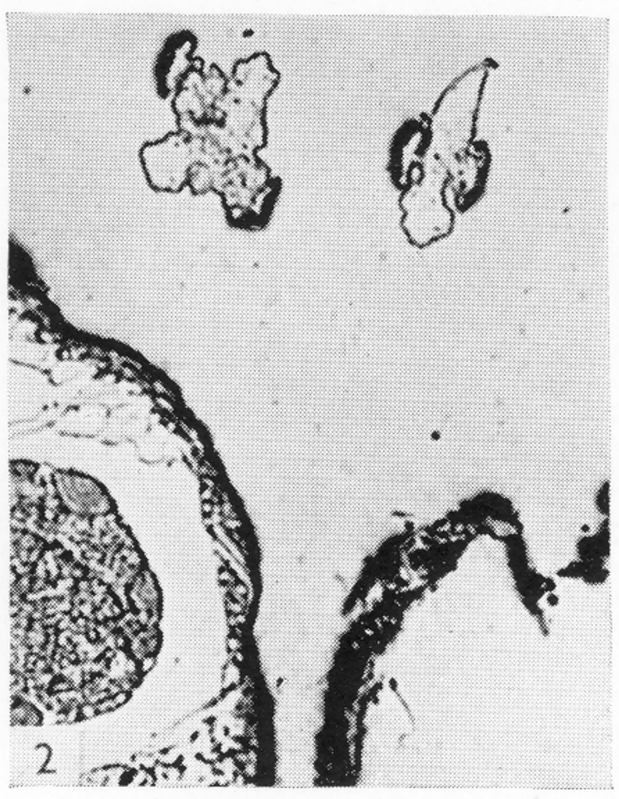
Fig. 9. Lateral border of a dorsal languet; a small amount of secretion overlies the columnar cells, and is associated with their cilia. PAS.

Fig. 10. Autoradiograph of an adjacent section. Iodine is concentrated over the surface of the ciliated columnar epithelium (see text), but is absent from the unciliated cubical epithelium below. The dense image at the top centre is associated with secretion overlying the cells (compare fig. 9, in which some of this secretion is visible).

Fig. 11. Autoradiograph of zone 7 (below) and zone 8 (in the upper third of the photograph). Iodine is equally distributed over the former, but is concentrated at the surface of the latter (see text, and compare figs. 6 and 10).

Fig. 12. Zone 7 epithelium. Thyroidal granules are in focus at intervals; see particularly the large dark granule in the lighter area where the epithelium is bending to the left in the upper part of the photograph. PAS.





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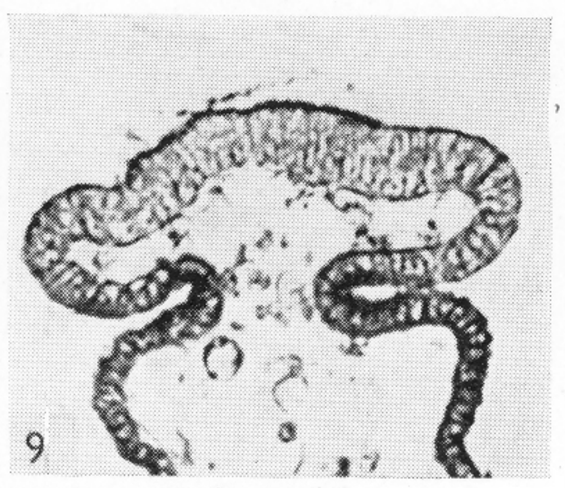

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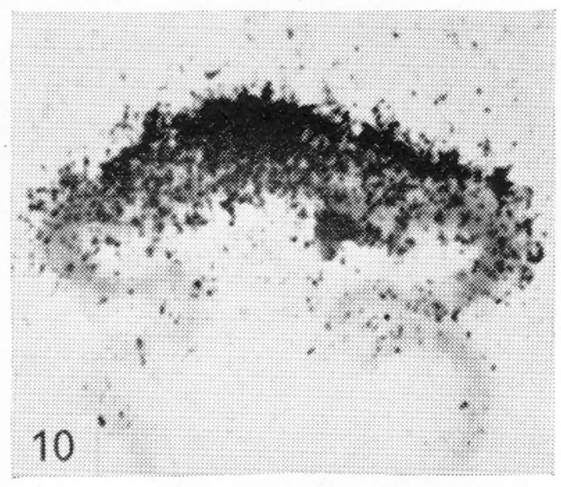

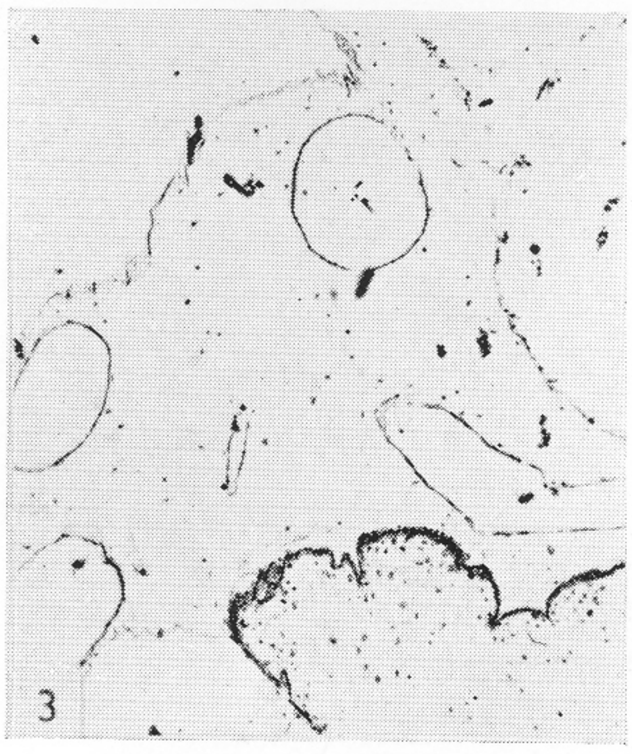

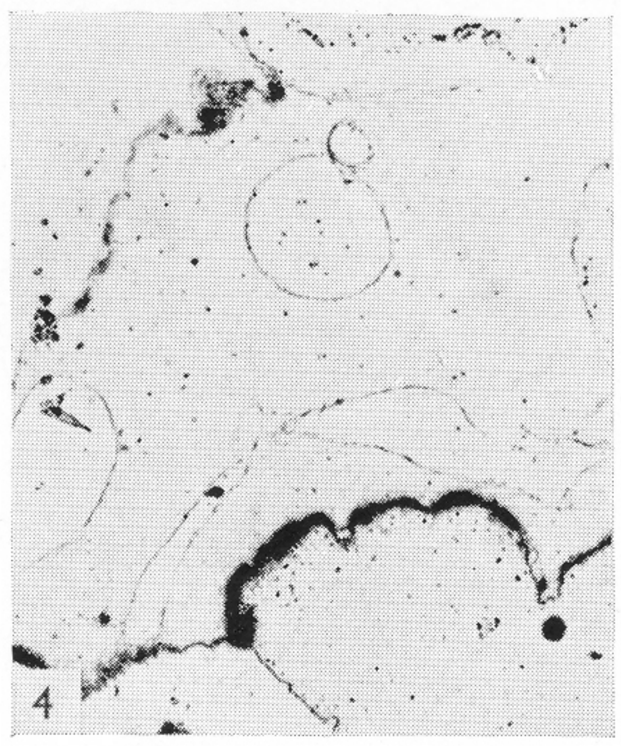

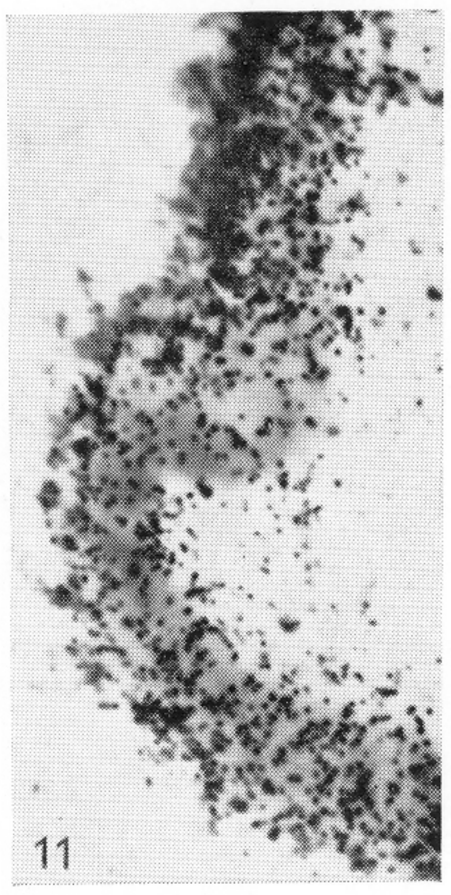

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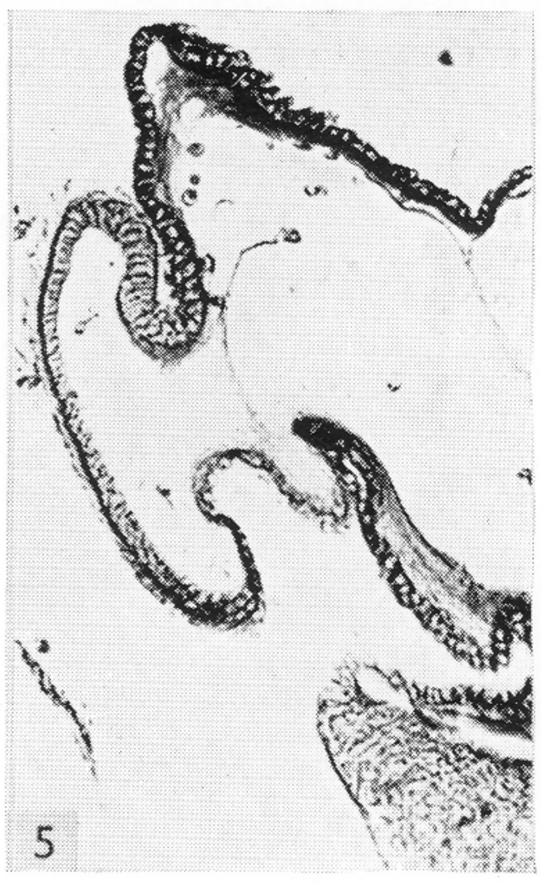

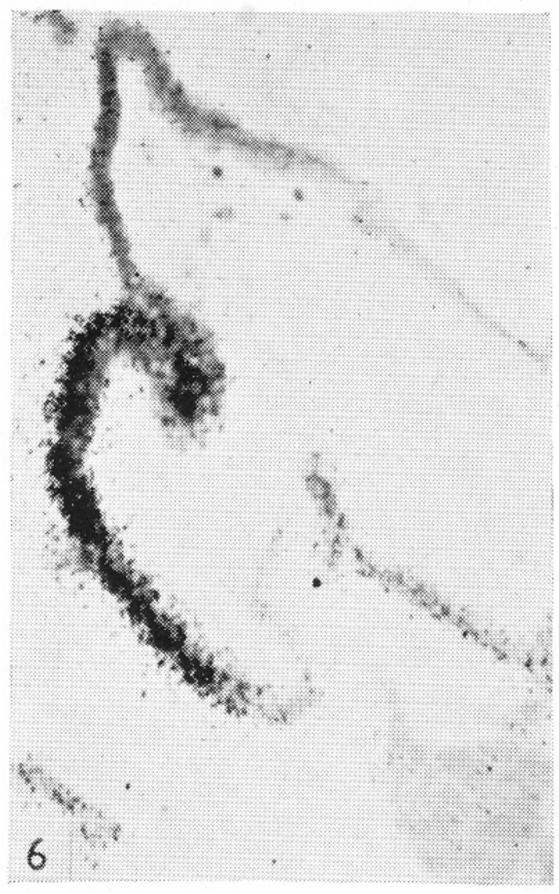

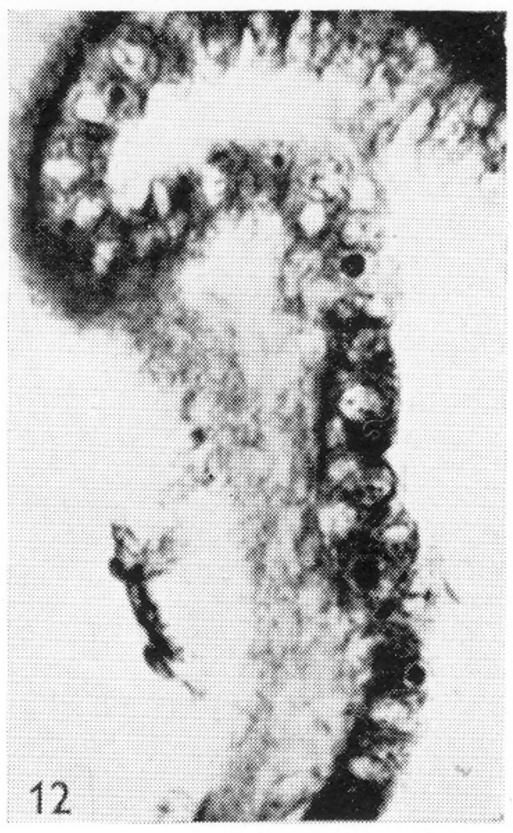

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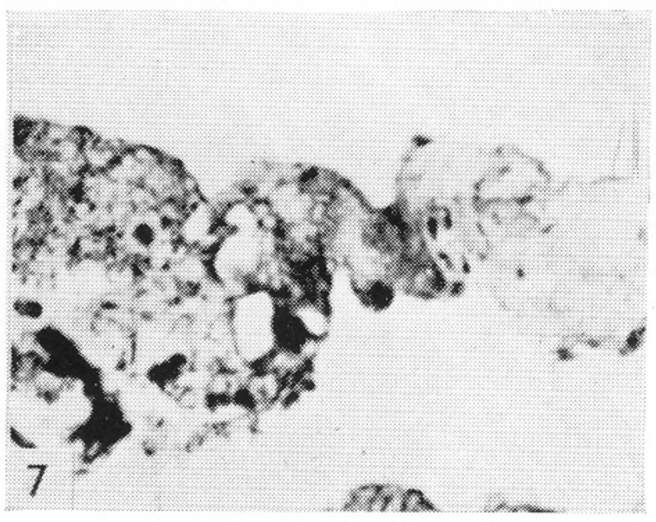

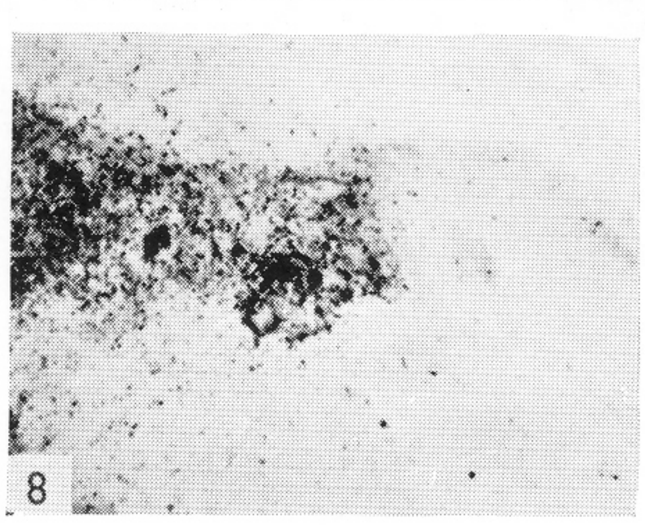

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A NEW QUANTITATIVE PLANKTON NET

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(Text-figs. 1–6)

INTRODUCTION

In the study of the production of organic matter in the ocean, it is necessary to find out not only how quickly organic matter is produced, but also how the material is distributed in the various stages of the organic cycle.

Ignoring for the moment difficulties created by the patchiness of plankton distribution, it is possible by taking a suitably sized sample of sea water to obtain a fairly representative sample of small organisms such as the unicellular algae. With the larger organisms constituting the zooplankton, the problem of sampling becomes more complex. Very few of the planktonic animals are small enough or abundant enough to be sampled adequately by a sample of water, and it is necessary to resort to the use of nets, filtering a large quantity of water, to obtain sufficient numbers of organisms. Further complications are introduced by the immense size range of the zooplankton organisms, some of the smaller ones being even smaller than some of the larger phytoplankton, while there is also the fact that some of the larger ones are apparently quite capable of evading capture by their greater powers of swimming.

The present work was aimed at studying the distribution and fluctuations of the biomass of the zooplankton. Although it was realized that no one sampling device could meet all the difficulties involved and give an accurate picture of the zooplankton as a whole, it was hoped that some single means might be found for sampling the bulk organisms of the zooplankton, and thereby collect material from which some valid inferences might be drawn with minor reservations as to the more active forms.

Furthermore, it was hoped to extend this study into both polar and tropical waters, and this also impinged on the method of sampling adopted. It is a well-known fact that organisms filling the same ecological niches are generally of much larger size in cold polar waters than in warmer waters. As an illustration of this, in temperate surface waters an averaged size Chaetognath, say *Sagitta enflata*, may reach a length of about 20 mm, whereas in the Antarctic the size of a similar creature, *Sagitta gazellae*, may be about 80–90 mm. This point was rather important if the studies made in these different regions were to be comparable.

As most of the work was designed to take place in oceanic waters, another

requirement was some form of closing device to enable the different water layers to be sampled.

The final and principal requirement for the investigation was to be able to relate the catch of the net to the actual amount of water from which it was filtered.

It was evident that no existing net could fulfil all of these requirements. The Clarke–Bumpus net (Clarke & Bumpus, 1950) was probably the nearest answer, but it was obviously too small to cope with the larger organisms, and also suffered the disadvantage of being designed for oblique or horizontal hauls. The practical difficulties of using an oblique net in deep water are rather great, especially as its operation requires so much time. Moreover, a vertical haul has the special advantage that it can conveniently be dovetailed with other work. So far as we are aware no suitable vertical net was in existence to satisfy our requirements, and it appeared to us, therefore, that a new piece of equipment would have to be designed.

From the results of expeditionary work in the past, it is apparent that the vertically hauled Nansen net (Künne, 1929) is a remarkably good sampling device. As it is only 70 cm diameter at the mouth, it obviously cannot be expected to sample such large and active organisms as *Euphausia superba* but, nevertheless, towed at a speed of 1 m/sec (2 knots) it is an adequate sampler of the normal bulk organisms of the zooplankton. Furthermore, the net has been used extensively in various parts of the world, and seems to produce reasonably comparable samples from a wide variety of regions.

The main disadvantages of the Nansen net are first, that there is no device to determine how much water it filters and, secondly, even if this were known, the mesh of the net is of two different sizes, namely 40 meshes per linear inch in the forepart of the net and 74 m.p.i. in the after part, and it would be impossible to tell how much water went through each mesh section.

The obvious course seemed to be to adapt the Nansen net so that it would be suitable for quantitative work, but it was clearly advantageous to retain in any altered version as many as possible of the desirable features of the Nansen net.

The first question of mesh size had to be settled experimentally. First, the front French netting section was replaced with canvas and then in consideration of Wiborg's (1948) findings relating to the advantages of certain meshes, various meshes were tried in the filtering part of the net. Eventually a size of 74 m.p.i. throughout was chosen as being the the best compromise between filtering capacity and smallness of organisms taken. The size of aperture of this silk is similar to that of no. 8 standard grade Dufour bolting silk, and in the dry silk averages 200 μ while in the wet silk it shrinks slightly to between 160 and 180 μ.

The next, and most important point, was the measurement of water flow through the net. This was particularly desirable for the deeper hauls where

there was no indication of the angle at which the net may be hauled up through the different water layers (that is when the drift of the ship causes the wire to stray from the vertical). It was intended that the net would be closed by the Nansen (1915) method, and so it was also desirable that the actual depth of closure might be known. It was evident that to suit the purpose, no available flowmeter existed. What was really required was a meter capable of showing the performance of the net and depth at which it fished. In other words, a meter which could give a graph of the amount of water filtered plotted against depth. The advantages of such a device would be enormous. Any clogging of the net by superabundance of plankton would at once be apparent and the depth at which it occurred would be known. The angle of hauling of the net would not matter since the actual volume filtered between two levels could be determined, and also any irregularity of performance of the net would be obvious.

Even if such a meter were designed, how was it to be fitted in the net? On hydrodynamic considerations it appeared most reasonable to measure the water after it had come through the net. To this end some trials were made. A net was enveloped in a cylindrical canvas sleeve and the meter placed behind the net. This apparatus proved quite unmanageable, as it sank so slowly through the water. The idea had to be abandoned and the meter was then mounted in the mouth of the net where it functioned excellently. The closing problem was later overcome by mounting the meter in a brass drum between a front canvas sleeve and the silk filtering part of the net. The net could then be closed in the normal manner by the Nansen method, in front of the meter.

Some 160 hauls have been made in all types of weather with this apparatus and so far the results have been extremely satisfactory.

THE DEPTH-FLOWMETER

The depth-flowmeter is shown in Figs. 1 and 2. The instrument consists basically of a flowmeter, similar in many of its features to that described by Harvey (1934), in which has been incorporated a depth-recording unit which, like the depth gauges of Hermann (1949) and others, utilizes a Bourdon tube. Flow is measured by the number of revolutions of a smoked glass cylinder which is marked by a stylus, while depth is measured by the vertical displacement of the stylus arm.

Water flowing into the net turns a six-bladed rotor, and through a double worm gear train, giving a 100:1 reduction, the rotor drives a slotted metal barrel mounted vertically in a plain bearing. Both the vertical rotor shaft and the horizontal shaft are adjusted to run freely on hardened stainless steel points set in jewel cups. The blades of the rotor are set at an angle of 45° and the whole is finely balanced.

The smoked glass cylinders, on which the trace of depth and flow is marked by the phosphor-bronze point of the stylus arm, are a push fit on the metal barrel. The stylus arm is connected through a twin strip flexure system (Geary, 1954) made of beryllium-copper to the free end of the Bourdon tube. The fixed end of the tube, which is sealed with a brass plug, is securely mounted on

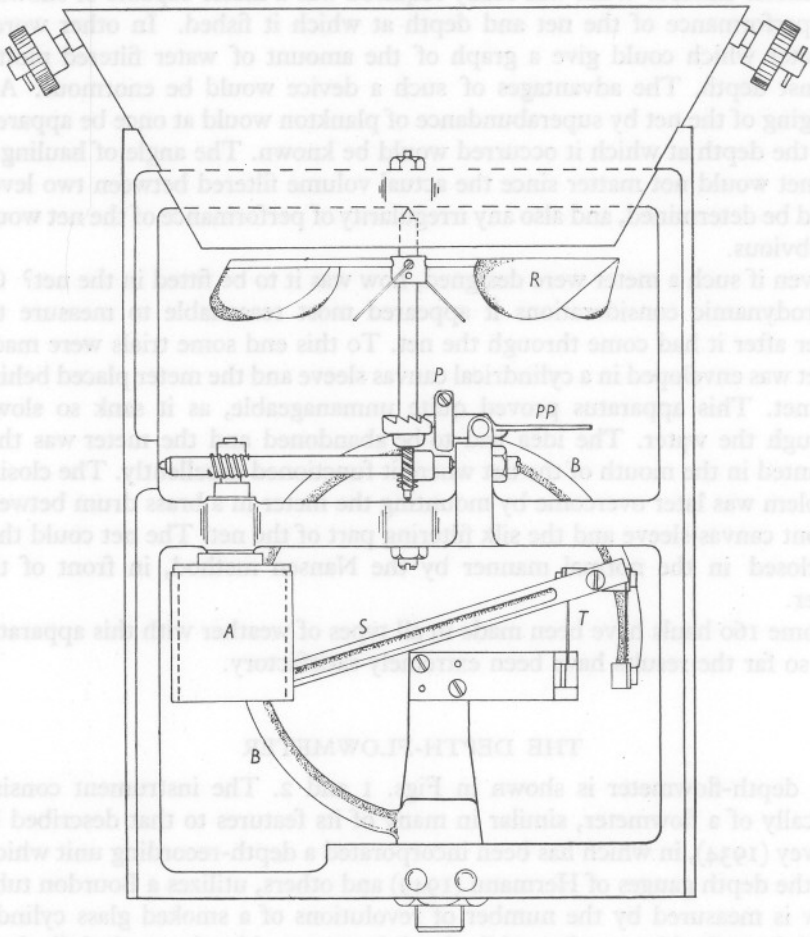

Fig. 1. The depth-flowmeter, one half actual size. *A*, smoked glass cylinder; *B*, Bourdon tube; *P*, pawl and ratchet mechanism; *PP*, pawl plate; *R*, rotor blade; *S*, stylus arm; *T*, twin strip flexure system.

the bottom bracket of the cast gun-metal meter frame. The Bourdon tube is sealed at normal atmospheric pressure so that increase of external pressure (with increased depth) causes the arc of the tube to decrease, resulting in a movement of the free end of the tube which produces a displacement of

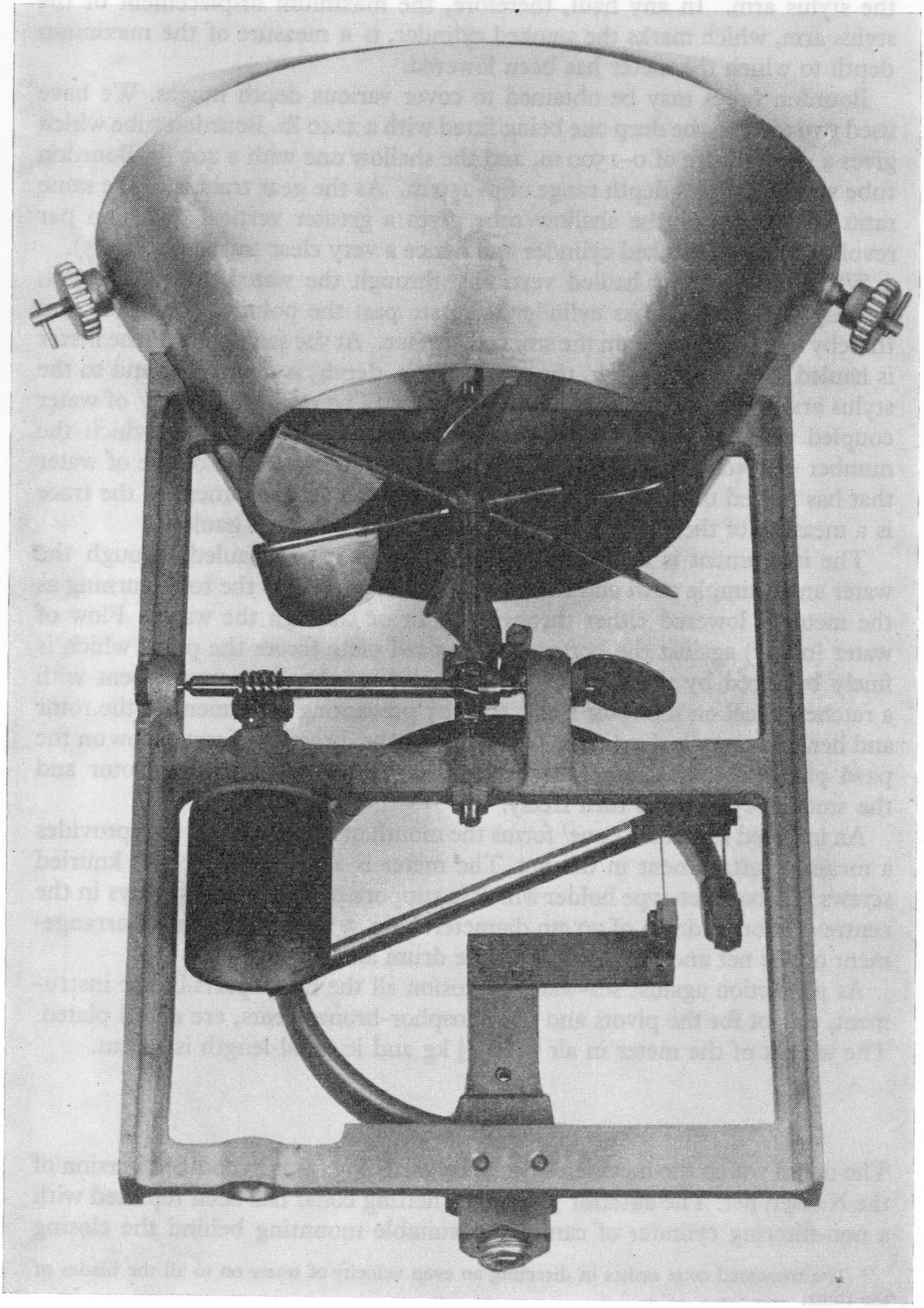

Fig. 2. The depth-flowmeter.

the stylus arm. In any haul, therefore, the maximum displacement of the stylus arm, which marks the smoked cylinder, is a measure of the maximum depth to which the meter has been lowered.

Bourdon tubes may be obtained to cover various depth ranges. We have used two meters, the deep one being fitted with a 2240 lb. Bourdon tube which gives a depth range of 0–1500 m, and the shallow one with a 400 lb. Bourdon tube which covers a depth range of 0–250 m. As the gear train is of the same ratio in each meter the shallow tube gives a greater vertical deflexion per revolution of the smoked cylinder and hence a very clear trace (see Fig. 5).

When the meter is hauled vertically through the water the rotor turns, causing the smoked glass cylinder to rotate past the point of the stylus arm thereby marking a trace on the smoked surface. At the same time as the meter is hauled through the water, the pressure (i.e. depth) is decreasing and so the stylus arm moves in an arc along the cylinder. The resultant of flow of water coupled with change in depth thus produces a helical trace in which the number of revolutions of the cylinder is proportional to the volume of water that has flowed through the meter, and the vertical displacement of the trace is a measure of the depth from which the meter has been hauled.

The instrument is designed to operate only as it is hauled through the water and a simple pawl and ratchet mechanism prevents the rotor turning as the meter is lowered either through the air or through the water. Flow of water (or air) against the bottom of the pawl plate forces the pawl, which is finely balanced by the position of a counter-weight, into engagement with a ratchet wheel on the rotor shaft, thereby preventing movement of the rotor and hence the smoked cylinder. On hauling, the direction of water flow on the pawl plate is reversed and the ratchet disengaged, allowing the rotor and the smoked cylinder to turn freely.

An inverted truncated cone[1] forms the mouth of the meter and also provides a means of attachment in the net. The meter is secured by the two knurled screws in a bayonet-type holder which is supported on three brass stays in the centre of a brass drum of 70 cm diameter. Fig. 3 shows the general arrangement of the net and the position of the drum and meter.

As protection against sea-water corrosion all the metal parts of the instrument, except for the pivots and the phosphor-bronze gears, are nickel plated. The weight of the meter in air is *ca* $1\frac{1}{3}$ kg and its total length is 24 cm.

THE NET

The net in which the flowmeter has been used (Fig. 3) is a modified version of the Nansen net. The anterior $\frac{1}{4}$ in. mesh netting collar has been replaced with a non-filtering cylinder of canvas. A suitable mounting behind the closing

[1] The truncated cone assists in directing an even velocity of water on to all the blades of the rotor.

point was inserted to take the flowmeter, and the graded mesh of the filtering part of the Nansen net has been restricted to the one mesh throughout.

The flowmeter net has a mouth diameter of 70 cm. Attached to the net ring is a cylindrical tube of canvas 4 ft. (122 cm) in length, around the lower end of which is sewn a piece of log line. This canvas tube slips over a brass drum, 70 cm diameter and 1 ft (30·5cm) in length, which carries the flowmeter, and the canvas is held on to the drum by a large metal band with a screw clamp which grips it immediately above the log line.

A similar fixture is provided for the attachment of the filtering part of the net at the lower end of the brass drum. The silk netting is sewn into a 6 in. (15 cm) wide band of canvas with a log line sewn around the top, and the first silk section is a 1 m cylindrical length of 74-mesh per linear inch bolting silk. At its after end it joins the second silk section which is a $1\frac{1}{2}$ m length of the same grade of silk tapering conically from a diameter of 70 cm to a diameter of 8 cm, where it joins a 3 in. (7·5 cm) wide canvas band that forms the attachment to the bucket. The bucket is of the type used with the 'Discovery' net, the N 70 V (Kemp, Hardy and Mackintosh, 1929).

The net is towed vertically by three bridles which are $\frac{1}{4}$ in. thick brass rods, 32 in. (81 cm) long joining together in a ring which fits into the release gear.

Supporting the flowmeter drum, there are three 3 mm phosphor bronze stays with brass eyes in the ends which are shackled to the net ring and to the flowmeter drum. These stays are seized to three of the six brass rings which are sewn around the anterior canvas collar, about 20 in. (50 cm) behind the net ring, to carry the throttling rope.

Three further stays of the same wire extend from the flowmeter drum down the whole length of the net. They are joined to the collar above the net bucket, and continue for about 3 ft. (92 cm) below the bucket to support the weight. A stout brass ring, 7 in. (17·8 cm) in diameter, encircles the net above the bucket, and is seized on to the three stays, to prevent their twisting.

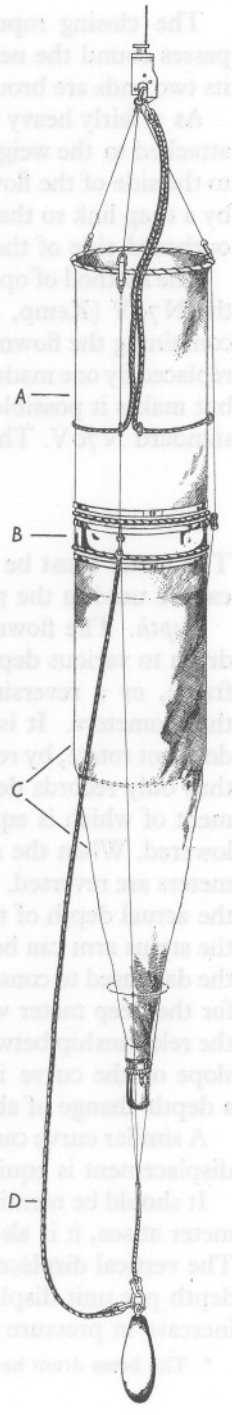

Fig. 3. The flowmeter net. *A*, canvas fore section encircled by closing rope; *B*, brass drum containing flowmeter; *C*, silk filtering section; *D*, handling line for weight.

The closing rope is a 16½ ft. (5·03 m) length of 2 in. (5 cm) rope. It passes round the net through the rings on the canvas collar and stay wires; its two ends are brought up and shackled to the closing mechanism.

As a fairly heavy weight (20–40 lb., 10–20 kg) is used, an additional line is attached to the weight to facilitate its handling at the surface. It is shackled to the side of the flowmeter drum. The three stays are attached to the weight by a snap link so that the weight can be detached easily and hooked up on the outboard side of the ship's rail.

The method of operation of this net is similar to that of the 'Discovery' net, the N70V (Kemp, Hardy & Mackintosh, 1929). Although the brass drum containing the flowmeter is somewhat bulky and heavy and may profitably be replaced by one made of fibre glass,* it not only affords protection to the meter, but makes it possible to pay out the net much faster than is possible with the standard N70V. This is a convenient saving of time on very deep hauls.

CALIBRATION OF THE METER

The meter must be calibrated both for depth and for flow of water before it can be used in the plankton net.

Depth. The flowmeter may be calibrated at sea by lowering it in the brass drum to various depths, having on the wire above it a reversing thermometer frame, or a reversing water-bottle, fitted with protected and unprotected thermometers. It is necessary to immobilize the smoked cylinder so that it does not rotate, by removing either the horizontal shaft or the rotor. The meter thus only records depth, giving a slightly curved trace, the vertical displacement of which is equal to the maximum depth to which the meter has been lowered. When the requisite amount of wire has been paid out, the thermometers are reversed. From the thermometer readings it is possible to calculate the actual depth of the meter and from the trace the vertical displacement of the stylus arm can be measured. The meter is lowered to different depths and the data used to construct a depth calibration curve. Fig. 4 shows such a curve for the deep meter which has been in use since 1954. As would be expected the relationship between depth and stylus displacement is linear, and from the slope of the curve it is seen that 1 mm stylus displacement is equivalent to a depth change of about 50 m.

A similar curve can be constructed for the shallow meter where 1 mm stylus displacement is equivalent to 7·5 m.

It should be mentioned that although it has been our practice to calibrate the meter at sea, it is also possible to do the calibration ashore in a pressure tank. The vertical displacement at different pressures gives a curve from which the depth per unit displacement can be calculated using the relationship that an increase in pressure of 1 atmosphere is equivalent to an increase in depth of

* The brass drum has since been replaced by one made of anodized aluminium alloy.

about 10 m. Although this is a convenient method it is important to be able to check any calibration from time to time, particularly if any repairs or adjustments have been made to the instrument. Reversing thermometers provide a convenient and accurate method of doing this in the field.

From experiments which have been performed in a pressure tank, it has been found that the accuracy of the depth reading from the flowmeter is limited, not by the accuracy of the Bourdon tubes, but rather by the accuracy with which the trace can be read. Under normal conditions with a clear trace we find this limit is $\pm 0{\cdot}25$ mm, which corresponds to a depth of ± 2 m with the shallow pattern flowmeter and ± 15 m with the deep pattern flowmeter.

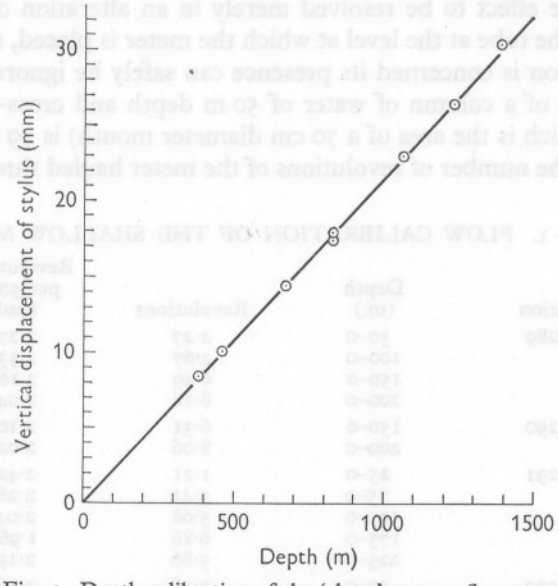

Fig. 4. Depth calibration of the 'deep' pattern flowmeter.

Flow. For similar reasons it is important to be able to calibrate the meter for flow of water at sea. The most practical way of doing this is to haul the meter, fitted in the brass drum with canvas forepart attached—but without the silk filtering section—vertically through a column of water of known length at a constant speed of 1 m/sec. It is then possible to relate the number of revolutions of the smoked cylinder to the flow past the meter of a volume of water equal to the length of the column times the area of the mouth of the net. This assumes that the axial velocity of the water (1 m/sec) is constant across the diameter of the canvas tube. This is not strictly true because the roughness of the walls of the tube (canvas and brass) exerts a drag on the water which results in a loss of velocity near the walls. For a canvas tube whose length is at least 40 times as great as its diameter it can be calculated (Goldstein, 1950) that the overall loss in the volume of water passing through the tube may be as

great as 10%. In the case of the flowmeter net, however, the total length of the canvas tube and the brass drum is only about twice the diameter, and although no definite figure can be given it seems probable that the loss will be less than 2–3% and that it can safely be ignored. Furthermore, although the meter itself offers little resistance to the passage of water, it undoubtedly must modify the flow pattern to some extent. It is unlikely, however, that this will have a significant effect on the calibration. Both the net hauls and calibration are done with the meter in the same position and so it is only the absolute value of the calibration that the presence of the meter could affect. It is considered, however, that the meter lies sufficiently far behind the mouth of the net, for any obstructive effect to be resolved merely in an alteration of the velocity profile across the tube at the level at which the meter is placed, and that as far as the calibration is concerned its presence can safely be ignored.

The volume of a column of water of 50 m depth and cross-sectional area of 0·38 m^2 (which is the area of a 70 cm diameter mouth) is 19 m^3 and this is equivalent to the number of revolutions of the meter hauled through the same depth.

TABLE 1. FLOW CALIBRATION OF THE SHALLOW METER

Station	Depth (m.)	Revolutions	Revolutions per 50 m haul
3289	50–0	2·27	2·27
	100–0	4·67	2·33
	150–0	6·49	2·16
	200–0	8·16	2·04
3290	150–0	6·31	2·10
	200–0	8·06	2·02
3291	25–0	1·21	2·42
	75–0	3·41	2·28
	125–0	5·08	2·04
	175–0	6·86	1·96
	225–0	9·86	2·19
3292	50–0	2·16	2·16
	100–0	4·06	2·03

Mean revolutions per 50 m haul = 2·15

The results of thirteen calibration hauls made with the shallow meter are given in Table 1. The result of each haul has been expressed as the number of revolutions per standard haul of 50 m, and the calibration value is taken as the mean number of revolutions per 50 m haul. It is seen from Table I that the mean revolutions per 50 m haul is 2·15; which is equivalent to a flow of 19 m^3 of water.

THE PREPARATION OF THE SMOKED CYLINDERS AND TREATMENT OF THE TRACES

The glass cylinders on which the trace is recorded are made from precision-bored glass tubing of 28 mm bore, 2 mm wall thickness and 35 mm length. These cylinders cost about 1*s*. each but they can be used time and time again.

The problem of storing many smoked cylinders, as is the practice with bathythermograph slides, makes it more convenient to smoke the requisite number of cylinders just prior to use. The method is basically the same as that used for bathythermograph slides except that the cylinder is rotated in the smoky flame, and the deposit of carbon is made rather heavy. The procedure will be described in detail because it is essential to have a uniform deposit of soot which will not wash off as the meter is hauled through the water.

(1) If the cylinder has been used before, it is washed first in acetone to remove the cellulose varnish (see below) and secondly in carbon tetrachloride and then dried with a clean rag.

(2) A very thin even film of tallow (vaseline or petroleum jelly will also do) is applied to the outer surface of the cylinder.

(3) The cylinder is rotated at about 30 rev/min in the smoky part of a spirit flame, the spirit lamp being filled with a 15% solution of cellulose thinner in methylated spirit. A cylinder-holder geared to a small electric motor has proved particularly suitable for rotating the glass cylinder.

(4) Smoking is continued until the deposit is a fairly dense even black, which gives the best trace for photographic reproduction.

(5) After use the trace is preserved by first washing the cylinder in tap water, drying it, and then dipping it in clear cellulose varnish and drying again.

For ease of storage and also to facilitate the measurement of the flow and depth, a photographic record is then made of the trace. This is simply and quickly done by wrapping a piece of bromide photographic paper round the cylinder which is then illuminated from the inside. Even illumination is ensured by slipping the cylinder over a piece of Perspex rod which is slightly roughened on its surface. A torch bulb shines into the highly polished end of the rod. The resulting photograph, which is a reverse image of the trace, can be analysed and stored as a record of the haul, and the cylinder can be used again.

DESCRIPTION OF THE TRACES

The prototype flowmeter was first taken to sea in September 1954, and it gave most encouraging results. Only minor modifications were necessary, principally to improve the method of attachment of the meter inside the net, and since then it has been used successfully on four short cruises of the R.R.S. *Discovery II*. In all, about 160 hauls have been made, of which only three have yielded traces which were unreadable. The only major repair which has been necessary was the replacement of two jewelled cup bearings.

It has already been noted that the spiral traces obtained on the smoked-glass cylinders are photographed on to a piece of bromide paper wrapped round the outside of the cylinder. Fig. 5 shows a typical series of these photographic records of the traces. It will be clear from the manner in which they are made

that the oblique lines in those figures represent one long continuous spiral. The traces which are shown for the 50–0, 100–50 and 200–100 m hauls were made with the shallow flowmeter, while those for the 500–200 and 1000–500 m were made with the deep pattern of flowmeter.

To understand the significance of the traces, let us first consider the one representing the 200–100 m haul at station 3312 (Fig. 5). The top of the trace represents the sea surface, and descending across the trace there is a gently

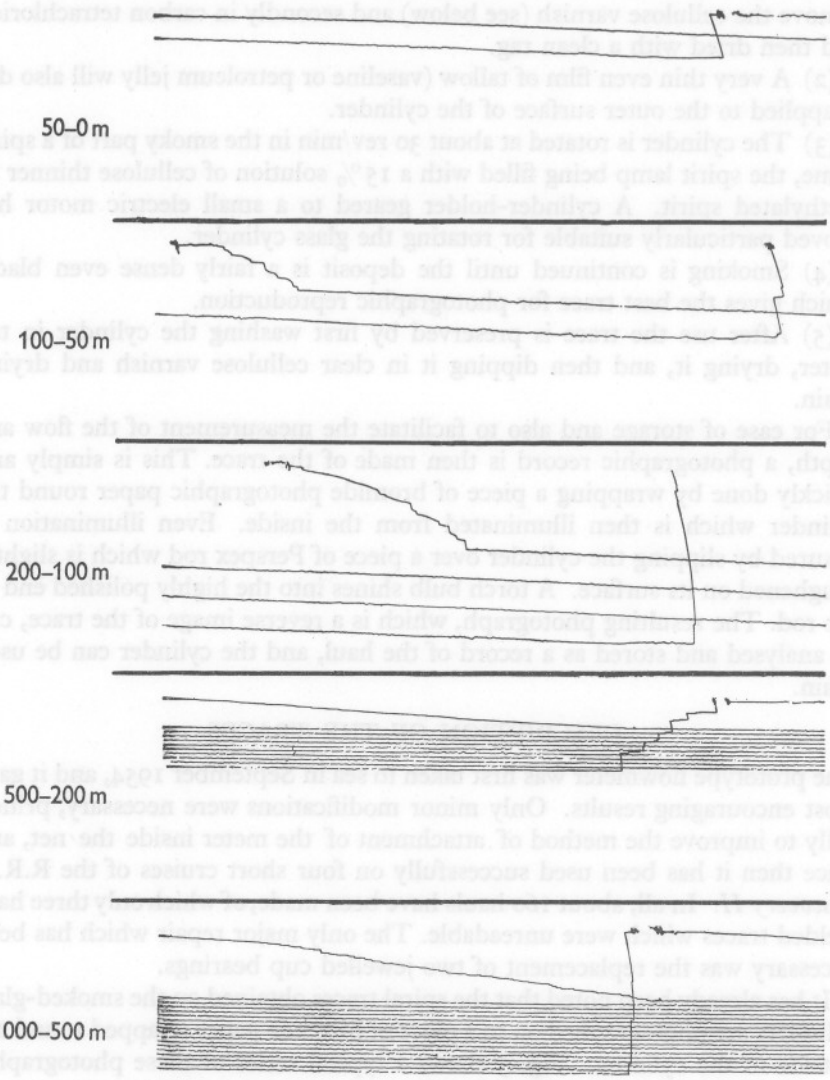

Fig. 5. Flowmeter traces, from hauls made at station 3312. ×0·86.

curved vertical line. This is the arc made by the stylus as the net was paid out. At this station the weather was very calm, and the net apparently went straight down without any surging which would be reflected in a rotation of the glass cylinder. From the lowest point of the trace, hauling was started, and the gently rising oblique line is formed as the glass cylinder is slowly turned by the rotor and the stylus rises with the decreasing pressure. In this particular trace, three complete rotations of the glass cylinder took place, and part way through the fourth revolution the net was closed and filtration stopped. The trace then rises upwards to begin with and then moves over to the left slightly as the closed net comes up to the surface. Theoretically the trace should have gone straight up to the surface after closure. This small amount of 'apparent filtration' which occurs after the net is closed is probably the result of a slight percolation of water through the canvas which is closing the net above the flowmeter. There must be quite a considerable pressure on this canvas as the closed net is hauled upwards, and no doubt a small amount of water is forced through the weave of the canvas.

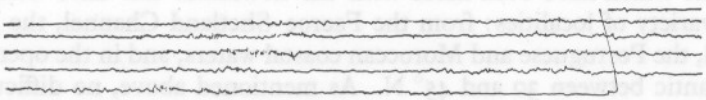

Fig. 6. Flowmeter trace from 100–0 m haul at station 3290, showing the effect of stray on the wire in the latter part of the haul.

From this explanation it will be easy to see what the other traces in Fig. 5 represent. The spiral trace made by the deep pattern flowmeter is of course much closer than that of the shallow version, since the vertical displacement of the stylus per unit of depth is not so great, but it is still quite clear to read. On most occasions it will be seen that there appears to be a certain amount of filtration when the net is lowered down. This is clearly seen, for example, in the 500–200 m trace in Fig. 5. Instead of the straight descending line as in the 200–100 m trace, there is a step-ladder effect on the 500–200 m trace. This is probably caused by water surging in and out of the mouth of the net since the rate of lowering cannot be adjusted to the movements of the ship. The authors observed this taking place under water and photographed it in the course of making a film of the closing of the Nansen net. It is unlikely that the net actually filters any appreciable quantity of water as it makes these surges, and if it did, anything it caught would be promptly washed out again.

A certain amount of short-period vibration can be seen in the traces, and this is probably caused by vibration of the stylus arm as the water flows over it.

So far, the hauls with the flowmeter net have been made on board the R.R.S. *Discovery II*, a ship which normally works stations hove to, into the wind. Consequently, the wire to the net is usually fairly near the vertical, all

the time. At one or two stations, however, the ship has fallen off the wind in the middle of a haul with the result that the wire to the net has acquired a considerable angle of stray, and an examination of the traces from the flowmeter on these occasions has shown that the stray is reflected in the amount of water filtered by the net. In the 100–0 m haul at station 3290 a close inspection of the flowmeter trace (Fig. 6) shows that the spacing of the oblique lines becomes less nearer the surface. In other words, the amount of water filtered per unit depth near the surface was greater than the amount filtered per unit of depth, earlier in the haul. During this haul the wire took on a stray partway through the haul. Obviously on such occasions the net is towed through the water as well as being hauled, and it filters more water per unit depth than it would in a truly vertical haul.

So far no traces have given any indication of reduction of filtration on account of clogging of the net by phytoplankton.

DISCUSSION

The hauls which have been made with the flowmeter net have come from a wide variety of localities: from the Faeroe–Shetland Channel, the English Channel, the Portuguese and Moroccan coastal waters, and in the open north-east Atlantic between 30 and 45° N. As mentioned above, no difficulty has been experienced with the net clogging, although many hauls have been made in waters visibly discoloured by the density of the phytoplankton, and also in areas of dense salp concentrations.

With the entire assembly of net and meter the measured flow of water, as indicated by the number of revolutions of the smoked cylinder, has been found to be only slightly less than the flow observed when the meter is hauled in the brass drum without the net attached, i.e. as in the calibration hauls (see p. 25). For instance the mean revolutions per 50 m haul for twelve shallow hauls with the complete net was found to be 1·96 compared with 2·15 given by the results in Table I. This difference in flow represents that part of the theoretical column of water that is prevented from flowing into the apparatus by the back pressure created by the presence of the silk netting, and can thus be used as a measure of the filtering efficiency of the net. Using the values quoted above we find that the filtration coefficient of the net when hauled at 1 m/sec has the surprisingly high value of about 90%. This is, of course, on the fairly reasonable assumption that the presence of the net does not appreciably alter the pattern of flow that pertains in the drum during the calibration (i.e. when the net is absent). One point which no doubt contributes towards the high filtration coefficient is that the total length of the silk part of the net is $2\frac{1}{2}$ m, and as the first section is cylindrical the net has a very high ratio of filtering surface to mouth area.

As the filtration coefficient of the net is so high there will be little or no frontal wave preceding the net; this fact must add to the ability of the net to

capture more active organisms: undoubtedly there will be less warning of approach of the net to them. The hauls which have been examined appear to take a very good representative sample of the bulk of the zooplankton. For a vertical net, the samples are relatively large, and this, of course, is clearly an advantage in attaining a reasonable degree of accuracy in their subsequent treatment.

The main advantage of the net, however, is its ability to sample different water layers in a quantitative manner. That this is essential in the study of the standing crop in the ocean is borne out by Foxton's (1956) results. These show that the extent of the seasonal change in the standing crop of zooplankton in the Antarctic could not have been properly understood without having divided hauls to a depth of at least 750 m available. Recent observations with the flowmeter net also bear out the fact that a large part of the standing crop is to be found in deep water. As an example, at station 3216 (April 1955) in the Faeroe–Shetland Channel, in a depth of 900 m, about 40% of the standing crop lay below 250 m and about 25% below 500 m. It would be possible to quote many more observations in subtropical waters where a similar distribution holds good.

The flowmeter itself may well find more extensive applications. Besides its adaptability to other vertical nets, it may be possible to use the meter on obliquely hauled nets. In this case it would probably only be possible to use the 'shallow pattern', as the spiral trace produced by an oblique tow of the 'deep pattern' meter would no doubt be too compressed to be interpreted accurately. The meter is also a useful tool for determining the filtration coefficients of other nets.

We should like to express our thanks to Mr R. Dobson, who was entirely responsible for producing the design of a working instrument from our inadequate sketches of the flowmeter. The meters were manufactured in the workshop of the National Institute of Oceanography. Mr R. Burt of R.R.S. *Discovery II* carried out all the rigging of the net and contributed many helpful practical ideas. Mr H. Charnock gave us guidance on matters concerning the water flow of the net. We are indebted to Mr P. M. David for the photograph of the depth-flowmeter in fig. 2, and also to Mr A. Style for the perspective drawing of the net in Fig. 3.

SUMMARY

The apparatus described in this paper was constructed for the purpose of sampling the zooplankton standing crop in the ocean in a quantitative manner.

A meter is described, which will record both the depth and amount of water which a net filters as it is hauled vertically through the water. The meter records on a smoked glass cylinder.

The meter is mounted in a brass drum which is positioned in front of the silk filtering section of the net. In front of the meter a long canvas sleeve carries the throttling rope which closes the net by the Nansen method.

Calibration of the meter for depth is done by using reversing thermometers and lowering to a series of suitable depths. Flow calibration is easily accomplished in the field by towing the canvas sleeve and meter without the silk net.

The method of smoking the glass cylinders and taking photographic records of them is described.

From the records it is possible to study the complete behaviour of the net below water—what depth range it has fished, how much clogging occurred, the depth of closure, etc.

REFERENCES

CLARKE, G. L. & BUMPUS, D. F., 1950. The plankton sampler—an instrument for quantitative plankton investigations. *Amer. Soc. Limnol. Oceanogr.*, Special Publication, no. 5. 8 pp.

FOXTON, P., 1956. The distribution of the standing crop of zooplankton in the Southern ocean. '*Discovery*' *Rep.*, Vol. 28, pp. 191–236.

GEARY, P. J., 1954. Flexure Devices. Pivots. Movements. Suspensions. *British Scientific Instrument Research Assoc., Res. Rep.*, M. 18.

GOLDSTEIN, S., 1950. *Modern Developments in Fluid Dynamics.* 2 vols. London: Oxford University Press.

HARVEY, H. W., 1934. Measurement of phytoplankton population. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 761–74.

HERMANN, F., 1949. A recording depth gauge for use in horizontal hauls with stramin nets and other pelagic fishing implements. *Medd. Komm. Havundersøg., Kbh., Ser. Fisk.*, Bd. 11, No. 5.

KEMP, S., HARDY, A. C. & MACKINTOSH, N. A., 1929. Discovery Investigations: Objects, equipment and methods. '*Discovery*' *Rep.*, Vol. 1, pp. 141–232.

KÜNNE, CL., 1929. Vergleich der Fangfähigkeit verschiedener Modelle von plankton-Netzen. *Rapp. Cons. Explor. Mer*, Vol. 59.

NANSEN, F., 1915. Closing nets for vertical hauls and for horizontal towing. *Publ. Circ. Cons. Explor. Mer*, No. 67.

WIBORG, K. F., 1948. Experiments with the Clarke-Bumpus plankton sampler and with a plankton pump in the Lofoten area in northern Norway. *Fiskeridir. Skr. Havundersøk.*, Bd. 2, No. 2.

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NOTES ON *MYTILUS GALLOPROVINCIALIS* LAMARCK IN GREAT BRITAIN

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(Plate I and Text-figs. 1, 2)

Mytilus galloprovincialis Lmk., the 'Mediterranean' mussel, was first reported, as *M. ungulatus* L., from Britain by Donovan (1802), who recorded that several specimens were found in Cornwall (for details of synonymy see below). He noted that it was known at that time as a Mediterranean but not hitherto as a British species. Jeffrey (1863) also recorded *M. ungulatus* L. from Cornwall and the Channel Islands, probably referring to *M. galloprovincialis* Lmk. This author also included a *M. galloprovincialis*, but this was not the mussel referred to in the present paper.

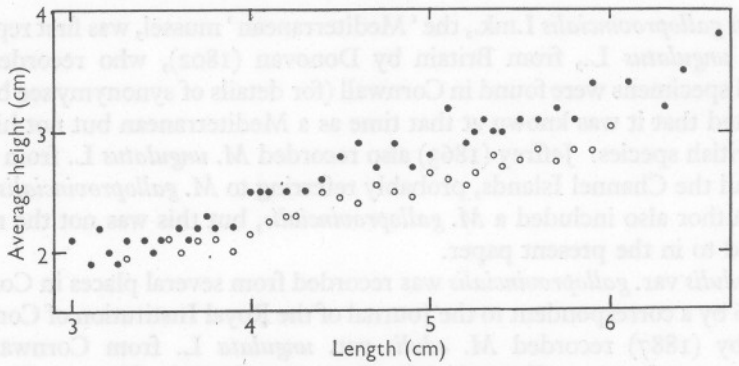
M. edulis var. *galloprovincialis* was recorded from several places in Cornwall in 1866 by a correspondent to the Journal of the Royal Institution of Cornwall. Sowerby (1887) recorded *M. edulis* var. *ungulata* L. from Cornwall and Guernsey and var. *galloprovincialis* from the English Channel. Tregellis (1896) recorded *M. edulis* var. *galloprovincialis* from Par, Falmouth, Helford and Hayle, and var. *ungulata* from Hayle and St Minver (all in Cornwall). Winckworth (1932) included the species in his list of British Marine Mollusca, but gave no locality. Dean recorded *M. galloprovincialis* as 'abundant in Cardiff Docks' (Conchological Soc., 1929), and Gardiner (1945) reported the species as present at Pendine, Carmarthenshire, and St Ives Bay and the Helford River, Cornwall.

In spite of these references to *M. galloprovincialis* in Britain, there seems to be no comprehensive account of the distribution of this species on our shores, or of its abundance relative to our common native mussel *M. edulis* L., and the present paper attempts to remedy these deficiencies.

The lack of information about this species was brought to light in the course of work on the mussel parasite *Mytilicola intestinalis* Steuer, when it was noticed that although the copepod often established a high level of infection in *Mytilus edulis* in north-west Europe, and frequently caused heavy mortalities, such infestations were less frequently reported from the Mediterranean and no disastrous mortalities are recorded. *Mytilicola* has been present in the Mediterranean for over 50 years (Steuer, 1902), but was not reported from north-west Europe until 1937 (Cole, 1951). These facts suggested that the Mediterranean mussel was in some way more resistant to the

parasite than *Mytilus edulis*. If this were so, and if *M. galloprovincialis* would thrive in Britain, it might be of value for restocking mussel beds decimated by *Mytilicola*.

In 1952 Dr H. A. Cole drew my attention to a sample of mussels received from Padstow for bacteriological examination. These mussels differed in many respects from the normal *Mytilus edulis* of our shores. On examination it was found that 85% of the mussel population at Padstow was of the 'unusual' type of mussel, the remaining 15% being normal *M. edulis*. It was decided to investigate this 'Padstow-type' of mussel and to determine whether it was in fact *M. galloprovincialis* Lmk.



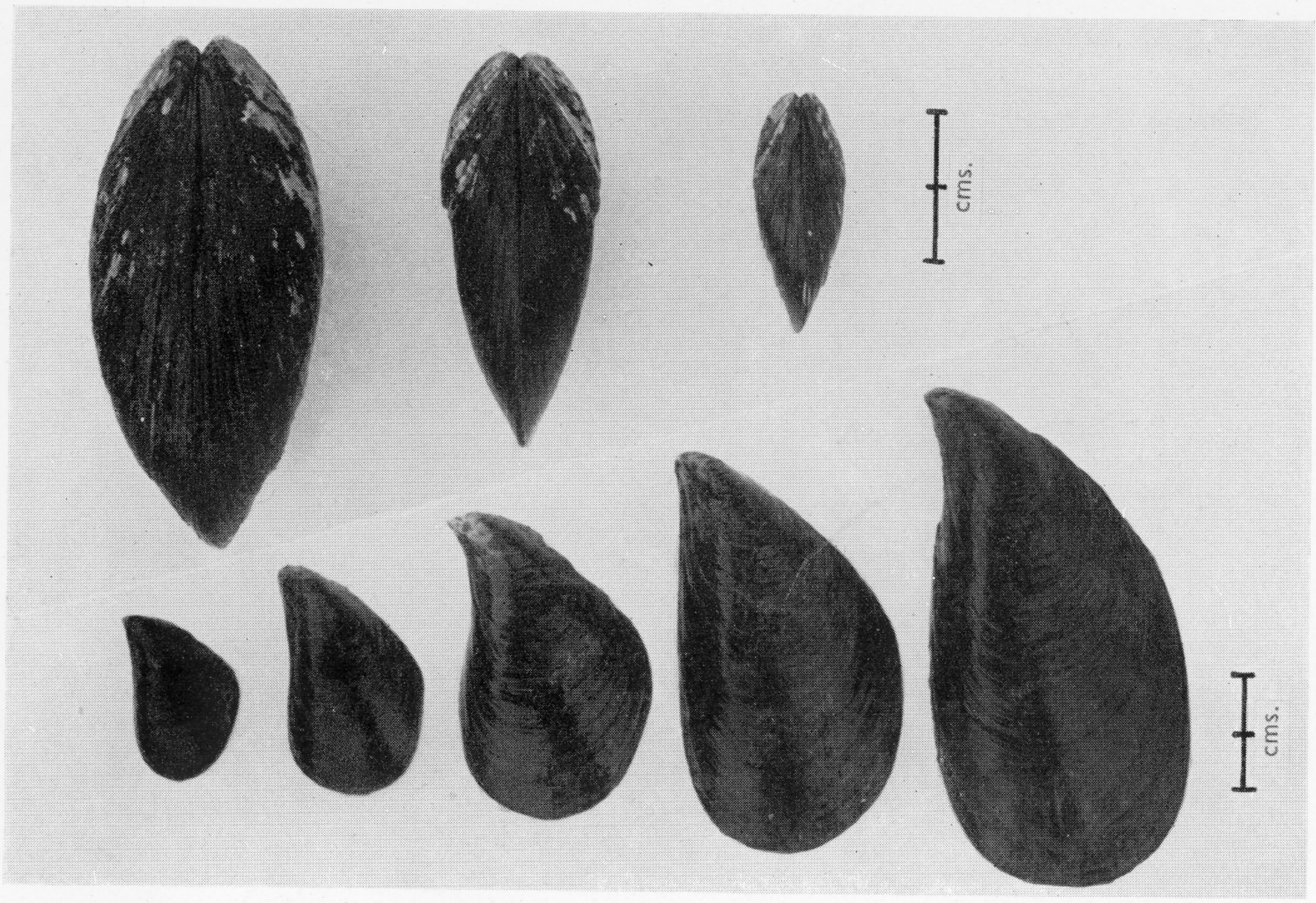
Text-fig. 1. Comparison of mean values of height with length for *Mytilus galloprovincialis* (●) and *M. edulis* (○).

THE 'PADSTOW-TYPE' MUSSEL

The mussels from Padstow were found to differ from *Mytilus edulis* in the following respects. (1) The umbones were more pronounced, more pointed and down-turned (see Pl. I). (2) The shells were proportionately higher, the length/height ratio for *M. edulis* being 1·95 and 1·77 for the Padstow mussels (see Text-fig. 1); these average figures were obtained from measurements of many specimens of each type. (3) The shells were less angular, and the distinct angle where the front and upper margins meet, which is often seen in *M. edulis*, was less evident in the Padstow mussels (see Pl. I). (4) They attained a larger size than *M. edulis*; individuals of 10–12 cm in length were quite common: in southern England *M. edulis* does not exceed 8–10 cm except under very favourable conditions. (5) The mantle edge was usually very dark, either blue, purple or violet, often appearing almost black, whereas in *M. edulis* it is much lighter, white to brown, usually appearing a straw colour.

EXPLANATION OF PLATE I

A. *Mytilus galloprovincialis* Lmk. from Padstow, Cornwall. Note the pronounced down-turned umbones, the high shells and the shape of the dorsal margin. B. *M. edulis* from Conway for comparison with A.



A

B

(*Facing p* 34)

The literature on the Mytilidae appears to contain no detailed comparison of *M. edulis* and *M. galloprovincialis*, but the Padstow mussel seems to correspond to the description and figures of *M. galloprovincialis* given by List (1902) who describes the species in great detail. In both forms the umbones are pronounced and down-turned, the length/height ratio is very similar, being 1·77 for Padstow mussels and 1·8, calculated from List's measurements, for seventeen specimens of *M. galloprovincialis*, while the general shell outline of the Padstow mussel is similar to both the illustrations and descriptions given by List. The dark-coloured mantle edge of the Padstow mussel closely resembles that illustrated in colour by List for *M. galloprovincialis*.

Of other authors who have compared *M. edulis* and *M. galloprovincialis*, Sowerby (1887) states that *M. edulis* var. *galloprovincialis* is 'broader and flatter, beaks incurved', and gives an illustration of this mussel which is very similar in outline to the Padstow mussel, and appears larger than *M. edulis*. Forbes & Hanley (1848), referring to the mantle edge of mussels, state '...usually in British specimens a yellowish white colour though sometimes...tinged with brown and in the foreign variety *galloprovincialis* they are deeply tinged with purple'. Lambert (1950), in a brief comparison of *M. edulis* and *M. galloprovincialis*, states '...the latter is larger, wider and its umbone is more pronounced'. Width in this context is taken to mean shell height.

Since the Padstow mussel corresponds so closely to the descriptions of *M. galloprovincialis* and also resembles closely specimens of this species in the Norman collection of the British Museum and comes from an area in which Mediterranean species are known to occur (Yonge, 1949), it must be concluded that it is in fact *M. galloprovincialis* Lmk.

SYSTEMATICS

In the earlier literature there appears to have been some confusion between *M. ungulatus* L. and *M. galloprovincialis* Lmk. Lamy (1920) states that the two are synonymous, and List (1902) appears to have been of the same opinion. However, it appears that *M. ungulatus* is a form of *edulis*, and in fact Linnaeus (1758, 10th ed.) himself suggested this, and the figures to which he referred appear to be of distorted specimens of *M. edulis*. In the 12th edition of the *Systema Naturae* Linnaeus (1767) enlarged on his description of *M. ungulatus* given in the 10th edition. Lamarck (1819) appears to have been fully aware of both Linnaeus's *M. ungulatus* and *M. edulis* when describing *M. galloprovincialis*. Dodge (1952) considers *M. ungulatus* to be a species of doubtful validity and this appears to be the opinion of most conchologists at the present time. In view of this confusion it seems probable that the *M. ungulatus* which Jeffrey (1863) recorded from the coasts of Cornwall and the Channel Islands was in fact *M. galloprovincialis* Lmk. Donovan (1802) recorded *M. ungulatus* from the coast of Cornwall and his colour illustrations

of this species so closely resemble in all respects the *M. galloprovincialis* taken from Cornwall, that it must be concluded that he was referring to the last-named species.

DISTRIBUTION

To study the distribution of *M. galloprovincialis* in Britain, and to determine whether stocks of this species were available for restocking mussel beds decimated by *Mytilicola*, surveys were made along the south-west peninsula of England, and on the south coast of Wales. *Mytilus galloprovincialis* was known to be absent from North Wales and the south coast of England from Teignmouth eastwards. At each site visited a sample of mussels was collected covering a wide range of sizes. The number of mussels varied according to quantities available. The mussels in each sample were sorted into groups of *M. edulis* and *M. galloprovincialis* on the criteria given above, and the percentage of the latter was calculated.

Results are shown in Table I, and in Text-fig. 2 the proportion of *M. galloprovincialis* and its distribution are shown.

It will be noticed that the mussel populations of north-west Devon and north and south-west Cornwall are dominated by *M. galloprovincialis*, with occasional specimens of this species occurring on the south coast as far east as Plymouth. In South Wales *M. galloprovincialis* occurs in small numbers at many points along the coast, being most common at Cardiff where it forms about half of the mussel population.

The absence of this mussel from the inlets of the Carmarthen coast can be explained on the grounds that it is intolerant of estuarine conditions (see below).

The well-defined zone of distribution of this mussel, with the rapid fall in the proportions of *M. galloprovincialis* over a short distance at the edges of the zone, suggests that this species would not be successful elsewhere in Britain. Although adult *M. galloprovincialis* have been kept in aquarium tanks at the laboratory at Burnham-on-Crouch in circulating River Crouch water for over a year, without any apparent ill effects, young specimens of 1–2 cm in length died within a few days of being placed in the River Crouch.

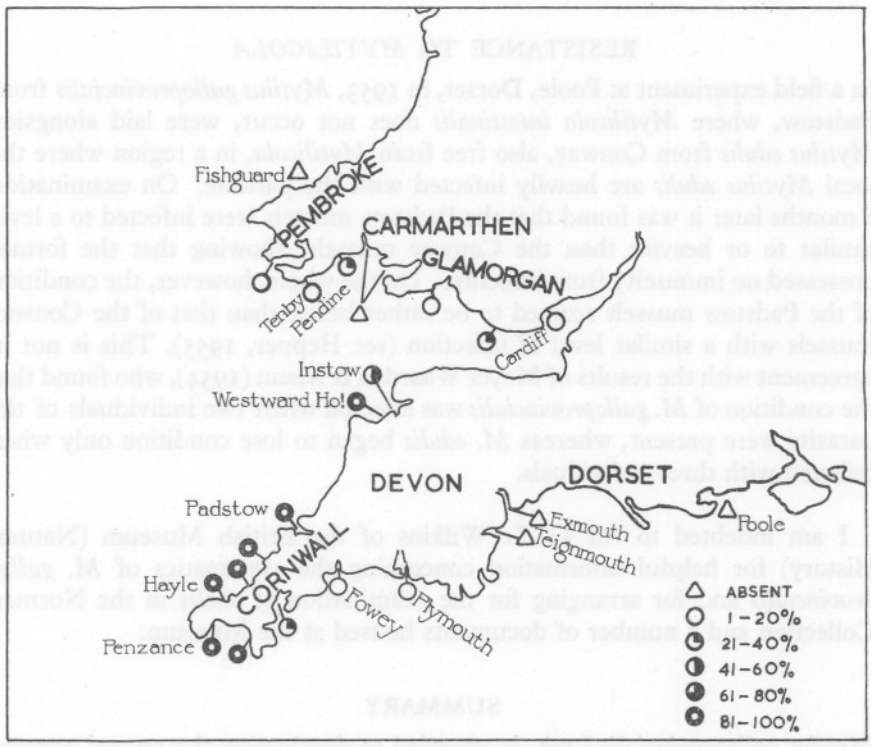
Some mussels received from Castletownbere in Southern Eire, collected by my colleague, Mr R. H. Baird, were found to be *M. galloprovincialis*, but it has not been possible to make a detailed study of its ecology in that area.

M. galloprovincialis has been reported from Concarneau, Finistère, by Bouxin (1955). Sinel (1906) mentions a *M. angulata*, 'the hoof mussel', and since he worked largely in the Channel Isles it may be assumed that the mussel he mentions was found there, and that he was in fact referring to *M. galloprovincialis*, '*angulata*' being a corruption of '*ungulatus*'.

TABLE 1. PERCENTAGE OF *MYTILUS GALLOPROVINCIALIS* AT SITES SURVEYED

Locality	No. in sample	*Mytilus gallo-provincialis* (%)	Locality	No. in sample	*Mytilus gallo-provincialis* (%)
Angle Bay	60	30	Newlyn	104	89
Tenby	147	1	Penzance	112	94
Pendine	56	40	Porthleven	68	87
Mumbles	72	13	Helford R.*	113	32
Aberthaw	45	26	Falmouth	260	75
Cardiff	110	60	Fowey	100	20
Instow*	20	35	Plymouth	200	2
Appledore	99	53			
Westward Ho!	58	88	Estuarine area of the Fal		
Padstow	180	85	Falmouth Harbour	260	75 Marine
Newquay	89	88	St Just	40	60 ↓
Gwithian	145	95	King Harry Reach	40	10
Hayle	73	97	Malpas	40	0 Brackish

* Estuarine areas



Text-fig. 2. Map of distribution of *Mytilus galloprovincialis* in Britain, showing the proportion of *M. galloprovincialis* in the mussel population.

ECOLOGY

M. galloprovincialis appears to occur in all types of habitat where salinity is not greatly reduced. On rocks on open exposed shores the mussel is stunted and thick-shelled, but at all times the characteristic features are maintained. In less turbulent waters, as in harbours and inlets, the mussel occurs in dense beds or clusters and grows to a very large size.

In surveys in the Fal and Truro rivers, and at Looe, *M. galloprovincialis* was found predominantly at the mouth of the estuary, being gradually replaced by *M. edulis* farther upstream (Table I). A similar situation was observed in the Camel estuary, at Padstow, but there the proportion of *M. edulis* was so small, and so very few mussels occurred in the less saline regions, that it would perhaps be unwise to draw any conclusions from this observation. It was noticeable that the few *M. galloprovincialis* found at Fowey and Plymouth were taken from the lower regions of the estuaries.

On a suitable substratum *M. galloprovincialis* appears to cover about the same vertical range as *M. edulis*.

RESISTANCE TO *MYTILICOLA*

In a field experiment at Poole, Dorset, in 1953, *Mytilus galloprovincialis* from Padstow, where *Mytilicola intestinalis* does not occur, were laid alongside *Mytilus edulis* from Conway, also free from *Mytilicola*, in a region where the local *Mytilus edulis* are heavily infected with the parasite. On examination 6 months later it was found that the Padstow mussels were infected to a level similar to or heavier than the Conway mussels, showing that the former possessed no immunity from infection. On the whole, however, the condition of the Padstow mussels seemed to be rather better than that of the Conway mussels with a similar level of infection (see Hepper, 1955). This is not in agreement with the results of Meyer Waarden & Mann (1954), who found that the condition of *M. galloprovincialis* was affected when two individuals of the parasite were present, whereas *M. edulis* began to lose condition only when infected with three individuals.

I am indebted to Mr G. L. Wilkins of the British Museum (Natural History) for helpful information concerning the systematics of *M. galloprovincialis* and for arranging for the examination of shells in the Norman Collection and a number of documents housed at the Museum.

SUMMARY

Mytilus galloprovincialis Lmk. is recorded as dominating the mussel population on the north coast of Devon and the north and south-west coasts of Cornwall from Instow to the Lizard. It was also found less frequently on the

coast of South Wales from Angle to Cardiff and on the south coast of Cornwall from the Lizard to Plymouth.

The chief distinguishing features of *M. galloprovincialis* from Cornwall are summarized, in comparison with *M. edulis* L.

M. galloprovincialis is apparently intolerant of estuarine conditions, but otherwise appears to be ecologically similar to *M. edulis*.

M. galloprovincialis is not resistant to infection by *Mytilicola intestinalis*, but appears to be less affected by the parasite than is *Mytilus edulis*.

The synonymy of the species is briefly discussed and it is concluded that, although earlier workers tended to confuse *M. ungulatus* L. (recorded also as *M. ungulata* and *M. angulata*) with *M. galloprovincialis* Lmk., the two types are distinct. *M. ungulatus* is a name of doubtful validity applied by Linnaeus to a distorted form of *M. edulis*.

REFERENCES

ANON., 1866. Cornish marine shells. *J. roy. Instn Cornwall*, Vol. 2, pp. 65–72.

BOUXIN, H., 1955. Observations sur le frai de *Mytilus edulis* var. *galloprovincialis* (Lmk.) *Rapp. Cons. Explor. Mer*, Vol. 140, pp. 41–4.

COLE, H. A., 1951. Le *Mytilicola* en Angleterre. *Rev. Trav. Off. Pêches marit.*, T. 17, Fasc. 2, pp. 59–61.

CONCHOLOGICAL SOCIETY, 1929. Recorder's Report (Marine molluscs). *J. Conch.*, Vol. 18, p. 300.

DODGE, H., 1952. A historical review of the mollusks of Linnaeus. Pt. I. The class Loricata and Pelecypoda. *Bull. Amer. Mus. nat. Hist.*, Vol. 100 (1).

DONOVAN, E., 1802. *The Natural History of British Shells*. London: F. and C. Rivington.

FORBES E. & HANLEY, S. 1848. *A History of British Mollusca*. London: John Van Voorst.

GARDINER, A. P., 1945. British marine records. *J. Conch.*, Vol. 22, p. 148.

HEPPER, B. T., 1955. Environmental factors governing the infection of mussels, *Mytilus edulis*, by *Mytilicola intestinalis*. *Fish. Invest. Lond.*, Ser. 2, Vol. 20, no. 3.

JEFFREY, J. G., 1863. *British Conchology*. London: John Van Voorst.

LAMARCK, J. B. M., 1819. *Histoire Naturelle des Animaux sans Vertèbres*. Paris.

LAMBERT, L., 1950. *Les Coquillages Comestibles*. Paris: Presses Universitaires de France.

LAMY, E., 1920. Notes sur les espèces de *Mytilus* décrites par Lamarck. *Bull. Mus. Hist. nat. Paris*, T. 26, pp. 520–6.

LINNAEUS, C., 1758. *Systema Naturae*. 10th ed. Stockholm.

—— 1767. *Systema Naturae*. 12th ed. Stockholm.

LIST, T., 1902. Die Mytilieden des Golfes von Neapel. *Fauna u. Flora Neapel*, Monogr. 27, 312 pp.

MEYER WAARDEN, P. F. & MANN, H., 1954. Untersuchungen über die Bestande von *Mytilus galloprovincialis* an der italienischen Küste auf ihren Befall mit *Mytilicola intestinalis* (Copepoda parasitica). *Boll. Pesca Piscic. Idrobiol.*, Ann. 29, N.S. Vol. 8, Fasc. 2, pp. 5–24.

SINEL, J., 1906. *An Outline of the Natural History of our Shores*. London: Swan Sonnenschein.

SOWERBY, G. B., 1887. *Illustrated Index of British Shells.* London: Sowerby and Simpkin Marshall.

STEUER, A., 1902. *Mytilicola intestinalis*, n.gen. n.sp., aus dem darme von *Mytilus galloprovincialis* Lam. *Zool. Anz.*, Bd. 25, p. 635.

TREGELLIS, J., 1896. The marine Mollusca of Cornwall. *J. Conch.*, Vol. 8, No. 6, pp. 191–200, 209–25.

WINCKWORTH, R., 1932. The British marine Mollusca. *J. Conch.*, Vol. 19, pp. 211–52.

YONGE, C. M., 1949. *The Sea Shore.* London: Collins, New Naturalist Series.

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THE GROWTH OF *SCROBICULARIA PLANA* (DA COSTA) IN THE GWENDRAETH ESTUARY

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(Text-figs. 1–3)

Scrobicularia plana (da Costa) is an abundant inhabitant of intertidal estuarine muds. Populations up to 1000 per m^2 have been recorded from the Tamar Estuary (Spooner & Moore, 1940). Similar population densities have been found in the Gwendraeth Estuary in South Wales. In one patch a population of 1025 per m^2 was found; the average length of the shells was 30 mm. Individual lengths up to 54 mm were found in other parts of the estuary. The large size of the specimens from the Gwendraeth prompted an investigation into the rate of growth and the age reached in this locality.

METHODS

Random samples of *Scrobicularia* were taken by digging up 1 m^2 to a depth of 30 cm in a region where the average population was 500 per m^2. A square metal frame with sides 0·5 m long and 15 cm deep was used to prevent mud slipping into the area being sampled. Four samples taken using such a frame made up the total sample of 1 m^2. Samples were collected in December 1953, April 1954, August 1954, April 1955 and July 1955.

The large specimens were sorted by hand and the small specimens by sieving. A sieve with a mesh of about 6 mm was used for the early samples, but for the last sample a 1 mm-mesh sieve was used. As there were some empty shells in the mud, care was taken to collect only the living animals; these were recognized in the field by the slightly protruding mantle lobes and by the presence of an oxidized layer of soil immediately next to the shell even when the animals were taken from a black layer. The specimens were washed and then boiled until the shells gaped widely and the animals dropped out. The shells were then washed and dried; one valve was kept from each. In this way a large number of shells could be stored and their lengths measured at leisure. The length of each shell, to the nearest mm, was written in pencil on its inner surface for future reference and size grouping. In all some 2500 shells were sorted and measured.

Like many other bivalved molluscs, *S. plana* has growth rings on its shell, but often only the last one or two are visible. The earlier growth rings become obliterated and only those left by the last few winters can be found. It is thus

not possible to determine the age of a shell directly from its growth rings. It is, however, possible to deduce an approximate age by using a series of shells such as that shown in Fig. 1. By homologizing the rings on each shell with those on larger and smaller shells, and by working back to progressively smaller shells an estimation can be made of the age of any shell in the series. Examination of the growth rings on some of the large specimens was found to be facilitated by immersion in dilute hydrochloric acid for a short period. Immediately after such treatment the growth rings were very clear, but when the treated shells were stored the rings tended to disappear, and, of course, the treated shells were much more fragile than the untreated shells.

The environmental conditions in the sampling area were studied by measuring the salinity of the water over the area during a tide and by a grade analysis of the soil.

The salinity of the water was estimated with a glass hydrometer; the temperature of the water was measured at the same time and the density reading converted to salinity in ‰ using the graph given by Harvey (1945, fig. 12).

The sample of soil for analysis was taken by pushing a stout glass tube into the ground and then lifting it out after clearing the soil away from its sides. The ends of the tube were then closed with rubber bungs. The sample was taken about 3 h after the tide had uncovered the sampling area so that most of the superficial water had drained away. In the laboratory the soil was pushed out of the tube and chopped into 2 cm lengths which were weighed and then dried for 16 hr at 105° C. The subsequent treatment of the samples was identical with that of Holme (1949) except that the treatment with hydrochloric acid to remove carbonates was omitted and sieves with the following meshes were used: 30, 60, 90 and 100 I.M.M. In practice it was found that over 95% of the soil passed through the 100 I.M.M. sieve.

CONDITIONS IN THE SAMPLING AREA

The area from which the samples were taken was about 70 m long, with the length parallel to the edge of the tide, and about 4 m wide. Neap tides covered the area for about 2 h; on a spring tide the time was increased to about 4 h. The depth of water over the surface of the mud on a neap tide was 0·5 m. The depth over the mud on a spring tide was not measured but is estimated at 2·0 m. The times given for coverage by the tide do not represent the limits of the time available to *Scrobicularia* for feeding since the surface is poorly drained and still wet for several hours after the tide has receded. The inhalant siphons of *Scrobicularia* can be seen actively sucking up the surface mud 3 or 4 h after being uncovered by the tide.

The salinity of the water over the area during a neap tide on 29 July 1955 is shown in Table 1. The salinity is probably somewhat higher than usual, due to the hot dry summer and the low state of the river. It was not practic-

able to measure the salinity during a spring tide, but a few measurements made during the winter 1952–53 showed a salinity of 18‰ at a level just above the sampling area about an hour before high tide. This somewhat lower salinity on a spring tide in winter when compared with a neap tide in summer indicates that the main factor governing salinity over the sampling area is the strength of flow of the river. Other measurements made during winter, as the tide was just uncovering the area, showed a salinity as low as 2‰.

TABLE 1. SALINITY OF THE WATER COVERING THE SAMPLING STATION DURING A NEAP TIDE IN JULY 1955

The tide covered the area at 12.44 G.M.T. and uncovered it at 14.45 G.M.T.

Time (G.M.T.)	Salinity (‰)	Time (G.M.T.)	Salinity (‰)
12.30	25	14.15	12
13.00	25	14.30	11
13.30	25	15.00	11
14.00	15		

TABLE 2. GRADE ANALYSIS OF THE SOIL IN THE SAMPLING AREA

Depth (cm.)	% water content	Fine sand as % dry weight	Silt + clay as % dry weight
1–2	31	75	24
2–4	28	78	21
4–6	30	80	19
6–8	29	81	17
8–10	27	84	13
10–12	26	88	10
12–14	24	90	8
14–16	24	90	8

Grade analysis of the soil showed that most of the particles passed through the 100 I.M.M. sieve and that of the total dry soil between 8 and 24% by weight belonged to the silt + clay fraction (Table 2). The most significant variation with depth is in the amount of silt + clay; the surface layers contain three times as much as the soil at a depth of 14–16 cm. The water content, as estimated by the present method, may not be quite the same as that of the mud *in situ*, but it does give a measure of the water-holding capacity of the soil. It is clear from Table 2 that this capacity increases with increasing silt + clay content, so that the surface layers have a higher water content.

AGE ESTIMATED FROM GROWTH RINGS

From a series such as that shown in Fig. 1 it is possible to construct a growth curve. Such a curve, based on the specimens in Fig. 1, is given by the broken line in Fig. 2. The middle part of the curve is based on several specimens at each length (Table 3). Other series of shells give slightly different curves, and if the whole range of variation is considered it is possible to draw up a table giving the upper and lower limits of length for a given year group

(Table 5). This range of variation, based on the December 1953 sample, is shown in Fig. 2. Attempts to gain greater precision in defining the growth curve do not seem to be very profitable. The details of the curve can only apply to this locality, and further examination of the shells with several

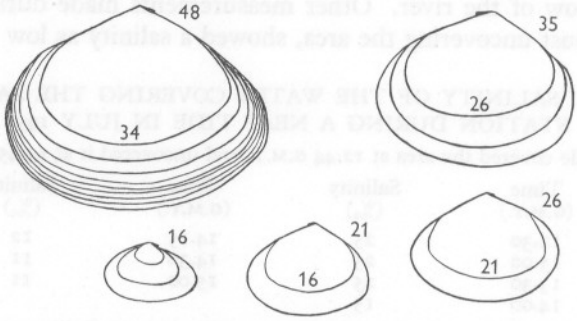

Fig. 1. *S. plana.* Series of shells used to construct the growth curve given by the broken line in Fig. 2. All are drawn to the same scale. The numbers above each shell and on some of the rings give the length in mm.

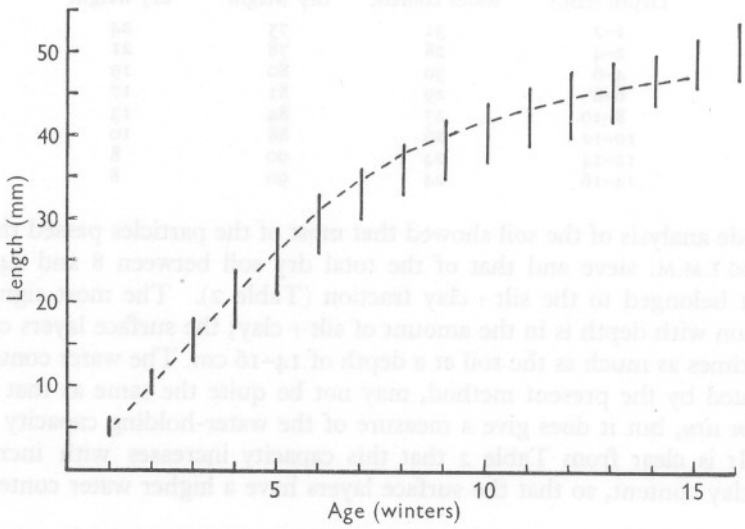

Fig. 2. *S. plana.* Growth curve constructed using the specimens in Fig. 1 (broken line), and the range of variation (shown by vertical lines) found in other series of shells.

growth rings often reveals a narrow interval between two wider ones, indicating that some years are better for growth than others.

If the mid-point in the range of variation given for each year group in Table 5 is taken as representative of an 'average' animal of that year the following summary can be given of the growth of *Scrobicularia*. A length of

5 mm is reached by the first winter. During each of the next two years another 5 mm is added to the length. Between the third and fourth winters 6 or 7 mm are added, thereafter the growth rate steadily decreases until the ninth or tenth winter, after which a fairly steady addition of about 2 mm per year is made.

TABLE 3. GROWTH RINGS ON MEDIUM-SIZED *SCROBICULARIA PLANA*

Sample collected in December 1953

Length of shell (mm)	No. examined	Mean length of growth rings (mm)	
		Last	Penultimate (when visible)
21	10	15·4	—
26	10	21·0	—
30	10	26·4	21·5 (4)*
35	10	32·2	26·5 (7)
38	5	35·0	30·0 (2)

* The numbers in parentheses give the number with two growth rings visible.

TABLE 4. GROWTH RINGS ON LARGE *SCROBICULARIA PLANA*

Sample collected in December 1953

Length 46 mm with 7 rings between one at 36 mm and the shell edge
Length 46 mm with 5 rings between one at 38 mm and the shell edge
Length 46 mm with 5 rings between one at 39 mm and the shell edge
Length 46 mm with 6 rings between one at 35 mm and the shell edge
Length 46 mm with 6 rings between one at 33 mm and the shell edge
Length 50 mm with 7 rings between one at 36 mm and the shell edge
Length 50 mm with 5 rings between one at 43 mm and the shell edge
Length 50 mm with 6 rings between one at 37 mm and the shell edge

TABLE 5. VARIATION IN LENGTH WITH AGE IN *SCROBICULARIA PLANA* FROM THE GWENDRAETH ESTUARY

Age (winters)	Length (mm)	Age (winters)	Length (mm)
1	4–6	9	35–42
2	9–12	10	37–44
3	13–18	11	39–46
4	17–24	12	40–48
5	21–29	13	42–49
6	26–33	14	44–50
7	30–36	15	46–52
8	33–39	16	47–54

Raymont (1955) has studied the early growth of *S. plana* in Kyle Scotnish, Scotland. Here the shells reach a length of 6 mm by the first autumn and 16 mm by the second. This is clearly a much greater increase during the second season than that found in the Gwendraeth, and may be due to the artificial fertilization of Kyle Scotnish.

Table 5 and Fig. 2 indicate that the age of shells above 20 mm in length cannot be determined accurately. For instance, a shell with a length of 33 mm might be 6, 7 or 8 years old. When even larger shells are considered the accuracy diminishes still further. A shell with a length of 46 mm may be anywhere between 11 and 15 years old. Some idea of the variation in large shells can be deduced from Table 4. Some individuals appear to live for about 18 years.

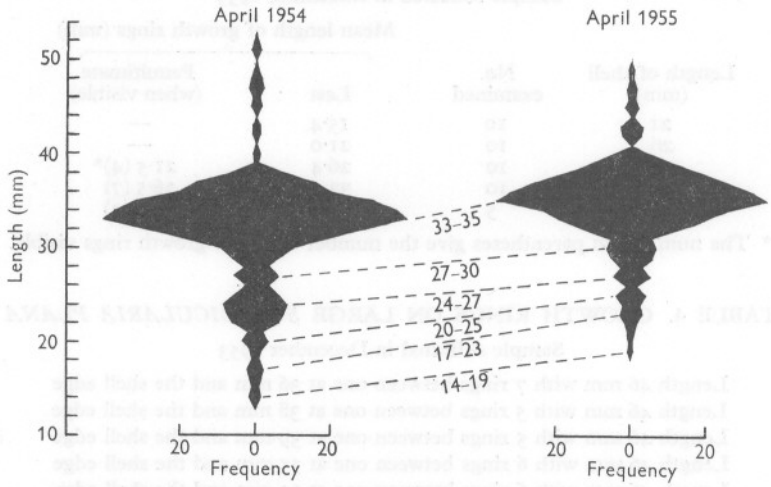

Fig. 3. *S. plana.* Shift in the peaks of length frequency distribution during one year.

LENGTH FREQUENCY DISTRIBUTION

In the preceding section it was assumed that the growth rings on the shell are annual rings. In order to test this assumption two samples were taken at a year's interval (April 1954 and 1955) to see how the peaks of the length frequency kites moved (Fig. 3). The shifts of the peaks are in fair agreement with the intervals between growth rings and all lie within the range of variation given in Table 5. If the mean size of the whole sample is considered there is a shift from 32·1 to 34·6 mm which is of a similar order to the increase in length between the seventh and eighth winters.

The earlier samples give no indication of the numbers of small shells so that no idea could be gained of the numbers of spat surviving. The July 1955 sample, taken with a 1 mm-mesh sieve, and sieved very gently so as not to destroy the delicate shells, showed a very small percentage of small shells. Only seven of the 483 shells in the sample were below 10 mm in length. This indicates that there is at present very little successful settlement of spat in the area. The dense adult population is probably the cause of this. Five

hundred inhalant siphons working in a square metre would make the area a most difficult one for a recently metamorphosed *Scrobicularia* to survive in. Nevertheless, a few do survive, and it may be that when the present population bulge at a length of 30–40 mm dies out there will be heavier successful settlements of spat.

SUMMARY

Populations of *Scrobicularia plana* with densities up to 1025 per m^2 are found in the Gwendraeth Estuary.

In a region where the population density was about 500 per m^2 it was found that some specimens lived for 16–18 years and reached a length of 54 mm. An approximate growth curve is given.

There is very little successful settlement of spat in the sampling area; less than 2% of the shells were under 10 mm in length in July 1955. This low figure is attributed to the feeding activities of the dense adult population making the area difficult to settle in.

REFERENCES

HARVEY, H. W., 1945. *Recent Advances in the Chemistry and Biology of Sea Water*. Cambridge University Press.

HOLME, N. A., 1949. The fauna of sand and mud banks near the mouth of the Exe Estuary. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 189–237.

RAYMONT, J. E. G., 1955. The fauna of an intertidal mud flat. *Deep-Sea Res.*, suppl. to Vol. 3, pp. 178–203.

SPOONER, G. M. & MOORE, H. B., 1940. The ecology of the Tamar Estuary. VI. An account of the macrofauna of the intertidal muds. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 283–330.

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THE DISTRIBUTION OF POLYCHAETA IN OFFSHORE DEPOSITS IN THE IRISH SEA

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Marine Biological Station, Port Erin[1]

(Text-figs. 1 and 2)

The bottom fauna of the Irish Sea around the Isle of Man and off the coast of Cumberland has been investigated by Dr N. S. Jones, who has described the fauna in general (1940, 1951, 1952) and the Amphipoda in particular (1948). Apart from the Amphipoda the smaller animals have remained relatively unknown. To extend this earlier work a survey of the polychaetous worms was carried out during 1952–54, and this paper deals with their distribution and ecology in the offshore bottom deposits. New records and details of taxonomic interest have been published elsewhere (Southward, 1956).

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THE AREA INVESTIGATED

The area investigated during the 1952–54 survey was roughly the same as that studied by Jones (1951). To begin with all the collections were made within 10 miles of Port Erin but later the area was extended to include the deeper water to the west (Fig. 1).

The coastline of the southern half of the Isle of Man is steep and rocky and, except in the bays, the sea bottom slopes sharply from low-water mark to 10 fm. Below this depth the slope is more gradual and on the west side of the island the 50 fm. line is reached about 10 miles offshore. There is a channel about 70 fm. deep between the Isle of Man and Ireland. The sea to the south and east is shallower, the bottom is more irregular, and between the Isle of Man and England the depth rarely exceeds 20 fathoms.

The offshore grounds present a variety of habitats, most of which can be found within 10 miles of Port Erin. The deposits have been classified by Jones (1951) into four main types: (*a*) coarse sands, gravels including shell

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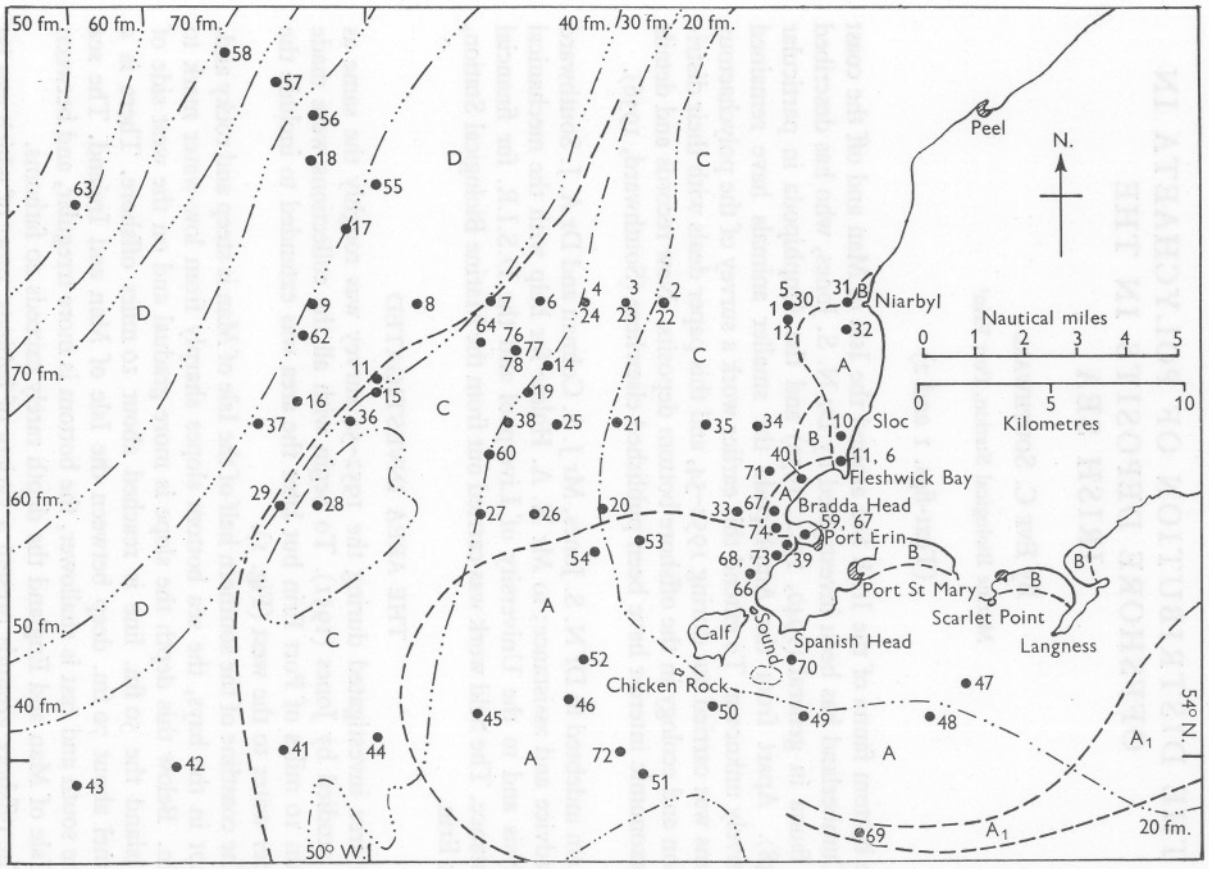

Fig. 1. Map of the south end of the Isle of Man, showing positions of offshore stations (numbers as in Table II). The heavy broken lines mark the approximate limits of the various deposits. A gravels (A_1 *Modiolus* epifauna); B clean sand; C muddy sand; D mud.

and stony gravels; (*b*) fine sand; (*c*) muddy sand; (*d*) mud. The approximate limits of these types of deposit (as determined by Jones) are marked by the heavy broken lines in Fig. 1. The deposits grade into one another and there are no sharp boundaries between them.

In this area the main factor controlling the grade of a deposit appears to be the amount of water movement over it. The tidal currents reach $3\frac{1}{2}$ knots at spring tides along the south coast of the island, and in places $4\frac{1}{2}$ knots. There are weaker currents inshore along the west coast, but in a large area to the west of the island they are too slight to be perceptible (*West Coast of England Pilot*, 1948). It is in this area that soft deposits are present in relatively shallow water. Wave action may be felt down to 25 fm., as this is the least depth at which mud is found (Jones, 1951).

The surface temperature of the sea off the south-west coast had a mean annual range of 6·5° C during 1903–12, according to Matthews (1914). The monthly mean was lowest in February, at 7·5° C (all depths) and highest in August at 13° C. At this time of year the bottom temperature was less than 10° C; it reached its maximum in November when the temperature at all depths was 12° C. During 1953 (Slinn, 1956) the temperature was lower than the mean in February (6–7° C at all depths) and higher than the mean in August (13·5–15° C at the surface and 11·5° C at 50 fm.). The maximum bottom temperature was recorded in October (13·3–13·7° C).

The salinity of the sea in the same area is about 34‰ with an annual variation of about 0·2‰ (Matthews, 1914).

METHODS

GEAR USED

Several different types of gear were used for obtaining bottom samples. The most useful was a van Veen type of grab sampler taking a sample of one-tenth of a square metre (Thamdrup, 1938). The depth to which this instrument would dig depended on the hardness of the deposit. It was most efficient on mud and muddy sand, where it probably dug to at least 15 cm. Dredges were used on deposits too hard for the grab sampler. A small naturalist's dredge, with a bowed frame (1 ft. 6 in. wide) and a stramin bag, was used for fine gravel or gravel mixed with sand; judging from the fragments of *Ensis* sp. sometimes brought up it could dig 4–8 cm into these deposits. A larger naturalist's dredge (2 ft. 6 in.) was occasionally employed on sand and fine gravel but was less efficient than the smaller one. Where the bottom was composed of coarse gravel a scallop dredge (4 ft. wide and lined with $\frac{3}{8}$ in. netting) was found most useful since the stramin of the small dredge was easily torn. It brought up only the coarse gravel and large stones, any fine material being washed out while the dredge was being hauled to the surface.

Depths were measured with an echo sounder and the positions of the stations calculated from compass bearings. A list of stations is given in Table II, and their positions are marked on Fig. 1.

TREATMENT OF COLLECTIONS

The contents of the scallop dredge were examined on board the boat, larger specimens being picked out and samples of stones, shells and hydroids kept for more detailed examination. The samples taken in the naturalist's dredges were not sorted on board, but the whole or part of the sample was taken back to the laboratory. Grab samples were washed through a 2 mm round-holed sieve with a jet of water from a hose, on board the boat, after which the entire residue on the sieve was retained for examination in the laboratory.

At the laboratory samples and specimens were either examined fresh, soon after arrival, or preserved, without sorting, in 5% sea water formalin.

Weeds, hydroids and shells were searched carefully, usually under a dissecting microscope, and the worms picked out. Gravel was sorted in two stages: first, the larger animals were picked out by hand, then the gravel was washed several times and the washing waters strained through fine bolting silk. In this way many small animals were retained, that would otherwise have been lost. The first residue from the grab samples was treated in the same way if it contained much gravel. If not, it was sorted by hand.

In the case of the grab samples, and those of the small dredge, all the macrofauna was preserved and the animals other than polychaetes identified at least as far as phyla. When the scallop dredge was used only the polychaetes were picked out, but notes were made on the abundance of other animals.

After identification the polychaetes were preserved in 70% alcohol, and a representative collection of species has been stored at Port Erin.

GRADE ANALYSIS OF DEPOSITS

Small samples of most types of offshore deposit were taken from hauls of the grab sampler and small dredge. These samples were dried and later subjected to the method of grade analysis used by Holme (1954). This method separates the sample into eight grades of particles: (i) over 2 mm diameter; (ii) 2–1 mm; (iii) 1–0·5 mm; (iv) 0·5–0·25 mm; (v) 0·25–0·21 mm; (vi) 0·21–0·124 mm; (vii) 0·124–0·0313 mm; (viii) less than 0·0313 mm. In addition, fragments over 10 mm in diameter were separated from the first group.

TAXONOMY

The names and classification used here are mainly those used by Fauvel (1923, 1927). Some of the species identified during the present survey are not included by Fauvel; references to descriptions of these are given in the taxonomic paper referred to above (Southward, 1956).

BOTTOM DEPOSITS

During the field work bottom deposits were identified fairly readily as belonging to the four main groups described by Jones (1951), but several subgroups could also be recognized.

The coarse grounds (Fig. 1, A) include more than one type of gravel. A wide area to the south of the Isle of Man is occupied by large stones, shells and coarse shell gravel and the same type of coarse shell gravel also occurs on

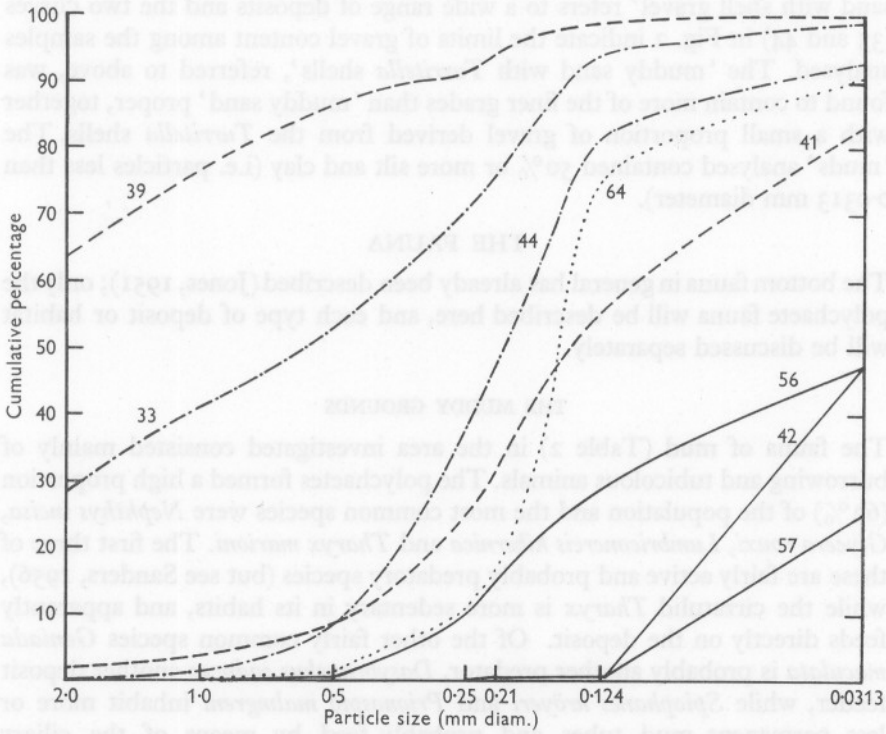

Fig. 2. The composition of various bottom deposits shown by means of cumulative curves. Station numbers as in Table II. ----, fine stony gravel; -·-·-·-, muddy sand with shell gravel; ·····, muddy sand; - - - - -, muddy sand with *Turritella* shells; ————, mud.

the west side of the island, off Bradda Head. Further north, between Fleshwick Bay and Niarbyl, there are deposits of fine stony gravel, which in places is mixed with larger stones or with nodules of coralline algae (*Lithothamnion* sp.). This algal deposit, mixed with muddy sand, also occurs to the west of Niarbyl.

Towards the outer limits of the coarse grounds the gravel is mixed with muddy sand, the proportion of which increases with depth until the pure

muddy sand grounds are reached (Fig. 1, C). The muddy sand in turn grades into mud in deeper water, and along the edges of the mud grounds (Fig. 1, D) the muddy sand may contain large numbers of empty shells of *Turritella communis* Lamarck.

Mechanical analysis (see p. 52) of the deposits from fourteen stations has shown that the differences noticed in the field were definitely differences in grade composition. The composition of the various deposits (Table 1) can be shown graphically by means of cumulative curves (Fig. 2). The term 'muddy sand with shell gravel' refers to a wide range of deposits and the two curves (33 and 44) in Fig. 2 indicate the limits of gravel content among the samples analysed. The 'muddy sand with *Turritella* shells', referred to above, was found to contain more of the finer grades than 'muddy sand' proper, together with a small proportion of gravel derived from the *Turritella* shells. The 'muds' analysed contained 50% or more silt and clay (i.e. particles less than 0·0313 mm diameter).

THE FAUNA

The bottom fauna in general has already been described (Jones, 1951); only the polychaete fauna will be described here, and each type of deposit or habitat will be discussed separately.

THE MUDDY GROUNDS

The fauna of mud (Table 2) in the area investigated consisted mainly of burrowing and tubicolous animals. The polychaetes formed a high proportion (62%) of the population and the most common species were *Nephthys incisa*, *Glycera rouxi*, *Lumbriconereis hibernica* and *Tharyx marioni*. The first three of these are fairly active and probably predatory species (but see Sanders, 1956), while the cirratulid *Tharyx* is more sedentary in its habits, and apparently feeds directly on the deposit. Of the other fairly common species *Goniada maculata* is probably another predator, *Dasybranchus caducus* another deposit feeder, while *Spiophanes kröyeri* and *Prionospio malmgreni* inhabit more or less permanent mud tubes and probably feed by means of the ciliary currents of the tentacles. A few species seem to be confined to mud; these are *Leanira tetragona*, *Paraonis gracilis*, *Dasybranchus caducus*, *Clymene gracilis* and *Rhodine loveni*, but all except *Dasybranchus* were rare, even in mud.

Thus, the majority of the polychaetes found in mud were either active, predatory species or non-selective deposit feeders. Selective deposit feeders, such as the spionids, were few, and the specialized filter-feeding sabellids and serpulids were absent.

THE MUDDY SAND GROUNDS

Where the bottom deposit was muddy sand with little or no gravel the main members of the polychaete fauna were the same as those of mud, but the

number of species present and the total population were much greater (Table 3). *Nephthys incisa* and *Glycera rouxi* were as abundant as in the mud, but *Lumbriconereis hibernica* and *Tharyx marioni* were rather less common. *Goniada maculata*, *Lumbriconereis gracilis*, *Spiophanes kröyeri*, *Chaetozone setosa*, *Diplocirrus glaucus*, *Owenia fusiformis*, *Myriochele heeri*, *Amphicteis gunneri*, *Trichobranchus glacialis* and *Terebellides stroemi* were all fairly common and characteristic species of this deposit. Most of these are sedentary species feeding on the deposit. *Owenia* has a crown of short ciliated tentacles with which it collects food as well as building its tube (Watson, 1900); *Myriochele* (a closely related species), though without tentacles, has a ciliated oral funnel with which it sets up a feeding current, and by closing this funnel it is able to reject unwanted particles. *Spiophanes*, *Diplocirrus*, *Amphicteis* and the terebellids probably feed by means of their ciliated tentacles (Blegvad, 1915; Hunt, 1925; Nicol, 1930; Mare, 1942; Dales, 1955). The cirratulid *Chaetozone* appears to feed directly on the deposit.

Among this largely burrowing and tubicolous polychaete population surface-living species were few, but probably all the aphroditids, except *Panthalis*, live on the surface of the deposit. Some of the tubicolous forms are dependent on sand grains or shell fragments for the formation of their tubes, for example *Owenia*, *Myriochele* and *Pectinaria* spp.; these are absent from mud and most common in the coarser muddy sands. Moreover, *Pectinaria* has a specialized method of feeding through a funnel in the deposit (Watson, 1928), which probably requires a fairly firm deposit for its construction.

MUDDY SAND WITH SHELL GRAVEL

Where shell gravel was mixed with the muddy sand the fauna was much richer, since it included most of the muddy sand species together with many of those from shell gravel; the number of species of Polychaeta (122) found in this deposit was greater than in any other investigated. The average density of the population was also fairly high (221 per m^2) though it formed less than 50% of the macrofauna. Most of the common species were burrowing or tubicolous, but with a greater gravel content the number of wandering and fixed species increased.

The most abundant species were *Lumbriconereis gracilis*, *L. hibernica* and *Owenia fusiformis*; the latter occurred at all stations and sometimes exceeded 100 per m^2 (Table 4). The other two were almost equally widely distributed but were less numerous. The other characteristic species of this mixed deposit were mainly burrowers and included *Nephthys incisa*, *Glycera rouxi*, *Goniada maculata*, *Eone nordmanni*, *Notocirrus scoticus* (all probably predators), *Laonice cirrata*, *Heterocirrus zetlandicus*, *Myriochele heeri*, *Amphicteis gunneri* and *Pectinaria auricoma* (deposit feeders of various types). The serpulid *Hydroides norvegica* was common on the larger shell fragments.

Polynoids, phyllodocids and syllids were more common in this mixed

deposit than in muddy sand alone and many species of the two latter groups were found in crevices in shell fragments. The sabellid *Potamilla reniformis* was also found in crevices, two others (*Euchone rubrocincta* and *Chone suspecta*) were found, apparently free-living, among gravel.

THE FINE GRAVEL GROUNDS

Fine shell gravel

Of the fine gravels the fine shell had the richest fauna, and the polychaetes formed a higher proportion of the total macrofauna than in the other two. This deposit also contained the greatest number of polychaetes per square metre of any of the grounds investigated with the grab sampler, although this was the ground on which the grab obtained the smallest samples. Thus, the population may be considerably greater than that shown by this survey.

The most common polychaetes (Table 5) were *Pholoë minuta*, *Syllis armillaris*, *Glycera lapidum*, *Nematonereis unicornis*, *Laonice cirrata*, *Aonides paucibranchiata*, *Owenia fusiformis*, *Polycirrus denticulatus* and *Hydroides norvegica*. The first four of these are active and may be carnivorous, *Laonice* does not seem to form a permanent tube and its large eye-spots suggest that it also has an active mode of life, but its feeding is probably ciliary, as in other spionids. The only common tubicolous polychaete among the infauna was *Owenia fusiformis*. *Onuphis conchylega* and *Eunice harassi* were found occasionally among the epifauna; these two species build tubes of gravel and shell fragments but, unlike *Owenia*, they can move about, dragging their tubes with them (Watson, 1903).

Sabellids and serpulids were common in fine shell gravel, the serpulids being attached to shell fragments. These two groups appear to be most common where there is some water movement.

Fine stony gravel

The fine stony gravels provide a rather similar habitat to the shell gravel but the density of the population appears to be lower. However, as the grab sampler was not used, this conclusion is only tentative. The percentage of polychaetes in the total population was lower than in either of the other fine gravels. *Glycera lapidum*, *Capitomastus minimus*, *Aonides paucibranchiata* and *Pista cristata* were the most common species (Table 5), except at station 34, where the gravel was mixed with muddy sand and large stones. Here many of the species were more characteristic of muddy sand than of stony gravel. *Scalisetosus pellucidus* was probably commensal with the ophiuroid *Ophiothrix fragilis*, which was present in large numbers.

Coralline gravel

The 'coralline' gravels were composed of irregular nodules of a species of *Lithothamnion*, mixed with muddy sand. While some of the animals were

characteristic of muddy sand, the fauna as a whole had more in common with that of the fine gravels. The polychaetes formed nearly 60% of the total macrofauna and the most common species were *Pholoë minuta*, *Lumbriconereis gracilis*, *Glycera lapidum*, *Nematonereis unicornis*, *Laonice cirrata*, *Capitomastus minimus* and *Pista cristata* (Table 5). *Lumbriconereis gracilis* appeared to be most common in mixed deposits of muddy sand and gravel, while *Capitomastus* and *Pista cristata* were more characteristic of stony gravels. The other species were common in fine shell gravel.

Thus, the polychaete fauna of all the fine gravels consisted mainly of active species, some burrowing (notably *Glycera lapidum*) and some living on the surface. Tubicolous species were few, except for the serpulids of the shell gravels. Few of the species feed directly on the deposit, without selection, and many of the active ones are probably predators.

THE COARSE GRAVEL GROUNDS

The fauna of coarse gravels appeared to be poorer than that of the fine gravels, but it is possible that further sampling and more careful examination of the gravel would reveal more species. Many tubicolous polychaetes were found in cracks and holes, but the external species were rather few and corresponded to the epifauna of the fine gravels. The common species of Polychaeta were mainly polynoids, sabellids and serpulids. *Halosydna gelatinosa* was fairly common, *Platynereis dumerilii* was frequent where there were fragments of algae among the gravel (it has been observed feeding on these), and the terebellid *Polymnia nebulosa* was common on large shell fragments. Both *Platynereis* and *Polymnia* inhabit semi-permanent mucous tubes. The serpulids occurred on shells and stones; *Pomatoceros triqueter* was the most common, being found on most shells and stones in every haul, while *Serpula vermicularis* and *Hydroides norvegica* were less common than *Pomatoceros* but nevertheless fairly frequent. *Sabellaria spinulosa* formed tubes of sand on shells and was widely distributed though not abundant.

Large stones had a surface fauna of *Pomatoceros* and *Hydroides* together with some polynoids, but the latter were more common in holes and crevices (Table 6). An examination of several lumps of limestone bored by *Hiatella* (station 50) showed the most common crevice-living species to be *Lepidonotus squamatus*, *Syllis armillaris*, *Autolytus aurantiacus* and *Polydora caeca*. The bigger holes in a large rock from station 74 contained many *Polymnia nebulosa* and several *Dasychone bombyx*, but the smaller species were not fully investigated. At station 32 the coarse stony gravel was mixed with muddy sand and the fauna as a whole was rather sparse, consisting mainly of muddy sand species, though the polynoid *Harmothoë impar* was common on the stones.

Crevices and holes in shells

The fauna of crevices in shells included many of the species found in holes in stones and also some boring forms. Of the polychaetes, only some species of *Polydora* and *Dodecaceria* are definitely known to bore into shells (e.g. Söderström, 1923), but it is possible some other species may do so. The burrows of *Polydora*, *Cliona* (the boring sponge) and *Phoronis ovalis* Wright were common in shells and shell fragments, and these were utilized by many other animals, some of which form their own tubes inside. The polychaetes appeared to be the most common animals in this habitat and thirty-two species were found (Table 7). The commonest and most widespread were *Syllis armillaris*, *Nematonereis unicornis*, *Polydora caeca*, *Polycirrus denticulatus* and *Potamilla reniformis*, but *Syllis variegata*, *Autolytus aurantiacus*, *Polydora ciliata*, *P. flava* and *Lumbriclymene minor* were sometimes common. Syllids and phyllodocids were abundant in this habitat but only one aphroditid, *Pholoë minuta*, was found. Oyster shells (*Ostrea edulis* L.) contained a large number of species but those of other molluscs (*Modiolus*, *Pecten* and *Glycimeris*) had a poorer fauna. Many of the species found in shells also inhabited the empty tubes of serpulids.

Hydroids

Several species of hydroids were common on shells and stones in the gravel deposits. They had a characteristic fauna, consisting mainly of polychaetes (Table 8), which differed from the gravel fauna. The serpulids *Hydroides norvegica* and *Spirorbis spirillum* were common, the latter often abundant. Many syllids lived among the branches, usually in their own mucous tubes, and several aphroditids were found, though some of these may have been inhabitants of the gravel on which the hydroids grew. Members of these two families may feed on the hydroids, but most of the other polychaetes are detritus—or filter—feeders.

Sponges

Sponges as well as hydroids are part of the epifauna of coarse gravel, and they also have a typical fauna. They were most frequent on and among the shells of *Modiolus modiolus* (L.), which are common in an area to the south-west of the Isle of Man (Fig. 1, A_1). This fauna has not been much investigated, but *Syllis spongicola* seems to be the most characteristic species. A few other syllids were found, notably *S. armillaris*, but they were also common species on hydroids and gravel.

THE CLEAN SAND GROUNDS

The offshore clean sand (Fig. 1, B) was sampled only in Port Erin Bay and off Niarbyl. A series of grab samples taken in Port Erin Bay (Table 9,

station 59) indicated a fauna fairly rich in species of polychaetes and similar to that found at E.L.W.S. on the beach (c.f. Pirrie, Bruce & Moore, 1932; Moore, 1933). The grab sampler did not dig very deeply on this sand and the samples cannot have included the deeper burrowing animals; however, the number of animals per m^2 at 3–4 fm. exceeded the number found at E.L.W.S. on the beach. Two dredge hauls in the bay (65) brought up rather more surface-living species than the grab sampler. The common polychaetes, on or near the surface, were *Sthenelais limicola*, *Kefersteinia cirrata* and *Pista cristata*, while the common tubicolous and burrowing species were *Scoloplos armiger*, *Chaetozone setosa* and *Clymene oerstedii*. None of the three surface-living species has been found in the sandy beach, but *Scoloplos* is a characteristic intertidal species and *Clymene* is fairly common around and below M.L.W.S.

A small area of sand near Niarbyl was also sampled (31) and *Scoloplos armiger* and *Heterocirrus zetlandicus* were found to be common. Single specimens of *Nephthys cirrosa* were taken at Niarbyl and Port Erin, and the species may be common in the lower layers of the sand, which were not reached by either the grab sampler or the dredge.

DISCUSSION

The results of this survey indicate that many species of Polychaeta are widely distributed in the various types of habitat examined, but that, nevertheless, the polychaete fauna of each is distinctive. The density of the population varies very much with the type of deposit, as does the number of species present (Table 10). Environmental factors, apart from differences in the deposit, appear to have little effect on the fauna. Any direct effect of water movement on the fauna is masked by its effect on the composition of the deposit and these two factors cannot be separated. Differences in depth, unaccompanied by differences in the deposit, appear not to have much influence on the composition of the fauna. However, the depths in the area studied are comparatively small.

Most of the habitats examined were composite. That is, the softer deposits contained gravel and the gravels contained some sand or muddy sand. This may account for the wide distribution of some species. For example, seven species were found in all four main types of deposit but, of these, four glycerids, *Glycera rouxi*, *G. gigantea*, *Goniada maculata* and *Eone nordmanni*, are more characteristic of soft deposits and only occur in gravel where it is mixed with sand or muddy sand. *Nematonereis unicornis* occurs in all four deposits but is always associated with gravel, often living in crevices in shell fragments. The other two species, *Mystides limbata* and *Glycera convoluta*, are not common in any deposit and their distribution is rather sporadic.

The extent of the distribution of each species appears to depend mainly on

its mode of life and feeding habits. Thus, carnivorous species are found in all habitats, but the burrowing forms, such as *Nephthys*, are restricted to the softer deposits, while the surface-living forms, such as the aphroditids, are more common on the harder grounds. Tubicolous species, such as *Owenia* and *Pectinaria*, are limited in their distribution by their dependence on definite sizes of particles for the formation of their tubes, while the serpulids require hard substrata for the attachment of their calcareous tubes. The distribution of filter-feeders may be limited by high concentrations of silt in the water, for no serpulids or sabellids were found in the mud, and few were found in the muddy sand (cf. Loosanoff & Tommers, 1948).

There were many examples of related species, of similar body form, being found in very different types of deposit. Thus, the Capitellidae were represented by *Dasybranchus caducus* in mud, *Notomastus latericeus* in muddy sand, *Capitomastus minimus* in fine gravel and *Capitella capitata* in sand. Again, the common species of *Lumbriconereis* (Eunicidae) in mud and muddy sand was *L. hibernica*, while *L. fragilis* was characteristic of coarse gravel and *L. gracilis* was common in the intermediate types of deposit. The factors involved in the distribution of these species may include the method of reproduction and the larval development and settlement (cf. Thorson, 1950; Wilson, 1952), as well as possible differences in feeding habits or food. Unfortunately, very little information is available on the reproduction of any species, since sampling was insufficient for detailed investigation of the breeding period or annual fluctuations in the population of the various species.

The numerical proportion of polychaetes was high in comparison with the other main faunistic groups at all stations where the animals were counted (Table 10). However, on the muddy grounds the heart-urchin *Brissopsis* and the crustacean *Calocaris* appear to form the greater part of the population by weight, though present in small numbers, and in the coarser grounds the Mollusca (or sometimes the Ophiuroidea) probably form the greatest proportion.

The density of the polychaete population on the grounds surveyed is low compared with that of some other British localities, and the species rarely attain the maximum size recorded by other authors. The maximum population found was 575 polychaetes per m^2 (station 19), and this compares badly with, for example, the 2000 per m^2 in the Rame mud near Plymouth (Mare, 1942).

The paucity of the fauna may be correlated with the low biomass found by Jones (1951, 1952) for the offshore grounds of the Isle of Man, compared with the grounds off the Cumberland coast. He considered that the low biomass was associated with a low run-off from the land in the area, and a low concentration of organic matter in the bottom deposits.

SUMMARY

During 1952–54 a survey was made of the Polychaeta living in the offshore bottom deposits of the south of the Isle of Man. These deposits range from stones and coarse gravel to soft mud; samples were obtained with a van Veen grab sampler and various dredges.

It was found that, although some species were widely distributed, each bottom deposit had a typical fauna, and that where the deposits graded into one another the polychaete fauna was also mixed. The distribution of each species appears to depend mainly on its mode of life and feeding habits; some can exist in several types of deposit or habitat, while others are very much restricted in their distribution.

The Polychaeta formed a high proportion, numerically, of the macrofauna, exceeding any other animal group, except possibly in the coarse gravels. However, the density of the polychaete population was low compared with other areas of the British Isles, and this may be correlated with the comparatively low biomass in the area.

REFERENCES

ADMIRALTY, HYDROGRAPHIC DEPARTMENT, 1948. *West Coast of England Pilot*. 9th ed. London.

BLEGVAD, H., 1915. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. *Rep. Danish biol. Sta.*, Vol. 22, pp. 43–78.

DALES, R. P., 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

FAUVEL, P., 1923. Polychètes errantes. *Faune Fr.*, No. 5, 488 pp.

—— 1927. Polychètes sédentaires. *Faune Fr.*, No. 16, 494 pp.

HOLME, N. A., 1954. The ecology of British species of *Ensis*. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 145–72.

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 560–99.

JONES, N. S., 1940. The distribution of the fauna and bottom deposits off Port Erin. *Proc. Lpool biol. Soc.*, Vol. 53, pp. 1–34.

—— 1948. The ecology of the Amphipoda of the south of the Isle of Man. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 400–39.

—— 1951. The bottom fauna off the south of the Isle of Man. *J. Anim. Ecol.*, Vol. 20, pp. 132–44.

—— 1952. The bottom fauna and the food of flatfish off the Cumberland coast. *J. Anim. Ecol.*, Vol. 21, pp. 182–205.

LOOSANOFF, V. L. & TOMMERS, F. D., 1948. Effect of suspended silt and other substances on rate of feeding of oysters. *Science*, Vol. 107, pp. 69–70.

MARE, M. F., 1942. A study of a marine benthic community, with special reference to the micro-organisms. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 517–554.

MATTHEWS, D. J., 1914. The salinity and temperature of the Irish Channel and the waters south of Ireland. *Sci. Invest. Fish. Br. Ire.*, Vol. 4, pp. 1–26.

MOORE, H. B., 1933. A comparison of the sand fauna of Port Erin Bay in 1900 and 1933. *Proc. malac. Soc. Lond.*, Vol. 20, pp. 285–294.

NICOL, E. A. T., 1930. The feeding mechanism, formation of the tube and physiology of digestion in *Sabella pavonina*. *Trans. roy. Soc. Edinb.*, Vol. 56, pp. 537–98.

PIRRIE, M. E., BRUCE, J. R. & MOORE, H. B., 1932. A quantitative study of the sandy beach at Port Erin. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 279–96.

SANDERS, H. L., 1956. The biology of marine bottom communities. Oceanography of Long Island Sound. X. *Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 345–414.

SLINN, D. J., 1956. Phosphate and oxygen in sea water off the Isle of Man during the years 1952–54. *Rep. mar. biol. Sta. Pt Erin*, No. 68, pp. 30–8.

SÖDERSTRÖM, A., 1923. Über das Bohren der *Polydora ciliata*. *Zool. Bidr. Uppsala*, Bd. 8, pp. 319–26.

SOUTHWARD, E. C., 1956. On some Polychaeta of the Isle of Man. *Ann. Mag. nat. Hist.* Ser. 12, Vol. 9, pp. 257–79.

THAMDRUP, H. M., 1938. Der van Veen-Bodengreifer. Vergleichsversuche über die Leistungsfähigkeit des van Veen- und des Petersen-Bodengreifers. *J. Cons. int. Explor. Mer*, Vol. 13, pp. 206–12.

THORSON, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, Vol. 25, pp. 1–45.

WATSON, A. T., 1900. On the structure and habits of the Polychaeta of the family Ammocharidae. *J. Linn. Soc.* (*Zool.*), Vol. 48, pp. 230–60.

—— 1903. Observations on the habits of the Onuphidae and on the internal structures with which they fortify their homes. *Proc. Lpool biol. Soc.*, Vol. 17, pp. 303–18.

—— 1928. Observations on the habits and life history of *Pectinaria koreni* Malmgren. *Proc. Lpool biol. Soc.*, Vol. 42, pp. 25–60.

WILSON, D. P., 1952. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis*. *Ann. Inst. océangraph. Monaco*, T. 27, pp. 49–156.

TABLE 1. GRADE COMPOSITION OF SOME OFFSHORE DEPOSITS

Station	Particle size (mm diameter) >10 %	10–2·0 %	2·0–1·0 %	1·0–0·5 %	0·5–0·25 %	0·25–0·21 %	0·21–0·124 %	0·214–0·0313 %	<0·0313 %	Type of deposit	Depth (fm.)
39	16·8	49·69	12·77	10·28	4·57	3·45	4·22	0·84	0·28	Fine stony gravel	5
33	14·17	14·592	12·633	11·972	17·934	5·432	17·618	4·407	1·253	Muddy sand and shell	17
35	6·2	2·737	1·935	5·895	28·228	11·790	30·609	8·225	4·380		18
60	0	0·592	3·069	8·375	30·538	12·549	33·850	5·385	5·639		31
45	0	6·070	9·844	9·811	21·766	9·926	23·571	9·734	9·269		32
44	0	0·020	1·270	6·803	29·468	10·143	32·542	12·623	7·127		34
64	0	0	0·165	1·437	9·125	6·139	58·652	15·126	9·349	Muddy sand	37
41	0	2·162	1·948	3·934	17·029	6·592	24·136	27·609	16·586	Muddy sand and *Turritella* shells	35
61	0	0·082	0·601	2·405	10·032	4·756	30·836	28·403	22·881		41
43	0	0	0·080	0·040	0·080	0·040	0·240	50·061	49·503	Mud	35
42	0	0	0·035	0·035	0·105	0·035	0·140	47·377	52·270		38
56	0	0·141	0·100	0·463	9·651	5·138	13·439	19·021	52·043		58
57	0	0·033	0·067	0·067	0·067	0·033	0·336	24·789	74·604		65
58	0	0·028	0·056	0·056	0·085	0·056	0·226	29·553	69·935		70

TABLE 2. POLYCHAET FAUNA OF MUD GROUNDS

(As number per square metre.)

Station number	*43*	*42*	*15*	*7*	*16*	*8*	*9*	*17*	*37*	*62*	*55*	*18*	*56*	*63*	*57*	*58*
Depth (fathoms)	35	38	40	40	45	45	50	50	50	50	53	55	58	60	65	70
Number of grab hauls	3	3	5	5	5	5	5	5	3	3	2	5	2	3	2	2
Species																
Panthalis oerstedi	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Leanira tetragona	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	—	—	—	—	—	7	3	—	—	—	—	—	—
Ophiodromus flexuosus	—	—	—	—	—	—	2	—	—	—	—	—	5	—	—	—
Ancistrosyllis groenlandica	—	3	4	—	—	—	—	2	3	—	—	—	—	—	—	—
Nephthys incisa	7	20	18	10	10	12	2	12	23	10	15	2	15	7	—	15
Glycera convoluta	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—
G. gigantea	—	—	—	—	—	—	2	2	—	—	—	—	—	—	—	—
G. rouxi	7	7	2	6	10	8	6	2	13	7	5	2	5	7	—	5
Goniada maculata	3	—	4	4	2	4	—	—	3	—	—	—	—	—	—	—
Eone nordmanni	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—
Nematonereis unicornis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Lumbriconereis hibernica	33	17	4	12	8	14	10	6	23	7	15	8	5	10	5	15
L. gracilis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Spiophanes kroyeri	—	—	4	2	12	4	—	4	7	3	—	—	—	3	—	10
Polydora sp.	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
Prionospio malmgreni	—	—	—	—	—	—	2	2	7	—	5	6	—	—	—	10
Paraonis gracilis	—	—	—	—	—	—	—	—	—	2	3	2	—	7	—	—
Tharyx marioni	13	10	—	4	12	18	22	6	20	13	15	6	25	37	20	35
Diplocirrus glaucus	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Scalibregma inflatum	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Dasybranchus caducus	—	3	6	4	2	10	—	2	3	3	—	—	—	—	10	—
Ammotrypane aulogaster	—	—	—	4	—	2	2	2	—	—	—	—	5	—	—	—
Clymene gracilis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Rhodine loveni	—	—	—	—	—	—	—	2	—	—	—	—	5	7	10	—
Ampharete grubei	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Pectinaria sp.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 3. POLYCHAET FAUNA OF MUDDY SAND

(As number per square metre.)

Station number	*3*	*23*	*4*	*24*	*19*	*6*	*41*	*64*	*29*	*36*	*61*
Depth (fathoms)	25	25	30	30	30	35	35	37	40	40	41
Number of grab samples	5	5	5	5	5	5	3	5	3	3	3
Species											
Aphrodite aculeata	2	—	2	—	—	—	—	—	—	—	—
Harmothoë antilopis	—	—	2	—	—	—	—	—	—	—	—
Lagisca extenuata	—	—	—	—	—	—	—	2	3	—	—
Polynoë kinbergi	—	—	2	—	—	—	—	—	—	—	—
Panthalis oerstedi	—	—	—	—	—	—	—	—	3	—	—
Phyllodoce mucosa	—	—	—	—	—	—	—	—	—	3	—
Eulalia sanguinea	—	—	—	—	2	—	—	2	—	—	—
Protomystides bidentata	2	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	—	—	—	3	—	3	7	3
Phyllodocidae: unidentified	—	—	—	—	—	—	—	2	—	—	—
Ophiodromus flexuosus	—	—	2	2	—	—	—	—	—	—	—
Hesionidae: unidentified	—	—	—	—	—	—	—	—	3	—	—
Ancistrosyllis groenlandica	—	2	—	6	2	4	—	2	—	—	—
Exogone gemmifera	—	—	—	—	2	—	—	—	—	—	—
Autolytus aurantiacus	—	2	—	—	—	—	—	—	—	—	—
Nephthys incisa	14	10	10	12	3	16	20	10	17	30	13
N. hombergi	—	2	2	—	—	—	—	—	—	—	—
Glycera rouxi	2	2	2	8	—	18	7	—	13	7	10

TABLE 3 (*continued*)

Station number ...	*3*	*23*	*4*	*24*	*19*	*6*	*41*	*64*	*29*	*36*	*61*
G. gigantea	—	—	—	—	—	2	—	—	—	—	—
G. convoluta	—	—	—	—	—	—	—	—	—	3	—
Goniada maculata	—	6	6	2	6	2	3	14	—	3	—
Eone nordmanni	—	—	—	—	—	4	—	—	—	7	—
Lumbriconereis hibernica	4	4	2	14	—	14	7	2	3	10	3
L. gracilis	6	8	2	6	4	2	7	2	—	—	—
Notocirrus scoticus	—	6	2	2	2	—	—	4	—	—	—
Drilonereis filum	—	—	—	—	—	2	—	—	—	—	—
Nerinides tridentata	—	2	—	2	—	2	—	2	—	3	—
Laonice cirrata	12	—	—	—	—	—	—	—	—	—	—
Spiophanes bombyx	—	10	—	—	4	6	—	—	—	3	—
S. kröyeri	—	14	12	10	24		10	2	—	3	3
Polydora flava	—	—	—	—	—	—	3	2	—	—	—
Prionospio malmgreni	—	10	—	6	2	6	—	6	—	10	—
P. steenstrupi	—	—	4	—	—	—	—	—	—	—	—
Aricidea branchiata	—	2	—	—	—	—	—	—	—	—	—
Paraonis lyra	—	—	—	—	—	—	3	—	—	—	—
P. gracilis	—	—	—	—	—	—	—	—	—	—	3
Chaetopterus variopedatus	—	—	—	—	—	—	—	2	—	—	—
Heterocirrus zetlandicus	—	—	—	—	4	—	—	8	—	—	—
Heterocirrus sp.	—	8	—	—	—	—	—	—	—	—	—
Chaetozone setosa	2	10	6	4	6	—	—	—	—	3	—
Tharyx marioni	—	2	—	6	2	4	10	2	10	20	43
Diplocirrus glaucus	2	4	4	—	10	—	3	8	—	13	—
Scalibregma inflatum	—	—	—	—	—	—	—	2	—	3	—
Notomastus latericeus	—	6	—	6	—	—	—	2	—	—	—
Dasybranchus caducus	—	—	—	—	—	—	—	—	—	—	3
Owenia fusiformis	26	192	16	2	386	—	—	12	—	—	—
Myriochele heeri	8	12	20	2	46	—	3	62	—	3	—
Ammotrypane aulogaster	2	—	—	—	—	—	—	—	—	—	—
Rhodine loveni	—	—	—	—	2	—	—	—	—	—	—
Clymene affinis	—	2	—	2	8	—	—	12	—	6	—
Clymene sp.	—	2	—	—	—	—	—	—	—	—	—
Leiochone clypeata	—	2	—	—	—	—	—	—	—	—	—
Ampharete grubei	—	—	—	2	6	—	—	—	—	3	—
Amphicteis gunneri	6	6	8	2	2	4	—	—	3	—	—
Sabellides octocirrata	—	—	—	—	4	—	—	—	—	—	—
Pectinaria auricoma	8	8	4	—	10	—	—	—	—	3	—
P. koreni	2	6	2	—	14	—	—	—	—	—	—
Pista cristata	—	2	—	—	—	—	—	—	—	—	—
Thelepus cincinnatus	—	—	—	—	2	—	—	—	—	—	—
Polycirrus denticulatus	—	—	2	—	—	2	—	—	—	—	—
P. plumosus	—	—	2	—	6	—	—	—	3	—	—
Trichobranches glacialis	—	10	10	—	6	2	—	2	—	7	—
Terebellides stroemi	2	10	8	2	2	—	—	4	—	7	—
Sabella pavonina	—	—	—	—	2	—	—	—	—	—	—

TABLE 4. POLYCHAET FAUNA OF MUDDY SAND MIXED WITH SHELL GRAVEL

(Number per square metre or relative abundance.)

Station number ...	*33*	*35*	*2*	*22*	*13*	*25*	*28*	*14*	*38*	*60*	*45*	*44*	*76*	*77*	*78*
Depth (fathoms) ...	17	18	20	20	20	25	29	30	30	31	32	34	35	35	35
Dredge (D) or grab (G) ...	D	D	G	G	G	G	G	G	G	G	D	D	G	G	G
Number of samples ...	1	1	5	4	5	5	3	5	3	3	1	1	10	10	10
Species															
Aphrodite aculeata	—	p	—	—	2	—	—	—	—	—	—	p	2	—	—
Lepidonotus squamatus	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Harmothoë impar	p	p	—	—	—	—	—	—	—	—	p	—	—	—	—

TABLE 4 (*continued*)

Station number	... 33	35	2	22	13	25	28	14	38	60	45	44	76	77	78
H. antilopis	—	—	6	—	—	—	7	2	—	—	—	—	1	—	2
H. haliaeti	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
H. longisetis	—	p	—	3	—	2	—	—	—	—	—	—	—	—	1
H. sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Lagisca extenuata	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Sthenelais minor	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
Pholoë minuta	p	p	2	3	2	—	3	—	—	—	p	—	—	—	—
Phyllodoce mucosa	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Eulalia bilineata	p	—	—	—	12	—	—	—	—	—	—	—	—	—	—
E. fucescens	p	p	—	—	—	—	—	—	—	—	—	—	—	—	—
E. sanguinea	p	—	2	—	—	2	—	—	—	—	—	—	—	—	2
E. macroceros	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
Notophyllum foliosum	—	—	—	3	—	—	—	—	—	—	p	—	—	—	—
Eteone longa	p	p	2	—	—	—	—	—	—	—	—	—	—	—	—
Protomystides bidentata	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	6	6	2	—	—	3	7	—	—	—	—	—
Phyllodocidae: unidentified	—	—	—	—	—	2	—	—	—	—	—	—	1	1	—
Podarke pallida	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Castalia punctata	p	p	—	5	—	2	—	—	—	—	—	—	—	—	—
Ancistrosyllis groenlandica	—	—	—	—	—	—	—	2	3	—	—	—	—	—	—
Syllis variegata	—	p	—	—	2	—	—	—	—	—	—	—	—	—	—
S. armillaris	c	—	4	—	28	—	—	—	—	—	p	—	—	—	—
S. cornuta	—	—	—	—	4	2	3	2	7	—	—	—	—	—	—
Eusyllis blomstrandi	—	p	—	3	—	—	—	—	—	—	—	—	—	—	—
Odontosyllis fulgurans	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O. gibba	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eurysyllis tuberculata	p	—	—	—	—	—	—	—	—	3	—	—	—	—	—
Exogone gemmifera	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
E. verugera	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
E. hebes	p	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Sphaerosyllis hystrix	p	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Autolytus aurantiacus	p	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Nereis pelagica	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nephthys incisa	—	p	4	3	—	6	3	4	—	—	—	p	19	36	10
N. hombergi	—	—	—	—	—	—	—	—	—	—	—	p	—	—	—
N. rubella	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Ephesia gracilis	p	p	—	—	—	—	—	—	—	—	—	—	—	—	—
E. periphatus	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glycera rouxi	—	p	—	5	12	—	27	2	13	—	p	p	6	2	3
G. gigantea	—	—	—	—	—	4	—	—	7	—	—	—	—	2	1
G. convoluta	—	p	6	—	—	—	—	—	—	—	—	p	—	—	—
G. lapidum	c	—	8	8	4	—	—	—	—	3	p	—	—	—	—
G. capitata	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Goniada maculata	—	p	—	3	6	—	27	—	7	—	p	c	6	2	9
G. norvegica	—	—	—	3	2	—	—	—	—	—	p	—	—	—	—
Eone nordmanni	p	p	4	3	2	—	—	2	—	—	—	p	2	1	3
Eunice harassi	p	p	—	—	—	—	3	—	—	—	—	p	—	—	1
Onuphis conchylega	p	p	—	3	—	2	3	2	7	—	—	p	—	—	3
Nematonereis unicornis	c	c	4	3	46	2	—	—	3	23	p	—	—	—	—
Lumbriconereis gracilis	c	c	4	20	12	4	13	10	33	7	—	p	9	1	6
L. latreilli	p	—	—	—	—	—	3	—	7	—	—	—	—	—	—
L. impatiens	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—
L. hibernica	p	p	8	8	14	4	17	2	17	3	—	p	3	4	4
L. fragilis	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—
Notocirrus scoticus	—	p	2	8	4	—	23	6	7	—	—	p	5	—	4
Drilonereis filum	—	—	2	—	—	—	—	—	—	—	p	—	—	—	—
Eunicidae: unidentified	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Aricia cuvieri	—	—	—	—	—	—	—	—	3	—	p	—	—	1	—
Aricidea suecica	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
A. minuta	—	—	—	—	—	—	—	—	—	—	—	p	—	—	—
A. branchiata	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—
Laonice cirrata	—	p	14	30	6	4	3	2	7	7	—	—	9	—	11

TABLE 4 (*continued*)

Station number	... 33	35	2	22	13	25	28	14	38	60	45	44	76	77	78
Aonides paucibranchiata	p	—	4	—	4	—	—	—	—	—	—	—	—	—	—
Spiophanes bombyx	—	—	—	—	4	2	—	8	—	—	—	—	4	—	—
S. kröyeri	—	—	—	8	—	6	23	—	3	—	p	—	4	1	5
Polydora flava	p	p	—	—	2	—	—	—	—	—	—	—	1	—	—
P. ciliata	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—
P. caulleryi	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Polydora sp.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Prionospio malmgreni	—	—	—	—	—	—	7	—	—	—	—	—	2	—	1
P. steenstrupi	—	—	—	—	—	—	—	2	—	—	—	—	2	—	2
Poecilochaetus serpens	—	—	—	3	—	—	—	—	—	—	p	—	1	1	—
Phyllochaetopterus socialis	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Heterocirrus zetlandicus	c	c	—	8	32	4	3	—	3	7	p	p	1	—	—
H. caput-esocis	p	p	—	—	2	2	—	—	—	—	—	—	—	—	—
Tharyx marioni	—	—	—	8	2	2	13	—	3	1	p	—	—	1	—
Chaetozone setosa	—	—	6	3	2	2	3	6	—	—	—	—	10	—	11
Dodecaceria concharum	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Macrochaeta clavicornis	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Zeppelinia dentata	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Diplocirrus glaucus	—	—	—	—	—	2	—	2	3	—	—	—	1	7	7
Scalibregma inflatum	p	—	—	—	—	—	—	—	—	—	—	p	—	—	—
Notomastus latericeus	—	p	—	10	2	2	17	—	17	—	p	—	1	1	—
Owenia fusiformis	p	c	6	95	4	114	3	26	43	10	p	c	42	12	56
Myriochele heeri	—	p	—	13	2	24	40	8	3	—	—	p	4	6	42
Ammotrypane aulogaster	p	—	—	—	2	—	—	—	—	—	—	—	—	2	2
Clymene affinis	—	—	2	—	2	—	—	—	—	—	—	—	—	—	—
C. robusta	—	—	—	3	8	—	3	—	7	—	—	—	—	—	—
Clymene sp.	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nicomache lumbricalis	—	p	—	—	—	2	—	—	—	—	—	—	—	—	—
Praxillura longissima	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Leiochone clypeata	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—
Ampharete grubei	p	—	—	—	6	—	7	—	3	10	—	—	—	1	—
Amphicteis gunneri	—	p	2	20	10	2	10	8	7	—	p	—	9	—	15
Anobothrus gracilis	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Sabellides octocirrata	—	—	—	—	2	—	—	4	—	—	—	—	—	—	—
Melinna cristata	—	p	—	—	—	—	—	2	—	—	p	—	—	—	—
M. palmata	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Sabellaria spinulosa	—	p	4	—	—	—	—	—	—	—	—	—	—	—	—
Pectinaria auricoma	—	p	4	—	2	6	—	34	10	—	—	c	23	10	16
P. koreni	—	—	—	5	—	2	—	8	3	—	—	p	7	—	—
Amphitrite gracilis	p	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Phisidia aurea	p	—	—	4	—	2	3	—	—	3	p	—	—	—	—
Pista cristata	—	p	—	20	—	2	—	—	7	—	p	—	—	—	—
Thelepus cincinnatus	—	—	—	3	6	—	—	—	—	—	—	—	—	2	2
Polycirrus denticulatus	p	—	—	5	—	—	—	—	—	—	—	—	1	—	—
P. plumosus	—	—	—	—	—	2	—	—	—	—	—	—	4	—	—
Amaea trilobata	—	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Lysilla loveni	—	—	—	3	2	—	—	—	—	—	—	—	—	—	—
Trichobranchus glacialis	—	p	2	3	2	—	—	4	—	—	—	—	12	—	8
Terebellides stroemi	—	p	2	10	2	4	3	2	—	—	—	p	6	—	6
Terebellidae: unidentified	—	—	—	—	—	—	—	—	—	7	p	—	2	1	—
Sabella pavonina	—	—	—	—	20	—	—	12	—	—	p	—	—	—	—
Dasychone bombyx	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—
Potamilla reniformis	p	—	—	—	10	—	—	—	—	—	—	—	—	—	—
Chone suspecta	p	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Euchone rubrocincta	p	p	—	10	2	4	—	—	3	—	—	—	—	—	—
Myxicola infundibulum	—	—	—	—	—	—	—	2	3	—	—	—	—	—	—
Serpula vermicularis	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Hydroides norvegica	p	—	—	15	22	2	3	2	13	20	p	p	—	—	1
Pomatoceros triqueter	p	—	4	—	—	—	—	—	—	—	p	—	—	—	—
Spirorbis spirillum	—	—	3	—	—	—	—	—	—	—	—	p	—	—	—

p = present c = common.

TABLE 5. POLYCHAET FAUNA OF FINE GRAVELS

(Expressed as numbers per square metre, or relative abundance.)

	Fine shell gravel					Fine stony gravel					Coralline gravel			
Station number ...	*71*	*20*	*21*	*26*	*27*	*75*	*10*	*11*	*39*	*34*	*1*	*5*	*12*	*30*
Depth (fathoms) ...	18	20	20	25	30	6	9	10	10	15	15	15	15	15
Dredge (D) or Grab (G)...	D	G	G	G	G	D	D	D	D	D	G	G	G	G
Number of hauls	1	3	3	5	3	1	1	1	1	1	4	5	5	1
Species														
Aphrodite aculeata	p	—	—	—	—	—	—	—	—	p	—	—	—	—
Malmgrenia castanea	—	—	—	2	—	—	—	—	—	—	—	—	2	—
Gattyana cirrosa	—	—	—	—	—	—	—	—	—	p	—	2	—	—
Lepidonotus squamatus	p	—	—	—	—	—	p	—	—	p	—	—	—	—
Harmothoë impar	p	17	7	—	—	—	—	—	—	—	—	—	—	—
H. antilopis	—	3	—	—	—	—	—	—	—	—	—	2	4	—
H. haliaeti	—	—	—	—	—	p	—	—	—	—	—	—	—	p
H. longisetis	—	—	—	—	—	p	p	—	—	—	—	—	—	—
H. lunulata	—	—	3	—	—	—	p	—	—	—	—	—	—	p
Harmothoë sp.	—	—	—	—	—	—	p	—	—	—	3	2	2	—
Halosydna gelatinosa	p	—	—	—	—	—	—	—	—	p	—	—	—	—
Lagisca extenuata	p	—	—	—	—	—	p	—	—	c	—	—	—	p
Scalisetosus pellucidus	—	—	—	—	—	—	—	—	—	c	—	—	—	—
Pholoë minuta	p	3	3	4	10	p	p	—	—	p	5	6	10	c
Pisione remota	—	—	—	—	—	—	p	p	—	—	—	—	2	—
Phyllodoce kosteriensis	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Notophyllum foliosum	p	—	3	6	—	—	—	—	—	—	—	—	—	—
Eulalia viridis	—	—	—	4	—	—	—	—	—	—	—	—	—	p
E. bilineata	—	—	—	6	—	—	—	—	—	—	—	—	—	—
E. sanguinea	—	23	3	2	—	—	p	—	—	p	—	—	—	—
Eulalia sp.	—	—	—	—	3	—	—	p	—	—	—	—	—	—
Eteone foliosa	—	3	—	—	3	—	—	—	—	—	—	—	—	—
E. longa	—	—	—	—	—	—	—	—	p	—	—	—	—	—
Mystides limbata	—	—	7	—	—	—	p	—	—	—	—	—	—	—
Podarke pallida	—	—	—	—	—	—	—	—	—	p	—	—	—	p
Castalia punctata	—	7	63	10	—	—	—	—	—	p	—	—	—	p
Kefersteinia cirrata	—	—	—	—	—	—	p	—	—	p	—	—	—	p
Syllis armillaris	—	17	23	24	3	—	p	—	—	—	—	—	2	—
S. cornuta	—	7	—	4	—	—	—	—	—	—	—	2	2	—
Syllis sp.	—	—	—	6	—	—	—	—	—	—	—	—	—	—
Trypanosyllis coeliaca	—	3	—	6	—	p	—	—	—	—	—	—	—	—
Odontosyllis fulgurans	—	—	—	—	—	—	p	—	—	c	—	6	—	—
Eusyllis blomstrandi	p	13	—	—	—	—	—	—	—	—	—	—	—	p
Eurysyllis tuberculata	—	13	3	—	—	—	—	—	—	—	—	—	—	—
Exogone gemmifera	—	3	3	—	—	—	—	—	—	—	—	—	—	—
Sphaerosyllis hystrix	—	—	3	—	3	—	p	p	—	—	—	—	—	—
S. bulbosa	—	—	—	2	—	—	p	—	—	—	—	—	—	—
Autolytus rubropunctatus	—	—	3	—	—	—	—	—	—	—	—	—	—	—
A. prolifer	—	3	—	2	3	—	—	—	—	—	—	—	—	—
A. aurantiacus	—	—	—	—	—	—	—	—	—	—	—	8	—	—
Nereis zonata	—	7	—	—	—	—	—	—	—	—	—	—	—	—
N. pelagica	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Platynereis dumerilii	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nephthys incisa	p	—	—	—	—	—	—	—	—	p	—	4	2	—
N. rubella	—	—	—	—	—	—	—	—	—	—	—	2	—	—
Ephesia gracilis	—	3	—	—	—	—	—	—	—	p	3	2	—	c
E. peripatus	—	—	3	—	—	p	p	—	—	—	—	—	2	p
Glycera lapidum	—	20	7	18	27	a	a	a	a	p	10	32	34	a
G. capitata	p	—	—	—	—	—	—	—	—	—	—	—	—	—
G. rouxi	—	—	—	—	3	p	—	—	—	p	3	2	6	p
G. gigantea	—	—	—	4	—	—	—	—	—	—	—	2	—	p
G. convoluta	—	—	—	—	—	—	—	—	—	c	—	—	—	—
Goniada maculata	—	—	—	—	3	—	—	—	—	—	—	2	—	p
Eone nordmanni	—	—	3	4	—	—	—	—	—	c	—	4	2	—

TABLE 5 (*continued*)

Station number	... *71*	*20*	*21*	*26*	*27*	*75*	*10*	*11*	*39*	*34*	*1*	*5*	*12*	*30*
Eunice harassi	p	—	—	2	—	—	—	—	—	—	—	—	—	—
Onuphis conchylega	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nematonereis unicornis	—	10	20	14	10	—	p	—	—	p	35	18	10	p
Lumbriconereis gracilis	—	10	—	4	13	p	p	—	—	c	35	96	40	c
L. fragilis	—	—	—	2	—	p	p	—	—	—	—	2	2	c
L. hibernica	—	—	—	—	3	—	—	—	—	p	—	—	—	—
Drilonereis filum	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Staurocephalus neglectus	—	—	—	—	—	p	p	p	—	—	—	—	—	—
Aricia cuvieri	—	3	—	—	—	—	—	—	—	—	—	—	4	—
Laonice cirrata	p	7	7	34	17	p	c	—	p	p	13	10	22	c
Aonides paucibranchiata	—	30	3	42	7	c	c	c	c	—	—	—	2	p
Spiophanes bombyx	—	—	—	—	—	—	—	—	p	p	—	—	—	—
Polydora ciliata	—	—	7	—	—	—	—	—	—	—	—	—	—	—
P. flava	—	3	—	2	—	—	—	—	—	—	—	—	—	—
P. caulleryi	—	—	—	2	—	—	p	—	—	—	—	—	—	—
Aricidea jeffreysi	—	—	—	4	—	—	—	—	—	—	3	—	—	—
A. branchiata	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Paraonis lyra	—	—	—	2	—	—	—	—	—	—	—	—	6	—
Heterocirrus zetlandicus	—	—	26	4	—	—	—	—	—	c	—	4	—	—
H. caput-esocis	—	—	3	4	—	—	—	—	—	—	—	—	—	—
H. bioculatus	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Tharyx marioni	—	—	3	2	3	—	—	—	—	p	—	—	—	—
Dodecaceria concharum	—	—	20	—	—	—	—	—	—	—	—	—	—	—
Macrochaeta clavicornis	—	3	—	—	—	—	p	—	—	—	—	—	10	p
Stylarioides plumosa	—	—	—	2	3	—	—	—	—	—	—	—	—	—
Diplocirrus glaucus	—	—	—	—	—	—	—	—	—	—	3	—	—	—
Flabelligera affinis	—	—	—	—	—	—	—	—	—	—	—	—	—	p
Scalibregma inflatum	—	—	—	—	—	—	—	—	—	p	—	2	2	p
Notomastus latericeus	—	—	—	—	—	p	p	p	—	—	—	2	—	p
Capitomastus minimus	—	3	—	—	—	c	a	c	—	a	40	48	50	—
Maldane sarsi	—	—	7	—	—	—	—	—	—	—	—	—	—	—
Clymene affinis	—	—	—	—	6	—	p	—	—	c	—	4	—	—
Leiochone clypeata	—	—	—	—	—	p	p	—	—	—	—	—	—	—
Nicomache trispinata	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Maldanidae: unidentified	—	3	—	—	—	—	—	—	—	p	—	—	—	—
Owenia fusiformis	p	—	3	6	3	—	—	—	—	a	—	2	—	—
Myriochele heeri	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Pectinaria auricoma	—	—	—	—	—	—	—	—	—	p	3	2	—	—
P. koreni	—	—	—	—	—	—	—	—	—	—	3	—	—	—
Sabellaria spinulosa	p	—	—	2	3	—	—	—	—	—	—	—	—	—
Ampharete grubei	p	—	—	—	3	—	—	—	—	p	—	—	—	—
Anobothrus gracilis	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Amphicteis gunneri	p	—	—	—	—	—	—	—	—	—	—	—	2	p
Melinna palmata	—	—	—	—	—	—	—	—	—	—	8	—	—	—
M. cristata	—	—	—	—	—	—	—	—	—	—	—	—	—	p
Phisidia aurea	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Pista cristata	—	—	—	2	—	a	a	c	a	p	3	10	22	c
Polymnia nebulosa	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nicolea zostericola	—	3	—	—	—	—	—	—	—	—	—	—	—	—
Thelepus cincinnatus	—	—	—	12	—	—	—	—	—	—	—	—	—	p
Polycirrus denticulatus	—	13	10	2	10	p	p	—	—	p	—	2	4	p
Trichobranchus glacialis	p	3	—	—	—	—	p	—	—	p	—	2	2	—
Terebellides stroemi	—	—	—	—	—	—	—	—	—	p	3	10	—	p
Lysilla loveni	—	3	—	—	—	—	—	—	—	—	—	—	—	—
Potamilla reniformis	—	—	27	8	3	—	—	—	—	p	—	—	—	—
Fabricia sabella	—	—	—	—	3	—	—	—	—	—	—	—	—	—
Jasminiera caudata	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Chone suspecta	—	7	3	38	—	p	p	—	—	—	3	—	2	p
Euchone rubrocincta	—	—	—	—	3	—	—	—	—	—	—	—	2	—
Hydroides norvegica	—	17	57	26	3	—	—	—	—	p	—	—	—	p
Pomatoceros triqueter	—	30	3	38	—	—	p	—	—	a	—	—	—	—
Spirorbis spirillum	—	170	33	—	—	—	—	—	—	—	—	—	—	—

p = present c = common a = abundant.

TABLE 6. POLYCHAET FAUNA OF COARSE GRAVELS

		Shell gravel										Stones		
Station number	...	*67*	*40*	*47*	*48*	*53*	*54*	*52*	*46*	*69*	*72*	*74*	*32*	*50*
Depth (fathoms)	...	10	16	20	21	21	21	24	24	25	27	10	10	25
Species														
Hermione hystrix		p	—	—	—	—	—	—	—	—	—	—	—	—
Gattyana cirrosa		—	—	—	—	—	—	p	—	—	—	—	p	—
Lepidonotus squamatus		p	—	—	—	—	—	—	—	—	—	p	—	a
Harmothoë impar		—	—	—	—	—	—	—	—	—	p	—	a	—
H. haliaeti		—	—	—	—	—	—	—	—	—	—	—	p	—
H. reticulata		—	—	—	—	—	—	—	—	—	—	—	—	p
Halosydna gelatinosa		c	—	—	—	—	—	—	—	—	—	p	—	—
Lagisca extenuata		—	—	—	—	—	—	p	—	—	—	—	—	p
Polynoë scolopendrina		p	—	—	—	—	—	—	—	—	—	—	—	—
Scalisetosus assimilis		p	—	—	—	—	—	—	—	—	—	—	—	—
S. pellucidus		—	—	—	—	—	—	—	—	—	—	—	a	—
Pholoë minuta		—	—	—	—	—	—	—	—	—	—	—	—	p
Phyllodoce laminosa		—	—	—	—	—	—	—	—	—	p	—	—	—
Eulalia viridis		—	—	—	—	—	—	—	—	—	—	—	—	p
E. fucescens		—	—	—	—	—	—	—	—	—	—	—	p	p
E. macroceros		—	—	—	—	—	—	—	—	—	—	—	—	p
Castalia punctata		—	—	—	—	—	—	—	—	—	—	—	c	p
Kefersteinia cirrata		—	—	—	—	—	—	—	—	—	—	p	—	p
Syllis armillaris		p	—	—	—	—	—	—	—	—	p	—	—	a
S. variegata		—	—	—	—	—	—	—	—	—	—	—	—	p
Trypanosyllis coeliaca		—	—	—	—	—	—	—	—	—	—	—	p	—
Eusyllis blomstrandi		—	—	—	—	—	—	—	—	—	—	—	—	p
Exogone gemmifera		—	—	—	—	—	—	—	—	—	p	—	—	—
Autolytus pictus		—	—	—	—	—	—	—	—	—	—	—	—	c
A. rubropunctatus		—	—	—	—	—	—	—	—	—	—	—	—	p
A. longeferiens		—	—	—	—	—	—	—	—	—	—	—	—	p
A. aurantiacus		—	—	—	—	—	—	—	—	—	—	—	—	a
Autolytus sp.		—	—	—	—	—	—	—	—	—	p	—	—	p
Myrianida pinnigera		p	—	—	—	—	—	—	—	—	—	—	—	—
Nereis pelagica		—	—	—	—	—	—	—	—	—	—	—	—	p
Platynereis dumerilii		p	—	—	—	—	—	—	—	—	—	—	p	—
Nephthys incisa		—	—	—	—	—	—	—	—	—	—	—	p	—
Ephesia gracilis		—	—	—	—	—	—	—	—	—	—	—	p	—
Glycera lapidum		p	—	—	—	—	—	—	—	—	—	—	p	—
Goniada maculata		—	—	—	—	—	—	—	—	—	—	—	p	—
Eone nordmanni		—	—	—	—	—	—	—	—	—	—	—	p	—
Lumbriconereis fragilis		p	—	—	—	—	—	—	—	—	—	—	—	—
Polydora caeca		—	—	—	—	—	—	—	—	—	—	—	p	c
Chaetopterus variopedatus		—	—	—	—	—	—	p	p	—	—	—	—	—
Heterocirrus zetlandicus		—	—	—	—	—	—	—	—	—	—	—	p	—
Flabelligera affinis		—	—	—	—	—	—	—	—	—	c	—	p	—
Clymene robusta		—	—	—	—	—	—	—	—	—	—	—	p	—
Owenia fusiformis		—	—	—	—	—	—	—	—	—	—	—	c	—
Petta pusilla		—	—	—	—	p	—	—	—	—	—	—	—	—
Pectinaria auricoma		p	—	—	—	—	—	—	—	—	—	—	—	—
Sabellaria spinulosa		—	—	—	—	—	—	p	—	p	—	—	—	—
Polymnia nebulosa		p	—	—	—	—	—	p	c	—	—	c	—	—
Pista cristata		—	—	—	—	—	—	—	—	—	—	p	—	—
Polycirrus denticulatus		—	—	—	—	—	—	—	—	—	—	—	—	p
Potamilla reniformis		—	—	—	—	—	—	—	—	—	—	—	p	—
Dasychone bombyx		—	—	—	—	—	—	—	—	—	—	c	—	p
Sabellidae: unidentified		—	—	—	—	—	—	—	—	—	—	—	—	p
Serpula vermicularis		—	—	p	—	—	—	p	p	p	—	p	—	—
Hydroides norvegica		—	p	—	—	p	p	p	—	p	—	c	—	—
Pomatoceros triqueter		p	p	p	p	c	p	—	—	—	—	a	p	—
Protula tubularia		p	—	—	—	—	—	—	—	—	—	—	—	—
Filograna implexa		—	—	p	—	—	—	—	—	—	—	p	—	—
Spirorbis sp.		—	—	—	p	—	—	p	—	—	—	—	p	—

p = present c = common a = abundant.

TABLE 7. POLYCHAETA BORING OR INHABITING CREVICES IN EMPTY SHELLS OR SERPULID TUBES (RELATIVE ABUNDANCE)

Shell	...	*Ostrea*			*Modiolus*	*Glycimeris*		Serpulid tubes				
Station number	...	*40*	*54*	*54*	*52*	*53*	*46*	*68*	*34*	*48*	*46*	*69*
Depth (fathoms)	...	16	21	21	24	21	24	10	15	21	24	25
Species												
Pholoë minuta		—	p	—	—	—	—	—	p	—	—	—
Eulalia viridis		—	p	—	—	—	—	—	—	—	—	—
E. viridis var. *aurea*		—	—	—	—	—	—	—	—	—	p	—
E. bilineata		—	p	p	—	—	—	—	—	—	—	—
E. sanguinea		—	—	—	—	—	—	—	p	—	—	—
E. fucescens		—	—	p	—	—	—	—	—	—	—	—
E. pusilla		p	—	p	—	—	—	—	—	—	—	—
E. macroceros		p	—	—	—	—	—	—	—	—	—	—
Notophyllum foliosum		—	p	—	—	—	p	—	—	p	—	—
Castalia punctata		—	p	—	—	—	—	—	—	—	—	—
Syllis armillaris		—	c	c	—	—	—	p	—	—	—	p
S. amica		—	—	—	—	—	—	p	—	—	—	—
S. variegata		—	p	p	—	—	—	p	—	—	—	—
Trypanosyllis coeliaca		p	—	—	—	—	—	—	—	—	—	—
Odontosyllis fulgurans		—	—	—	—	—	—	—	p	—	—	—
Eusyllis blomstrandi		—	p	p	—	—	—	p	—	—	—	—
Exogone gemmifera		—	p	—	—	p	—	—	—	—	—	—
Autolytus rubropunctatus		—	p	p	—	—	—	p	—	—	—	—
A. longeferiens		—	p	—	—	—	—	—	—	—	—	—
A. aurantiacus		—	c	p	—	—	—	p	—	—	—	—
Nereis zonata		—	p	—	—	—	—	—	—	—	—	—
Nematonereis unicornis		p	p	—	p	p	p	—	—	—	—	—
Polydora ciliata		—	—	—	—	—	—	p	—	—	—	—
P. caeca		—	p	p	—	—	p	—	—	c	—	—
P. flava		—	—	p	—	—	—	—	c	—	—	—
P. giardi		—	—	—	p	—	—	—	—	—	—	—
Heterocirrus zetlandicus		—	p	p	—	—	—	—	p	—	—	—
Dodecaceria concharum		—	—	—	—	—	—	p	—	—	—	p
Lumbriclymene minor		—	—	c	—	—	c	—	—	—	—	—
Polycirrus denticulatus		p	p	p	—	—	—	—	—	—	—	—
Potamilla torelli		—	—	—	—	—	—	p	—	—	—	—
P. reniformis		—	p	c	—	p	p	—	—	—	—	p

p = present c = common.

TABLE 8. POLYCHAET FAUNA OF HYDROIDS

(Relative abundance.)

Station number	...	*47*	*48*	*49*	*53*	*52*	*46*
Depth (fathoms)	...	20	21	21	21	24	24
Species							
Lepidonotus squamatus		p	—	—	p	p	—
Harmothoë impar		—	—	—	p	p	—
Halosydna gelatinosa		—	—	—	p	—	—
Lagisca extenuata		—	—	p	—	p	p
Eulalia bilineata		p	—	—	p	—	p
E. fucescens		—	—	—	p	—	—
E. pusilla		—	—	—	p	—	—
Notophyllum foliosum		—	—	—	—	—	p
Mystides limbata		—	—	—	—	—	p
Castalia punctata		p	—	—	p	p	p
Syllis armillaris		a	p	p	a	—	p
S. variegata		p	p	—	—	—	—

TABLE 8 (*continued*)

Station number	... 47	48	49	53	52	46
Eusyllis blomstrandi	p	c	c	c	c	a
Trypanosyllis zebra	—	—	—	p	—	—
Exogone gemmifera	—	—	—	c	p	p
Autolytus pictus	—	—	—	p	—	—
A. rubropunctatus	p	—	—	p	—	—
A. aurantiacus	p	c	—	p	c	—
A. longeferiens	—	a	—	p	a	c
A. prolifer	—	—	—	—	p	—
Autolytus sp.	—	—	—	p	—	—
Procerastea perrieri	—	—	—	—	p	—
Nereis pelagica	—	—	—	p	p	—
N. zonata	—	—	—	p	—	—
Ephesia peripatus	p	—	—	—	p	—
Glycera lapidum	—	—	—	—	p	—
Lumbriconereis fragilis	—	p	—	—	—	—
Polydora caeca	—	—	—	p	—	—
Melinna cristata	—	—	—	—	p	—
Pista maculata	—	—	—	—	p	—
Nicolea zostericola	—	—	—	—	—	p
Polymnia nebulosa	—	—	—	—	p	—
Polycirrus denticulatus	—	—	p	p	p	p
Trichobranchus glacialis	—	p	—	—	—	—
Hydroides norvegica	—	p	—	—	c	c
Spirorbis spirillum	—	a	—	—	a	c

a = abundant c = common p = present.

TABLE 9. POLYCHAET FAUNA OF CLEAN SAND

(Number per square metre or relative abundance.)

Station number ...	65	59	31
Depth (fathoms) ...	2–5	3–4	10
Dredge or grab ...	D	G	D
Number of samples ...	1	5	1
Species			
Harmothoë imbricata	p	—	—
Sthenelais limicola	c	—	—
Eteone foliosa	—	2	—
E. longa	—	4	—
Mystides limbata	—	2	—
Kefersteinia cirrata	p	—	—
Exogone verugera	—	4	—
Nereis pelagica	p	—	—
Platynereis dumerilii	p	—	—
Nephthys cirrosa	p	—	p
Glycera convoluta	—	6	—
G. gigantea	—	2	—
G. rouxi	—	2	—
Goniada maculata	—	2	—
Eone nordmanni	—	2	—
Nematonereis unicornis	—	2	—
Scoloplos armiger	—	58	p
Spiophanes bombyx	—	6	—
Polydora caulleryi	—	2	—
Magelona papillicornis	—	2	—
Chaetozone setosa	—	10	—
Heterocirrus zetlandicus	—	—	p
Capitella capitata	—	8	—
Clymene oerstedii	—	10	—
Owenia fusiformis	—	2	—
Pista cristata	c	2	—
Polycirrus denticulatus	p	—	—

c = common p = present.

TABLE 10. COMPOSITION OF THE MACROFAUNA OF VARIOUS DEPOSITS

Habitat	Percentage of total population per station					Polychaeta				
						No. of specimens per m²		No. of species per station		
	Polychaeta Mean	Crustacea Mean	Mollusca Mean	Echinodermata Mean	Others Mean	Range	Mean	Range	Mean	Total species
Mud	62	15·3	9·7	4·3	8·7	26–109	56	4–12	7·9	27
Muddy sand (no shell)	43·6	7·3	31·5	6·5	11·1	66–574	174	8–31	19·3	64
Muddy sand (with shell)	43·3	15·5	13	11·9	16·3	95–368	221	14–50	31·6	122
Fine shell gravel	64	5·2	9·2	11·4	10·2	150–466	342	21–39	29	85
Fine stony gravel	49	17·7	7·7	16	9·6	—	—	6–45	24	66
Coralline gravel	58	8·4	7	22·1	4·5	180–292	244	18–31	27	58
Coarse shell gravel	—	—	—	—	—	—	—	2–13	—	27
Large stones	—	—	—	—	—	—	—	10–24	18	41
Clean sand	60	21·5	17·5	1	—	120	—	3–19	—	27
Boring in shells	—	—	—	—	—	—	—	1–19	6·4	32
Sponges	—	—	—	—	—	—	—	6	—	6
Hydroids	—	—	—	—	—	—	—	4–20	11·5	37

TABLE 11. STATION LIST

No.	Date	Position	Depth (fm.)	Bottom deposit	Gear used	No. of hauls
1	16. ii. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
2	16. ii. 53	4 miles W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	5
3	16. ii. 53	5 miles W. of Niarbyl	25	m.s. & sh.gr.	V-V.G.	5
4	16. ii. 53	5½ miles W. of Niarbyl	30	m.s.	V-V.G.	5
5	9. iii. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
6	9. iii. 53	6½ miles W. of Niarbyl	35	m.s.	V-V.G.	5
7	9. iii. 53	7 miles W. of Niarbyl	40	m.	V-V.G.	5
8	9. iii. 53	8½ miles W. of Niarbyl	45	m.	V-V.G.	5
9	9. iii. 53	10½ miles W. of Niarbyl	50	m.	V-V.G.	5
10	20. iv. 53	½ mile N. of Fleshwick Bay	9	f.st.gr.	S.N.D.	1
11	20. iv. 53	Fleshwick Bay	10	f.st.gr.	S.N.D.	1
12	18. iv. 53	1½ miles S. 80° W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
13	18. iv. 53	4 miles S. 80° W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	5
14	18. iv. 53	5½ miles S. 80° W. of Niarbyl	30	m.s. & sh.gr.	V-V.G.	5
15	18. iv. 53	9 miles S. 80° W. of Niarbyl	40	m.	V-V.G.	5
16	18. iv. 53	10 miles S. 80° W. of Niarbyl	45	m.	V-V.G.	5
17	27. viii. 53	12 miles S. 80° W. of Peel	50	m.	V-V.G.	5
18	27. viii. 53	13 miles N.W. of the Sound	55	m.	V-V.G.	5
19	27. viii. 53	6 miles N. 55° W. of the Sound	30	m.s.	V-V.G.	5
20	14. x. 53	3 miles W. of Bradda Head	20	sh.s. & sh.gr.	V-V.G.	3
21	14. x. 53	4½ miles W. of Sloc	20	sh.s. & sh.gr.	V-V.G.	3
22	14. x. 53	4 miles W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	4
23	14. x. 53	5 miles W. of Niarbyl	25	m.s. & sh.gr.	V-V.G.	5
24	14. x. 53	5½ miles W. of Niarbyl	30	m.s.	V-V.G.	5
25	14. x. 53	5½ miles W. of Sloc	25	m.s. & sh.gr.	V-V.G.	5
26	15. x. 53	5 miles W. of Bradda Head	25	sh.s. & sh.gr.	V-V.G.	5
27	15. x. 53	6 miles W. of Bradda Head	30	sh.s. & sh.gr.	V-.VG.	3
28	15. x. 53	9 miles W. of Bradda Head	29	m.s. & sh.gr.	V-V.G.	3
29	15. x. 53	10 miles W. of Bradda Head	40	m.s. & *Turr*.sh.	V-V.G.	3
30	24. xi. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	S.N.D.	1
31	24. xi. 53	½ mile W. of Niarbyl	10	s.	S.N.D.	1
32	24. xi. 53	½ mile S.S.W. of Niarbyl	10	coarse st.gr.	S.N.D.	1
33	9. ii. 54	1 mile W. of Bradda Head	17	m.s. & sh.gr.	S.N.D.	1
34	9. ii. 54	2 miles W. of Sloc	15	large stones, f.st.gr. & m.s.	S.N.D.	1
35	9. ii. 54	3 miles W. of Sloc	18	m.s. & sh.gr.	S.N.D.	1
36	9. ii. 54	9½ miles W. of Sloc	40	m.s. & *Turr*.sh.	V-V.G.	3
37	9. ii. 54	11 miles W. of Sloc	50	m.	V-V.G.	3
38	9. ii. 54	7 miles W. of Sloc	30	m.s. & sh.gr.	V-V.G.	3
39	11. iii. 54	Off P.E. breakwater	5	f.st.gr.	N.D.	1
40	11. iii. 54	¼ mile W. of Charran	16	st.gr.	N.D.	1
41	1. iv. 54	8 miles S. 80° W. of Chicken R.	35	m.s. & *Turr*.sh.	V-V.G.	3
42	1. iv. 54	10 miles S. 80° W. of Chicken R.	38	m.	V-V.G.	3
43	1. iv. 54	12 miles S. 80° W. of Chicken R.	35	m.	V-V.G.	3
44	1. iv. 54	6 miles S. 80° W. of Chicken R.	34	m.s. & sh.gr.	S.N.D.	1
45	1. iv. 54	4 miles S. 80° W. of Chicken R.	32	m.s. & sh.gr.	S.N.D.	1
46	1. iv. 54	2 miles S. 80° W. of Chicken R.	24	shells & sh.gr.	4 ft. D.	1
47	19. iv. 54	1½ miles S. 10° W. of Scarlet P.	20	stones	4 ft. D.	1
48	19. iv. 54	3 miles S. 60° W. of Langness	21	shells & stones	4 ft. D.	1
49	19. iv. 54	2¾ miles S. 15° W. of P. St. M.	21	shells	4 ft. D.	1
50	19. iv. 54	1 mile S.E. of Chicken R.	25–30	stones	4 ft. D.	1
51	19. iv. 54	2 miles S. 22° W. of Chicken R.	24	sh.gr. & stones	4 ft. D.	1
52	19. iv. 54	2 miles N. 80° W. of Chicken R.	25	sh.gr. & shells	4 ft. D.	1
53	19. iv. 54	2¾ miles S. 80° W. of Bradda Head	21	shells	4 ft. D.	1
54	19. iv. 54	3½ miles S. 80° W. of Bradda Head	21–24	shells	4 ft. D.	1
55	26. iv. 54	11 miles N. 40° W. of the Sound	53	m.	V-V.G.	2
56	26. iv. 54	13 miles N. 40° W. of the Sound	58	m.	V-V.G.	2
57	26. iv. 54	14 miles N. 40° W. of the Sound	65	m.	V-V.G.	2
58	26. iv. 54	15 miles N. 40° W. of the Sound	70	m.	V-V.G.	2

TABLE 11 (*continued*)

No.	Date	Position	Depth (fm.)	Bottom deposit	Gear used	No. of hauls
59	26. iv. 54	Port Erin Bay	3–4	s. & f.st.gr.	V-V.G.	5
60	12. v. 54	6 miles N. 60° W. of the Sound	31	m.s. & sh.gr.	V-V.G.	3
61	12. v. 54	9 miles N. 60° W. of the Sound	41	m.s. & *Turr.*sh.	V-V.G.	3
62	12. v. 54	10½ miles N. 60° W. of the Sound	50	m.	V-V.G.	3
63	12. v. 54	15 miles N. 60° W. of the Sound	60	m.	V-V.G.	3
64	12. v. 54	7½ miles N.W. of the Sound	37	m.s.	V-V.G.	5
65	13. xi. 53	Port Erin Bay	2–3	s.	N.D.	2
66	20. i. 54	½ mile N. of the Sound	15	*Chlamys opercularis*	4 ft. D.	1
67	14. x. 52	¼ mile W. of Bradda Head	12	shells & sh.gr.	4 ft. D.	2
68	24. x. 52	Bay Fine	12	shells	4 ft. D.	1
69	5. xi. 52	5 miles S. of P. St. M.	27	*Modiolus* epif.	4 ft. D.	1
70	11. xi. 52	1 mile S. of Spanish Head	17	sh.gr. & m.s.	S.N.D.	1
71	11. xi. 52	1 mile N.N.W. of Bradda Head	18½	sh.gr. & m.s.	S.N.D.	1
72	11. xi. 52	2 miles S.W. of Chicken R.	26	shells & sh.gr.	S.N.D.	1
73	28. vii. 53	Bay Fine	15	*Chlamys opercularis*	4 ft. D.	1
74	6. x. 53	Off P. E. breakwater	10	rock	Trawl	
75	1. xii. 52	Fleshwick Bay	6	f.st.gr.	S.N.D.	1
76	30. ix. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10
77	17. xi. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10
78	23. xii. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10

Abbreviations

Gear used: V-V.G., van Veen bottom sampler (1/10 m^2); N.D., naturalist's dredge (2 ft. 6 in.); S.N.D., small naturalist's dredge (1 ft. 6 in.); 4 ft. D., 4 ft. scallop dredge.

Bottom deposit or contents of haul: c.gr., coralline gravel; f.st.gr., fine stony gravel; m., mud; m.s., muddy sand; s., sand; sh.s., shell sand; sh.gr., shell gravel; *Turr.*sh., empty shells of *Turritella communis*.

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THE OCCURRENCE AT PLYMOUTH OF *DICTYOCOTYLE COELIACA* NYBELIN, 1941 (TREMATODA: MONOGENEA)

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(Plate I)

Dictyocotyle coeliaca is a somewhat unusual monogenetic trematode in that it is endoparasitic and not ectoparasitic, as are most monogeneans. It was first described by Nybelin (1941) from two specimens, one of which had been collected by Swenander in 1905 from the coelom of *Raia radiata* caught at Trondhjem, and the other collected by Nybelin himself from *R. lintea* caught at Göteborg. Later, according to Sproston (1946), Mr Quinten Geering at Cambridge found three trematodes firmly attached to the liver of a young *Raia* sp. from Plymouth. These parasites were deposited in the British Museum, where they were provisionally identified as damaged specimens of *Calicotyle krøyeri*, a common parasite of the cloaca of various species of *Raia*. However, these specimens were subsequently examined by Dr Brinkmann (1952) of Bergen, and he, having seen the type material, was able to identify the Plymouth specimens as *Dictyocotyle coeliaca*.

Two parasites collected in the Zoology Department at Sheffield University from the outside of the stomach of *Raia clavata*, believed to have been landed at Brixham, were identified by Dawes (1948) as Nybelin's species, but Dawes regarded the species as belonging to the genus *Calicotyle*.

Hunter & Kille (1950) examined rays brought from Scottish fishing grounds into the Zoology Department at Edinburgh University, and found *Dictyocotyle coeliaca* in sixteen of fifty-five specimens of *Raia naevus* and in thirty-one of seventy-three specimens of *R. radiata*, but none in *R. batis*, *R. montagui*, *R. brachyura*, *R. clavata* and *R. fullonica*. These authors gave a description of *Dictyocotyle coeliaca* and compared the parasite with *Calicotyle krøyeri*.

In the present observations, examinations have been made of specimens of the four species of *Raia* commonly landed at Plymouth, in the period May 1955 to August 1956 inclusive. The coelom and viscera of each host specimen were searched for the presence of *Dictyocotyle coeliaca*, and the results are included in Table 1.

Of the thirty-five infected specimens of *Raia naevus*, nineteen were males and sixteen were females. The mean infestation of parasites per fish was 3·97, and the maximum number of parasites collected from a single host specimen was twenty-two. There appeared to be no particular site of infestation within the coelom, and living specimens of the trematode were collected, at periods varying from 1 to 18 hr after the death of the host, from all the inner surfaces of the body wall, and less frequently, from the viscera.

TABLE 1

Host	No. of specimens examined	No. infected
Raia naevus	135	35
R. clavata	478	0
R. montagui (=*R. maculata*)	420	0
R. brachyura	34	0

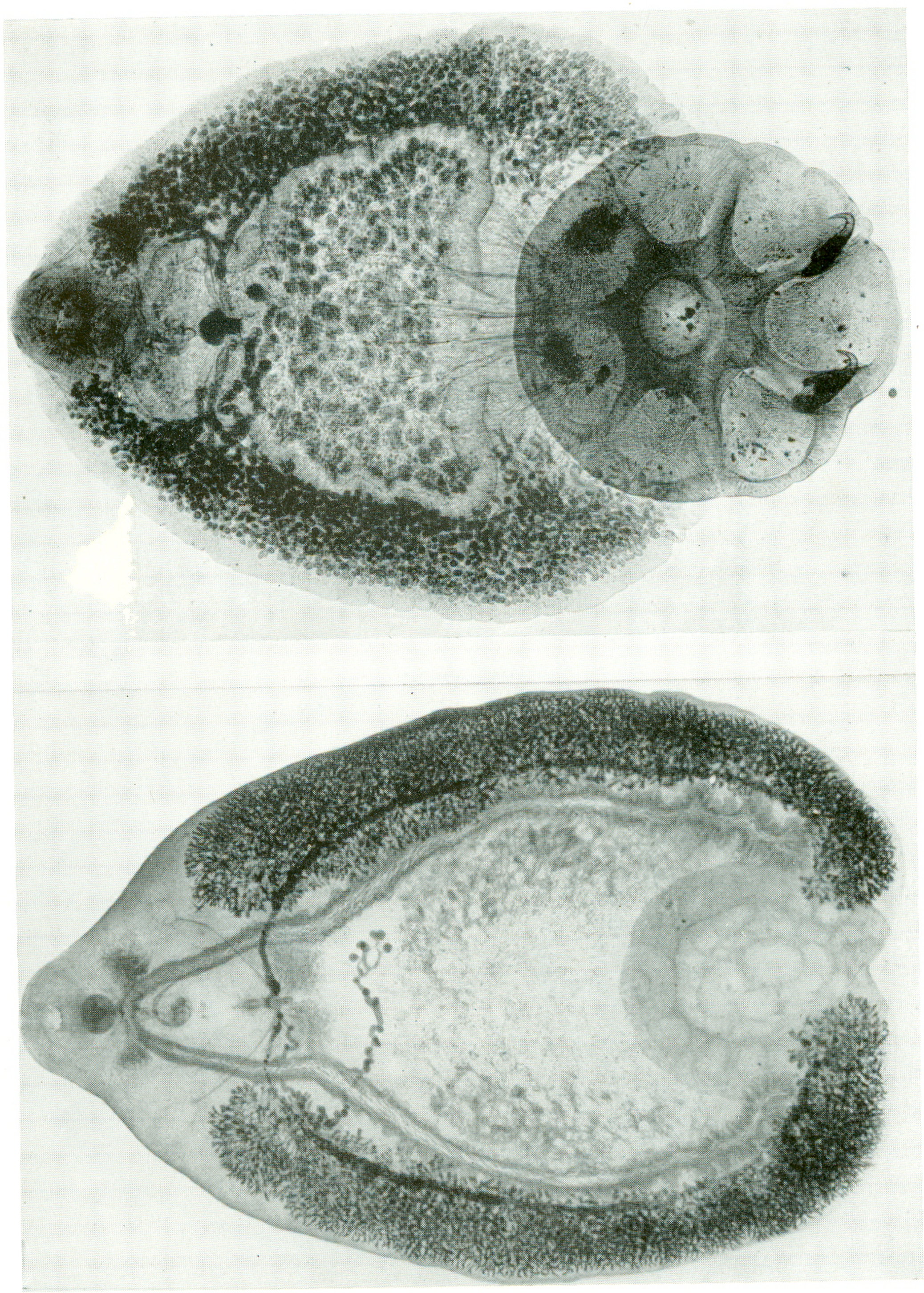
On several occasions specimens of both *Dictyocotyle coeliaca* and *Calicotyle krøyeri* have been found in the same individual *Raia naevus*, the former in the coelom and the latter in the cloaca. Photographs illustrating the differences between the two parasites, previously noted by Hunter & Kille, are included in Pl. I, figs. 1 and 2.

It may be concluded then that *Dictyocotyle coeliaca* is at least as common in *Raia naevus* at Plymouth (about 26% infestation) as are most other monogeneans on their respective hosts (see table of frequencies in Llewellyn, 1956); the remarkable feature is that it has escaped attention, since *R. naevus*, along with other rays, is frequently supplied to University Departments for class dissection.

In view of the absolute restriction of *Dictyocotyle coeliaca* to *Raia naevus* in over 1000 *Raia* specimens examined at Plymouth in the present study, and to *R. naevus* and *R. radiata* in Hunter & Kille's (1950) study of 181 specimens of rays from Scottish waters, it seems distinctly possible that the Brixham host ('*Raia clavata*') of the material sent to Dawes (1948) had been mis-identified.

SUMMARY

In a sample of over 1000 rays belonging to four species of *Raia* examined at Plymouth between May 1955 and August 1956, the 'very rare' monogenetic trematode *Dictyocotyle coeliaca* Nybelin, 1941 (total of four previous records) was found in the coelom of thirty-five out of 135 specimens of *Raia naevus*, but absent from 478 *R. clavata*, 420 *R. montagui* and 34 *R. brachyura*.



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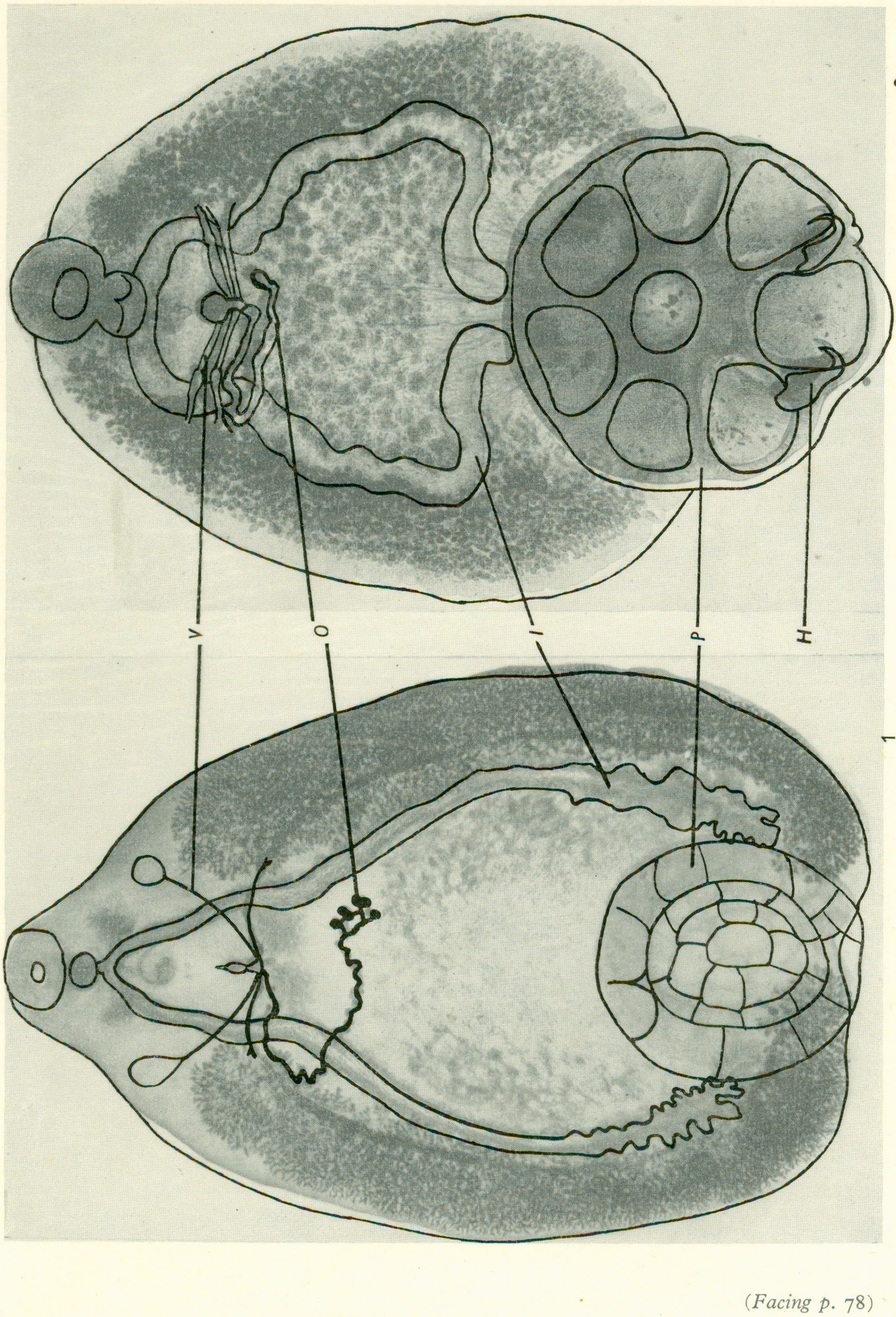

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REFERENCES

BRINKMANN, A., 1952. Fish trematodes from Norwegian Waters. *Univ. Bergen Årb. naturv. R.*, No. 1.

DAWES, B., 1948. A rare coelom-dwelling trematode. *Nature, Lond.*, Vol. 161, p. 642.

HUNTER, G. C. & KILLE, R. A., 1950. Some observations on *Dictyocotyle coeliaca* Nybelin, 1941. *J. Helminth.*, Vol. 24, pp. 15–22.

LLEWELLYN, J., 1956. The host specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

NYBELIN, O., 1941. *Dictyocotyle coeliaca* n.g. n.sp. Ein leibeshöhlebewohnender monogenetischer Trematode. *Göteborgs VetenskSam. Handl.*, Följ. VI, Ser. B, Bd 1, No. 3, 19 pp.

SPROSTON, N. G., 1946. A synopsis of the monogenetic trematodes. *Trans. zool. Soc. Lond.*, Vol. 25, pp. 185–600.

EXPLANATION OF PLATE I

A comparison of the morphology of *Dictyocotyle coeliaca* Nybelin, 1941 with that of *Calicotyle krøyeri* Diesing, 1850. Fig. 1. *Dictyocotyle coeliaca*. Fig. 2. *Calicotyle krøyeri*. *I*, posterior ends of intestinal caeca of *Dictyocotyle* sacculated and end well behind anterior edge of posterior adhesive organ; not sacculated in *Calicotyle*, and end in front of anterior edge of posterior adhesive organ. *H*, hooks present in *Calicotyle*, absent in *Dictyocotyle*. *O*, proximal region of ovary 5–7 lobed in *Dictyocotyle*, simple in *Calicotyle*. *P*, posterior adhesive organ of *Dictyocotyle* with numerous irregular shallow loculi of various sizes; of *Calicotyle* with a central stalk and seven peripheral loculi all of similar shape and size; posterior adhesive organ in *Dictyocotyle* reaches posterior border of body, in *Calicotyle* it projects well beyond the posterior margin of the body. *V*, vaginae pass obliquely anteriorly to open in front of vitellaria in *Dictyocotyle*, but pass transversely in *Calicotyle*.

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THE FEEDING MECHANISM AND STRUCTURE OF THE GUT OF *OWENIA FUSIFORMIS* DELLE CHIAJE

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(Text-figs. 1 and 2)

In 1901 Arnold Watson published a paper on the habits of *Owenia fusiformis* delle Chiaje (=*Ammochares filiformis* Watson) in his now classic series of papers on the tube-building habits of various polychaetes. There is little to be added to his description in this respect, but Watson was not concerned with the feeding mechanism, which receives but brief mention. *O. fusiformis* has previously been studied by a number of workers, but none has given much attention to the morphology of the gut or to the method of feeding which are the main considerations of the present paper.

O. fusiformis has been studied from other aspects in some detail, notably by von Drasche (1885), who, while dealing little with the gut, did note that the mid-gut region has a green colour. Claparède (1868, 1873) and Saint-Joseph (1898) also mention this. Some account of other features of the morphology of *Owenia* will be found in the papers of Gilson (1893, 1897), Lo Bianco (1893), Eisig (1887) and McIntosh (1915), while the development and metamorphosis of the mitraria larva belonging to this species is described in the well-known paper of Wilson (1932).

O. fusiformis is not uncommon in clean sand below low water of neap tides on British shores, often in beaches subject to some disturbance and not tenable by polychaetes with a permanently fixed tube or burrow. Though local in occurrence, the species is cosmopolitan in distribution. The worms studied in the present work were collected from Tor Abbey sands, Torquay, Devon, and from near St Mawes in Cornwall. Elwes (1910) first reported *Owenia* as the commonest polychaete in Tor Abbey sands; Wilson collected worms from this beach for his developmental studies (1932), and it is certainly still numerous there. The worms construct long flexible tubes up to 15–20 cm long and 2–5 mm across, consisting of flat grains arranged like roof tiles with the free edge upwards; white or lightly coloured grains seem to be preferred, even when relatively scarce, so that the tubes are often conspicuous, as in the reddish sand of Torquay. The flexibility and probably also the arrangement of the sand grains enables the worm to work the tube up and down in the sand and to rebury itself when necessary. Worms can withdraw the tube completely

beneath the surface, but when feeding in the laboratory the tube may project 2–3 cm above the sand. Possibly the tubes do not project as much as this in nature. *Owenia* is very hardy, as Watson (1901) found, and will live in the laboratory for long periods without aeration.

STRUCTURE OF THE CROWN AND THE FEEDING MECHANISM

The tip of the tube forms an elastic cone of transparent material which almost closes when the worm retracts; during feeding the crown and the most anterior segments are pushed through the minute opening at the tip of the cone which is then distended, and worms when undisturbed may remain for long periods in this position. On other occasions the crown may be seen within the cone, but it sems unlikely that feeding takes place to any significant extent at this time, though some respiratory exchange may continue. The cone is probably of great protective value when the worm is moving within the tube through the sand. The crown has an elaborate blood supply, and is light red, brown or greenish in colour; while there is no doubt that it is respiratory in function, it also participates in feeding.

The crown is a bilaterally symmetrical, branched structure developed from the prostomium, and has some mobility. Though short it is twice the diameter of the body when expanded (Fig. 1). There are four main branches or units on each side (Fig. 1 A), and each unit (Fig. 1 B) is divided into 4–6 branches, each of which ends in 2–4 small bifid lobes (Fig. 1 C). While there is some variation in arrangement, these lobes are almost always arranged in pairs, strongly ciliated on their oral surface, with the stem of each pair raised at the edges to form a gutter (Fig. 1 C). There are long cilia on these edges which beat inwards towards the axis of the stem, and shorter cilia in the gutter itself which beat towards the mouth. The long cilia on the edges of the gutter cause swirling movements between adjacent branches sweeping particles into the crown. Particles which are too large to be ingested usually do not come to rest in the gutter at all, or, if arrested, collect along the edges and are eventually cleaned off by muscular movements, either by convulsive movements of the whole crown, or by more local movements. Cilia are entirely absent from the aboral surface of the crown, but mucus gland cells are distributed over both oral and aboral surfaces.

The crown forms a funnel at the base closed by three lips, a single dorsal lip, and two ventro-lateral in position. These lips are all expansions of the prostomium, and when dilated close the mouth by coming together at the centre (Fig. 1 A). They are hollow and extremely mobile; dilation is caused by the inflow of coelomic fluid, but each lip has a complicated musculature and is capable of a variety of rippling movements. The crown contains an extensive coelomic cavity. Closure of the crown is brought about by the circular crown muscles acting against the hydrostatic pressure of the body fluid; when

the crown is expanded these crown muscles are relaxed (Fig. 1 B). These circular muscles extend into all the branches except into the bifid tips, so that each gutter is capable of a certain amount of inrolling which can assist both in directing the strings of particles towards the mouth, and in freeing the crown of unwanted matter. While convulsive closure of the whole crown takes place

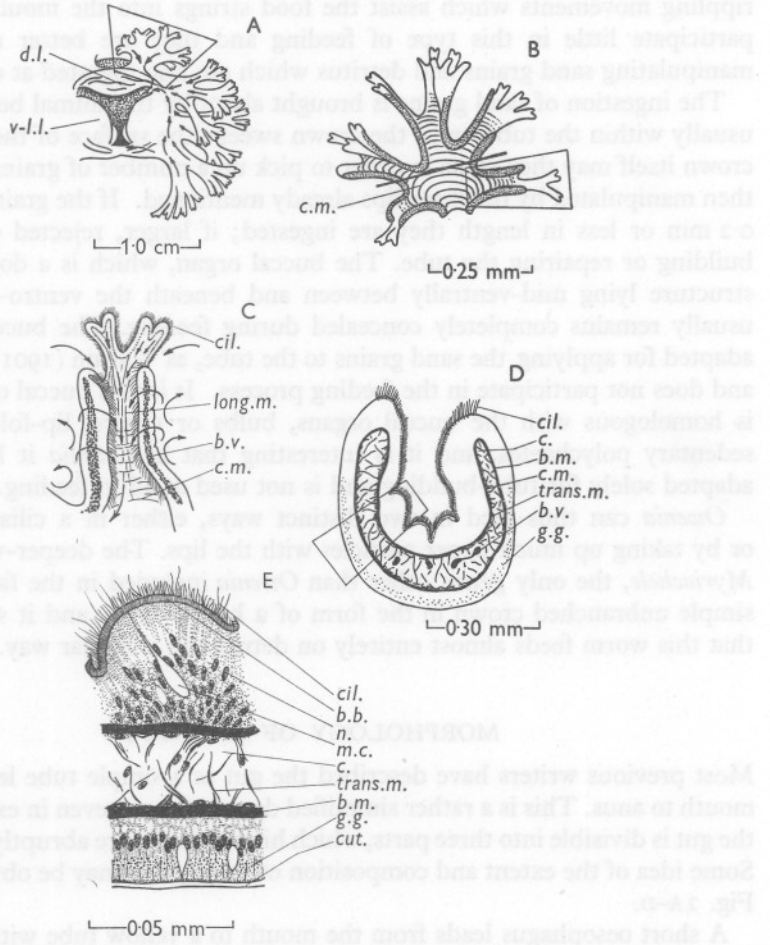

Fig. 1. The crown and feeding mechanism. A, the left half of the crown seen from above; arrows indicate direction of ciliary currents; the area marked is shown in B. B, a single unit showing the gutter and circular muscles; the area marked is shown in C. C, a single lobe showing arrangement of cilia and direction of ciliary currents. D, section through a unit corresponding to the plane of the line shown in A; the area marked is shown in E. E, section through the axis of a unit. *b.b.* brush-border; *b.m.* basement membrane; *b.v.* blood vessel; *c.* coelom; *c.m.* circular muscle; *cil.* cilia; *cut.* cuticle; *d.l.* dorsal lip; *g.g.* granules of green pigment; *long.m.* longitudinal muscle; *m.c.* mucus gland cell; *n.* nuclei; *trans.m.* transverse muscle; *v-l.l.* ventro-lateral lip.

at intervals during feeding, different parts of the crown are capable of independent movement. There are also muscle fibres across the coelomic space between aboral and oral surfaces (Fig. 1 D, E) and longitudinal fibres in the bifid tips (Fig. 1 C).

Ciliary feeding, which is continued with the worm in a more or less erect posture may continue for many hours. During this time the lips may undergo rippling movements which assist the food strings into the mouth, but they participate little in this type of feeding and they are better adapted for manipulating sand grains and detritus which are also ingested at other times.

The ingestion of sand grains is brought about by the animal bending over, usually within the tube, until the crown sweeps the surface of the sand. The crown itself may then close in order to pick up a number of grains, which are then manipulated by the three lips already mentioned. If the grains are about 0·2 mm or less in length they are ingested; if larger, rejected or used for building or repairing the tube. The buccal organ, which is a double-lipped structure lying mid-ventrally between and beneath the ventro-lateral lips, usually remains completely concealed during feeding. The buccal organ is adapted for applying the sand grains to the tube, as Watson (1901) described, and does not participate in the feeding process. It is this buccal organ which is homologous with the buccal organs, bulbs or ventral lip-folds in other sedentary polychaetes, and it is interesting that in *Owenia* it has become adapted solely for tube-building and is not used at all in feeding.

Owenia can thus feed in two distinct ways, either in a ciliary manner, or by taking up much larger particles with the lips. The deeper-water genus *Myriochele*, the only genus other than *Owenia* included in the family, has a simple unbranched crown in the form of a hollow cone, and it seems likely that this worm feeds almost entirely on detritus in a similar way.

MORPHOLOGY OF THE GUT

Most previous writers have described the gut as a simple tube leading from mouth to anus. This is a rather simplified description, as even in external view the gut is divisible into three parts, which histologically are abruptly separated. Some idea of the extent and composition of these parts may be obtained from Fig. 2 A–D.

A short oesophagus leads from the mouth to a yellow tube with thick and much involuted walls. Farther back the gut assumes a deep green colour, and this region has somewhat thinner walls and is less infolded; between the level of the 5th and 6th pairs of tori the green colour diminishes, and the hind gut is very thin-walled and capable of great distension.

The floor of the crown has ciliated tracts leading down into the mouth as already described. The whole of this buccal region is ciliated as is the oesophagus, the tall epithelial cells of which are characterized by a brush-border,

long cilia, finely granular cytoplasm and elongate nuclei (Fig. 2E). Basal granules beneath the brush-border and converging fibres characteristic of cells of this kind may be seen leading from the granules towards the middle of the cell where the nucleus lies. Gland cells, staining blue with Azan, occur in the epithelium of the buccal region and in the oesophagus, between the

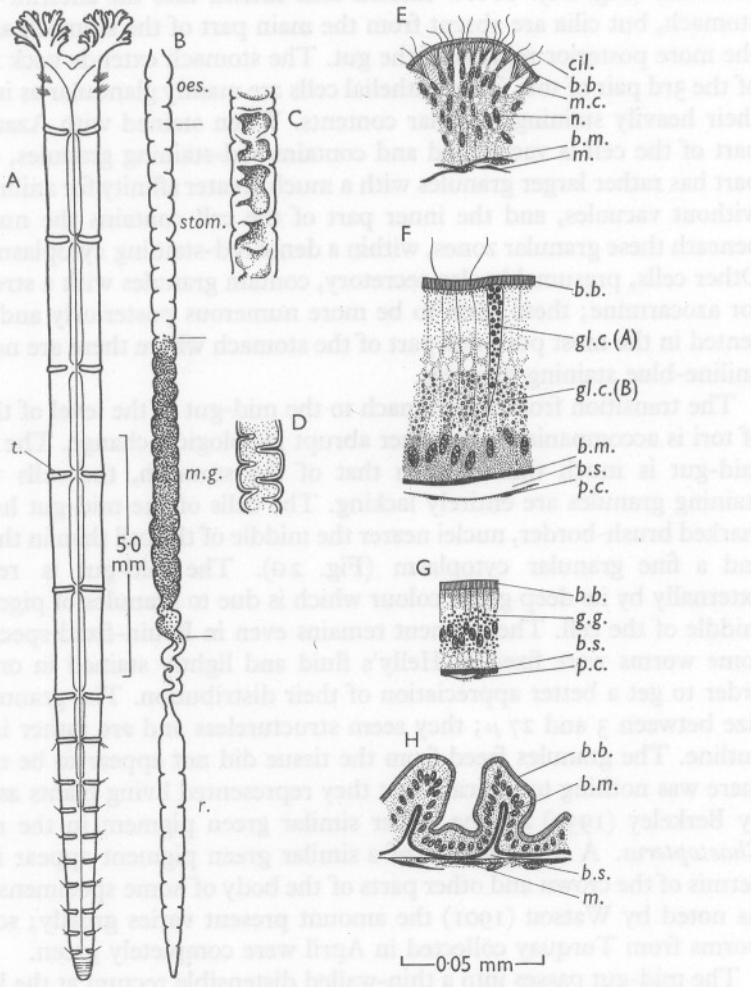

Fig. 2. The morphology and histology of the gut. A, the whole worm in ventral view. B, external appearance of the whole gut; anterior part of rectum is empty, the hinder part filled. C, part of the stomach showing thick walls and involutions. D, part of the mid-gut showing the thin walls and simpler infoldings. E–H, transverse sections of the oesophagus (E), stomach (F), mid-gut (G) and rectum (H), all drawn to the same scale. Abbreviations not used in Fig. 1: *b.s.* blood sinus; *gl.c.*(*A*), gland-cell with granules with azocarmine affinity; *gl.c.*(*B*), with granules with aniline-blue affinity; *m*, muscle; *m.g.* mid-gut; *oes.* oesophagus; *p.c.* peritoneal cell; *r.* rectum; *stom.* stomach; *t.* torus.

ciliated cells. These mucus cells are scattered throughout the length of the oesophagus which extends back to the level of the 1st pair of tori; externally the oesophagus is distinguishable by its lighter colour from the yellow stomach, but internally there is a sharp histological transition.

The stomach has much thicker walls than the oesophagus and is much involuted (Fig. 2C). A few ciliated cells extend into the anterior part of the stomach, but cilia are absent from the main part of the stomach and from all the more posterior regions of the gut. The stomach extends back to the level of the 3rd pair of tori. The epithelial cells are mainly glandular as indicated by their heavily staining granular contents. When stained with Azan the outer part of the cell is vacuolated and contains red-staining granules, the middle part has rather larger granules with a much greater affinity for aniline blue, but without vacuoles, and the inner part of the cell contains the nucleus lying beneath these granular zones, within a dense red-staining cytoplasm (Fig. 1F). Other cells, presumably also secretory, contain granules with a strong affinity for azocarmine; these seem to be more numerous posteriorly and are represented in the most posterior part of the stomach where there are no cells with aniline-blue staining granules.

The transition from the stomach to the mid-gut at the level of the 3rd pair of tori is accompanied by another abrupt histological change. The wall of the mid-gut is much thinner than that of the stomach, the cells with blue-staining granules are entirely lacking. The cells of the mid-gut have a well-marked brush-border, nuclei nearer the middle of the cell than in the stomach, and a fine granular cytoplasm (Fig. 2G). The mid-gut is recognizable externally by its deep green colour which is due to granules of pigment in the middle of the cell. The pigment remains even in Bouin-fixed specimens, but some worms were fixed in Helly's fluid and lightly stained in orange G in order to get a better appreciation of their distribution. The granules vary in size between 3 and 27 μ; they seem structureless and are rather irregular in outline. The granules freed from the tissue did not appear to be motile, and there was nothing to indicate that they represented living plants as suggested by Berkeley (1930) for the rather similar green pigment in the mid-gut of *Chaetopterus*. A few granules of a similar green pigment appear in the epidermis of the crown and other parts of the body of some specimens (Fig. 1D). As noted by Watson (1901) the amount present varies greatly; some of the worms from Torquay collected in April were completely green.

The mid-gut passes into a thin-walled distensible rectum at the level of the 5th–6th pairs of tori (Fig. 2H). It is here that sand grains and other matter collect before being defaecated in discrete pellets. Defaecation seems to be an intermittent process, and a great deal of matter may collect in the extensive rectum before the worm reverses in its tube to void the contents. The faecal pellets are surrounded by a thin membrane which stains blue with Azan. There appears to be no mid-ventral gutter so commonly present in this region

in other polychaetes, and the rather short cells of the hind-gut appear to have a brush-border without cilia, and with a fine granular cytoplasm. Transmission of the gut contents is presumably mainly muscular, both by the action of the gut musculature itself, though this is very poorly developed in all regions, and by the squeezing action of the body-wall muscles acting through the coelomic fluid. Strong peristaltic waves of contraction may often be seen in the hind part of the body in worms removed from their tubes.

Worms continued feeding when iron saccharate was added to the water, and these were fixed in Bouin's fluid 3 h after the addition. The whole gut of several worms was sectioned, and the sections submitted to the Prussian blue reaction and then lightly counterstained with orange G. Iron particles were found only in the mid-gut cells. It is interesting to note that the region in which the iron had been absorbed coincided exactly with that occupied by the green pigment. No tests have been made for enzymes, but the histology suggests that the stomach is the main, if not the only, secretory part of the gut, the mid-gut the absorptive part, and the rectum serves to store and elaborate the faecal pellets.

ANALYSIS OF THE GREEN PIGMENT

The mid-gut region of over fifty worms was carefully dissected out and extracted in ether: acetic acid, 5:1 mixture, after grinding in a small Griffeth-pattern tissue homogenizer. The dark green solution had a brilliant deep red fluorescence in ultra-violet light. The acetic acid was washed out by shaking repeatedly with distilled water (the first three washings buffered with sodium acetate) until the washings were no longer acid to blue litmus paper. The ethereal solution was then roughly dried by passing through ether-soaked filter paper, and concentrated *in vacuo*. The filtrate was then examined by long-paper chromatography (Kennedy, 1953) using 2:6 lutidine-water system. Two spots were obtained, one with an R_F value of 0·53, suggesting a tetra-carboxyl porphyrin such as coproporphyrin, the other with an R_F value of 0·89, suggesting a mono-carboxyl porphyrin compound such as phaeophorbide.

The ethereal solution was further concentrated *in vacuo* in the cold and extracted with 0·1 % w/v HCl, when a red-fluorescent extract was obtained; this further suggested coproporphyrin which has an HCl value of 0·08. The porphyrin was driven back into ether, neutralized with saturated potassium acetate, and the ether solution washed carefully with distilled water, three times. It was then roughly dried by passing through ether-soaked filter paper as before, and evaporated to dryness *in vacuo*. The pigment was then esterified by dissolving the residue in $MeOH:H_2SO_4$, 19:1 mixture. This was left for 48 h at room temperature, after which time the solution was diluted with water and the esterified porphyrin extracted with chloroform. The chloroform extract was washed free from acid with water, roughly dried by filtering

through chloroform-soaked paper and redissolved in dry chloroform. The solution was examined with the Hartridge reversion spectroscope and also by the chromatography method of Chu, Green & Chu (1951) when the pigment was found to consist of coproporphyrin III only (Kennedy & Vevers, 1954; Kennedy & Dales, unpublished). The spectrum showed the following maxima (in mμ):

I	II	III	IV
622	566·6	533	500·1

The epiphase from the extract of the ethereal solution with HCl was washed repeatedly with water until free from acid. The solution was examined in the Hartridge reversion spectroscope and also in the 'Unicam' S.P. 600 quartz spectrophotometer when the following maxima characteristic of phaeophorbide-*b* were shown (in mμ):

	I	II	III	IV	Sorêt
Hartridge reversion	652	602	537·5	504·5	
'Unicam'	655	603	537	505	408

The ethereal solution was then extracted with 0·2 % w/v sodium bicarbonate (Willstätter & Stoll, 1913). The hypophases were red-fluorescent, the epiphases were not; this indicates the presence of phaeophorbide-*b* only. There was also a residual yellow pigment in the epiphase after extraction with sodium bicarbonate, which when concentrated had a very slight yellowish fluorescence and which was possibly a carotenoid.

Extracts of green specimens from which the gut had been removed were similarly treated, and the green pigment in the epidermis was also found to be phaeophorbide-*b* alone.

This work was mostly done at Plymouth, and I am indebted to the Director and staff of the Laboratory for their help, and to Dr G. Y. Kennedy of the Department of Cancer Research, Sheffield, in particular, for analysing the green pigment.

SUMMARY

Owenia may feed either by ciliary means or by swallowing sand and detritus. The gut consists of three main parts, a fore-gut or stomach which is secretory, a non-secretory mid-gut region which is absorptive, and a hind-gut which is non-secretory, non-absorptive and serves to elaborate and store the faecal pellets. The mid-gut contains coproporphyrin III and also phaeophorbide-*b*, to which the green colour is due. The green pigment in the epidermis is also phaeophorbide-*b*, but phaeophorbide-*a* is apparently absent from the body.

REFERENCES

BERKELEY, C., 1930. The green bodies of the intestinal wall of certain Chaetopteridae. *Quart. J. micr. Sci.*, Vol. 73, pp. 465–76.

CHU, J. C., GREEN, A. A. & CHU, E. J., 1951. Microchromatography of porphyrin esters. *J. biol. Chem.*, Vol. 190, pp. 643–6.

CLAPARÈDE, É., 1868. Les Annélides Chétopodes du Golfe de Naples. *Mém. Soc. Phys. Genève*, T. 19, pp. 313–584.

—— 1873. *Recherches sur la structure des Annélides Sédentaires.* 199 pp. Geneva: Georg.

DRASCHE, R. VON, 1885. *Beiträge zur feineren Anatomie der Polychaeten. Zweites Heft: Anatomie von* Owenia filiformis *delle Chiaje.* 22 pp. Wien: C. Gerold's Sohn.

EISIG, H., 1887. Monographie der Capitelliden des Golfes von Neapel. *Fauna u. Flora Neapel*, Monogr. 16, 906 pp. Berlin: Friedländer.

ELWES, E. V., 1910. Notes on the littoral Polychaeta of Torquay. Part 3. Other families. *J. mar. biol. Ass. U.K.*, Vol. 9, pp. 59–81.

GILSON, G., 1893. Recherches sur les cellules sécrétantes. II. Les glandes filières de l'*Owenia fusiformis* delle Chiaje. *Cellule*, T. 10, pp. 299–325.

—— 1897. Les valves septales de l'*Owenia*. *Cellule*, T. 12, pp. 377–416.

KENNEDY, G. Y., 1953. Partition paper chromatography of the prophyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KENNEDY, G. Y. & VEVERS, H. G., 1954. The occurrence of porphyrins in certain marine invertebrates. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 663–76.

LO BIANCO, S., 1893. Gli annellidi tubicoli trovati nel Golfo di Napoli. *Atti Accad. Sci. fis. mat. Napoli*, Ser. 2, Vol. 5, pp. 1–97.

MCINTOSH, W. C., 1915. *A Monograph of the British Marine Annelids*, Vol. 3, Part 1, 368 pp. London: Ray Society.

SAINT-JOSEPH, A. de, 1898. Les Annélides Polychètes des côtes de France (Manche et Océan). *Ann. Sci. nat.*, Sér. 8, T. 5, pp. 209–464.

WATSON, A. T., 1901. On the structure and habits of the Polychaeta of the family Ammocharidae. *J. Linn. Soc. (Zool.)*, Vol. 28, pp. 230–60.

WILLSTÄTTER, R. & STOLL, A., 1913. *Untersuchungen über Chlorophyll.* 424 pp. Berlin: Springer.

WILSON, D. P., 1932. On the Mitraria larva of *Owenia fusiformis* delle Chiaje. *Phil. Trans.*, B, Vol. 221, pp. 231–334.

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PRELIMINARY OBSERVATIONS ON THE ROLE OF THE COELOMIC CELLS IN FOOD STORAGE AND TRANSPORT IN CERTAIN POLYCHAETES

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(Text-figs. 1, 2)

INTRODUCTION

To what extent food may be stored, and where such stores may be found in the polychaete body is virtually unknown. There are four main sites where the more detailed economy may take place: in the wall of the gut, in the epidermal layers, in the peritoneum, and in the cells or tissue found within the coelom. The mechanism for the removal of waste products is no better understood than the stages in the utilization and transport of absorbed nutrients. What little information exists is for the most part contradictory and unsupported by quantitative measurement.

While it is hoped to review the function of the chloragocytes in connexion with excretion in a later paper, some discussion of the function of these cells is necessary here in view of their possible relation with food storage and with the identity of the different cells found in the coelomic fluid.

It will be appreciated that most of the work on chloragocytes has been done on oligochaetes, and the identification of these cells in polychaetes has been made by analogy. While there is an extensive literature on coelomic cells (see the bibliographies of Romieu (1923) and Liebman (1946)), the only detailed work on polychaetes is that of Schaeppi (1894) on *Ophelia*, apart from brief descriptions of chloragocytes and general information in the papers of Schneider (1897, 1899) and Picton (1898). There seems to be little agreement about the chemical nature of the chloragosomes or granules occurring within the chloragocytes, and almost any tissue of a yellow or green colour associated with blood vessel or gut has been described as 'chloragogenous' by different writers.

Briefly, there are two main views regarding the function of the chloragocytes and the identity of the chloragosomes. According to one view, the chloragosomes are excretory substances which are liberated by the chloragocytes into the coelomic fluid where they may be taken up by amoebocytes and transported to the nephridia, the gut epithelium, or stored as 'brown bodies'. Supporters of this theory (Cuénot, 1898; Rosa, 1896, 1898; Schneider, 1896, 1899; Willem & Minne, 1900; Kollman, 1908; Romieu, 1923; Abdel-

Fattah, 1955) have described the chloragosomes as consisting of guanin, urates, urea or chitin. Opponents of this view maintain that the chloragosomes represent stored food materials—fat, glycogen, or albumin—and have no relation to excretion. The main exponent of this idea in recent years is Liebman (1946) working on earthworms.

Liebman recognizes two types of cell which he calls *lymphoidocytes* (here called *amoebocytes*) which are small, amoeboid cells, and *trephocytes* which produce, distribute and release nutritive substances into the blood and tissues. Liebman departs from the interpretation of others in identifying the trephocytes with chloragocytes which have become free; in them he traces three stages: (1) cells in which neutral fat is present, (2) those in which phospholipid appears, and (3) cells in which the lipid granules are released into the coelomic fluid or tissues. Liebman infers that the blood in such animals participates little in the transport of nutrients through the body, and that on the contrary it is the 'chloragogue' or trephocyte system which has been elaborated to serve this function. Liebman, however, uses the term 'chloragogue' in a very wide sense to include all the cells covering the gut. He has found in *Eisenia foetida* that wound healing is aided by the aggregation of coelomic trephocytes in the region of the wound, the cells liberating their lipid granules which are then taken up and utilized by the proliferating tissue. Liebman equates the trephocytes with the 'eleocytes' of polychaetes. To what extent this may be justified is discussed later. Issel (1905), working on the enchytraeid *Henlea*, found that the chloragogen covering of the gut was entirely lost at the time of the development of the oocytes, and concluded that this was evidence for the trophic nature of the chloragocytes. Freudweiler (1905) came to the same conclusion in her work on another enchytraeid, *Stercutus*, visualizing the liberation of the chloragosomes (which she also identifies as lipid) into the coelomic fluid with their subsequent uptake by the oocytes.

Those supporting the excretory hypothesis agree that the chloragocytes are modified peritoneal cells in contact with some branch of the intestinal blood system, and that they are not modified amoebocytes (Rosa, 1898; Rice, 1902; Sterling, 1908). The chloragosomes were thought to be guanine by Willem & Minne (1900), who strongly oppose the existence of any fat in the chloragocytes, though both Schneider (1896) and Freudweiler (1905) describe fat in these cells. Cuénot (1898) argued that glycogen, which occurs in large amounts in the peritoneum, is the main storage substance in earthworms, and that the view that the chloragosomes are lipid in nature does not justify the conclusion that they represent important food stores. More recently, Abdel-Fattah (1955) identifies urea in the chloragocytes, and opposes the views of Willem & Minne (1900) in identifying the chloragosomes as guanine. Abdel-Fattah (1955) finds no evidence for the chloragocytes becoming free.

Clearly much of this confusion has resulted from differences in interpretation in the origin and history of the coelomic amoebocytes and trephocytes.

Willem & Minne (1900) regard the chloragocytes as storing the waste products which are later taken up by amoebocytes, and they oppose the view that the chloragocytes themselves become free as Liebman maintains. Romieu (1923), Kollman (1908) and Rosa (1898) derive the trephocytes from amoebocytes, and Romieu identifies in various polychaetes, both fat and excretory substances in these cells.

In polychaetes the increase in abundance of the trephocytes during the early development of the gametes has been noted by a number of observers (Claparède, 1868, 1873; Cuénot, 1891; Romieu, 1921; Fauré-Fremiet, 1929; Herpin, 1921; Dales, 1950), and this implies a nutritive function. None of these writers has identified these trephocytes with chloragocytes or suggested a connexion with excretion.

Some knowledge of the quantities of fat and glycogen in different parts of the body, especially in the trephocytes at different seasons, would aid the elucidation of these problems in polychaetes, and this is the main purpose of the present paper. It is hoped to extend the work in much greater detail.

The species studied were selected for various reasons. Both *Amphitrite johnstoni* Malmgren and *Arenicola marina* (L.) are large and have few septa, so that the collection of the coelomic fluid and contained corpuscles can be reasonably complete. While both species spawn in autumn, *Amphitrite* has a large number of trephocytes at all times, but *Arenicola* has very few. *Nereis diversicolor* O. F. Müller does not lend itself well to this type of study, not only because of its smaller size, but because of the virtual impossibility of collecting the whole coelomic contents owing to the septa. Some determinations of the fats in the body wall and gametes have been made, however, in the light of earlier work (Dales, 1950; Dales & Kennedy, 1954). A few observations have been made on *Terebella lapidaria* (Kahler) for comparison with *Amphitrite*, as the trephocytes differ in containing haemoglobin. The *Amphitrite* were all collected at Noss Mayo, near Plymouth, *Arenicola* from Plymouth and from Chalkwell, Essex, where the *Nereis* were also collected.

Much of this work has been done in the Plymouth Laboratory, and I wish to thank the Director, and Dr L. H. N. Cooper and other members of the staff for help in various ways. In particular I have to thank Mr R. Tozer for collecting *Amphitrite* at times when I could not be at Plymouth, and Dr G. Y. Kennedy for assistance with the analysis of the coelomic cells in *Amphitrite* and *Terebella*. I also wish to thank Mr M. A. Gross for taking the photographs illustrated in Fig. 2.

METHODS

Bloor's methods (1928, 1929) for the analysis of the fat constituents of blood plasma were adapted for the estimation in the different tissues of total fat (estimated as total fatty acids plus sterols) and phospholipid. Cholesterol was

estimated by Sperry's method (1938). After some practice total fats could be estimated to an accuracy of $\pm 2\%$ with samples of 50–100 mg of tissue (2–5 mg fat); cholesterol could be estimated rather more accurately; values for phospholipid were found to be less reliable, and have been mostly disregarded. Full details of the procedure adopted in the analysis of the fat are given in the Appendix; the main principles are outlined below.

Extraction was effected with boiling 95% ethanol:ether 3:1 mixture; the extract was made up to a known volume which was then sampled for estimation of total fat, phospholipid, and sterol fractions. For the estimation of total fats, a portion of the extract was saponified, the solvent evaporated off and the pasty residue treated with hot H_2SO_4 to liberate the fatty acids which were then re-extracted with boiling light petroleum. A known quantity of the solution was evaporated to dryness and the fatty acids estimated by oxidation with a chromic acid mixture against a control, followed by back titration with sodium thiosulphate. Phospholipids were estimated by evaporating a portion of the original extract to dryness, re-extracting with light petroleum and then precipitating the phospholipids with acetone and magnesium chloride. The precipitate was washed repeatedly with acetone, and redissolved in ether; a measured quantity evaporated to dryness, and the residue estimated by the chromic acid procedure as before. Total sterols were estimated by treating a portion of the original extract with caustic alkali, and after neutralization, precipitating the sterols as their digitonides. After purification the amount present was estimated by the Liebermann-Burchard reaction, the density of the colour produced being measured in a spectrophotometer against a cholesterol standard at 670 mμ.

For the estimation of glycogen the sample of tissue (about 50 mg) was placed in ethanol at 0° C, or directly in 3 ml. of 30% KOH and refluxed for 2 h. An excess of 95% alcohol was then added to the solution, the tube dipped into a boiling water-bath for 1 min, and then allowed to stand overnight. The precipitate was washed twice in absolute alcohol, and the precipitate repeatedly centrifuged. The walls of the tube were finally washed down with 10 ml. of approximately 0·6 N-HCl and then heated under reflux for 3 h. After cooling, the solution was neutralized with 1·0 N-NaOH against thymol blue, made up to a known volume, and the glucose so formed estimated by the method of Hagedorn & Jensen (1923). For this a Rehberg microburette was used; Krogh pipettes were used for the more critical measurements.

The histochemical work was done in the light of the recent work of Baker (1946) and Cain (1947) for fats, and Smyth & Hopkins (1948) for glycogen. While living coelomic cells in all forms were examined whenever possible, permanent preparations for fat distribution were made of tissue fixed in Baker's formal-calcium, and for glycogen in Best's picro-formal fixative at 0° C. For the study of glycogen, tissues were kept in absolute alcohol at 0° C until embedded in ester wax as recommended by Smyth & Hopkins (1948).

Best's carmine method was used for subsequent staining against diastase controls as recommended by Pearse (1953). In the study of fats, frozen sections were cut of whole tissues fixed in formal-calcium. Smears were made of the living coelomic cells (without addition of albumin) and fixed in the same way. Sudan black B, acetylated according to the technique of Casselman (1954) was used to localize the fats, the stain being dissolved either in 70% alcohol, or in propylene glycol (Chiffelle & Putt, 1951), against hot pyridine controls. The latter method seemed to give more delicate results. Baker's acid haematin method (1946) for phospholipids, and Cain's nile blue method (1947), staining and differentiating at 37° C, were also used, Gurr's glycerine jelly being employed as mountant in most cases.

RESULTS

Amphitrite johnstoni

Fats

Determinations of total fats in various tissues at different seasons are given in Table 1, and the arithmetic means are compared graphically in Fig. 1. The values for all tissues are highest in August, and the difference between the August values and those for November are significantly different for all tissues. The differences in the other values for body wall and gut are not statistically significant, and the higher April value compared with the February value for the coelomic cells only possibly significant. It can be concluded from these figures that the gut wall has the highest concentration of fat in the body at all times, and that the value is highest in August and lowest in November shortly after spawning. This suggests that the gut is to a certain extent a fat store. The body has the lowest value for fat concentration, but this may be due to the large proportion of muscular tissue which has a lower fat content than the epidermis. It is possible that the fat content of the epidermal layers is very similar to that of the gut, and it is this layer which may cause the seasonal change in values for body wall. The variation in value for coelomic contents reflects the quantity of gametes present; the value is highest in August owing to the large quantity of mature gametes, and low in November after spawning. The fat content of the nearly ripe oocytes and the trephocytes is similar. In August-September the cells and the oocytes may be separated almost completely by centrifugation. Spermatids and sperm contain much less fat, but the total fat in the coelom of a ripe male is much the same as that of a ripe female. The results of determinations of twelve randomly selected worms is shown in Table 2. The mean wet weight of the females was 9·35 g, of which 2·86 g was represented by the coelomic fluid and 0·95 g by the coelomic cells. In a ripe female, therefore, about 36 g of fat is represented by the oocytes and trephocytes, which is about half the total quantity in the remainder of the body at this time. During the early stages of maturation of the oocytes (January–February) when they are similar in size to the trephocytes, sudan black B

TABLE 1. CONCENTRATION OF FAT IN *AMPHITRITE JOHNSTONI* AT DIFFERENT SEASONS (MG/G WET WEIGHT)

	August (1955)	November (1955)	January–February (1956)	April (1956)
Body wall	7·37	10·60	3·83	5·00
	16·60	10·30	7·00	8·90
	11·45	8·05	7·95	10·00
	15·00	3·65	6·25	15·90
	16·00	7·20	7·80	6·30
	15·20	—	8·10	5·90
Mean	13·60	7·96	6·80	8·70
Gut	38·40	31·80	42·50	30·50
	42·50	23·20	26·80	39·40
	55·20	14·90	35·10	44·50
	47·40	41·70	49·40	51·50
	54·70	19·90	30·10	47·20
	40·30	25·70	25·30	—
Mean	46·40	26·20	34·90	42·60
Coelomic cells	45·20	15·70	12·10	21·30
	35·90	12·00	12·40	21·10
	29·50	6·80	8·50	14·40
	52·80	1·20	9·30	7·60
	51·20	—	10·20	26·90
	36·50	—	11·60	—
Mean	41·85	8·90	10·68	18·30

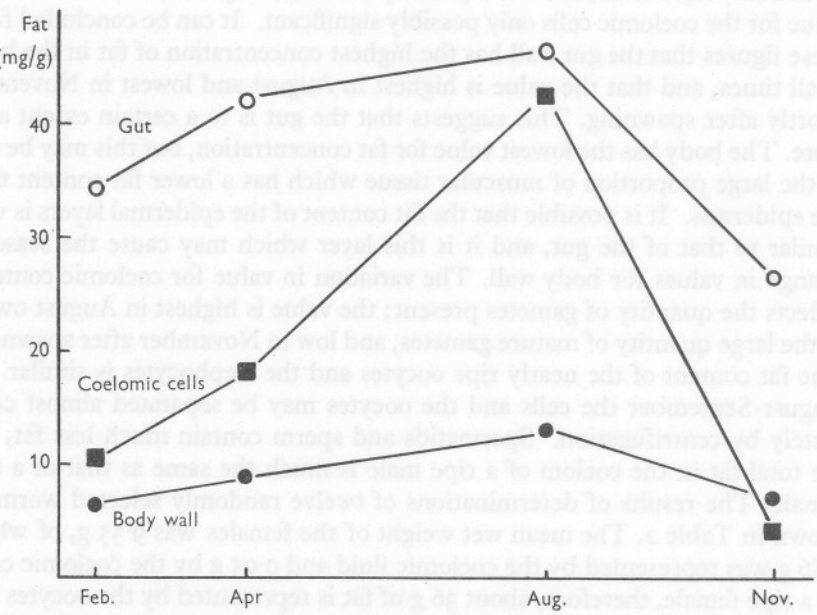

Fig. 1. Mean concentration of fat in *Amphitrite johnstoni* at different seasons (mg/g fresh weight)

staining showed no more fat in the oocytes than in the trephocytes. At this time, six worms of mean weight 9·0 g gave a mean total value of 6·1 mg of fat represented by the coelomic cells. There is about six times this quantity in the gut. The proportion by weight of the different parts of the body of three randomly selected worms is shown in Table 3.

TABLE 2. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF FAT IN COELOMIC CONTENTS (MG/G WET WEIGHT)

	Trephocytes	Oocytes	Sperma-tids/sperm
Weight analysed	(1) 2·985 g (female) (2) 3·667 g (male)	(1) 4·927 g (2) 7·332 g	— (2) 334 g
Total fat	(1) 45·2 (2) 35·9	(1) 31·8 (2) 42·2	10·2 —
Phospholipid	(1) 12·0 (2) 15·3	(1) 15·0 (2) 19·4	6·9 —
Total sterols	(1) 3·3 (2) 3·4	(1) 3·5 (2) 4·1	1·8 —

TABLE 3. *AMPHITRITE JOHNSTONI*, PROPORTION OF PARTS ANALYSED (G WET WEIGHT)

	Gross weight	Body wall	Gut	Coelomic cells
	10·6	3·6	0·76	1·25
	9·6	3·0	0·56	1·20
	9·1	2·4	0·70	1·20
Mean	9·8	3·0	0·67	1·22

TABLE 4. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF FAT IN DIFFERENT PARTS OF THE GUT (MG/G)

Fore stomach	Hind stomach	Intestine
61·8	14·0	82·5
87·3	18·0	56·2

The concentration of fat in different parts of the gut was investigated, and the results of two determinations each containing guts from six worms, are shown in Table 4. The values for the fore stomach and intestine are not significantly different, but the hind stomach, which is almost entirely muscular (Dales, 1955), is much lower and this value is interesting in giving some idea of the fat content of muscle alone. The values for fore stomach and intestine are higher than for the trephocytes, though the greater weight of the trephocytes tends to equate the total amount of fat represented in the gut and the trephocytes in any worm.

Glycogen

The concentration of glycogen in different parts of the body is shown in Table 5. The low concentration in all parts of the gut contrasts strongly with the high fat values, though the concentrations in the body wall and the coelomic cells are roughly equivalent to the concentration of fats. Sections of body

wall stained with Best's carmine showed most of the glycogen in the peritoneum, and as there is much less in the muscular layers which constitute a large proportion of the weight, the actual concentration in the peritoneum may be high. It is interesting that the values for the coelomic cells are not very different from the values for fat.

TABLE 5. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF GLYCOGEN IN DIFFERENT PARTS OF THE BODY (MG/G WET WEIGHT)

			Gut		
	Body wall	Coelomic cells	Fore stomach	Hind stomach	Intestine
	13·7	37·4	3·5	2·3	6·2
	11·6	18·1	5·8	1·4	2·0
	6·9	35·0	3·6	1·7	2·3
	10·5	13·3	3·6	1·4	3·5
	19·2	29·2	7·8	3·3	2·8
	14·2	36·0	5·8	2·0	10·4
	5·3	15·5	5·3	2·2	1·5
	10·5	44·9	2·8	2·3	3·7
	14·7	18·9	6·3	1·8	2·8
	9·7	26·4	6·7	1·9	5·5
	13·1	16·8	—	1·0	3·5
	17·4	8·4	—	—	2·7
	—	16·8	—	—	—
	—	32·0	—	—	—
Mean	12·2	25·7	5·1	1·8	3·6

Histochemical data

Smears of the coelomic cells were made at all seasons and stained for fats and glycogen. Their appearance when stained with sudan black B is shown in Fig. 2. All the granules stain a dark black and the same granules stain dark blue with Cain's 0·02% nile blue and give a positive reaction with Baker's acid haematin, so that they are at least partly phospholipid in nature. No red-staining granules were ever found, either in the trephocytes or in other parts of the body with Cain's method, and positive results were always obtained with Baker's acid haematin test. From the quantitative data it is known that 30–50% of the total fatty acids in most tissues is phospholipid, so that these granules are presumably a mixture of neutral fat and phospholipid or possibly consist of neutral fat surrounded by a phospholipid shell. Purple-staining granules were in fact sometimes obtained with Cain's method, and the smaller granules of fat nearer the nucleus in the trephocytes often gave a rather greenish colour with 0·02% nile blue. These granules are about 2 μ in diameter, the largest about 10 μ in diameter. Glycogen is scattered throughout the cytoplasm and these granules are only 1–2 μ in diameter. Frozen sections of gut and body wall were made and stained with acetylated sudan black B. In the gut most of the fat is present in the epithelial cells rather than in the very thin peritoneum, and in the body wall most of the fat is in the epidermal layer.

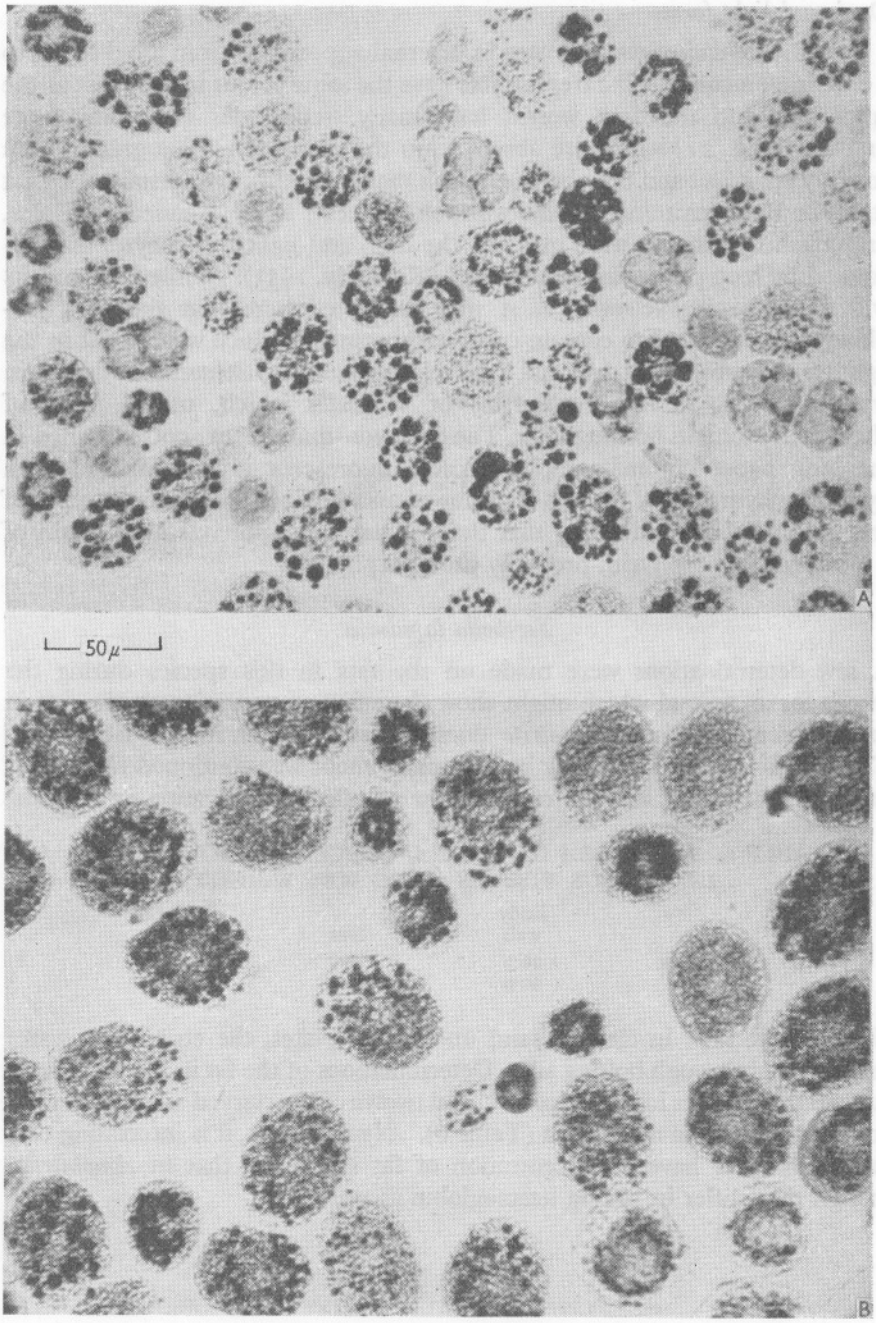

Fig. 2. Coelomic trephocytes of *Amphitrite johnstoni*, stained with acetylated sudan black B in propylene glycol. A: cells from the dark form showing the large unstained spherical granules. B: cells from the light form, lacking the spherical granules.

Dark and light forms

Adult *Amphitrite johnstoni* vary in external appearance from a light pink to a dark brown colour. The trephocytes have the same colour in the mass as the epidermis, and the dark worms have many trephocytes containing large granules (Fig. 2A) which are absent from the pink form. The granules are roughly spherical and may be 12–15 μ in diameter; they are unstained by fat stains or Best's carmine. In the work on the heart body (Kennedy & Dales, unpublished) extracts were made of the dark and light trephocytes and examined by long paper chromatography (Kennedy, 1953). While these results will be published elsewhere, it may be noted here that the dark trephocytes were found to contain coproporphyrin III, which was absent in the light trephocytes. Both dark and light cells contained a haematin. A haematin was found also in the trephocytes of *Terebella* which, unlike those of *Amphitrite*, contain haemoglobin. The dark non-fluorescent spots obtained in the long paper chromatography became fluorescent after treatment with hydrazine hydrate and gave an R_F value of 0·81 in *Terebella* and both types of cell in *Amphitrite*, indicating that the original dark spot was a haematin of a dicarboxylic porphyrin, probably protoporphyrin.

Terebella lapidaria

A few determinations were made on the fats in this species during the search for an animal which might show the effect of starvation on the fats in the different tissues. *Amphitrite* does not starve well in the laboratory; *Terebella* is rather more hardy but the experiment was continued for a short time only (10 days), and the results show no effect of starvation on the fats.

TABLE 6. *TEREBELLA LAPIDARIA*, CONCENTRATION OF FAT IN VARIOUS TISSUES (MG/G WET WEIGHT)

	Body wall	Gut	Coelomic cells
Controls	26·3	35·7	32·8
'Starved'	20·0	32·9	32·5

Worms were kept in cleaned sand under glass plates, the circulating water being passed through bolting silk. Determinations of the fat in the body wall, gut and trephocytes in twelve control and twelve of the starved animals showed no difference in the fat content (Table 6). Nevertheless, it is interesting that the trephocytes have a concentration of fat similar to that in *Amphitrite*, though they differ in having haemoglobin as well.

Arenicola marina

Fats

The quantity of coleomic cells is very small at all seasons, though there is some increase during the maturation of the gametes, though even then the quantity of cells in the coelomic fluid is small compared with the quantity of gametes. The quantity of coelomic cells was measured at different times by filtering the whole of the coelomic fluid through oven-dried and weighed filter-paper and reweighing after drying at 100° C. It was found that the value did not differ significantly over most of the year, and that the increase in weight of coelomic contents towards October was due entirely to the oocytes, which may be separated from the other cells by centrifugation. The detailed results are of little relevance in the present context, but it may be noted here that of sixteen worms of mean weight 3·15 g sampled in January, the mean dry

TABLE 7. *ARENICOLA MARINA*, CONCENTRATION OF FAT IN VARIOUS TISSUES (MG/G WET WEIGHT)

(November–December: six worms in each determination.)

		Body wall	Gut	Coelomic cells
Normal		13·95	33·20	15·80
		11·20	39·70	26·00
		11·95	13·10	39·00
	Mean	12·33	28·67	26·93
Starved (6 weeks)		21·75	123·50	23·60

weight of the coelomic cells was 5·6 mg, or less than 0·2 % of the gross weight of the worm. While the quantity of coelomic cells is much less than in *Amphitrite*, the concentration of fat is not so much less (Table 7). *Arenicola* starves better in the laboratory than most worms, and in November–December worms were kept for 6 weeks at room temperature without food. Those that survived had lost considerable weight, though much of this reduction was due to loss of coelomic fluid. Determinations were made on the fats of normal and starved worms, and it was found that in starved worms there was a greatly increased fat-concentration in the gut and body wall, but the coelomic cells did not seem to be affected (Table 7). In a normal non-breeding worm with a gross weight of 8·0 g, a total of 47·5 mg of fat is located in the body wall, 18·1 mg in the gut and less than 0·2 mg in the coelomic cells.

Glycogen

The results of determinations of the quantity of glycogen present in various tissues in six randomly selected worms is shown in Table 8. The values for the coelomic cells were negligible and have not been included. It will be seen that the concentration of glycogen is highest in the body wall, the value being of the same order as that in the trephocytes of *Amphitrite*.

TABLE 8. *ARENICOLA MARINA*, CONCENTRATION OF GLYCOGEN IN VARIOUS TISSUES (MG/G WET WEIGHT), AND PROPORTION OF PARTS (APRIL)

	Gross weight (g)	Weight coelomic contents (g)	Weight body wall (g)	Concentration glycogen in body wall (mg/g)	Weight gut (g)	Concentration glycogen in gut (mg/g)
	3·2	1·6	1·3	25·1	0·3	11·4
	9·7	4·0	5·3	22·5	0·6	10·6
	6·0	3·1	2·6	20·0	0·3	6·3
	11·0	4·7	5·3	20·7	1·0	15·6
	11·8	4·5	6·2	12·1	1·1	11·7
	5·3	2·5	2·4	39·4	0·4	13·9
Mean	—	—	3·8	23·3	0·6	11·6

Histochemical data

Sudan black B staining showed that fat granules were present in some of the ceolomic cells, but never in great quantities. The oocytes, too, contain little fat as compared with either *Amphitrite* or *Nereis*. Glycogen was not demonstrable by Best's carmine in the coelomic cells. In sections of body wall considerable deposits were found in the peritoneum, while fat occurs mainly in the gut epithelium and the epidermis.

Nereis diversicolor

Fats

In a previous paper (Dales & Kennedy, 1954) the seasonal variation in colour in this species was described and it was suggested that the greener specimens most usually encountered in early spring owed their colour to the withdrawal of carotenoids from the epidermis thereby unmasking the biliverdin to which the green colour is due. Determinations of the fat content of the body wall in different worms have been made before and after spawning in order to test the hypothesis that the carotenoid removal is merely a visible manifestation of the withdrawal of the epidermal fats in connexion with the maturation of the oocytes. The results are not conclusive, but are presented here because of the interest of the values for fat and glycogen in the general economy of the animal.

Completely green males just before spawning have an extremely thin body wall and this has a fat content below that measurable in a single worm by the method used. While fats have been removed along with other substances in the tissues phagocytized, there is no evidence that they are withdrawn specifically for utilization in the maturation of the sperm. In ripe females, which are usually greener soon after the spawning period, the fat content is still high. The values for November, January (pre-spawning), and April (post-spawning) do not differ significantly (Table 9). There may still be a real utilization of epidermal fat in the maturation of the oocytes, but the high proportion of muscle included in the body-wall samples analysed may mask the change, for

there is no doubt that a green male has negligible amounts of fat in the epidermis while a non-breeding worm with an orange hue has values in the order of 16 mg/g. The amount of fat represented by the sperm in the coelom is roughly equivalent to that represented by the oocytes in a mature female of similar size. In April it was found that the gut contains about 20 mg/g of fat.

TABLE 9. *NEREIS DIVERSICOLOR*, CONCENTRATION OF FAT IN THE BODY WALL AT DIFFERENT TIMES (MG/G WET WEIGHT). FEMALES

	November (1955)	January (1956)	April (1956)
	17·00	18·40	10·50
	15·70	15·40	13·50
	17·90	7·96	15·90
	27·50	23·70	20·00
	27·10	22·40	11·50
	11·50	12·85	15·75
	13·70	20·10	13·10
	12·00	20·90	—
	21·00	—	—
	9·80	—	—
Mean	17·32	17·70	14·32

TABLE 10. *NEREIS DIVERSICOLOR*, ANALYSIS OF OOCYTES

	Fat concentration (mg/g)	Glycogen concentration (mg/g)	Water (% wet weight)
16 Jan. (coelomic)	59·1	32·9	20·9
19 Feb. (released)	97·0	57·5	57·5

TABLE 11. *NEREIS DIVERSICOLOR*, TOTAL WEIGHT OF OOCYTES IN RIPE FEMALES (g)

	Gross weight of worm	Wet weight oocytes
	0·489	0·0423
	0·790	0·0498
	0·602	0·0577
	0·621	0·0550
Mean	0·625	0·0510

In a ripe female of 625 mg gross weight, the coelomic fluid weighs about 75 mg. The tissues have a fat content of about 15·7 mg/g and the ripe eggs 90 mg/g (Table 9). A worm of this size has about 50 mg of eggs, or a total of 4·5 mg of fat in the eggs—about half the quantity in the body wall (Table 9). If all the fat required for the maturation of the oocytes were withdrawn from the body wall alone, a much greater variation in the body wall values would be expected. The fat and glycogen content of the oocytes are shown in Table 10. Clearly, either only some of the fat is withdrawn from the body wall or there is a continual replacement of fat, implying a resorption of tissue from other sites or continued feeding by the animal prior to spawning. It has been remarked already (Dales & Kennedy, 1954) that females remain much more healthy prior to spawning than the males, in which much more phagocytosis occurs

and which may well be incapable of feeding just before the spawning period. As far as the males are concerned all the fat required for the maturation and nourishment of the sperm could easily be provided by the amount in the body wall alone.

Glycogen

Some determinations of the concentration of glycogen in the body wall were made in January. The mean value of ten determinations was 34·3 mg/g, a value which is high compared with *Arenicola* or *Amphitrite*.

TABLE 12. FAT CONCENTRATION IN DIFFERENT TISSUES (MG/G)

	Body wall	Gut	Coelomic cells (excluding oocytes)
Amphitrite	9·26	37·50	40·50
Arenicola	12·33	28·67	26·93
Terebella	26·30	35·70	32·80
Nereis	15·70	21·40	—

DISCUSSION

In Table 12 are summarized all the mean values of fat concentration in the four species studied. It will be noticed that there is a rough correspondence in each species between the values for gut and coelomic cells. While *Arenicola* and *Amphitrite* are similar in this respect, the proportion which each part represents in the total body weight is different. If the total quantities of fat are calculated for each species in worms of similar size (say 10 g) it appears that the total quantity of fat in each is similar, and the lack of a well-developed trephocyte system in *Arenicola* is compensated for by a larger proportion of body wall in the total body weight. This is shown in Table 13. The same comparison may be made for the total quantity of glycogen present, and these calculations are shown in Table 14.

It is difficult to conclude from these preliminary results how far these variations reflect different food depots, but the increase in fat concentration in all tissues in late summer in *Amphitrite* and a corresponding decrease in winter suggests that the fats are both deposited and withdrawn from the gut, the body wall, and the trephocytes during the maturation of the gametes. The high fat values for the gut and the histochemical evidence of its distribution in the epithelial cells suggests that the physiological difficulty of transporting fats in the blood is partly overcome by storing them primarily in the epithelial cells of the gut itself, though perhaps later the fat may be transferred by the blood stream to the epidermis, by the peritoneum to the coelomic fluid, or may be removed by amoebocytes. Except in the event of direct removal by amoebocytes, the fat must first enter the blood stream, since the epithelial cells are separated from the muscular and peritoneal layers by the gut sinus. This

interpretation would concur with the views of Romieu (1923) and Kollman (1908) that the trephocytes are amoebocytes which have taken up fat granules.

The similar appearance of the epidermis and coelomic cells that has been noted in *Nereis* (Dales & Kennedy, 1954), *Owenia* (Dales 1957) and in the dark and light forms of *Amphitrite* (Kennedy & Dales, unpublished), suggests a close interrelationship between them. Fretter (1953) found that the amoebocytes in *Platynereis dumerilii* took up radioactive strontium and yttrium from the sea water, both through the gut and the epidermis, and that amoebocytes containing these isotopes were most frequent in the epidermis. Fretter, following Liebman (1946), distinguishes these amoebocytes from trephocytes which did not, apparently take up the isotopes. In *Amphitrite* trephocytes in the coelom certainly show no amoebic powers, though they may be kept healthy

TABLE 13. TOTAL QUANTITY OF FAT IN 10 G WORMS (MG) (NOVEMBER–FEBRUARY)

	Body wall	Gut	Coelomic cells	Total
Arenicola	55·3	20·0	0·135	75·4
Amphitrite	27·8	25·1	17·400	70·3

TABLE 14. TOTAL QUANTITY OF GLYCOGEN IN 10 G WORMS (MG) (APRIL)

	Body wall	Gut	Coelomic cells	Total
Arenicola	88·5	7·0	—	95·5
Amphitrite	37·0	1·9	36·6	74·5

in a hanging drop in a sealed slide for many days. Fauré-Fremiet's observations (1929) confirm this. The amount of fat increases with increasing size of the trephocyte, but the largest contain few granules. This suggests that the fat is either synthesized by the cell or taken up directly from the coelomic fluid, and eventually discharged for the benefit of younger cells or growing gametes. It is just possible that there is some correspondence between the glucose in the body fluid, and the glycogen and fat in the trephocyte, and it is planned to investigate this further. Seton & Wilbur (1949) have made some determinations of the glucose in the coelomic fluid, which was found to increase with rising temperature. In some preliminary determinations in *Amphitrite* in the present work, the glucose content was found to increase under various adverse conditions at different temperatures, but insufficient observations have been made as yet to make any conclusions about these experiments.

The trephocytes in *Amphitrite* undoubtedly contain substances other than glycogen and fat, but these have not yet been determined and are beyond the scope of this paper. Both Fauré-Fremiet (1929) and Romieu (1923) state that albuminoids are present, and Romieu (1923) also identifies various excretory

substances. Von Brand (1927), Fauré-Fremiet (1929) and Wilbur & Bayors (1947) are the only previous workers who have published determinations of fat content in polychaete tissues. Fauré-Fremiet (1929) found a total fat content in the trephocytes of *Amphitrite* of 1·23% (wet weight), and Wilbur & Bayors (1947) found a total lipid content in *Amphitrite ornata* of 3·15%, *Arenicola marina* 1·22% and *Nereis pelagica* of 2·17% of the total wet weight. Von Brand (1927) found that fat accounted for 3·81% in *N. pelagica*, 0·54 in *A. marina* (wet weight). These values are not dissimilar to those obtained in the present work, but they have little relevance to the present problem since they are overall values, and no information is given about the state of the worms. Alscher (1949) has also made observations on the trephocytes in *Arenicola marina* and *Amphitrite ornata* and has made counts of the number of fat granules in different types and size of coelomic cell. The counts show that the amount of fat present within each size-group of cell is very variable, but in both species the smaller cells have fewer granules, and the largest cells have the widest spread in number with a possible inference of loss of granules in the larger and older cells.

While the experiments on the effect of starvation are not conclusive, the rise of fat concentration after starving in *Arenicola* is very reminiscent of the 'fatty liver' condition in vertebrates, but it may be due directly to substances other than fats being withdrawn at times of inanition. On the other hand, in the male *Nereis diversicolor* the gut and the body wall are eroded almost completely immediately before the spawning period, and the total fat content is considerably less than its normal value. It is proposed to examine the effects of starvation more closely, especially in relation to glycogen which has not yet been measured in starving worms.

The increase in the number of trephocytes during the maturation of the gametes in *Nereis* (Dales, 1950) suggests that these cells are primarily, if not solely, concerned with this function. The terebellids, on the other hand, maintain a large number of trephocytes which does not vary appreciably during the year, though there is a considerable loss when spawning occurs. While Liebman (1946) may be correct in assuming a dual origin for the amoebocytes and trephocytes in earthworms, there is no evidence for this in *Amphitrite*. The assumption that the trephocytes in earthworms are chloragocytes which have become free may be correct, but these trephocytes may not be comparable with the trephocytes in *Amphitrite*. Terebellids do not appear to have any cells on the outside of the gut or on the dorsal vessel which could be described as chloragocytes, and green or yellow granules are found only in the heart body (Kennedy & Dales, unpublished). There is no evidence that the trephocytes in *Amphitrite* are derived from the heart body, or that this organ ever liberates cells into the blood stream in which they are virtually absent. Ashworth (1904) describes intravasal tissue in the heart of *Arenicola*, but this is very small compared with that in *Amphitrite*. Kermack (1955) has given some account of

the activity of amoebocytes after injection of ink particles into the gut of *Arenicola*, amoebocytes laden with ink particles collecting in the intravasal tissue and in the coelom. This is interesting in demonstrating one way in which substances may be removed directly from the gut. The intravasal tissue contains chloragosomes, but chloragocytes are found also in the coelomic epithelium (Schneider, 1899). The relationships of the chloragocytes and the amoebocytes and trephocytes are not known. Some of these problems are now being investigated.

SUMMARY

Determinations of the concentration of fat and glycogen in the body wall, in different parts of the gut and in the coelomic cells are described in *Amphitrite* and *Arenicola*. It is suggested that the trephocyte system constitutes a store of fat and glycogen derived from a primary store in the absorptive parts of the gut itself. In *Arenicola* and *Nereis* surplus fat is removed from the gut itself through the blood or directly by amoebocytes; the fat deposited in the epidermis and the glycogen in the peritoneum. In these worms the coelomic trephocytes are solely concerned with the maturation of the gametes. No relationship can be established between these cells and the chloragocytes. In *Amphitrite* and *Terebella* fat is stored also in the coelomic trephocytes which may derive their contents directly from the gut or from the body wall. Glycogen is stored in the trephocytes in *Amphitrite*, and in *Arenicola* in the peritoneum. Thus while large amounts of fat and glycogen are found in the trephocytes in *Amphitrite*, the total amount present in the body is no more than in *Arenicola* which lacks a well-developed trephocyte system, and in this species a larger proportion of fat and glycogen is found in the body wall.

REFERENCES

ABDEL-FATTAH, R. F., 1955. The chloragogen tissue of earthworms and its relation to urea metabolism. *Proc. Egypt. Acad. Sci.*, Vol. 10, pp. 36–50.

ALSCHER, R. P., 1949. Description of, and localization in, cells of body cavity fluid of *Arenicola marina* (Lamarck) and *Amphitrite ornata* (Leidy). *Biol. Bull., Woods Hole*, Vol. 97, pp. 253–4.

ASHWORTH, J. H., 1904. Arenicola. *L.M.B.C. Memoirs*, Vol. 11, 118 pp. London: Williams and Norgate.

BAKER, J. R., 1946. The histochemical recognition of lipine. *Quart. J. micr. Sci.*, Vol. 87, pp. 441–70.

BLOOR, W. R., 1928. The determination of small amounts of lipid in blood plasma. *J. biol. Chem.*, Vol. 77, pp. 53–73.

—— 1929. The oxidative determination of phospholipid (lecithin and cephalin) in blood and tissues. *J. biol. Chem.* Vol. 82, pp. 273–86.

BRAND, T. F. VON, 1927. Stoffbestand und Ernährung einiger Polychaëten und anderer mariner Würmer. *Z. vergl. Physiol.*, Bd. 5, pp 643–98.

CAIN, A. J., 1947. The use of nile blue in the examination of lipoids. *Quart. J. micr. Sci.*, Vol. 88, pp. 383–92.

CASSELMAN, W. G. B., 1954. Acetylated sudan black B as a more specific histochemical reagent for lipides. *Quart. J. micr. Sci.*, Vol. 95, pp. 321–2.

CHIFFELLE, T. L. & PUTT, F. A., 1951. Propylene and ethylene glycol as solvents for sudan IV and sudan black B. *Stain Tech.*, Vol. 26, pp. 51–6.

CLAPARÈDE, E., 1868. *Les annélides chétopodes du Golfe de Naples.* 500 pp. Geneva: Georg.

—— 1873. *Recherches sur la structure des Annélides Sédentaires.* 200 pp. Geneva: Georg.

CUÉNOT, L., 1891. Études sur le sang et le glandes lymphatiques dans la série animale. *Arch. Zool. exp. gén.*, Sér. 9, T. 2, pp. 365–475.

—— 1898. Études physiologiques sur les Oligochètes. *Arch. biol.*, T. 15, pp. 79–124.

DALES, R. P., 1950. The reproduction and larval development of *Nereis diversicolor* O. F. Müller. *J. mar biol. Ass. U.K.*, Vol. 29, pp. 321–60.

—— 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

—— 1957. The feeding mechanism and morphology of the gut of *Owenia fusiformis* delle Chiaje. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 81–9.

DALES, R. P. & KENNEDY, G. Y., 1954. On the diverse colours of *Nereis diversicolor*. *J. mar. biol. Ass. U.K.*, Vol. 33. pp. 699–708.

FAURÉ-FREMIET, E., 1929. Constitution et proprietes physico-chimiques des éléocytes d'*Amphitrite johnstoni* (Malmgren). *Protoplasma*, Vol. 5, pp. 321–37.

FRETTER, V., 1953. Experiments with radioactive strontium (^{90}Sr) on certain molluscs and polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 367–84.

FREUDWEILER, H., 1905. Studien über das Gefäßsystem niederer Oligochäten. *Jena. Z. Naturw.*, Bd. 40, pp. 383–422.

HAGEDORN, H. C. & JENSEN, N., 1923. Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.*, Bd. 135, pp. 46–58.

HERPIN, R., 1921. Sur l'origine et le rôle des cellules à résèrves de la cavité générale chez *Perinereis cultrifera* (Gr.) et *Perinereis Marioni* (Aud. et Edw.) et la différenciation précoce de leurs œufs. *C.R. Acad. Sci., Paris.*, T. 173, pp. 249–52.

ISSEL, R., 1905. Intorno agli escreti dei linfociti. *Arch. Zool. (ital.), Napoli*, Vol. 2, pp. 125–35.

KENNEDY, G. Y., 1953. Partition paper chromatography of the porphyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KERMACK, D. M., 1955. The anatomy and physiology of the gut of the polychaete *Arenicola marina* (L.). *Proc. zool. Soc. Lond.*, Vol. 125, pp. 347–81.

KOLLMAN, M., 1908. Notes sur les réserves albuminoides des Insectes et des Annélides. *Bull Soc. zool. Fr.*, T. 34, pp. 149–55.

LIEBMAN, E., 1946. On trephocytes and trephocytosis; a study on the role of leucocytes in nutrition and growth. *Growth*, Vol. 10, pp. 291–330.

PEARSE, A. G. E., 1953. *Histochemistry, Theoretical and Applied*, 530 pp. London: Churchill.

PICTON, L. J., 1898. On the heart body and coelomic fluid of certain polychaetes. *Quart. J. micr. Sci.*, Vol. 41, pp. 263–302.

RICE, W. J., 1902. Studies in earthworm chloragogue. *Biol. Bull., Woods Hole*, Vol. 3, pp. 88–94.

ROMIEU, M., 1921. Les inclusions cristallines des éléocytes de *Nereis* et leurs relations avec la granulation éosinophile. *C.R. Acad. Sci., Paris*, T. 168, pp. 367–9.

—— 1923. Recherches histophysiologiques sur le sang et sur le corps cardiaque des Annélides polychètes. *Thès. Fac. Sci. Univ. Paris*, 336 pp., Paris: Doin.

ROSA, D., 1896. I linfociti degli Oligocheti. Ricerche istologiche. *Mem. R. Accad. Torino*, Vol. 46, pp. 149–78.

ROSA, D., 1898. I pretesi rapporti genetici tra i linfociti ed il chloragogeno. *Atti Accad., Torino*, Vol. 33, pp. 612–37.
SCHAEPPI, T., 1894. Das Chloragogen von Ophelia radiata. *Jena Z. Naturw.*, Bd. 28, pp. 249–93.
SCHNEIDER, G., 1896. Über phagocytäre Organe und Chloragogenzellen der Oligochäten. *Z. wiss. Zool.*, Bd. 61, pp. 363–92.
—— 1897. Über die Segmentalorgane und den Herzkörper einiger Polychäten. *Arb. d. Kaiserl. Naturf. Ges. St. Petersburg*, Vol. 27, pp. 135–7.
—— 1899. Über Phagocytose und Excretion bei den Anneliden. *Z. wiss. Zool.*, Bd. 66, pp. 497–520.
SETON, E. & WILBUR, G. G., 1949. Glucose metabolism in marine annelids. *Biol. Bull., Woods Hole*, Vol. 97, p. 246.
SMYTH, J. D. & HOPKINS, C. A., 1948. Ester wax as a medium for embedding tissue for the histological demonstration of glycogen. *Quart. J. micr. Sci.*, Vol. 89, pp. 431–5.
SPERRY, W. M., 1938. A micromethod for the determination of the total and free cholesterol. *Amer. J. clin. Path., Tech. Suppl.*, Vol. 2, pp. 91–9.
STERLING, S., 1908. Das Blutgefäßsystem der Oligochäten. *Jena Z. Naturw.*, Bd. 44, pp. 253–352.
WILBUR, C. G. & BAYORS, W. M., 1947. A comparative study of the lipids in some marine annelids. *Biol. Bull., Woods Hole*, Vol. 93, pp. 99–101.
WILLEM, V. & MINNE, A., 1900. Recherches sur l'excrétion chez quelques Annélides. *Mém. Acad. R. Belg. Cl. Sci.*, T. 58, pp. 1–73.

APPENDIX

ANALYSIS OF FATS

Extraction

Tissues were dried on filter-paper and the quantity extracted weighed by difference before being macerated in a Griffith pattern tissue grinder. Extraction was effected by refluxing with fifty times the weight of the tissue with 95% ethanol:peroxide-free ether 3:1 mixture overnight. Larger quantities of tissue were extracted in a Soxhlet apparatus. The extract was filtered through fat-free paper and made up to 50 ml. The efficiency of extraction was tested with known quantities (50–100 mg) of pure oleic acid absorbed on filter paper.

Estimation of total fatty acids plus sterols

A 20 ml. portion of the extract was measured accurately into a wide-necked Ehrlenmeyer flask, and 2 ml. of sodium ethoxide added. This was then placed in a ventilated electric oven at 90° C and allowed to evaporate to about 2 ml. This was found more satisfactory than evaporating on a water-bath owing to loss by spitting. The pasty residue was dried in a current of air, and the fatty acids liberated by the addition of 1 ml. of dil. H_2SO_4 and heating on a boiling water-bath for 1 min. The fatty acids were then dissolved in successive 3 ml. portions of boiling 40–60 light petroleum by rotating over the water-bath for 3 min, the combined extracts being made up to 25 ml. A 10 ml. portion of this was carefully measured into a 150 ml. conical ground glass-stoppered flask and evaporated to dryness, the last traces being blown off in a current of air. A control flask was started at this stage. 5 ml. of the silver chromate: H_2SO_4 reagent and exactly 3 ml. of 1·0 N potassium dichromate were then added, the contents mixed, the flask stoppered and placed in the oven at 90° C for 90 min. In the event of all the oxidant becoming used up after 5 min, the light

petroleum extract was diluted, and the process repeated. The flasks were placed on a bed of sand in the oven to maintain a more even temperature. After 90 min the contents were diluted to about 100 ml. with cold distilled water and carefully washed out into a 250 ml. conical flask; 10 ml. of 10% potassium iodide added and the mixture immediately titrated against 0·1 N sodium thiosulphate, using starch indicator. The difference in the titration values between the samples and the control is a measure of the amount of oxidant reduced by the fatty acids. Usually four flasks were run at once. Values within ±2% were obtained with pure oleic acid. All steps in the procedure were tested, and most loss occurs in the oxidation.

Estimation of phospholipids

A 20 ml. portion of the ethanol:ether extract was evaporated to dryness in a 50 ml. beaker. The residue was then extracted with 3 ml. portions of boiling 40:60 light petroleum and the extracts combined in a 15 ml. centrifuge tube. Any sediment was centrifuged out and the clear liquid decanted into another tube if necessary and allowed to evaporate to 2 ml. To the solution was then added 7 ml. of acetone and 0·1 ml. of magnesium chloride in ethanol (saturated solution), the solutions mixed and the phospholipid precipitate centrifuged down. The supernatant liquid was then carefully drawn off, the gummy precipitate kneaded with successive portions of acetone, and finally allowed to dry by inverting the tube on filter-paper. The precipitate was then dissolved in moist ether, with warming, and the solution transformed to the 150 ml. oxidation flask, the ether evaporated in a current of air and the phospholipid estimated by the chromic acid oxidation mixture as before. Bloor's correction factor was applied in the calculations.

Estimation of total sterols (free and combined)

Exactly 1 ml. of the ethanol:ether extract was transferred to a 15 ml. centrifuge tube, 0·05 ml. of 50% KOH added and the mixture warmed to 40° C for 30 min. After cooling the mixture was neutralized with 5% HCl using phenolphthalein as indicator. 1 ml. of 1% digitonin in 50% ethanol was added, stirred with a glass rod and allowed to stand 24 h. The precipitate was centrifuged down at 2500 r.p.m. for 15 min, the supernatant carefully removed, the rod replaced and the tube vigorously washed down with 2 ml. acetone:ether 1:2 mixture from a syringe; after further centrifugation, the precipitate was washed and centrifuged twice with pure ether, the precipitate being then dried at 40° C in a current of air. The tube was kept in a desiccator until ready to develop the colour reaction.

The precipitate was then dissolved in 1 ml. glacial acetic acid; warming to 40° C and stirring with original glass rod was usually necessary. Exactly 2·1 ml. of acetic anhydride:H_2SO_4 mixture was added and the tube with controls stirred well and placed in an incubator at 25° C for exactly 30 min. The density of the colour was measured at 670 mμ using a 'Unicam' S.P. 600 Quartz spectrophotometer, and the amount of sterol present calculated from a standardization curve obtained by successive dilution of a known solution of pure cholesterol.

The above description includes all the data on the variations in the general methods of Bloor (1928, 1929) and Sperry (1938). Reference should be made to the original papers for details of making and purity of the reagents required.

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A NOTE ON *STICHOCOCCUS BACILLARIS* NAEG. AND SOME SPECIES OF *CHLORELLA* AS MARINE ALGAE

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(Text-fig. 1)

The recent publication (Butcher, 1952) of some new marine algal species belonging to genera largely non-marine arouses interest as there are relatively few genera ranging from marine to freshwater habitats. Two of the genera concerned, *Chlorella* and *Stichococcus*, are notable for their very wide habitat range in fresh water and on land.

Three marine *Stichococcus* strains were examined and compared with *S. bacillaris* Naegeli, 1849, and with various non-marine strains of that species. The marine strains were (i) *Stichococcus cylindricus* Butcher, type strain; (ii) another strain identified by Butcher as *S. cylindricus*; and (iii) Plymouth no. 82 = Cambridge no. 379/5, also identified by Butcher as *S. cylindricus*. None of these three strains differed appreciably from Naegeli's description of *S. bacillaris*. The shape and dimensions agree, while the presence or absence of a pyrenoid cannot be regarded as decisive. Fritsch (1935) and Smith (1950) say that *Stichococcus* has no pyrenoid, while Prescott (1951) states there is one pyrenoid but does not depict it in his illustrations. Forest (1954) is probably nearer the truth when, referring to *Ulothrix* Kütz. em. Forest (including *Ulothrix*, *Hormidium* and *Stichococcus*), he writes: 'In only one species was the pyrenoid distinctive enough to be of possible critical value'. And that was not a species of *Stichococcus* Naeg. Though the tendency to fragment is strong in these strains the method of cell division appears typical of the genus and not intermediate between it and *Nannochloris* as Butcher suggests. The type strain when grown in a sea water and soil biphasic medium produces filaments of usually four to eight cells and occasionally up to 18 cells; while in the other strains seldom are more than two cells seen in a filament.

That Butcher's alga is both aquatic and marine does not warrant its position in a separate species. Though *S. bacillaris* is frequently found in damp terrestrial places it is often also aquatic and like *Chlorella* is one of the more usual colonists of neglected stock bottles of various solutions and of distilled water. It is not surprising therefore that this alga should be found in estuarine and off-shore marine waters.

To test the impression that *S. bacillaris* is an unexacting plant regarding salinity three strains were grown in (*a*) Föyn's erdschreiber medium, (*b*) the

same but with half-strength sea water, and (*c*) a similar medium with no sea water added. The strains were, a freshwater strain, Cambridge 379/1c; a strain isolated from the contents of a cow's rumen, Cambridge 379/1e; and the marine Cambridge 379/5. They were grown in the different media for 10 days before being inoculated into the cultures for estimation. Haemacytometer counts were made covering the logarithmic phase of growth. Irregularity in

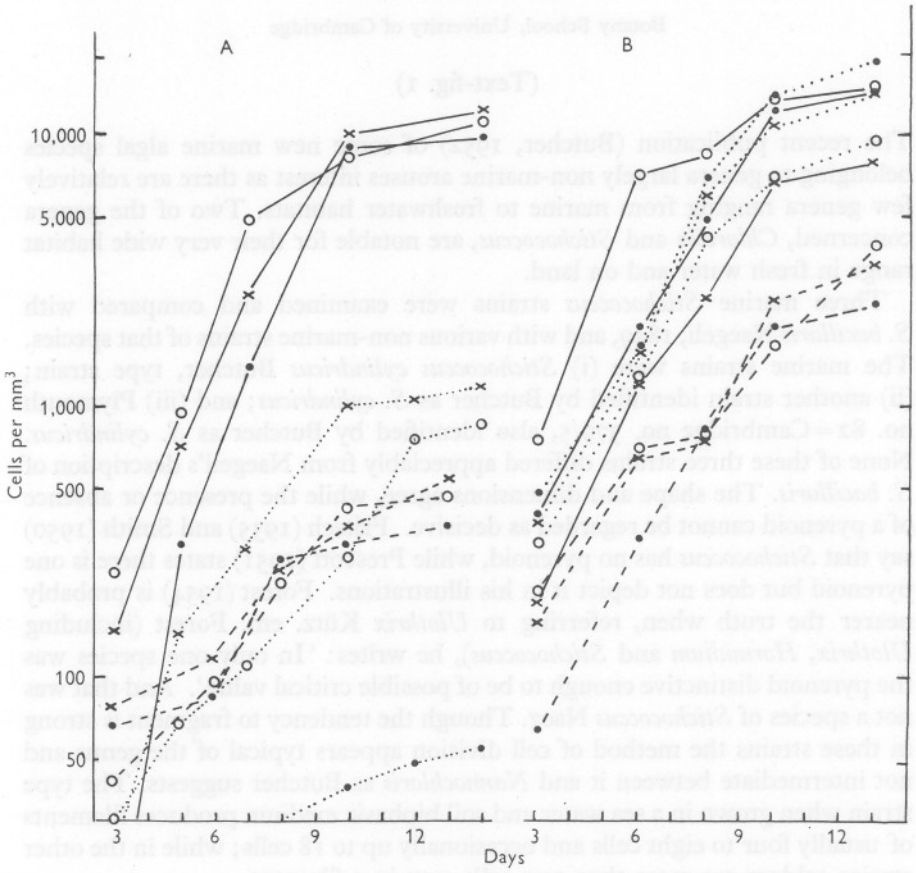

Fig. 1. Growth of (A) *Stichococcus* cultures and (B) *Chlorella* cultures in (●) sea water, (×) half-strength sea water, and (○) fresh water. A: ....., *Stichococcus bacillaris* 379/1e; - - - - -, *S. bacillaris* 379/1c; ——, *S.* '*cylindricus*' 379/5. B: ..., *Chlorella stigmatophora*; - - - - -, *C.* 'Oslo' strain; ——, *C. ovalis*.

some of the counts was due largely to the cells clumping or adhering to the glass despite vigorous shaking. The growth curves (Fig. 1A) suggest that the marine form grows equally well in fresh or sea water. The freshwater form is equally tolerant but with lower maximum cell counts. Strain 379/1e grew

perhaps slightly better in half-strength sea water than in fresh water but much more slowly in full-strength sea water.

A similar experiment was done with five *Chlorella* strains—*C. stigmatophora* Butcher, Cambridge 211/20; *C. ovalis* Butcher, 211/21; *Chlorella* sp. from Oslo Fiord; *C. vulgaris* 211/11n and *C. pyrenoidosa* 211/8c; the two latter being freshwater strains. The three marine strains (Fig. 1B) show no strong preference for sea water against fresh water. Further investigation would be necessary to ascertain whether there are slight differences of growth due solely to salt concentration. The curves for *C. vulgaris* and *C. pyrenoidosa* showed no measurable growth in sea water and much less growth in half-strength sea water than in freshwater media.

Both these genera are as far as we know completely non-sexual. This implies that every surviving mutation starts a new clonal line and that the micro-evolution of these forms is entirely a divaricating system. In sexual organisms, on the other hand, there is a frequent recombination of characters within the population, the micro-evolutionary system is reticulate within the breeding group and pure lines are only obtained by artificial methods. Thus the species (or subspecies) in sexual forms is in general the potential breeding group, while in non-sexual forms there will be a large number of clonal lines in the conventional species. These clonal lines will perforce differ slightly from one another either physiologically, or morphologically, or both. In *S. bacillaris* differences of growth rate, tendency to form filaments and tendency to clump irregularly have been noted. To name each of these lines as a separate species would seriously overburden taxonomy, as practically every strain isolated seems slightly different and hence of a different clonal line. All that seems necessary in these forms is an aggregate species, perhaps with subspecies, and the clonal lines, where necessary, known by the reference numbers of a recognized culture collection. Thus it seems best to regard *Stichococcus bacillaris* as an (aggregate) species including many clonal lines all of which fit Naegeli's description and all of which grow well in fresh or sea water. With the *Chlorella* strains we have a more difficult problem because the taxonomy of the genus is in need of thorough investigation. However, there are small but constant differences and we may tentatively at least accept Butcher's species while noting that they are very unexacting about salinity.

SUMMARY

Several strains of *Stichococcus bacillaris* Naeg. and of the marine *S. cylindricus* Butcher were all found to fit morphologically in Naegeli's species. Both the marine and non-marine strains grow well in either fresh- or sea-water media. *S. cylindricus* must therefore be regarded as a synonym of *S. bacillaris*. Three marine *Chlorella* strains including *C. ovalis* Butcher and *C. stigmatophora* Butcher were also found to be quite unexacting about salinity. The significance

of small differences, particularly of growth rate, is discussed with regard to non-sexual organisms. In general, widely based species are advocated for such organisms, with the clonal lines, where appropriate, designated by the reference number of a recognized culture collection.

REFERENCES

BUTCHER, R. W., 1952. Contributions to our knowledge of the smaller marine algae. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 175–190.

FOREST, H. S., 1954. Discussion of a portion of the Ulotrichaceae. *Castanea*, Vol. 19, pp. 61–75.

FRITSCH, F. E., 1935. *Structure and Reproduction of the Algae*, Vol. 1. Cambridge University Press.

NAEGELI, C., 1849. *Gattungen einzelliger Algen*. Zurich.

PRESCOTT, G. W., 1951. *Algae of the Western Great Lakes Area*. Cranbrook Inst. Sci.

SMITH, G. M., 1950. *Freshwater Algae of the United States*. New York: McGraw Hill.

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OBSERVATIONS ON THE RESISTANCE OF *TIGRIOPUS FULVUS* (FISCHER) TO CHANGES IN TEMPERATURE AND SALINITY

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(Text-figs. 1 and 2)

The harpacticid copepod *Tigriopus fulvus* (Fischer) has a wide distribution along the European coast, generally occurring in pools at or above high-water mark, where the environmental conditions are liable to sudden and extreme changes. Fraser (1936), while studying the life history and ecology of *T. fulvus*, has quoted Issel (1914): 'A few weeks of periodical observations of the pools since 1912 have drawn my attention to a phenomenon worth studying; as soon as the density of the water reaches a certain degree the copepod *T. fulvus* falls into a state of apparent death, from which it can awake even after a very long time and regain normal activity when the water is sufficiently diluted'. The experiments described in this paper were designed to investigate this phenomenon and other reactions of *T. fulvus* to changes in salinity and temperature.

EFFECTS OF SALINITY CHANGES

T. fulvus were collected from rock pools above high-water neaps at Port St Mary, Isle of Man, and transferred to sea water of salinity 34·0‰ in which they were conditioned for 2 days, to overcome the effects of the fluctuations in the salinity of the rock pools. Lower salinities were obtained by diluting the sea water with distilled water and higher salinities were obtained by adding Tidman's sea salt. The salinities were determined by titration with silver nitrate.

A graded series of twenty-three solutions was prepared with salinities ranging from 0·0 to 118·0‰. Approximately fifty specimens of conditioned *T. fulvus* were pipetted into 50 c.c. of each solution and left for a period of up to 15 days.

It was found that in distilled water the animals died after 84 h, while in solutions from salinity 4·2‰ up to 90·0‰ they were living normally after 15 days. In salinities above 90·0‰ *T. fulvus* fell into a state of apparent death, as described by Issel (1914); as soon as the animals were introduced into the solutions of salinity above 90·0‰ they ceased their activity and sank to the

bottom of the dish and lay motionless. But when they were transferred back to sea water of salinity 34·0‰ they regained their normal activity after a period depending on the strength of the previous solution and the duration of immersion in it.

In order to investigate this behaviour a further series of experiments was conducted. For this a set of four solutions was made with salinities of 98·0, 135·0, 180·0 and 225·0‰ respectively. Fifty specimens of conditioned *T. fulvus* were transferred to each of the above solutions. At definite intervals animals were transferred from the high salinity solutions to sea water of salinity 34·0‰ and the time taken for complete recovery was noted. The temperature during

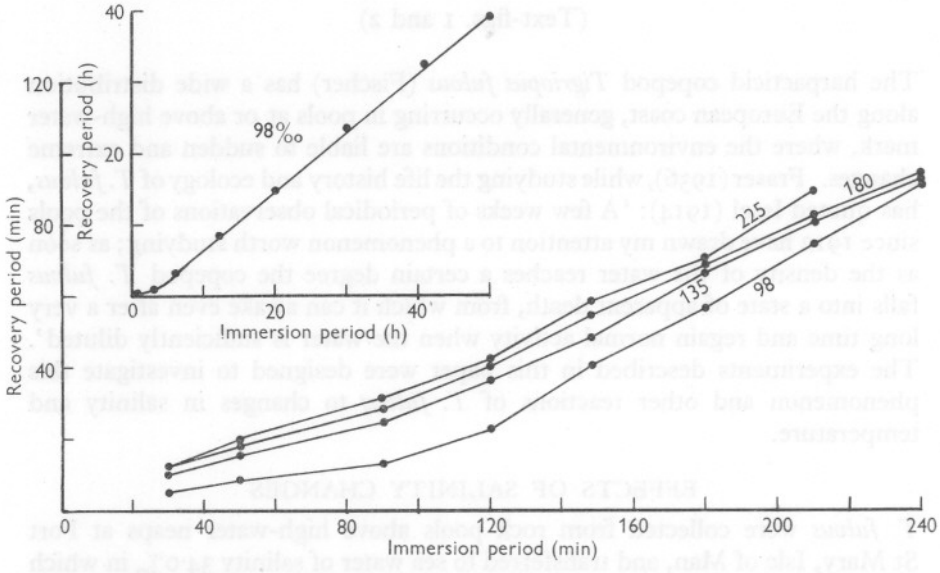

Fig. 1. *Tigriopus fulvus*. Relation between immersion period and the recovery period at salinities of 98, 135, 180, and 225‰, for various immersion periods up to 4 h. Inset: the same for a salinity of 98‰, when the immersion period was taken up to 50 h.

the experiments varied between 16·0 and 18·0° C. The relation between the immersion period and the recovery period is shown in Fig. 1. The points plotted are the means of two experiments; variation between the repeat experiments was very small. It will be seen that the strength of the high salinity solutions had some effect on the period required to regain normal activity after transfer, those which were immersed in stronger solutions requiring a rather longer recovery period. This factor was, however, of much less importance than the immersion period, and in cases of immersion for more than 4 h may be virtually neglected. The species was found to be unable to survive indefinite immersion in any of the solutions in which the 'apparent death' response was observed, the survival period varying with the salinity.

The maximum immersion periods, after which no recovery took place, were as follows:

Solution	Salinity (‰)	Maximum immersion period (h)
1	98	60
2	135	30
3	180	30
4	225	3

EFFECTS OF TEMPERATURE CHANGES

Two batches of twenty *T. fulvus* in 20 c.c. of sea water of salinity 34·0‰, immersed in a water-bath, were subjected to a slow rise in temperature at the rate of approximately 2° C per hour by means of a thermostat and heater. The animals behaved normally up to 34° C, but their movements became more rapid with increasing temperature until heat coma set in at 36° C when they became motionless and sank to the bottom of the dish. Death occurred when

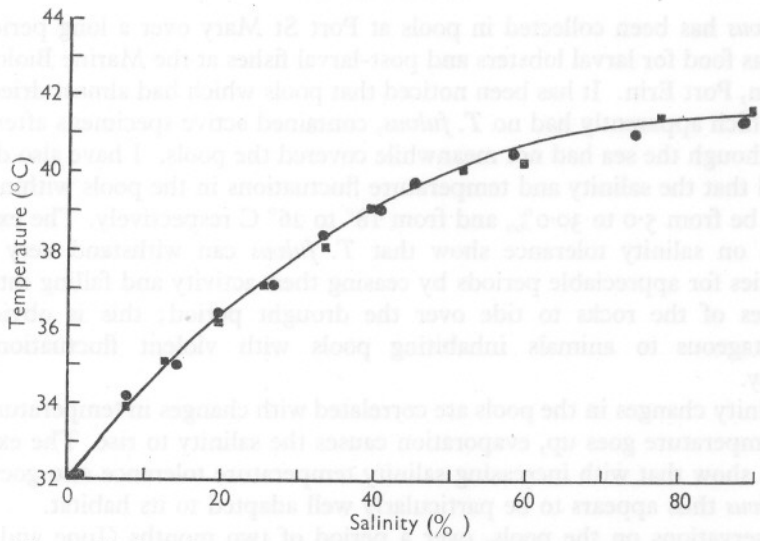

Fig. 2. *Tigriopus fulvus*. Lethal temperatures at different salinities. ■ First experiment, 18 August 1956. ● Second experiment, 26 September 1956.

the temperature reached 38° C. The death-point was determined when no recovery took place after transferring the animals to room temperature (16° C) for a period of 24 h.

In rock pools at high water, high temperatures are usually accompanied by increasing salinities on account of evaporation. An experiment, therefore, was set up to investigate the relation between the salinity of water and temperature tolerance of *T. fulvus*.

For this a series of eleven solutions of different salinities ranging from distilled water to 90·0‰ were made. Twenty specimens of *T. fulvus* were transferred from sea water to 20 c.c. of each solution in small dishes. These dishes were floated on a water-bath at 16° C and the temperature of the bath increased at a rate of approximately 2° C per hour. The *T. fulvus* were kept under continuous observation and the temperature of the water in each dish was taken at the 'death point'. This point was remarkably clear-cut, for about three-quarters of the animals always died simultaneously; the rest died within 3 min, during which the temperature never rose more than 0·1° C.

This experiment was repeated a month later with a fresh set of solutions and a new stock of *Tigriopus*. The second set of results agreed very closely with the first set, and both are shown in Fig. 2, which shows that the lethal temperatures varied over a range of 9·8° C depending on the salinity of the solutions. In distilled water death occurred at 32·0° C, while in salinity 90·0‰ the animals died at 41·8° C.

DISCUSSION

T. fulvus has been collected in pools at Port St Mary over a long period to serve as food for larval lobsters and post-larval fishes at the Marine Biological Station, Port Erin. It has been noticed that pools which had almost dried up, and which apparently had no *T. fulvus*, contained active specimens after rain even though the sea had not meanwhile covered the pools. I have also determined that the salinity and temperature fluctuations in the pools within 24 h could be from 5·0 to 30·0‰ and from 18° to 26° C respectively. The experiments on salinity tolerance show that *T. fulvus* can withstand very high salinities for appreciable periods by ceasing their activity and falling into the crevices of the rocks to tide over the drought period; this is obviously advantageous to animals inhabiting pools with violent fluctuations in salinity.

Salinity changes in the pools are correlated with changes in temperature; as the temperature goes up, evaporation causes the salinity to rise. The experiments show that with increasing salinity temperature tolerance also goes up; *T. fulvus* thus appears to be particularly well adapted to its habitat.

Observations on the pools, over a period of two months (June and July 1956) have shown that the fluctuations in salinity and temperature in the pools were from 5·0 to 42·0‰ and 13° to 27° C respectively. These changes are well within the tolerance limits of *T. fulvus* as shown in the foregoing experiments.

I am greatly indebted to Mr A. B. Bowers for his help in the collections and experimental work. I also wish to thank Mr J. S. Colman and Dr D. I. Williamson for their help in preparing the manuscript.

SUMMARY

Tigriopus fulvus can live normally within a salinity range of from 4·2 to 90‰.

In waters of salinities above 90‰ it falls into a state of apparent death from which it can recover if transferred back to lower salinities.

The relation between immersion period and the recovery period is only slightly different at different salinities.

The lethal temperatures vary between 32·0 and 41·8° C depending on the salinity of the sea water, the lethal temperature being higher in higher salinities.

The fluctuations in salinity and temperature in the pools where the animals were collected are well within their tolerance limits.

T. fulvus is well adapted to its habitat.

REFERENCES

FRASER, J. H., 1936. The occurrence, ecology and life history of *Tigriopus fulvus* (Fischer). *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 523–536.

ISSEL, R., 1914. Vita latente per concentraz. dell'acqua e biologia delle pozze de scogliera. *Mitt. Zool. Sta. Neapel*, Bd. 22, pp. 191–255.

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THE RESPONSE OF THE LIMPET, *PATELLA VULGATA* L., TO WATERS OF DIFFERENT SALINITIES

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(Text-figs. 1–5)

During a study of the behaviour of the common British limpet, *Patella vulgata* L., undertaken in this laboratory, it was found that when dry this species reacts to splashing in a manner which can be correlated with the salinity of the water used. Since no experimental work relating to the chemosensitivity of the limpet could be traced in the available literature, these reactions were further studied in an attempt to identify the receptor regions responsible for detecting variations in salinity. The results so far obtained, while necessarily preliminary in nature, have indicated that in *Patella* the graded response to different salinities is mediated by receptors in the cephalic tentacles and mantle fringe.

MATERIALS AND METHODS

The limpets used in these experiments were obtained from bare or weed-covered surfaces at the East Rocks, St Andrews. They were removed by chipping away fragments of the rock on which they were lodged and any animals which appeared to have been damaged in the process were discarded. In the laboratory they were placed in running sea water for some hours, then left dry for about 8 h before the start of the experiments, most of which were performed during the morning or early afternoon of the day following collection. After use the limpets were kept for a further 3 or 4 weeks under circulation with prolonged periods of dryness each day; they survived well, moving and feeding with every appearance of normality.

On being brought into the laboratory, many of the limpets abandoned the rock to which they were attached and were then placed on the bases of inverted solid watch-glasses, to which the majority readily adhered. Those which did not leave the rock of their own accord were studied without being detached. Stimulation was provided by dropping about 2 ml. of water on to the apex of the shell, a small amount passing beneath the shell and affecting the animal. The response, a vertical movement of the front edge of the shell, was recorded by means of a heart lever (arm ratio 12:1). Further details are given in the accounts of the experiments concerned.

OBSERVATIONS AND EXPERIMENTS

THE RESPONSE TO SEA AND FRESH WATER

When dry, *Patella* remains quiescent, with only occasional slight upward movements of the shell, but, as upon the shore, the animal is not clamped hard down upon its support except when subjected to stimuli of a potentially dangerous nature. In this quiescent state the head is almost completely withdrawn into the mantle cavity, the foot spread over most of the surface beneath the shell and the fringe of the mantle extended almost, but not quite, as far as the edge of the shell (Fig. 1*a*).

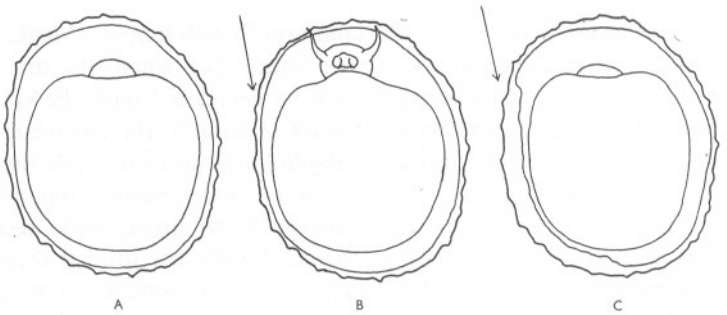

Fig. 1. The response of *Patella* to stimulation by sea and fresh water. A, ventral view of quiescent animal, drawn through glass; B, the same animal, after stimulation with sea water; C, the same animal after stimulation with fresh water. Arrows show direction of flow of water.

If a limpet in this condition was splashed with sea water, it responded by lifting the front edge of the shell, advancing the head and extending the cephalic tentacles up to or beyond the edge of the shell (positive response, Fig. 1*b*). The vertical movement of the shell was often considerable, being as much as 5 mm in a high-tide limpet of 50 mm length. The time elapsing between the stimulus and the commencement of the response varied from less than 30 sec to about 2 min. The response to a single stimulus was normally followed by a sharp contraction of the muscles and a return to the quiescent state. Under repeated stimulation the majority of limpets began to wander and once this had commenced would no longer give clear responses to splashing with sea water. The few animals encountered which gave no response to sea water were later found to have been damaged during or subsequent to collection.

Stimulation by splashing with fresh water elicited the opposite response. As the water flowed beneath the edge of the shell the mantle fringe contracted before it (Fig. 1*c*), the head was withdrawn even further into the mantle cavity than when the limpet was quiescent and the shell was clamped hard down to exclude the noxious liquid (negative response). Similar stimulation

of limpets extended in response to sea water resulted in an immediate contraction, begun as soon as the water touched the body and completed in less than 30 sec. No animal failed to respond to fresh water in this manner.

SENSORY REGIONS RESPONSIBLE FOR THE REACTION

An inverted limpet will extend fully in an attempt to right itself, even if it is out of water at the time. This enables the body to be explored for the localization of sensory regions. Exploration by touch revealed five regions characterized by different local reactions to slight contact (Fig. 2). The sole of the foot (1) contracted at the point touched, leaving a deep pit. A similar light touch at the side of the foot (2) induced an outward movement which would

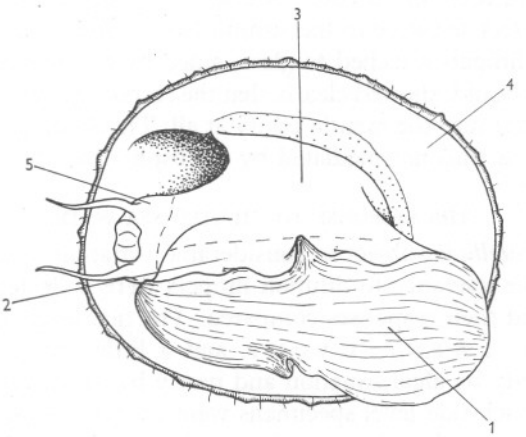

Fig. 2. Ventro-lateral view of extended limpet, showing major sensory regions. For explanation, see text.

ultimately bring the sole of the foot over the object touched. The body and the walls of the mantle cavity (3) were almost insensitive to touch, but the mantle fringe (4) and its tentacles were extremely sensitive and reacted to the slightest contact by immediate withdrawal. The head and cephalic tentacles (5) also recoiled sharply from contact with any object unfamiliar to the animal.

A similar exploration was conducted by dropping water from a fine pipette on to these various regions. When sea water was used on a well-extended limpet regions 1–4 gave no recognizable local response, nor did the behaviour of the animal change. The cephalic tentacles, however, would explore the surface of the pipette, if this could be reached, withdrawing only when the pipette ran dry. The foot and general body surface proved equally insensitive to contact with fresh water, but both the mantle fringe and the cephalic tentacles contracted sharply when stimulated in this manner.

While an inverted limpet is endeavouring to right itself it is easy to remove almost all the sea water from the mantle cavity without affecting the activity

of the animal. Fresh water dropped into the drained cavity was found not to elicit a response, provided that neither the mantle fringe nor the cephalic tentacles were touched in the process. Only when the cavity had been completely filled with fresh water did the activity of the limpet slow and the head withdraw into the cavity. This contraction did not have the appearance of a response to stimulation, but seemed rather a general 'malaise'.

The osphradia, for which both chemoreceptive and tactile functions have been proposed (Hulbert & Yonge, 1937), merited special attention, but, owing to their position far back in the floor of the mantle cavity, could not be removed from intact limpets, while shell-less animals, though they lived, remained inactive. Attempts to destroy the osphradia by cautery were also unsuccessful and therefore it could not be determined directly whether these organs were in fact sensitive to the stimuli used. However, close observation from below of limpets attached to glass, aided by the addition of carmine to the stimulating liquid, showed clearly that the responses were obtained without the entry of water into the mantle cavity at all. The response of *Patella* to sea and fresh water is thus not mediated by the osphradia.

THE RESPONSE TO DILUTED SEA WATER

On the shore *Patella* is subject to considerable variation of salinity. In order to determine the tolerance to dilution limpets were collected from six levels on the shore and their responses compared. The tide levels used (H.W.O.N.T., H.W.E.N.T., M.T., L.W.E.N.T., L.W.O.N.T. and a little above L.W.O.S.T.) were determined partly by algal zonation and partly by direct observation during collection. At each tide level specimens were chosen within two size ranges, *c.* 25 and *c.* 45 mm. length, in order to determine whether or not there was a difference in response ascribable to age. In fact, no difference was found.

Each animal was tested with a range of salinities produced by diluting sea water in 10% steps with fresh water. As 'fresh' water, rain, tap and distilled water were used, but in parallel experiments gave similar results. Stimuli were given at approximately 5 min intervals and were at random, with the exception that the same stimulus was not given twice in succession. After each stimulus the limpet was washed briefly with fresh water. If a response was given its magnitude was determined by measuring the height of the trace in respect to a superimposed base-line determined from the positions of maximum contraction at the beginning and end of the experiment. Animals on glass frequently moved slightly during the course of an experiment, and when wandering took place to any great extent that sequence of observations was rejected. If no positive response was given the stimulus was marked as 0, irrespective of whether the limpet did or did not contract to the maximum extent.

All limpets tested were tolerant to some at least of the dilutions, and the

magnitude of their responses was found to correspond in fair degree with the salinity used. The responses of a given animal to similar stimuli were quite consistent (Table 1), though there was a tendency to greater variability as the salinity decreased. The largest responses were invariably given to undiluted sea water, but in several animals the size of the response did not fall off regularly with dilution, but showed an additional secondary peak. The reason for this is unknown. Comparison of limpets taken from different tide levels showed that while there was individual variation within each group, especially

TABLE 1. VERTICAL MOVEMENT IN MM OF THE FRONT EDGE OF THE SHELL OF TWO LIMPETS FROM DIFFERENT TIDE LEVELS IN RESPONSE TO SPLASHING WITH NORMAL AND DILUTED SEA WATER

Tide level	Length of shell (mm)	Relative salinity of sea water						
		100%	90%	80%	70%	60%	50%	40%
H.W.O.N.T.	26	2·00	1·33	1·25	0·83	0·58	0·25	0
		2·00	1·58	1·17	0·75	0·50	0·17	0
		1·91	1·75	1·42	1·25	1·25	0·42	0
		2·09	—	—	—	—	—	—
	Mean	2·00	1·55	1·28	0·94	0·78	0·28	0
L.W.E.N.T.	24	1·75	1·42	1·17	1·50	0·92	0·25	—
		2·00	1·25	1·08	1·42	1·17	0	—
		1·75	1·58	1·42	1·42	0·75	0	—
	Mean	1·83	1·42	1·22	1·45	0·95	0·08	—

obvious in those from the mid-tide region, several distinct trends could easily be recognized when passing from the upper to the lower tide limits. Limpets taken from H.W.O.N.T. (the upper limit of colonization) gave very consistent and clear-cut responses and were tolerant to salinities below 50% sea water (Fig. 3) and in one case even as low as 20%. Their movements were quick to commence, large and smooth. In contrast, limpets collected a little above L.W.O.S.T. (the lower limit of colonization) were negative to salinities of 80% sea water or less and gave much smaller and more irregular responses than did high-tide animals of similar size. These extremes were linked by the range of behaviour of animals drawn from intervening tide levels (Fig. 4).

THE RESPONSE TO SOLUTIONS OF SODIUM CHLORIDE

Some limpets from H.W.O.N.T. were tested with solutions of sodium chloride. This was of Analar grade made up in distilled water in concentrations ranging from 100 g/l. down to 20 g/l. The responses given to these solutions resembled those given to natural and diluted sea water, except that the movements were more jerky and less rapid, while the limpets soon became refractory. Consistent results could only be obtained by allowing the animals frequent and lengthy periods for recovery during the course of each experiment.

Responses to undiluted sea water and to a sodium chloride solution of equivalent salinity were of similar magnitude, while abnormally large responses

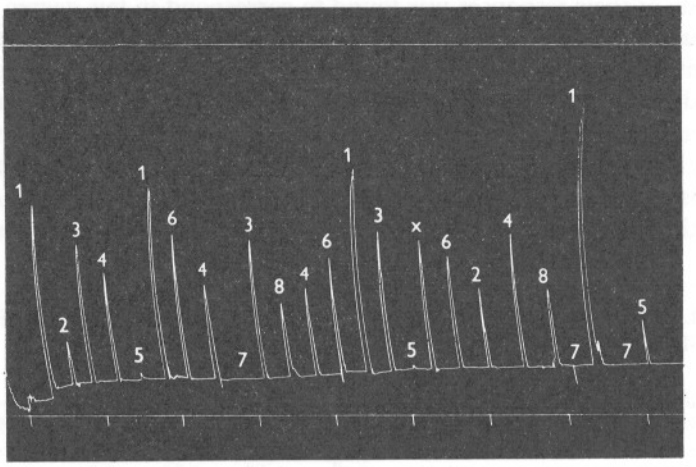

Fig. 3. Response of limpet, taken from H.W.O.N.T., 46 mm long, to normal and diluted sea water. Time at 15 min intervals. *Key to numbering*: 1 = normal sea water; 2 = 60 % sea water; 3 = 90 % sea water; 4 = 70 % sea water; 5 = 40 % sea water; 6 = 80 % sea water; 7 = 30 % sea water; 8 = 50 % sea water. × = spontaneous movement.

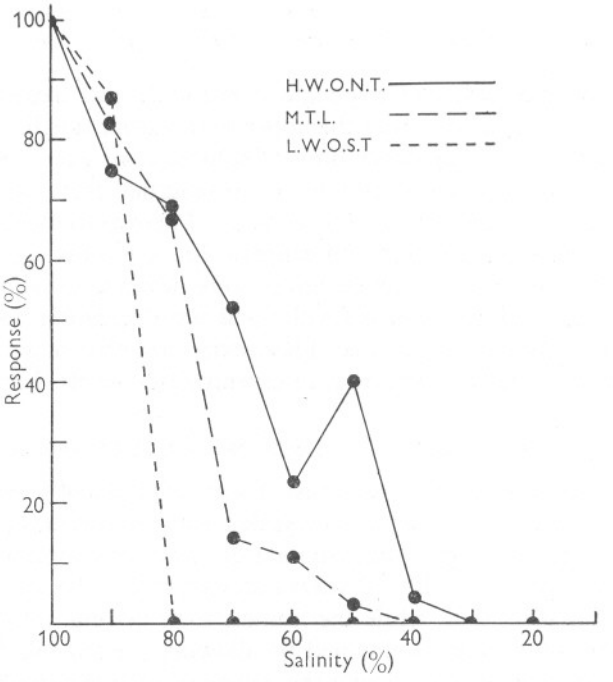

Fig. 4. The response of limpets from different tide levels to various salinities. Salinities expressed as percentages of undiluted sea water, responses as percentages of response to undiluted sea water.

were given to solutions containing 40g/l. NaCl (Fig. 5). Above this concentration the responses once more diminished in size.

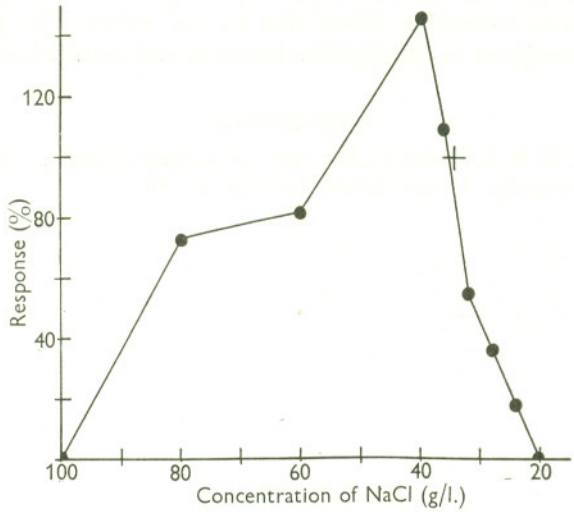

Fig. 5. The response of a limpet from H.W.O.N.T. to various NaCl concentrations. Concentrations expressed as g NaCl/l., responses as percentages of response to undiluted sea water (salinity *c.* 34·5‰), the position of which is shown by a cross.

DISCUSSION

These experiments have yielded the results which are to be expected on *a priori* considerations. The negative response of *Patella* to water of low salinity, the increasing tolerance as one ascends the shore and the more immediate and larger responses to splash shown by the high-tide limpets, are all characters which are to be expected as adaptations to a progressively more rigorous environment in which activity is curtailed by ever longer periods of dryness. Whether these potentialities are possessed by all limpets and expressed according to their position on the shore, or whether the possession of certain powers of activity under adverse conditions determines the position ultimately occupied by the adult limpet is not known.

The perception of salinity by the mantle fringe and cephalic tentacles is also to be expected, since these are the parts of the animal which are most exposed to the environment.

SUMMARY

Patella vulgata gives a positive response to splashing with sea water or water of high salinity, and a negative response to fresh water or water of low salinity. The intensity of the positive response broadly corresponds with the salinity of the water used. There is a greater response to splash and a greater tolerance

to reduced salinity in limpets from high-tide than in those from low-tide regions. A positive response is given to NaCl solutions of concentrations approximately equivalent to sea water, while a supra-normal response is given to concentrations somewhat above that of sea water. The perception of salinity is by receptors in the cephalic tentacles and mantle fringe.

REFERENCE

HULBERT, G. C. E. B. & YONGE, C. M., 1937. A possible function of the osphradium in the Gastropoda. *Nature, Lond.*, Vol. 139, pp. 840–1.

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CHROMOSOME NUMBERS OF GAMMARIDS

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(Plate I and Text-figs. 1–4)

The taxonomy of gammarid amphipods is a notoriously difficult subject. The group comprises a large number of recognized species whose identification depends on an expert knowledge of morphological minutiae. We began the study to be described in the present paper with the hope that a knowledge of the chromosome numbers of gammarids might be of taxonomic value. This hope has proved in the event to be largely ill-founded. We also undertook the present study in order to resolve a dispute as to whether polyploidy has played a part in gammarid evolution. This question has been clearly settled in the negative sense.

The nuclear cytology of most Crustacea presents severe technical problems. All crustaceans so far examined have relatively small, often extremely small, chromosomes. Most of the higher Crustacea have high chromosome numbers, those of decapods being the highest chromosome numbers in the whole animal kingdom. Gammarids, with small chromosomes and diploid numbers up in the fifties, are by no means easy cytological material.

From the literature describing previous work on gammarid cytology it was abundantly clear that we would make little progress using stained paraffin sections of testes of the various species. Aceto-carmine-stained squash preparations of gammarid testes have been used by the most reliable previous workers, but in our limited experience such preparations are frequently defective as regards quality of fixation and, critically and regularly, in the absence of analysable side views of first meiotic metaphase. Staiger & Bocquet's paper demonstrating female heterogamety in certain isopods (Staiger & Bocquet, 1954) suggested to us that it might be more profitable to base our cytological study of gammarids on oocyte meiosis. By making squash preparations of oocytes just prior to oviposition Staiger & Bocquet obtained analysable side views of first meiotic metaphase, and it is clear from their illustrations that the chromosomes of *Jaera* oocytes are considerably larger than those of spermatocytes. There are comparable advantages in studying oocyte as opposed to spermatocyte meiosis in the prosobranch gastropod *Nucella lapillus* (L.) (Staiger, 1954, as *Purpura*) and the earthworm *Eisenia foetida* (Savigny) (Callan, unpublished).

Previous determinations of the chromosome numbers of gammarids are listed by Le Calvez & Certain (1951). Their list, with minor modifications, is

reproduced in Table 1. Within this list the haploid numbers of 13 for *Gammarus chevreuxi* and *G. duebeni*, on the authority of Palmer and Le Roux,[1] respectively, stand apart from all the other determinations, which range from $n=22$ to $n=27$. At first sight this incongruity might appear to indicate that *G. chevreuxi* and *G. duebeni* are diploid members of a predominantly tetraploid group. However, Le Calvez & Certain (1951) redetermined the chromosome numbers of *G. chevreuxi* and *G. duebeni* (specimens of the latter species from the same locality as those with which Mme Le Roux had previously worked),

TABLE 1. PUBLISHED CHROMOSOME NUMBERS OF GAMMARIDS

Species	Haploid number	Authority
Gammarus chevreuxi Sexton	*ca.* 22	Huxley, 1923
G. chevreuxi Sexton	13	Palmer, 1926
G. chevreuxi Sexton	26	Le Calvez & Certain, 1951
G. duebeni Lilljeborg	13	Le Roux, 1933*
G. duebeni Lilljeborg	27	Le Calvez & Certain, 1951
G. zaddachi Sexton	26	Le Calvez, 1949†
G. pulex pulex (L.)	26	Poisson & Le Calvez, 1948
G. locusta (L.)	26	Poisson & Le Calvez, 1948
G. pungens Milne Edwards	24	Le Calvez & Certain, 1951
G. annandalei Tattersall	27	Niiyama, 1935
Marinogammarus marinus (Leach)	26	Le Calvez & Certain, 1951
Echinogammarus berilloni (Catta)	26	Le Calvez, 1949
Carinogammarus roeseli (Gervais)	26	Le Calvez, 1949
Niphargus plateaui var. *elongatus* Chevreux	25	Le Calvez, 1949
N. tatrensis var. *lunzensis* Schell.	25	Le Calvez, 1949
Melita palmata (Montagu)	22	Le Calvez, 1949
Maera othonis (Milne Edwards)	23	Le Calvez & Certain, 1951
M. grossimana (Montagu)	23	Le Calvez & Certain, 1951
Metacrangonyx longipes Chevreux	26	Le Calvez & Certain, 1951

* In all probability Le Roux's species was really *Gammarus z. zaddachi*.

† *G. zaddachi* was incorrectly designated *G. duebeni* in Le Calvez's 1949 paper: the error was corrected in the 1951 paper by Le Calvez & Certain.

and found haploid numbers of 26 and 27. With considerable justification they suggested that both Palmer's and Le Roux's determinations were based on inadequate techniques and were erroneous. Nevertheless, some uncertainty still remained: Palmer's *G. chevreuxi* material originated from Plymouth, whereas Le Calvez & Certain's *G. chevreuxi* came from Roscoff, and thus the alternative chromosome numbers might conceivably reflect genuine interracial differences.

By working with oocytes we have been able to make accurate chromosome counts in nine gammarid species. However, since in at least three of these species chromosome number varies from individual to individual, and since the chromosome numbers of different species overlap, it is clear that chromosome counts will not be of great value to students of gammarid systematics.

[1] According to G. M. Spooner (personal communication) the illustrations and comparative measurements of limbs given in Le Roux's paper indicate that she was dealing with the typical subspecies of *Gammarus zaddachi* Sexton and not with *G. duebeni*.

Reliance can be placed on the identification of material which we have studied cytologically since we have enjoyed the invaluable assistance of two experts on gammarid taxonomy, Dr C. Edwards of the Scottish Marine Biological Association at Millport, and Mr G. M. Spooner of the Marine Biological Association of the United Kingdom at Plymouth.

MATERIALS AND METHODS

We started our cytological work on gammarids by making aceto-orcein squash preparations of fresh and of acetic-alcohol prefixed testes of several species. In well-flattened preparations equatorial plates of first meiotic metaphase are only to be seen in polar view: the bivalents are crowded so close together (the first metaphase equatorial plates of *G. chevreuxi*, *G. locusta* and of *Marinogammarus marinus* are only some 7 or 8 μ in diameter) that it proved quite impossible to make accurate chromosome counts. At second meiotic metaphase the difficulties are still greater: in *Gammarus chevreuxi*, for example, the diameter of a second metaphase equatorial plate is only some 4–5 μ. Occasional spermatogonial prometaphases can be counted with fair accuracy, but in most preparations these are not sufficiently frequent to allow of adequate cross-checking. In short, we would not have been able to establish with certainty the existence of chromosomal polymorphism in gammarid species by working with testis preparations alone.

In order to work on oocyte meiosis it is necessary to know the state of maturation of the eggs relative to the macroscopic cycle of events connected with oviposition. As is well-known, male and female gammarids associate in precopula several days before egg-laying. The female moults, and immediately thereafter the male turns the female round so that the pair lie transverse to one another: the male then ejects sperm into the female's brood-chamber. The pre-oviposition moult is an event which can be readily observed and it forms a useful reference point within the maturation cycle. Thirty to ninety minutes after the moult, dependent on species and on temperature, the first eggs drop down the paired oviducts into the brood-pouch. In pale species with well-pigmented eggs, e.g. *Marinogammarus pirloti*, this event can also be readily observed. The eggs collect in the brood-pouch in two thin-walled transient structures, the so-called 'mucous sacs', which slowly dissolve and disappear completely some 2 hr later: sperm penetration takes place at this time.

We have established the time-scale of the cytological events taking place during maturation of the eggs in *Gammarus pulex* L., the animals being maintained in good conditions at a constant temperature of 15° C. This time-scale is shown in Table 2.

Chromosome numbers can be established with certainty at three stages: first and second meiotic metaphase, and first cleavage metaphase (later cleavage metaphases are also of value, though they become progressively

more difficult to analyze the older the embryo). Typical photographs of these three stages are shown in Pl. I, figs. 2–5; for the sake of comparison a photograph of a particularly clear *G. pulex* spermatocyte first meiotic metaphase is also included in this plate (fig. 1). The great superiority of the egg preparations is evident. Of these three stages, oocyte first meiotic metaphase is the most informative: unfortunately it presents greater technical difficulties than do second meiotic metaphase or first cleavage mitosis in that the eggs in oviduct and ovary are very fragile, they stick together and cannot be entirely freed from ovarian wall tissue, and the stage is of short duration. We have intentionally worked with first meiotic metaphase only in *G. pulex* (first meiotic metaphase of *Marinogammarus marinus* was obtained by accident). Studies on the other species have been confined to second meiotic metaphase, in several respects the most easy stage with which to work.

TABLE 2. *GAMMARUS PULEX* (L.): TIME-SCALE OF CYTOLOGICAL EVENTS AT 15° C

Time from pre-oviposition moult of female (min)	Time from oviposition (min)	Stage
15	—	Diplotene
30	—	Diakinesis
45–60	—	Prometaphase I
75	0	Metaphase I
90	15	Anaphase I
100	25	Telophase I
110	35	Prometaphase II
115–195	40–120	Metaphase II
210	135	Anaphase II
225	150	Telophase II: sperm penetration
225–325	180–25	Approximation of pronuclei
425–525	350–450	Metaphase of 1st cleavage

For preparations of first meiotic metaphase in *Gammarus pulex* isolated pairs in precopula were watched for moulting. Sixty to ninety minutes after moulting the female was removed, slit open along its dorsal side and fixed entire in Carnoy's fluid (three parts absolute alcohol:one part glacial acetic acid). Soon thereafter the ovaries and oviducts were dissected out and transferred to fresh Carnoy's fixative for 12–18 h.

For preparations of second meiotic metaphase in all species maintained at a temperature of 15° C. the coupled pairs were watched for moulting and for the subsequent start of oviposition. Two hours after the beginning of oviposition the eggs were dissected out from the brood pouch and mucous sacs, washed in sea or tap water as appropriate, fixed in Carnoy's fluid, transferred to fresh fixative a few minutes later, and left for 12–18 h. For preparations of embryonic mitoses the embryos were similarly treated, being however allowed to remain for longer periods in the female's brood pouch prior to fixation.

After fixation the ovaries, eggs or embryos were transferred with as little fixative as possible to a freshly filtered 1% orcein solution made up in 45%

acetic acid and there left to stain for 4–6 h. Natural orcein gave us considerable trouble since it forms a noticeable precipitate within a few hours. Synthetic orcein supplied by G. T. Gurr later proved to be much superior both in staining qualities and freedom from precipitate.

The stained material was arranged on a clean coverslip over which an albuminized slide was subsequently lowered. Between folded filter-papers slide and coverslip were squashed very firmly together, and the edges of the coverslip then sealed with paraffin wax.

Most of the observations were made on such temporary preparations, which keep in good condition for several weeks if stored in a refrigerator just above freezing-point. Valuable preparations were later made permanent. The wax was soaked off in xylol and the preparation inverted in a ridged dish containing 90% alcohol. After a day or two in 90% alcohol such coverslips as had not separated freely from their slides were prized off with a razor blade. The material generally remained attached to the albuminized slide, which was then dehydrated in absolute alcohol and mounted in Euparal.

Gammarus pulex (L.)

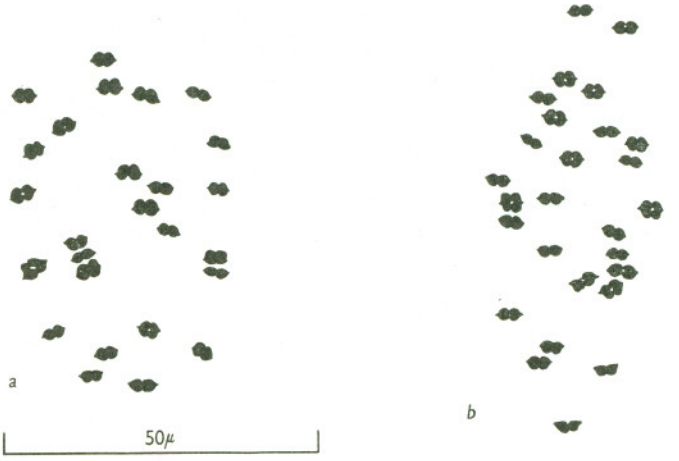
Although we did not start our study of gammarid chromosomes with the freshwater *Gammarus pulex*, it is convenient to deal with this species first. Our material was supplied by the Freshwater Biological Association, Ambleside.

Chromosome numbers found in oocytes at second meiotic metaphase are shown in Table III. From this table it will be seen that five females produced oocytes each containing 26 chromosomes, two females produced equal numbers of oocytes with 26 or 27 chromosomes, and one female produced oocytes each containing 27 chromosomes. Characteristic second meiotic metaphase chromosome groups, one with 26 and the other with 27 chromosomes, are shown in Text-fig. 1 and in Pl. I, figs. 3 and 4.

These observations suggested that *G. pulex* may have alternative diploid chromosome numbers of 52, 53 or 54. Our attention was now directed towards mitotic divisions in embryos. The existence of 52- and 53-type embryos was confirmed, though no 54-type was encountered amongst forty-four embryos from twelve females. 53-type embryos were present amongst the egg-batches of five different females, but since we do not know whether any of these egg-batches were uniformly of 53-type the chromosome constitutions of the parents, male or female, cannot be certainly stated.

We next examined first meiotic metaphase in oocytes, and for this purpose fixed the ovaries of some eighty females at the appropriate stage. Amongst these females six were definitely established as 53-type and three as 54-type. The majority of the remaining females were certainly 52-type, but the absolute ratio is not worth quoting since the constitution of too many animals within the group could not be definitely established.

In 52-type females the oocytes regularly show 26 bivalents: *G. pulex* chromosomes have median or submedian centromeres, and most of the bivalents appear to be associated by single chiasmata in both arm pairs (Pl. I, fig. 2; Text-fig. 2*a*, *b*). In 53-type females the oocytes regularly show 26 bivalents and a single univalent (Text-fig. 2*c*, *d*). Trivalent associations were not observed in any of the sixty-two cells of this type which were fully analysed. In 54-type females the oocytes generally show 27 bivalents (Text-fig. 2*c*, *f*),



Text-fig. 1. Camera lucida drawings of second meiotic metaphase chromosome groups from oocytes of *Gammarus pulex*: (*a*) with 26 and (*b*) with 27 chromosomes.

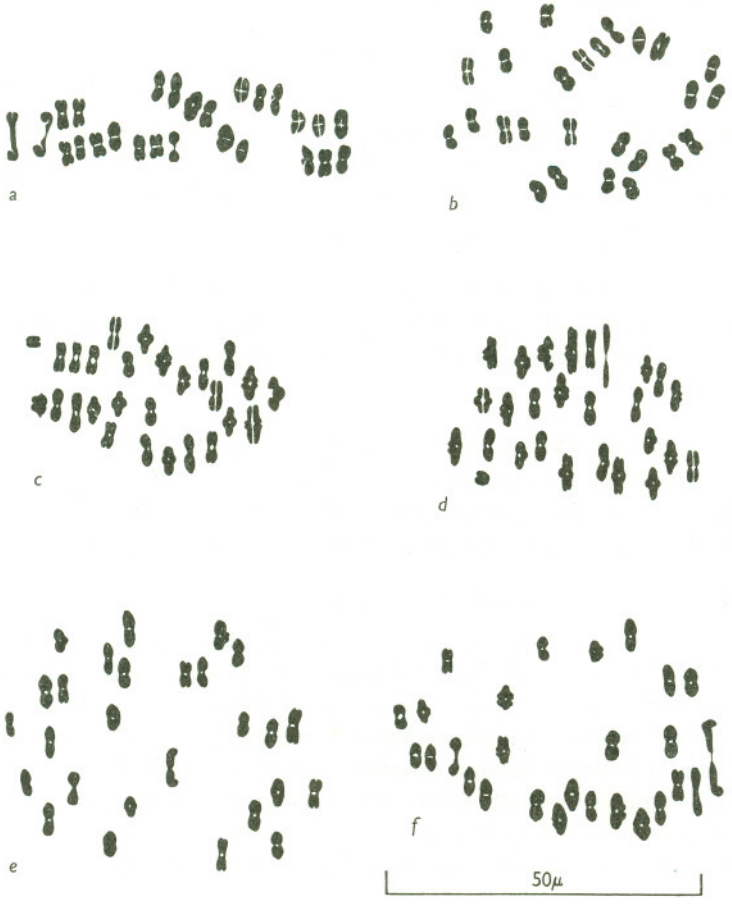
TABLE 3. CHROMOSOMES OF *GAMMARUS PULEX* (L.)

Specimen number	Number of secondary oocytes with chromosome numbers of		Assessed diploid chromosome number
	26	27	
5	8	10	53
7	12	—	52
9	—	16	54
10	6	7	53
11	8	—	52
12	6	—	52
15	5	—	52
29	5	—	52

though in four cells out of the twenty-seven of this type which were fully analyzed there was some uncertainty as to the status of one pair of chromosomes, which had either disjoined precociously or, less probably, which had failed to associate earlier in meiosis. No multivalent chromosome associations were observed in oocytes from 54-type females.

To judge from the chromosome counts made at second meiotic metaphase, chromosome disjunction at first anaphase is regular in 52- and 54-type oocytes,

whilst in 53-type oocytes the univalent appears normally to pass undivided into secondary oocyte or polar body with equal frequency. The picture is complicated, however, by the occasional presence in 52- 53- or 54-type females of minute fragment chromosomes which, at least on occasion, lag



Text-fig. 2. Camera lucida drawings of first meiotic metaphase chromosome groups from oocytes of *Gammarus pulex*: (*a*) and (*b*) with 26 bivalents; (*c*) and (*d*) with 26 bivalents plus one univalent (top left in *c*, bottom left in *d*); (*e*) and (*f*) with 27 bivalents.

behind on the equator at first anaphase. We do not know the fate of such fragment chromosomes nor their relationship to the chromosomes of the regular complement. They are not the outcome of crossing-over within inversion heterozygotes since they are not accompanied by bridges and, when present, are to be seen in every oocyte of a particular female.

The chromosomes of *G. pulex* are considerably larger than those of the other gammarids which we have studied and from a cytological standpoint

this is not a difficult species with which to work. It would be of interest to study the chromosome constitutions of different populations of *G. pulex* and also to determine whether, since in this animal several generations succeed one another within the year, there is any seasonal variation in chromosome constitution within a given population.

Gammarus duebeni Lilljeborg

A few oocytes from four examples of this species collected at the East Rocks, St Andrews, gave chromosome counts of 27 at second meiotic metaphase. This number agrees with the determination of Le Calvez & Certain (1951) and it corroborates their suggestion that Madame Le Roux's determination was erroneous.

Gammarus locusta (L.)

A few oocytes from six examples of this species supplied by the Dove Marine Laboratory, Cullercoats, gave chromosome counts of 26 at second meiotic metaphase. This number agrees with the determination of Poisson & Le Calvez (1948).

Gammarus zaddachi Sexton subsp. *salinus* Spooner

A few oocytes from two examples of this species collected at the East Rocks, St Andrews, gave chromosome counts of 26 at second meiotic metaphase. This number again agrees with the determination of Le Calvez (1949).

Gammarus chevreuxi Sexton

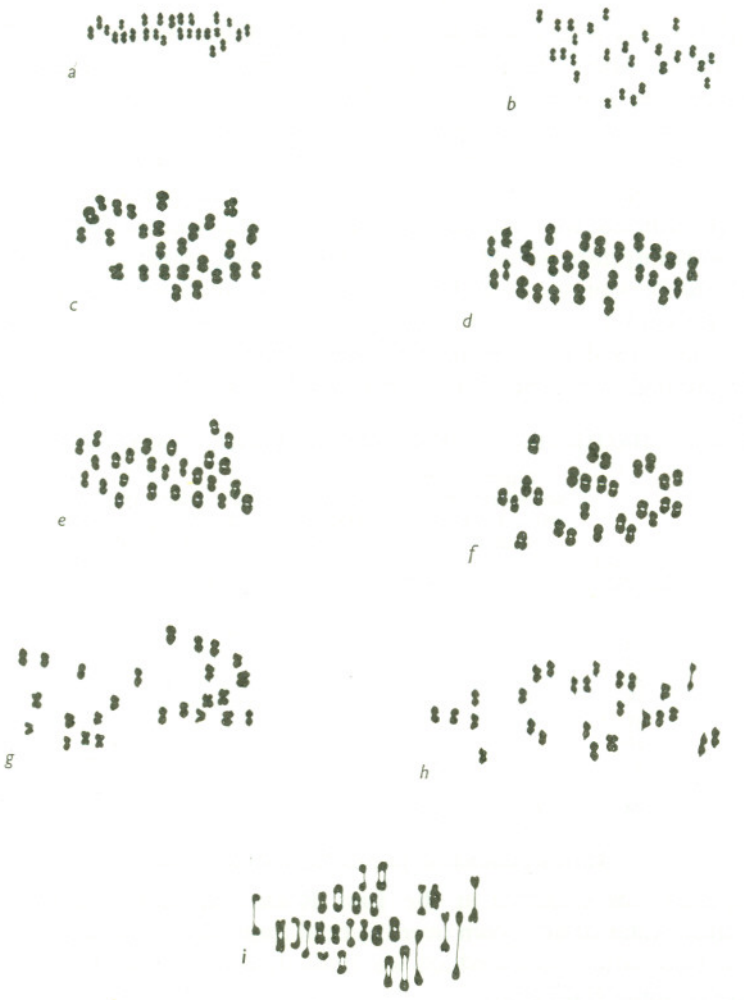
Specimens of *G. chevreuxi* from Chelson Meadow, Plym Estuary, were supplied by the Marine Biological Association's laboratory at Plymouth and were thus from the same site as those which Palmer (1926) studied. Forty oocyte second meiotic metaphase chromosome groups from ten females were counted and a constant haploid number of 26 established. As can be seen in Text-fig. 3 *a* and *b*, the chromosomes of *G. chevreuxi* are very small indeed. The determination of Le Calvez & Certain (1951) has been corroborated, and their suggestion that Palmer's determination was erroneous upheld.

Marinogammarus obtusatus (Dahl)

This species was collected at the East Rocks, St Andrews. Eighty-nine oocyte second meiotic metaphase chromosome groups from fifteen females were counted and a constant haploid number of 26 established. (See Pl. I, fig. 6; Text-fig. 3 *c*, *d*).

Marinogammarus finmarchicus (Dahl)

This species was collected at the East Rocks, St Andrews. Twenty-two oocyte second meiotic metaphase chromosome groups from four females each gave counts of 26 (see Text-fig. 3 *e*, *f*).



Text-fig. 3. (*a*) and (*b*) second meiotic metaphase chromosome groups from oocytes of *Gammarus chevreuxi*, both with 26 chromosomes. (*c*) and (*d*) second meiotic metaphase chromosome groups from oocytes of *Marinogammarus obtusatus*, both with 26 chromosomes. (*e*) and (*f*) second meiotic metaphase chromosome groups from oocytes of *M. finmarchicus*. both with 26 chromosomes. (*g*) and (*h*) second meiotic metaphase chromosome groups from oocytes of *M. marinus*, (*g*) with 25 and (*h*) with 26 chromosomes. (*i*) first meiotic metaphase chromosome group from oocyte of *Marinogammarus marinus* with 25 bivalents plus one univalent. All same magnification as Text-fig. 1.

Marinogammarus marinus (Leach)

This species was collected at the East Rocks, Eden Estuary and Harbour, St Andrews. Apart from one exceptional observation on a delayed first metaphase, the chromosomes of this species were studied at first meiotic anaphase and second meiotic metaphase: the counts are listed in Table 4.

The chromosomes of *M. marinus* (Text-fig. 3*g*, *h*) are nearly as small as those of *G. chevreuxi*, and a great many preparations could not be analysed. However, sufficient were scored to establish that this species shows a chromosomal polymorphism similar to that of *G. pulex*, though with alternative diploid numbers of 50, 51 and 52. By good fortune specimen 33 contained one oocyte with a delayed and well-spread first meiotic metaphase, and this preparation showed 25 bivalents plus one univalent (Text-fig. 3*i*). Of the ten females studied four were 50-type, five were 51-type and one 52-type.

TABLE 4. CHROMOSOMES OF *MARINOGAMMARUS MARINUS* (LEACH)

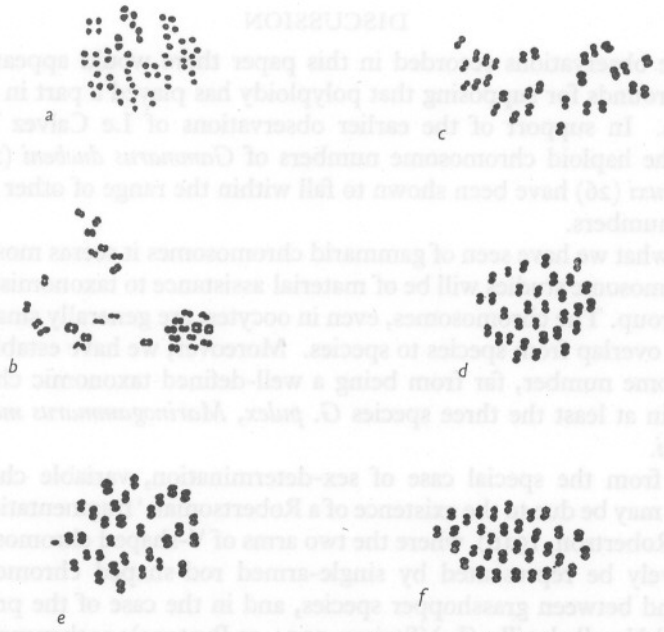
Specimen number	Numbers of secondary oocytes with chromosome numbers of		Numbers of first polar bodies with chromosome numbers of		Assessed diploid chromosome number
	25	26	25	26	
5	—	10	—	—	52
23	3	3	—	—	51
33	1	2	2	1	51
36	1	1	1	1	51
38	2	3	—	—	51
39	5	—	5	—	50
40	2	2	—	—	51
41	3	—	—	—	50?
43	2	5	—	—	51
44	10	—	—	—	50

Marinogammarus pirloti Sexton & Spooner

This species was collected at the East Rocks, St Andrews, in a region where fresh water drains down over the tidal zone. *M. pirloti* happened to be the first gammarid species which we studied, and it presents a cytological problem of some complexity.

By virtue of the pale coloration of the females and the intense brown or black pigmentation of the eggs, the species provides admirable material for following the cycle of events at reproduction. Unfortunately, however, its chromosomes are as small as those of *M. marinus*, and there are more of them (Text-fig. 4). Counts were made at second meiotic metaphase in secondary oocytes (Pl. I, figs. 7, 8), and the species is exceptional in that at this stage the polar body chromosomes are still condensed and also frequently countable. The counts are summarized in Table 5, numbers in brackets being counts made on polar body chromosome groups. It will be seen that in the six individuals studied haploid numbers range from 29 to 32 and assessed diploid

numbers from 59 to (?) 63. The assessed diploid numbers could only be established when complementary oocyte and polar body chromosome groups were both countable (Text-fig. 4*a*, *b*), and even in such cases there were sometimes grounds for uncertainty. To judge from the appearance of some second meiotic metaphase chromosome groups, notably two of 32 in specimen 15 and one of 32 in specimen 19, chromosomes present as univalents during first meiosis may on occasion divide equationally at first anaphase. The normal second metaphase chromosomes are dumb-bell shaped, the constriction



Text-fig. 4. (*a*) Early second meiotic anaphase and (*b*) first polar body chromosome groups from oocytes of *Marinogammarus pirloti*: (*a*) with 30 × 2 chromosomes; (*b*) with 29 chromosomes. (*c*), (*d*), (*e*) and (*f*) second meiotic metaphase chromosome groups from oocytes of *M. pirloti*: (*c*) with 29 chromosomes; (*d*) with 31 chromosomes; (*e*) with 32 chromosomes, the one on the extreme left being the product of the equational division of a univalent during first meiosis; (*f*) with 32 chromosomes. All same magnification as Text-fig. 1.

TABLE 5. CHROMOSOMES OF *MARINOGAMMARUS PIRLOTI* SEXTON & SPOONER

Specimen number	Numbers of secondary oocytes (or 1st polar bodies) with chromosome numbers of				Assessed diploid chromosome number
	29	30	31	32	
1	—	2	1	—	61?
2	—	2	2 (1)	—	61
3	5 (2)	6 (2)	—	—	59
15	—	1	—	3 (1)	62
18	—	—	3	4	63?
19	2	2 (1)	2 (1)	2 (1)	61

marking the centromere: it is suggested that the small chromosome lacking a constriction shown in Pl. I, fig. 8, and in Text-fig. 4*e*, is the product of such an equational division.

It would clearly be of interest to work on the first meiotic division of *M. pirloti* and to carry out a much more extensive cytological survey. The species unquestionably shows chromosomal polymorphism, and higher chromosome numbers than any previously recorded for gammarids.

DISCUSSION

From the observations recorded in this paper there would appear to be no further grounds for supposing that polyploidy has played a part in gammarid evolution. In support of the earlier observations of Le Calvez & Certain (1951), the haploid chromosome numbers of *Gammarus duebeni* (27) and of *G. chevreuxi* (26) have been shown to fall within the range of other gammarid haploid numbers.

From what we have seen of gammarid chromosomes it seems most unlikely that chromosome studies will be of material assistance to taxonomists working on this group. The chromosomes, even in oocytes, are generally small, and the numbers overlap from species to species. Moreover, we have established that chromosome number, far from being a well-defined taxonomic character, is variable in at least the three species *G. pulex*, *Marinogammarus marinus* and *M. pirloti*.

Apart from the special case of sex-determination, variable chromosome numbers may be due to the existence of a Robertsonian 'fragmentation-fusion' system (Robertson, 1916), where the two arms of V-shaped chromosomes may alternatively be represented by single-armed rod-shaped chromosomes, as within and between grasshopper species, and in the case of the prosobranch gastropod *Nucella lapillus* (L.) (Staiger, 1954, as *Purpura*); or they may be due to the presence of supernumeraries extra to a basic complement. The variable chromosome numbers of gammarids do not appear directly related to a sex-determining mechanism, though it is certainly conceivable that they may in some fashion be indirectly related. Clear proof of a Robertsonian system in gammarids is also wanting. In such a system the heterozygotes generally show trivalents at first meiosis where V-shaped chromosomes are associated with their genetically related two rod-shaped chromosomes, and indeed balanced disjunction within such a system is only possible when in heterozygotes trivalent associations are formed with great regularity. No multivalent associations were observed throughout our reasonably extensive studies on *Gammarus pulex*.

It is therefore reasonable to assume that the variable chromosome numbers of gammarids are due to the presence of supernumeraries, and that crustaceans should be added to the already long list of organisms in which such chromosomes have been found (see White, 1954, p. 167). The genetic significance of

supernumerary chromosomes remains to a great extent undecided, and light might well be thrown upon this problem by further work on the easily reared and cytologically manageable *G. pulex*.

SUMMARY

The chromosomes of nine species of gammarids have been studied in oocytes and embryos. There is no evidence that polyploidy has played a part in gammarid evolution. Three of the species which we have studied, *Gammarus pulex* (L.), *Marinogammarus marinus* (Leach) and *M. pirloti* (Sexton & Spooner) show chromosomal polymorphism. There is no evidence that this polymorphism depends on the existence of a Robertsonian 'fragmentation-fusion' system; rather it appears to be due to the presence of supernumerary chromosomes.

REFERENCES

HUXLEY, J. S., 1923. Further data on linkage in *Gammarus chevreuxi*, and its relation to cytology. *J. exp. Biol.*, Vol. 1, pp. 79–96.

LE CALVEZ, J., 1949. Quelques nouveaux caryotypes de Crustacés Amphipodes. *C.R. Acad. Sci., Paris*, T. 228, pp. 427–428.

LE CALVEZ, J. & CERTAIN, P., 1951. *Gammarus chevreuxi* Sext. et la caryologie des Gammariens. *Arch. Zool. exp. gén.*, T. 88, pp. 131–141.

LE ROUX, M. L., 1933. Recherches sur la sexualité des Gammariens. *Bull. Biol.*, Suppl., Vol. 16, 138 pp..

NIIYAMA, H., 1935. The chromosomes of *Paralithodes camtschatica* and *Gammarus annandalei*. *Jap. J. Genet.*, Vol. 11, pp. 34–35.

PALMER, R., 1926. The chromosome complex of *Gammarus chevreuxi* Sexton. I. Spermatogenesis. *Quart. J. micr. Sci.*, Vol. 70, pp. 541–551.

POISSON, R. & LE CALVEZ, J., 1948. La garniture chromosomique de quelques Crustacés Amphipodes. *C.R. Acad. Sci., Paris*, T. 227, pp. 228–230.

ROBERTSON, W. R. B., 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae: chromosomes and variation. *J. Morph.*, Vol. 27, pp. 179–331.

STAIGER, H., 1954. Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. *Chromosoma*, Bd. 6, pp. 419–478.

STAIGER, H. & BOCQUET, CH., 1954. Cytological demonstration of female heterogamety in isopods. *Experientia*, T. 10, pp. 64–66.

WHITE, M. J. D., 1954. *Animal Cytology and Evolution*. Cambridge University Press.

EXPLANATION OF PLATE I

Photomicrographs of 'squash' preparations stained in aceto-orcein: all figures at the same magnification.

Fig. 1. Polar view of first meiotic metaphase in spermatocyte of *Gammarus pulex*.

Fig. 2. Side view of first meiotic metaphase in oocyte of *G. pulex*: 26 bivalents.

Fig. 3. Side view of second meiotic metaphase in oocyte of *G. pulex*: 26 chromosomes.

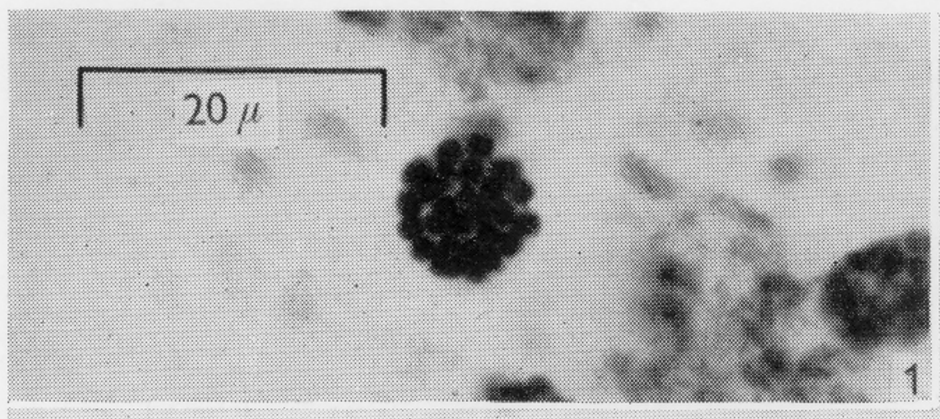
Fig. 4. Side view of second meiotic metaphase in oocyte of *G. pulex*: 27 chromosomes.

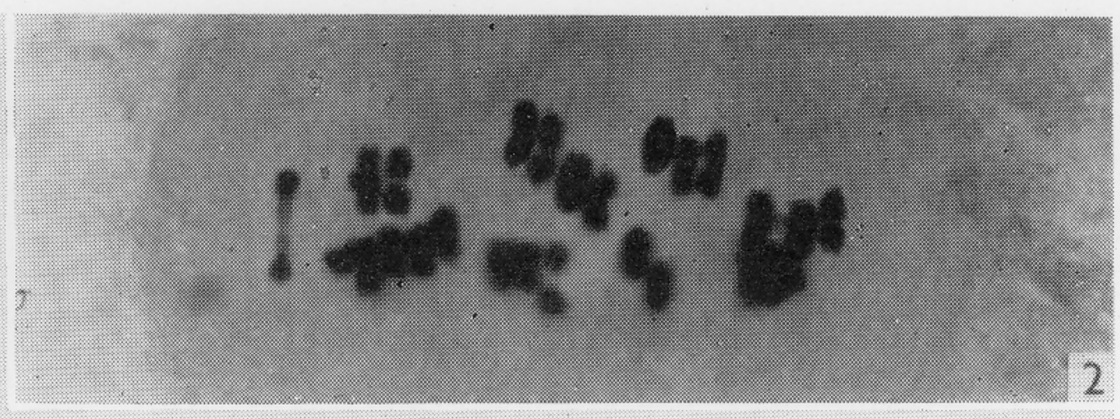
Fig. 5. Prometaphase of first mitotic division in fertilized egg of *G. pulex*: 52 chromosomes.

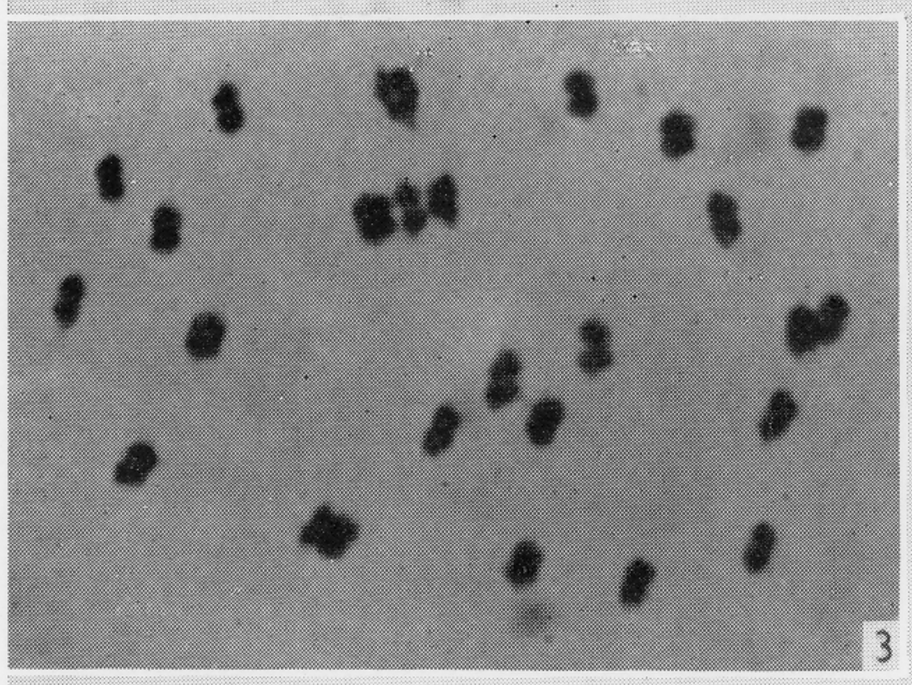
Fig. 6. Side view of second meiotic metaphase in oocyte of *Marinogammarus obtusatus*: 26 chromosomes.

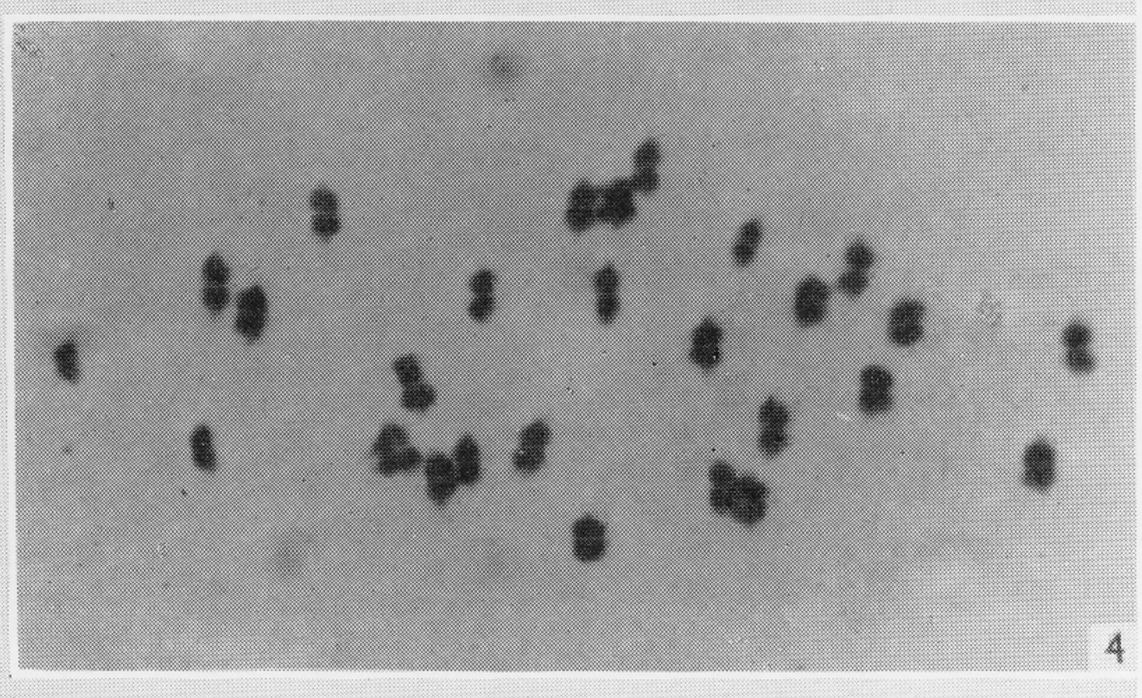
Fig. 7. Side view of second meiotic metaphase in oocyte of *M. pirloti*: 29 chromosomes.

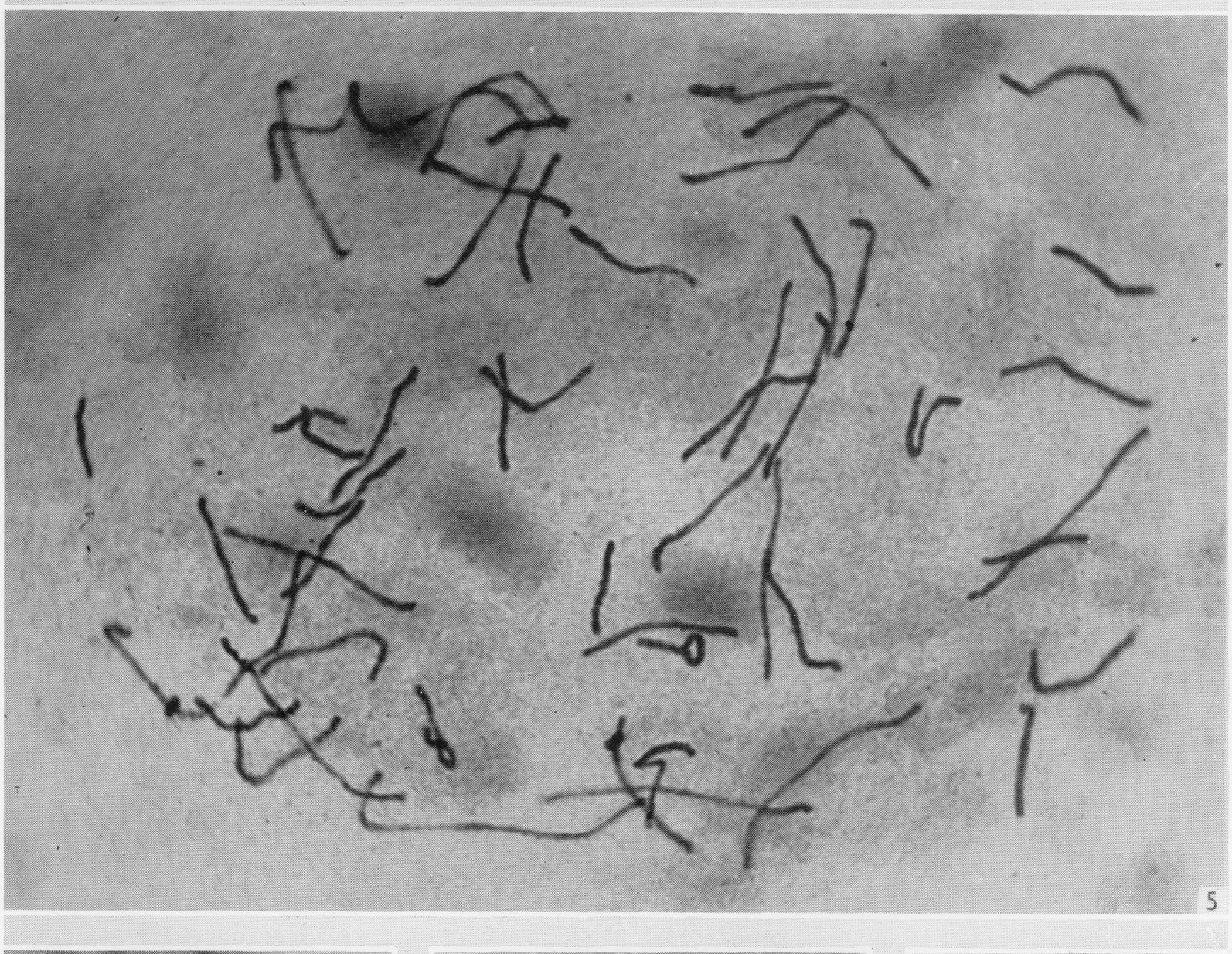
Fig. 8. Side view of second meiotic metaphase in oocyte of *M. pirloti*: 32 chromosomes, of which the one on the extreme right is the product of the equational division of a univalent during first meiosis.

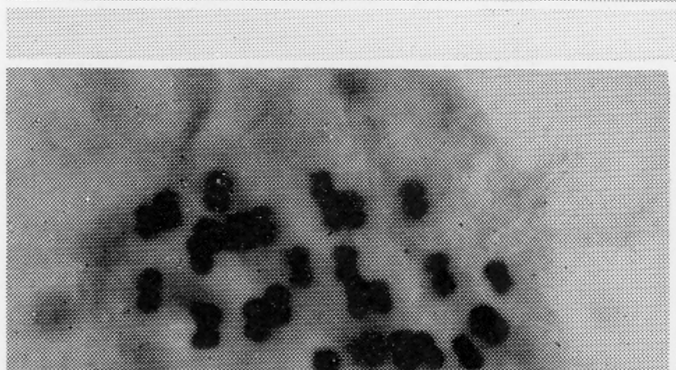


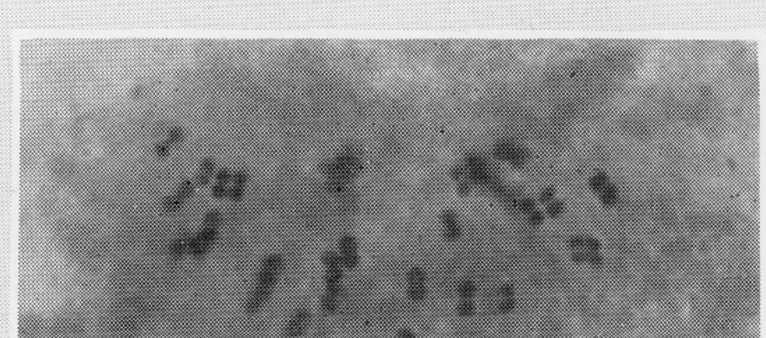


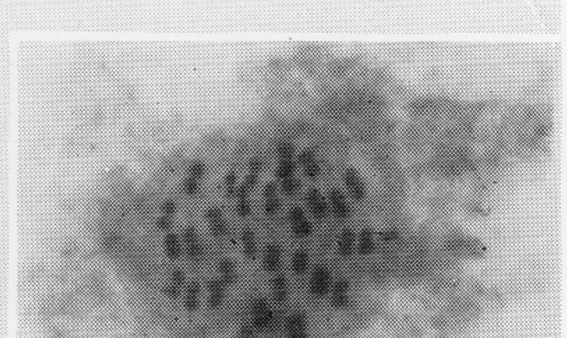












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SEASONAL VARIATIONS IN HOST-PARASITE RELATIONS BETWEEN FISH AND THEIR PROTOZOA

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(Text-fig. 1)

Most papers on the subject of parasitology have been confined to descriptions and lists of parasites and to clinical and economical considerations. Ecological studies are more subject to errors of interpretation, and they usually require more time to complete. For these reasons an ecological approach to the study of parasitology has not been popular, but through such an approach the parasitologist has an opportunity to extend the range of his contributions to an understanding of basic problems in biology.

The term 'parasite' generally implies injury to a host, but parasitologists usually assume the absence of injury when visible evidence of it is lacking. Physiological injury may well exist in a healthy-looking animal. Parasites such as *Entamoeba histolytica* are injurious only occasionally, and are normally commensal in their relationship to the host. For these reasons I shall employ the term 'parasite' in a broad sense to include all organisms which normally nourish themselves within or on the body of another organism without destroying the latter.

Only one year was at my disposal for the present study, but I felt that a regular and systematic examination of the Protozoa and their fish hosts during the seasons of that year should result in information of value. I wish to emphasize that this report is presented primarily to suggest an ecological approach, often neglected, to problems in parasitology. Any significant conclusions on seasonal variations should be based upon at least three full annual cycles. My results, therefore, can only serve as a basis for further studies. The work was done at the Laboratory of the Marine Biological Association of the United Kingdom, Plymouth, from 28 July 1955 to 1 June 1956. I am exceedingly grateful to the Director of the Laboratory, Mr F. S. Russell, and his staff, who generously placed all necessary equipment and supplies at my disposal. Dr C. E. Lucas of the Marine Laboratory, Aberdeen, and Mr E. Ford of the Marine Station, Millport, were very helpful in supplying material from Scottish waters. I wish also to acknowledge receipt, from the National Science Foundation, Washington, of a research grant which made my trip to England possible.

METHODS

My studies were restricted to the Protozoa because an adequate attention to these forms, especially to the living parasites, consumed all of the available time. The fish used were the Lemon Sole (*Microstomus kitt*), the Dragonette (*Callionymus lyra*) and the Whiting (*Gadus merlangus*). They were collected by means of an otter trawl within about 10 miles of the port of Plymouth. The fish were brought immediately to the laboratory, and were alive when I started work on them, or they were so recently dead that the blood flowed freely when the heart was cut. If the blood did not flow freely the fish was discarded. Blood for smears was obtained from the heart immediately after incision by a razor blade. In *M. kitt* the incision was made anterior to the liver after the peritoneal cavity was opened; in *G. merlangus* it was made between the gills before the body was opened; and in *C. lyra* it was made ventrally through the body wall. Before each incision the area to be cut was wiped with absorbent paper in order to reduce the possibility of contamination.

Macroscopic examinations of the following organs of each fish were made: skin, fins, mouth, pharynx, gills, viscera, kidney and peritoneal cavity. I recorded the appearance of all unusually heavy infections of worms, and gill infections by the copepod *Lernaea*. Other copepods were very seldom observed. Leeches were never found on the fish or in the water of the containers in which the fish were brought to the laboratory.

Microscopic examinations of the blood, gall bladder and gut contents were made for each fish. Occasionally, examinations of the stomach contents, urinary bladder and other organs were also made. The gut and gall bladder contents were examined in the fresh condition immediately after collection so that living parasites, if present, were easily observed. Relatively few permanent, stained preparations from these organs were prepared. Schaudinn's or Susa's fluids with iron haematoxylin were usually employed. The blood smears were fixed for from 5 to 10 min in methyl alcohol as soon as they were dry, then stained for from 30 to 60 min in Giemsa diluted with a phosphate buffer solution.

Abundant evidence from numerous workers indicates that although young fish may have a high percentage of parasitic infection, the intensity is low. Adult fish have the most parasites because of their intensive feeding habits during growing stages, including the feeding on intermediate hosts, and because of an accumulation of parasites (see Dubinin, 1936). For these reasons I generally selected the larger fish for study. No particular effort was made to separate males from females, but no differences in the degree or kind of infection between the sexes were noted. A maximum of fifteen fish (usually five of each species) was studied at one time because I found that more than this number required so much time to dissect and examine, that degeneration of host cells and death of the parasites (especially flagellates) reduced the chances of their detection in fresh preparations.

THE HOSTS

Of the fish studied, *Microstomus kitt* and *Callionymus lyra* are bottom feeders, and both are to be found in the same localities. If parasites of one have a wide range of host tolerance, the other fish might be expected to be host to the same parasites. *M. kitt* feeds primarily on polychaete worms which are the predominant fauna in the mud frequented by the fish. *C. lyra*, on the other hand, feeds upon practically any bottom-dwelling animal of suitable size; e.g. small crustaceans, annelids, arthropods and molluscs. It has been well established that differences in feeding habits are related to differences in parasites, especially when intermediate hosts are involved.

The two species of fish mentioned above are widely separated taxonomically. *Microstomus kitt* is a flatfish belonging to the family Pleuronectidae. *Callionymus lyra* is a member of the family Callionymidae, and is of a more conventional fish shape, except that its mouth is ventrally placed. It is brilliantly coloured, and the sexes are strikingly different in their external features. The third species of fish, *Gadus merlangus*, belongs to the family Gadidae. It is an active predator, feeding chiefly upon smaller fish, with a greater vertical range of habitat than is characteristic of the other two species.

Dr G. A. Steven of the Plymouth Marine Laboratory has been keeping records of the numbers of fish of each species taken by otter trawl during the past several years at Plymouth. Although the records are incomplete, the chart shows that *Callionymus lyra* tends to be found in greater numbers during the warmer months, but that the other two species of fish do not fluctuate in any consistent manner throughout the year. The records are tabulated in Table 1.

TABLE 1. NUMBER OF FISH PER HAUL OF ONE HOUR AT PLYMOUTH, ENGLAND

Figures show an average usually of three or four hauls. From the records of Dr G. A. Steven (unpublished).

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Callionymus lyra	201	161	185	169	151	370	335	572	373	548	419	241	1953
Gadus merlangus	>1	1	28	87	45	204	286	67	5	2	0	0	1953
Microstomus kitt	9	7	10	8	5	7	3	6	8	3	0	6	1953
C. lyra	239	4	42	236	N.R.*	N.R.	N.R.	N.R.	200	385	611	176	1954
G. merlangus	>1	0	7	33	N.R.	N.R.	N.R.	N.R.	9	37	0	11	1954
M. kitt	4	10	11	9	N.R.	N.R.	N.R.	N.R.	6	2	4	8	1954
C. lyra	94	91	98	79	168	437	377	294	N.R.	57	82	105	1955
G. merlangus	20	63	85	168	27	20	7	13	N.R.	5	1	0	1955
M. kitt	13	7	6	21	9	13	7	9	N.R.	1	2	0	1955
C. lyra	136	N.R.	N.R.	243	192	545	—	—	—	—	—	—	1956
G. merlangus	7	N.R.	N.R.	29	8	27	—	—	—	—	—	—	1956
M. kitt	4	N.R.	N.R.	15	4	11	—	—	—	—	—	—	1956

* N.R. = no record.

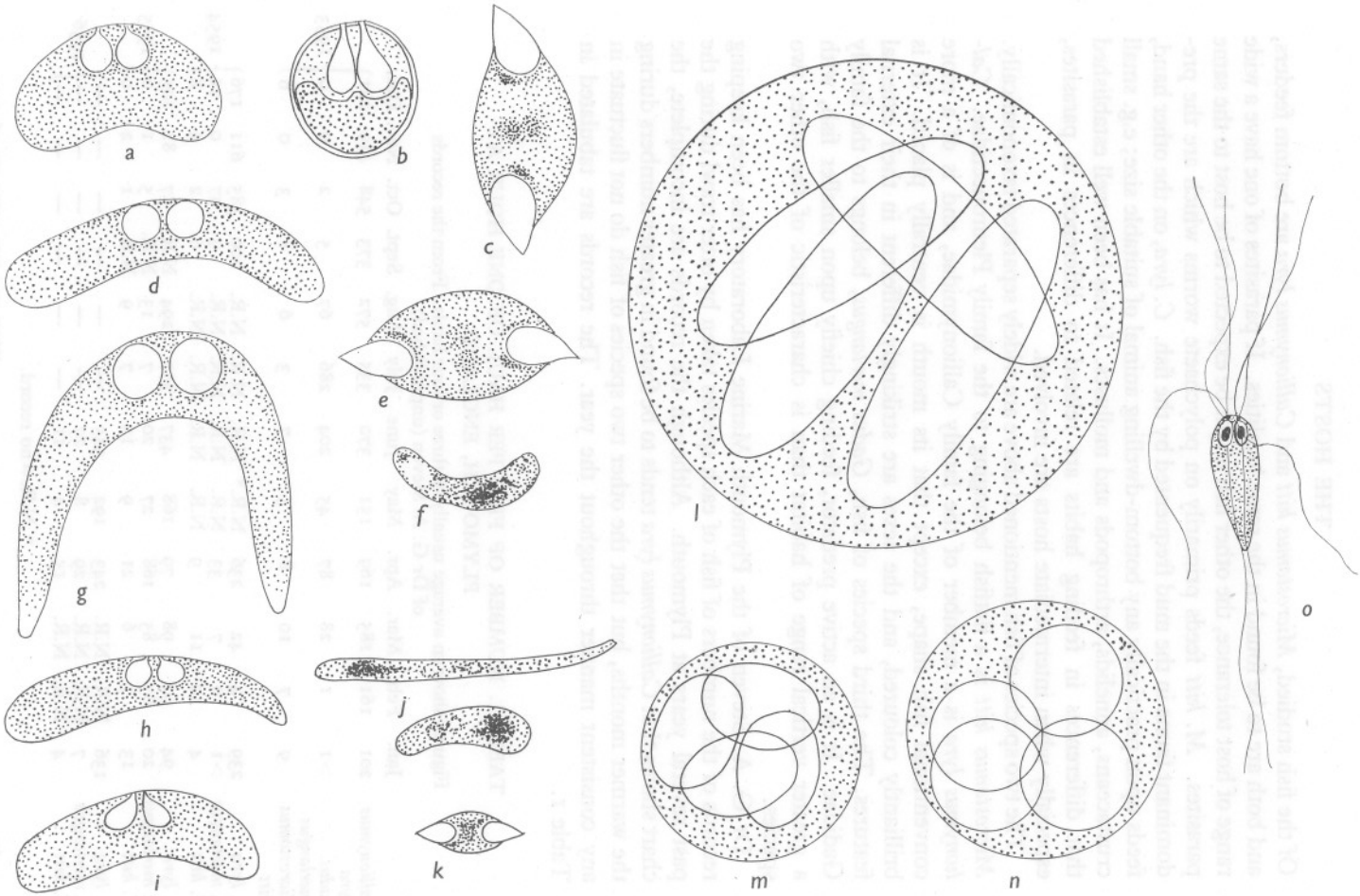

Fig. 1

THE PROTOZOAN FAUNA

It is distressing to find statements in the literature indicating that a new species name has been created solely or even primarily because the parasite is found in a new species of host. Such a practice is entirely unwarranted. It is well known that many species of parasites have a wide range of host tolerance, and unless there are obviously-marked differences in morphology, and unless the entire life cycle is known, and sufficient numbers of individuals have been observed and measured, the description of a new species is unjustified. Too little attention has been paid to physiological differences which should be as important as morphological ones in the distinctions among species. Physiological characteristics are, of course, more difficult to identify, but this fact should not result in their being ignored. For these reasons I shall not attempt, at this time, descriptions or species designations of the forms which appear to be new. The figures illustrated in Fig. 1 are semi-diagrammatic to show only general form and relative sizes of the Protozoa which I have found. Lists of the Protozoa for each of the three fish are shown in Tables 2–4.

I have seen many forms intermediate in appearance between the two species of *Callionymus*-infecting haemogregarines described by Brumpt & Lebailly, and I believe that only one species is present. Reichenow (1932) suggested that *Haemogregarina callionymi* is the sexual form of *H. quadrigemina*. *H. binucleata* was mentioned in 1910 by Henry but he did not describe it. It also is probably *H. quadrigemina*. The two unspecified *Ceratomyxa*, one from the gall bladder, the other from the urinary bladder, and the *Eimeria* may be identical with species reported from other hosts, but, as indicated above, I shall not attempt a description of them at this time. *Eimeria* in the liver of *Callionymus* was frequently abundant enough to give it a speckled-white appearance. In such heavy infections the oocysts were usually found in large numbers in the gall bladder.

As regards the whiting, Auerbach (1910) distinguished *Myxidium bergense* from *M. sphaericum* Thélohan chiefly on the basis of the trophic stages which

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Legend to Fig. 1

Fig. 1. Semi-diagrammatic drawings of Protozoa obtained from fish, intended only to show general outlines and relative size. Scale uniform. *a*, *Leptotheca informis* from gall bladder of *Gadus merlangus*. *b*, *Myxobolus aeglefini* from eye cartilage of *Gadus merlangus*. *c*, *Myxidium sphaericum* from gall bladder of *Gadus merlangus*. *d*, *Ceratomyxa lata* from gall bladder of *Microstomus kitt*. *e*, *Myxidium* sp. from gall bladder of *Microstomus kitt*. *f*, *Haemogregarina platessae* from blood of *Microstomus kitt*. *g*, *Ceratomyxa* sp. from urinary bladder of *Callionymus lyra*. *h*, *Ceratomyxa* sp. from gall bladder of *Callionymus lyra*. *i*, *Ceratomyxa arcuata* from gall bladder of *Callionymus lyra* and *Gadus merlangus*. *j*, *Haemogregarina quadrigemina* from blood of *Callionymus lyra*. *k*, *Myxidium incurvatum* from gall bladder of *Callionymus lyra* and *Gadus merlangus*. *l*, *Eimeria* sp. from gut of *Gadus merlangus*. *m*, *Eimeria* sp. from gut of *Gadus merlangus*. *n*, *Eimeria* sp. from liver of *Callionymus lyra*. *o*, *Hexamita* sp. from stomach and gut of *Gadus merlangus*.

in the former species has medium-pointed pseudopodia and a length up to 54 μ, while in the latter it is rounded, about 20 μ in diameter with lobed pseudopodia. My observations suggest that these are the same species because there is a great deal of variation in dimensions and appearance of the trophic stages, depending on temperature, freshness of the preparation, age of parasites, etc. I could find no evidence for two distinct kinds of *Myxidium* spores within the size ranges of *M. bergense* and *M. sphaericum*.

TABLE 2. PROTOZOA IN *CALLIONYMUS LYRA*

Previously reported	Found during present study	Percentage of hosts infected
Blood		
Haemogregarina quadrigemina Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	*H. quadrigemina* (Fig. 1*j*)	70
H. callionymi Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1910	—	—
H. binucleata Henry, 1910, from Plymouth	—	—
Trypanosoma callionymi Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	—	—
Gall bladder		
Ceratomyxa arcuata Thélohan, 1892 (Europe) Report from Plymouth by Dunkerley, 1920	*C. arcuata* (Fig. 1*i*)	62
	C. sp. (Fig. 1*h*)	
Myxidium incurvatum Thélohan, 1892 Report from Plymouth by Dunkerley, 1920	*M. incurvatum* (Fig. 1*k*)	92
Liver		
—	*Eimeria* sp. (Fig. 1*n*)	9
Urinary bladder		
—	*Ceratomyxa* sp. (Fig. 1*g*)	(4 out of 8)
Muscle		
Chloromyxum quadratum Thélohan, 1894 (Europe)	—	—
Nosema destruens (Thélohan, 1891), Labbé, 1899 (Europe)	—	—

The *Eimeria* of the gut of *Gadus merlangus* undoubtedly belong to two species because of the marked differences in shape and size of oocysts. *Hexamita* in the stomach and gut is very similar to several species which have been described from unrelated hosts (see Wenrich, 1935). This genus is remarkable in its lack of host specificity, and I hope to make a special study of the form in *Gadus merlangus*, and relate it to the more general problem of host-specificity and origin of parasitism. *Myxobolus aeglefini* was found in only one out of the 200 fish examined.

TABLE 3. PROTOZOA IN *GADUS MERLANGUS*

Previously reported	Found during present study	Percentage of hosts infected
Gall bladder		
Ceratomyxa arcuata Thélohan, 1892 (Europe) Report from Plymouth by Dunkerley, 1920	*C. arcuata* (Fig. 1*i*)	2
Leptotheca informis Auerbach, 1910 (Europe)	*L. informis* (Fig. 1*a*)	17
Myxidium bergense Auerbach, 1910 (Europe)	*M. incurvatum* (Fig. 1*k*) Thélohan, 1892	3
M. sphaericum Thélohan, 1894 (Europe) Report from Plymouth by Dunkerley, 1920	*M. sphaericum* (Fig. 1*c*)	58
Gills		
Amyloodinium ocellatum (Brown), 1934 (England)	—	—
Bone and cartilage		
Myxobolus aeglefini Auerbach, 1906 (Europe)	*M. aeglefini* (Fig. 1*b*)	0·5
Pyloric caecae		
Mrázesia piscicola Cépède, 1924 (Europe)	—	—
Stomach and intestine		
—	*Eimeria* sp. no. 1 in gut (Fig. 1*l*)	0·5
—	*Eimeria* sp. no. 2 in gut (Fig. 1*m*)	1·5
—	*Hexamita* sp. in gut and stomach (Fig. 1*o*)	4

TABLE 4. PROTOZOA IN *MICROSTOMUS KITT*

Previously reported	Found during present study	Percentage of hosts infected
Blood		
Haemogregarina platessae Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	*H. platessae* (Fig. 1*f*)	1·1
H. flesi Lebailly, 1904 (Europe)	—	—
Gall bladder		
Ceratomyxa awerinzewi Reichenow, 1929 (Europe)	*Myxidium* sp.	11
Ceratomyxa lata Dunkerley, 1920 (Plymouth)	*C. lata* (Fig. 1*d*)	97

The *Myxidium* from *Microstomus kitt* appears very similar to *M. bergense* Auerbach originally described from *Gadus virens*. *Ceratomyxa lata* seems to have two forms (possibly two species), one with relatively large (average 5·2 μ in diameter) polar capsules, and the other with smaller (average 3·2 μ) polar capsules. The size of the spore (9 by 25·7 μ) is somewhat larger than that

originally reported by Dunkerley. *Haemogregarina platessae* and *H. flesi* seem to be identical (see Reichenow, 1932). Ten schizonts from my preparations of blood smears averaged 2·4 by 8·4 μ.

SEASONAL VARIATIONS

The intensity of infection was estimated on the basis of relative numbers of individual parasites as seen through the microscope, using an oil-immersion objective and 10× ocular. One to about fifteen parasites in a field was designated as +. If the field was crowded with parasites it was designated as +++, and everything between these two was listed as ++. The obvious limitations of this method did not prevent a reasonably accurate indication of shifts of infection intensity, but for definite and convincing results a more precise method would have to be used, and the results subjected to a statistical analysis. I arbitrarily divided the year into three periods roughly corresponding to summer, winter and spring. The times of my arrival in and departure from Plymouth prevented collections of fishes during the month of June and most of July. The results are listed in Tables 5–7.

Material from a small number of the same three species of fish collected at Millport and at Aberdeen during the month of October 1955 indicated similar degrees of infection as those found at Plymouth during the same month.

Callionymus lyra (Table 5)

Some evidence for seasonal changes is to be found in this fish. Heavy infections of myxosporidia in the gall bladder occurred in 19% of the summer fish, and rose to 40% in winter fish. At the same time, however, and in the same fish, haemogregarines of the blood showed a reverse tendency. The blood in 24% of the fish was heavily infected during the summer, while only 7% were heavily infected during the winter.

TABLE 5. *CALLIONYMUS LYRA*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of infection	Summer 28 July to 31 Oct. 76 fish	Winter 1 Nov. to 29 Feb. 62 fish	Spring 1 Mar. to 26 May 62 fish	Total of 200 fish
Myxosporidia in gall bladder	–	0	2	0	0·7
	+	39	14	27	27
	++	42	44	46	44
	+++	19	40	27	28·3
Haemogregarines in blood	–	36	28	35	33
	+	40	66	48	51
	++	24	7	17	16

Twice as many spring fish were infected with gill copepods (*Lernaea*) and internal worms as were the summer and winter fish. Liver infection with *Eimeria* occurred in one summer fish, four winter fish and thirteen spring

fish. Worm infections were never heavy and no general correlations between the intensities of Protozoa and worms were noted in *Callionymus lyra*. The gall bladders of 99·3% of the fish contained myxosporidia, and 66% of these fish were infected with haemogregarines.

Microstomus kitt (Table 6)

There was a slight increase in numbers of winter fish with heavy gall bladder infections, but the small numbers involved preclude any generalization. Cysts of larval worms were occasionally found in the viscera. Of the total fish, over 99% were infected with myxosporidia.

TABLE 6. *MICROSTOMUS KITT*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of infection	Summer 28 July to 31 Oct. 70 fish	Winter 1 Nov. to 29 Feb. 51 fish	Spring 1 Mar. to 26 May 55 fish	Total of 176 fish
Myxosporidia in gall bladder	–	3	0	0	1
	+	93	53	62	69
	++	4	33	33	24
	+++	0	14	5	6

Gadus merlangus (Table 7)

No seasonal changes are indicated. Heavy worm infections (predominantly nematodes) appeared to be more abundant during the spring period. Of the total fish, 79% were infected with myxosporidia.

TABLE 7. *GADUS MERLANGUS*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of infection	Summer 28 July to 31 Oct. 61 fish	Winter 1 Nov. to 29 Feb. 74 fish	Spring 1 Mar to 26 May 65 fish	Total of 200 fish
Myxosporidia in gall bladder	–	23	16	26	21
	+	45	57	56	53
	++	23	19	12	18
	+++	9	8	6	8

PATHOLOGY

Protozoan parasites, in general, appear to be of a commensal nature in fishes, but very little work has been done on the effects of parasites on the growth and development of these hosts. The most commonly infected organ in all the fish examined was the gall bladder, containing myxosporidian parasites. Infected bile undergoes chemical changes as indicated by a yellow colour and changes in density and viscosity. In a heavily infected gall bladder the bile often becomes so viscous that it does not flow when the shrunken bladder is cut open. Its contents are frequently of a white, cheese-like consistency. Similar pathological changes, however, may appear when there is no visible

evidence of the presence of myxosporidia or of bacteria, and the pathological changes are presumably due, in these instances, to a virus infection.

The only other infected organs found to exhibit visible signs of pathology were the eyes of one specimen of *Gadus merlangus*, the cornea and sclera of which were spotted by opaque, white cysts of the myxosporidian, *Myxobolus aeglefini*; and the liver of *Callionymus lyra* which was occasionally speckled with cysts of *Eimeria*. One observation not indicated in the previous tabulations of results, and often noted by parasitologists, was the apparent deleterious effect on one species of parasite by the presence of unusual numbers of another species of parasite in the same host body. For example, during massive infections of nematode worms, Protozoa were greatly reduced in numbers, or they were absent.

DISCUSSION

If one may assume that myxosporidia are consistently abundant in the gall bladders of *Callionymus lyra* in summer, and that haemogregarines are consistently more abundant in the blood in winter (an assumption based on little evidence), one might correlate these changes with population fluctuations of the hosts (see Table 1). One might assume that the Plymouth area during the summer months is more favourable for intermediate hosts of haemogregarines and less favourable for infection with myxosporidian spores than during the winter months. But we do not know whether the fish that are caught during the summer months are those that are left behind when most of the population migrates to places unknown, or whether they represent fish which have moved into the Plymouth area during the summer season from elsewhere. We are not even sure of the identity of the intermediate hosts.

Nothing is known, as far as I am aware, of the migratory habits of the fish used in this study. Are the bottom-dwellers more restricted in their range than is *Gadus merlangus*? *Callionymus lyra* generally has blood Protozoa as well as gall bladder Protozoa, and occasionally also copepods on its gills. *Microstomus kitt* rarely has blood parasites, and I found no gill copepods. Are these differences correlated with differences in habitats? A basis for some hypotheses about their migratory habits might be found among conclusions drawn by other workers who have been interested in similar problems dealing with freshwater fish. Bychowskaja (1936), for example, found that fish in small lakes have fewer numbers and kinds of parasites than do fish in large lakes. It occurred to me that perhaps marine fish in a restricted habitat have fewer parasites than marine fish which roam over a wide area. Unfortunately, few studies have been made on migrations of marine fishes, and, while bottom-dwelling fish might be expected to be the more restricted in their range, I know of no proof for the assumption. One might predict, however, on the basis of their respective parasitic faunas, that *C. lyra* has a wider migratory range than has *M. kitt*. Gorbunova (1936) studied the variations of the

parasites of freshwater pike and roach with respect to their ages. She found that, in addition to an increase in numbers of parasitic species with the ages of the hosts, the fauna in fish of quieter habits is poorer than that in the predatory fish (i.e. pike). Again applying these findings to my own studies, one could correlate the relatively poorer protozoan fauna exhibited by *M. kitt* with the presumably more restricted and 'quieter' bottom habitat which the host occupies.

Gadus merlangus has a diet restricted chiefly to small fish, but there appeared to be more varieties of fish in its stomach than varieties of annelids in the stomach of *Microstomus kitt*. In addition, *G. merlangus* probably has a wider migratory range than has *M. kitt*. These factors would suggest a higher degree of parasitism in *G. merlangus*, and my records show that ten species of Protozoa have been reported from this fish as compared with four species from *M. kitt*. In addition, *G. merlangus* is much more heavily infected with worms than is either *Callionymus lyra* or *M. kitt*. *C. lyra*, on the other hand, consumes a wide variety of food, and it has demonstrated a marked seasonal fluctuation in population numbers. It has about the same numbers of kinds of protozoan parasites as has *G. merlangus*, but the percentage of hosts infected with Protozoa is considerably higher.

It is surprising that my search for blood parasites in 200 stained slides, each from a different *Callionymus lyra*, did not reveal a single trypanosome. The fact that in 1910 Henry found, at Plymouth, six out of fifteen of these fish to be infected with trypanosomes suggests that they are subject to a yearly fluctuation of trypanosome infection. The fluctuation may be due to a periodic disappearance of leeches which serve as intermediate hosts, but haemogregarines are also transmitted by leeches, and haemogregarines were common in *C. lyra*, and occasionally they were found in *Microstomus kitt*. In fact, the abundance of haemogregarines in *C. lyra*, with the absence of leeches, throws doubt on the assumption that these parasites in this fish are transmitted by leeches. A few copepods attached to the gills were examined for haemogregarines but the results were negative.

The results of my studies, together with those of other writers, suggest the following tentative conclusions. An active predatory habit predisposes to heavy parasitism, but this result may be off-set by a restriction of the diet to one or to a few kinds of food species. Likewise, a less active host may acquire numerous kinds of parasites because of a taste for a wide variety of foods. A sluggish bottom-feeder with little tendency toward migration, and with limited tastes for food variety, tends to acquire relatively few kinds and numbers of parasites.

I have raised more questions than I have answered, but these questions suggest several lines of research which should be of significance in the field of ecological parasitology. Mere descriptions of parasites and their life cycles (including those which I have previously authored) generally carry with them

implications of completeness which can be grossly misleading. Such descriptions fail to impress the reader with the impact of environmental changes, both internal and external, upon host-parasite relationships.

SUMMARY

From July 1955 to June 1956 a study of host-parasite relations between three species of marine fish and their Protozoa was made at Plymouth. *Callionymus lyra*, a bottom-dweller, tends to be more abundant during the summer months, consumes a wide variety of food, and was more heavily infected with Protozoa than were the other two species of fish. *Gadus merlangus* is an active predator, feeding on small fish, and although it has about the same numbers of kinds of Protozoa as does *C. lyra*, it was less heavily parasitized. *G. merlangus*, however, was more heavily parasitized with worms than were the other two fish. *Microstomus kitt* is a sluggish bottom-dweller, feeding on annelids, and, although over 99% were infected with myxosporidia, the intensity of infection was generally low. Very few other parasites were found in *M. kitt*.

An active predatory habit combined with a taste for a wide variety of food appears to predispose to heavy parasitism. Some evidence for seasonal variations in intensities of protozoan infection in *Callionymus lyra* was obtained, but for conclusive results more precise methods of measuring numbers of parasites, and further studies carried on over a period of at least three consecutive years, must be made. The report is presented primarily to emphasize the importance of an ecological approach to the study of parasitology.

REFERENCES

AUERBACH, M., 1906. Ein Myxobolus im Kopf von *Gadus aeglefinus* L. *Zool. Anz.*, Bd. 30, pp. 568–70.

—— 1910. Biologische und morphologische Bemerkungen über Myxosporidien. *Zool. Anz.*, Bd. 35, pp. 57–63.

BROWN, ELEANOR M., 1934. On *Oodinium ocellatum* Brown, a parasitic dinoflagellate causing epidemic diseases in marine fish. *Proc. zool. Soc. Lond.*, pp. 583–607.

BRUMPT, E. & LEBAILLY, C., 1904. Description de quelques nouvelles espèces de Trypanosomes et d'Hémogrégarines parasites des Téléostéens marins. *C.R. Acad. Sci., Paris*, T. 139, pp. 613–15.

BYCHOWSKAJA, I., 1936. The influence of the dimensions of the waterbasins on the parasitic fauna of fish. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 163–6. (In Russian.)

CÉPÈDE, C., 1924. *Mrázekia piscicola* n.sp. microsporidie parasite du merlan *Gadus merlangus* Linné. *Bull. Soc. zool. Fr.*, T. 49, pp. 109–13.

DUBININ, V. B., 1936. Investigation of the parasitic fauna of *Thymallus vulgaris* in various periods of its life. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 31–48. (In Russian.)

DUNKERLEY, J. S., 1920. Fish Myxosporidia from Plymouth. *Parasitology*, Vol. 12, pp. 328–33.

GORBUNOVA, M., 1936. Variations of the parasitic fauna of the pike and the roach with their age. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 5–30. (In Russian.)

HENRY, H., 1910. On the haemoprotozoa of British sea-fish (a preliminary note). *J. Path. Bact.*, Vol. 14, pp. 463–5.

HENRY, H., 1913. A list of blood parasites of sea-fish taken at Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 9, pp. 570–1.

LABBÉ, A., 1899. Sporozoa. *Das Tierreich*, Bd. 5, 180 pp.

LEBAILLY, C., 1904. Sur quelques hémoflagellés des téléostéens marins. *C.R. Acad. Sci. Paris*, T. 139, pp. 576–7.

REICHENOW, E., 1929. Sporozoa, Ciliata und Suctoria. In Doflein, F. & Reichenow, E., *Lehrbuch der Protozoenkunde* (Aufl. 5), Teil 2.

—— 1932. Sporozoa. *Tierwelt N.- u. Ostsee*, Teil 2, g, pp. 1–88 (Lief. 21).

THÉLOHAN, P., 1891. Sur deux sporozoaires nouveaux, parasites des muscles des poissons. *C.R. Acad. Sci. Paris*, T. 112, pp. 168–71.

—— 1892. Sur quelques coccidies nouvelles parasites des poissons. *J. Anat., Paris*, T. 28, pp. 151–71.

—— 1894. Recherches sur les myxosporidies. *Bull. sci. Fr. Belg.*, T. 26, pp. 100–394.

WENRICH, D. H., 1935. Host-parasite relations between parasitic Protozoa and their hosts. *Proc. Amer. phil. Soc.*, Vol. 75, pp. 605–50.

J. mar. biol. Ass. U.K. (1957) 36, 157–160
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BIO-ASSAY OF NITROGEN AVAILABLE TO TWO SPECIES OF PHYTOPLANKTON IN AN OFF-SHORE WATER

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In a series of preliminary trials, off-shore water, collected during the summer of 1955 and containing only small concentrations of nitrogen and phosphorus available to plants, was filtered, pasteurized, enriched with phosphate, manganese and iron, inseminated with washed *Phaeodactylum tricornutum* and portioned into a series of flasks. To these were added varying quantities of nitrate, raising the concentration, in steps, up to 40 μg nitrate-N per litre. The flasks were illuminated, either in a north window or in front of a 'warm daylight' fluorescent strip, precautions being taken to prevent contamination with ammonia or oxides of nitrogen in the atmosphere. Growth of the algae was followed by withdrawing samples at intervals, after shaking, and measuring their optical density in a 10 cm cuvette.

After an initial lag period and the following period of exponential growth, during which the nitrogen source was used and consequently synthesis of chlorophyll ceased, the rate of increase in optical density slowed, and persisted at a regular slow rate for several days.

During this post-exponential period of growth the increase in optical density due to added nitrate was almost directly proportional to the quantity of nitrogen source which had been added.

The observations of optical density (light path 10 cm), shown in Table 1, were made in an experiment after 7, 9 and 11 days illumination. The initial O.D. (10 cm) of the inseminated water was 0·007, and the inseminum was nitrogen-deficient and had a long lag period before growth started.

TABLE 1. INCREASE IN OPTICAL DENSITY DURING POST-EXPONENTIAL PERIOD

Flask no.	O.D. (10 cm) after ...	7 days	9 days	11 days
1	With no added nitrate	0·035	0·033	0·050
2		0·041	0·046	0·050
3	+20 μg/l. NO_3N	0·063	0·070	0·078
4		0·066	0·071	0·080
5	+40 μg/l. NO_3N	0·084	0·101	0·106
6		0·085	0·102	0·114
7	—	0·090	0·095	0·112
Increase due to addition:				
of 20 μg N/l.		0·026	0·030	0·029
of 40 μg N/l.		0·049	0·059	0·060

In order to assay the available nitrogen present in a sample of water the following reasoning was employed. Provided the washed plant cells added as inseminum were wholly deficient in nitrogen, and consequently incapable of further growth unless supplied with a nitrogen source, then, at any particular time T during post-exponential growth, the increase in optical density since the start of the experiment depends upon the concentration C of available nitrogen in the water, and equals Cy μg/N/l. if y is the increase in optical density due to the addition of 1 μg nitrate-N/l.

If, however, the plant cells added as inseminum were growing actively and capable of further carbon synthesis without a source of nitrogen, then the increase in optical density equals $Cy+x$. The value of x is directly proportional to the quantity of inseminum added. Therefore, x is equal to the difference in the increases in optical density which have taken place in samples to which one unit and two unit quantities of inseminum have been added.

Hence from observations of these two increases and of the increase due to a known addition of available nitrogen, the value of C can be calculated. Experiments indicated that throughout several days in which post-exponential growth was taking place, the value of C remained the same within the limits of experimental error.

A bio-assay was made in water collected 20 miles off shore from Plymouth on 13 July 1955, from a depth of 15 m.

The water was filtered, pasteurized, and enriched with 500 μg/l. of phosphate-P, 10 μg/l. of manganese and 10 μg/l. of iron as ferrous dipyridyl.

Samples of this enriched water were analysed by Dr J. P. Riley, and was found to contain 10 μg total inorganic nitrogen per litre by the method described by Riley and Sinhaseni (1957).

Part of the water was inseminated with *Phaeodactylum tricornutum*, the initial optical densities measured, and the flasks were kept in a north window. The optical densities were again measured on the fourth and sixth days, and from the observed increases on the sixth day the concentration C was calculated (Table 2).

On the fourth day the nitrate added to flasks 5–8 had not exerted its full effect upon carbon synthesis.

The remainder of the water was inseminated with *Chlorella stigmatophora* and treated in the same way, providing the observations shown in Table 3.

On the sixth day some of the cells had started to disintegrate; the post-exponential growth was of short duration compared with that of *Phaeodactylum*.

Since the average concentration of available nitrogen found by assay is similar to the concentration of total inorganic nitrogen found by analysis, it appears that only an insignificant quantity of organic nitrogen was used by

these plants in the presence of their associated bacteria, although sea water contains a material quantity of organic nitrogen.

It is remarkable that so much organic nitrogen should remain in solution unattacked by bacteria in the sea—Krogh (1934) found some 240 μg organic-N/l. down to depths of 4750 m—whereas organic phosphorus is not found at depths below 1000 m.

TABLE 2. BIO-ASSAY OF CHANNEL WATER WITH *PHAEODACTYLUM*

Flask no.		Optical density, 10 cm light path: Initial	On 4th day	On 6th day
1	Unit inseminum	0·013	0·035	0·036
2		0·012	0·036	0·035
3		0·013	0·036	0·036
4		0·013	—	0·034
5	Unit inseminum + 40 μg NO_3N/l.	0·013	0·072	0·081
6		0·014	0·079	0·085
7		0·014	0·078	0·088
8		0·017	—	0·088
9	Double inseminum	0·022	0·052	0·053
10		0·023	0·052	0·054
11		0·027	0·055	0·057
12		0·026	—	0·057

Calculated concentration of available nitrogen, $C = 11{\cdot}5$ μg N/l.

TABLE 3. BIO-ASSAY OF CHANNEL WATER WITH *CHLORELLA*

Flask no.		Optical density, 10 cm light path: Initial	On 4th day
1	Single inseminum	0·011	0·066
2		—	0·061
3		—	0·066
4		—	0·063
5	Single inseminum + 40 μg NO_3N/l.	—	0·127
6		—	0·129
7		—	0·132
8		—	0·142
9	Double inseminum	0·025	0·107
10		—	0·127
11		—	0·100
12		—	0·107

Calculated concentration, C, of available nitrogen 13 μg/l.

SUMMARY

A bio-assay of the nitrogen which was available to two phytoplankton species with their associated bacteria in an off-shore water is described.

The concentrations found by assay (11·5 and 13 μg N/l.) were similar to the concentration of total inorganic nitrogen compounds found by analysis (10 μg N/l.).

Of the organic nitrogen normally present in solution in sea water, an insignificant quantity appears to be available to the plant-bacteria community.

REFERENCES

KROGH, A., 1934. Conditions of life at great depths in the ocean. *Ecol. Monogr.*, Vol. 4, pp. 430–39.

RILEY, J. P. and SINHASENI, P., 1957. The determination of ammonia and total ionic inorganic nitrogen in sea water. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 161–68.

J. mar. biol. Ass. U.K. (1957) 36, 161–168
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THE DETERMINATION OF AMMONIA AND TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

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(Text-figs. 1 and 2)

Combined inorganic nitrogen occurs in sea water principally as nitrate, nitrite and ammonium ions, the concentrations of which lie in the ranges 1–600 mg NO_3^-–N/m^3, 0·1–50 mg NO_2^-–N/m^3 and 5–50 mg NH_4^+–N/m^3 respectively. Nitrogen occurring in any of these forms is readily assimilable by marine organisms, and its exhaustion in sea water is frequently a growth-limiting factor in the water. This paper describes a method for the determination of the total ionic inorganic nitrogen in sea water, based on preliminary reduction to ammonia followed by separation and estimation of the latter colorimetrically.

Riley (1953) has discussed the determination of ammonia in sea water, and has concluded that ammonia is best separated from the water, adjusted to pH 9·2, by distillation under reduced pressure in a current of air as described by Krogh (1934). The distillation process is rather time-consuming and requires a special apparatus. Experiments were therefore carried out to separate the ammonia by diffusion. It was found that the capacity of the concentric microdiffusion cells employed by Conway (1950, p. 8) was too small for the volume of sea water which was necessary if low concentrations of ammonia were to be determined. When diffusion was carried out in the flasks (Fig. 1) described by Cavett (1937) for the microdetermination of alcohol in blood, reproducible recoveries of approximately 73 % of added ammonium salt were obtained from 50 ml. of sea water after diffusion at 70° C for 24 h at pH 9·2 (Table 1).

Much of the earliest work on the determination of nitrate in sea water was based on its reduction to ammonia, which was separated by distillation and determined by Nessler's method. Few of the reducing agents (magnesium, Raben, 1905*a*, sodium amalgam, Raben, 1905*b*; aluminium amalgam, Raben, 1910, 1914; Devarda's alloy, Brandt, 1927; iron-zinc couple, Thorpe & Morton, 1871; aluminium and sulphuric acid, Gad-Andresen, 1923) which have been used for this purpose are satisfactory if the ammonia is to be separated by diffusion, owing to the evolution of hydrogen which causes pressure to develop in the Cavett flask. Attempts to carry out the reduction using ferrous hydroxide and titanous salts gave very poor recoveries of ammonia.

Raney nickel (semi-colloidal nickel prepared by the action of strong alkali on nickel-aluminium alloy) has been used by Van Dalen (1951) as a catalyst in the hydrogenation of macro amounts of nitrate to ammonia. Since the catalytic nickel contains up to 120 ml. H/g (Mozingo, Wolf, Harris & Folkes, 1943) it was thought that microgram quantities of nitrate could be reduced to

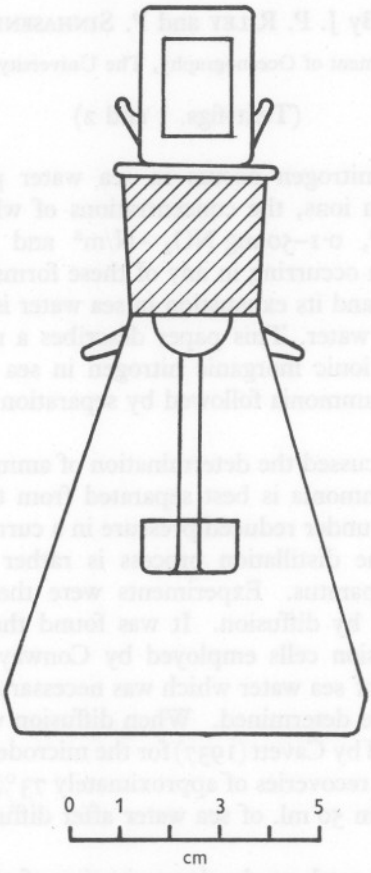

Fig. 1. A Cavett flask as used for the microdiffusion of ammonia.

TABLE 1. RECOVERY OF AMMONIA FROM 50 ML. OF SEA WATER CONTAINING ADDED AMMONIUM SULPHATE AND 10 ML. OF METABORATE BUFFER DIFFUSED AT 70° C FOR 20 H.

μg NH_4^+-N		Mean recovery (%)
Added	Found	
0·0	0·0	—
2·5	1·7 1·7 1·8	69
5·0	3·5 3·6 3·6 3·7	72
10·0	7·2 7·5	73

ammonia by the action of its adsorbed hydrogen. This proved to be the case. It was found that the reduction proceeded much more rapidly in the presence of ethylenediamine-tetraacetic acid (EDTA) which also served to reduce the quantity of magnesium and calcium salts precipitated from the sea water at the pH of 10·5 used in the reduction and diffusion.

COLORIMETRIC DETERMINATION OF AMMONIA

The advantages of the phenate-hypochlorite method over the Nessler method for the colorimetric determination of ammonia have been enumerated by Riley (1953). Crowther & Large (1956) have found that the indophenol-blue colour develops rapidly at room temperature if the sodium phenate reagent is made up in an aqueous mixture of acetone and methyl alcohol instead of water.

TABLE 2. EFFECT OF VARIATION OF VOLUMES OF SODIUM PHENATE AND SODIUM HYPOCHLORITE REAGENTS ON OPTICAL DENSITIES OF SOLUTIONS MEASURED AT 625 Mμ IN A 1 CM CELL (10 μG NH_4^+-N IN FINAL VOLUME OF 10 ML.)

ml. of hypochlorite*	0·5	1·0	1·5	2·0	2·5	3·0
0·5 ml. of phenate†	0·387	0·340	0·280	0·234	0·185	0·165
1·0 ml. of phenate†	0·372	0·380	0·373	0·370	0·350	0·328
1·5 ml. of phenate†	0·273	0·320	0·337	0·370	0·365	0·352
2·0 ml. of phenate†	0·163	0·280	0·310	0·340	0·355	—

* 0·9 % available chlorine.

† In methanol containing acetone.

In order to find the optimum conditions for the determination of ammonia using Crowther's reagent, determinations were carried out using 10 μg NH_4^+-N and varying the amounts of both sodium phenate (prepared according to Crowther & Large, 1956) and sodium hypochlorite added. The optical densities of the solutions were measured at 625 mμ after 1 h. The results, which are given in Table 2, indicated that 1 ml. of sodium phenate and 1·5 ml. of sodium hypochlorite were the most suitable volumes. Under these conditions, which were adopted for all subsequent work, the maximum optical density was attained after 45 min at 20° C, and remained constant for a further 60 min, after which time slow fading occurred.

A calibration curve was prepared using known amounts of ammonium salt (Fig. 2); it indicated that Beer's law is obeyed up to a concentration of 1·5 μg NH_4^+–N/ml. (in a final volume of 10 ml.). Above this concentration the change in optical density per unit weight of NH_4^+–N is considerably reduced. Replicate determinations (6) carried out with 5 and 10 μg NH_4^+–N gave mean optical densities (less blank) of 0·190 ± 0·001 and 0·377 ± 0·002.

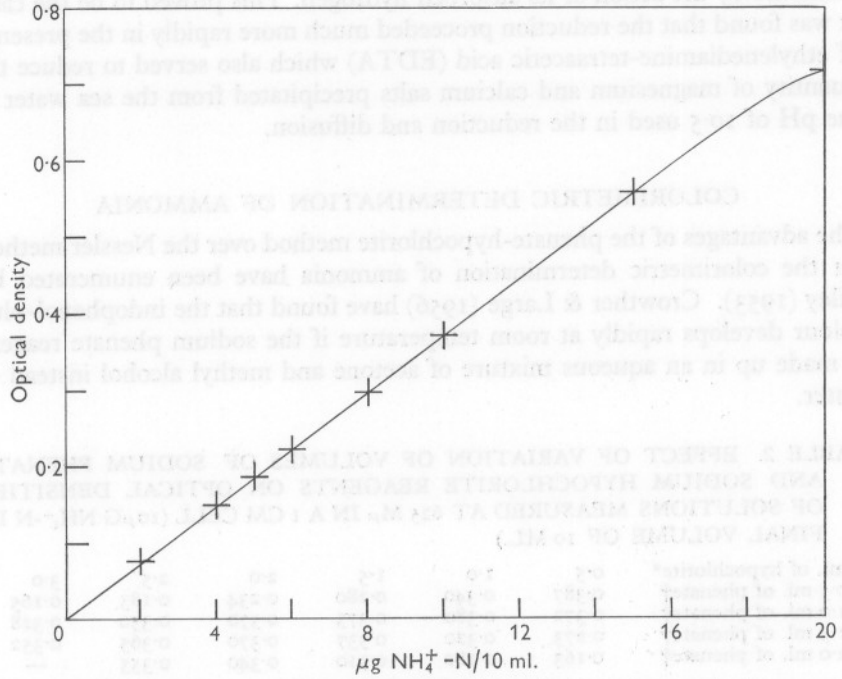

Fig. 2. Calibration chart of the method for the determination of ammonia in sea water, prepared by using known amounts of ammonia. Optical density at 625 mμ using a 1 cm cell.

METHOD

All measurements of optical density were made with a Unicam S.P. 500 spectrophotometer using 1 cm glass cells, against distilled water in the compensating cell.

REAGENTS

All reagents and solutions must be made up with water which has been freshly distilled from dilute sulphuric acid in an all-glass still.

1. *Metaborate buffer*

Dissolve 2·55 g of sodium metaborate and 2·0 g of sodium hydroxide in distilled water, dilute to 100 ml.

2. *Raney nickel*

Weigh out *c.* 5 g of powdered nickel-aluminium alloy, (50/50) and add it gradually to a 250 ml. beaker containing a solution of 10 g of sodium hydroxide in 70 ml. of distilled water. After the addition has been completed, heat the covered beaker on a hot plate for 30 min, when all effervescence should have

ceased. Wash the finely divided nickel with hot water by decantation, until the washings are free from alkali. Preserve it under water and do not allow it to become dry. Fresh Raney nickel should be prepared at least weekly.

Caution: care should be taken when disposing of waste Raney nickel as it is pyrophoric when dry.

3. *EDTA reagent*

Dissolve 4 g of ethylenediamine-tetraacetic acid (disodium salt) in 100 ml. of distilled water.

4. *Sodium hydroxide* (2N)

Gently boil a solution of 8 g of sodium hydroxide in 60 ml. of water for a few minutes. When cool, dilute to 100 ml.

5. *Hydrochloric acid* (0·02N)

Dilute 2 ml. of concentrated hydrochloric acid to 1000 ml.

6. *Sodium phenate reagent*

(*a*) Phenol solution (solution A). Dissolve 62·5 g of phenol B.P. in methyl alcohol, add 20 ml. of acetone and dilute to 100 ml. with methyl alcohol. The reagent, which should be prepared each week, must be kept in a refrigerator.

(*b*) Sodium hydroxide (solution B). Dissolve 27 g of sodium hydroxide A.R. in *c.* 60 ml. of distilled water, boil gently for a few minutes to remove ammonia, cool and dilute to 100 ml.

For use mix 20 ml. portions of solutions A and B and dilute to 100 ml. The mixed reagent should be prepared freshly as required.

7. *Sodium hypochlorite reagent*

The reagent solution is prepared by dilution of commercial sodium hypochlorite solution and contains 0·9 g of available chlorine per 100 ml. The strength of the stock solution should be checked periodically.

STANDARD SOLUTIONS

1. *Ammonium sulphate*

Weigh out 0·4716 g of ammonium sulphate A.R., dissolve in water and dilute to 1000 ml. This solution, which contains 100 μg NH_4^+-N/ml. is used for the preparation of the working solution containing 2·5 μg. NH_4^+-N/ml.

2. *Potassium nitrate*

Prepare a solution of potassium nitrate containing 10 μg NO_3^--N/ml. by dissolving 0·0722 g of the A.R. grade salt in water and diluting to 1000 ml. Prepare from this a working solution containing 2·5 μg NO_3^--N/ml.

3. *Sodium nitrite*

Dissolve 0·1099 g of recrystallized silver nitrite in about 10 ml. of boiling water, and add while still hot *c.* 0·10 g of sodium chloride. Shake until the silver chloride flocculates, set aside in the dark until the solution has cleared. Dilute to 1000 ml. From this solution, which contains 10 μg NO_2^--N/ml., prepare a working solution containing 2·5 μg. NO_2^--N/ml.

TREATMENT OF FLASKS

Wash all Cavett flasks and graduated flasks with concentrated hydrochloric acid. Rinse with distilled water, then with 0·1 N sodium hydroxide and again with water, allow them to drain. Lubricate the upper part of the ground surface of the Cavett flask stoppers with a mixture of equal weights of paraffin wax (congealing point 49° C) and medicinal paraffin.

DETERMINATION OF AMMONIA IN SEA WATER

Pipette 50 ml. of filtered sea water into a 150 ml. conical flask fitted with a B24 socket, add 2 ml. of 4% EDTA reagent and raise to boiling on the hot plate. Remove from the heater and add *c.* 2 ml. of sodium metaborate buffer from a fast running pipette. Immediately close the flask with a lubricated Cavett stopper (Quickfit and Quartz Catalogue No. BC3CH), the cup of which contains 1 ml. of 0·02 N hydrochloric acid. Gently shake the stoppered flask and then heat in an oven at 70° C. After 24 h remove the flask from the oven and allow to cool for a few minutes. Transfer the hydrochloric acid in the cup to a 10 ml. graduated flask by means of a drawn-out glass tube fitted with a rubber teat. Wash the cup well with distilled water and transfer the washings to the graduated flask. To the combined solutions add 1 ml. of sodium phenate reagent and 1·5 ml. of sodium hypochlorite solution, dilute to volume and mix thoroughly. After 45–60 min measure the optical density at 625 mμ. Run a reagent blank on 10 ml. of distilled water in the same manner. Calibrate the method by carrying out a determination on 50 ml. of the same sea water to which 5 μg NH_4^+-N has been added.

DETERMINATION OF TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

Pipette 50 ml. of sea water into a 150 ml. conical flask fitted with a B24 socket, add from a small scoop *c.* 0·2 g of Raney nickel and 1 ml. of 4% EDTA reagent. Heat the solution on a hot plate and allow to boil gently for 15 min. Remove from the heater and add rapidly 2 ml. of 2 N sodium hydroxide. Immediately close the flask with a well-lubricated Cavett stopper, the cup of which contains 1 ml. of 0·02 N hydrochloric acid. Place in an oven at 70° C to allow the ammonia to diffuse. After 24 h determine the ammonia as described above.

Carry out a blank determination on 10 ml. of distilled water and a calibration run on the same sea water, to which has been added 5 μg NO_3^- or NO_2^--N.

Great care must be taken to avoid contamination by extraneous nitrogen compounds during the determination; in particular, the apparatus should not be handled more than is absolutely necessary.

It is important also to take approximately the same amount of Raney nickel in each determination and in the blank, since it always yields small amounts of ammonia during the diffusion.

RESULTS

In order to test the recovery of nitrogen from sea water, samples of sea water very low in inorganic nitrogen were enriched by the addition of known amounts of nitrate, nitrite, or ammonium-nitrogen. These samples were then analyzed for total nitrogen as described on p. 166. The results are shown in Table 3. They show that the reduction is complete since a recovery of 73 ± 4% of nitrate, nitrite and ammonium-nitrogen can be obtained using a diffusion time of 24 h. Higher recoveries can be obtained with longer diffusion periods, but the results are less reproducible. Using the recommended conditions, replicate determinations (7) were carried out on a sample of Irish Sea water; they showed that the water contained 240 μg N/l. with a coefficient of variation of 2·5%.

TABLE 3. RECOVERY OF INORGANIC NITROGEN FROM SEA WATER

Nitrogen added	Nitrogen recovered (μg N)								Average recovery	Percentage recovery
Sea water only				0					0	—
2·5 μg NH_4^+-N	1·9	1·9	1·7	1·8	1·7	2·0	2·0		1·86	74
5·0 μg NH_4^+-N	3·5	3·4	3·3	3·6	3·5	3·6			3·48	70
10·0 μg NH_4^+-N	7·6	7·6	7·5	7·5	7·3	7·7			7·53	75
2·5 μg NO_3^--N	2·0	1·7	1·8	2·1	2·1	1·9			1·92	77
5·0 μg NO_3^--N	3·5	3·7	3·7	3·6	3·7				3·64	73
10·0 μg NO_3^--N	7·1	7·3	7·5	7·7	7·6	7·7	7·2	7·3	7·42	74
2·5 μg NO_2^--N	1·5	1·7	1·8						1·69	68
5·0 μg NO_2^--N	3·8	3·9	3·3	3·7	3·4	3·4	3·9		3·62	72
10·0 μg NO_2^--N	7·5	7·3	7·6	7·9					7·58	76
									Mean	73%

In order to investigate the possible interference which might be caused by the breakdown of organic nitrogen compounds during the diffusion, the determination was carried out in the presence of a number of typical organic nitrogen compounds. No interference was experienced using 20 μg of DL-α-alanine, L-arginine hydrochloride, cystine, glutamic acid, urea, or choline chloride.

SUMMARY

Microdiffusion using a Cavett flask has been employed instead of vacuum distillation for the separation of ammonia from sea water; diffusion is approximately 75% complete after 24 h at 70° C. The recovered ammonia is determined by a modification of the phenate-hypochlorite method.

Raney nickel in the presence of ethylenediamine-tetraacetic acid has been used for the reduction of nitrate and nitrite to ammonia. The latter is separated by microdiffusion and determined colorimetrically. The method showed a coefficient of variation of 2·5% on a sample of sea water containing

$$240\,\mu g\ NO_2^- + NO_3^- + NH_4^+ - N/l.$$

No interference was encountered from a variety of organic nitrogen compounds.

REFERENCES

BRANDT, K., 1927. Stickstoffverbindungen im Meere I. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 20, p. 212.

CAVETT, J. W., 1937. The determination of alcohol in blood and other body fluids. *J. Lab. clin. Med.*, Vol. 23, pp. 543–6.

CONWAY, E. J., 1950. *Microdiffusion Analysis and Volumetric Error*, 3rd ed. 391 pp. London: Crosby Lockwood.

CROWTHER, A. B. & LARGE, R. S., 1956. Improved conditions for the sodium-phenoxide-sodium hypochlorite method for the determination of ammonia. *Analyst*, Vol. 81, p. 64.

GAD-ANDRESEN, K. L., 1923. A method for quantitative determination of ammonia, nitrate and nitrite, together with other nitrogenous compounds in sea-water. *Publ. Circ. Cons. Explor. Mer*, No. 82, pp. 14–17.

KROGH, A., 1934. A method for the determination of ammonia in water and air. *Biol. Bull., Woods Hole*, Vol. 57, pp. 126–31.

MOZINGO, R., WOLF, D. E., HARRIS, S. A. & FOLKES, K., 1943. Hydrogenolysis of sulphur compounds by Raney nickel catalyst. *J. Amer. chem. Soc.*, Vol. 65, p. 1013.

RABEN, E., 1905*a*. Weitere Mitteilungen über quantitative Bestimmungen von Stickstoffverbindungen und gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 8, pp. 279–80.

RABEN, E., 1905*b*. Über quantitative Bestimmung von Stickstoffverbindungen im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 8, pp. 81–98.

RABEN, E., 1910. Dritte Mitteilung über quantitative Bestimmungen von Stickstoffverbindungen und von gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 11, pp. 305–19.

RABEN, E., 1914. Vierte Mitteilung über quantitative Bestimmungen von Stickstoffverbindungen im Meerwasser und Boden sowie von gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 16, pp. 207–29.

RILEY, J. P., 1953. The spectrophotometric determination of ammonia in natural waters, with particular reference to sea-water. *Anal. chim. acta*, Vol. 9, pp. 575–89.

THORPE, T. E. & MORTON, E. H., 1871. Über das Wasser des Irländischen Meeres. *Liebig's Ann. Chem.*, Bd. 158, dp. 122–31.

VAN DALEN, E., 1951. Schnelle Bestimmung von Nitrat in lösung mittels katalytischer Reduktion mitt Wasserstoff. *Anal. chim. acta*, Vol. 5, pp. 463–71.

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THE TOXIN FROM *GYMNODINIUM VENEFICUM* BALLANTINE

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(Text-figs. 1–8)

One of the authors (Ballantine, 1956) has recently described a new species of toxic dinoflagellate (*Gymnodinium veneficum*). Interest in the toxicity was stimulated by Bainbridge's observations (1953) on this species and by the well-known problem of mass mortality associated with 'red tide' outbreaks of poisonous flagellates. Much has been published on this latter subject, for which extensive bibliographies are available in the papers of Brongersma-Sanders (1948) and Hayes & Austen (1951).

Dinoflagellates in particular have long been associated with marine fish mortalities (see Davis, 1948, on *Gymnodinium brevis*, and the review by Nightingale, 1936) and with the problem of paralytic shellfish poisoning (Sommer, Whedon, Kofoid & Stohler, 1937, *Goniaulax catenella*; Koch, 1939, *Pyrodinium phoneus*; and Needler, 1949, *Goniaulax tamarensis*).

The published work on the mode of action of dinoflagellate toxins has been mainly concerned with the practical aspects linked with the cause, effects and prevention of paralytic shellfish poison, and has been directed towards mammalian and amphibian tissues (e.g. Kellaway, 1935; Fingerman, Forester & Stover, 1953), with attempts to ascertain the chemical nature of the poisons and with bio-assay. As there is no 'red tide' problem here, we are not concerned with the essential practical preventative approach and have attempted, over a wide field, to determine the actual site and mode of action of the toxin in marine animals, in addition to trying to extract it and find the effect on whole animals.

All our experiments have made use of a uni-algal culture of *Gymnodinium veneficum* grown in Erdschreiber culture solution (Plymouth formula).[1] These cultures are not bacteria-free, but repeated attempts to grow the bacteria from these cultures in a variety of marine media have always resulted in non-toxic cultures. In view of this we feel confident that the toxin is either a product of the *Gymnodinium* itself or possibly a product of the action of bacteria on a waste substance produced by the *Gymnodinium*. This latter possibility does not seem probable, as the substance is an exotoxin secreted

[1] Erdschreiber. Filtered sea water 1 l., pasteurized. Soil extract (*w/v*) 50 ml., $NaNO_3$ 0·3 g, $Na_2HPO_4 . 12H_2O$ 0·02 g.

into the water, and whereas about 90% of the total toxin in any culture is present in the supernatant fluid, on a volume to volume basis the cells contain about 200–500 times as much toxin as the water. In addition, when the bacterial population of a culture becomes high the toxicity decreases.

In this research we had assistance and advice from many people. We cannot express our thanks to all personally, but in particular we mention the folowing: Dr Mary Parke for providing large volumes of *Gymnodinium* culture; Dr G. Y. Kennedy and his assistants of Sheffield University who have carried out all experiments using mice; Dr N. R. Stephenson of the Department of National Health and Welfare of Canada for a supply of paralytic shellfish poison; Dr J. P. Quilliam of St Bartholomew's Hospital, London, for carrying out experiments with isolated ganglia; Dr D. B. Carlisle of this laboratory for assistance with experiments on isolated hearts and much helpful discussion; Dr E. D. S. Corner, International Paints Research Fellow at Plymouth, for experiments on respiration rates, for a great deal of help and advice on extraction techniques and for continued encouragement; and to the Director and staff of the Plymouth Laboratory, especially workshop staffs, for their unfailing interest in this work, and the ready production of apparatus when required.

EXTRACTION

In studies on the effect of the *Gymnodinium* toxin on whole marine animals, whole cultures of the flagellate or supernatant water after the removal of the cells were used. The specific action of the toxin, however, could be investigated only on isolated tissues from animals and the use of sea water as a bathing medium severely limits the range of material. Extraction of the toxin in a more concentrated form free from salt therefore became necessary.

Most of the work on paralytic shellfish poison has been based on extraction from the digestive glands of poisonous but living shellfish. The procedure generally adopted in this extraction has been to mince the digestive glands and extract the toxin with acidified alcohol (4 ml. conc. HCl/l. alcohol), centrifuge, evaporate to dryness and wash with chloroform (Müller, 1935). The method has since been extended by other workers and purer extracts have been produced (Sommer & Meyer, 1937; Sommer, Monnier, *et al.* 1948; Sommer, Riegel, *et al.* 1948; and Riegel, *et al.* 1949*a*).

In view of the concentration of toxins from *Goniaulax* spp. by shellfish we fed *Mytilus edulis* with cultures of *Gymnodinium veneficum* in an attempt to obtain a similar effect. Strong cultures were lethal within a day and more dilute cultures eventually killed the *Mytilus* without any significant concentration of the toxin by the animal.

It was therefore necessary for us to extract the toxin from cultures. The methods of Sommer *et al.* referred to above have been extended to extraction

from the cells of *Goniaulax catenella* (Sommer, Whedon, *et al.* 1937; Riegel *et al.* 1949*b*). We followed this method using centrifuged cells of *Gymnodinium veneficum* and dissolved the solid residue in the appropriate bathing media for experiments on isolated tissues. Extracts prepared by this means were also dissolved in sea water, and it was found that immersion of fish in this solution did not give the same symptoms as the original culture (see p. 176). The cells were therefore extracted with neutral alcohol and under these conditions the characteristics of the original culture were completely retained. In all our work we find that 'acid extraction' alters the toxin and we have employed 'neutral extraction' in all cases except when stated. Hashimoto & Migita (1952) have noted a similar effect.

Extraction from cells only is very wasteful as most of the poison remains in the supernatant fluid, and large volumes of culture were needed in order to obtain significant quantities of cells. The supernatant fluid contains about 90% of the toxin in most cultures, but the problem is to separate it from the salt. Adsorption on carbon columns was attempted. Owing, however, to the large amount of salt present it was necessary to use a very active charcoal to adsorb the toxin in reasonable amounts. Elution from such carbon was very difficult; hot ethyl alcohol containing 5% pyridine or hot dioxan were the only successful eluants. These were removed by evaporation under reduced pressure. Owing to the variability between batches of carbon and to the difficulty of removing final traces of solvents dialysis was tried. Cell-free culture fluid was dialysed in tubes and the salt had gone in 6 h, leaving behind the toxin which was then evaporated to dryness under reduced pressure. Very little toxin is lost through the membrane indicating that the toxin molecule is large, probably of mol.wt. > 1000. By this process an active extract was obtained, although the product was contaminated by impurities from the soil extract used in growing the cultures. Work on the purification of these extracts will be attempted, but it is obvious even at present that this is a very potent poison.

EXPERIMENTS WITH WHOLE ANIMALS

Throughout this series of experiments marine animals were taken from the aquarium sea water and placed either in whole cultures of *Gymnodinium veneficum* or in the supernatant culture fluid from which the cells had been removed. Experiments with other non-toxic flagellates have shown that this treatment produces no ill-effects.

Various animals have been used (see Table 1) and they appear to fall into three main groups, as follows:

(A) *Not affected.* Animals of only one group, the Polychaeta, have been found to be completely unaffected by the toxin. Specimens of *Nereis diversicolor* and *Arenicola marina* survive in cultures apparently indefinitely. With

TABLE 1

Animal	Mode of treatment	Reaction group (see p. 171)	Time to death
COELENTERATA			
Calliactis parasitica	Imm., Imm. + inj.	B	2–3 days
Anemonia sulcata	Imm., Imm. + inj.	B	3–4 days
Ephyrae of *Aurelia aurita*	Imm.	C	< 1 min
ANNELIDA: POLYCHAETA			
Nereis diversicolor	Imm.	A	—
	Imm. + inj.	A	—
Nereis diversicolor	Imm. + inc.	A	—
Arenicola marina	Imm.	A	—
ARTHROPODA: CRUSTACEA			
Calanus finmarchicus (see Marshall & Orr, 1955)	Imm.	B	1–2 days
Tigriopus fulvus	Imm.	B	1–3 days
Hemimysis lamornae (see Bainbridge, 1953)	Imm.	B	2–3 days
Macromysis flexuosus	Imm.	B	3–4 days
Palaemon serratus	Imm.	B	>4 days
Eupagurus bernhardus	Imm.	B	2–3 days
Carcinus maenas	Imm.	B	>4 days
Cancer pagurus	Imm.	B	3 days
MOLLUSCA			
Mytilus edulis	Imm.	B	<3 days
M. galloprovincialis	Imm.	B	<3 days
Pecten maximus	Imm.	C	1 h
Lasaea rubra	Imm.	B	>1 day
Buccinum undatum	Imm.	C	1 h
Aplysia punctata	Imm.	C	1 h
Eusepia officinalis (cuttle fish)	Imm.	C	3 h
ECHINODERMATA			
Asterias rubens	Imm.	B	3–5 days
Ophiothrix fragilis	Imm.	B	<3 days
Ophiocomina nigra	Imm.	B	<3 days
TUNICATA			
Ciona intestinalis	Imm.	B	3 days
CEPHALOCHORDATA			
Amphioxus lanceolatus	Imm.	C	45 min
PISCES			
Scyllium canicula (2 ft.) (dogfish)	Imm.	C	3 h
Gadus pollachius (3 in.) (pollack)	Imm.	C	18 min
Blennius gattorugine (blenny)	Imm.	C	<45 min
Trachinus vipera (lesser weaver)	Imm.	C	30 min
Gobius niger (goby)	Imm.	C	5–15 min
G. virescens (goby)	Imm.	C	5–10 min
Pleuronectes platessa (5 in.) (plaice)	Imm.	C	30 min
Ctenolabrus rupestris (wrasse)	Imm.	C	15–20 min
AMPHIBIA			
Rana temporaria (frog)	Inj.	C	Strong rapid paralysis kills in *c.* 30 h
MAMMALIA			
Mus musculus (mouse)	Inj.	C	2–4 min

Controls using sea water and non-toxic cultures were made in all experiments, and the control animals survived much longer and were still healthy when the test animals had all died.

Imm., immersion in culture; inj., injection of extract; inc., incision.

Nereis some animals were injected and others cut down the ventral surface. They were then placed in cultures but showed no ill effects.

(B) *Slowly affected.* In this category are various animals including members of the Coelenterata, Crustacea, Mollusca, Echinodermata and Tunicata. It is felt that with all these, except the Mollusca, which can close their shells protectively, the reason for the slow effect of the toxin is due to the fact that the large toxin molecule cannot penetrate the animal; for Dr Sheina M. Marshall (personal communication) reports that *Calanus* which ate a large number of *Gymnodinium* from strong actively growing cultures died within 18 h, whereas poor feeders survived some days. We have found that this applies also to the other crustaceans, except *Eupagurus* where the increased sensitivity may be associated with the soft body.

Ophiothrix and *Ophiocomina* both fragment when immersed in *Gymnodinium* cultures, and the arms are finally broken up into short segments leaving the body which then dies.

In the molluscs of group B, i.e. *Lasaea* (see Ballantine & Morton, 1956) and *Mytilus*, the mechanism for prevention of entry of the toxin is simply shell closure. Both are intertidal bivalves capable of withstanding long exposure to unfavourable conditions and they close up when immersed in this culture. Eventually, however, sufficient poison enters to kill them. In all animals of this group, isolated excitable tissues are quickly blocked by the toxin (see p. 179).

(C) *Rapidly killed.* This last group is by far the most interesting and with it most of the work on whole animals has been carried out. Into this section fall molluscs without an adequate shell closure mechanism, *Amphioxus*, all the fish, frogs and mice—the last two after injection. In the marine animals the primary site of entry of the toxin is probably through the gills and not orally, as the supernatant culture fluid from which all cells have been removed is as quickly lethal as the whole culture, even with continuous aeration, and evidence from feeding experiments with mice suggests that such rapid and lethal penetration of the toxin cannot take place through the gut.

Gobius virescens was used as the test animal for comparison of the toxicity of cultures and extracts because of its availability and sensitivity to the toxin. A strong culture kills the fish in 5–6 min, the death time being prolonged as the toxin strength is reduced, and a bio-assay method has been worked out on this basis (Fig. 1). The symptoms shown by all fish when immersed in cultures, supernatant fluid from cultures, cells resuspended in sea water, and all toxic extracts produced at pH 7–8·5, were identical. The immediate reaction of the fish was a violent attempt to swim away from the unpleasant medium. In the goby this violent swimming, either forwards or backwards, continued for about 2 min and then subsided. Intense vasodilation and a strong expansion of the chromatophores to produce a marked colour pattern occur at this stage. At the same time the balance control mechanism is upset, and the fish

floats on its side or even upside down. Breathing slows considerably from a normal rate of 75–90/min to about 45/min after 2 min. Soon after immersion breathing is interrupted by a spasmodic violent 'vomiting reaction', which continues until death. Breathing continues to slow down, turning to irregular gasping at long intervals (up to 20 sec) and there is a lack of sensory response.

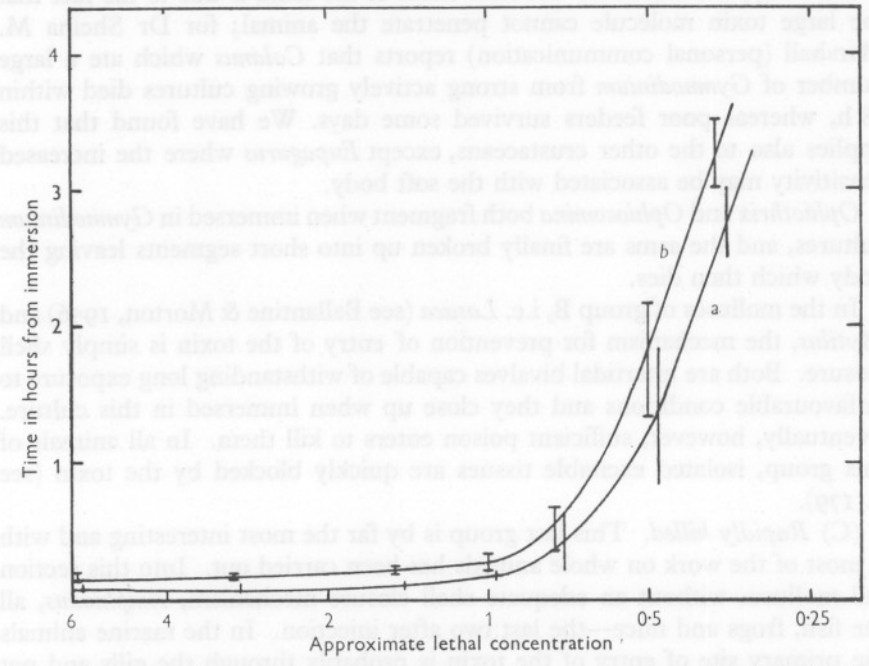

Fig. 1. Assay curve for toxin strength using gobies. Curve *a*, time to complete loss of balance in fish. Curve *b*, time to death. Extremes shown by vertical lines. Concentration in arbitrary units defined in text.

Reaction to touch or prodding disappears progressively from the head towards the tail, the base of the tail retaining sensitivity longest. Death occurs in quiescent paralysis, with the gills extended, apparently due to some sort of respiratory failure, but is preceded by a few irregular bursts of violent activity. If removed to clean sea water before the balance is upset the fish will recover; but there is no survival once the balance has gone.

The bio-assay technique we have developed using gobies is simple but only approximate. Fig. 1 shows the graphs obtained by plotting time to complete loss of balance (*a*) and time to death (*b*) against dose in arbitrary units. By using these two criteria it is possible to find an approximate lethal concentration per ml. of the sea water in which fish are immersed by using two or three different dilutions. This 'approximate lethal concentration' may be defined as the amount of toxin per ml. which causes permanent loss of balance

in 8–15 min and death in 10–20 min. A point which must be made about this assay is the fact that these times are constant with the gobies used (*G. virescens* and *G. niger*, where the loss of balance is interpreted as that stage when the fish can no longer right itself when inverted) regardless of the size of the goby. Fish from 17 mg to 7 g have been tested and body size has no effect on death time when a given dose of toxin is used. This is probably due to the fact that the toxin penetrates through the gills and the active gill surface area is roughly proportional to the size of the fish within a species. The average lethal concentration per ml. of a strongly toxic culture is about 6 units.

Because of its large size further experiments were carried out on the dogfish, and general symptoms were the same. Just before death (at the completely quiescent stage) the animal was removed and cut open. The heart was beating strongly, and there was no sign of haemolysis in blood samples taken. Electrical stimulation of the body muscles showed that these still responded to a direct stimulus. Although the muscles can themselves be poisoned by the toxin (p. 182), death is not due to failure of the muscles, and must result from a blockage in the nervous system.

Invertebrates of group C die with very few symptoms. Ephyrae of *Aurelia* pulsated about 5 times and then sank to the bottom fully contracted. Quick removal into clean sea water gave an eventual recovery in most cases. Molluscs died with their muscles relaxed, the bivalves with shells gaping.

During these experiments we have noted that, when agitated, the cultures produce an irritant vapour or mist (probably a suspension of droplets in air) which gives discomfort to throat and nose, of the type associated with an incipient cold. This phenomenon has also been observed as being associated with red tides in America (Woodcock, 1948; Ingle, 1954). One member of the staff of this laboratory, who is extremely susceptible to hay fever and allergic to many types of dust, is immediately and strongly affected by this mist. Since this type of reaction is associated with histamine release, several experiments were carried out on fish exposed to a variety of drugs. The histamine releaser '48–80' in sea water at strengths up to 100 μg/ml. has no effect at all on gobies. Histamine itself at 2·5 mg/ml. is not lethal but disturbs the animal and causes complete contraction of the chromatophores (greyish white) in contrast to the expansion in *Gymnodinium*. The addition of the antihistamine drug Phenergan at 2·5 μg/ml. to histamine-treated animals restored them to normal in a few minutes. On the other hand, Phenergan alone at 5 μg/ml. is lethal to gobies in 5 h. Phenergan gave no protection at all to gobies in *Gymnodinium* culture, and in fact hastened death. It therefore seems that there is no direct relation between the action of histamine and the toxin.

Evidence of protection against the toxin comes from two types of experiment: previous treatment in non-toxic flagellates, and addition of cholesterol. When gobies have been kept in cultures of non-toxic flagellates (e.g. *Isochrysis galbana* Parke) for 2 weeks, removed into clean sea water to clear the gills

of any possible clogging and then placed in *Gymnodinium* culture, time to death was prolonged from 15 min (control) to 2 hr. This protection was only slight. The influence of cholesterol was more dramatic, however. Cholesterol ground to a fine suspension in alcohol was added to sea water containing *Gymnodinium* culture. It was found that with a concentration of toxin of about 4 lethal units/ml (see Fig. 1), complete protection was provided if 20 mg. cholesterol was added per 100 ml sea water. Smaller doses prolonged the time to death. The actual amount of cholesterol which went into solution was uncertain.

Whereas extracts made at or near neutrality gave the same symptoms as the original culture, those made at pH 4, extracting from the centrifuged cells or from carbon columns with acidulated methyl alcohol (as described by Medcof *et al.*, 1947; and Müller, 1935), proved non-lethal to fish. The acid extract has a pronounced excitatory effect, does not slow breathing, and shows some resemblances to the action of acetylcholine. This difference in action between neutral and acid extracts is very marked. A similar observation has been made by Hashimoto & Migita (1952) on extracts from mollusc livers. Similar symptoms to these are shown by fish immersed in large doses of scallop liver extract (20 mg/100 ml.) which, if lethal, kills only after prolonged exposure (> 1 day).

All the experiments on mice reported below were done at the Cancer Research Department of Sheffield University by Dr G. Y. Kennedy and his assistants. Extracts were given to mice either orally or by intraperitoneal injection. Injection of neutral extracts in lethal doses caused death within 2 min, with immediate prostration and no other symptoms. There were no post-mortem aberrations. With sublethal doses the mice looked 'thoughtful' and slightly depressed, becoming moribund as the dose was increased. Eventual recovery was complete. Injection of the acid extract produced a slight excitation but was not lethal in the doses given.

We have been fortunate in obtaining a sample of lyophilized scallop liver extract containing the acid extract of paralytic shellfish poison from Dr N. R. Stephenson of the Department of National Health and Welfare of Canada (see also Stephenson *et al.*, 1955). This was also administered to mice during the series of tests. Injection of lethal doses produced prostration, and sub-lethal doses gave excitation. Oral administration also produces excitation of the animals, and is less toxic than injection. The *Gymnodinium* extracts were not toxic when administered orally, but again caused excitation. Thus it would appear that the neutral extract undergoes a change during digestion, probably due to the acid nature of the gastric juices, giving symptoms similar to those produced by acid extracts of *Gymnodinium* and by scallop liver extracts.

The evidence from fish experiments suggesting that death is due to respiratory failure is supported by injection of frogs. A quantity of neutral extract

sufficient to kill a mouse twice the weight was injected into the dorsal lymph sac of frogs. This caused immediate and complete paralysis, and the animals remained moribund for about 2 h. Some recovery then appeared to occur, but the animals died in 24–48 h. There was no sign of breathing over this period, but the oxygen supply through the skin must have been adequate to sustain life for this length of time. Prinzmetal, Sommer & Leake (1932) have also noted this greater resistance of frogs to paralytic shellfish poison.

Although death in vertebrates seems to be due to failure of the respiratory system to provide an adequate oxygen supply there is no doubt that the poison acts by blocking the nervous system.

EXPERIMENTS ON ISOLATED TISSUES

These were carried out to show the actual site of action of the toxin, and form a progressive series as information was accumulated. Unless it is otherwise stated, the tissues were treated with either the supernatant fluid from cultures if sea water was a suitable bathing medium, or neutral extracts of the toxin dissolved in an appropriate bathing fluid. 'Acid extracts' were used only in a few experiments, and a distinct difference in action was found in all of them, after which 'acid extraction' was abandoned in this project. The experiments will be grouped together under a series of general headings and for brevity only the salient points emerging will be discussed.

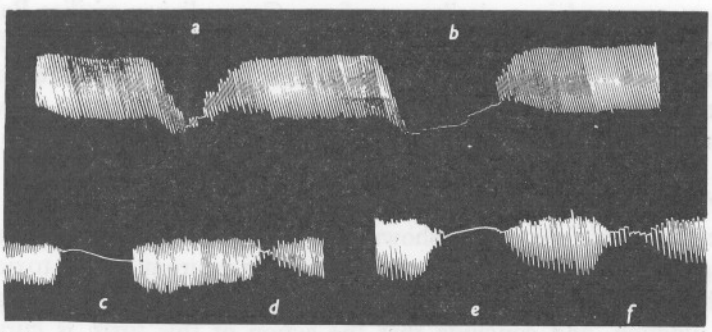

Fig. 2. Action of *Gymnodinium* toxin on perfused *Buccinum* heart. Contraction of heart (systole) downwards; perfusion fluid sea water; temp. 15° C. (*a*) Original culture diluted twofold; (*b*) neutral extract; (*c*) 'acid extract'; (*d*) 'acid extract' at half previous strength; (*e*) and (*f*) acetylcholine at concentrations of 5×10^{-7} and 2×10^{-7} respectively.

Isolated hearts.

Hearts from various animals were isolated and perfused. Recording was on a kymograph with a normal heart lever. Frog, *Loligo* and *Buccinum* hearts were all arrested in systole by the toxin, in contrast to the actions of acetylcholine and the 'acid extract', both of which cause arrest in diastole (Fig. 2).

Buccinum heart appears to be quite sensitive to the toxin and may be suitable for a comparative assay technique. The disadvantage of *Buccinum* heart when isolated and suspended is that the auricle is extremely friable, and the ligatures necessary for cannulation frequently sever it. A more reliable preparation results from cannulation of the heart *in situ*, via the efferent branchial vein. If a cannula with a long tip is used there is no need to ligate this vein. A small amount of citrate added to the perfusion fluid (sea water) prevents coagulation of the residual blood in the heart.

With *Maia* heart the 'acid extract' increases the heart rate whereas the neutral extract slows it.

Frog rectus abdominus muscle

Because of the similarity in action of the 'acid extract' and acetylcholine on isolated hearts we investigated the effect of our extracts on frog rectus abdominus muscle. The muscle was mounted in a chamber and connected to a simple lever which recorded on a kymograph. Bathing solutions were run into the chamber as necessary. The response to acetylcholine was first measured. Application of the 'acid extract' of the toxin caused a slow, steadily increasing contracture, but on the addition of acetylcholine the muscle contracted to the same final level as with acetylcholine alone. Relaxation of the contractured muscle is very slow. The neutral extract induces no contracture, but reduces the response of the muscle to acetylcholine, slowing the rate of contraction and almost stopping relaxation.

Extracts prepared in a similar way from *Gymnodinium vitiligo* Ballantine (1956), which is non-toxic to fish, were used on both hearts and muscles, and had no action at all. Thus it is assumed that any pharmacological reactions are due to the toxin and not to other cell products with which the extracts are undoubtedly contaminated.

Frog sartorius nerve-muscle preparation

Fingerman *et al.* (1953) have shown that paralytic shellfish poison extracted from digestive glands of poisonous shellfish is a neuromuscular toxin. They found that it eliminates the transmission of an impulse across the frog nerve-muscle junction, and conclude that the action of this toxin is curare-like. We have carried out similar experiments using neutral extracts of *G. veneficum*, and the preparation becomes inexcitable to stimulation through the nerve, without the nerve itself being affected. End-plate potentials can be recorded just after indirect excitability has disappeared, but these also decrease. The muscle does not respond to direct stimulation when the end-plate potential drops. In contrast to the conclusions of Fingerman *et al.* we find, as shown below (p. 182), that the membrane potential in the muscle has been destroyed and therefore the action of this toxin differs from that of curare. The 'acid extract' also renders the muscle inexcitable, but we have not followed this up.

Mechanical response of muscles.

The time course of the effect of the toxin on the mechanical response of frog and *Mytilus* muscles (Figs. 3, 4) shows that as the excitability decreases the rates of rise and fall of tension become slower.

The frog sartorius nerve-muscle preparation was mounted in a muscle bath and connected to an isometric lever. Tensions were recorded photo-electrically and displayed on a cathode-ray tube (Hill, 1949*a*). Stimulation could be applied to either nerve or muscle. When the anterior byssus retractor muscle of *Mytilus* was used the muscle was mounted on a multi-electrode assembly and again connected to the same recording apparatus (Abbott & Ritchie, 1951). Stimulation was by square wave pulses from an electronic stimulator.

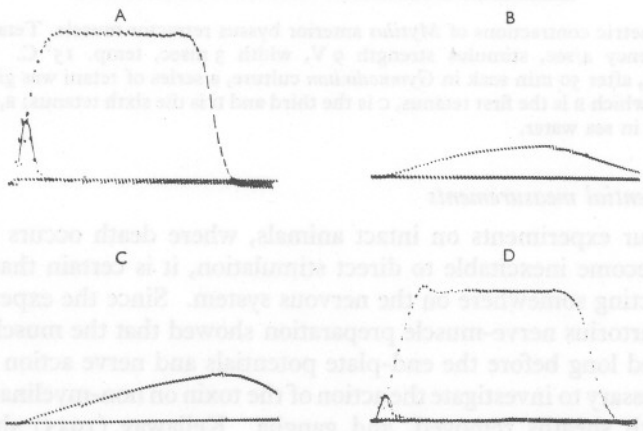

Fig. 3. Mechanical response of frog sartorius nerve-muscle preparation. Twitch and tetanic (25/sec) stimulation applied in each figure. Time base 1·5 sec, temp. 15° C. A, indirect maximal stimulation of untreated preparation; B, indirect stimulation after 10 min soak in neutral toxin extract; the twitch has disappeared; C, direct (muscle) stimulation immediately after B; D, indirect stimulation after 30 min soak out in normal Ringer.

Figs. 3, B, C show that even when the active isometric tension has been reduced to one third by the toxin (10 min immersion), the response by frog sartorius muscle to direct stimulation is identical with that to indirect stimulation, a fact not consistent with the action of a curare-like substance. It can also be seen that the twitch has by this time disappeared and that tetanic tension rises and falls very slowly. After 5 min soak-out in normal Ringer the tetanus tension had recovered to about 50% and was completely restored (Fig. 3D) in 30 min, together with the twitch.

Mytilus muscle (Fig. 4) similarly becomes inexcitable in the presence of the toxin. In a toxin-treated muscle successive tetani show facilitation (B, C, D) when stimulated by a voltage which gives a maximal response in the untreated

muscle (A). Increased voltage, however, gives a tetanus equivalent to that shown at the end of a series of facilitating tetani (D). Thus the voltage required to give a maximal response steadily increases as the toxin acts.

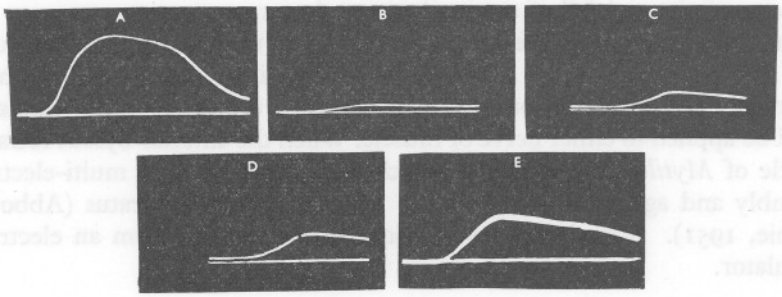

Fig. 4. Isometric contractions of *Mytilus* anterior byssus retractor muscle. Tetanus duration 5 sec, frequency 4/sec, stimulus strength 9 V, width 3 msec, temp. 15° C. A, untreated muscle; B–D, after 50 min soak in *Gymnodinium* culture, a series of tetani was given at 30 sec intervals, of which B is the first tetanus, C is the third and D is the sixth tetanus; E, tetanus after 3 h soak out in sea water.

Action potential measurements

From our experiments on intact animals, where death occurs before the muscles become inexcitable to direct stimulation, it is certain that the toxin must be acting somewhere on the nervous system. Since the experiments on the frog sartorius nerve-muscle preparation showed that the muscle response disappeared long before the end-plate potentials and nerve action potentials, it was necessary to investigate the action of the toxin on non-myelinated nerves, nerves with sheaths removed, and ganglia. Kellaway (1935) showed that sensory nerve endings in frog skin were quickly blocked by paralytic shellfish poison.

Lengths of nerve about 4 cm were dissected and mounted between forceps in an arrangement similar to that of Keynes & Lewis (1951), with the ends of the nerve higher than the central portion which was immersed in the bathing solution all the time. The whole assembly was on a Palmer stand so that the whole nerve could be immersed between records. A recorded action potential means that the whole immersed length of the nerve is conducting. Nerves were first soaked in their normal bathing solution and excitability checked. Toxin was then added and the effect with time noted.

Leg nerve of *Maia* (spider crab) was used as an example of a non-myelinated nerve with very little sheath (Fig. 5). The bathing solution was that described by Welsh (1936). Within 3 min of immersion in toxin the action potential had become spread out into a series of humps (B) and then steadily decreased in amplitude over about 45 min (C, D). Washing in normal Ringer restored the action potential.

When either ray wing motor nerve or frog sciatic nerve was tested the toxin was found to have only a small, slow effect on the intact nerve. When the nerve sheath is removed the nerves remain viable in normal Ringers (ray Ringer— Babkin, Bowie & Nicholls, 1933; frog Ringer— Hill, 1949*b*) for many hours, but conduction is quickly blocked in the presence of the toxin (less than 30 min). The inability of the toxin to block sheathed nerves is probably due to the fact that the molecule, which we believe to be large (p. 171), cannot penetrate the sheath.

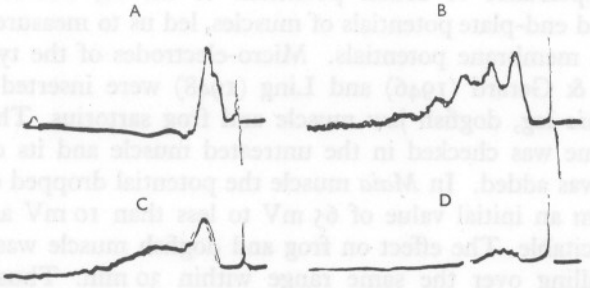

Fig. 5. Action potentials in *Maia* leg nerve. Time base sweep duration 150 msec. (right to left), temp. 15° C. A, untreated nerve; B, after 3 min in *Maia* Ringer with *Gymnodinium* toxin extract added; C, after 10 min in toxin; D, after 45 min in toxin.

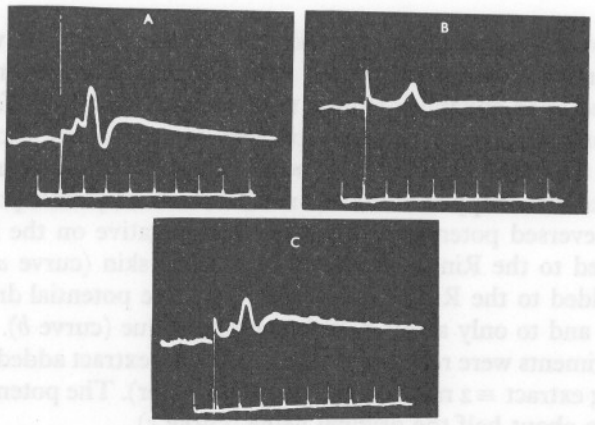

Fig. 6. Ganglionic potentials in isolated rat superior cervical ganglion. Time markers 50/sec, temp. 37° C. A, untreated ganglion; B, after 10 min in toxin; C, after 15 min soak out in normal Kreb's solution.

Ganglia

Preganglionic, ganglionic and postganglionic action potentials of isolated superior cervical ganglia of rats (at 37° C) were recorded for us by Dr J. P. Quilliam. Addition of toxin to the bathing solution slowly depressed the preganglionic potentials and more quickly depressed the postganglionic potentials,

both without appreciable change in their time course. The ganglionic potentials (Fig. 6), on the other hand, were depressed and eventually blocked, and their characteristics altered. In the untreated state there is an after-potential lasting nearly $\frac{1}{5}$ sec (A). As the toxin acts this after-potential is destroyed quicker than the spike (B). The potential returns to normal on soak-out of the toxin (C).

Membrane potentials

The disappearance of action potentials of nerves, and of both action potentials and end-plate potentials of muscles, led us to measure the effect of the toxin on membrane potentials. Micro-electrodes of the type described by Graham & Gerard (1946) and Ling (1948) were inserted into muscle fibres of *Maia* leg, dogfish jaw muscle and frog sartorius. The membrane potential value was checked in the untreated muscle and its change noted when toxin was added. In *Maia* muscle the potential dropped over a period of 1–2 h from an initial value of 65 mV to less than 10 mV and the fibres became inexcitable. The effect on frog and dogfish muscle was more rapid, potentials falling over the same range within 30 min. Thus, apparently, although the precise point at which the toxin acts to kill an animal is unknown, it functions by depolarising excitable membranes.

Frog skin

Further evidence that the toxin reduces cellular potentials was obtained with frog skin. Isolated pieces of frog skin taken from the abdominal surface were mounted in an apparatus similar to that described by Koefoed-Johnsen, Ussing & Zerahn (1952). The skin separated two chambers, each containing Ringer, and the potential difference across the skin was measured (Fig. 7). The skin potential dropped within 10 min from about 70 mV positive on the inside to a reversed potential of about 10 mV negative on the inside, when toxin is added to the Ringer on the inside of the skin (curve *a*). When the toxin was added to the Ringer outside the skin, the potential dropped much more slowly and to only about half the original value (curve *b*).

The experiments were repeated with scallop liver extract added to the inside Ringer (2 mg extract $\equiv$ 2 mouse units to 4 ml. Ringer). The potential again fell slowly and to about half the original value (curve *c*).

In all experiments the potential was restored to its original level within a few minutes of replacing the toxin with normal Ringer.

Ussing & Zerahn (1951) have explained the standing potential across frog skin in terms of an equivalent electrical circuit in which the sodium pump provides a driving potential in series with a resistance to the movement of sodium ions, with a passive resistance to other ions acting in parallel to this circuit. Disappearance of the potential could occur from a blockage of the sodium pump or from a greatly decreased passive skin resistance. A measure

of the skin resistance was obtained by forcing extra current through the skin from an outside source. The potential recorded across the skin varies with the current passed in a nearly linear manner and the slope of the potential–current relationship represents the effective total conductance of the skin. It was found that the skin conductance, and therefore the resistance, did not alter measurably when the skin potential was abolished by the toxin. It therefore appears that the toxin is acting on the sodium exchange mechanism. The brief description given recently by Koefoed-Johnsen & Ussing (1956) of a modified theory of the origin of frog skin potential is discussed later (p. 186).

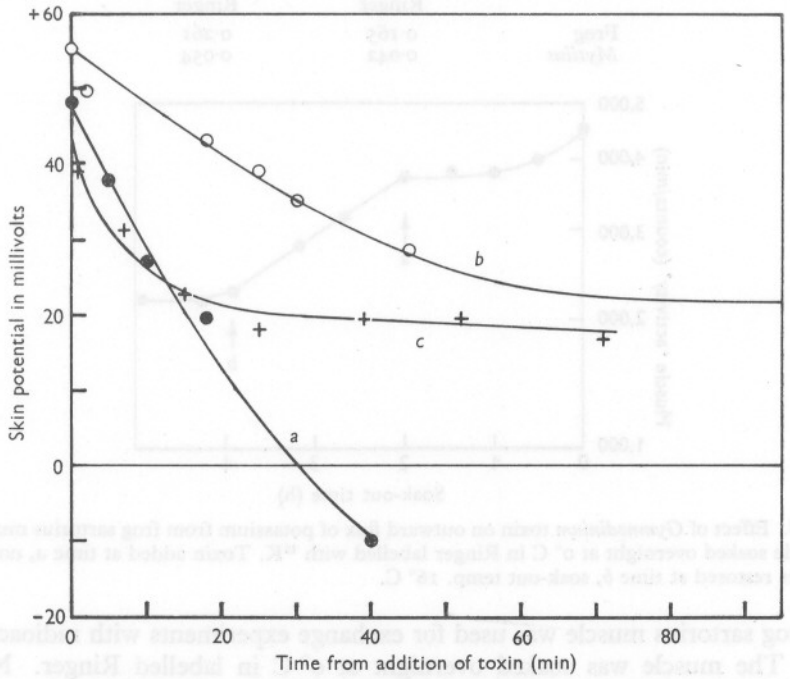

Fig. 7. Time course of decay of potential across isolated frog skin in the presence of toxins. Ringer on both sides of skin, temp. 15° C. Curve (*a*), neutral *Gymnodinium* toxin extract added to inside surface of skin; curve (*b*), neutral *Gymnodinium* toxin added to outside surface of skin; curve (*c*), scallop liver extract added to inside surface of skin.

Other experiments

Two other lines of approach have been made and preliminary results are quoted here, though further investigation is proceding.

The influence of the toxin on the rate of oxygen consumption by frog and *Mytilus* muscles has been measured, and we find that the rate is increased by 58 and 28% respectively (Table 2).

It is obvious that a great deal of information can be obtained from studying, with radioactive potassium and sodium, the influence of the *Gymnodinium*

toxin on the ionic exchanges across cell membranes. In resting nerve and muscle the membrane potential is closely related to the ratio of potassium concentrations on the two sides of the membrane. The speed with which the toxin acts, and also the rate of recovery, is surprisingly fast if the entire potential decrease is due to leakage of potassium out of the cell.

TABLE 2. RESTING RESPIRATION RATES OF FROG AND *MYTILUS* MUSCLE, AT 19·7° C

(μl O_2/mg wet wt/h)

	Normal Ringer	Toxin-treated Ringer
Frog	0·165	0·261
Mytilus	0·042	0·054

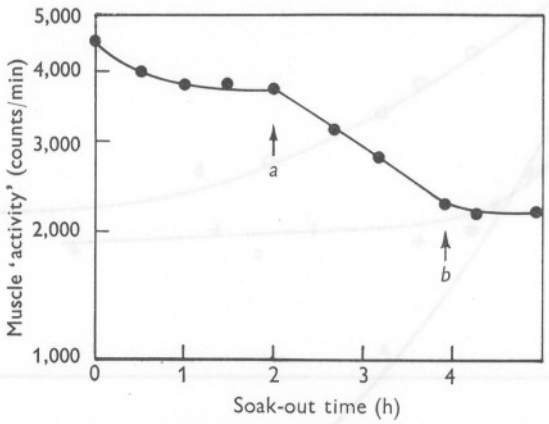

Fig. 8. Effect of *Gymnodinium* toxin on outward flux of potassium from frog sartorius muscle. Muscle soaked overnight at 0° C in Ringer labelled with ^{42}K. Toxin added at time *a*, normal Ringer restored at time *b*, soak-out temp. 16° C.

Frog sartorius muscle was used for exchange experiments with radioactive ^{42}K. The muscle was soaked overnight at 0° C in labelled Ringer. Next morning it was transferred to normal Ringer at room temperature to wash out the radioactivity. The activity of the muscle was counted at intervals as soaking out continued. When the extracellular potassium had diffused away, the activity (after correcting for decay of the isotope) decreased exponentially with time over several hours. The slope of the curve gives a measure of the potassium efflux, and the half-time of exchange (Fig. 8) is about 30 h. Toxin was then added to the soak solution, at the time indicated on the curve. The muscle was inexcitable within 20 min. The efflux rate of potassium increased greatly but the time for half exchange was about 3 h, so that the membrane potential disappeared long before the internal potassium level had fallen to the requisite value to account for the depolarization.

The experiments described in this section are summarized in Table 3.

TABLE 3. SUMMARY OF THE ACTION OF *GYMNODINIUM* TOXIN ON VARIOUS TISSUES

Preparation	Effect of 'Neutral' toxin extract	Page reference
Buccinum heart	Arrest in systole	177
Frog rectus abdominus	No contracture; reduction of sensitivity to acetylcholine: slowing of relaxation	178
Frog sartorius nerve-muscle	(1) indirect stimulation: muscle inexcitable, nerve slightly affected, end-plate potentials decrease and disappear. Muscle twitch quickly goes, tetanic tension decreases and becomes slower in rise and fall	178
	(2) direct stimulation: mechanical response decreases and is always identical with response to indirect stimulation	179
	Muscle respiration rate increased	183
	Efflux rate of potassium greatly increased	184
Mytilus anterior byssus retractor	Becomes inexcitable. Progressive threshold increase, mechanical response is slowed	179
Nerve, crab	Action potential spreads out into several humps, and disappears	180
Nerve, de-sheathed frog sciatic	Blocked	181
Superior cervical ganglion of rat	Ganglion potential depressed and then blocked. After-potential disappears first	181
Frog skin	Toxin on inside, potential decreases and reverses in sign. No measurable change in skin resistance. Toxin on outside, very slow partial drop in potential	182

DISCUSSION

Sommer & Meyer (1937) have suggested that the toxin produced by dinoflagellates and causing paralytic shellfish poisoning may be different from that liberated by other dinoflagellates associated with mass mortality of fish. Our observations on the toxin from *Gymnodinium veneficum* tend to confirm this view, as we are unable to render shellfish poisonous with it, and it differs from the poison in lyophilized scallop liver extract, both in action on fish (p. 176) and mice (p. 176) and on isolated frog skin (p. 182). Whereas Fingerman *et al.* have indicated that the paralytic shellfish poison operates in a curare-like manner, we have shown that the *Gymnodinium* toxin depolarizes the excitable membrane.

Our evidence from the frog skin, considered in the light of Ussing's original hypothesis of the origin of the skin potential, suggests that the toxin blocks the sodium pump—particularly as the skin resistance is not measurably altered. In the case of nerve and muscle, however, blockage of the sodium pump could only result in depolarization of the membrane after sufficient potassium had leaked out, in exchange for the sodium entering the fibre—a process which would take very much longer than is needed for depolarization with the toxin. In a similar way the recovery is much too rapid to be explained by a reversal of this process. Moreover, analysis of muscles which

have been rendered completely inexcitable by the toxin shows that they still contain considerable amounts of potassium. The tracer experiments mentioned above (p. 184) also show that although the potassium efflux increases in the presence of the toxin, the exchange is not rapid enough to explain the depolarization (in terms of a concentration cell).

Koefoed-Johnsen & Ussing (1956) have recently modified their hypothesis and now suggest that two separate membranes are concerned with the potential in frog skin: on the outer surface there is a membrane selectively permeable to sodium, and on the inner surface a membrane which is selectively permeable to potassium. A sodium-potassium exchange pump, located in the inner membrane, maintains a low sodium and high potassium concentration in the skin cells. The potential measured across the skin is the sum of the sodium diffusion potential across the outer membrane and the potassium diffusion potential across the inner membrane. Such a potential would decrease if the inner membrane became permeable to sodium ions; and if the permeability became greater than that of the outside membrane the potential could certainly temporarily reverse. We find that the toxin produces a reversal of potential. Certainly the selectivity of the toxin for the inside of the skin indicates that its site of action is there.

An increase in sodium permeability would also reduce the membrane potential in nerve and muscle cells. Such a permeability increase occurs during the rising phase of an action potential, when the potential drops rapidly and reverses in sign.

From these arguments we conclude that the *Gymnodinium* toxin is acting on the nervous system of animals by depolarizing excitable membranes. We feel that this probably occurs by interference with the sodium exchange mechanism. The speed of action of the toxin makes it improbable that it is only stopping sodium extrusion, and the more probable explanation is that it allows rapid entry of sodium into the cells.*

SUMMARY

Methods of extraction of the toxin from cultures of *Gymnodinium veneficum* are described. This is now done by dialysis and evaporation under reduced pressure. The toxin molecule must be large, as it cannot penetrate a dialysis membrane; it is soluble in water and the lower alcohols, but insoluble in ether and chloroform. It is unstable in acids, turning into another toxic product, and is decomposed by hot alkali, though in neutral solution is more or less thermostable. The toxin as it occurs in sea water is not the same as paralytic shellfish poison, but there are some resemblances between this and the 'acid extract', though much more work is needed to check this point.

The action of the toxin on a variety of animals is described, and an attempt

* See note at end of paper.

is made to devise an approximate assay technique using gobies as the test animals.

The action has also been observed on a range of isolated preparations, and although the final conclusion as to the mode of action is not quite clear, we feel confident that the site of action in whole animals is in the nervous system, probably acting on ganglion transmission. With regard to the mode of action it depolarizes nerve and muscle membranes. It also abolishes the potential across frog skin without measurably altering the skin resistance.

This depolarization probably occurs by interference with the sodium exchange mechanism, allowing rapid entry of sodium into the cells.

Note added in proof

Preliminary experiments have been carried out on the effect of this toxin on sodium permeability of frog sartorius muscle using the radioisotope ^{22}Na. Pairs of sartorius muscles were isolated. From each pair, one muscle was soaked in normal frog Ringer labelled with ^{22}Na and the other soaked in similar labelled Ringer to which toxin was added in sufficient quantity to render the muscle inexcitable. The exchange of sodium across the muscles was followed in terms of the radioactivity of the muscles: the muscles were removed at intervals, surface liquid blotted off and activity measured. Activity (and total sodium) of the toxin treated muscles increased more rapidly and to a higher final level than that of the untreated muscles. The tissues were later soaked in non-radioactive Ringer solutions and the decrease in activity followed. The activity of the normal muscle decreased rapidly at first corresponding to exchange in the extracellular sodium, and then more slowly as intracellular sodium exchanged. In the case of the toxin-treated muscle the exchange was very rapid and large, and consisted only of the one fast phase. It appeared that the membrane had become so permeable to sodium that the exchange from inside the cells had become similar in time course to that of the extracellular sodium.

The conclusions of this paper are of necessity preliminary and need verifying with a purified sample of the toxin. This, however, must await further development of the technique of producing large quantities of material and subsequent purification.

REFERENCES

ABBOTT, B. C. & RITCHIE, J. M., 1951. The onset of shortening in striated muscle. *J. Physiol.*, Vol. 113, pp. 336–45.

BABKIN, B. P., BOWIE, D. J. & NICHOLLS, J. V. V., 1933. Structure and reactions to stimuli of arteries (and conus) in the elasmobranch genus *Raja*. *Contr. Canad. Biol.*, Vol. 8, pp. 207–25.

BAINBRIDGE, R., 1953. Studies on the interrelationships of zooplankton and phytoplankton. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 385–447.

BALLANTINE, DOROTHY, 1956. Two new marine species of *Gymnodinium* isolated from the Plymouth area. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 467–74.

BALLANTINE, DOROTHY & MORTON, J. E., 1956. Filtering, feeding and digestion in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 241–74.

BRONGERSMA-SANDERS, M., 1948. The importance of upwelling water to vertebrate paleontology and oil geology. *Verh. Akad. Wet. Amst., Afd. natuurkunde*, Sect. 2, Deel 45, No. 4, 112 pp.

DAVIS, C. C., 1948. *Gymnodinium brevis* n.sp., a cause of discolored water and animal mortality in the Gulf of Mexico. *Bot. Gaz.*, Vol. 109, pp. 358–60. (Contrib. no. 17, Univ. Miami Mar. Lab.)

FINGERMAN, M., FORESTER, R. H. & STOVER, J. H., 1953. Action of shellfish poison on peripheral nerve and skeletal muscle. *Proc. Soc. exp. Biol., N.Y.*, Vol. 84, pp. 643–6.

GRAHAM, J. & GERARD, R. W., 1946. Membrane potentials and excitation of impaled single muscle fibres. *J. cell. comp. Physiol.*, Vol. 19, pp. 135–44.

HASHIMOTO, Y. & MIGITA, M., 1952. On the shellfish poisons. 1. Inadequacy of acidulated alcohols with HCl as solvent. *Bull. Tokai Fish. Res. Lab.*, Vol. 3B, pp. 77–84.

HAYES, HELEN L. & AUSTEN, T. S., 1951. The distribution of discolored sea water. *Tex. J. Sci.*, Vol. 3, pp. 530–41.

HILL, A. V., 1949*a*. Myothermic methods. *Proc. roy. Soc.* B, Vol. 136, pp. 228–41.

—— 1949*b*. Is relaxation an active process? *Proc. roy. Soc.* B, Vol. 136, pp. 420–35.

INGLE, R. M., 1954. Irritant gases associated with Red Tide. *Univ. Miami mar. Lab., Special Service Bull.*, No. 9. (Florida State Bd of Conservation.)

KELLAWAY, C. H., 1935. The action of mussel poison on the nervous system. *Aust. J. exp. Biol. med. Sci.*, Vol. 13, pp. 79–94.

KEYNES, R. D. & LEWIS, P. R., 1951. The resting exchange of radioactive potassium in crab nerve. *J. Physiol.*, Vol. 113, pp. 73–98.

KOCH, H. J., 1939. La cause des empoissonements paralytiques provoqué par les moules. *C.R. Ass. Franc. Avanc. Sci., Sean. Sess.* 63, *Liége*, pp. 654–7.

KOEFOED-JOHNSEN, V. & USSING, H. H., 1956. Nature of the frog skin potential. *XX Congr. int. Physiol.*, pp. 511–512.

KOEFOED-JOHNSEN, V., USSING, H. H. & ZERAHN, K., 1952. The origin of the short-circuit current in the adrenaline stimulated frog skin. *Acta physiol. scand.*, Bd. 27, pp. 38–48.

LING, G., 1948. Effect of stretch on membrane potential in frog muscle. *Fed. Proc.* Part 1, vol. 7, p. 72.

MARSHALL, S. M. & ORR, A. P., 1955. On the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 495–529.

MEDCOF, J. C., LEIM, A. H., NEEDLER, A. R., NEEDLER, A. W. H., GIBBARD, J. & NAUBERT, J., 1947. Paralytic shellfish poisoning on the Canadian Atlantic coast. *Bull. Fish. Res. Bd Can.*, Vol. 75, pp. 1–32.

MÜLLER, H., 1935. The chemistry and toxicity of mussel poison. *J. Pharmacol.*, Vol. 53, pp. 67–89.

NEEDLER, A. B., 1949. Paralytic shellfish poisoning and *Goniaulax tamarensis*. *J. Fish. Res. Bd Can.*, Vol. 7, pp. 490–504.

NIGHTINGALE, H. W., 1936. *Red Water Organisms: Their Occurrence and Influence upon Marine Aquatic Animals, with Special Reference to Shellfish in Waters of the Pacific Coast*, 24 pp. Seattle, Washington: Argus Press.

PRINZMETAL, M., SOMMER, H. & LEAKE, C. D., 1932. The pharmacological action of 'mussel poison'. *J. Pharmacol.*, Vol. 46, pp. 63–75.

RIEGEL, B., STANGER, D. W., WILKHOLM, D. M., MOLD, J. D. & SOMMER, H., 1949*a*. Paralytic shellfish poison. IV. Bases accompanying the poison. *J. biol. Chem.*, Vol. 177, pp. 1–6.

—— 1949*b*. Paralytic shellfish poison. V. The primary source of the poison, the marine plankton organism, *Gonyaulax catenella*. *J. biol. Chem.*, Vol. 177, pp. 7–11.

SOMMER, H. & MEYER, K. F., 1937. Paralytic shellfish poisoning. *Arch. Path.* (*Lab. Med.*), Vol. 24, pp. 560–98.

SOMMER, H., MONNIER, R. P., RIEGEL, B., STANGER, D. W., MOLD, J., WIKHOLM, D. M. & KIRALIS, E. S., 1948. Paralytic shellfish poison. I. Occurrence and concentration by ion exchange. *J. Amer. chem. Soc.*, Vol. 70, pp. 1015–18.

SOMMER, H., RIEGEL, B., STANGER, D. W., MOLD, J. D., WILKHOLM, D. M. & MCCAUGHEY, MARGARET B., 1948. Paralytic shellfish poison. II. Purification by chromatography. *J. Amer. chem. Soc.*, Vol. 70, pp. 1019–21.

SOMMER, H., WHEDON, W. F., KOFOID, C. A. & STOHLER, R., 1937. The relation of paralytic shellfish poison to certain plankton organisms of the genus Gonyaulax. *Arch. Path.* (*Lab. Med.*), Vol. 24, pp. 537–59.

STEPHENSON, N. R., EDWARDS, H. I., MACDONALD, B. F. & PUGSLEY, L. I., 1955. Biological Assay of the toxin from shellfish. *Canad. J. Biochem. Physiol.*, Vol. 33, pp. 849–57.

USSING, H. H. & ZERAHN, K., 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. scand.*, Bd. 23, pp. 110–27.

WELSH, J. H., 1936. Chemical mediation in crustaceans. I. The occurrence of acetylcholine in nervous tissues and its action on the decapod heart. *J. exp. Biol.*, Vol. 16, pp. 198–219.

WOODCOCK, A. H., 1948. Note concerning human respiratory irritation associated with concentrations of plankton and mass mortality of marine organisms. *J. mar. Res.*, Vol. 7, pp. 56–62.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

A NEW MUSCLE PREPARATION FOR THE STUDY OF OPTICAL CHANGES DURING CONTRACTION

By B. C. ABBOTT and J. LOWY

Nature, Lond., 1956, Vol. 177, pp. 788–9

Pieces cut from the mantle muscle of squid have been used to study transient optical changes in the muscle when stimulated. When alive and healthy this muscle is very transparent. Two types of changes are described. The first is a small increase in transparency which begins during the falling phase of the action potential. At room temperature the peak of this increase is at 22 msec after the stimulus. The first sign of tension increase occurs at 19 msec, with no evidence of a preceding latency relaxation.

This transparency increase is followed by a large opacity increase due to light scattering. The scattering closely follows the time course of the twitch, and about 30% of the light is scattered at the twitch peak. B.C.A.

RESTING TENSION IN SNAIL MUSCLE

By B. C. ABBOTT and J. LOWY

Nature, Lond., 1956, Vol. 178, pp. 147–8

The retractor pharynx of the snail extends to great lengths, even under a small load and its ability to exert a resting tension has been doubted.

The experiments described demonstrate that the muscle has a very wide range of working length. The isometric tensions produced during tetanus have been measured at various lengths, and it is demonstrated that although appreciable tension is produced at as little as 12 mm, the maximum active tension occurs at about 40 mm. Above this length active tension decreases again: as in vertebrate striated muscle.

At lengths above that for maximum active tension, the resting muscle exerts a true resting tension and tears with a breaking stress of about 1·5 kg/cm^2. B.C.A.

CILIARY FEEDING MECHANISMS OF BRACHIOPODS

By D. ATKINS

Nature, Lond., 1956, Vol. 177, pp. 706–7

A preliminary account is given of the ciliary feeding mechanisms of brachiopods based on the articulates *Terebratulina*, *Gryphus* and *Macandrevia* and the inarticulate *Crania*. In all these, the lateral cilia, beating across the length of the filaments and from the inside to the outside of the lophophoral spaces, create the main water current, which in adults, whether with plectolophus or with spirolophus lophophores, enters laterally and leaves the shell anteriorly. In young stages with trocholophus and with early schizolophus lophophores, the inhalant current is single and median, setting into the bell-shaped lophophore, while the exhalant current escapes between the filaments all around. As the anterior indentation increases with age, two lateral arms being formed, the median inhalant current becomes divided into two.

The frontal currents in all the species examined are capable of reversal. Small particles are conveyed by the frontal cilia toward the bases of the filaments and eventually reach the mouth; large particles and collections of particles are conveyed to the tips of the filaments and are finally rejected from the shell. In a thick culture of *Chlorella*, etc., the frontal cilia beat toward the tips of the filaments. This reversal of the frontal currents of the filaments appears to be due to reversal of the ciliary beat and not to adjacent tracts of cilia beating in opposite directions as in certain bivalves.

In *Terebratulina retusa*, *Gryphus vitreus* and *Macandrevia cranium*, abfrontal cilia are present on the filaments and these beat continuously toward their tips. D.A.

OBSERVATIONS ON LUMINESCENCE IN SEA PENS (PENNATULACEA)

By D. DAVENPORT and J. A. C. NICOL

Proc. roy. Soc., B, 1956, Vol. 144, pp. 480–96

Luminescent responses of a sea pen, *Leioptilus gurneyi*, obtained off the Pacific coast of the United States, proved favourable for physiological studies. Light is emitted by autozooids and siphonozooids. Mechanical and electrical stimuli evoke luminescent waves which pass over the rachis at a velocity of 26 cm/sec (20° C). Consecutive flashes increase in intensity owing to neuro-effector facilitation. A flash has a latent period of 0·18 sec (total duration of 1–2 sec) and reaches maximal intensity in 0·2 sec from first deflexion. Strong mechanical and prolonged electrical stimulation produce a refractory state in which transmission of luminescent waves ceases. Slow progressive spread of luminescence can then be induced by maintained repetitive stimulation. This effect is ascribed to internuncial facilitation overcoming fatigue in the nerve net. J.A.C.N.

SYSTEMATICS OF THE MARINE-BUG

By DENNIS LESTON

Nature, Lond., 1956, Vol. 176, pp. 427–8

Study of male and female genitalia, head and mouthparts, abdomen, dorsal glands and other structures shows that the bug, *Aepophilus bonnairei*, hitherto regarded as comprising a monotypic family of uncertain affinities, is a saldid. The lateral abdominal 'strigil' of males confirms this view. Other saldid species from widely separated genera show a tendency to live a submarine life, but *A. bonnairei* has progressed furthest along this path and has well-defined structural and behavioural modifications suiting it to life at the lowest *Fucus* zone. It can survive submergence for at least 49 h in sea water. D.L.

PRESSURE RECEPTORS IN THE FINS OF THE DOGFISH *SCYLLIORHINUS CANICULA*

By OTTO LOWENSTEIN

J. exp. Biol., 1956, Vol. 33, pp. 417–21

The presence of so-called terminal corpuscles in the connective tissues of the fins of *Scylliorhinus canicula*, first described by Wunderer in 1908, is confirmed.

It is demonstrated that they are pressure receptors with a slow rate of adaptation.

From their topographic distribution and from their mode of response to mechanical stimulation, it is postulated that the terminal corpuscles serve as proprioceptors in the widest sense of the term by signalling the spatio-temporal patterns of active or passive deformation of the fin.

Their topographic distribution makes it possible to distinguish their responses from those of sense endings associated with the muscles or tendons of the fin. O.L.

NERVOUS REGULATION OF LUMINESCENCE IN THE SEA PANSY *RENILLA KÖLLIKERI*

By J. A. C. NICOL

J. exp. Biol., 1955, Vol. 32, pp. 619–35

During a visit to the Scripps Institution of Oceanography, advantage was taken of the opportunity to study luminescence in the sea pansy *Renilla köllikeri*. When stimulated, *Renilla* produces luminescent waves, which are under control of a non-polarized nerve net. The response is subject to facilitation, which occurs terminally, at the neuro-photocyte junctions. Facilitation is analysed in detail, and a facilitation-decay curve presented. Between

successive bursts of stimuli, facilitation may persist for some 10 min. Measured response-parameters were as follows: latent period, 0·12 sec; local duration of response, 1 sec; time to reach maximal intensity, 0·22 sec; conduction speed of luminescent wave, 9 cm/sec (16–17° C). The refractory period of the response is about 0·2 sec, and increases under repetitive stimulation. When strongly excited the animal passes into a hyper-excitatory state, in which luminescent waves continue to arise long after stimulation has ceased.

J.A.C.N.

OBSERVATIONS ON THE ANATOMY AND MODE OF LIFE OF *LASAEA RUBRA* (MONTAGU) AND *TURTONIA MINUTA* (FABRICIUS)

By E. OLDFIELD

Proc. malacol. Soc. Lond., 1955, Vol. 31, pp. 226–49

The anatomy of *Lasaea rubra* and *Turtonia minuta* is described. Certain organs such as the gills and stomach show simplicity of structure which has probably been acquired secondarily in relation to small size.

In both genera the mode of reproduction is specialized for protection of the developing embryos and consequently relatively few eggs are produced, but these are large and heavily laden with yolk. *Lasaea* and *Turtonia* show entirely different methods for protecting the developing young; in *Lasaea* the embryos are incubated within the suprabranchial chamber, whereas in *Turtonia* the embroys develop within a gelatinous capsule which is attached to the byssus of the female. In both genera the free veliger stage is completely suppressed; a study of the embryology is in progress.

The systematic position of the two genera is discussed and it appears that *Turtonia* should not be included together with *Lasaea* and *Kellia* in the family Erycinidae.

E.O.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. 27 (p. 761) and Vol. 31 (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

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Members of the Association have the following rights and privileges: they elect annually the 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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