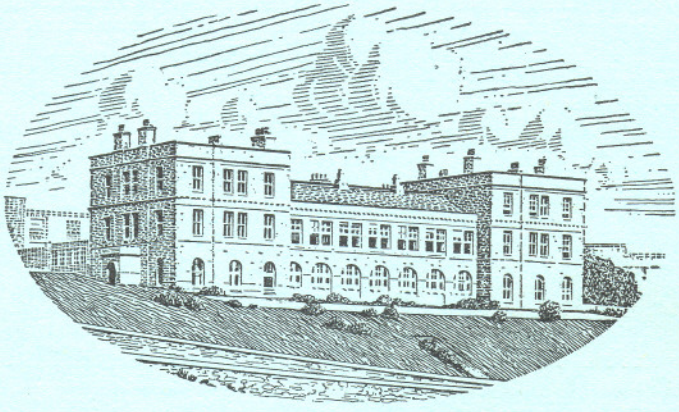
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



THE PLYMOUTH LABORATORY

VOLUME 33, No. 3

(issued October 1954 and completing Volume 33)

CAMBRIDGE
AT THE UNIVERSITY PRESS
1954

Price Forty Shillings net

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J. Mar. biol. Ass. U.K. (1954) 33, 565–85
Printed in Great Britain

OBSERVATIONS ON THE OPISTHOBRANCH MOLLUSC *ACTEON TORNATILIS* (L.).

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(Text-figs. 1–9)

CONTENTS

	PAGE
Habits and external features	565
The alimentary canal	570
The reproductive system	572
Discussion	576
Summary	583
References	583
Abbreviations used in the figures	585

Habits and External Features

Acteon tornatilis (L.), the most primitive British opisthobranch, may be collected in large numbers at Pendine Sands, Carmarthenshire, where, during early September, occasional individuals may be seen creeping over the shore near the water's edge at low tide. More conspicuous than the adult animals, however, at that time of the year are the gelatinous masses of spawn: these measure about 6 cm in length, are club-shaped and taper towards one end which is secured to a piece of weed, wood or stone, usually on the surface of the sand but often underneath. Sometimes molluscs are found with the egg mass trailing from the genital aperture: if these are followed they may be seen, when near an object projecting from the sand, to burrow a short distance, and, whilst the mollusc is out of sight, the last secretions are poured from the female opening and the mass fastened to the buried object, presumably by means of the foot.

During the ebb tide most of the specimens of *Acteon* which live in the area are not on the surface of the sand because they then tunnel through the upper 6 in. Their burrows open to the surface by a hole 4–5 mm in diameter, and, once this is realized, it is an easy matter to collect them. The method of burrowing may be followed in the laboratory. An individual placed on sand will creep over the surface leaving behind it a characteristic trail which is formed by the head lobes (i.e. expanded and flattened cephalic and labial tentacles) and by the widely expanded foot, the former lying in front of the

latter (see Fig. 1). These structures act like a snow-plough, pushing aside the sand and heaping it on either side of the path; strong ciliary currents beat towards the free edges of the head and posteriorly over the sole of the foot to keep these surfaces clean, and the tentacles are reflexed over the edge of the shell so as to close off the mantle cavity. After creeping for a short distance over the surface the mollusc soon presses deeper into the sand and disappears from sight, the sand closing over behind it. What happens then may be seen if

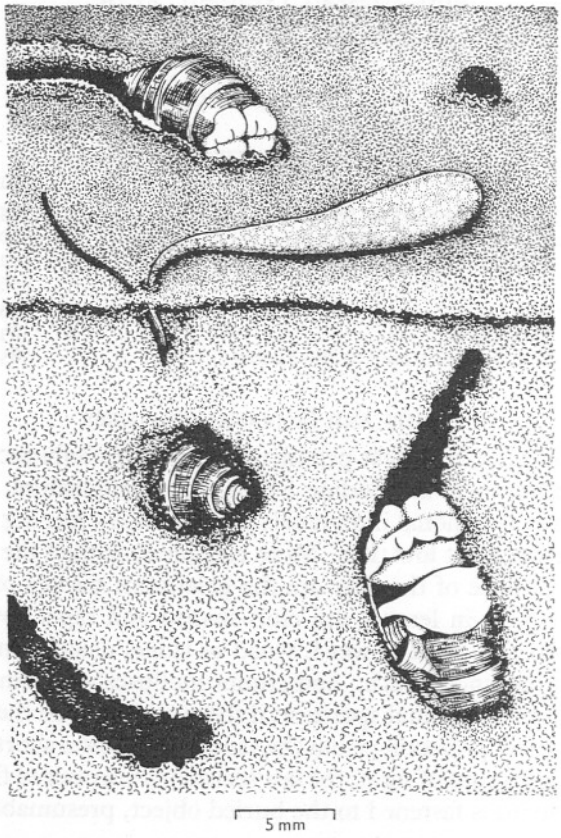

Fig. 1. *A. tornatilis* in its natural habitat. In the upper part of the drawing an animal is seen creeping over the surface of the sand, and the opening of a burrow is in the top right-hand corner. In the middle a mass of spawn lies on the surface attached to weed a little below. In the lower half of the drawing a mollusc is shown in its burrow. The exhalant siphon shows on its left.

a tunnel be made against the side of an aquarium. The mollusc will travel vertically or diagonally downwards for an inch or two, then horizontally, and, sooner or later, will start towards the surface again. Its progress is slow. As it moves the burrow does not collapse behind it, for secretion from the head

lobes and pedal gland is pressed against the sand and agglutinates the particles. On reaching the surface the head is pressed back against the rim of the opening to make the edges firm with secretion and the animal may remain in this position for some time. Alternatively it may retreat, moving backwards as it goes, so that the head is kept facing the mouth of the burrow, and it may come to rest a few inches down with the semi-contracted foot holding firmly. The right margin of the foot overhangs the conspicuous exhalant siphon, which is partially protected, too, by the edges of the shell, and a slow, continuous current of water, apparently free from sand particles, passes out of the siphon. When the animal emerges fully from its shell this exhalant siphon comes to occupy the posterior corner of the mouth of the shell.

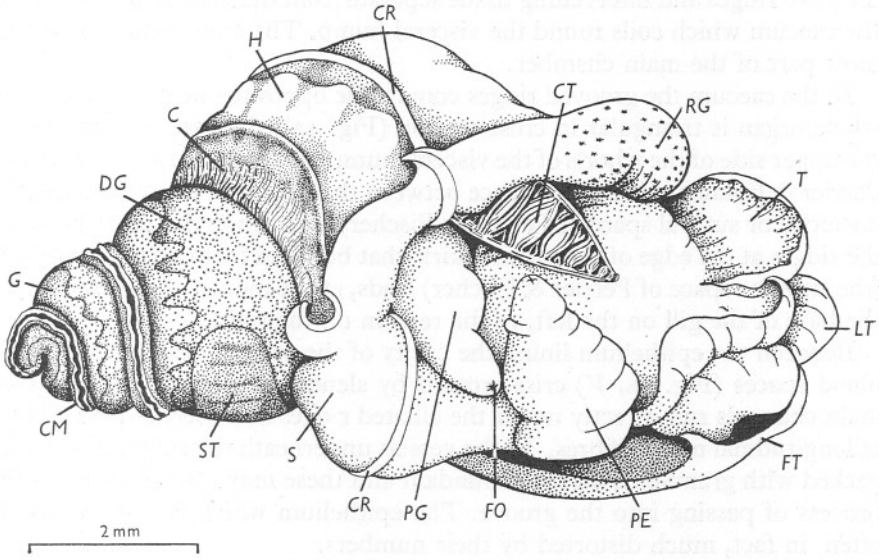

Fig. 2. *A. tornatilis* with shell removed. For explanation of lettering see p. 585.

Some of the primitive features of *Acteon* may be correlated with the large size of the shell, which permits the animal to withdraw completely and seal itself with an operculum. The body whorl is deep, its aperture an elongated slit along the right side with a length equal to about two thirds the height of the shell. This may be correlated with the fact that the mantle cavity of *Acteon* is at least as large as in any other opisthobranch and resembles that of a prosobranch in having to accommodate such structures as a large penis (Fig. 2, *PE*) which cannot be invaginated, and pallial genital ducts (*PG*) which in many gastropods have sunk into the haemocoel. The shape of the cavity differs from that of the typical prosobranch in two important respects: the opening, though large and anterior, is markedly twisted to the right so that there is no opening left of the mid-line, where the gill is attached; and the cavity is drawn out

posteriorly into a narrow extension (*CM*) which runs separately alongside the main spiral of the visceral hump almost or quite to its apex.

The posterior extension of the mantle cavity lies right of the gill, but left of the rectum and genital tract, and is, therefore, a derivative of the hypobranchial region. Along it run two grooved and ciliated ridges (*CR*), both of which lead to the free edge of the mantle skirt anteriorly. The grooves were not described by previous workers. The left of these, the larger of the two, lies parallel to the ctenidium and is placed just right of the mid-line; the right lies left of the rectum and leads on to the expanded flap of pallial tissue used as an exhalant siphon (*S*). Glandular tissue intermixed with ciliated cells lines the wall along and between these ridges and represents the hypobranchial gland. The two ridges and intervening tissue separate from the main mantle cavity as the caecum which coils round the visceral hump. The anus lies in the innermost part of the main chamber.

In the caecum the grooved ridges come to lie opposite one another and the whole organ is triangular in cross-section (Fig. 4A), the base resting against the upper side of the whorls of the visceral hump, not flattened as described by Perrier & Fischer (1911). The space between the ridges on the outer side (the posterior or sutural space of Perrier & Fischer) leads on to the space between the ridges at the edge of the mantle skirt; that between them on the inner side (the anterior space of Perrier & Fischer) leads, at the mouth of the caecum, to the base of the gill on the left, to the rectum on the right.

Between the epithelium lining the cavity of the caecum and the mantle lie blood spaces (Fig. 4B, *V*) criss-crossed by slender strands of muscle. Two main channels run directly under the ciliated ridges and there is here a band of longitudinal muscle fibres. In the vessels underneath the ridges blood cells packed with granules (*AM*) are abundant and these may often be seen in the process of passing into the groove. The epithelium which lies at its base is often, in fact, much distorted by their numbers.

The glands of the caecum are of two sorts, one a 'mucous' cell (*MC*), secreting mucoitin sulphuric acid, the other (*GC*) producing granules of a substance staining intensely with iron haematoxylin. Both are of types commonly encountered in the hypobranchial gland of other gastropods.

Ciliary currents (Fig. 3) run across the ridges into the posterior space of the caecum, where they set in an outward direction so that particulate matter (including extruded blood cells) is swept to the region of the exhalant siphonal fold on to which currents from the anus and surrounding parts also converge. A compensating ingoing current travels up the anterior space of the caecum so that a circulation of water is maintained.

The edge of the mantle skirt in *Acteon*, in its left half, is greatly thickened by the presence of elaborate glands called by Pelseneer (1894) the semilunar gland. These form a belt, parallel to the edge, which has a pitted aspect, each depression being the mouth of a compound tubular or flask-shaped gland. If

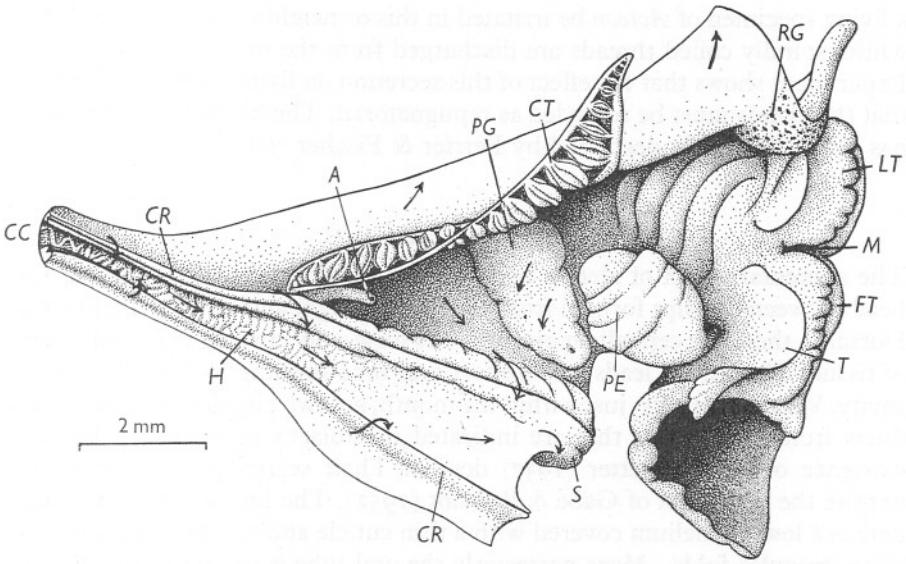

Fig. 3. *A. tornatilis.* Mantle cavity and base of caecum opened by a longitudinal slit to the right of the ctenidial axis. Arrows show direction of ciliary currents. For explanation of lettering see p. 585.

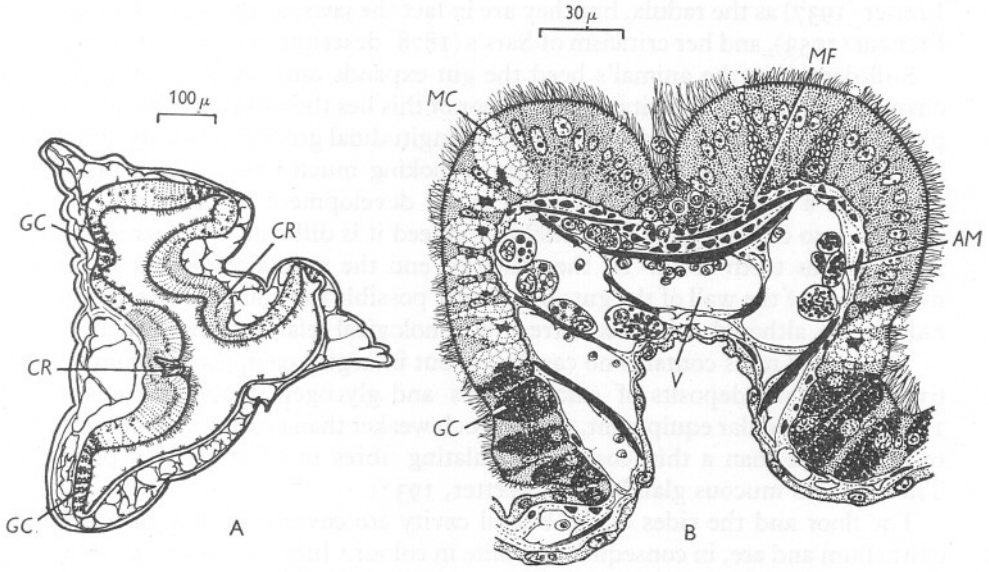

Fig. 4. *A. tornatilis.* A: transverse section of the caecum; B, transverse section of ciliated ridge of the caecum. For explanation of lettering see p. 585.

a living specimen of *Acteon* be irritated in this or neighbouring regions small, white, spirally coiled threads are discharged from the mouths of the glands. Experiment shows that the effect of this secretion on living animals is toxic so that the glands must be regarded as repugnatorial. The histology of the gland has been adequately described by Perrier & Fischer (1911).

The Alimentary Canal

The alimentary tract of *Acteon* opens at the mouth placed anteriorly on the head between two lips formed from the bases of right and left labial tentacles. Dorsal to these the cephalic tentacles lie as forwardly curving flattened sheets of tissue. The mouth leads into a short oral tube running back to the buccal cavity. Ventro-laterally, just within the mouth, are two papillae on which open ducts from oral glands that are indicated in Guiart's plate (1901), but the existence of which Fretter (1937) denied. Their secretion is not mucous, despite the allegation of Gabe & Prenant (1952). The lining of the oral tube here is a low epithelium covered with a thin cuticle and it is flung into two or three irregular folds. More posteriorly the oral tube is narrowed to a dorso-ventral slit passing between two bulges, one in each lateral wall. These are muscular and the epithelium over them bears a cuticle made of small oval plates each with a denticulate edge. These are the structures described by Fretter (1937) as the radula, but they are in fact the jaws, as shown by Gabe & Prenant (1953), and her criticism of Sars's (1878) description is not warranted.

Still deeper in the animal's head the gut expands once more to form the chamber of the buccal cavity. On the floor of this lies the rather small odontophore, deeply cleft on its dorsal side by a longitudinal groove. Over its dorsal and lateral surfaces is spread the radula, looking much like a cuticle raised slightly in a regular pattern, so slight is the development of the teeth. No division into different kinds is possible—indeed it is difficult to recognize the structures as teeth at all. At the posterior end the radula originates in the merest fold of the wall of the gut; it is hardly possible to regard it as a separate radular sac, although it has the correct morphological relationships of such.

The buccal mass contains no cartilages, but is largely composed of connective tissue with deposits of calcium salts and glycogen (Gabe & Prenant, 1952). Its muscular equipment, too, is much weaker than is usual and amounts to little more than a thin coat of reticulating fibres in its superficial parts. There are no mucous glands here (Fretter, 1937).

The floor and the sides of the buccal cavity are covered with a glandular epithelium and are, in consequence, white in colour. Into the cavity, dorsally, discharge two salivary glands, the histological structure of which has already been described fully by Fretter (1937).

The oesophagus shows no trace of the glandular equipment of a prosobranch, nor of torsion. It is a simple tube, with many parallel folds on its

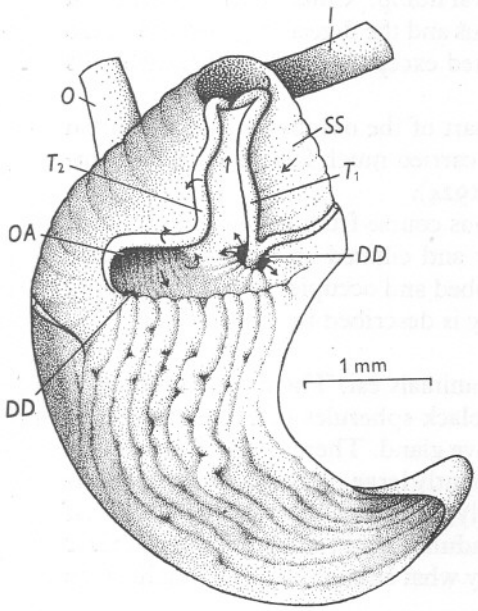

Fig. 5.

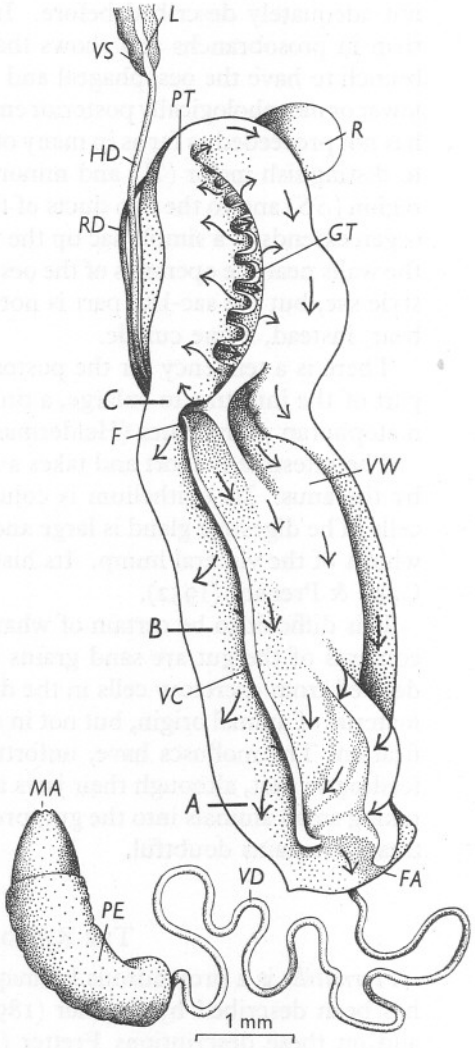

Fig. 6.

Fig. 5. *A. tornatilis*. Stomach opened dorsally. Arrows show direction of ciliary currents. For explanation of lettering see p. 585.

Fig. 6. *A. tornatilis*. The genital ducts. The mucous gland has been opened along its length. Arrows show the direction of ciliary currents. *A*, *B*, *C*, mark positions of sections diagrammatically represented in Fig. 7.

inner epithelium, which leads back to the stomach (Fig. 5), a part of the gut not adequately described before. It is, like the oesophagus, much simpler than in prosobranchs and shows the characteristic tendency of the opisthobranch to have the oesophageal and intestinal apertures close together at the lower or morphologically posterior end. The process of simplification, however, has not proceeded as far as in many other opisthobranchs and it is still possible to distinguish major (T_1) and minor (T_2) typhlosoles related to the style sac region (SS) and to the two ducts of the digestive gland (DD). The bulk of the organ extends as a simple sac up the visceral hump. Ciliary currents occur on the walls near the openings of the oesphagus and the digestive gland and in the style sac, but the sac-like part is not ciliated except in small strips. The cells bear, instead, a fine cuticle.

There is a tendency for the posterior part of the oesphagus and the initial part of the intestine to enlarge, a process carried much farther in the basommatophoran pulmonates (Heidermanns, 1924).

The intestine is short and takes a sinuous course from left to right to open by the anus. Its epithelium is columnar and ciliated and contains mucous cells. The digestive gland is large and bilobed and occupies most of the upper whorls of the visceral hump. Its histology is described by Fretter (1937) and Gabe & Prenant (1952).

It is difficult to be certain of what the animals eat. The most conspicuous contents of the gut are sand grains and black spherules of excretory matter derived from excretory cells in the digestive gland. There is also fragmentary material of animal origin, but not in sufficiently large pieces to allow of identification. The molluscs have, unfortunately, not been seen in the process of feeding so that, although their jaws and radula suggest the possibility of their raking small animals into the gut, precisely what animals it is that might so be treated remains doubtful.

The Reproductive System

A. tornatilis is a protandrous hermaphrodite. The course of the genital ducts has been described by Bouvier (1893), Pelseneer (1893) and Guiart (1901), and on these descriptions Fretter (1946) has based a comparison with the genital ducts of other gastropods. Pelseneer's figure of the ducts is adopted by Guiart. It shows the little hermaphrodite duct passing forwards from the gonad and joining the duct from a copulatory pouch in which sperms from another individual are stored. The common duct then leads to a large glandular structure associated with the female system, and comprising an albumen gland and a mucous gland. It is stated that on their way through this the outgoing sperm are confined to a gutter between two folds which, near the opening to the mantle cavity, close to form a vas deferens running forwards to the penis. Near the divergence of the vas deferens the female duct opens to the exterior.

This description may have been put together from a study of dissections (Fig. 6) for when the glandular pallial oviduct is opened by a longitudinal slit, there can be seen a gutter (*VC*) which leads from near the opening of the little hermaphrodite duct (*HD*) to the point of origin of the vas deferens (*VD*). Indeed this channel and the deferent duct appear to be confluent. Sections, however, show that this is not so. The longitudinal gutter, a ciliated and muscular channel, where gland cells are rare, arises anteriorly (without connexion with the vas deferens) near the genital aperture (*FA*) and leads posteriorly to the duct (*RD*) of the copulatory pouch or receptaculum seminis (*R*). It receives this duct, and, immediately beyond this point, opens to a small pouch which is the focal area of the female reproductive system since it also communicates with the hermaphrodite gland and with the albumen gland.

In addition to this connexion the little hermaphrodite duct, which bifurcates at its lower end, connects to a prostate gland (*P*), the existence of which was not known to Pelseneer, Guiart, or to Gabe & Prenant (1952). The prostate lies in the same position as the glandular oviduct, for the most part beneath it (*VW*), the two being so intimately connected that it is difficult to separate them. Anteriorly it is the *prostate* that narrows to the vas deferens (Figs. 6, 7, *VD*) which runs forward to the penis (Fig. 6, *PE*) on the right of the head. Thus from the point of bifurcation of the little hermaphrodite duct near its lower end male and female channels are entirely separated.

The structure of the hermaphrodite system may now be followed in greater detail. The description is based on animals which were collected during early September, whilst spawning.

The tubules of the gonad are hermaphrodite, each giving rise to eggs and sperm. The little hermaphrodite duct into which the gonad opens is ciliated throughout its course and has a moderately thick coat of circular and longitudinal muscle fibres. On functional and histological grounds it is divisible into three regions: short proximal and distal parts for conduction only, and an intermediate one which is swollen with unorientated sperm even when eggs are passing to the oviduct. The entrance to this seminal vesicle (Fig. 6, *VS*) is by way of a muscular papilla, and there is a valve which controls the passage through the duct near the glandular pallial ducts. Dissections of animals which have just spawned show the dilated vesicle as a broad duct, brown in colour, except for a longitudinal streak (*L*) marking a thin ciliated tract on the wall through which can be seen the iridescence of the underlying sperm. The streak has the same simple ciliated epithelium as the conducting regions of the duct; elsewhere the histology is more complex. Bordering the ciliated tract are gland cells with exceptionally large nuclei and containing protein spherules. The glands are placed between ciliated cells and do not seem to be present in younger animals in which only the male system is mature. Covering a much greater area on the wall opposite the ciliated tract are cells with fine, short

cilia and vacuolated cytoplasm, the vacuoles containing spherules. The important feature of these cells is that they can ingest spermatozoa; within the cytoplasm ingested gametes lose their identity and are apparently digested. Absorption of effete sperm by the epithelium of the vesicula seminalis has been recorded for *Littorina* (Linke, 1933), the Stenoglossa (Fretter, 1941) and the tectibranch *Onchidella celtica* (Fretter, 1943). The two branches of the hermaphrodite duct, after its bifurcation into male and female channels, embrace the duct of the receptaculum seminis as it opens to the ventral channel.

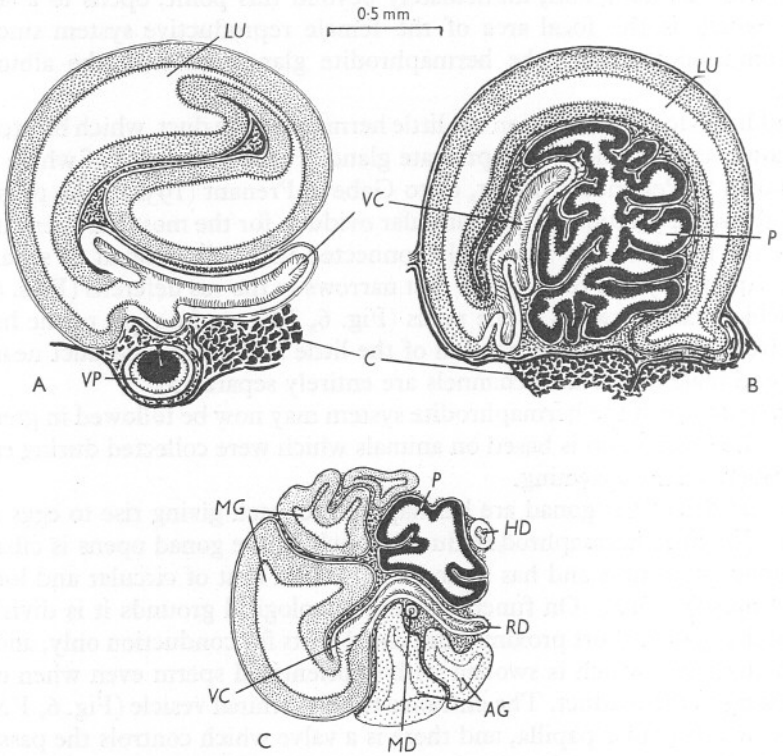

Fig. 7. *A. tornatilis*. Diagrammatic transverse sections through pallial genital ducts at levels indicated in Fig. 6. For explanation of lettering see p. 585.

The prostate (*P*) constitutes the broad pallial region of the male duct. Here the epithelium is thrown into deep longitudinal folds and it has, therefore, a large secreting area. The gland cells are all of one sort, each containing a number of protein spherules, and they alternate with small ciliated cells. This part of the male duct lies immediately above the columellar muscle and under the ventral wall of the oviduct (Figs. 6, 7, *VW*), to which it is joined by a sheet of connective tissue. Anteriorly, near the origin of the vas deferens,

the prostate narrows, and its ventral wall becomes a strongly ciliated channel without glands. In this transitional region the typical secreting cells of the upper portion are confined to the dorsal wall where they form a ridge projecting deeply into the lumen (*VP*). The deferent duct (*VD*), which passes anteriorly, has the same diameter throughout until it approaches the tip of the penis where it contracts. It is ciliated and not glandular. The penis, which it penetrates, is not invaginable as in other opisthobranchs and is unusually large. When the penis is at rest the duct coils in the haemocoel at its base and in the blood space which lies centrally within it. It is excessively muscular. The tip of the penis forms a glans kept turgid by a core of connective tissue; when the organ is relaxed the tip is slightly sunk into the surrounding tissues which form a shallow fold around it like a prepuce. The vas deferens has a straight course through the connective tissue of the glans.

The female system has characteristic tectibranch features which concern, among other things, the histology of its glands and the relative position of its parts. The gland cells alternate with ciliated cells and are never subepithelial in position. Most of them secrete mucus. The secreting areas of the oviduct comprise two distinct masses: a posterior one (*PT*) referred to by previous workers as the albumen gland and a larger and more anterior one, the mucous gland. The lumen of the former is complex, that of the latter is a simple channel. The ventral ciliated gutter (*VC*) is folded off from the secreting epithelium and it is presumably along this that the penis lies to reach the duct of the receptaculum. The penis, when extended, can attain a length which would permit this, and the channel is broad enough to contain it.

The posterior part of the pallial oviduct, the so-called albumen gland, is a composite structure: some of its tubules open into a centrally placed space which almost certainly functions as a fertilization chamber, whereas the remainder communicate with the upper part of the mucous gland since this extends alongside the albumen gland as a straight channel, reduced in size, as far as the posterior end of the oviduct. In sections the true albumen gland can be distinguished from the surrounding mucous tunic on account of the different histological features of the two. The mucous gland has a variety of secreting cells in its epithelium, though the kind which covers most of its surface is the mucous cell filled with uniform spherules. There are, however two places where the gland cells do not produce mucus. These lie along the ventral wall, where they form a narrow strip bordering the ciliated channels and anteriorly, in the epithelium which precedes the genital papilla and spread posteriorly for a short distance along the lateral walls of the gland. The secretion from this anterior end of the mucous gland is more viscid than that produced posteriorly.

Near the genital aperture the glandular epithelium stops rather abruptly, and the ventral channel enlarges to form the small papilla on which the female opening is placed. The epithelium here is ciliated as in the channel.

The plan of the various parts of the female system suggests that the site of fertilization of the eggs is at the confluence of the albumen and mucous glands. Here is the pouch, already referred to, which receives the female branch of the hermaphrodite duct and also secretion from the albumen gland; it leads directly into that part of the mucous gland into which the receptacular duct opens. It has already been noted by Bouvier (1893) dealing with *Acteon solidulus* that the receptacular duct may be filled with sperm like the receptaculum itself. In *A. tornatilis*, when spawning, sperm are orientated along the duct, dilating it considerably if they are present in large numbers, but they do not appear to occur in the receptaculum. That pouch is filled with a yellowish brown, viscous fluid, most of which responds to stains for mucus in the same way as secretion from the female tract whilst the other contents stain like prostatic fluid. From these facts it may be deduced that the receptaculum and its duct have a dual function as in the stenoglossans *Nucella* and *Buccinum* (Fretter, 1941) where it acts as a storeplace for sperm and, in distally placed outgrowths, for the destruction of superfluous sperm and granules of yolk. There is, however, no obvious absorption in *Acteon*.

The mouth of the receptaculum and its duct bear a ciliated epithelium; in the pouch, on the other hand, cells with large nuclei and cytoplasm filled with spherules in vacuoles form a columnar epithelium.

The embryos contained in each mass of spawn are individually supplied with albumen and may be seen rotating in this when they have reached the veliger stage. A membrane separates the albumen from the common jelly in which all are embedded, and a more viscid outer layer gives shape to the spawn and forms the cord for attachment. Presumably each egg is fertilized and enclosed in albumen within the fertilization chamber, and is then freed to the general cavity of the mucous gland. Dissection of an animal which is spawning shows that there are large quantities of secretion filling this gland prior to the entry of the eggs, and also shows how the eggs, enclosed in albumen, are forced into this secretion. The spawn mass is retained in the mucous gland until it is complete. The rounded distal end is the first to emerge from the genital aperture, the stalk for attachment leaving last. The glands in the part of the oviduct nearest the mouth produce much of the fluid of which the stalk is composed. The spawn swells when exposed to sea water and superficially resembles that of other tectibranchs such as *Haminea* and *Philine*. As in these genera the young of *Acteon* escape at the veliger stage.

Discussion

The earliest fossil representatives of *Acteon* are shells of *Acteon* (= *Cylindrites*) *acutus* (Sow.) from the calcareous portion of the Great Oolite (Bathonian). *A. tornatilis* does not appear until the Miocene when it occurs in deposits in France, Austria and northern Italy. These may well have been the most

ancient of opisthobranchs and have had a considerable range in European waters. A century ago Forbes & Hanley (1853) gave the distribution of *A. tornatilis* as throughout the British Isles, with a general range from Norway to the Mediterranean. How abundant the animal then was the authors do not say, but our general conception of the distribution of *Acteon* to-day is that of a rather uncommon animal living in sand at L.W.S.T. and to a depth of sixty or more fathoms, since only very occasional specimens are dredged at Plymouth, Port Erin or Millport. Such infrequent capture, however, may have little meaning since an aquarium known to contain many specimens may exhibit few on account of their burrowing habit; and at Pendine sands the numbers obtained without digging give no idea of their real frequency. They are, in fact, abundant at this spot and presumably in similar habitats throughout Britain.

The habit of burrowing is shared with other opisthobranchs such as *Haminea*, *Scaphander* and *Philine*. As adaptations for this the foot is powerful and the tentacles form lobes round the mouth for shovelling sand away; the cephalic tentacles become flat and spoon-shaped and are used for the same purpose and for blocking the entrance to the mantle cavity, and the eyes sink into the tissues of the head. It may be partly in connexion with this habit, too, that these animals (along with *Scaphander* and *Acera*) have evolved the pallial caecum which runs up the spiral of the shell alongside the visceral hump. The most obvious function for such a structure would be to aid respiration, not only by itself providing a respiratory surface, but also by leading a current of water to the neighbourhood of the upper parts of the visceral hump. It may be noted that the gill is small and is built on the usual opisthobranch rather than upon the usual prosobranch pattern. This may well be a less efficient type of gill since in most cases where it occurs the body of the mollusc is naked or only partially covered by shell so that secondary respiratory surfaces are large. *Acteon*, however, is a type of opisthobranch in which the body may be completely withdrawn into a shell and in which a great deal of the surface of the body is covered even at maximal expansion. In these circumstances accessory respiratory surfaces within, or mechanisms for accelerating the flow of water through, the mantle cavity may be necessary and the caecum may be such a device. In the pyramidellids (Fretter & Graham, 1949), in *Omalogyra* and *Rissoella* (Fretter, 1948) there is no gill at all, and it is fundamentally the same method which is used in each of these animals to compensate for its absence—longitudinal ridges of ciliated epithelium in the neighbourhood of the hypobranchial gland, which produce a strong exhalant current. These animals, therefore, differ from most molluscs in replacing an inhalant by an exhalant force as the main driving power of their respiratory water-current in the mantle cavity. This strong exhalant stream is exploited to some extent in all these animals for the evacuation of excretory matter contained in amoebocytes. These abound in the blood vessels of the pallial caecum in *Acteon*, from which they escape to the ciliated grooves by diapedesis and are

swept down to the exhalant siphon. A similar extrusion of blood cells laden with excreta was noted by Fretter & Graham (1949) in the pyramidellids. It may partly be due to the strength of the current, too, that the anus has retained its primitive position in the innermost part of the mantle cavity instead of extending to near the edge of the mantle skirt. The similarity of these molluscs as regards their production of pallial water currents offers further evidence that pyramidellids and *Omalogyra* and *Rissoella* are opisthobranchs.

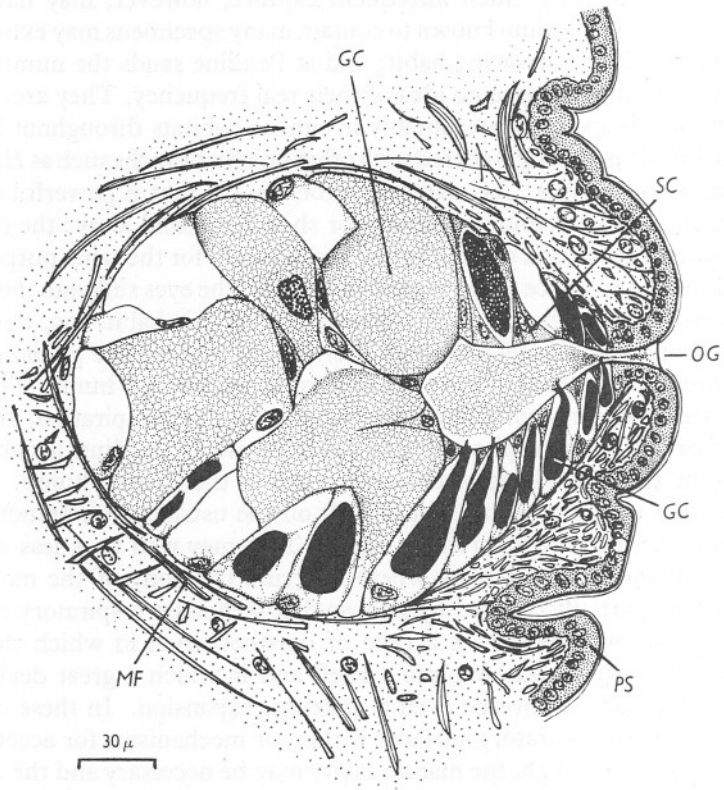

Fig. 8. *Siphonaria* sp. Vertical section of lateral pedal gland. For explanation of lettering see p. 585.

Like many opisthobranchs *Acteon* has a number of special glands associated with the mantle cavity and the mantle itself. When irritated it liberates a purple secretion from the hypobranchial gland and toxic secretions from the repugnatorial glands on the mantle skirt. Glands of almost identical histological nature may be found at the pallial edge or on the sides of the foot of many nudibranchs, opisthobranchs and marine pulmonates. Fig. 8 illustrates such a gland in *Siphonaria*. *Onchidella celtica* (Joyeux-Laffuie, 1882; von Wissel, 1898; Fretter, 1943) possesses very similar ones except that they discharge by

nger ducts. Many limpet-like prosobranchs, too, in which the conical shape f the shell and the absence of operculum prevent the animal from shutting up ntirely when disturbed, possess similar or even more elaborate glands. These ıave been described for *Calyptraea* and *Crepidula* by Graham (1954), but perhaps the most complex arrangement is to be met in *Patelloida virginea*. If the mantle edge of this animal be examined it will be found to be thickly set with a series of unicellular glands round its entire length (Fig. 9). The margin of the mantle also carries, at regular intervals, groups of special cells carrying

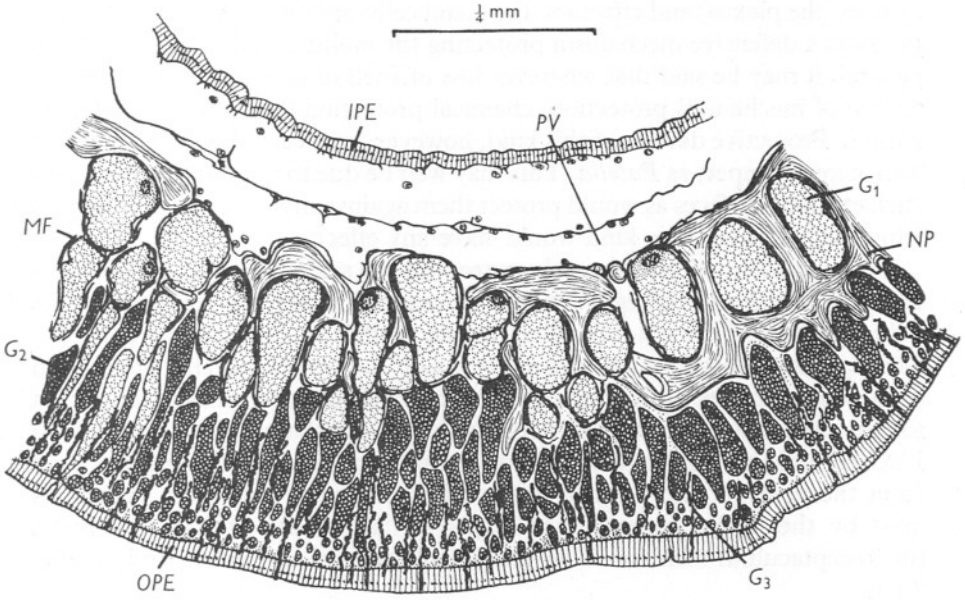

Fig. 9. *Patelloida virginea*. Tangential section of part of edge of the mantle skirt showing the nerve plexus, gland cells and muscle fibres around gland cells. The sensory organs on the outer pallial epithelium do not occur at this level. For explanation of lettering see p. 585.

long, motionless cilia set on slight projections. Even when the mollusc is closely pressed to the underlying rock these ciliated structures project a little beyond the edge of the shell. There are three types of gland cell. The first, the most conspicuous and the commonest, is a flask-shaped gland, bright white in colour, the body of the flask set deep in the pallial tissue and communicating with the edge by a long duct. The body of each of these cells is surrounded by a basketwork of muscle fibres and the cytoplasm is granular. On contraction of the muscles threads of white secretion are squeezed into the water where they retain their identity for some time. Exactly similar secretion comes from the glands in *Acteon*, *Onchidella* and the calyptraeids, and experiment by one of us (V.F.) has shown that this is rapidly toxic to small animals.

The two other types of gland in *Patelloida* lie in banks successively nearer the mantle edge and they have correspondingly shorter ducts. The second is also a large, pear-shaped gland, but it lacks the muscular coat of the first type, its cytoplasm is homogeneous and uncoloured and its secretion appears to disperse. The third type is a bright red in a living mollusc and the gland is short and tubular. All these glands, but the first type in particular, are surrounded by a great plexus of nerves connected to the main pallial nerve, which also involves the groups of sensory cilia set on the mantle edge. It seems likely that here is a local arrangement of sense organs (the ciliated cells), nerve centres (the plexus) and effectors (the gland cells and their muscle coat) which provides a defensive mechanism protecting the mollusc against predators. In general, it may be said that wherever loss of shell or operculum deprives the animal of mechanical protection, chemical protection has appeared as a substitute. Protective devices of this kind, however, are noticeably absent in such well-known limpets as *Patella*. This may well be due to their ability to live on such exposed surfaces as would protect them against any potential predator on which secretions of this kind would have any effect.

In the majority of prosobranch gastropods the sexes are separate, fertilization is internal and the eggs are laid in capsules which are secreted by the pallial region of the oviduct. This region traffics in both sperm and ova: although its elaborations are apparently mainly concerned with the production of secretions for capsules, they also comprise well-defined tracts and pouches for the manipulation of sperm received during copulation. In *Littorina* and *Nucella*, and in other genera, the bursa copulatrix receives the seminal fluid from the penis of the partner and a ciliated groove, free from glands, is used by the sperm to travel the length of the pallial oviduct and reach the receptaculum seminis. The receptaculum is at the inner end of this duct.

In the opisthobranchs hermaphroditism is the rule. A hermaphrodite gland replaces the unisexual gonad, and the ducts which pass its products to the exterior must deal with outgoing as well as incoming spermatozoa, and produce the gelatinous secretions which characterize the spawn. In detorted gastropods, both pulmonates and opisthobranchs, the pallial duct has separated from the body-wall which restricted its further evolution in the prosobranchs and, freed to the haemocoel, it attains a further degree of complexity and a relatively larger size. The tectibranch *Acteon tornatilis* is an exception. Although it has an assemblage of opisthobranch characters which include hermaphroditism, its genital ducts are still linked to the musculature of the body-wall and have a straight course from gonad to genital aperture. In some tectibranchs such as *Philine*, *Scaphander* (Lloyd, 1952) and *Aplysia* (Eales, 1921) the plan of the hermaphrodite duct is similar to that of the female prosobranch in that it is single from gonad to genital aperture, and is simply divided by longitudinal ridges into sperm-conducting and egg-conducting

grooves; whilst the groove which conducts sperm to the exterior passes forwards from the female opening to the penis on the right side of the head, giving an open vas deferens—a condition which may be primitive for the prosobranchs (Fretter, 1946) as well as the opisthobranchs. *Acteon* is not primitive in this respect since from its origin at the distal end of the gonadial duct the vas deferens is closed, and, throughout its course, is separated from the oviduct. The vas deferens leads to the penis which is, of course, unaffected by detorsion. In *Acteon* the mantle cavity is large and, as in prosobranchs, offers protection to the penis, whereas in other tectibranchs with a reduced mantle cavity, the penis, when not in use, lies retracted in a sheath sunk into the haemocoel.

Perhaps it is on account of the separation of pallial vas deferens and oviduct, and the possession of a penis of exceptional size, that the arrangement of sperm pouches in the female tract of *Acteon* differs from that of *Philine* and *Scaphander* in which the prosobranch plan is adhered to. In these two genera the homologue of the bursa copulatrix of prosobranchs is not far from the female opening, and is characterized by the possession of a long, muscular duct and pigmented contents of the pouch. If copulation be reciprocal the insertion of the penis into the duct, which receives the seminal fluid, would not block the passage of the hermaphrodite tract nor interfere with the flow of spermatozoa to the recipient's own penis. A deeper insertion of the penis to the vicinity of the receptaculum might well do so, however. Now in *Acteon* male and female tracts are separate ducts and the penis is long enough to extend to the inner end of the pallial oviduct: a bursa near the female opening is not developed and it is assumed that the penis uses the ventral channel of the oviduct to deposit sperm in the duct of the receptaculum, where they may be found orientated soon after copulation. In structure and in function the receptaculum resembles the bursa and not the receptaculum of *Philine* and *Scaphander*—the duct long and muscular, the pouch spherical and conspicuous on account of its size and pigmented contents. These comprise prostatic fluid received during copulation and sometimes other secretions from the genital ducts. Secretion is apparently also produced by the epithelium of the pouch itself: this may agglutinate the debris or cause the breakdown of the proteins (Lloyd, 1952). The ultimate fate of the contents is unknown: it may be that they are passed into the surrounding haemocoel and so utilized by the animal. A receptaculum and a bursa (the spermatheca of Eales) are developed in *Aplysia*, the former receiving sperm directly from the penis, the latter accumulating unwanted material introduced with the sperm and perhaps absorbing it or discharging it to the exterior (Eales, 1921).

As in other tectibranchs like *Onchidella* (Fretter, 1943), *Aplysia* (Eales, 1921), *Philine* and *Scaphander* (Lloyd, 1952) the albumen gland is an appendage of the female tract: this contrasts with prosobranchs where the eggs traverse the cavity of the gland.

In a comparison between the genital ducts of prosobranchs and opisthobranchs Fretter (1946) regarded the arrangements of the ducts in *Acteon* as showing remarkable agreement with those of the female *Littorina* and the Stenoglossa. The comparison was based on Guiart's (1901) description of *Acteon tornatilis* in which the prostatic region of the vas deferens underneath the pallial oviduct was not mentioned and the ventral channel of the oviduct was regarded as the vas deferens. Now that another investigation of that species has been made it appears that the reproductive system displays a mixture of primitive and advanced characters. The former, which are shared with the prosobranchs, relate to the large and uninvaginable penis and the association of the pallial ducts with the body-wall. The latter concern the loss of the large hermaphrodite duct with the separation of male and female channels, a character which is unusual in the tectibranchs, though found in higher opisthobranchs.

The alimentary tract shows, in general, an apparent simplicity, even the radular structure having a superficial suggestion of extreme primitiveness. When, however, the gut is compared with that of a prosobranch the loss of all trace of oesophageal glands, of all signs of torsion, the complex histology of the salivary and digestive glands and the modification of the stomach indicate that the simplicity must be secondary and the radular structure advanced in relation to a (presumably) carnivorous way of life. The position of the anus is, in fact, the sole genuinely primitive character which the gut appears to possess, and there is no very obvious reason why that might not also be secondary.

Acteon, therefore, is a gastropod with a medley of prosobranch and opisthobranch characters. The external facies is chiefly prosobranch, with its emphasis on shell and operculum, its large mantle cavity still facing well forward and the uninvaginable penis. Apart from the gill, which is opisthobranch in organization, the main departures from the usual prosobranch appearance might well be directly due to adaptation for burrowing.

So far as internal anatomy is concerned the nervous system, with its streptoneury and lack of fusion of ganglia, is frankly prosobranch in plan; and so is the auricle lying anterior to the ventricle. The reproductive system, on the other hand, shows a mixture of characters and the gut is distinctly advanced.

Acteon and the Acteonidae, in view of this mixture of characters, must always occupy a debatable position in any clear-cut classification. There is, however, a gentler passage, with animals showing more intermediate patterns of structure, from *Acteon* to the more characteristic opisthobranchs than from *Acteon* to the more characteristic prosobranchs. As a matter of convenience, therefore, it seems appropriate to leave the animal where it has long been placed, as the most primitive type of living opisthobranch. This work on *Acteon*, and recent work on *Siphonaria* (Hubendick, 1947), on the ellobiid pulmonates (Morton, 1954), on *Onchidella* (Fretter, 1946), on *Omalogyra* and *Rissoella* (Fretter, 1948), on the pyramidellids (Fretter & Graham, 1949) and

on other tectibranchs (Lloyd, 1952), demonstrate, however, with great emphasis, how close the relationship of all the three major groups of gastropods is at some central point such as this. It seems likely that further research will show that it is from some archaeogastropod nucleus, represented to-day by such molluscs as the trochids, that all these groups have arisen.

This work was carried out at the Plymouth Laboratory of the Marine Biological Association. Our thanks are due to Birkbeck College for a grant towards the cost of publication.

Summary

Acteon tornatilis is an opisthobranch mollusc which burrows in sand, using foot, labial and cephalic tentacles in the process. The last also help to exclude sand from the mantle cavity, which is extended into a caecum coiling alongside the visceral hump, presumably used for respiration but also for excretion. The main pallial water current is an exhalant one on the right.

The mantle skirt carries, on the left, numerous repugnatorial glands with toxic secretions.

Labial glands lie at the mouth and the buccal cavity contains jaws and a reduced buccal mass and radula. Into it open salivary glands the structure of which is like that of the pyramidellids. The oesophagus shows traces neither of glands nor of torsion whereas the stomach, though simplified, has resemblances to that of a prosobranch.

The reproductive system is shown to be different from previous descriptions. Male and female ducts are separate from the lower end of the little hermaphrodite duct. The former passes to a prostate from which a vas deferens leads to a large uninvaginable penis; the latter has associated albumen and mucous glands and there is a receptaculum seminis to the duct of which a ventral channel leads from the female aperture in the mantle cavity.

Discussion of these aspects of the animal's structure confirms its position as the most primitive of the opisthobranchs.

References

Bouvier, E. L., 1893. Sur l'organisation des Actéons. *C.R. Soc. Biol., Paris*, T. 5, pp. 25–30.

Eales, N. B., 1921. *Aplysia*. L.M.B.C. Memoir xxiv, 84 pp. Liverpool: University Press.

Forbes, E. & Hanley, S., 1853. *A History of British Mollusca and their Shells*. London: van Voorst.

Fretter, V., 1937. The structure and function of the alimentary canal of some tectibranch molluscs, with a note on excretion. *Trans. Roy. Soc. Edinb.*, Vol. 59, pp. 599–646.

—— 1941. The genital ducts of some British stenoglossan prosobranchs. *J. Mar. biol. Ass. U.K.*, Vol. 25, pp. 173–211.

FRETTER, V., 1943. Studies in the functional morphology and embryology of *Onchidella celtica* (Forbes & Hanley) and their bearing on its relationships. *J. Mar. biol. Ass. U.K.*, Vol. 25, pp. 685–720.

—— 1946. The genital ducts of *Theodoxus*, *Lamellaria* and *Trivia*, and a discussion on their evolution in the prosobranchs. *J. Mar. biol. Ass. U.K.*, Vol. 26, pp. 312–51.

—— 1948. The structure and life-history of some minute prosobranchs of rock pools: *Skeneopsis planorbis* (Fabricius), *Omalogyra atomus* (Philippi), *Rissoella diaphana* (Alder) and *Rissoella opalina* (Jeffreys). *J. Mar. biol. Ass. U.K.*, Vol. 27, pp. 597–632.

FRETTER, V. & GRAHAM, A., 1949. The structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *J. Mar. biol. Ass. U.K.*, Vol. 28, pp. 493–532.

GABE, M. & PRENANT, M., 1952. Quelques particularités histologiques d'*Acteon tornatilis* L. *Bull. Soc. zool. Fr.*, T. 77, pp. 220–8.

GABE, M. & PRENANT, M., 1953. Données morphologiques sur la région antérieure du tube digestif d'*Acteon tornatilis* L. *Bull. Soc. zool. Fr.*, T. 78, pp. 36–44.

GRAHAM, A., 1954. The anatomy of the prosobranch *Trichotropis borealis* Broderip & Sowerby, and the systematic position of the Capulidae. *J. Mar. biol. Ass. U.K.*, Vol. 33, pp. 129–44.

GUIART, J., 1901. Contribution à l'étude des gastéropodes opisthobranches et en particulier des céphalaspides. *Mém. Soc. zool. Fr.*, T. 14, pp. 5–219.

HEIDERMANNS, C., 1924. Über den Muskelmagen der Süsswasserlungenschnecken. *Zool. Jb. Physiol.*, Bd. 41, pp. 335–424.

HUBENDICK, B., 1947. Phylogenie und Tiergeographie der Siphonariidae. Zur Kenntnis der Phylogenie in der Ordnung Basommatophora und des Ursprungs der Pulmonatengruppe. *Zool. Bidr. Uppsala*, Bd. 24, pp. 1–216.

JOYEUX-LAFFUIE, J., 1882. Organisation et développement de l'oncidie *Oncidium celticum* Cuv. *Arch. Zool. exp. gén.*, T. 10, pp. 225–383.

LINKE, O., 1933. Morphologie und Physiologie des Genitalapparates der Nordseelittorinen. *Wiss. Meeresuntersuch. Abt. Helgoland* (N.F.), Bd. 19, No. 5, 60 pp.

LLOYD, H. M., 1952. A study of the reproductive systems of some opisthobranchiate molluscs. Ph.D. thesis, University of London.

MORTON, J. E., 1954. The evolution of the Ellobiidae, with a discussion on the origin of pulmonates. *Proc. zool. Soc. Lond.* (in press).

PELSENEER, P., 1893. Sur le genre *Actaeon*. *Ann. Soc. roy. malacol. Belg.*, T. 28, 3 pp.

—— 1894. Recherches sur divers opisthobranches. *Mém. Sav. étr. Acad. R. Belg.*, T. 53, 157 pp.

PERRIER, R. & FISCHER, H., 1911. Recherches anatomiques et histologiques sur la cavité palléale et ses dépendances chez les Bulléens. *Ann. Sci. nat. Zool.*, 9th ser., T. 14, pp. 1–189.

SARS, G. O., 1878. *Bidrag til Kundskaben om Norges Arktiske Fauna. I. Mollusca regionis arcticae Norvegiae*. Christiania.

WISSEL, K. VON, 1898. Beitrage zur Anatomie der Gattung *Oncidiella*. *Zool. Jb.*, Suppl. 4, pp. 583–640.

Abbreviations used in the Figures

A anus
AG tubules of albumen gland
AM amoebocyte
C columellar muscle
CC cut end of pallial caecum
CM pallial caecum
CR ciliated ridge
CT ctenidium
DD opening of duct of digestive gland
DG digestive gland
F ventral channel leading to duct of receptaculum and to fertilization chamber
FA lips of female aperture
FO female opening
FT foot
G gonad
G_1, G_2, G_3 different types of pallial gland cells in *Patelloida*
GC gland cell
GT opening of glandular tubules into upper part of mucous gland
H hypobranchial gland
HD hermaphrodite duct
I intestine
IPE inner pallial epithelium
L longitudinal strip of ciliated epithelium in hermaphrodite duct
LT labial tentacle
LU lumen of mucous gland
M mouth
MA opening of vas deferens at tip of penis
MC mucous cell
MD male deferent channel, a bifurcation of the little hermaphrodite duct
MF muscle fibre
MG tubules of mucous gland
NP nerve plexus
O oesophagus
OA oesophageal aperture
OG opening of repugnatorial gland
OPE outer pallial epithelium
P prostate gland
PE penis
PG pallial genital duct
PS pedal surface
PT posterior part of pallial oviduct formed of albumen gland and tubules of mucous gland
PV pallial vein
R receptaculum seminis
RD duct of receptaculum seminis
RG repugnatorial gland
S exhalant siphon
SC supporting cell
SS style sac region of stomach
ST stomach
T cephalic tentacle
T_1, T_2, major and minor typhlosoles
V blood space of caecum
VC ventral channel
VD vas deferens
VP vas deferens with prostatic gland cells projecting from the dorsal wall
VS vesicula seminalis
VW ventral wall of mucous gland overlying prostate

J. Mar. biol. Ass. U.K. (1954) 33, 587–588
Printed in Great Britain

THE POSITION OF *PONTOPHILUS ECHINULATUS* (M. SARS) IN THE CRANGONIDAE

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On 26 November 1953 the new research ship *Sarsia* on her first cruise collected a number of *Pontophilus echinulatus* (M. Sars) (with an Agassiz trawl, depth 100 fm., La Chapelle, Bay of Biscay). Mr G. R. Forster, of the Plymouth Laboratory, noted that these did not fit into the key given by Kemp (1910) in that they have in the male a distinct appendix interna at the base of the four last pairs of pleopods, while in the female this is absent, the inner ramus being usually undivided, although occasionally an indistinct division can be made out. Otherwise they agree with Kemp's diagnosis.

Kemp (1910) notes M. Sars 1861 as authority, but this is only a preliminary description. In Sars (1868), given in Kemp's (1910) bibliography but not under the species, it is seen that he describes both male and female exactly as in the specimens from *Sarsia*, the male having a distinct appendix interna on the last four pairs of pleopods and an appendix masculina also on the second pair, the female pleopods having very short inner rami, except in the first which is long, and there is no appendix interna. Sars's figures of these limbs are very good. Kemp (1911) joins *Philocheras* with *Pontophilus*, and in this he is now followed by most authors.

Kemp (1916) reviewed the pleopods in the genus *Pontophilus*, as far as he could with the material he had in India, and he proposed certain groups according to the presence or absence of the appendix interna. His material was deficient in males of some species, including *P. echinulatus* which he suggests would probably belong to group V in which the appendix interna is absent in both sexes.

This problem was referred to Dr I. Gordon, who very kindly examined some of the material in the British Museum and found that both male and female agreed with Sars's figures. She also found that most of Kemp's Irish Fisheries' material is immature, although one (Helga CXXI), presumably that at the foot of p. 145 in Kemp (1910), had an appendix interna on pleopods 2–5, probably not noted by Kemp. Thus the immaturity of these Irish specimens accounts for the fact that *P. echinulatus* (as *Philocheras*) is placed among those species which have no appendix interna (Kemp, 1910, pp. 135, 146).

As it is now certain that *P. echinulatus* has in the male a distinct appendix interna and none at all in the female, it follows that of the groups formed by

Kemp *P. echinulatus* should be placed in group III in which are included his four new Indian species *P. lowisi*, *P. pilorus*, *P. candidus* and *P. plebs*. It is interesting to find that Holthuis (1952) describes the pleopods of *P. prionolepis* n.sp. exactly as in Sars's description of *echinulatus*. He states that *P. prionolepis* is closely related to *P. lowisi* Kemp which is in group III, thus emphasizing the probable correct position of *P. echinulatus*. However, *P. sculptus* (Bill) seems very close to *echinulatus* except for the pleopods. Kemp (1911) revised his diagnosis of *P. sculptus*, and again in 1916, as it was found to have an appendix interna on the last four pairs of pleopods in the male and on some of them in the female. In Kemp's (1911) table, if we substitute the presence of an appendix interna instead of the absence in *P. echinulatus*, we see that *echinulatus* and *sculptus* agree in every other point. Kemp (1916) shows that the female *sculptus* has an appendix interna on the second and third pleopods and a rudimentary one on the fourth, thus differing from *echinulatus*, and therefore that species cannot be included in group II.

It follows from the above notes that *P. echinulatus* should take a different position and no longer be classified with *P. bispinosus* and *P. trispinosus*, if the appendix interna is as important in showing relationship as Kemp suggests. *P. echinulatus* does not fit into group II and must, if placed in any of the groups, be included in group III with Kemp's four Indian and Holthuis's African species, none of which appear to be so near it as do *P. sculptus*, *P. bispinosus* and *P. trispinosus*. Except for the appendix interna, *P. echinulatus* seems to agree very well with these species.

The best one can do at present seems to be to keep Kemp's groups I–V, emphasizing the fact that *P. echinulatus* seems to be nearest *P. sculptus*, *P. bispinosus* and *P. trispinosus*, although these are in different groups.

Group I (with appendix interna in both sexes).

Group II (with appendix interna in ♂ and partly in ♀): *P. sculptus*, etc.

Group III (with appendix interna in ♂ only): *P. echinulatus*, etc.

Groups IV and V (no appendix interna): *P. bispinosus*, *P. trispinosus*, etc.

REFERENCES

HOLTHUIS, L. B., 1952. Crustaces decapodes, Macrures. *Résult. sci. Expéd. océanogr. Belge dans l'Atlantique sud* (1948–49). Vol. 3, Fasc. 2, 88 pp.

KEMP, S., 1910. The Decapoda Natantia of the coasts of Ireland. *Sci. Invest. Fish. Br. Ire.*, 1908, No. 1, 190 pp.

—— 1911. Notes on Decapoda in the Indian Museum. II. Description of two new Crangonidae with observations on the mutual affinities of the genera *Pontophilus* and *Philocheras*. *Rec. Indian Mus.*, Vol. 6, pp. 5–12.

—— 1916. Notes on Crustacea Decapoda in the Indian Museum. VI. Indian Crangonidae. *Rec. Indian Mus.*, Vol. 12, pp. 355–384.

SARS, M., 1861. Bemerkongen om Crangonidae. *Forh. VidenskSelsk. Krist.*, Aar 1861, pp. 179–187. [Not seen.]

—— 1868. Bidrag til kundskab om Christiania-Fjordens Fauna, Crustacea. *Nyt Mag. Naturv.*, Bd 15, 104 pp.

J. Mar. biol. Ass. U.K. (1954) 33, 589–612 *N.I.R.D. Paper No.* 1536
Printed in Great Britain

VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

II. STUDIES OF SEASONAL VARIATIONS IN SOME MARINE CRUSTACEA

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(Text-figs. 1–4)

Introduction

We have previously discussed the richness in vitamin A of the euphausiids, *Meganyctiphanes norvegica* (M. Sars) and *Thysanoessa raschii* (M. Sars), (Fisher, Kon & Thompson, 1952, 1953). We found that astaxanthin was the principal carotenoid pigment and that β-carotene was present in minute amounts, or not at all, in free-swimming animals of these two species, in *Meganyctiphanes norvegica* taken from the stomachs of arctic baleen whales (Kon & Thompson, 1949*a*) and in *Euphausia superba* Dana from the stomachs of antarctic baleen whales (Kon & Thompson, 1949*b*).

Our findings contradicted those of Wagner (1939), who had claimed to have found high concentrations of β-carotene in euphausiids from the stomachs of arctic whales, but made no mention of the typical crustacean pigment, astaxanthin.

Moore (1950), in discussing our work, stated that 'in view of Goodwin's observations on the variations in the relative concentrations of astaxanthin and β-carotene in locusts it might be unwise to discredit Wagner's claim to have isolated β-carotene from krill without fully exploring the effects of age and season'. During his study of locusts, Goodwin (1950) found that, in developing eggs, β-carotene was present initially but was gradually replaced from the 6th or 7th day of incubation by astaxanthin. As the hoppers approached maturity, however, the astaxanthin content remained steady whereas β-carotene increased, especially so in the adult phases.

The work described in this paper was designed among others to study whether similar variations in carotenoids occurred in some euphausiid and decapod Crustacea, and whether in this way Wagner's findings could be reconciled with ours.

Material and Methods of Collection

A series of collections of *Meganyctiphanes norvegica* and *Thysanoessa raschii* was taken from Loch Fyne at about monthly intervals for 18 months, and the

analytical results have now provided information about possible seasonal and developmental variations in the concentrations of vitamin A and carotenoids. Similar studies were made on *Nephrops norvegicus* Leach and *Crangon allmani* Kinahan taken during the same period from the Clyde Sea area, on *C. vulgaris* L. from Plymouth and Conway and on the eyes of *Homarus vulgaris* M.-Edwards.

The euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, were taken from lower Loch Fyne with a 1 m stramin net at depths of 80–90 fathoms (146–165 m) at approximate intervals of 1 month from February 1951 until July 1952. Before the animals were killed each monthly haul was separated into size-groups by length, measured from the tip of the rostrum to the base of the telson, at 2 mm intervals. Intermediate lengths were grouped with the next smaller size.

Collections of *Crangon allmani* and *Nephrops norvegicus* were also made during these visits to Loch Fyne, the former caught with the Agassiz trawl and the latter with the otter trawl. Groups of the brown shrimp, *Crangon vulgaris*, were sent to us at frequent intervals, at first from the Plymouth Laboratory and later from the Fisheries Experiment Station at Conway, during the period from August 1951 to February 1953. One group was collected at Burnham-on-Crouch in March 1952.

All these specimens were preserved as soon as possible after catching by boiling in sea-water, as described by Fisher *et al.* (1952). The euphausiids and *Crangon* spp. were separated into eyes and bodies before analysis and the specimens of *Nephrops* were dissected into several parts. We were also supplied with eyes of *Homarus vulgaris* taken from lobsters boiled for catering.

Analytical Methods

Fat was extracted, and vitamin A and carotenoids separated from it by saponification and chromatography on alumina columns, as outlined by Fisher *et al.* (1952). Total carotenoids were measured with the photoelectric spectrophotometer of Thompson (1949), and vitamin A was determined by measurement with this instrument of the intensity of the blue colour produced in the Carr-Price reaction.

Vitamin A ester and alcohol were separated by chromatography before saponification (see Fisher *et al.*, 1952) in the analyses of groups of *Thysanoessa raschii* collected on 5–6 September 1951, and of both this species and *Meganyctiphanes norvegica* collected on 17–18 October 1951, and in subsequent months. Similar treatment was given to some groups of the decapod Crustacea.

We have also analysed groups of eyes of *M. norvegica* for retinene by a method used successfully to separate this compound from cephalopod eyes, to be fully described in a later paper. No retinene was detected in the eyes of *Meganyctiphanes*.

Results

Euphausiacea

The graphs in Figs. 1 and 2 show the vitamin A and astaxanthin concentrations in relation to the length of the specimens in each size-group of *M. norvegica* (Fig. 1) and *Thysanoessa raschii* (Fig. 2). The average weight per specimen of each size-group is also plotted. The detailed results of all the analyses on which these graphs are based have been recorded by Fisher (1953).

Vitamin A was found predominantly in the eyes of both species, varying from 66 to 100% of the total quantity in *Meganyctiphanes norvegica* and from 65 to 98% in *Thysanoessa raschii*, and amounting usually to over 90%. Astaxanthin or its esters were the only carotenoids in any of the size groups of either species. In *Meganyctiphanes* the eyes contained from 18 to 77% of this pigment, the average proportion being about 50% of the total in the whole animal; in *Thysanoessa*, from 33 to 94% of the total astaxanthin was in the eyes, the average value being about 65%.

As mentioned already, most of the specimens were subjected to the full analytical treatment of Fisher *et al.* (1952) and the relative proportions of vitamin A alcohol and ester were determined. In *T. raschii* collected in September the ratio of vitamin A ester to alcohol varied between 6:1 and 28:1 in the eyes of different size-groups and between 1:1 and 2:1 in the bodies; in October the eyes had from 6:1 to 11:1 and the bodies 1:1 to 3:1. In the eyes of *Meganyctiphanes norvegica* in October, the ratio varied between 2:1 and 16:1, being about 6:1 in most groups, whereas in the bodies it was 1:1 in all groups except one in which it was 3:1. These considerably higher ratios of vitamin A ester to vitamin A alcohol in the eyes than in the bodies observed during September and October were also found in all the remaining groups analysed until the investigation ended in July 1952.

The size-groups studied included the later furcilia stages through to the largest adult forms. In none of the groups of either species was β-carotene ever found in more than minute traces. There was certainly never enough β-carotene to measure, and the measurement would have been possible even if only 1 μg of β-carotene had been present in an extract from several thousand euphausiids. It is unlikely, therefore, that β-carotene is ever present in *M. norvegica* and *Thysanoessa raschii* in quantities that would justify Wagner's (1939) claim.

In many of the groups of *Meganyctiphanes norvegica* collected from January to July 1952 and in those of *Thysanoessa raschii* collected from March to July 1952 the sexes were analysed separately, but no consistent differences between them in their contents and concentrations of vitamin A and astaxanthin were found and indeed, on many occasions, the results for the two sexes were remarkably close. We have obtained similar results for *Meganyctiphanes norvegica* collected at Monaco (Fisher *et al.*, 1953).

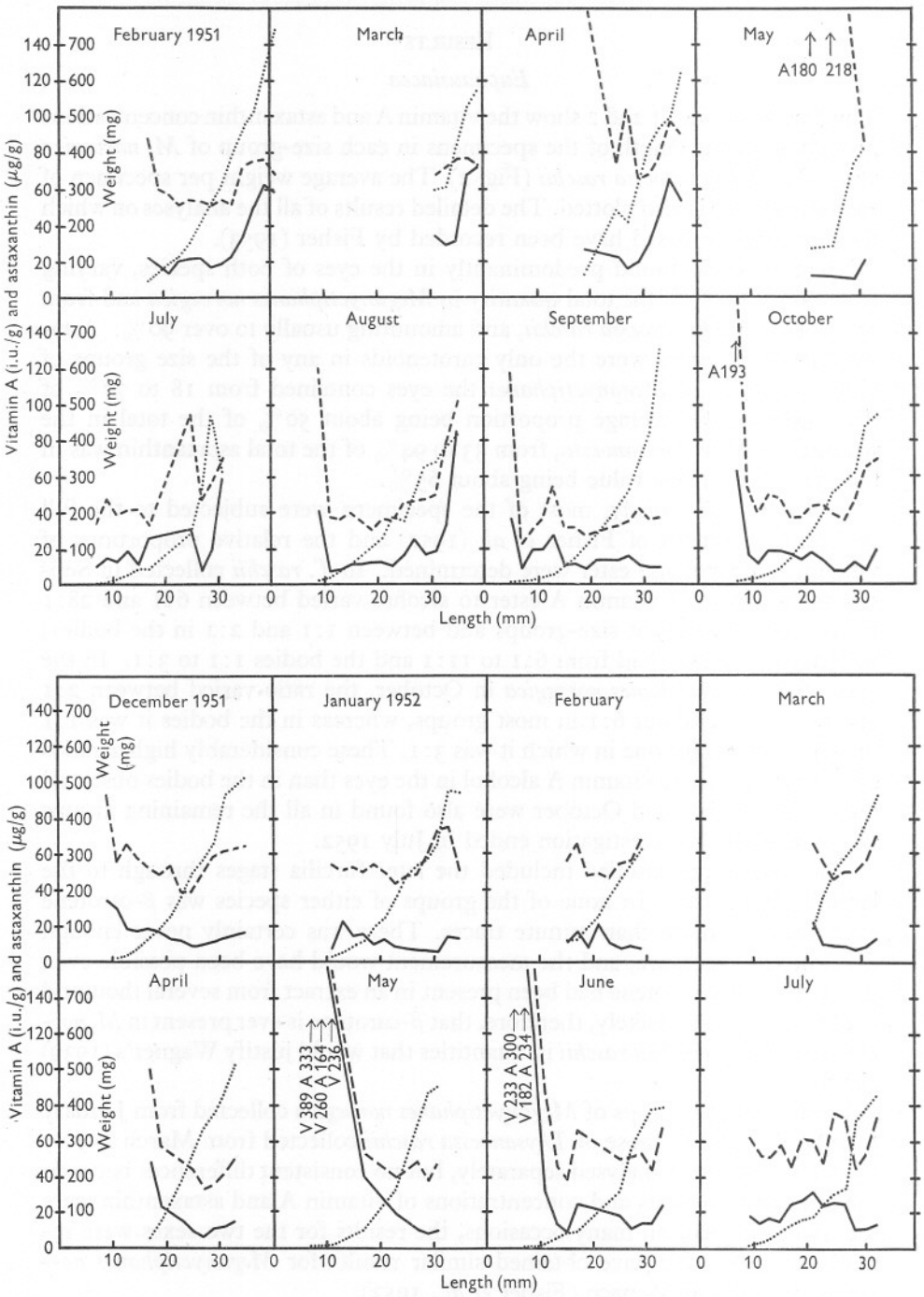

Fig. 1. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Meganyctiphanes norvegica*. V, vitamin A; A, astaxanthin; ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight.

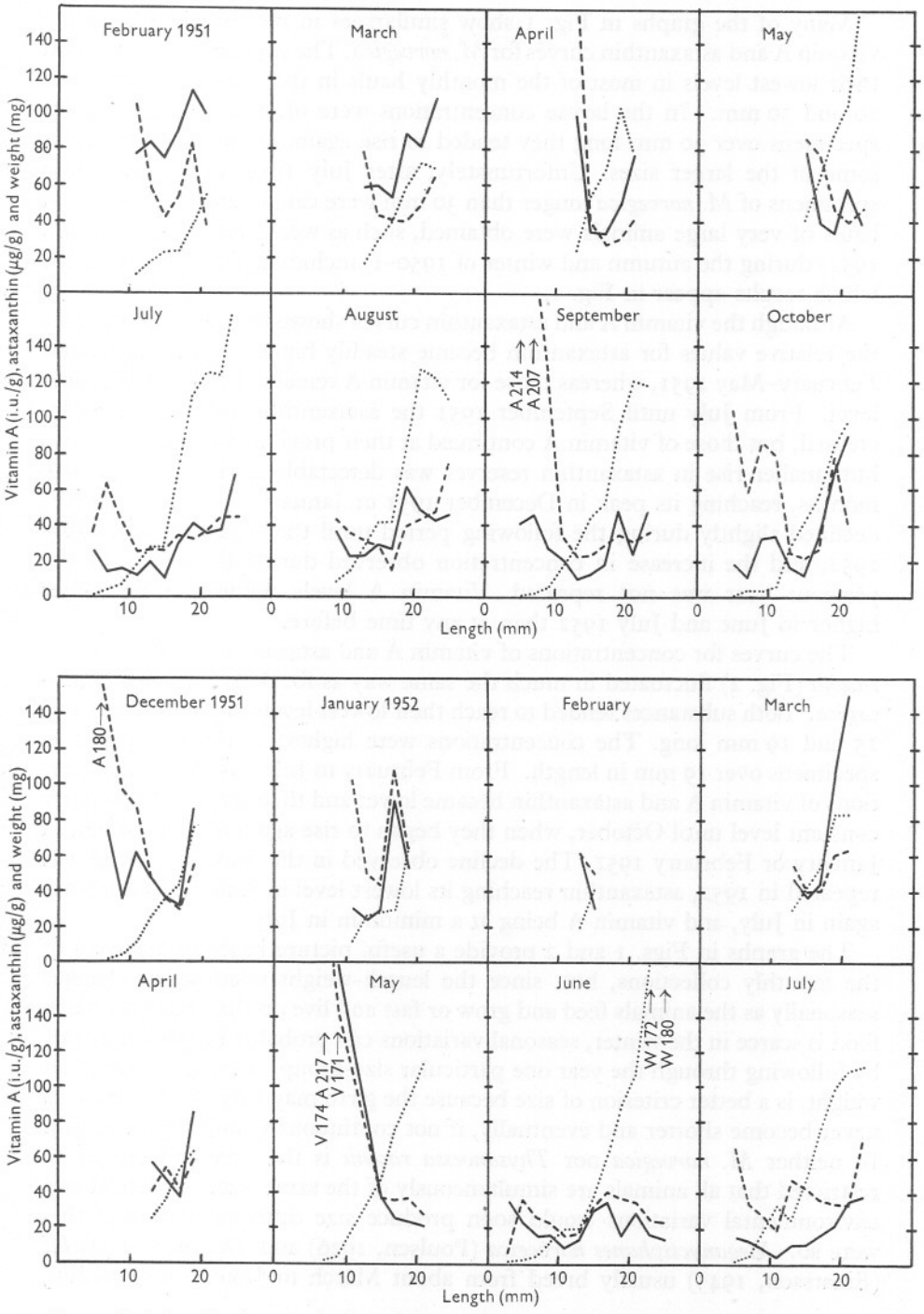

Fig. 2. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Thysanoessa raschii*. V, vitamin A; A, astaxanthin; W, weight; ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight.

Many of the graphs in Fig. 1 show similarities in the fluctuations of the vitamin A and astaxanthin curves for *M. norvegica*. The concentrations reached their lowest levels in most of the monthly hauls in the size-groups between 20 and 30 mm. In the larvae concentrations were often very high, and in specimens over 30 mm long they tended to rise again, often quite steeply in some of the larger sizes. Unfortunately, after July 1951, only a very few specimens of *M. norvegica* longer than 30 mm were caught and no more good hauls of very large animals were obtained, such as were taken (Fisher *et al.*, 1952) during the autumn and winter of 1950–1, including February 1951, for which results appear in Fig. 1.

Although the vitamin A and astaxanthin curves showed parallel fluctuations, the relative values for astaxanthin became steadily higher during the period February–May 1951, whereas those for vitamin A remained at about the same level. From July until September 1951 the astaxanthin concentrations decreased, but those of vitamin A continued at their previous values. A second, but smaller rise in astaxanthin reserves was detectable during the next few months, reaching its peak in December 1951 or January 1952. The reserves declined slightly during the following period until the study ended in July 1952, and the increase in concentration observed during the spring of the previous year was not repeated. Vitamin A levels tended to be rather higher in June and July 1952 than at any time before.

The curves for concentrations of vitamin A and astaxanthin in *Thysanoessa raschii* (Fig. 2) fluctuated in much the same way as for *Meganyctiphanes norvegica*. Both substances tended to reach their lowest levels in animals between 15 and 19 mm long. The concentrations were highest in the larvae and in specimens over 19 mm in length. From February to July 1951 the concentrations of vitamin A and astaxanthin became lower and then remained at a fairly constant level until October, when they began to rise again to a maximum in January or February 1952. The decline observed in the previous spring was repeated in 1952, astaxanthin reaching its lowest level in June and increasing again in July, and vitamin A being at a minimum in July.

The graphs in Figs. 1 and 2 provide a useful picture based on analyses of the monthly collections, but, since the length-weight relationship changes seasonally as the animals feed and grow or fast and live on their reserves when food is scarce in the winter, seasonal variations can probably be studied better by following through the year one particular size-group. Length, rather than weight, is a better criterion of size because the girth may vary but the animals never become shorter and eventually, if not continuously, increase in length. In neither *M. norvegica* nor *Thysanoessa raschii* is the breeding period so restricted that all animals are simultaneously of the same size. Genetical and environmental variations would soon produce size differences even if this were so. *Meganyctiphanes norvegica* (Poulsen, 1926) and *Thysanoessa raschii* (Einarsson, 1945) usually breed from about March to June. The breeding

periods appear to reach a peak, however, since, in our hauls of both species one size was almost always present in larger numbers than others. The 1 m stramin net was, therefore, regarded as a random sampler of the available population since it is likely that only very small larvae would escape from it. All the specimens in each haul were sorted into size-groups, as far as practicable, except when vast numbers of tiny larvae were taken. This method of selection did not take into account vertical or horizontal movements of populations within, and to and from, Loch Fyne, but euphausiids are caught there regularly and abundantly only in the deepest parts, and it is likely that a population would not move out entirely from an obviously favourable environment. With these reservations, we have considered the values for the size-group with the largest number of specimens in each monthly haul and regarded the series as representing the development of a homogeneous population. The fact that the length of the animals in the maximum-number groups either increased steadily or remained stationary for certain periods, but never regressed, during the year speaks for the soundness of this supposition. From the values so selected graphs were drawn showing the variations in vitamin A and astaxanthin content and concentration in *Meganyctiphanes norvegica* (Fig. 3) and *Thysanoessa raschii* (Fig. 4). Table I gives the average length of the specimens forming the selected group on each date. It was difficult to follow maximum-number groups from the larval stages since these, when present, were always in enormous numbers. The study covered the period from July 1951 to July 1952. In the first month, there were three size-groups of *Meganyctiphanes norvegica* with larger numbers of specimens than those next to them above or below, but in subsequent months only one group predominated in numbers. It appeared to correspond to the 15 mm size-group for July 1951 and this group was, therefore, used as starting-point in the graphs (Fig. 3). The group was probably hatched in the spring of 1951. Values for specimens of 13 mm length, which MacDonald (1927) regarded as first-year spawners, were the starting-points in the graphs for *Thysanoessa raschii* (Fig. 4). In all the graphs body-weight and fat concentration were also plotted.

The information contained in these graphs can now be considered in more detail under the headings of the various characteristics studied.

Size

In *Meganyctiphanes norvegica* (Fig. 3) the weight increased steadily from 47 mg in July to 246 mg in December, with a corresponding increase in length from 15 to 25 mm. From January to April 1952 both weight and length remained stationary, and then increased further to 434 mg and 33 mm by July. The two main periods of growth coincided with the spring and autumn diatom outbursts.

Thysanoessa raschii (Fig. 4) did not grow in the autumn to the same extent as *Meganyctiphanes norvegica*. The maximum-number group remained at 13 mm and 28 mg from July 1951 until October 1951 when the weight rose to 42 mg with no further change till March 1952. The length increased to 15 mm in December and remained so until March 1952. In April the length was 17 mm and the weight 54 mg; in May and

TABLE I. AVERAGE LENGTH IN MM OF SPECIMENS IN SELECTED GROUPS OF *MEGANYCTIPHANES NORVEGICA* AND *THYSANOESSA RASCHII* PRESENTED IN FIGS. 3 AND 4.

Date	*M. norvegica*	*T. raschii*
5. vii. 51	15	13
16. viii. 51	17	13
6. ix. 51	21	13
18. x. 51	25	15
12. xii. 51	25	15
16. i. 52	25	15
13. ii. 52	25	15
13. iii. 52	25	15
24. iv. 52	27	17
22. v. 52	29	19
19. vi. 52	31*	19†
9. vii. 52	33‡	19§

* Largest group was of specimens 15 mm long, but the series considered here was 1 year older and, among these, animals 31 mm long formed the largest group.

† Largest group was of specimens 7 mm long, but the series considered here was 1 year older and, among these, animals 19 mm long formed the largest group.

‡ Corresponding maximum number for series was actually in 29 mm group, but specimens were both shorter and lighter than their predecessors in the series under consideration. The larger size-group completed the series more logically.

§ Largest group was of specimens 9 mm long, but series considered here was 1 year older and, among these, animals 19 mm long formed the largest group.

June the length was 19 mm and the weight 92 and 90 mg, and in July the length remained 19 mm and the weight had increased to 99 mg. As with *M. norvegica*, growth occurred during the spring diatom increase.

Fat

The fat concentration of the sample of *M. norvegica* (Fig. 3) collected in July 1951 (6·7 %, or 3 mg/specimen) was the highest for the whole year. There was a sharp drop in August to 2·4 % (2 mg/specimen) followed by a steady rise to 16 mg/specimen (6·4 %) in October and then a slight drop in December (5·6 % or 12 mg/specimen) and January (4·0 % or 10 mg/specimen), and an increase in February to 5·7 % (13 mg/specimen). In March the fat concentration dropped to a minimum for the year of 0·9 % (2 mg/specimen), increasing irregularly to 4·3 % (19 mg/specimen) by July 1952. Thus the general picture is one of increasing fat reserves during the autumn and spring feeding periods.

In *Thysanoessa raschii* (Fig. 4) the fat percentage increased from 7·0 % (2 mg/specimen) in July 1951 to 9·2 % (4 mg/specimen) in October 1951 and then steadily declined to a minimum of 1·3 % (0·5 mg/specimen) in February 1952. During the spring the fat reserves were replenished until they reached 5·2 % (5 mg/specimen) in July 1952. Autumn and spring feeding appear to have led to accumulation of fat which was used during the intermediate winter period when food was scarce.

The relatively sharp drop between February and March in *Meganyctiphanes norvegica* and between January and February in *Thysanoessa raschii* may have been associated with spawning.

Vitamin A

The content of vitamin A in *Meganyctiphanes norvegica* (Fig. 3) increased from 0·5 i.u./specimen in July 1951 to 1·9 i.u./specimen in September. Until April 1952 the

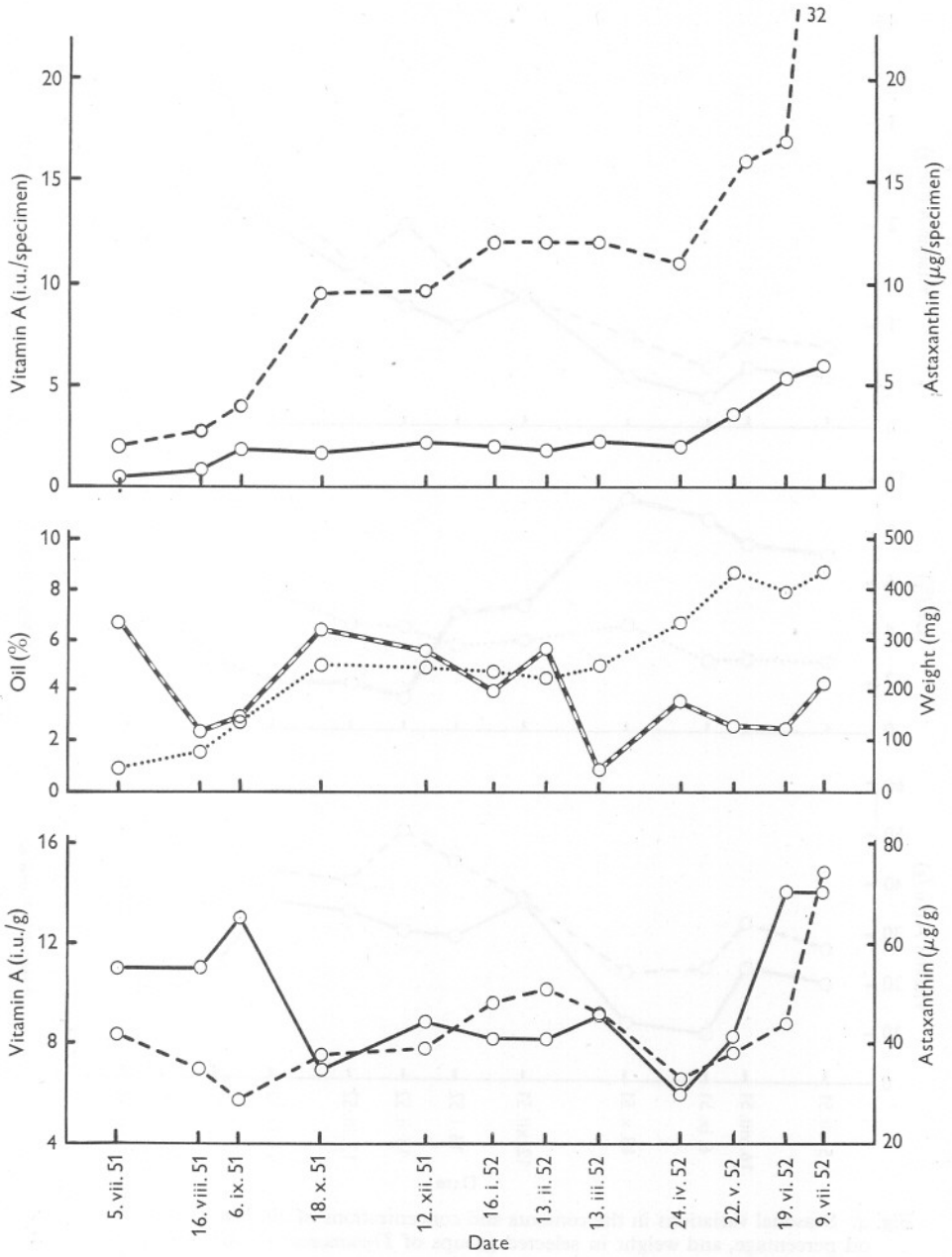

Fig. 3. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Meganyctiphanes norvegica*. ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight; ▬▬, oil.

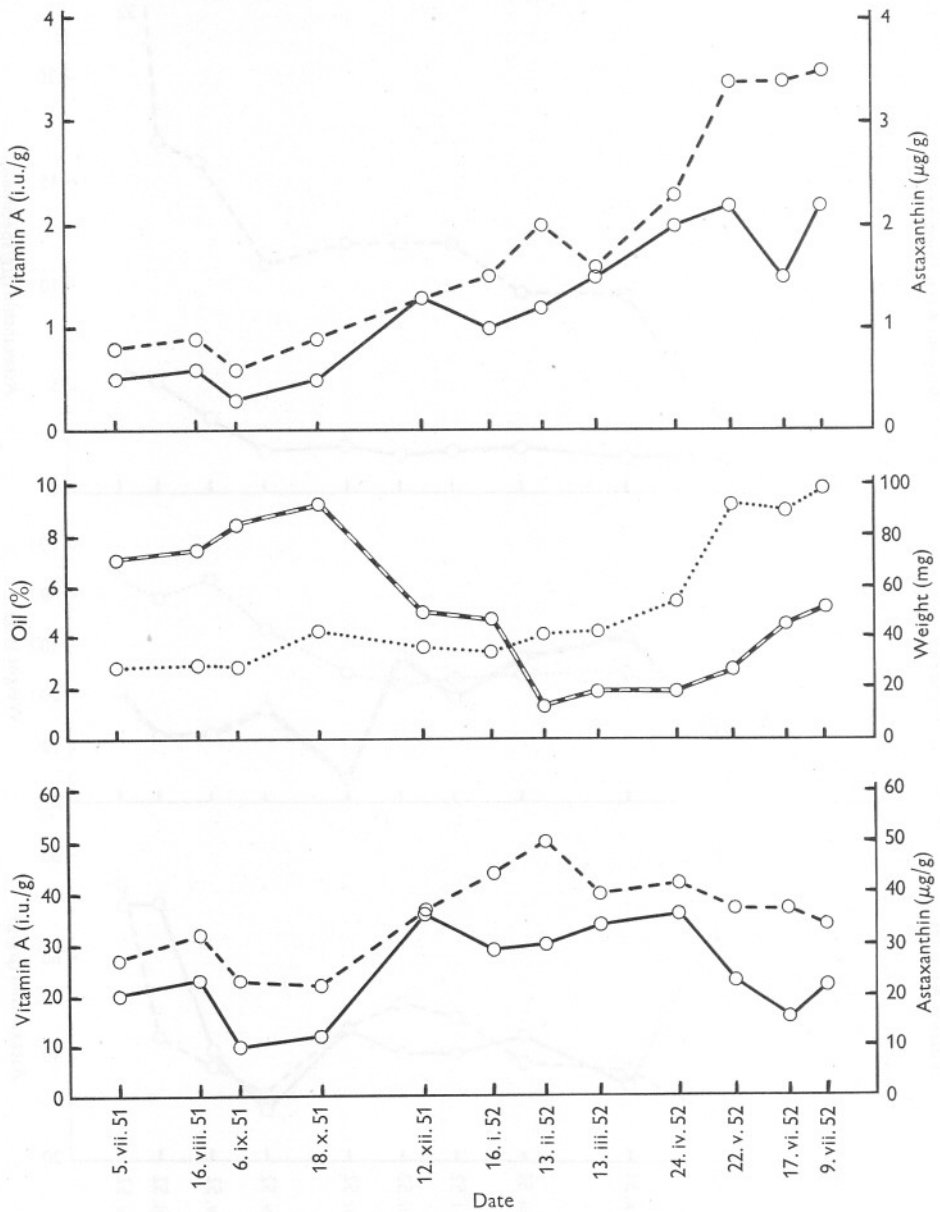

Fig. 4. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Thysanoessa raschii*. ——, vitamin A; – – –, astaxanthin; ....., weight; ▬▬, oil.

value fluctuated around 2·0 i.u./specimen. It then climbed steadily to 6·0 i.u./specimen in July. The vitamin A concentration of *M. norvegica* (Fig. 3) increased from 11 i.u./g in July 1951 to 13 i.u./g in September, but decreased sharply in October to 7 i.u./g. During the following months it remained around 8–9 i.u./g, and then, in April, reached its lowest level, of 6 i.u./g; thereafter it climbed steeply to 14 i.u./g in June and July. The final concentration is rather low for animals of this size (see Fisher *et al.*, 1952).

The vitamin A content of *Thysanoessa raschii* (Fig. 4) was 0·5 i.u./specimen in July 1951 and remained at around this level until October. By December it had reached 1·3 i.u./specimen but increased no further until April, when it went up to 2·0 i.u. In May it was 2·2 i.u., regressed to 1·5 i.u. in June, and recovered to 2·2 i.u./specimen in July. The vitamin A concentration in the July 1951 sample of *T. raschii* (Fig. 4) was 20 i.u./g, and rose to 23 i.u./g in August. By September, it had fallen to 10 i.u./g and then increased, through 12 i.u./g in October to 36 i.u./g in December.

The concentration was somewhat lower in January and February but reached the December level again in March and April. Thereafter it declined to 23 i.u./g in May and to 16 i.u./g in June, climbing once more to 22 i.u./g in July 1952. As in *Meganyctiphanes norvegica* the concentrations of vitamin A in *Thysanoessa raschii* are also somewhat lower than found by us previously (Fisher *et al.*, 1952).

Astaxanthin

The astaxanthin content of *Meganyctiphanes norvegica* (Fig. 3) increased 16-fold from 2 μg/specimen in July 1951 to 32 μg/specimen in July 1952 compared with a 12-fold increase in vitamin A during the same period. This increase in astaxanthin content occurred in two periods, one during the autumn months, reaching 10 μg/specimen in December, and the second from 11 μg in April to 17 μg in June, with a final steep climb to 32 μg in July. The concentration of astaxanthin in *M. norvegica* (Fig. 3) decreased from 42 μg/g in July 1951 to 29 μg/g in September, when it rose once more, reaching its highest level in February of 51 μg/g. It then steadily declined until April to 33 μg/g. Thereafter it increased to 44 μg/g by June and to 74 μg/g in July.

In *Thysanoessa raschii* (Fig. 4) the astaxanthin content in July 1951 was 0·8 μg/specimen and remained at around this level until October. It increased steadily during the next few months and by February was 2·0 μg/specimen. After a slight drop in March, the increase continued to 3·4 μg/specimen in May when the content levelled off for the rest of the period until July. Astaxanthin concentrations in *T. raschii* (Fig. 4) fluctuated during the autumn months from 27 μg/g in July 1951 to 22 μg/g in October, and then steadily increased to attain their peak in February 1952 at 50 μg/g. In March 1952 the concentration fell to 40 μg/g and decreased slightly during the next few months to 34 μg/g when the study ended in July.

Caridea

Crangon allmani was collected from Loch Fyne less regularly than the euphausiids. The results are given in Table II. Vitamin A was present, always in the ester form, in all groups except that collected in September 1951 and, when found, was chiefly, and often exclusively, in the eyes, but in concentrations much lower than in the euphausiids. The carotenoids of *C. allmani* included astaxanthin or its esters, carotenes and xanthophylls.

C. vulgaris (Table III) had vitamin A in all groups, confined exclusively to the eyes. The ester predominated in all those groups in which the two forms were separated. The concentrations of vitamin A in this species were usually

lower than those found in *C. allmani*, but β-carotene was found in a larger proportion of, though not in all, groups of *C. vulgaris*.

The principal carotenoid in both species of *Crangon* was astaxanthin and its concentration in the eyes, not given in Table III, was about 10 times that in the bodies, although the eyes contained only about 5% of the total carotenoids in the whole animal, the bulk of the pigments being xanthophylls.

There was no indication of any seasonal variation in either vitamin A or carotenoids in these two species of *Crangon*.

TABLE II. DISTRIBUTION OF OIL PERCENTAGE, VITAMIN A AND CAROTENOIDS PER SPECIMEN AND PER GRAM IN WHOLE ANIMAL (A) AND PAIRS OF EYES (E) OF *CRANGON ALLMANI* COLLECTED FROM LOCH FYNE

Date	Tissue	No. of specimens	Wt. (mg)	Oil (%)	Vitamin A		Carotenoids*	
					i.u./specimen	i.u./g.	μg/specimen	μg/g
6. ix. 51	A	17	403	2·1	0	0	1·2	3·0
	E	17	2	1·6	0	0	0·05	25
18. x. 51	A	11	260	4·2	0·28	1·1	1·1	4·2
	E	11	1	2·9	0·15	150	0·05	50
13. iii. 52	A	18	1109	1·8	0·15	0·1	1·8	1·6
	E	18	5	0·4	0·15	32	0·08	17
24. iv. 52	A	10	514	0·1	0·14	0·3	0·6	1·2
	E	10	3	5·5	0·14	50	0·4	132
22. v. 52	A	71	829	0·7	0·11	0·1	0·6	0·8
	E	71	4	11	0·11	27	—	—

* β-carotene absent.

Astacura

Nephrops norvegicus was collected in small groups from Loch Fyne during the visits made to collect euphausiids between July 1951 and July 1952. The animals were dissected and various organs were analysed in separate groups. The results are given in Table IV. From February 1952, the specimens were separated into eyes, gizzard, hepatopancreas and rest of body. As previous experience had shown that this last portion contained little or no vitamin A, expenditure of large volumes of solvents for the extraction of the large bulk of material in a number of bodies seemed unnecessary and to study any seasonal variation we confined our attention to the organs mentioned separately above.

In the group collected on 14 August 1951 the eye-stalks were separated from the eyes to determine whether the vitamin A in the eyes was associated with the sinus gland of the eye-stalk, but all the vitamin was in the eyes themselves. In contrast to the shrimps, the carotenoid pigment in the eyes was in concentrations of a similar order to those in the rest of the body and was exclusively astaxanthin. Carotenes and xanthophylls were present with astaxanthin in the other parts of the animals and small measurable amounts of β-carotene were found in some groups, usually in the hepatopancreas. There

TABLE III. OIL PERCENTAGE, VITAMIN A PER SPECIMEN AND PER GRAM, CAROTENOIDS PER SPECIMEN, PER GRAM AND PER GRAM OIL, AND β-CAROTENE PER GRAM OIL IN GROUPS OF *CRANGON VULGARIS* FROM SEVERAL LOCALITIES

Specimens analysed as bodies and eyes, but all Vitamin A was in the eyes and only total figures are given here

Date	Locality	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Total carotenoids			β-carotene, μg/g oil
					i.u./specimen	i.u./g	μg/specimen	μg/g	μg/g oil	
21. viii. 51	Plymouth	93	680	1·1	0·05	0·08	5·2	7·6	691	41
19. ix. 51	Plymouth	52	1055	0·8	0·06	0·06	2·3	2·2	276	28
27. xi. 51	Colwyn Bay	161	1176	1·2	0·08	0·07	4·9	4·1	357	17
12. xii. 51	Abergele	164	1239	1·0	0·06	0·05	4·2	3·4	340	59
2. i. 52	Conway	148	1360	1·4	0·06	0·05	6·2	4·5	326	33
16. i. 52	Conway	62	873	1·6	0·10	0·11	2·9	3·3	212	—
16. i. 52	Conway	92	568	1·4	0·06	0·11	1·8	3·1	224	—
16. i. 52	Conway	72	292	1·8	0·06	0·19	1·2	4·1	225	—
26. i. 52	Colwyn Bay	79(*a*)	1819	1·9	0·07	0·04	8·1	4·5	224	11
26. i. 52	Colwyn Bay	91(*b*)	647	1·2	0·08	0·12	2·5	3·8	325	0
28. i. 52	Holyhead	58(*a*)	1499	1·3	0·08	0·05	6·7	4·5	338	22
28. i. 52	Holyhead	114(*b*)	798	1·2	0·08	0·10	2·4	3·0	250	4·6
11. ii. 52	Colwyn Bay	40	2204	1·8	0·09	0·04	7·0	3·2	175	9·0
11. ii. 52	Colwyn Bay	80	1032	1·8	0·09	0·08	3·8	3·7	208	7·1
11. ii. 52	Colwyn Bay	70	549	1·5	0·04	0·07	1·5	2·7	187	1·9
25. ii. 52	Pendwffyn	18(*a*)	1223	2·0	0·09	0·07	3·8	3·1	157	—
25. ii. 52	Pendwffyn	57(*b*)	600	1·5	0·06	0·10	2·0	3·3	215	—
26. ii. 52	Colwyn Bay	37(*a*)	1706	1·7	0·09	0·05	8·0	4·5	271	—
26. ii. 52	Colwyn Bay	53(*b*)	707	1·7	0·09	0·13	2·5	3·6	211	—
25. iii. 52	Burnham-on-Crouch	92	1125	1·2	0·06	0·05	5·7	5·8	497	1·9
18. iv. 52	Conway	130	1229	2·2	0·13	0·11	6·1	5·0	226	1·0
22. v. 52	Colwyn Bay	165	1021	1·4	0·09	0·09	4·0	3·9	277	0·7
5. viii. 52	Llanfairfechan	129	1352	1·4	0·04	0·03	7·1	5·3	370	0·7
21. viii. 52	Colwyn Bay	107	1003	1·1	0·05	0·05	4·5	4·5	420	0·3
4. x. 52	Pendwffyn	190	686	0·6	0·04	0·05	2·3	3·4	544	25
23. x. 52	Pendwffyn	119	1088	0·9	0·06	0·06	4·3	3·9	443	15
17. xi. 52	Rhyl	101	1406	1·8	0·07	0·05	5·3	3·8	211	0
18. xii. 52	Pendwffyn	148	1053	0·8	0·07	0·06	7·1	6·7	413	0
9. ii. 53	Conway	192	1105	1·8	0·04	0·04	5·5	5·5	272	—

(*a*) 'Berried' ♀. (*b*) Mixed ♂ and ♀ without eggs.

TABLE IV. DISTRIBUTION OF OIL PERCENTAGE, VITAMIN A PER SPECIMEN AND PER GRAM, CAROTENOIDS PER SPECIMEN, PER GRAM AND PER GRAM OIL, AND β-CAROTENE PER GRAM OIL IN *NEPHROPS NORVEGICUS* FROM THE CLYDE SEA AREA

For eyes and eye-stalks all values are per pair

Date	Organ	No. of specimens	Av. wt. (g)	Oil (%)	Vitamin A		Total carotenoids			β-carotene, μg/g oil
					i.u./specimen	i.u./g	μg/specimen	μg/g	μg/g oil	
3. vii. 51	Whole animal	5	76	1·5	3·0	0·04	673	8·9	593	7·8
	Eyes	5	0·4	1·3	1·2	2·8	4·1	9·5	715	0
14. viii. 51	Whole animal	6	121	0·9	3·4	0·03	965	8·0	889	18
	Eyes	6	0·5	1·5	2·2	4·7	5·7	12	793	0
	Eye-stalks	6	0·07	8·5	0	0	0·8	12	137	0
4. ix. 51	Whole animal	6	132	2·0	12	0·09	741	5·6	280	4·7
	Eyes	6	0·6	0·9	2·4	4·0	5·6	9·2	1022	0
	Hepatopancreas	6	7·8	29	9·3	1·2	61	7·9	27	5·2
	Alimentary canal	6	2·5	0·7	0	0	23	9·4	1343	0
16. x. 51	Whole animal	6	108	1·3	3·2	0·03	792	7·3	551	12
	Eyes	6	0·6	0·4	2·0	3·5	2·4	4·2	1141	0
	Hepatopancreas	6	6·0	20	1·2	0·2	36	6·0	30	12
11. xii. 51	Whole animal	6	203	1·3	6·1	0·03	1218	6·0	453	8·8
	Eyes	6	0·7	0·3	4·1	5·7	5·5	7·6	2523	0
	Gizzard	6	2·6	0·3	0	0	51	19	5972	0
	Hepatopancreas	6	10	20	0·9	0·1	62	6·1	31	11
15. i. 52	Whole animal	6	211	0·6	4·6	0·02	916	4·3	767	13
	Eyes	6	0·8	0·2	3·9	5·1	5·8	7·5	3165	0
	Gizzard	6	2·9	0·2	0	0	17	6·1	2568	0
	Hepatopancreas	6	8·1	10	0·7	0·1	22	2·7	27	18
12. ii. 52	Whole animal	6	176	—	1·8	0·01	—	—	—	—
	Eyes	6	0·7	0·9	1·8	2·5	4·4	6·1	686	0
	Gizzard	6	3·8	0·5	0	0	4·7	1·2	239	0
	Hepatopancreas	6	8·9	13	0	0	26	2·9	22	4·4
11. iii. 52	Whole animal	3	144	—	2·6	0·02	—	—	—	—
	Eyes	3	0·6	0·8	2·6	4·1	6·7	11	1329	0
	Gizzard	3	2·2	0·8	0	0	11	5·0	632	0
	Hepatopancreas	3	7·1	19	0	0	69	9·6	52	0·6
24. iv. 52	Whole animal	4	200	—	2·1	0·01	—	—	—	—
	Eyes	4	0·8	0·2	2·1	2·6	2·8	3·4	2298	0
	Gizzard	4	3·1	0·3	0	0	19	6·0	1742	0
	Hepatopancreas	4	9·5	9·8	0	0	50	5·8	59	19
23. v. 52	Whole animal	9	143	—	4·7	0·03	—	—	—	—
	Eyes	9	0·6	0·2	4·0	6·4	5·7	9·1	5120	0
	Gizzard	9	2·6	0·3	0	0	13	5·0	1527	0
	Hepatopancreas	9	7·5	8·3	0·8	0·1	46	6·1	74	16
17. vi. 52	Whole animal	11	165	—	1·4	0·01	—	—	—	—
	Eyes	11	0·7	0·2	1·4	1·9	1·9	2·6	1340	0
	Gizzard	11	3·3	0·3	0	0	18	5·4	1736	0
	Hepatopancreas	11	8·1	4·6	0	0	22	2·8	59	17
9. vii. 52	Whole animal	4	169	—	4·8	0·03	—	—	—	—
	Eyes	4	0·6	0·7	4·8	7·7	6·7	11	1611	0
	Gizzard	4	2·7	0·5	0	0	20	7·5	1520	0
	Hepatopancreas	4	8·1	6·7	0	0	28	3·5	53	20

was no consistent seasonal variation in the concentrations of vitamin A, carotenoids or fat in any of the organs investigated. The hepatopancreas was much richer in fat than the other parts of the animal.

Of *Homarus vulgaris*, only groups of eyes were analysed and the results are shown in Table V. Concentrations of both vitamin A and carotenoids, the latter predominantly astaxanthin with a little xanthophyll, were fairly high during the early months of 1951, but by July the vitamin A level had dropped to a value from which it deviated little during the remaining period of investigation up to April 1952. The content and concentration of carotenoids was fairly uniform throughout 1951, but in January 1952 it dropped to about half the previous level and persisted so in the following months until April.

TABLE V. OIL PERCENTAGE AND VITAMIN A AND CAROTENOIDS PER PAIR AND PER GRAM IN THE EYES OF *HOMARUS VULGARIS* OF UNKNOWN ORIGIN

Date	No. of eyes	Wt. (mg/pair)	Oil (%)	Vitamin A		Carotenoids	
				i.u./pair	i.u./g	μg/pair	μg/g
15. i. 51	57	686	0·4	10	15	40	54
19. ii. 51	50	743	0·7	9·9	13	44	59
28. iii. 51	52	646	0·8	12	17	43	66
16. iv. 51	50	739	0·7	15	20	44	59
vi. 51	56	713	0·7	6·5	9·1	38	53
vii. 51	16	599	1·6	3·7	6·2	51	85
10. ix. 51	55	710	0·5	4·2	6·0	28	39
5. xi. 51	58	735	0·6	2·8	3·8	24	33
5. xii. 51	50	488	0·9	3·0	6·1	30	62
19. xii. 51	55	534	1·4	3·0	5·4	32	58
28. i. 52	51	655	1·3	3·5	5·3	12	19
ii. 52	50	656	0·6	4·4	6·7	16	25
iii. 52	50	688	1·0	4·1	5·9	17	25
7. iv. 52	50	804	0·4	4·9	6·1	17	22

DISCUSSION

The foregoing observations indicate that, whereas the fat, vitamin A and astaxanthin reserves of the euphausiids varied markedly with the season, no such changes occurred in the shrimps, *Crangon allmani* and *C. vulgaris*, or in *Nephrops norvegicus*. Vitamin A and carotenoids in the eyes of *Homarus vulgaris* varied to some extent with the season. These eyes weighed between 0·5 and 0·8 g per pair and were all preserved, stored and analysed in the same way, but the origin of the lobsters was unknown and may have varied through the year. This might account for the absence in 1952 of the trend observed in the previous year, when vitamin A and carotenoids increased during the spring to a maximum in April.

An interesting comparison between the two species of *Crangon* is noteworthy. The concentration of vitamin A in the eyes of *C. allmani* was higher than in those of *C. vulgaris*. The difference may be associated with the relative visual requirements of the two animals, as *C. allmani* lives at much greater depths and, therefore, in much darker surroundings than *C. vulgaris*, although

both are benthic animals in which vision may be comparatively unimportant. On the other hand, it is possible that *C. allmani* from Loch Fyne also inhabited by large populations of vitamin A-rich euphausiids may have, by feeding on their living or dead bodies, accumulated its reserves at a faster rate than *C. vulgaris* from an environment where its food contains much less vitamin A. Admittedly, though *C. allmani* is carnivorous, we do not know whether it in fact preys on euphausiids. Another possibility is that both obtain their vitamin A by the conversion of a precursor such as β-carotene and that the process operates more efficiently in *C. allmani*, which usually had no β-carotene, than in *C. vulgaris* which contained it in most parts of the body.

We have established for a considerable range of sizes that the presence of the bulk of the vitamin A and of at least half the total astaxanthin in the eyes of both *Meganyctiphanes norvegica* and *Thysanoessa raschii* is a characteristic independent of the season. We have confirmed our earlier findings (Kon & Thompson, 1949*a*; Fisher *et al.*, 1952) that euphausiid vitamin A occurs mainly in the ester form, but we now know that this form predominates only in the eyes. Ester and alcohol were usually present in about equal amounts in the small quantity of vitamin A found in the bodies.

The ester is the form of vitamin A stored in the liver by vertebrates and the alcohol is the active form passed to the rest of the body in the blood stream. Thus most of the vitamin A in the euphausiid eye is in the storage form with only a small amount in the active state.

In the studies reported here and previously (Fisher *et al.*, 1952, 1953), we found the concentration of vitamin A in the eyes of *Meganyctiphanes norvegica* to be within the range of 2000–20,000 i.u./g dry weight and even if 95% of this is ester, the concentration of vitamin A alcohol would be between 100 and 1000 i.u./g. Such quantities would certainly be adequate for visual purposes, since, as we have previously pointed out (Fisher *et al.*, 1952), Wald (1935) found that in mammalian retinas the concentration of vitamin A adequate for vision is about 70 i.u./g dry weight and in frog retinas about 1200 i.u./g, and Morton & Rosen (1949) recorded a maximum value for whole eyes of frogs equivalent to 300 i.u./g dry weight.

Retinene (vitamin A aldehyde) which participates in the visual cycle of higher animals (Wald, 1945) is absent. Thus vitamin A in the eyes of euphausiids is apparently in excess of that required for vision. What, therefore, is its function, if any? In Crustacea, the hepatopancreas is a digestive organ and not a storage organ like the vertebrate liver. Both vitamin A ester and astaxanthin are found in the euphausiid eye in much higher concentrations than in the eyes or bodies of other animals and there is no obvious function for these large quantities. It may be that they are no more than metabolic end-products accumulating there during the life of the euphausiids, like vitamin A and carotenoids in, for example, fish livers (Macpherson, 1933).

Figs. 3 and 4 show that *M. norvegica* and *Thysanoessa raschii* accumulated

vitamin A and astaxanthin mainly during the autumn and spring when diatom outbursts stimulated feeding. In *Meganyctiphanes norvegica* the concentrations of vitamin A reached maxima in June–July and in September, falling during the winter when growth continued but the vitamin A was not being accumulated. The astaxanthin concentration remained fairly high all winter in *M. norvegica*, dropping with that of vitamin A in April, a month after the fat concentration reached its minimum. Thus there was a time-lag between the resumption of growth at the faster spring rate, which probably began between February and March in *M. norvegica* and between January and February in *Thysanoessa raschii*, and an increase in the fat reserves, which began a month later, and another month passed before the contents of vitamin A and astaxanthin began to increase. Spawning may have occurred at around this time and depleted the stocks of fat, vitamin A and astaxanthin.

Meganyctiphanes began accumulating vitamin A and astaxanthin again in April (Fig. 3), whereas in *Thysanoessa* the concentration of vitamin A did not begin to rise until June or July (Fig. 4) and, even then, that of astaxanthin was still falling.

The difference between the two species may possibly be explained in terms of their food. *Meganyctiphanes norvegica* of the size considered here would be carnivorous (Macdonald, 1927), whereas the smaller *Thysanoessa raschii* would probably be mainly herbivorous, although less is known about the feeding of this species. Gillam, el Ridi & Wimpenny (1939) found that both phytoplankton and zooplankton reached their highest growth in May and August but the plant concentrations were much more transitory than those of animals. Zooplankton densities remained fairly high for 2 or 3 months after the peaks, whereas those of the phytoplankton soon became much lower. *Meganyctiphanes norvegica* might thus have a continued supply of adequate animal food but *Thysanoessa raschii* would have to subsist on rapidly diminishing stocks of plants.

Further evidence of a difference in feeding habits between *Meganyctiphanes norvegica* and *Thysanoessa raschii* is that the former (Fig. 1) accumulated astaxanthin much more rapidly than vitamin A, during spring 1951, whereas in *T. raschii* (Fig. 2) the two substances increased at a more closely similar rate. A greater proportion of zooplankton, composed predominantly of copepods and thus rich in astaxanthin, in the diet of *Meganyctiphanes norvegica* would account for its increased stores of this carotenoid. Another explanation may be found in the larger pigmented areas of the body in *M. norvegica* than in *Thysanoessa raschii*. Our analytical results have shown that a much higher percentage of the total astaxanthin is in the eyes, and, therefore, less is in the bodies, of *T. raschii*, than in those of *Meganyctiphanes norvegica*, which, therefore, requires more astaxanthin for its pigmentation.

In neither species of euphausiid was the accumulation of vitamin A and astaxanthin as marked in the spring of 1952 as in the previous year. The

lower concentrations of these substances found during the winter months of 1951–52 (Figs. 1, 2) may, in some way, have been associated with the absence from our hauls of very large specimens of *M. norvegica* and the reduction in the numbers of both this species and *Thysanoessa raschii* during these months and the following spring. It is interesting that, in February 1952, fishermen at Monaco were complaining that *Meganyctiphanes norvegica* appearing in the port were smaller than usual at that time of year.

Examination of Fig. 4 shows that in *Thysanoessa raschii* there was a marked inverse relationship between the concentrations of fat and vitamin A through the year, and a similar relationship was also detectable in *Meganyctiphanes norvegica* (Fig. 3). The feeding habits of the animals probably account for the phenomenon. Fig. 4 indicates that *Thysanoessa raschii* lived mainly on its reserves during the period from October to March. Body-weight remained fairly constant but the stores of fat were reduced considerably. Continued accumulation of vitamin A and astaxanthin either from food eaten or by conversion from a precursor within the body resulted in increased concentrations of these substances. With the resumption of large-scale feeding in the spring, growth was more rapid and fat was again stored with the result that the concentrations of vitamin A and astaxanthin fell as these accumulated less rapidly than fat owing to the time-lag discussed above. Cause and effect are less easily elucidated in *Meganyctiphanes norvegica*, but there is no doubt that the inverse relationship is also found in this species. Gillam *et al.* (1939) noted a similar inverse relationship in their study between the vitamin A concentration and fat-free solids of gross plankton over the period of a year, apart from the spring increase when vitamin A increased as well. They suggested that the relationship was indicative of the type of behaviour expected from an autocatalyst of growth. It may, however, merely be a measure of the proportions in the total plankton of various planktonic organisms. At the peak periods for fat-free solids, the plankton would be composed mainly of plants and smaller Crustacea, such as copepods which, we know, lack vitamin A. This mixed population would be grazed down by larger animals, especially fish larvae and euphausiids, which form or accumulate vitamin A and concentrate it in their bodies. The population density of these organisms would be relatively much smaller than that of copepods and diatoms, so that the concentration of fat-free solids would be reduced, especially as most of the diatoms die and sink to the bottom soon after their outbursts, thereby further reducing the solid material present.

All our evidence points to the richness in vitamin A of euphausiids in comparison with other Crustacea. In Loch Fyne, *Meganyctiphanes* and *Thysanoessa* were taken in the same hauls as *Calanus finmarchicus* (Gunnerus) and *Euchaeta norvegica* Boeck and very often, near the bottom, with *Crangon allmani* and *Pandalus bonnieri* Caullery. Neither the copepods nor the decapods contained vitamin A in amounts approaching those in the euphausiids. The

question arises what source of the vitamin or its precursors is available to these animals and closed to their neighbours. We believe that our study may have shed a little light on the problem. An examination of the monthly graphs for *Meganyctiphanes* in Fig. 1 shows that the highest concentrations of vitamin A and astaxanthin were found in the smallest and largest specimens, although the content of both vitamin A and astaxanthin increased throughout life. It seems very likely that in the former the vitamin and the pigment are passed on to the larva from the egg. But why do the concentrations increase steeply in animals over 30 mm long? This size is not associated with the achievement of maturity, since adult animals are found upwards of 20 mm long. The high concentrations were observed in large specimens at all times of year and so were not connected specifically with the breeding period.

Thysanoessa raschii showed certain of the trends noted in *Meganyctiphanes norvegica*. The increase in vitamin A and astaxanthin concentrations usually appeared in animals between 15 and 20 mm long. Again these are not related to the attainment of sexual maturity which occurs in this species at about 13 mm length (Macdonald, 1928).

We have examined the gut contents of *Meganyctiphanes norvegica* of different sizes. Smaller animals (15–25 mm long) eat much detritus of vegetable origin, together with diatoms, of which fragments were found in the gut. In the larger specimens (over 30 mm long), detritus was also present, but accompanied there by crustacean fragments, mainly of copepod origin and often identifiable as parts of *Calanus finmarchicus* and *Euchaeta norvegica*. Macdonald (1927) made a more systematic investigation of the food of *Meganyctiphanes norvegica* and reached the following conclusions:

'(1) Organic detritus is eaten most abundantly during the first months of the year; (2) *Meganyctiphanes*, ranging from 21–29 mm, feed more extensively on vegetable detritus than do larger or smaller specimens (it should be noted that this size was by far the most abundant in the Clyde Sea area); (3) Copepods are eaten most extensively by the larger specimens viz. 31–39 mm and (4) the smaller specimens, 13–19 mm, feed most extensively on diatoms and "wet dust"'.

'Wet dust' was also called, by Macdonald, flocculent detritus and consisted of a mass of greenish brown unidentifiable particles, including shells of diatoms and peridinians, spores of algae and possibly argillaceous particles. The species of diatoms he found most abundantly in *M. norvegica* were *Paralia sulcata* (Heiberg) Cleve, *Thalassiosira nordenskïoldi* Cleve, *T. gravida* Cleve and *Coscinodiscus* spp. Other diatoms occasionally eaten in quantities included *Nitzschia* and *Skeletonema*. We have analysed pure cultures of several diatoms, including *Thalassiosira gravida* Cleve, *Coscinodiscus concinnus* W. Sm., *Phaeodactylum tricornutum* Bohlin, and *Skeletonema costatum* (Grev.) Cleve and the dinoflagellate, *Peridinium trochoideum* (Stein) Lemm., and found no vitamin A in any of these organisms.

Little is known about the feeding of *Thysanoessa raschii*. Our own experience has been to find the gut empty except for occasional detritus or other unidentifiable material. It is difficult to postulate a change of diet, as in *Meganyctiphanes norvegica*, in animals so much smaller when the concentrations of vitamin A and astaxanthin begin to rise, unless the larger prey on larval copepods. We have never found, nor did Macdonald (1927), parts of euphausiids in the guts of others, so that it is very unlikely that larger euphausiids obtain their vitamin A preformed by eating smaller ones.

The change-over from mainly vegetable to mainly animal food, observed by both Macdonald and ourselves in specimens of *M. norvegica* over 30 mm long, might well be the cause of their increased rates of storage of vitamin A and astaxanthin, resulting from differences in the carotenoid constituents in the diet. The increased uptake and storage of astaxanthin would be thus explained because this pigment is the principal carotenoid in copepods. Vitamin A and astaxanthin are both absent, however, from diatoms and there is no vitamin A in *Calanus finmarchicus* (Euler, Hellström & Klussmann, 1934; Lederer, 1938; Fisher *et al.*, 1952), and only rarely in *Euchaeta norvegica* (Fisher *et al.*, 1952). Direct uptake of both substances is thus unlikely to account for their presence in the young herbivorous stages of *Meganyctiphanes*, and that of vitamin A for its presence in larger predominantly carnivorous animals. We must, therefore, look for precursors that these euphausiids can convert to astaxanthin and vitamin A. The plant food of the herbivores contains β-carotene, a known provitamin A and possibly also a precursor for astaxanthin. Neither β-carotene nor any other known provitamin A is found in more than trace amounts in the copepods and yet the larger stages of *M. norvegica* feeding on them appear capable of storing vitamin A much more rapidly than those living on plant material. They must, therefore, utilize as precursors either known carotenoids or other substances, normally not regarded as vitamin A precursors.

Let us consider the carotenoids present in the plankton organisms involved in these food-chains. The principal carotenoids in diatoms are β-carotene and xanthophylls, including certain pigments peculiar to these plants, such as diatoxanthin (Strain, Manning & Hardin, 1944). This carotenoid has an absorption spectrum very similar to that of zeaxanthin which, according to Goodwin (1952), is probably 3:3′-dihydroxy-β-carotene. Goodwin believes that diatoxanthin may be a *cis*-isomer of zeaxanthin and so possess the same structural formula. Astaxanthin is 3:3′-dihydroxy-4:4′-diketo-β-carotene (Kuhn & Sörensen, 1938), so that zeaxanthin, and, therefore, probably diatoxanthin, are structurally intermediate between β-carotene and astaxanthin. The oxidation of β-carotene to astaxanthin, if it occurs at all, may thus go as far as diatoxanthin in the diatoms and be completed in the zooplankton, giving rise to astaxanthin and vitamin A in euphausiids and to astaxanthin only in copepods. Zeaxanthin itself is inactive as a provitamin A for pigs (Braude *et al.*, 1941), but invertebrate carotenoid metabolism may be different,

and it is interesting to note that recently Lenel (1953) found in the organs of the shore-crab, *Carcinus maenas* (Pennant), not only β-carotene and astaxanthin or their stereoisomers but also more or less oxidized pigments intermediate between these two. It is generally believed that Crustacea are unable to synthesize astaxanthin *de novo* (Sörensen, 1936; Fox, 1947). Fox suggested that pigments similar to astaxanthin may be formed in coelenterates, sponges, molluscs, echinoderms and other invertebrates, and that the common occurrence of acidic or acidogenic carotenoids among many invertebrate animals indicates a unique ability to effect a partial oxidation of commoner polyene molecules without actually splitting them. However, the only carotenoid molecule available in any quantity in its copepod diet to *Meganyctiphanes norvegica* is astaxanthin. If *Meganyctiphanes* converts this pigment to vitamin A, a reduction is involved but, as Fox (1953) points out, the general tendency in the animal body is towards the oxidation of carotenoids rather than to their reduction. The presence of oxygen in the β-ionone rings of carotenoids has always been considered by biochemists as a bar to their conversion to vitamin A, although Goodwin (1951) has suggested that this criterion may have to be modified since the work of Karrer, Jucker, Rutschmann & Steinlin (1945) on the production of carotenoid epoxides indicates that 5:6 epoxides, e.g. 5:6–5′:6′-diepoxy-β-carotene, are vitamin A precursors. As Goodwin pointed out, the formation of vitamin A may occur after initial conversion of the epoxides to β-carotene, in other words, after their reduction.

Our evidence certainly suggests that euphausiids utilize a vitamin A precursor not available to other Crustacea living around them, and it is difficult to ignore the coincidence between the appearance of astaxanthin in their diet and the appreciable increase in their vitamin A reserves.

The suggestion that astaxanthin may be a precursor for vitamin A is not entirely new; Drummond & MacWalter (1935) thought that astacin from krill might be converted to vitamin A by the whale. We now know (Kon *et al.*, 1949*a*, *b*) that there is no need for such a hypothesis since the vitamin is already present preformed in krill; Morton (1940) believed it possible that certain carnivorous animals, such as fishes, may be able to use astaxanthin from Crustacea in their food as a basis for conversion to vitamin A; and more recently Collins, Love & Morton (1953) have suggested astaxanthin as a possible precursor for vitamin A_2. In dealing with possible sources of vitamin A for euphausiids a further factor must be considered. Detritus is eaten by euphausiids of all sizes and is, therefore, unlikely to account for increased accumulation of vitamin A in larger euphausiids. Nevertheless, the importance of detritus as a source of carotenoids must not be overlooked (see Fox, 1950). Fox, Isaacs & Corcoran (1952) have estimated that in the waters off the coast of California living cells form only 1·5–4% of the total colloidal or otherwise finely particulate organic matter, called leptopel, and its carotenoid

content is almost unknown. Earlier, Fox (1937) filtered off detritus and microplankton from 4000 l. of sea water and extracted from the deposit 0·1 mg of xanthophylls and 0·02 mg of carotenes. Marine muds are also rich in carotenoids (Fox, Updegraff & Novelli, 1944), especially carotenes. This part of their diet could, therefore, provide by conversion at least part of the vitamin A found in euphausiids.

Be it as it may, Euphausiacea seem unique in their ability to accumulate vitamin A in the sea and the interesting possibility remains that they, of all Crustacea, may be able to utilize the ubiquitous crustacean pigment, astaxanthin, as a precursor of vitamin A. The joint presence in the euphausiid eye of large amounts of vitamin A and astaxanthin in similar proportions in different size-groups, as indicated in Figs. 1 and 2, strongly suggests a close interrelationship of these substances in the carotenoid metabolism of euphausiids.

The Development Commission has continued to support our work. We are much indebted to Mr E. Ford and his staff at Millport for making arrangements for, and to Captain R. E. Souter and the crew of M.F.V. *Calanus* for their invaluable assistance in, the collection of specimens. We are grateful to Mr F. S. Russell, F.R.S., at Plymouth and Mr R. E. Savage at Conway and their staffs for the supply of *Crangon vulgaris* and to Mr R. D. Waugh and his staff of the Oyster Research Station, Burnham-on-Crouch, for allowing us facilities for collecting specimens. Our thanks are due to Messrs Lyons Laboratories, Hammersmith, and their chemists, Dr E. B. Hughes and Mr D. H. F. Clayson, for their co-operation in providing us with the lobster eyes. Dr M. Parke of the Plymouth Laboratory kindly supplied the pure cultures of diatoms. We have had much analytical assistance from Mrs W. M. Henschel.

Summary

Results are given for measurements of fat, vitamin A and carotenoids in groups of *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *Crangon allmani*, *C. vulgaris*, *Nephrops norvegicus* and eyes of *Homarus vulgaris*, collected at regular intervals for a year or longer.

In both species of euphausiids vitamin A was consistently present mainly in the ester form and concentrated chiefly in the eyes. More than half the total astaxanthin was in the eyes. No other carotenoids were detected.

Astaxanthin and vitamin A were accumulated by the euphausiids more rapidly during the spring and autumn-feeding periods associated with diatom outbursts than at other seasons. There was a delay in *Thysanoessa raschii*, but not in *Meganyctiphanes norvegica*, between resumption of more rapid growth during spring and autumn and increase in concentration of vitamin A and astaxanthin. The difference was possibly due to differences in diet of the two species in relation to the seasonal biological composition of the plankton.

In the euphausiids, concentrations of vitamin A and astaxanthin were much

higher in larvae and in adults over 30 mm long of *M. norvegica*, and in larvae and adults over 15 mm of *Thysanoessa raschii*, than in mature adults of *Meganyctiphanes norvegica* of 20–30 mm and *Thysanoessa raschii* of 13–15 mm, respectively. An inverse relationship between fat and vitamin A concentration was noted in both species throughout the year.

In *Crangon* spp. and *Nephrops norvegicus* there was no evidence of any seasonal variation of either vitamin A or carotenoids. Differences in the carotenoid metabolism of the two species of *Crangon* are discussed.

In the eyes of *Homarus vulgaris*, vitamin A and carotenoids reached their highest concentrations in the spring, gradually decreasing during the rest of the year.

The metabolism of vitamin A and carotenoids in euphausiids and other Crustacea is considered. The origin of the large reserves of vitamin A in the eyes of euphausiids is discussed, and special attention is given to the importance of astaxanthin as a possible precursor.

REFERENCES

BRAUDE, R., FOOT, A. S., HENRY, K. M., KON, S. K., THOMPSON, S. Y. & MEAD, T. H., 1941. Vitamin A studies with rats and pigs. *Biochem. J.*, Vol. 35, pp. 693–707.

COLLINS, F. D., LOVE, R. M. & MORTON, R. A., 1953. Studies in vitamin A. 25. Visual pigments in tadpoles and adult frogs. *Biochem. J.*, Vol. 53, pp. 632–6.

DRUMMOND, J. C. & MACWALTER, R. J., 1935. Pro-vitamin A in the food of whales. *J. exp. Biol.*, Vol. 12, pp. 105–7.

EINARSSON, H., 1945. Euphausiacea. I. Northern Atlantic species. *Dana Report*, No. 27, 185 pp.

EULER, H. v., HELLSTRÖM, H. & KLUSSMANN, E., 1934. Über den Carotinoidgehalt einiger Evertebraten. *Hoppe-Seyl. Z.*, Bd. 223, pp. 77–89.

FISHER, L. R., 1953. Vitamin A and carotenoids in some marine invertebrates. Ph.D. Thesis, London University.

FISHER, L. R., KON, S. K. & THOMPSON, S. Y., 1952. Vitamin A and carotenoids in certain invertebrates. I. Marine Crustacea. *J. Mar. biol. Ass. U.K.*, Vol. 31, pp. 229–58.

——— 1953. Vitamin A and carotenoids in some Mediterranean Crustacea: with a note on the swarming of *Meganyctiphanes*. *Bull. Inst. océanogr. Monaco*, T. 50, No. 1021, 19 pp.

FOX, D. L., 1937. Carotenoids and other lipoid-soluble pigments in the sea and in deep marine mud. *Proc. nat. Acad. Sci., Wash.*, Vol. 23, pp. 295–301.

—— 1947. Carotenoid and indolic biochromes of animals. *Annu. Rev. Biochem.*, Vol. 16, pp. 443–70.

—— 1950. Comparative metabolism of organic detritus by inshore animals. *Ecology*. Vol. 31, pp. 100–8.

—— 1953. *Animal Biochromes and Structural Colours*. 379 pp. Cambridge University Press.

FOX, D. L., ISAACS, J. D. & CORCORAN, E. F., 1952. Marine leptopel, its recovery, measurement and distribution. *J. Mar. Res.*, Vol. 11, pp. 29–46.

FOX, D. L., UPDEGRAFF, D. M. & NOVELLI, D. G., 1944. Carotenoid pigments in the ocean floor. *Arch. Biochem.*, Vol. 5, pp. 1–23.

GILLAM, A. E., EL RIDI, M. S. & WIMPENNY, R. S., 1939. The seasonal variation in biological composition of certain plankton samples from the North Sea in relation to their content of vitamin A, carotenoids, chlorophyll, and total fatty matter. *J. exp. Biol.* Vol. 16, pp. 71–88.

GOODWIN, T. W., 1950. The biochemistry of locusts. 2. Carotenoid distribution in solitary and gregarious phases of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and the desert locust (*Schistocerca gregaria* Forsk.). *Biochem. J.*, Vol. 45, pp. 472–9.

—— 1951. Vitamin A-active substances. *Brit. J. Nutr.*, Vol. 5, pp. 94–100.

—— 1952. *The Comparative Biochemistry of the Carotenoids.* 356 pp. London: Chapman and Hall.

KARRER, P., JUCKER, E., RUTSCHMANN, J. & STEINLIN, K., 1945. Zur Kenntnis der Carotinoid-epoxyde. Natürliches Vorkommen von Xanthophyll-epoxyd und α-Carotin-epoxyd. *Helv. chim. Acta*, Vol. 28, pp. 1146–56.

KON, S. K. & THOMPSON, S. Y., 1949*a*. Preformed vitamin A in northern krill. *Biochem. J.*, Vol. 45, p. xxxi.

—— 1949*b*. Preformed vitamin A in Crustacea. *Arch. Biochem.*, Vol. 24, pp. 233–4.

KUHN, R. & SÖRENSEN, N. A., 1938. Über Astaxanthin und Ovoverdin. *Ber. dtsch. chem. Ges.*, Bd. 71, pp. 1879–88.

LEDERER, E., 1938. Recherches sur les caroténoïdes des invertébrés. *Bull. Soc. Chim. biol., Paris*, T. 20, pp. 567–610.

LENEL, R., 1953. Localisation et métabolisme des pigments caroténoïdes chez *Carcinus maenas* Pennant. *C.R. Acad. Sci., Paris*, T. 236, pp. 1448–50.

MACDONALD, R., 1927. Food and habits of *Meganyctiphanes norvegica*. *J. Mar. biol. Ass. U.K.*, Vol. 14, pp. 753–81.

—— 1928. Life-history of *Thysanoessa raschii*. *J. Mar. biol. Ass. U.K.*, Vol. 15, pp. 57–79.

MACPHERSON, N. L., 1933. Vitamin A concentration of cod liver oil correlated with age of cod. *Nature, Lond.*, Vol. 132, p. 26.

MOORE, T., 1950. Fat-soluble vitamins. *Annu. Rev. Biochem.*, Vol. 19, pp. 319–38.

MORTON, R. A., 1940. The constitution and physiological significance of carotene and allied pigments. *J. Soc. chem. Ind., Lond.*, Vol. 59, pp. 301–7.

MORTON, R. A. & ROSEN, D. G., 1949. Carotenoids, vitamin A and 7-dehydrosteroid in the frog (*Rana temporaria*). *Biochem. J.*, Vol. 45, pp. 612–27.

POULSEN, E. M., 1926. Om den store Lyskrebs (*Meganyctiphanes norvegica*). Betydning som Fiskeføde i Skagerak. *Dansk. Fisk-Tid.*, Bd. 24, pp. 286–9.

SÖRENSEN, N. A., 1936. Über die Verbreitung des Astacins unter den Evertebraten. Lipochrome mariner Tiere. VII. *K. norske vidensk. Selsk. Skr.*, No. 1, 14 pp.

STRAIN, H. H., MANNING, W. M. & HARDIN, G., 1944. Xanthophylls & carotenoids of diatoms, brown algae, dinoflagellates and sea anemones. *Biol. Bull., Woods Hole*, Vol. 86, pp. 169–91.

THOMPSON, S. Y., 1949. A photoelectric spectrophotometer suitable for the measurement of vitamin A by the antimony-trichloride reaction. *Brit. J. Nutr.*, Vol. 3, pp. 43–50.

WAGNER, K.-H., 1939. *Vitamin A und β-Carotin des Finn-, Blau- und Spermwals*, 70 pp. Leipzig: Johann Ambrosius Barth.

WALD, G., 1935. Vitamin A in eye tissues. *J. gen. Physiol.*, Vol. 18, pp. 905–15.

—— 1945. The chemical evolution of vision. *Harvey Lect.*, Ser. 41, pp. 117–60.

J. Mar. biol. Ass. U.K. (1954) 33, 613–625
Printed in Great Britain

FURTHER NOTES ON A MARINE MEMBER OF THE SAPROLEGNIACEAE, *LEPTOLEGNIA MARINA* N.SP., INFECTING CERTAIN INVERTEBRATES

By D. Atkins, D.Sc.

(Text-figs. 1–5)

Twenty-five years ago I published a note (Atkins, 1929) on a then unnamed member of the Saprolegniaceae, a destructive fungal parasite of the pea-crab, *Pinnotheres*; the name *Leptolegnia marina* is now proposed for this fungus.

Sexual Reproduction

In the previous note what appeared to be young oogonia were figured (Atkins, 1929, fig. 5), but sexual organs were not then identified with certainty. In 1943, while preserved material of *Pinnotheres pisum* was being re-examined, the eggs beneath the abdomen of one female were seen to be crowded with round bodies, which proved to be the oogonia and oospores of a fungus (Fig. 1). Identification of these plants with the fungus previously described rests on the presence of that fungus in its asexual reproductive stage in the crab bearing the eggs, and in one of a cluster of sixty-eight eggs otherwise containing plants in the sexual stage. In some eggs containing few oospores the mycelium had in part been emptied, following the formation of zoospores, by short emission tubes resembling those of *Leptolegnia marina*, but definite proof that the oospores are the sexual organs of that species would need evidence from pure cultures. This was the only occurrence of the sexual stage of the fungus in more than 100 infected crabs.

The crab taken on 6 August 1928 from a *Mytilus edulis*, received 6 days earlier from the Camel Estuary, near St Issey Cliff, Padstow, north Cornwall, and which had been kept in a finger-bowl of sea water changed at intervals, was noticed to be moribund on 23 August. The cause of death was infection by *Leptolegnia marina*, the gills being crowded with hyphae. In one gill the mycelium was already empty except for a few cysts, in the others production of zoospores was proceeding; no oogonia were present. The crab was fully berried when taken from the mussel. The eggs, brown in colour, contained advanced embryos, most of which hatched before the death of the crab. The eggs remaining were dirty and degenerate, with a rich growth of bacterial filaments, while many bore clusters of vorticellids. Nearly all of them contained the oogonia and oospores of the infecting fungus, up to 200 in a single

egg. Most plants with sexual organs had not previously produced zoospores; this was evident from the absence of projecting tubes on the surface of the *Pinnotheres* egg.

From 6 to 23 August 1928 the temperature of the laboratory sea water was about 16–17° C.

When the ovum of *Pinnotheres pisum*, which is only about 0·3 mm in diameter, is packed with a mass of tangled hyphae and with oogonia, it is exceedingly difficult to tease out the oogonia with mycelial connexions intact, so as to be able to discern the disposition of the sexual organs and especially the origin of the antheridia. That the fungus had attacked eggs containing pre-zoeae added to the difficulty, for the cuticle of these resembled the wall of empty hyphae. The hyphae tended to keep within the cuticle of the zoeae, even of the appendages.

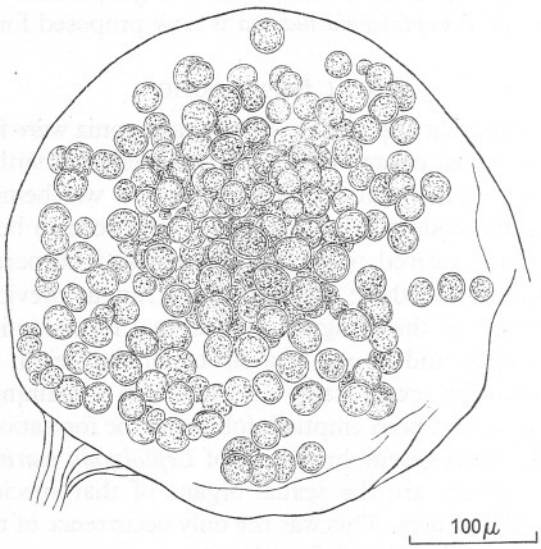

Fig. 1. *Leptolegnia marina*. Immature oospores, thin-walled and with food reserve in scattered drops or granules, within the egg of *Pinnotheres pisum*. Unstained, in 10% formalin.

In some *Pinnotheres* ova the mycelium was young and the oogonia immature (Fig. 2A–F). Other eggs contained plants with numerous oogonia in various stages of maturity. In these the mycelium was mainly empty, but where a few short lengths contained protoplasm these bore young oogonia. In yet others the mycelium was entirely empty and all oospores were ripe (Fig. 3). Septa were frequent.

Oogonia are borne on short side branches (Fig. 2B, E, F), sometimes sessile in diverticula of the hyphae (Figs. 2A; 3D, E), sometimes terminal (Fig. 2C). Fig. 3D is reminiscent of Butler's fig. 19 pl. V (1907) of the sexual apparatus,

of *Pythium rostratum*. In *Leptolegnia marina* the frequently sessile position of the oogonium on the hypha may be due to the restricted condition of growth in the *Pinnotheres* ovum. The oogonia are smooth, thin-walled and spherical, although some tend to be pyriform or oval and an occasional one is irregular in shape. Very rarely there may be one or two papillae above the oogonium.

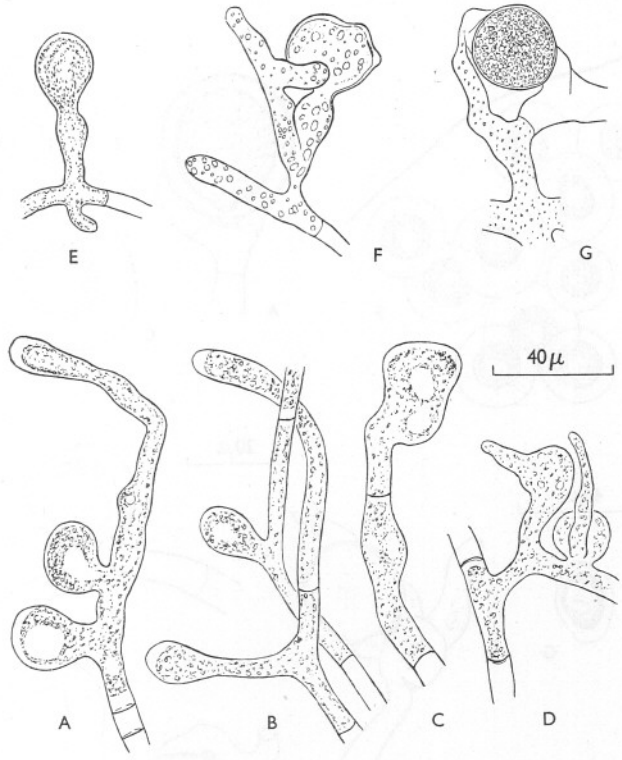

Fig. 2. *Leptolegnia marina*. Young oogonia and immature oospore. In E and F the swelling in the stalk below the oogonium is probably the initial of an hypogynous antheridium. Unstained, in 10% formalin.

Each oogonium contains a single oosphere which fills it completely, and measures 17·5–30μ and occasionally up to 37μ in diameter. The antheridia are hypogynous (Fig. 3A, D, E); those seen were mostly empty. The oogonium is produced into a slight beak where the hypogynous antheridium is applied, and such a structure has not been seen in any other position. It would appear that the antheridia are not always cut off by septa when the oogonium is sessile (see Fig. 3E). Diclinous antheridia have not been observed near young oogonia in the present material and I am not satisfied as to the interpretation of the portions of empty hyphae seen attached to the distal end of a number of

oogonia containing ripe oospores, as, for instance, in Fig. 3E. They may be the remains of elongated protrusions similar to those described on the oogonia of *Leptolegnia subterranea* by Harvey (1925), or, more probably, merely a continuation of the growth of the hypha beyond the oogonium, the latter being intercalary; this is suggested by the formation of the oogonia in Fig. 2D. The oogonium depicted in Fig. 2G appears to have an androgynous antheridium,

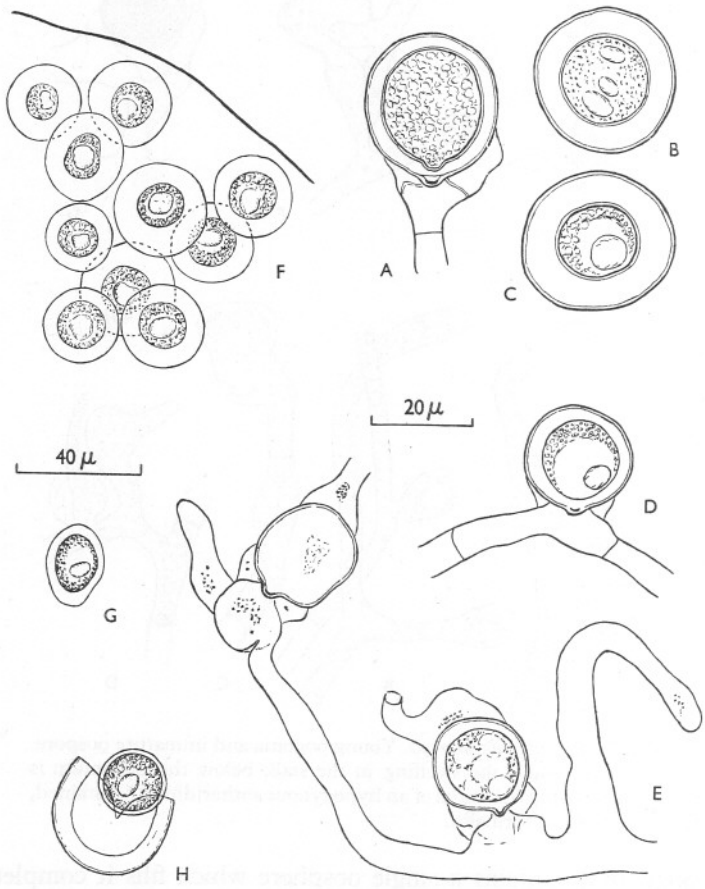

Fig. 3. *Leptolegnia marina*. A: oogonium with oospore and hypogynous antheridium: food reserve scattered. B and C: oospores containing three and one reserve food bodies respectively. D: sessile oogonium with mature oospore and hypogynous antheridium. E: two sessile oogonia on the same branch, both with hypogynous antheridia and both with a portion of hypha of problematical nature attached apically (see pp. 615–6). One oogonium is empty and the oospore in the other is highly vacuolated and perhaps abnormal. Small collections of shining residual granules are present in otherwise empty hyphae. F: fully mature oospores lying beneath membrane of *Pinnotheres* egg. G: pyriform oospore. H: oospore burst by pressure on cover-slip. Unstained, in 10% formalin. A–E to scale on right, F–H to scale on left.

but it may be merely a hyphal branch passing behind the oogonium, and certainly most of the oogonia seen had clearly hypogynous antheridia.

When the oospore is immature the wall is thin (Fig. 2G). As it matures the protoplasmic contents become reduced and the wall thickens greatly (Fig. 3); in fully ripe oospores it is about 7·5μ thick, and is two-layered. The extremely thick outer layer is smooth, colourless, transparent and very faintly stratified, too faintly to be shown in the figures. When oospores are burst by pressure on the cover-slip, sometimes the contents emerge entire, surrounded by a thin wall, the endospore (Fig. 3H). The thin oogonial wall is still distinguishable. In the immature oospore with thin wall, the fat or oily reserve is in scattered droplets (Figs. 2G; 3A); the mature oospore contains one to three reserve bodies, probably when fully mature there is but one, eccentric in position (Fig. 3C, D, F–H). In some oospores this has the appearance of a refringent body, in others of a large oil drop.

The specific characters of *L. marina* are given on p. 622.

Additional Records of Occurrence

In the Body, Eggs and Embryos of Pinnotheres pisum *and in the Body of one Individual of* Pinnotheres pinnotheres (=veterum)

In a previous paper (Atkins, 1929) this fungus was recorded as occurring in crabs taken from *Mytilus edulis* obtained from the Camel Estuary near St Issey Cliff, Padstow, north Cornwall, on 25 November 1927, 24 February, 25 April, 1 August and 14 September 1928. Crabs obtained from *Mytilus* from the same locality on 11 October 1928 were apparently free from infection. The disease was also recorded in crabs taken near the junctions of the Tamar and Tavy (Weir Point, on the Cornwall–Devon border) on 22 March, 10 and 20 April 1928; and from the Yealm Estuary, S. Devon, on 21 July 1928. Subsequently the fungus was again found in female *Pinnotheres pisum* from Padstow mussels received on 27 February, 6 June and 9 August 1929. These mussels were opened over a period of days: the crabs taken from them, and in particular the adult females, were mostly killed and examined the same day, or within a few days, of being found. This rapid examination no doubt explains the small number of crabs in which the fungus was found as compared with previous batches which had been kept together in bowls for several weeks and probably infected each other. In some crabs the fungus was only discovered when they were being searched for an entoniscid.

In June 1952 *Leptolegnia marina* developed in eggs of *Pinnotheres pisum*, ovigerous when obtained from Conway, North Wales; as the eggs were kept in filtered sea water, they probably brought the disease with them.

As stated previously (Atkins, 1929, p. 204), in some few instances it is certain that the crabs were already infected when taken from mussels on arrival from the beds. One instance may be given: among mussels received from Padstow at midday on 1 August 1928, one which was opened the same

day contained an adult female carrying a few dead and degenerate eggs. By 2 August the crab was dead, the gills of the left side opaque and invaded by the fungus.

Information on the rate of development of the fungus and the length of time before death of the host ensues is lacking. The time varies no doubt with the disposition of the fungus in the body of the host: once it has invaded the gills then death is rapid. Death is probably more rapid under laboratory than under natural conditions.

A few crabs have shown the infection and have died within 8 days of reaching the laboratory, but more have died of the disease between the 15th and 57th days. In an exceptional instance one died after 78 days in the laboratory. For some time before death occurred this crab had been a milky orange colour, owing to the breaking down of the gonad among which hyphae were found; there were also patches of fungus in the abdomen, and hyphae were in the pleopods and the gills. Whether crabs could harbour the vegetative stage of the fungus for such a length of time before it became fatal seems doubtful, but they may have carried resting bodies caught in hairs, which germinated after the crabs reached the laboratory. On the other hand, it is possible, or probable, that the fungus is widespread and that some crabs became infected in the laboratory tanks. More females than males have been observed to be infected but more females than males have been examined, the proportion of female to male *P. pisum* in a total of 508 taken from mussels received on 27 February, 6 June and 9 August 1929 being approximately four to one.

Leptolegnia marina in vegetative and spore-producing stages has been found in crabs in the laboratory in all months of the year, except February and November, when its absence was probably due to chance. The temperature range of the laboratory sea water was between 8 and 17° C.

The only occurrence of infection of *Pinnotheres pinnotheres* was of a tiny female (2·1 mm carapace width) obtained on 15 March 1928 from Teignmouth and found dead on 4 May with the fungus visible in the walking legs. It most probably became infected in the laboratory.

In the previous paper, although attention was paid mostly to attacks on the crab itself, an infection was recorded in a few embryos which had hyphae radiating from them (Atkins, 1929). The formation of zoospores was not observed and the fungus was assumed to be that already known, as the crab carrying the embryos later developed the disease.

A few further instances of infection of the eggs of *P. pisum* by *Leptolegnia marina* have been observed, namely two egg-masses of 6 June 1929 and one of 1 September 1930. A number have probably been overlooked, for re-examination of preserved material in 1943 revealed the presence of the fungus in the egg-mass of two more crabs; in one the fungus had produced numerous oogonia, as already described.

The following observations were made in September 1930 on the living fungus in *Pinnotheres* eggs. The unbranched hyphae radiating from some of the eggs were up to 600μ long and 7·5–10μ broad, those within the egg were generally broader, 10–40μ. Spore formation was observed in fine external hyphae: the contents divided into a single row of more or less rectangular blocks, which gradually lost their angles and escaped from the small aperture at the tip of the hypha as pear-shaped, biflagellate zoospores. In the intra-matrical sporangia the spores were in more than one row. As in the fungus in the crab itself, sporangia were formed from unchanged hyphae.

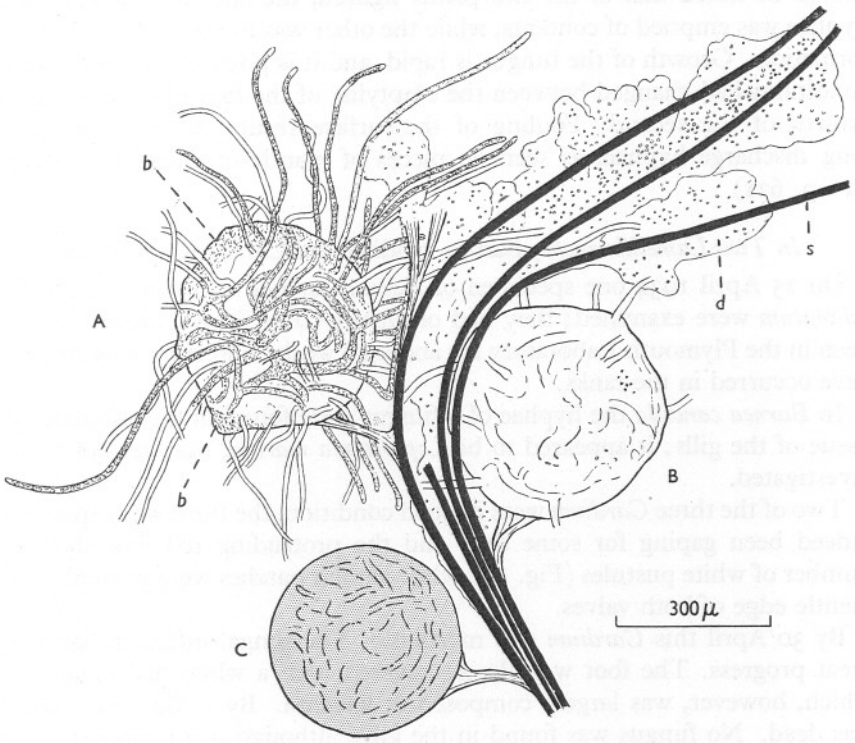

Fig. 4. *Leptolegnia marina*. Three ova of *Pinnotheres pisum* attached to the setae of a pleopod and all infected with the fungus. A: ovum with considerable extra-matrical growth of the fungus. The intra-matrical hyphae are clearly visible as the contents of the ovum have been mostly absorbed. Some hyphae contain zoospores, others are empty after their discharge. B: ovum with mycelium intra-matrical and entirely emptied by the formation of zoospores; the efferent hyphae are all short. C: ovum with mycelium entirely intra-matrical; no efferent hyphae yet formed: ovum opaque as indicated by stippling. *b*, bacteria; *d*, flocculent matter caught on setae (*s*), and in which are embedded numerous encysted spores of the fungus, some of which are indicated. Unstained, in 70 % alcohol.

The formation of zoospores was very prolific, and collections of encysted spores on the crab eggs reached a size of 160 by 100μ. Spores also collected

in piles on pleopod hairs; these reached a size of 700 by 300μ. It seems improbable that in the event of primary infection taking place soon after the extrusion of the crab eggs any would escape destruction.

There appeared to be a greater tendency to the formation of long discharge tubes when the egg was the substratum than when the crab itself was the host; many infected eggs, however, showed only short discharge tubes. Of plants in two adjacent eggs one may have all efferent hyphae short, while the other may have extra-matrical hyphae of considerable length (Fig. 4, p. 619), so that apparently external conditions are not responsible for the difference. But it should be noted that of the two plants figured, the one with short external hyphae was emptied of contents, while the other was in the midst of zoospore formation. Growth of the fungus is rapid, and it is possible that the external conditions had changed between the emptying of the first plant and external growth of the second. Fouling of the surface results in the formation of long discharge hyphae, as seen on pieces of mantle of *Cardium echinatum* (see p. 621).

In Two Lamellibranchs, Barnea candida *and* Cardium echinatum

On 25 April 1934 one specimen of *Barnea candida* and three of *Cardium echinatum* were examined; they had originally come from Torquay but had been in the Plymouth Laboratory for about 10 weeks; infection may therefore have occurred in the tanks.

In *Barnea candida* the hyphae of a fungus were found in the subfilamentar tissue of the gills; it appeared to be *Leptolegnia marina*, but was not further investigated.

Two of the three *Cardium* were in good condition, the third was gaping, had indeed been gaping for some days and the protruding red foot showed a number of white pustules (Fig. 5A), while similar patches were present on the mantle edge of both valves.

By 30 April this *Cardium* was moribund. The fungal infection had made great progress. The foot was almost covered with a white flocculent layer, which, however, was largely composed of bacteria. By 1 May the *Cardium* was dead. No fungus was found in the gills, although it was present round the point of their attachment to the siphons. In the mantle the tissue surrounding the pustules was invaded by a network of hyphae, but there was little extra-matrical growth. On the foot the extra-matrical hyphae reached a length of about 150μ only.

In the mantle edge broad vegetative hyphae were present, such as previously described and figured from the roof of the gill chamber of *Pinnotheres pisum* (Atkins, 1929, p. 206, fig. 2, p. 205). The hyphae farthest away from those discharging zoospores contained clear, transparent cytoplasm, with shining droplets, probably oil globules.

The fungus lived for about 12 days in mantle fragments placed on 1 May in

dishes of sea water; by the end of the period the hyphae had become emptied of their contents by zoospore formation. By 5 May bacteria coated the fragments to some depth, and, apparently because of this fouling, the external hyphae were unusually long, up to about 400μ in length, allowing the zoospores to be liberated beyond the bacterial layer (see Fig. 5B). From

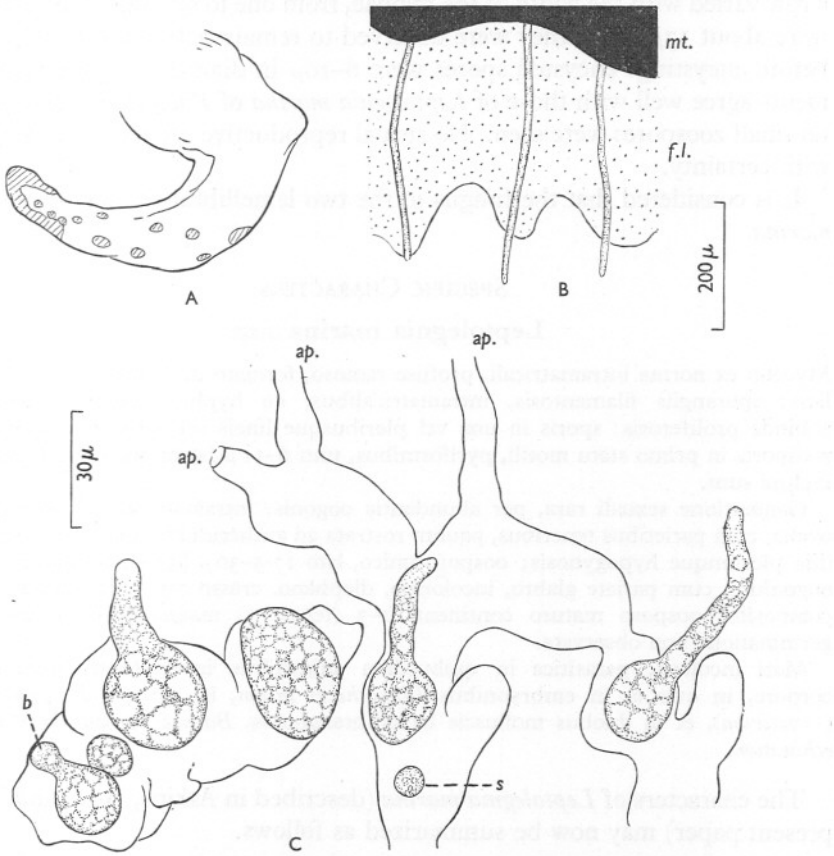

Fig. 5. *Leptolegnia marina* infecting *Cardium echinatum*. A: sketch of areas of infection on foot of the *Cardium*. B: efferent hyphae extending from the mantle (*mt.*) and passing through a flocculent layer (*f.l.*) composed of mucus, sloughed off cells, protozoa, bacteria, etc. C: unusually coarse hyphae (in mantle) most of the contents of which have been used up in zoospore formation, but with a number of rounded bodies of vacuolated protoplasm remaining. Several of the bodies have sent out hyphae; note bulge of tip (*b*) where pressed against wall. *ap.* aperture through which zoospores from old sporangium have been released; *s*, encysted spore. From living material.

7 May onward there was a great tendency for rounded masses and short lengths of protoplasm to remain when the rest of the hypha had emptied its contents in zoospore formation. Each of these bodies then sent out a hypha which eventually penetrated the wall of the old hypha; they were not thick-

walled, and possibly were vegetative portions reconstituting themselves for renewed growth (Fig. 5C).

The liberation of biflagellated pear-shaped zoospores was seen a number of times during the 12 days the fungus lived in the mantle fragments. The sporangia were formed from unchanged hyphae; the number of spores in a row varied with the width of the hyphae, from one to several. The zoospores were about 13μ long; they were observed to remain active for up to 30 min before encysting: encysted spores were 6–10μ in diameter. These measurements agree well with those of *Leptolegnia marina* of *Pinnotheres*, except that no small zoospores were seen. No sexual reproductive organs were identified with certainty.

It is considered that the fungus in the two lamellibranchs was *Leptolegnia marina*.

Specific Characters

Leptolegnia marina n.sp.

Mycelio ex norma intramatricali, profuse ramoso, formato de hyphis 7·5–20 μ [40 μ] latis; sporangiis filamentosis, intramatricalibus, de hyphis immutatis formatis, subinde proliferosis; sporis in una vel pleribusque lineis (de latitudine sporangii); zoosporis in primo statu motili, pyriformibus, tum 6–11 μ latitudine cum in capsulas inclusa sunt.

Generatione sexuali rara, per abundantia oogonia, intramatricalia, sphaeralia vel ovalia, cum parietibus teneribus, paulim rostrata ad antheridium adiunctum; antheridiis plerumque hypogynosis; oosporo unico, lato 17·5–30 μ [37 μ], complenti totum oogonium, cum pariete glabro, incolorato, diaphano, crasso 7·5 μ, de duobus stratis composito; oosporo maturo continenti 1–3 (ferme 1) magna corpora reservata; germinatione non observata.

Mari incolens; parasitica in quibusdam animalibus invertebratis marinis; in corpore, in ovis et in embryonibus *Pinnotheres pisum*, in corpore *P. pinnotheres* (=*veterum*), et in duobus molluscis lamellibranchiatis, *Barnea candida* et *Cardium echinatum*.

The characters of *Leptolegnia marina* (described in Atkins, 1929, and in the present paper) may now be summarized as follows.

Mycelium almost entirely intra-matrical and then freely branched. Hyphae 7·5–20 μ in diameter, sometimes up to 40 μ in diameter. Sporangia filamentous, formed from unchanged hyphae within the tissue of the host; occasionally proliferous: short branches for the discharge of zoospores lead to the exterior of the host. Number of rows of zoospores within the sporangium vary with its width, from one to several. When active within the sporangium the zoospores may be oval, pyriform or rod-shaped. First zoospores 8–14 μ in length, pyriform—when free swimming—biflagellate, swimming away upon emergence and encysting after an interval varying from a few to 50 min. Encysted spores 6–11 μ in diameter. Is probably diplanetic (Atkins, 1929, pp. 213–14). Sexual reproduction rare, then oogonia abundant. Oogonia formed within tissue of host: borne on short side branches, frequently sessile in diverticula of the hyphae, perhaps intercalary, generally spherical, sometimes pyriform or oval, rarely irregular in shape; thin-walled, smooth, very occasionally

with one or two small papillae. A slight beak present where hypogynous antheridium attached. Antheridia generally hypogynous. Oospores single, completely filling oogonium, 17·5–30 μ in diameter, occasionally up to 37 μ in diameter. Wall smooth, colourless, transparent, exceedingly thick (up to 7·5 μ), two-layered, very faintly stratified and all surrounded by oogonial wall. Mature egg with 1 to 3, generally one, large eccentric oil or fat body. Germination not observed.

Habitat: marine, parasitic in certain marine invertebrates, being so far known from the body, eggs and embryos of *Pinnotheres pisum*, from the body of *P. pinnotheres* (=*veterum*), and from two lamellibranchs, *Barnea candida* and *Cardium echinatum*.

Affinities

The saprolegniacean fungus which attacks certain marine invertebrates, is considered to belong to the genus *Leptolegnia* with which it has the following characters in common: (i) sporangia filamentous, formed from unchanged hyphae; (ii) zoospores on emerging swim away; (iii) single oospore completely filling the oogonium, eccentric; (iv) ripe oospore wall exceedingly thick. (It differs from the genus *Saprolegnia* in characters i, iii and iv.)

Leptolegnia marina is distinguished from the three known species of the genus, *L. caudata* de Bary, *L. subterranea* Coker and Harvey and *L. eccentrica* Coker in its marine habitat, in which indeed it differs from all known members of the Saprolegniaceae,[1] although one member *Synchaetophagus balticus*, parasitic in the rotifer, *Synchaeta monopus*, has been recorded from brackish water, salinity 5·3–11‰, in the Baltic (Apstein, 1911). *Leptolegnia marina* is also peculiar in that the mycelium is almost entirely intra-matrical, although when *Pinnotheres* eggs are the substrata there is some tendency to extra-matrical growth, not exceeding 1·0 mm in length, the external hyphae being slender, 7·5–10μ thick, and rarely branched, with zoospores in a single row.

The mycelium of *Leptolegnia marina* is not only more freely branched than in the other three species, but is of somewhat coarser growth (7·5–20μ in diameter, sometimes up to 40μ), although Couch (1932, p. 584) records that strains of *L. caudata* vary considerably in their robustness, the hyphae from some often approximating a well-developed *Saprolegnia* in thickness and length. In *Leptolegnia caudata* the hyphae are about 10–18μ thick (Coker & Matthews, 1937, p. 29), in *L. subterranea* 9·4–11·8μ thick (Harvey, 1925, p. 158) and in *L. eccentrica* 4·8–7·2μ thick (Coker, 1927, p. 215).

L. marina differs from the other species in that the zoospores are formed typically in more than one row in the sporangium, the number varying with its width from one to several. In *L. caudata* and *L. subterranea* the zoospores are said to be typically in a single row (Coker & Matthews, 1937, pp. 29, 30), from which it would appear that they are not invariably so. The peculiar bending back or reshaping of the spores before swimming away, which

[1] Since this was written a second marine member of the Saprolegniaceae, *Plectospira dubia* n.sp., has been found (Atkins, 1954).

is characteristic of the other species (Petersen, 1910; Coker, 1923, 1927; Harvey, 1925; Mathews, 1932) was not observed in *L. marina*.

In *L. caudata* the antheridia are diclinous (Coker, 1923, p. 158; Couch, 1932, p. 596; Coker & Matthews, 1937, p. 30), in *L. eccentrica* closely androgynous (Coker, 1927) and in *L. subterranea* lacking (Harvey, 1925), while in *L. marina* they are generally hypogynous.

L. marina agrees with *L. caudata* in being a destructive parasite of an animal, but the latter species is also found saprophytic on animal and vegetable substrata (Couch, 1932, p. 584). It moreover agrees with *L. caudata* in the occasional renewal of the sporangium within an empty one (for *L. caudata* see Coker, 1923; Coker & Matthews, 1937; and for *L. marina* see Atkins, 1929); in the rarity of sexual reproduction (for *L. caudata* see Petersen, 1910; Coker 1923; Couch, 1932) and the formation of oogonia within the tissue of the animal host (for *L. caudata* see Petersen, 1910, p. 513).

In *L. marina* the greater part of the protoplasm of the mycelium may be absorbed in zoospore formation (Atkins, 1929, pp. 209–210), agreeing in this with *L. caudata* (Petersen, 1910) and *L. eccentrica* (Coker, 1927). As in *L. eccentrica* and *L. subterranea* so in *L. marina* spore discharge occurs commonly at night.

L. marina in the great thickness of the mature oospore wall (up to $7{\cdot}5\mu$ thick) resembles most nearly *L. eccentrica* (5μ or more, Coker, 1927) and *L. subterranea* ($3{\cdot}7$–$5{\cdot}5\mu$, rarely $6{\cdot}5\,\mu$, Harvey, 1925) but differs from them in that the wall is smooth. The oospore wall is stated by Couch (1932) to be very thick in *L. caudata*, but he gives no measurements: according to Coker (1927) it is only about one-third as thick as in the other two species.

In *L. marina* the food reserve in the fully mature oospore is in the form of a single large eccentric refringent body or oil drop, thus agreeing with *L. eccentrica* (Coker, 1927) and differing from *L. caudata* (Coker, 1923; Couch, 1932) and *L. subterranea* (Harvey, 1925) in both of which it takes the form of a lunate cap of small oil drops.

I gratefully acknowledge the kindness of the late Prof. J. H. Orton, F.R.S., Dr W. B. Turrill, Miss E. M. Wakefield and the late Miss M. A. Sexton in procuring for me excerpts from the *Journal of the Elisha Mitchell Scientific Society* which I was unable to obtain on loan. Mrs A. H. Beers of the Botany Department, University of North Carolina, kindly supplied me with references to descriptions of two species of *Leptolegnia*. I wish to record my appreciation of the great help the Surrey County Library—with its associated network of libraries—has been to me in supplying on loan many necessary publications.

I am indebted to Dr W. R. G. Atkins, F.R.S., for the interest he has taken in this marine fungus, and to Dr B. Barnes for reading the manuscript. Dr J. Morton has most kindly turned the diagnosis into Latin.

Type material has been deposited in the British Museum.

REFERENCES

APSTEIN, C., 1911. *Synchaetophagus balticus*, ein in *Synchaeta* lebender Pilz. *Wiss. Meeresuntersuch., Abt. Kiel*, N.F., Bd. 12, pp. 163–6.

ATKINS, D., 1929. On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. *J. Mar. biol. Ass. U.K.*, Vol. 16, pp. 203–19.

—— 1954. A marine fungus, *Plectospira dubia* n.sp. (Saprolegniaceae) infecting crustacean eggs and small Crustacea. *J. Mar. biol. Ass. U.K.*, Vol. 33, pp. 721–32.

BUTLER, E. J., 1907. An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dep. Agric. India, Bot.*, Vol. 1, pp. 1–160.

COKER, W. C., 1923. *The Saprolegniaceae, with Notes on other Water Molds*. 201 pp. University of North Carolina Press.

—— 1927. Other water molds from the soil. *J. Elisha Mitchell sci. Soc.*, Vol. 42, pp. 207–26.

COKER, W. C. & MATTHEWS, V. D., 1937. Saprolegniales. *N. Amer. Flora*, Vol. 2, pp. 15–76.

COUCH, J. N., 1932. The development of the sexual organs in *Leptolegnia caudata*. *Amer. J. Bot.*, Vol. 19, pp. 584–99.

HARVEY, J. V., 1925. A study of the water molds and pythiums occurring in the soils of Chapel Hill. *J. Elisha Mitchell sci. Soc.*, Vol. 41, pp. 151–64.

MATHEWS, A. C., 1932. Cytological observations on zoospore formation in *Leptolegnia caudata* de Bary. *J. Elisha Mitchell sci. Soc.*, Vol. 47, pp. 281–92.

PETERSEN, H. E., 1910. An account of Danish freshwater Phycomycetes, with biological and systematic remarks. *Ann. mycol., Berl.*, Bd. 8, pp. 494–560.

J. Mar. biol. Ass. U.K. (1954) 33, 627–636
Printed in Great Britain

LEG DISPOSITION IN THE BRACHYURAN MEGALOPA WHEN SWIMMING

By D. Atkins, D.Sc.

(Text-figs. 1 and 2)

As long ago as 1852 Dana carefully described and figured the position of the legs of certain megalopae whilst swimming. Later writers having apparently overlooked his observations, his remarks are worth quoting in full, remembering that he is writing of five pairs of legs: 'In the genera *Monolepis*, *Marestia* and *Cyllene*, and probably also in *Tribola*,* the posterior legs are capable of overlying the posterior angle of the carapax, and there is a corresponding depression in this surface, which depression is sometimes abrupt and channel-like. The penult pair of legs also admits of being thrown forward over the border of the carapax, and extends above the base of the eyes, so that the tarsus hangs down in front. The second and third pairs fold up and partially overlap the sides of the carapax, beneath the fourth or penult pair, or, as is sometimes seen, the third pair is thrown forward like the fourth. A species very near *Megalopa mutica*, abundant near the Cape of Good Hope is figured on plate 31, with the legs in the position they had *while the animal was swimming*. This position is not often observed, since the animal when disturbed, is almost sure to swim with the legs extended; and the hinder legs are mostly like the others in form and habit. On the same plate, this position is shown for another species from the Sooloo Sea; in the former, the surface of the carapax is simply a little depressed or concave for the folded posterior legs (genus *Marestia*) while in the latter (genus *Monolepis*), as shown in figure 5*b*, there is an abrupt channel' (Dana, 1852*b*, pp. 485–6, pl. 31, figs. 2*a*, 5*a*).

Unaware of Dana's work I have recently made observations on the swimming of the megalopae of a number of species of crabs at Plymouth.

The brachyuran megalopa when walking (e.g. *Carcinides*), or when clinging to an object (e.g. *Macropodia*), uses its legs as in the crab stage. When swimming, by means of the pleopods, with abdomen outstretched, two different methods of disposing of the legs have been observed. In the more spectacular method, found in all but three of the species examined at Plymouth, the legs are packed away in a curious manner so that the body is compact and the outline approximately rectangular in dorsal and ventral

* Megalopae respectively of *Ocypode*, *Grapsids?*, *Lupa* (*Portunus*) and *Plagusia?*, see Gurney, 1939, pp. 118, 119.

view, but wider in front than behind because of the eyestalks. This arrangement of the legs when swimming is common to the following species: *Portunus depurator*, *P. puber*, *P. arcuatus*, *P. pusillus*, *P. marmoreus*, *P. holsatus*, *Carcinides maenas*, *Portumnus latipes*, *Cancer pagurus*, *Atelecyclus septemdentatus*, *Pilumnus hirtellus*, *Pinnotheres pisum* (but not *P. pinnotheres*) (Brachyrhyncha); *Ebalia tuberosa*, *E. cranchii* (Oxystomata); *Maia squinado*, *Eurynome aspera*, *Hyas* sp., and several species of *Inachus* and *Macropodia* (Oxyrhyncha). It is briefly that, while the chelipeds and first two pairs of walking legs are folded beneath the body, the third and fourth pairs are wrapped over the dorsum, the third being stretched out anteriorly (Figs. 1 and 2).

The chelipeds are carried on the ventral surface, bent upon themselves at the carpus, the chelae pointing posteriorly and touching in the mid-line of the thorax (Fig. 2B). The exact disposal of the chelipeds varies somewhat according to their length and size. In the megalopa of *Carcinides* there is a curiously large hook on the ischium of the cheliped, and when the chelipeds are folded for swimming the chela lies to the inner side of this (Fig. 2A). It appears to be present in most of the British Brachyrhyncha, although not in the Oxyrhyncha and Oxystomata megalopae (Lebour, 1928), and Lebour (1944, p. 13) noted in *Portumnus latipes* that these large hooks help in holding fast the food while the megalopa swims.

The proximal segments of the first and second walking legs are directed anteriorly and dorsally close to the side of the body, even slightly overlapping the dorsum in long-legged megalopae (Fig. 1C), bent sharply at the carpus, the propodus and dactylus being on the ventral surface, lying close together and to the chelipeds (Fig. 2B).

The proximal segments of the third walking legs are curved round the side of the body on to the dorsum and the leg is stretched out anteriorly, fitting in a groove by the side of the gastric hump. The dactylus curves over the eye stalk and, according to the length of the leg, projects slightly beyond it, as in *Eurynome aspera*, *Ebalia* (Fig. 1B); or is looped round it on to the ventral surface, the tip directed posteriorly, as in *Hyas*, *Inachus*, *Macropodia* (Fig. 1C), with long slender legs. In *Carcinides maenas* the dactylus is sometimes stretched out straight and sometimes curved round the eye (Figs. 1A, 2A) as it is also in *Portunus* spp.

The fourth walking legs, which are curved over the dorsum, are bent back upon themselves at the carpus, the ischium and merus being directed anteriorly and medially, the carpus and propodus posteriorly and outward and the dactylus posteriorly and inward, its tip reaching the base of the ischium. The tip projects slightly beyond the thorax, fitting into the angle between it and the abdomen. In *Macropodia* and *Inachus* the proximal ends of the carpi of the two sides touch or almost touch in the mid-dorsal line (Fig. 1C), while there is a small space between them in *Eurynome* and a wider space in

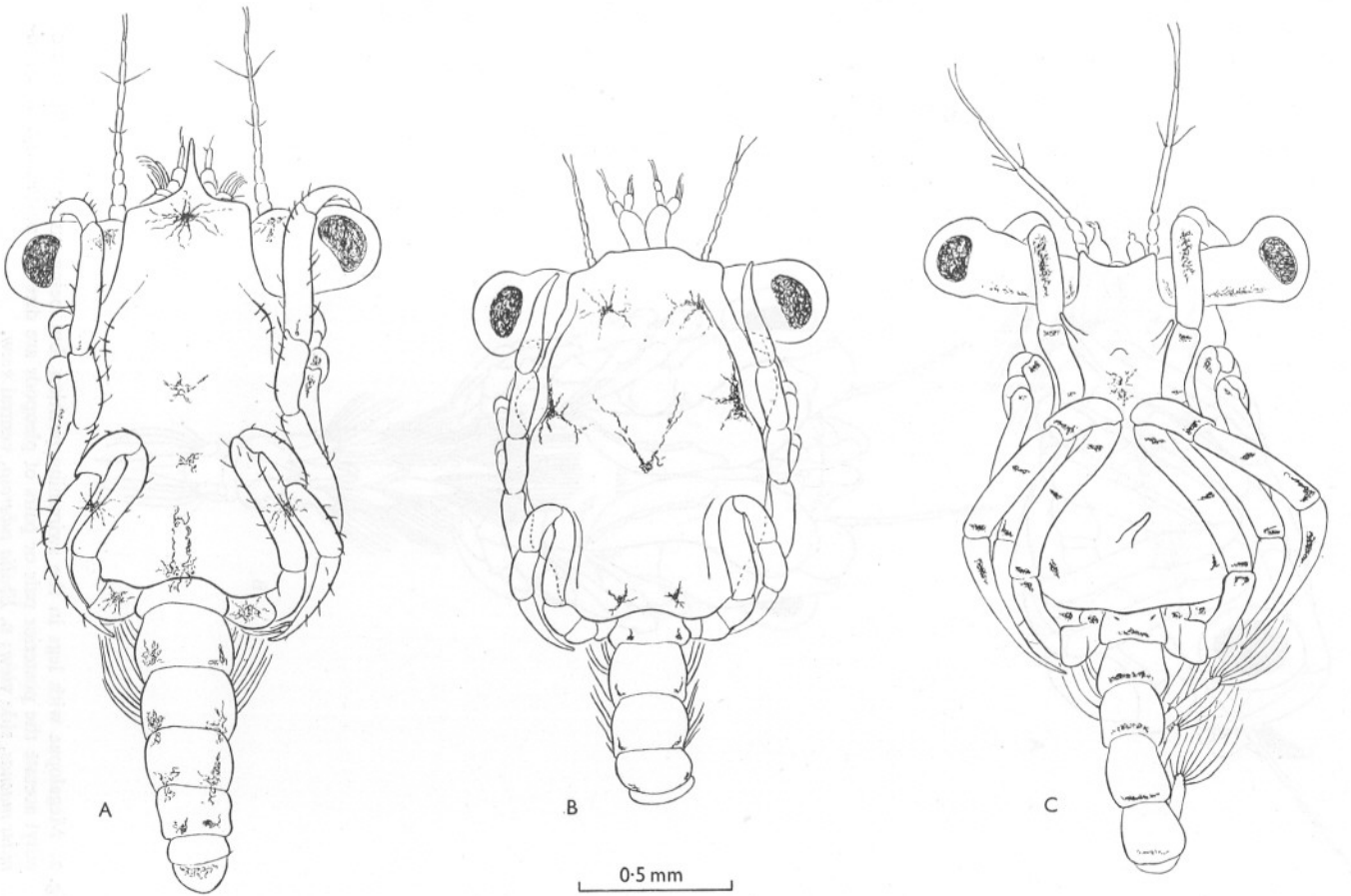

Fig. 1. Megalopae with legs in the swimming position, dorsal view: drawn alive. A, *Carcinides maenas*; B, *Ebalia tuberosa*; C, *Macropodia* sp.

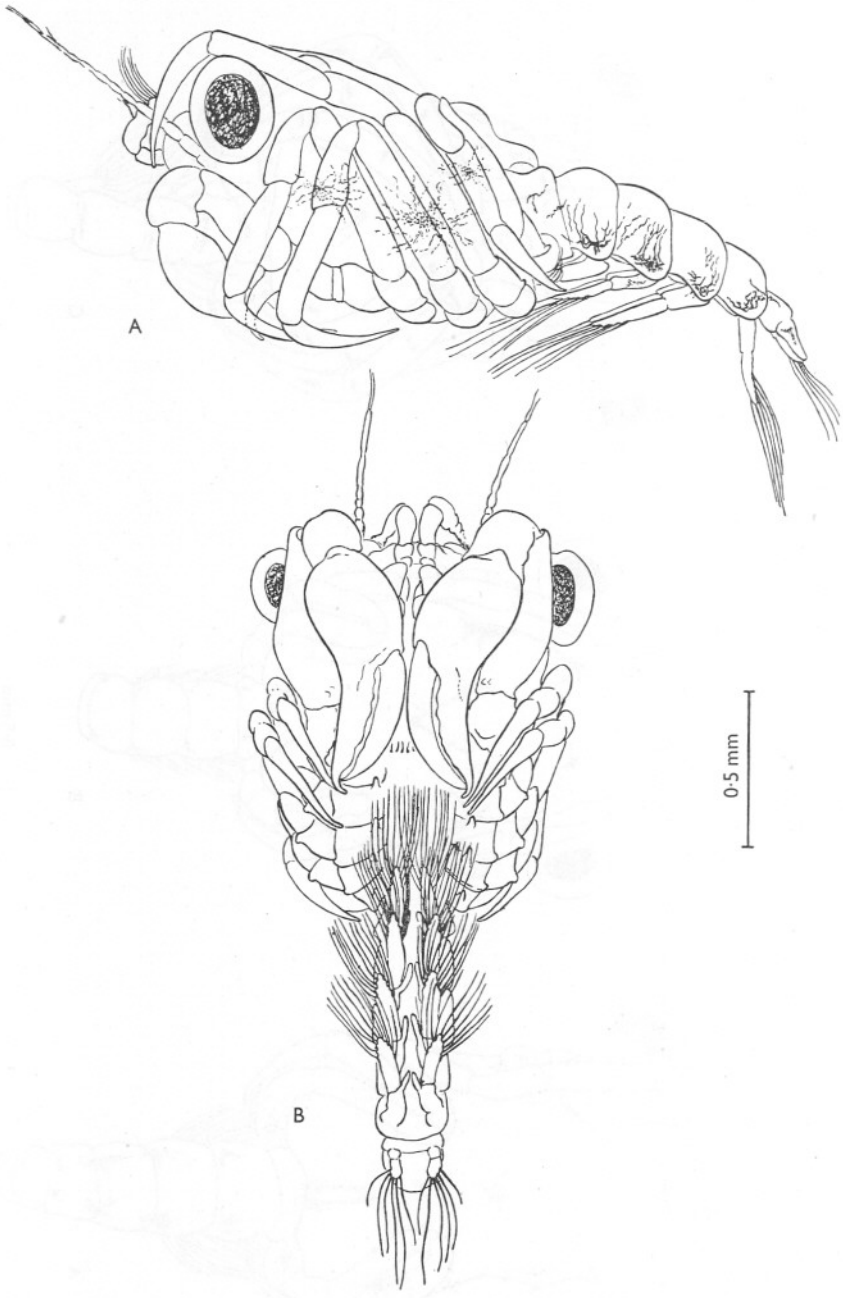

Fig. 2. Megalopae with legs in the swimming position, but being under the influence of ethyl acetate the posterior pair or pairs of pleopods are directed posteriorly. A, *Carcinides maenas*, side view; B, *Ebalia tuberosa*, ventral view.

Portunus, *Carcinides* and *Ebalia*. In *Ebalia* the body is thick and the fourth walking legs fit into depressions, while the third are more to the side than in most megalopae and curve round the dorsal hump (Fig. 1B).

The arrangement of the legs is most easily seen in *Macropodia* and *Inachus* in which the body is nearly transparent and little pigmented, and the legs banded with dark pigment, so that the third and fourth walking legs are conspicuous on the dorsum. Then, perhaps owing to the length of the legs or conditions of captivity, these megalopae and those of *Eurynome*, arrange their legs slowly enough for the eyes to follow the movement. Also some of them were observed frequently to rest on the bottom after a swim with their legs in the swimming position.

It was at first difficult to see the manner in which the *Carcinides* megalopa carried its legs, for the legs are transparent and almost colourless. When the third and fourth walking legs are folded over the back they are practically invisible. Moreover, these megalopae are particularly rapid in their movements, folding the legs and jumping into their swim in an instant. The forward and upward leap is coincident with the sudden straightening of the abdomen from the bent position beneath the thorax.

Unlike Williamson's (1903, p. 158) observation that in the megalopa of *Carcinides* the abdomen remains extended and is never tucked in under the thorax, the few specimens seen walked with it curved beneath the thorax; when molested the animal curled up with the legs in the swimming position and the abdomen tucked under the thorax, taking up the position shown in Crane's figure 2 (1940) of *Ocypode albicans*. Several other species behaved in the same manner (see also Kemp, 1915, p. 219): it was recorded of *Calappa marmorata* (Oxystomata) by Smith (1880, p. 266), but with the difference that chelipeds and all legs were folded very compactly beneath the carapace. This behaviour possibly varies with age, the abdomen being more apt to be tucked under the body as the moult to the crab stage approaches. The megalopae of *Macropodia* and *Inachus*, on the other hand, were rarely seen with the abdomen bent under, even during the day or two preceding the moult, although they momentarily flexed it. This habitual extension of the abdomen may be because these megalopae rarely seem to walk, but will cling to objects with all legs.

That the described arrangement of the legs when swimming is probably common to many other megalopae than those observed at Plymouth would seem to be indicated by many published figures of this post-larval stage, the proximal segments of the third and fourth walking legs projecting well beyond the carapace, either posteriorly or laterally (Connolly, 1923, fig. 11; 1925, fig. 6; Hart, 1935, figs. 2A, 3K, 6A, 7H; Lebour, 1928, many figures) and some even showing the fourth pair of legs folded over the dorsum, for instance Connolly's figure 11 of the megalopa of *Cancer amoenus* and Lebour's figure 8, pl. xi, of *Xantho incisus*. In some instances, no doubt, the protrusion of the

proximal segments of the last two legs, as shown in the figures mentioned above, is partly due to the preservative, especially if formalin had been used, but Dr Lebour informs me that her figures were of living or moribund specimens so that the proximal segments of the last two pairs of legs would be in the normal position.

This comparative freedom of the last two pairs of legs from the carapace as compared with the other legs, which allows of their being wrapped over the dorsum in the swimming megalopa, is more pronounced in some megalopae and crabs than in others. In certain species the last two pairs of legs, which are said to arise dorsally, have a specialized function if not specialized formation, and their freedom of movement is utilized to hold objects over the carapace. For instance in the Dorippidae (Oxystomata), *Ethusa investigatoris* (Menon, 1937), *Dorippe dorsipes* and *D. facchino* (Hornell, 1922, p. 932) have the claws of the last two pairs of legs modified so that they can hold shells, etc., on their backs, and this habit may even be exhibited by the megalopa (Menon, 1937, p. 42). The last two pairs of legs of the first and second young crab stages of *Pilumnus hirtellus* have great freedom of movement and can be stretched over the dorsum when clasping objects. The last pair, moreover, can be stretched directly backward and the young crabs frequently stand on their heads, ventral surface uppermost, supported on the front and the tips of the posteriorly and dorsally stretched last pair of legs. Dressing of the dorsum by the last pair of legs was not observed; it may be that debris collects on it because young crabs so frequently lie on their backs.

Among the megalopae observed at Plymouth three exceptions occurred to the usual method of disposing of the legs. In these, all the legs including the chelipeds were bent up under the body while swimming, as described by Gurney (1903, p. 464) for *Corystes cassivelaunus*. In addition to *Corystes*, *Thia polita* and *Pinnotheres pinnotheres* (=*veterum*) swam in this manner. These megalopae appeared to swim less rapidly than those which were more finely stream-lined. They were obtained by moulting from zoeae in captivity, but seemed healthy enough; they were watched swimming a number of times, and it would appear to be their normal method, although it would have been more satisfactory if the megalopae had been taken direct from the plankton. As Dana (1852*b*, p. 486) has cautioned, megalopae may need to be watched for some time before they show their characteristic method of swimming. Those which normally swim with the third and fourth legs over the dorsum may on occasion swim without packing the legs tightly away, but holding them loosely bent about the middle, with the chelipeds in a loose bow, tips close together in mid-line. They then swim slowly by comparison with their rapid movement when the legs are compactly stowed away.

A reason for the legs being held close to the body, the third and fourth folded over the dorsum, is no doubt that the stream-line effect offers less resistance to movement in water; moreover, the arrangement may have some-

thing to do with improved balance whilst swimming. If it allowed of increased speed in swimming it would be an advantage in escaping from danger. Although megalopae are known to eat fast-moving larvae, including zoeae and other megalopae (Lebour, 1927, p. 807; 1928, p. 500), it is not known if they actively hunt them. Incidentally the removal of the last two pairs of legs on to the dorsum prevents any possibility of their interfering with the action of the anterior pleopods, the swimming setae of which extend well forward on to the thorax at the beginning of the effective stroke, which is backward with the plumose setae spread out fanwise. Less effective is the arrangement in which all legs are bent beneath the body, as can be assessed by watching the swimming of megalopae with the two different arrangements of the legs.

The three species of megalopae which swim with all legs folded beneath the body possibly have a pelagic existence of short duration. The megalopa of *Corystes cassivelaunus* is very crab-like and, as noted by Gurney (1903, pp. 463–4), has most of the characteristics and habits of the adult, burrowing into fine sand. According to him it appears not to be a pelagic form properly speaking, and was only once obtained in the tow-net, and then within a fathom or so of the bottom, in deep water. Very occasionally the megalopae have been taken in swarms at the surface (Russell, 1927, p. 602): as the zoeae apparently have a preference for the surface layers (Russell, 1927, p. 602) it is possible that the swarms of megalopae had not long moulted into that stage. The megalopa of *Thia polita* (which incidentally has rather short legs) if provided with muddy gravel, on which the adult is taken, soon backs beneath it: this megalopa also may possibly have a short swimming stage. *Pinnotheres pinnotheres* possibly enters its host (*Ascidia mentula* at Plymouth) in the megalopa stage, for it is of a good size with a carapace length of 0·96 mm. and width of 0·8 mm.; the megalopae of *Pinnixa faba* (Pinnotheridae) are known to do so (Wells, 1940). These megalopae once in the host would feed after the manner of the adult and would be in a protected position. The smaller megalopa of *P. pisum*, with carapace length of 0·69 mm. and width of 0·56 mm. swims with the third and fourth walking legs over the dorsum and because of its small size it is doubtful whether this species enters the mollusc host in the megalopa stage; the risk of its being smothered in mucus and expelled may be too great.

Previous References to the Disposition of the Legs in Megalopae

The megalopae of crabs occurring at Plymouth are of no great size. With the outstanding exception of *Corystes cassivelaunus*, with carapace length 3·36–3·6 mm and width 3·1–3·24 mm, the largest Plymouth megalopa, that of *Atelecyclus septemdentatus*, has a carapace length of 2·7 mm. Although a few references to the position of the legs whilst swimming of megalopae of this size occur (Hyman, 1920, p. 497; 1922, p. 455; Lebour, 1927, p. 807), they are

not detailed, merely mentioning that the legs are folded against the body. They do not describe the peculiar posture noted in this paper. Hyman (1920, p. 497), however, mentioned that the last pair of legs in *Uca* (*Gelasimus*) are carried folded over the back and kept in that position even when clinging or walking.

The only account of the peculiar posture as occurring during swimming is that of Dana (1852*a*, *b*) already quoted (see p. 627). He observed it of megalopae of three genera, and of carapace length 3·3–11·0 mm. In addition several detailed descriptions occur of the complicated folding of the legs and of the grooving of the sides of the body and of the carapace posterio-laterally for the reception of the legs, and of the thorax for the repose of the abdomen, in a group of relatively gigantic megalopae, with carapace length 4·0–9·4 mm, with thick hard cuticle, some of which belong to the Ocypodidae (Say, 1817; Smith, 1873; Kemp, 1915; Miyake, 1939, pp. 193–5, as a crab; Crane, 1940; Edmonson, 1949). Most of these descriptions are of museum material, for curiously enough these large megalopae appear frequently to retain their legs folded against the body after death. Except for Dana, who actually observed them swimming, their peculiar characteristics have been considered as adapting them for life on sandy shores where certain of them have been taken, and some are known to moult into the first crab stage (Say, 1817, p. 158; Smith, 1873; 1880, p. 255; Kemp, 1915, p. 219; Edmonson, 1949, p. 235), and where the adults live (Rathbun, 1918, pp. 369, 372, 374). Such are rotund, have thick hard cuticle and the body so grooved for the reception of the legs and abdomen that they may be rolled about like tiny balls without appreciable injury (Crane, 1940, pp. 66, 81; Edmonson, 1949, p. 235). It may be recalled here that the megalopa of *Ebalia*, although tiny with a carapace length of about 1·2 mm, is also rotund and has marked depressions for the reception of the last pair of legs (p. 631): the adult crabs occur in sand. It appears to me that in all probability the stream-lining of the body was originally an adaptation for swimming, for these large megalopae have the pleopods fully developed and a number have been taken in the open sea (Dana, 1852*a*; 1852*b*; Smith, 1880, p. 255; Crane, 1940, p. 81; Edmonson, 1949, p. 243). Moreover, the posture is common to many megalopae the adults of which do not inhabit sandy beaches, and grooving of the body for the reception of the legs has been described in a megalopa of *Plagusia* of carapace length 4·6 mm (Rathbun, 1924; Boone, 1927, p. 271; Crane, 1940, p. 70) the adults of which live among rocks awash at high tide and on drift timber in the open sea (Rathbun, 1918, p. 332). No doubt the grooving of the body for the reception of the legs when swimming is more apparent in large megalopae with thick cuticle than in those with thin cuticle or of small size.

This work was made possible by the kindness of the Board of Studies in Zoology of London University in granting me the use of the London

University Table at the Laboratory of the Marine Biological Association at Plymouth.

I was unable to obtain on loan the volume of plates of Dana's Crustacea U.S. Navy (1852*b*) and am indebted to Dr J. Morton for kindly making sketches from pl. 31 of two megalopae in the swimming attitude.

Summary

Many brachyuran megalopae swim with the legs disposed in a curious manner. The chelipeds and first two pairs of walking legs are folded tightly beneath the body, while the two posterior pairs are wrapped over the dorsum, the third pair being stretched out anteriorly, the dactylus, according to its length, touching or being looped round the eye-stalk. It is suggested that this posture gives stream-lining to the megalopa and improves its swimming. The described arrangement has been observed in all but three of some twenty-five species occurring at Plymouth.

In the three exceptions, *Corystes cassivelaunus*, *Thia polita* and *Pinnotheres pinnotheres* (=*veterum*), all legs are carried folded beneath the body.

Reference is made to previous descriptions of the folding of the legs in the brachyuran megalopa.

References

Boone, L., 1927. The littoral crustacean fauna of the Galapagos Islands. Part 1 Brachyura. *Zoologica, N.Y.*, Vol. 8, pp. 127–288.

Connolly, C. J., 1923. The larval stages and megalops of *Cancer amoenus* (Herbst.). *Contr. Canad. Biol.*, N.S., Vol. 1, pp. 337–52.

—— 1925. The larval stages and megalops of *Rhithropanopeus harrisi* (Gould). *Contr. Canad. Biol.*, Vol. 2, pp. 327–34.

Crane, J., 1940. On the post-embryonic development of brachyuran crabs of the Genus *Ocypode*. *Zoologica, N.Y.*, Vol. 25, pp. 65–82.

Dana, J. D., 1852*a*. Conspectus of the Crustacea of the exploring expedition under Capt. Wilkes, U.S.N. Paguridea, continued, and subtribe Megalopidea. *Proc. Acad. nat. Sci. Philadelphia*, Vol. 6, pp. 6–10.

—— 1852*b*. Crustacea. *U.S. Exploring Expedition during the years* 1838–42 *under the Command of C. Wilkes, U.S.N.*, Vol. 13, pt. 1.

Edmonson, C. H., 1949. Some brachyuran megalopa. *Occ. Pap. Bishop Mus.*, Vol. 19, pp. 233–46.

Gurney, R., 1903. The metamorphosis of *Corystes cassivelaunus* (Pennant). *Quart. J. micr. Sci.*, Vol. 46, pp. 461–78.

—— 1939. Bibliography of the larvae of decapod Crustacea. *Ray Soc. Publ.*, Vol. 125.

—— 1942. Larvae of decapod Crustacea. *Ray Soc. Publ.*, Vol. 129.

Hart, J. F. L., 1935. The larval development of British Columbia Brachyura—I, Xanthidae, Pinnotheridae (in part) and Grapsidae. *Canad. J. Res.*, Vol. 12, pp. 411–32.

Hornell, J., 1922. Some commensals of Indian alcyonarians and crabs. *J. Bombay nat. Hist. Soc.*, Vol. 28, pp. 926–36.

HYMAN, O. W., 1920. The development of *Gelassimus* after hatching. *J. Morph.*, Vol. 33 (2), pp. 485–501.

—— 1922. Adventures in the life of a fiddler crab. *Rep. Smithson. Instn*, 1920 (1922), pp. 443–60.

KEMP, S., 1915. Fauna of the Chilka Lake: Crustacea Decapoda. *Mem. Ind. Mus.*, Vol. 5, pp. 199–325.

LEBOUR, M. V., 1927. Studies of the Plymouth Brachyura. I. *J. Mar. biol. Ass., U.K.*, Vol. 14, pp. 795–821.

—— 1928. The larval stages of the Plymouth Brachyura. *Proc. zool Soc. Lond.*, 1928, pp. 473–560.

—— 1944. The larval stages of Portumnus (Crustacea Brachyura) with notes on some other genera. *J. Mar. biol. Ass., U.K.*, Vol. 26, pp. 7–15.

MENON, M. K., 1937. Decapod larvae from the Madras plankton. *Bull. Madras Govt Mus.*, (*nat. hist.*) N.S., Vol. 3, No. 5.

MIYAKE, S., 1939. Notes on Crustacea Brachyura collected by Professor Teiso Esaki's Micronesia Expeditions 1937–1938. *Rec. oceanogr. Wks Jap.*, Vol. 10, pp. 168–247.

RATHBUN, M. J., 1918. The grapsoid crabs of America. *Bull. U.S. nat. Mus.*, No. 97.

—— 1924. Brachyuran crabs collected by the Williams Galapagos expedition, 1923. *Zoologica, N.Y.*, Vol. 5, pp. 153–9.

RUSSELL, F. S., 1927. The vertical distribution of marine macroplankton. V. The distribution of animals caught in the ring-trawl in the daytime in the Plymouth area. *J. Mar. biol. Ass., U.K.*, Vol. 14, pp. 557–608.

SAY, T., 1817. An account of the Crustacea of the United States. *J. Acad. nat. Sci. Philadelphia*, Vol. 1, pp. 155–69.

SMITH, S. I., 1873. The megalops stage of *Ocypoda*. *Amer. J. Sci.*, Vol. 6 (3), no. 1.

—— 1880. Occasional occurrence of tropical and sub-tropical species of decapod Crustacea on the coast of New England. *Trans. Conn. Acad. Arts Sci.*, Vol. 4, pp. 254–67.

WELLS, W. W., 1940. Ecological studies on the pinnotherid crabs of Puget Sound. *Univ. Wash. Publ. Oceanogr.*, Vol. 2, pp. 19–50.

WILLIAMSON, H. C., 1903. On the larval and early young stages, and rate of growth of the shore-crab (*Carcinus maenas* Leach.). *Rep. Fish. Bd Scot.*, Ann. 21 (1902), pp. 136–79.

J. Mar. biol. Ass. U.K. (1954) 33, 637–643
Printed in Great Britain

A PENETROMETER FOR USE ON WATER-COVERED BEACHES

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(Text-figs. 1 and 2)

The primary use of penetrometers has been in the road-building industry for testing materials such as tar and asphalt which are virtually homogenous in texture. They have also been used extensively and in wide variety in the food industry to determine optimum textures of products being canned, and an important application has been the determination of the toughness of cheese.

The idea underlying the use of devices to measure resistance to penetration has, of course, been to get away from dependence upon mere qualitative descriptions of firmness, and where interest attaches to investigating that of estuary bottoms and of other grounds covered by modest depths of water, quantitative description has become essential. Only so can conditions at places far apart be acceptably compared.

The importance attached in marine-biological literature to the nature of beaches in connexion with the burrowing powers of animals which inhabit them, has prompted the thought that it might be useful to make known the existence of an instrument which has been successfully used in shallow water to ascertain the firmness of bottoms *in situ*.

It will be convenient in what follows to speak of 'dry beaches' simply in contradistinction to water-covered beaches, by using the expression in the usual hydrographical sense to convey that the falling tide has uncovered them, and not with any connotation of aridity at all. As regards those striking sand properties dilatancy and thixotropy upon consideration of which Kendall thirty years and more ago based his stimulating theory of fossil earthquakes which alone could account for the large and economically important 'wash outs' which occur as amorphous sand infillings in certain coal seams of the West Riding, it does seem that students of sea and sand interrelationships might sometimes make useful small-scale application of Kendall's views on the events due to earthquake shock which can attend the quasi-liquefaction of vast stretches of sand traversed by a watercourse. There surely must be an important gradation of magnitudes between the mammoth physiographical events pictured by Kendall, and those on the micro scale seen by a man who contemplates the aureole of whitening and drying around his feet as he walks on a beach not far from the water's edge, and who finds that if he pats the ground repeatedly he produces a small quicksand.

In this connexion it is suggestive to think of the quicksands of the Solway shore which are in dangerous evidence at times when a rising tide effects a rapid extension of water cover landwards, and of such events as the extensive migration of buoys near the Goodwins which, after remaining firmly fixed for many years, suddenly lose their hold at a time of strong spring tides as very shallow water cover is quickly succeeded by deep. There is abundant reason why ever-increasing attention ought to be given to the study of the firmness and constitution of beach soils both when water-covered and dry.

The interest of the marine biologist in the dilatancy and thixotropy of the sand on beaches wherein animal communities live, has been discussed by Yonge (1950), by Chapman (1949) and by Chapman & Newell (1947).

In the two earliest of these papers there is an account of a device used for measuring the resistance to penetration of marine soils *in situ* on dry beaches.

Use of the small penetrometer in question produced results which were always expressed as the pressures in g/cm^2 necessary to thrust a disc 0·125 cm^2 in area 3 cm deep into a soil. Presumably where attention is limited to recording the resistance to a penetration of such modest amount as 3 cm the data collected can be accepted as measures of the resistance to burrowing which small animals would experience at the same place. It is to be assumed that the users of the small penetrometer just referred to (and apparently little longer than 40 cm overall), found no need to suppose that their results might have been somewhat falsified in point of intended applicability, by sand compaction changes occasioned by their own weight.

The concern of marine scientists with the texture, make-up, and morphology of beaches goes far beyond that of marine biologists of course, and very many factors have to be considered by those who study the potency of wave-sculpting, and the nature and magnitude of other causes of sea erosion, to say no more. Quite often it is necessary to be very chary of applying what is learnt regarding erosion trends, the effects of waves, the magnitudes of sand transports, etc., at one place, to the making of inferences in respect of other beaches even though exposures and wave conditions may be very similar. That very much indeed depends upon the actual make-up and natural compaction of the sands on a beach, is most impressively attested by the remarkable detail with which the Dutch have found it necessary to investigate the grain composition and the mineral constitution of their beach materials.

Where, as with them, the issues are so vitally important, it is risky indeed to assume that information gleaned at one place will necessarily apply adequately elsewhere.

There is in Britain nothing like the school of sedimentology of Wageningen led by Doeglas, nor has our country yet seen beach studies made in anything like the detail of those featuring so much in French marine-geological literature, but there are solid signs of an awakening interest.

It would be of interest (were this the place) to consider the findings of

Emery (1945) on the effects of the entrapment of air in different grades of beach sand, and those of Trask & Rolston (1950) regarding the relation of sediment strength to water content and grain size. Most of the considerations which were in mind when the large penetrometer to be described was designed, are set out in an excellent article by Kindle (1936). He describes beaches which exhibit all degrees of firmness and lack of it within the distance of a fraction of a mile, and dwells upon the abruptness of the transition from firm to soft sand which often exists. He cites such a contrast in the case of an English beach at Skegness, and gives a discussion of the effects upon beach sand firmness as the 'tide comes in'.

A purpose of the foregoing remarks has been to show the desirability of possessing a simple and rugged means of determining the firmness of beaches (whether dry or water-covered up to neck depth for instance), in such fashion that investigators far removed from each other can record their findings in common terms. It has been thought that such a possibility might make some appeal to marine biologists as well as seeming obligatory upon students of beach and sea-bottom topography.

The device to be described was constructed for use with both instrument and user completely submerged in a connexion which need not concern us here, but, since the instrument is tall it can easily be used by an observer within all wading depths. In these days when some marine biological laboratories possess their own frogmen, wading depths need not be the limit—though of course, the greater the degree of immersion of the user, the less becomes his power to force any instrument into the sea bed beneath his feet. The tallness of the instrument was a deliberate choice to aid the user to effect a vertical approach to the ground being investigated.

In the case of bottoms carrying more than wading depth of water, unless probing from some firm platform like a jetty be possible, no acceptable means is known of recording firmness *in situ* other than by invoking the services of divers of frogmen type, and they often find it far from easy to operate corers and penetrometers specially made for them except when water movements are very weak.

Description of the Penetrometer

For the accompanying drawing (Fig. 1) and the brief description which follows referring to it, I am indebted to Mr H. J. Garrood, A.M.I.M.E., who made the device to my instructions.

The penetrometer consists of a rod (1) pointed at the lower end (2). This rod is forced into the ground being investigated by pulling down by hand on the handles (6).

To the handles are attached two chains (7). These run over sprockets (8) and are fastened at their opposite end to a guide (9). The guide is connected through springs (10) to an anchor bracket (11). The guide (9) is able to slide on the rod (1).

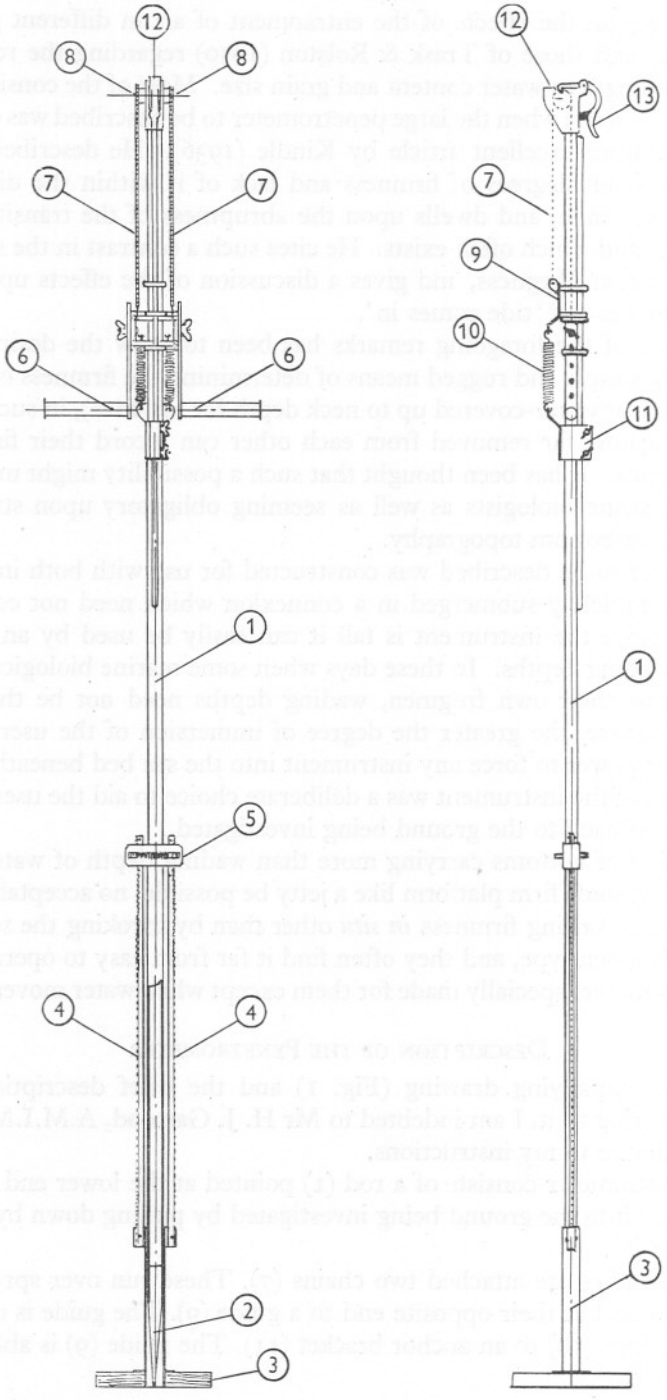

Fig. 1. Diagram showing construction of the penetrometer. For explanation see text.

When the handles are pulled downwards, the rod penetrates the ground and the springs are stretched proportionately to the tension applied. A spring-loaded ratchet lever (13) engaging a toothed wheel (12) mounted between the

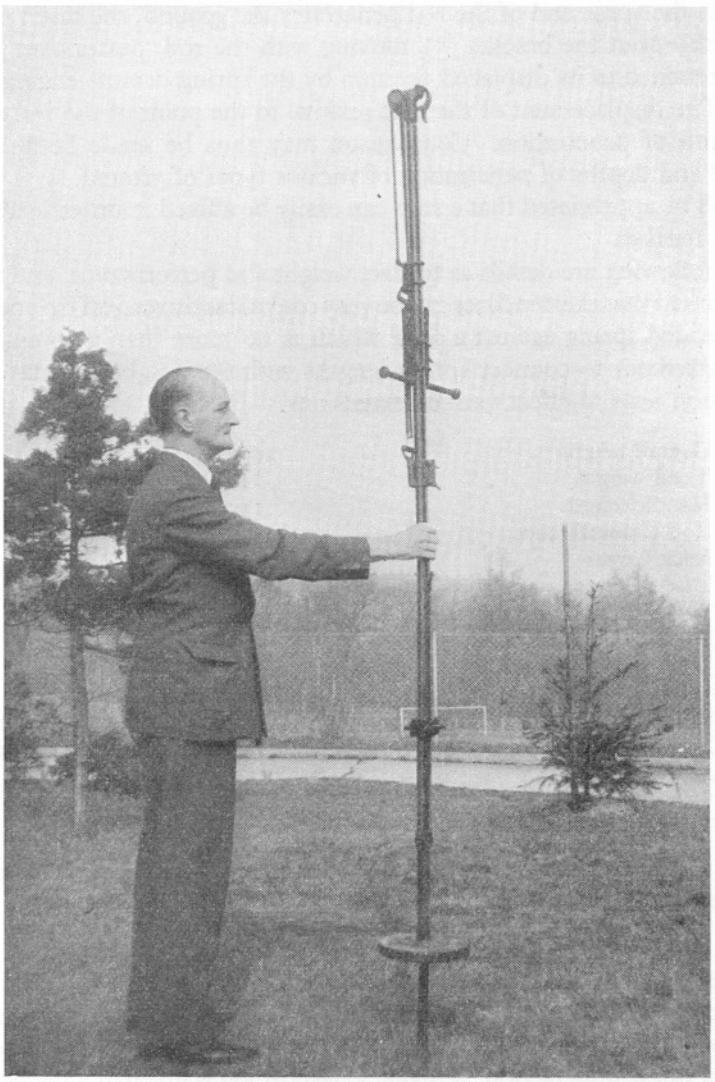

Fig. 2. Photograph of the submerged-beach penetrometer.

chain sprockets and fixed to them, prevents the springs from relaxing when the pull is released. By measuring the stretch of the spring, the force which has been exerted on the rod may be ascertained.

At the foot of the instrument a wooden disc attached to a tube (3) is arranged to slide on the rod. Moving with the disc and attached to it are two toothed racks (4) which pass through a bracket (5) fixed to the rod and having spring-loaded detents which engage in the ratchet teeth of the racks.

When the spear end of the rod penetrates the ground, the disc remains on the surface, but the bracket (5), moving with the rod, passes over the racks and is retained in its displaced position by the spring detents engaging in the teeth. The displacement of the disc relative to the point of the rod measures the depth of penetration. Comparison may thus be made between forces exerted and depths of penetration of various types of ground.

It will be appreciated that a stop can easily be affixed in order to always use a given tension.

The following are details as to size, weight and performance, and it should be remarked that exerted force can be very conveniently read off by 'measuring' the extended spring against a scale which is no more than a common white lath marked out to connect spring lengths with the weight loadings found in calibration tests to effect various extensions.

Overall height	265 cm
Total weight	11.3 kg
Handle length	15·2 cm
Rod circumference	9·1 cm
Rack length	71 cm
Disc diameter	22·9 cm
Spring length unstretched	30·5 cm
Spring length stretched:	
(*a*) by 14·7 kg weight	50·8 cm
(*b*) by 16·8 kg weight	58·4 cm
Penetration into loose recently dug soil	41·9 cm for 9·9 kg tension
Penetration into firm lawn	19·0 cm for 9·9 kg tension

N.B. The 'odd' dimensions and weights are due to conversion from British quantities into metric.

The accompanying photograph (Fig. 2) was kindly taken for me by my colleague Mr J. Darbyshire.

SUMMARY

Comments on the uses of penetrometers in general are followed by remarks upon an earlier one for use on dry beaches. Some discussion is given of the behaviour of beds of sand disturbed by shocks and converted thereby into a state of quasi-liquefaction. Finally, a description is given of a tall pull-down penetrometer which can be used on beaches covered by modest depths of water.

REFERENCES

CHAPMAN, G., 1949. The thixotropy and dilatancy of a marine soil. *J. Mar. biol. Ass. U.K.*, Vol. 28, pp. 123–40.

CHAPMAN, G. & NEWELL, G. E., 1947. The role of the body fluid in relation to movement in soft-bodied invertebrates. I. The burrowing of *Arenicola*. *Proc. roy. Soc.*, B., Vol. 134, pp. 431–55.

EMERY, K. O., 1945. Entrapment of air in beach sand. *J. sediment. Petrol*, Vol. 15, pp. 39–49.

KINDLE, E. M., 1936. Dominant factors in the formation of firm and soft sand beaches. *J. sediment. Petrol.*, Vol. 6, pp. 16–22.

TRASK, P. D. & ROLSTON, J. W., 1950. Relation of strength of sediments to water content and grain size. *Science*, Vol. 3, pp. 666–7.

YONGE, C. M., 1950. Life on sandy shores. *Sci. Progr. Twent. Cent.*, Vol. 38, pp. 430–44.

J. Mar. biol. Ass. U.K. (1954) 33, 645–662
Printed in Great Britain

THE MIXING AND MOVEMENT OF WATER IN THE ESTUARY OF THE THAMES

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(Text-figs. 1–9)

In Great Britain many estuaries are seriously polluted by discharge of untreated or partially treated sewage and industrial waste waters. In addition to the unpleasant effects produced in the estuary itself, fisheries in the upper reaches may be damaged, as a barrier of deoxygenated or poisoned water may be formed through which migratory fish, such as salmon, are unable to pass. In some estuaries conditions are so bad that at times the water is completely deoxygenated; reduction of sulphate may then occur, resulting in the evolution of hydrogen sulphide which may cause nuisance from smell, blacken paint-work, and increase corrosion of metals.

When an effluent is discharged into a freshwater stream flowing in one direction only, the concentration in the water, at a given distance from the point of discharge, of any soluble component which decays at a known rate, can be calculated with reasonable accuracy. It is also possible to predict, although with greater error, the effect of oxidizable substances in the effluent on the dissolved oxygen content of the river water, provided the rate of re-oxygenation from the air is known (Phelps, 1947). It is much more difficult, however, to predict the effects of pollution in an estuary where the movement of water resulting from the inflow of fresh water and from tidal action is much more complicated. As estuaries are important economically the problem is being investigated in this country and in America (Ketchum, 1950, 1951).

In an estuary the inflow of fresh water tends to carry sea water seawards by displacement. On the other hand, sea water is carried towards the head of the estuary by the mixing brought about by tidal action. These two processes determine the salinity of the water in the estuary at any time, and the present paper suggests a method of calculating the degree of mixing in an estuary from observations of the flow of fresh water and the resultant salinities.

Mixing in Estuaries

Types of estuaries

Stommel & Farmer (1952) divide estuaries into four types according to the degree of vertical mixing: (1) *Vertically mixed estuaries* are so thoroughly mixed vertically that there is no measurable difference in the salinity from the surface

to the bottom. (2) *Slightly stratified estuaries* are characterized by only slight vertical stratification; the vertical mixing is intense. (3) *Highly stratified estuaries* show big differences of salinity between the surface and bottom; often there is no fresh water in the lower layer. In (4) *Salt-wedge estuaries*, the sea water intrudes up-stream as a wedge.

In this paper only estuaries of type 1 or 2 will be considered—and only those in which the salinity is sufficiently constant, both vertically and across the stream, to permit the intermingling of salt and fresh water to be regarded as a one-dimensional problem without appreciable error. An extension of the theory would be required for other types of estuaries.

The Thames Estuary (which is now being investigated by the Water Pollution Research Laboratory) is of type 2; not only the salinity but also the concentration of dissolved oxygen and other dissolved substances is almost constant in a cross-section. Pillsbury (1939) considered the relations between erosion of the bed and the tidal flow in estuaries which have little vertical salinity stratification. He showed that the width of such an estuary should increase exponentially towards the sea, and that the distance of tidal flow should be constant. The Thames is a good example of an estuary of this type. The width and the area of cross-section increase exponentially except in a region near Tilbury where the estuary is narrow and deep. The average length of tidal flow is everywhere about 8 or 9 miles except near Teddington Weir, and the mixing during one tidal period must be confined in a length of this order. The theory developed in this paper probably applies to all estuaries of the Pillsbury type.

Causes of mixing

The entry of water into an estuary from land sources displaces the water of the estuary towards the sea, the magnitude of the displacement at any point depending on the area of cross-section. If the estuary water moved without any mixing between adjacent sections, each unit of water pushing the unit in front of it without overtaking in any way, only the displacement and the oscillation due to the tide would occur, and the sea water would be driven from the estuary. As a result, however, of movement caused by both tidal and land-water flow, much mixing in fact takes place between the waters at different distances along the estuary. It may be expected that the dispersal of the water by mixing depends on many factors. One of these factors is the difference in the pattern of flow between the flood and ebb tides. If the area of cross-section is large, water which travels farthest up the estuary in the fastest currents may not mix completely at slack water, and the amount which is returned to the original neighbourhood will depend on the difference in position of the fastest currents of the ebb and flood tides. Turbulence, wind, waves, shipping, obstacles, and the nature of the bed will also influence the amount of mixing. At one place on the side of the Thames Estuary the flow of

water is said to be always in one direction because of the formation of a very large eddy on the ebb tide; such eddies must also influence the amount of mixing. It is also to be expected that there will be a difference in the amount of dispersion of the water for spring and neap tides. In this paper the influence of the different tides will not be considered, but only the average amount of mixing during a long period will be calculated.

The movement of the water may be regarded as composed of three parts: displacement due to the flow from the land, tidal oscillation, and dispersal by mixing. Consider a unit of water which is contained between two cross-sections of the estuary which are very close to each other. Let the point, B, be midway between the cross-sections. At the corresponding time of the next tidal cycle this water will have been dispersed over the maximum distance of flow, to give a distribution curve similar to that shown in Fig. 1 *a*, in which R, the amount of the unit of water per unit of length, is plotted against distance along the estuary. The curve represents a dispersal which is additional to the displacement due to the entry of water from the land. R must be zero beyond the limits of the length of flow of the fastest currents during the tidal cycle, and is likely to be small beyond the mean distance of flow. Such a distribution curve exists for every point along the estuary. The water which has been dispersed during one tidal cycle will, during the next tide, be further dispersed according to the dispersion curves appropriate to the positions reached after the first tide. The result will be the dispersion curve corresponding to an interval of two tidal cycles; the sets of curves corresponding to different intervals of time can be calculated from each other.

The second moment of the curve is $\int_{-\infty}^{\infty} Rx^2\,dx$, where x is the distance from the original position. It is well known that if a dispersal according to a distribution curve of second moment M_1 is followed by another dispersal of second moment M_2, the resulting distribution has a second moment M_1+M_2. A set of dispersion curves exists for an estuary, and represents the mixing which causes sea water to move into the estuary against the general direction of flow. But if the water were repeatedly mixed in a way represented by a second set of dispersion curves, which differed in detail but had the same second moments, the movement would be almost identical. Hence it is not possible to calculate the exact shape of the curves from a consideration of the salinity balance during a long period. However, a complicated dispersion can be represented by a simpler dispersion which is repeated a number of times. For example, a binomial distribution approaches a normal distribution as the index increases. Hence a simplified form of the dispersion curves, with only a small number of arbitrary constants, can be taken, and the constants calculated, so that repeated mixing according to the simplified form is almost the same as the actual mixing in the estuary.

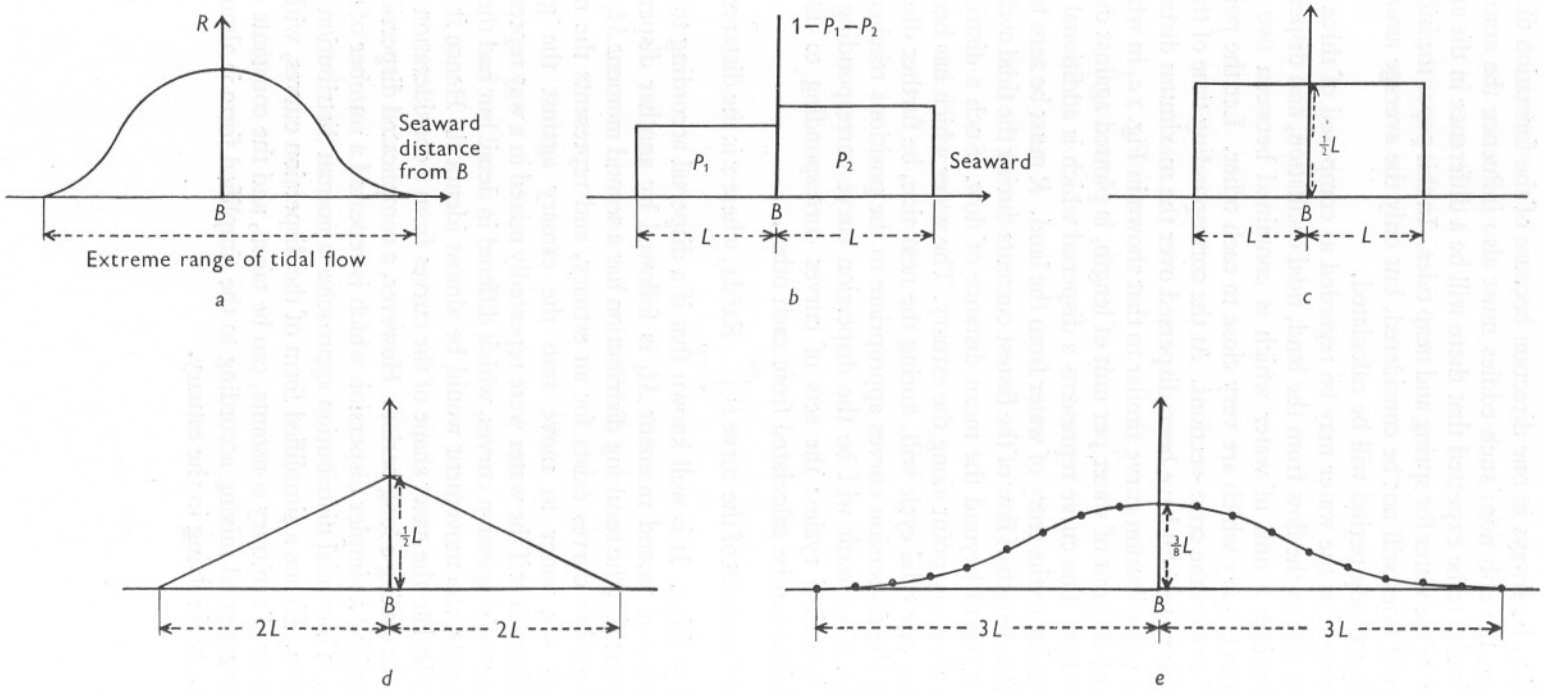

Fig. 1. Diagrams illustrating distribution of a unit volume of water in an estuary. *a*, distribution caused by tidal mixing; *b*, assumed equivalent distribution. Successive dispersals when $P_1=P_2=\frac{1}{2}$; *c*, after time T; *d*, after time $2T$; *e*, after time $3T$.

Asymmetry of mixing

In an estuary of which the area of cross-section decreases in a direction away from the sea, finally dwindling into that of the river at the head, the dispersion curves, whether actual or the equivalent simplified form, cannot be symmetrical. The mixing causes some of the water from any particular section to move landwards into the adjacent, smaller, section, in which the salinity is lower. To make space for the more saline water, the water already in the upper section must move towards the sea by mixing. Hence it is necessary to take a form of the simplified dispersion curves which allows the centre of the water to move towards the sea. This need not be so in an inlet of constant area of cross-section, in which a unit of water might disperse symmetrically until it was unable to continue to do so because some of the water had reached the end of the inlet.

An example may make the equilibrium clear. Consider an estuary which can be divided into adjacent sections 1, 2, 3, ..., m of equal length, whose volumes are v, $2v$, $4v$, ..., $2^{m-1}v$, respectively, the last section being next to the sea. The mixing can be considered as an exchange of water between the sections. If the amount exchanged is proportional to the volumes, and 1 unit is exchanged between the 1st and 2nd sections, then 2 units are exchanged between the 2nd and 3rd sections, 4 units between the 3rd and 4th sections, and so on, the last section exchanging 2^{m-1} units with the sea. The volume of water in each section remains constant, but the centre of gravity of the water of each section moves seaward.

Representation of the Mixing

Since the detailed shape of the dispersal curves cannot be calculated from the salinity data, it is necessary to represent them in a simplified manner. It will be shown later that two conditions have to be satisfied at every point along the estuary, and hence each curve will have two arbitrary constants which can be determined from the measured salinities. As there are many families of curves which produce almost identical effects, if the water is dispersed many times, a method of representation which simplifies the mathematics may be selected. Consider a unit of water which is contained in a narrow cross-section of the estuary at B (Fig. 1*b*). After a lapse of time, T, the mixing may be represented as shown in the figure. P_1 is the proportion of the water which is distributed uniformly over a distance L towards the sea, and P_2 the proportion of the water distributed uniformly over the distance L in the opposite direction. The remainder of the water $1-P_1-P_2$ is left in its original position.

P_1 and P_2 have mathematical existence only. It is assumed that values exist for P_1, P_2 and L such that if the water is repeatedly mixed in accordance with these values, the dispersion of a unit of water is almost the same as the actual dispersion.

It is desirable that the assumed equivalent mixing should resemble the actual mixing in the estuary as closely as possible, so that agreement between the two systems of mixing occurs quickly and long calculations are avoided. This is the reason why P_1+P_2 has not been equated to 1. A proportion, $1-P_1-P_2$, of the water is unmixed. If there is little mixing P_1+P_2 is small and the proportion $1-P_1-P_2$ is large, corresponding to the large amount of water which must remain very near its original position in the estuary. It has already been shown that if T is equal to the tidal period, say T_0, then L should be of the same order as the mean length of flow of the tide. If $nT=T_0$, then L should be of the same order as the mean length of flow of the tide divided by $n^{\frac{1}{2}}$, by the theorem of addition of second moments. P_1+P_2 must not be greater than 1. If the values of L taken lead to calculated values of P_1+P_2 which are greater than unity in a section of the estuary, it follows that the mixing in this section is substantial over a greater distance than the assumed value of L. Hence L should be increased in the section, and the values of P_1 and P_2 recalculated. If at some point there is little mixing, then the actual dispersion curve for this point will have high values at its centre and low values at its extremes. This corresponds to low values of P_1 and P_2, and the assumed equivalent mixing curve also has high values at its centre, since the proportion of the water, $1-P_1-P_2$, which remains in its original position, is large. If the calculated values of P_1 and P_2 are exceedingly small, the actual mixing in the estuary can be substantial only in a shorter distance than L, and it is better to decrease L and recalculate P_1 and P_2.

An example of repeated mixing in accordance with numerical values of P_1 and P_2 may clarify the assumption which has been made. The simplest case is that when $P_1=P_2=\frac{1}{2}$ for all points in the estuary. The water, which was initially at the point B is, after an interval of time T, distributed as in Fig. 1*c*. During the next interval of time T, this water is further scattered from the position it has already reached, and its distribution becomes that of Fig. 1*d*. After a total lapse of time $3T$ the water has reached a position as shown in Fig. 1*e*, and the shape of its distribution curve is approaching that of a normal error curve, as, of course, it must.

Equations relating P_1 and P_2 to observable data

The two following conditions must be satisfied. (i) During any period the net amount of salt carried upstream past any point is equal to the amount present above this point at the end of the period, *minus* the amount present at the beginning, *minus* any amount discharged into the estuary above the point during the period considered. (ii) The volume of water carried upstream past the point during the period must conform to a similar condition.

The volume of fresh water entering the estuary is completely accounted for by the displacement mentioned above, and hence the values of P_1, P_2 and

L must be such that the mixing process alone does not cause a net flow of water either upstream or downstream.

The salinity data available for the Thames Estuary made it convenient to consider conditions at half tide, which is defined as the time at which the height of the water is the mean of the heights at high and low tide.

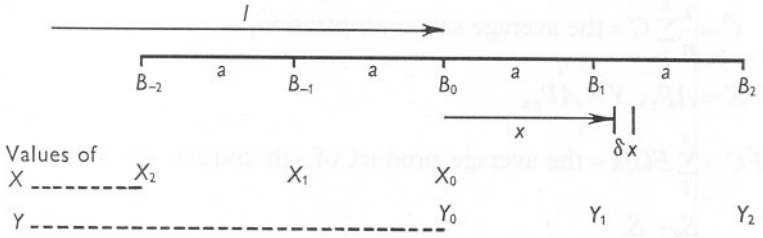

Fig. 2. Diagram of symbols used in calculation by relaxation.

Consider a point, B_0, at a distance l along the estuary (Fig. 2), and let A be the average area of cross-section of the river at half-tide. During a tidal cycle, salt in the water in the neighbourhood of B_0 is caused to flow past B_0 by the mixing. From a section between distances of x and $x+\delta x$ from B_0, the amount of salt which is carried past B_0 by the mixing during time T is

$$\frac{L-x}{L} CAP_2 \delta x,$$

where C is the concentration of salt in the water, and the amount of salt which flows past B_0 during n intervals of time T is

$$\sum_1^n CAP_2 \frac{L-x}{L} \delta x.$$

The values of C, A, P_2 and L are, of course, those at the point $1+x$.

The net amount of salt which is carried upstream past B_0 during the n intervals of time, T, is

$$\int_0^L \sum_1^n CAP_2 \frac{L-x}{L} dx + \int_0^{-L} \sum_1^n CAP_1 \frac{L+x}{L} dx. \qquad (1)$$

The amount carried seaward by the flow of water along the estuary is $\sum_1^n FC$, where F is the net flow of land water during 1 interval of time, T. From condition (i)

$$\frac{1}{n}\left[\int_0^L \sum_1^n CAP_2 \frac{L-x}{L} dx + \int_0^{-L} \sum_1^n CAP_1 \frac{L+x}{L} dx\right]$$

$$= \frac{1}{n}\left[\sum_1^n FC + S_2 - S_1\right], \qquad (2)$$

where S_2 and S_1 are the amounts of salt above B_0 at the end and the beginning of the period respectively. Or

$$\int_0^L \overline{C} Y \frac{L-x}{L} \mathrm{d}x + \int_0^{-L} \overline{C} X \frac{L+x}{L} \mathrm{d}x = \overline{FC} + S, \tag{3}$$

where $\overline{C} = \frac{1}{n} \sum_1^n C =$ the average salt concentration,

$$X = AP_1, \quad Y = AP_2,$$

$$\overline{FC} = \sum_1^n FC/n = \text{the average product of salt concentration and flow},$$

$$S = \frac{S_2 - S_1}{n}.$$

Similarly, from condition (ii)

$$\int_0^L Y \frac{L-x}{L} \mathrm{d}x + \int_0^{-L} X \frac{L+x}{L} \mathrm{d}x = 0. \tag{4}$$

From records of the salinities and of the flows of fresh water into the estuary, the numerical values of $\overline{C}$ and $\overline{FC}$ can be computed for the places of sampling and can be obtained at intermediate points by interpolation. The period for which the means are found should be long enough to give average conditions. The individual readings are not required to obtain X and Y, which are calculated from equations (3) and (4); only the averages $\overline{C}$ and $\overline{FC}$ are necessary. The values of $\overline{C}$ for the Thames Estuary from 1 January to 18 December are shown in Fig. 3. This graph, and a similar graph of $\overline{FC}$, provided the only experimental data of salinities which were used in the calculation of the values of X and Y for the Thames Estuary.

Solution by relaxation

Equations (3) and (4) have to be solved for X and Y numerically. This can be done by the relaxation method.

Let $B_{-2}, B_{-1}, B_0, B_1, B_2$ be points along the estuary, each separated by the distance a (Fig. 2), let X_2, X_1, X_0 be the values of X at B_{-2}, B_{-1}, B_0 respectively, and let Y_0, Y_1, Y_2 be the values of Y at B_0, B_1, B_2, respectively.

As a first approximation, X, Y and $\overline{C}$ may be considered as linear functions of distance within the range of the mixing length L and

$$X = X_0 - (X_1 - X_0)x/a \quad \text{from } B_0 \text{ to } B_{-1},$$

$$Y = Y_0 + (Y_1 - Y_0)x/a \quad \text{from } B_0 \text{ to } B_1,$$

$$\overline{C} = C_0 + C_1 x \quad \text{from } B_{-1} \text{ to } B_1,$$

where C_0 and C_1 are constants, and x is the distance from B_0.

Substitution in equations (3) and (4), and simplification, gives

$$2X_0L^2+2Y_0L^2+\frac{(Y_1-Y_0)L^3}{a}+\frac{(X_1-X_0)L^3}{a}=\frac{12Q}{C_1}, \tag{5}$$

and

$$3Y_0L-3X_0L+\frac{(Y_1-Y_0)L^2}{a}-\frac{(X_1-X_0)L^2}{a}=0, \tag{6}$$

where $Q=\overline{FC}+S$.

Equations (5) and (6) exist for all the points in the estuary, and can be used to obtain the approximate values of X and Y at a series of points by the relaxation method. If $a=L$, equations (5) and (6) become

$$X_0+Y_0+Y_1+X_1=12Q/L^2C_1, \tag{7}$$

$$2Y_0-2X_0+Y_1-X_1=0. \tag{8}$$

To obtain greater accuracy it is necessary to take $\overline{C}$, X and Y as quadratic functions of distance in the range of the mixing lengths and put

$$X=X_0-\frac{(4X_1-3X_0-X_2)x}{2a}+\frac{(X_2+X_0-2X_1)x^2}{2a^2},$$

$$Y=Y_0+\frac{(4Y_1-3Y_0-Y_2)x}{2a}+\frac{(Y_2+Y_0-2Y_1)x^2}{2a^2},$$

$$\overline{C}=C_0+C_1x+C_2x^2,$$

where C_0, C_1 and C_2 are constants. On substitution, equations (3) and (4) become

$$X_0[10C_1L^2-5C_2L^3]+Y_0[10C_1L^2+5C_2L^3]+[4Y_1-3Y_0-Y_2]\,[5C_1L^3+3C_2L^4]/2a+[4X_1-3X_0-X_2]\,[5C_1L^3-3C_2L^4]/2a+[X_2+X_0-2X_1]\times[3C_1L^4-2C_2L^5]/2a^2+[Y_2+Y_0-2Y_1]\,[3C_1L^4+2C_2L^5]/2a^2=60Q, \tag{9}$$

and

$$6X_0L-6Y_0L+(4X_1-3X_0-X_2)L^2/a-(4Y_1-3Y_0-Y_2)L^2/a+(X_2+X_0-2X_1)L^3/2a^2-(Y_2+Y_0-2Y_1)L^3/2a^2=0. \tag{10}$$

If $2a=L$, equations (9) and (10) become

$$C_1[X_0+Y_0+8X_1+8Y_1+X_2+Y_2]+LC_2[4Y_1-4X_1+Y_2-X_2]=60Q/L^2 \tag{11}$$

and

$$2X_1-2Y_1+X_0-Y_0=0. \tag{12}$$

These equations can be used to improve the values obtained from the linear equations. It is advisable to obtain the first approximate solution for a set of points whose distances apart are L and then to introduce the values of X and Y at points midway between the first points when the quadratic equations are used. Group relaxation greatly shortens the work. Decreasing the distance between the points does not produce greater accuracy, unless the values at all the points within the range of the integral are used to give more accurate

relaxation formulae. If $3a \leqslant L$, cubic or higher equations have to be used, and the relaxation becomes very cumbersome and difficult. If greater accuracy than that obtainable by the quadratic equations is required, it is better to use numerical integration or some other method. A little experience will show how to diminish the remaining error when this is found by numerical integration.

Any values of X and Y may be assumed at the start of the relaxation, but time will be saved if these values are reasonably accurate. For the period selected for the Thames Estuary it was found that for a long distance both $\overline{C}$ and Q were approximately linear functions of distance given by

$$\overline{C}=\theta_1(l+2), \tag{13}$$

$$Q=\theta_2(l+2), \tag{14}$$

where θ_1 and θ_2 are constants, and l is the distance in miles from London Bridge. Substitution in equations (3) and (4) shows that the following equations are good approximate solutions:

$$X=\frac{3l+6+L}{L^2}\,\frac{\theta_2}{\theta_1}, \tag{15}$$

$$Y=\frac{3l+6-L}{L^2}\,\frac{\theta_2}{\theta_1}. \tag{16}$$

The values given by equations (15) and (16) were used as the initial values in the relaxation for the Thames Estuary.

Sources of Information for the Thames Estuary

Salinity

The London County Council has a very extensive record of the salinities of the Thames Estuary from London Bridge to the Nore, from 1920 to the present day. Salinities of samples taken at a depth of 6 ft. in mid-stream are recorded weekly at many sampling positions. Times of sampling vary, but most of the samples from any one position have been taken at about the same state of the tide. The London County Council very kindly allowed full use to be made of these records.

Tides

The Port of London Authority has supplied much information regarding the tides, including the results of simultaneous tidal surveys at many points in the estuary. Data from the Admiralty Tide Tables have also been used.

Flow

The Thames Conservancy records the daily flow over Teddington Weir. Low flows are measured with accuracy, but there may be greater error in the high winter flows. Figures for the flow of tributaries and effluents have been

provided by River Boards, Local Authorities, and industries. Much information has also been obtained from the survey of the estuary now being made.

Correction of salinity position

The readings of the L.C.C. cannot be used directly to find the salinity gradient along the estuary, as samples from different points are taken at different states of the tide. The fact that there is little salinity variation in a cross-section of the estuary, suggests that when the salinities of the estuary

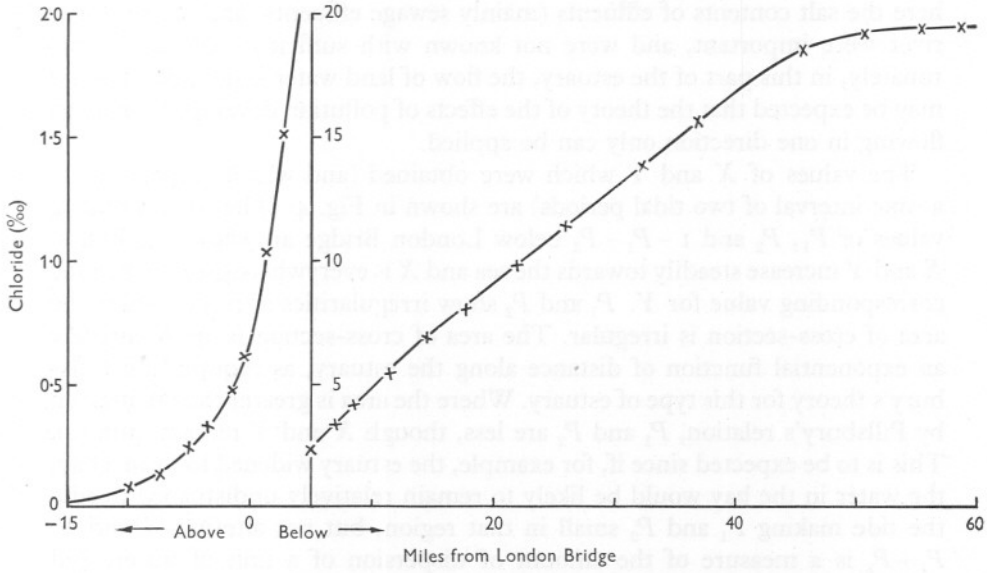

Fig. 3. Average contents of chloride in the Thames Estuary, 1 January to 18 December 1948. Chloride given as parts per thousand.

are in equilibrium, water of a constant salinity moves with the tide so that the volume of water between it and Teddington Weir is constant. If this is so, then the position of a sample can be adjusted to a half-tide position so that the volume of water between the sample and Teddington Weir remains the same. This has been done for a great number of observations and it has been found that salinities and concentrations of chemicals dissolved in the water then fall on a smooth curve. The correction may be large compared with the distance between the sampling points. Fig. 3 shows a corrected curve for the average values of the salinities from 1 January to 18 December 1948 plotted against distance along the estuary.

Results for the Thames Estuary

In calculating the extent of mixing in the Thames estuary, L was given the value 4·5 miles for mixing during half of the average tidal period, i.e. for $T = 6$ h $12\frac{1}{2}$ min. For $T = 24$ h 50 min, the time of two tides, L was given the

value of 9 miles. The calculation was made from the average values of salt concentration, and the average values of the product of salt concentration and volume of land water entering the landward section of the estuary, from 1 January to 18 December 1948. A longer period could not be used because of lack of information regarding the flows of the tributaries of the estuary at other times. Above London Bridge the average value of salt concentration became small, and in the section of the estuary near to Teddington (19 miles above London Bridge) X and Y could not be obtained accurately because here the salt contents of effluents (mainly sewage effluents) and of the upper river were important, and were not known with sufficient accuracy. Fortunately, in this part of the estuary, the flow of land water is dominant, and it may be expected that the theory of the effects of pollution developed for rivers flowing in one direction only can be applied.

The values of X and Y which were obtained (and which correspond to a time interval of two tidal periods) are shown in Fig. 4. The corresponding values of P_1, P_2 and $1-P_1-P_2$ below London Bridge are shown in Fig. 5. X and Y increase steadily towards the sea and X is everywhere greater than the corresponding value for Y. P_1 and P_2 show irregularities at places where the area of cross-section is irregular. The area of cross-section is approximately an exponential function of distance along the estuary, as required by Pillsbury's theory for this type of estuary. Where the area is greater than that given by Pillsbury's relation, P_1 and P_2 are less, though X and Y remain uniform. This is to be expected since if, for example, the estuary widened to form a bay, the water in the bay would be likely to remain relatively undisturbed during the tide making P_1 and P_2 small in that region, but not altering X and Y. P_1+P_2 is a measure of the amount of dispersion of a unit of water, and this varies along the estuary, being a maximum 25 miles below London Bridge.

X, Y, P_1 and P_2 probably vary with different ranges of tide. The values obtained are averages for the tides of the period considered.

Verification

If the salinities at a given time and the flows of fresh water during the following period are known, the salinity at the end of the period may be predicted from a knowledge of X and Y obtained from equations (3) and (4). The period must be divided into intervals of time for which the mixing values have been found, and the salinity at the end of each successive interval can be found by: (1) displacing the water to allow for the natural flow, and then (2) calculating the new salinities at a number of points by numerical integration of the expression

$$\frac{1}{A}\left[\int_0^L \frac{CY}{L}\,dx+\int_{-L}^0 \frac{CX}{L}\,dx+C(A-X-Y)\right], \qquad (17)$$

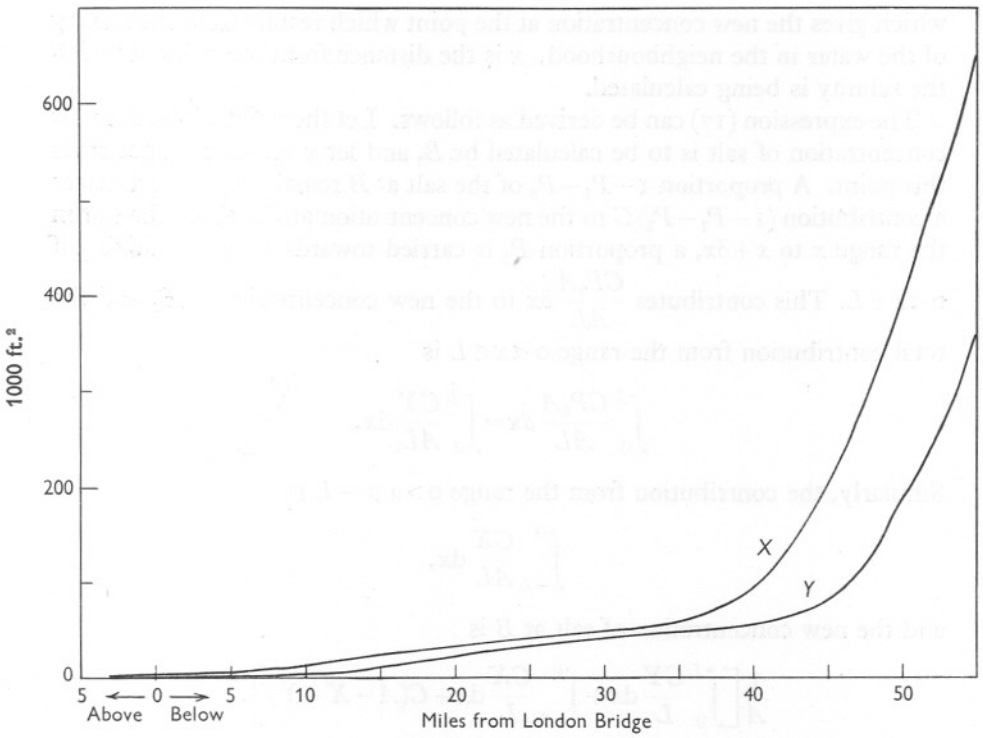

Fig. 4. Values for X and Y when T equals two tidal periods.

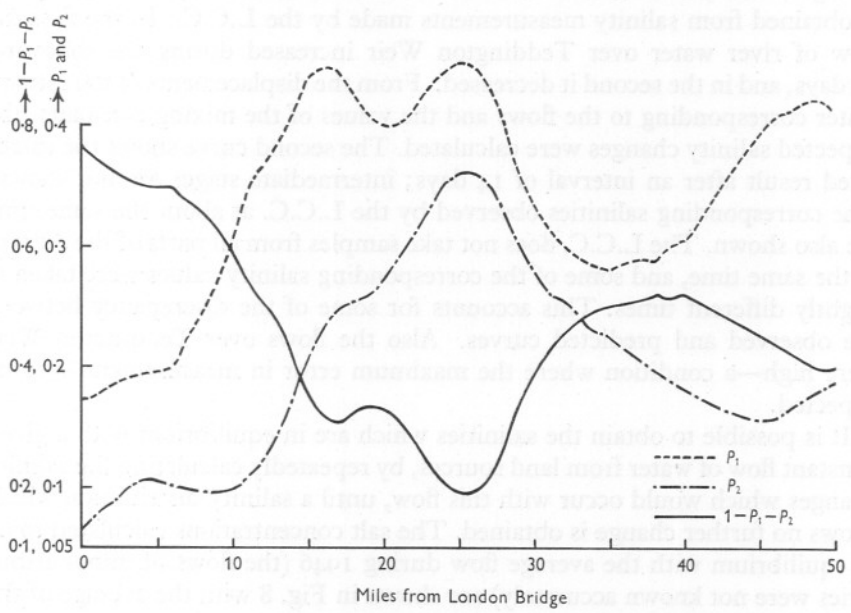

Fig. 5. Values of P_1 and P_2 for a period of two tides.

which gives the new concentration at the point which results from the mixing of the water in the neighbourhood. x is the distance from the point at which the salinity is being calculated.

The expression (17) can be derived as follows. Let the point where the new concentration of salt is to be calculated be B, and let x be the distance from this point. A proportion $1-P_1-P_2$ of the salt at B remains there and makes a contribution $(1-P_1-P_2)C$ to the new concentration at B. From the salt in the range x to $x+\delta x$, a proportion P_2 is carried towards B by the mixing if $0<x\leqslant L$. This contributes $\dfrac{CP_2A}{AL}\delta x$ to the new concentration at B, and the total contribution from the range $0<x\leqslant L$ is

$$\int_0^L \frac{CP_2A}{AL}dx=\int_0^L \frac{CY}{AL}\,\mathrm{d}x.$$

Similarly, the contribution from the range $0>x\geqslant -L$ is

$$\int_{-L}^0 \frac{CX}{AL}\,\mathrm{d}x,$$

and the new concentration of salt at B is

$$\frac{1}{A}\left[\int_0^L \frac{CY}{L}\,\mathrm{d}x+\int_{-L}^0 \frac{CX}{L}\,\mathrm{d}x+C(A-X-Y)\right].$$

Figs. 6 and 7 show the results of two calculations. Curve 1 of both figures is obtained from salinity measurements made by the L.C.C. In the first the flow of river water over Teddington Weir increased during the following 14 days, and in the second it decreased. From the displacements of the estuary water corresponding to the flows and the values of the mixing constants, the expected salinity changes were calculated. The second curve shows the calculated result after an interval of 14 days; intermediate stages are not shown. The corresponding salinities observed by the L.C.C. at about the same time are also shown. The L.C.C. does not take samples from all parts of the estuary at the same time, and some of the corresponding salinity values were taken at slightly different times. This accounts for some of the discrepancy between the observed and predicted curves. Also the flows over Teddington Weir were high—a condition where the maximum error in measurement is to be expected.

It is possible to obtain the salinities which are in equilibrium with a given constant flow of water from land sources, by repeatedly calculating the salinity changes which would occur with this flow, until a salinity distribution which shows no further change is obtained. The salt concentrations calculated to be in equilibrium with the average flow during 1946 (the flows of minor tributaries were not known accurately) are shown in Fig. 8 with the average of the

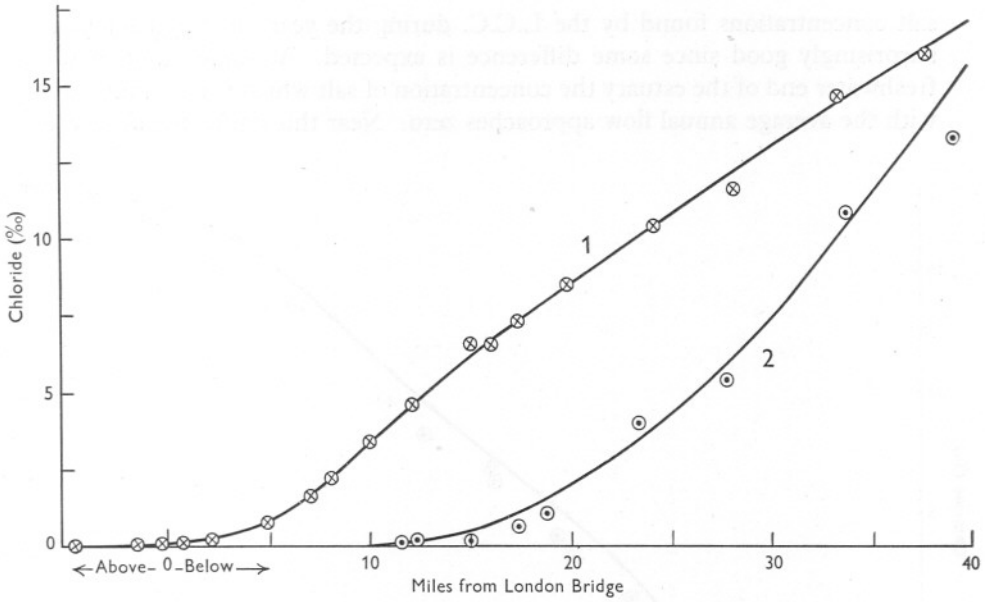

Fig. 6.

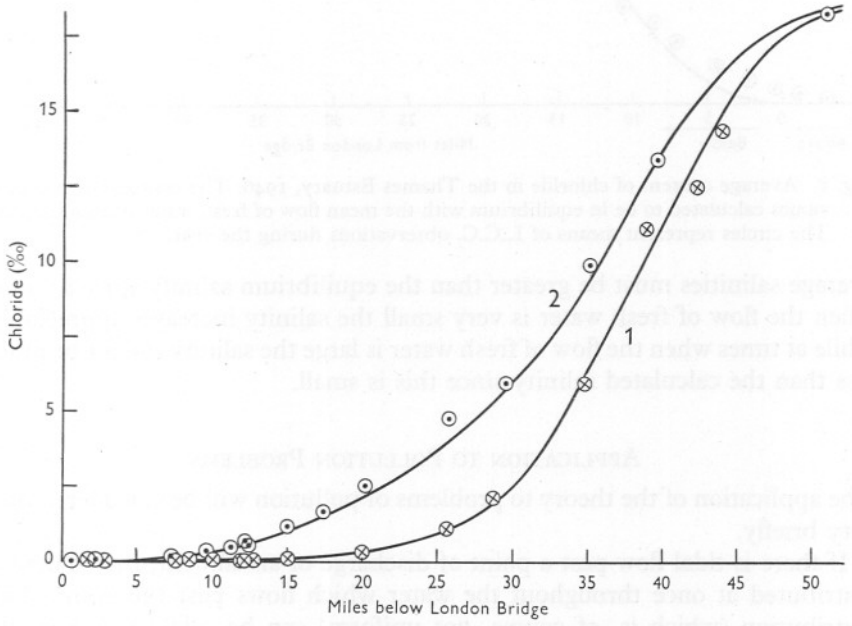

Fig. 7.

Figs. 6 and 7. Curves showing accuracy of calculation of changes in salinity in the Thames Estuary. Curve 1 was plotted through the readings ⊗ taken on a zero date. Curve 2 was calculated from curve 1, being an estimate for the conditions 14 days later. The readings ⊙ were the observed values on that day.

salt concentrations found by the L.C.C. during the year. The agreement is surprisingly good since some difference is expected. At some point in the freshwater end of the estuary the concentration of salt which is in equilibrium with the average annual flow approaches zero. Near this point, the observed

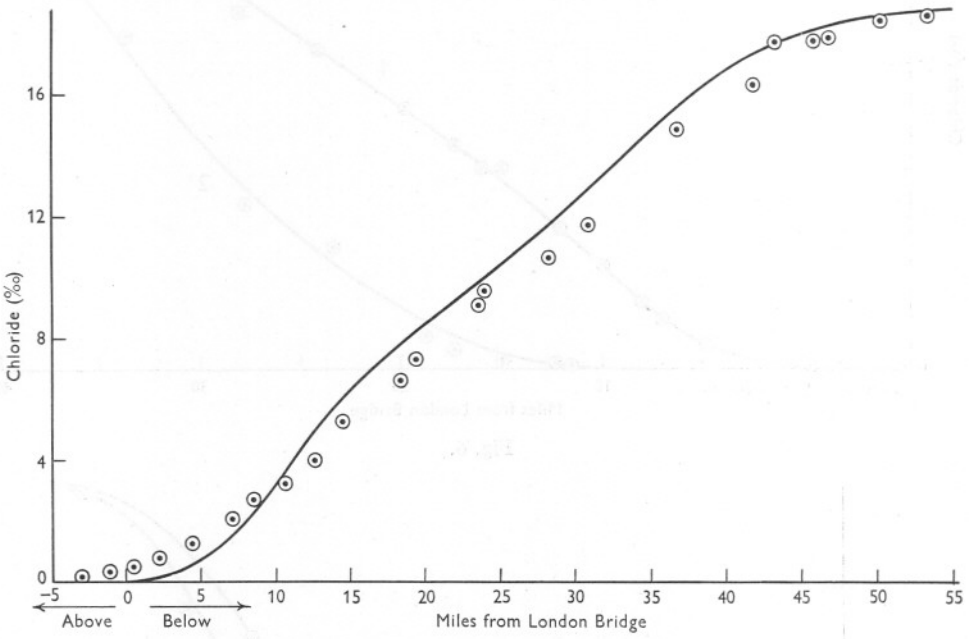

Fig. 8. Average content of chloride in the Thames Estuary, 1946. The continuous line plots values calculated to be in equilibrium with the mean flow of fresh water during the year. The circles represent means of L.C.C. observations during the year.

average salinities must be greater than the equilibrium salinity since at times when the flow of fresh water is very small the salinity increases appreciably, while at times when the flow of fresh water is large the salinity cannot be much less than the calculated salinity since this is small.

Application to Pollution Problems

The application of the theory to problems of pollution will be considered only very briefly.

If there is tidal flow past a point of discharge of an effluent, the effluent is distributed at once throughout the water which flows past the point. This distribution (which is, of course, not uniform) can be calculated from the tidal data and is equivalent to a discharge over a length $2L$ at half-tide. Hence the initial concentration of the effluent in the water can be found and the concentration at any point at a later time can be calculated by the process already

given for salinities. Fig. 9 shows the calculated distributions along the Thames Estuary of the effluent discharged from the Northern Outfall of the L.C.C. when the flow of fresh water over Teddington Weir is 500 million gallons per day. The extent of pollution of the water, as measured by the biochemical oxygen demand, can be calculated from these curves if the rate at which the

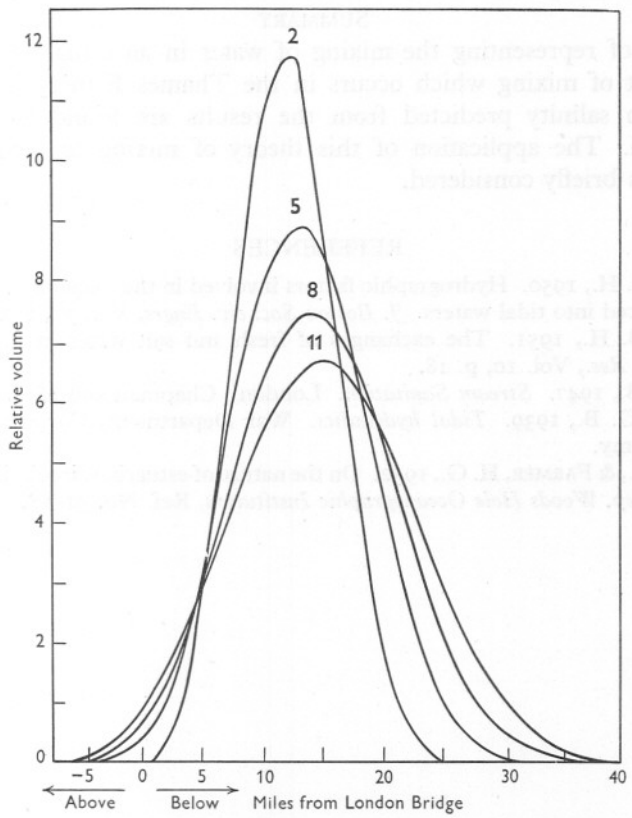

Fig. 9. Computed distribution of a liquid discharged into the Thames Estuary, during a period between low and high water, at the Northern Outfall of the London County Council sewage-disposal works. The number of tidal cycles after discharge is shown against each curve. The vertical scale shows the comparative volume of added liquids per unit length of river. Flow of fresh water at Teddington = 500 m.g.d.

demand is satisfied is known. If the rate of solution of oxygen from the air is also known, it is then possible to calculate the level of oxygenation of the water, using the mixing constants. For the Thames Estuary, where there are a great number of discharges, the calculation by this method is very tedious, but it seems likely that methods can be developed which will simplify the arithmetical work involved.

Much of the numerical calculation for this work was done by Mr L. J. Jeeves, who also collected many of the data, and made many useful suggestions. I should like to thank Mr C. J. Regan, Chief Chemist of the L.C.C., for many valuable discussions.

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SUMMARY

A method of representing the mixing of water in an estuary is given, and the amount of mixing which occurs in the Thames Estuary is calculated. Changes in salinity predicted from the results are found to agree with observation. The application of this theory of mixing to some pollution problems is briefly considered.

REFERENCES

KETCHUM, B. H., 1950. Hydrographic factors involved in the dispersion of pollutants introduced into tidal waters. *J. Boston Soc. civ. Engrs*, Vol. 37, No. 3, p. 296.

KETCHUM, B. H., 1951. The exchanges of fresh and salt water in tidal estuaries. *J. Mar. Res.*, Vol. 10, p. 18.

PHELPS, E. B., 1947. *Stream Sanitation*. London. Chapman and Hall.

PILLSBURY, G. B., 1939. *Tidal hydraulics*. War Department, Corps of Engineers, U.S. Army.

STOMMEL, H., & FARMER, H. G., 1952. On the nature of estuarine circulation. Part III. *Tech. Rep. Woods Hole Oceanographic Institution*, Ref. No. 52–88.

J. Mar. biol. Ass. U.K. (1954) 33, 663–576
Printed in Great Britain

THE OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

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(Text-fig. 1)

Kennedy & Vevers (1953*a*, *b*) confirmed the presence of a porphyrin in the integument of the starfish *Asterias rubens*, reported by MacMunn (1887), and showed that it was protoporphyrin. A yield of 33·6 mg of protoporphyrin dimethyl ester was obtained from 548·5 g of *Asterias* integument. Following this work, and the investigation of porphyrins in mollusc shells by Nicholas & Comfort (1949), it was naturally of interest to examine the distribution of porphyrin pigments in the soft and skeletal parts of other marine animals. A survey has therefore been made of the occurrence of porphyrins in certain invertebrates common in the waters off Plymouth.

Methods

The animals were examined first in the fresh state under ultra-violet light (Osira 125 W black glass lamp) and then homogenised (in an Atomix blender) and extracted by the methods used previously (Kennedy & Vevers, 1953*b*). The extracts in turn were examined by ultra-violet light and the occurrence of red fluorescence noted. Where extracts showed red fluorescence the source of this was investigated chemically and, where possible, the pigments responsible for this fluorescence were isolated and characterized by the following methods:

(1) *Solubility* in ether, chloroform, ethyl acetate, 0·1N hydrochloric acid, and 0·1N sodium hydroxide.
(2) *Partition* between ether and increasing concentrations of aqueous hydrochloric acid from 0·1% (w/v), this is the HCl number (Salzsäurezahl) of Willstätter & Stoll (1913).
(3) *Column chromatography*, Nicholas (1951) and Kennedy (1953*a*).
(4) *Paper partition chromatography* Nicholas & Rimington (1949), Kennedy (1953*b*) and Chu, Green & Chu (1951).
(5) *Absorption spectra*, using a Beck-Hartridge Reversion spectroscope, and a Unicam S.P. 500 spectrophotometer.
(6) *Formation of derivatives* and conversion to other porphyrins, when sufficient material was available.

TABLE I. OCCURRENCE OF RED FLUORESCENCE IN EXTRACTS FROM CERTAIN MARINE INVERTEBRATES

(Extracts of whole animal except where otherwise stated.)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
PORIFERA			
Sycon coronatum	−	−	
Grantia compressa	−	−	
Tethya aurantium	?+	−	Not yet identified
Halichondria panicea	−	+	Algae
COELENTERATA			
Velella spirans (skeleton)	−	−	
V. spirans (soft parts)	−	−	
Alcyonium digitatum	−	−	
Actinia equina	−	−	
Anemonia sulcata	−	+	Algal symbionts
Tealia felina	−	−	
Metridium senile	−	−	
Calliactis parasitica	−	−	
ANNELIDA			
Aphrodite aculeata	−	−	
Nereis diversicolor	+	+(*a*)	
Perinereis cultrifera	−	−	
Chaetopterus variopedatus	+	+(*b*)	
Arenicola marina	−	−	
Myxicola infundibulum	−	−	
M. infundibulum (purple tentacles)	−	−	
M. infundibulum (alimentary system)	+	−	
ARTHROPODA			
Portunus depurator	−	−	
Carcinides moenas (soft parts)	−	+	From diet in alimentary tract
C. moenas (carapace)	−	−	
Cancer pagurus (eggs)	−	−	
C. pagurus (viscera)	−	−	
C. pagurus (claw shell)	−	−	
C. pagurus (carapace)	−	−	
Eupagurus bernhardus (soft parts)	−	−	
MOLLUSCA			
Mytilus edulis	−	−	
M. edulis (shell)	−	−	
Chlamys opercularis	−	−	
C. opercularis (mantle)	−	−	
Cardium edule (soft parts)	−	−	
Buccinum undatum (soft parts)	−	−	
Scaphander lignarius (bursae)	−	−	
Philine aperta (bursae)	−	−	
Aplysia punctata (integument)	+	−	
Jorunna tomentosa	−	−	
Archidoris britannica	−	−	
A. britannica (eggs)	−	+	Chlorophyll from Algae
Duvaucelia plebeia (upper integument)	+	−	
Loligo forbesi	−	−	
Parasepia elegans	−	−	

TABLE I (*continued*)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
ECHINODERMATA			
Antedon bifida	–	–	.
Astropecten irregularis (integument)	+	–	.
Luidia ciliaris (integument)	+	–	.
Porania pulvillus (integument)	–	–	.
Palmipes membranaceus (integument)	–	–	.
Solaster papposus (integument)	–	–	.
Henricia sanguinolenta (integument)	–	–	.
Asterias rubens (integument)	+	–	.
Marthasterias glacialis (integument)	–	–	.
M. glacialis (spicules)	–	–	.
Ophiothrix fragilis	–	–	.
Ophiocomina nigra	–	–	.
Psammechinus miliaris (test)	–	+	Chlorophyll from Algae on test
Holothuria forskali (integument)	–	–	.
TUNICATA			
Ascidiella aspersa (viscera)	–	–	.
Ciona intestinalis (viscera)	–	–	.

(*a*) Phaeophorbide *a*; (*b*) Phaeophorbides *a* and *b*.

The results of this survey are given in Table I, and further details of those animals in which porphyrins were found are given in Table II. Where positive identification of pigments was possible the evidence upon which this was based is described under the name of the pigment.

TABLE II. OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

Phylum	Species	Porphyrin	Distribution
Annelida	*Nereis diversicolor*	Coproporphyrin III	Viscera
	Chaetopterus variopedatus	Coproporphyrin III and a pentacarboxylic porphyrin	Viscera
	Myxicola infundibulum	Coproporphyrin III	Viscera
Mollusca	*Aplysia punctata*	Uroporphyrin I	Integument
	Duvaucelia plebeia	Uroporphyrin I	Integument
Echinodermata	*Astropecten irregularis*	Chlorocruoroporphyrin and protoporphyrin	Integument
	Luidia ciliaris	Chlorocruoroporphyrin and protoporphyrin	Integument
	Asterias rubens	Protoporphyrin	Integument

IDENTIFICATION OF PIGMENTS

Coproporphyrin III

This pigment was noticed during the course of paper partition chromatography (Nicholas & Rimington, 1949) of chloroform extracts of the three polychaetes—*Nereis*, *Chaetopterus* and *Myxicola*—in an investigation of the

green pigment 'chaetopterin' (with J. A. C. Nicol), to be reported in the near future. The long-paper method of Kennedy (1953*b*) was employed, with 2:6-lutidine and water as solvent phases. A well defined spot appeared, with strong red fluorescence at R_F 0·65, which suggested a 4-COOH porphyrin, and coproporphyrin in particular. Further chromatograms were run under the same conditions, but including on each paper a spot of coproporphyrin I as a marker, in both adjacent and mixed spots. Only one spot appeared at R_F 0·65 on the mixed spot papers, and on the papers with adjacent spots there were two spots side by side at R_F 0·65. This confirmed the presence of a 4-COOH porphyrin, and strongly suggested coproporphyrin.

There was very little material for experiment so the spots of all the long-paper chromatography experiments were cut out carefully, the pieces soaked in a little dry pyridine and examined spectrophotometrically. The spectrum showed the maxima:

I	II	III	IV
623·6	569·0	533·0	499·0 mμ

further indicating that this porphyrin is indeed a coproporphyrin.

Concentrated solutions of the extracts from the three worms were then evaporated to dryness *in vacuo*, the residues esterified with methanol/hydrochloric acid and examined by the double-development paper-chromatography method of Chu *et al.* (1951), using chloroform: kerosene/*n*-propanol: kerosene at 19° C. Well-marked spots were observed with centre-dense red fluorescence at R_F 0·81, corresponding to coproporphyrin III. The test was repeated with markers of coproporphyrin I tetramethyl ester and coproporphyrin III tetramethyl ester (the latter very kindly supplied by Prof. C. Rimington, F.R.S.) in both adjacent and mixed spots. On those papers bearing the extracts and coproporphyrin I in mixed spots, two clear sets of spots were seen, indicating that the two porphyrins were not identical. On those papers bearing adjacent spots of coproporphyrin I and extract, there was a wide difference between the R_F values.

Papers carrying mixed spots of the worm extracts with authentic coproporphyrin III showed only one set of spots at R_F 0·80 confirming that the worm porphyrin was, in fact, identical with coproporphyrin III. Papers bearing adjacent spots of the coproporphyrin III ester and the worm extract showed that both pigments travelled together in parallel, and took up the same R_F position, 0·80. This established that the worm porphyrin was present as coproporphyrin III.

A Penta-carboxylic Porphyrin

In the course of these long-paper chromatography experiments just described, extracts of *Chaetopterus variopedatus* produced a spot above that of coproporphyrin with an R_F value of 0·5. This is indicative of a porphyrin with five carboxyl groups. This was repeated several times and confirmed, but there

was insufficient material for exact characterization. Further material is being collected for a detailed examination of this porphyrin.

Uroporphyrin

The upper integument of the nudibranch gastropod *Duvaucelia* (*Tritonia*) *plebeia* yielded no red fluorescent material to ether/acetic acid, but on placing the tissue in a mixture of absolute methanol (19 parts) and concentrated sulphuric acid (1 part) overnight the extract was found to be slightly red fluorescent to ultra-violet light. This fluorescence increased very much in intensity on diluting the extract with water, shaking up with chloroform and then examining the chloroform hypophase under ultra-violet light.

The chloroform extract was roughly dried by filtration and evaporated to dryness, and the residue was then re-dissolved in chloroform. This chloroform extract was passed through a column of alumina grade IV (Nicholas, 1951), packed by sprinkling into chloroform, and a broad red fluorescent band formed at the top of the column, with a diffuse pinkish non-fluorescent band below. The top band was immovable with ethanol/chloroform mixtures, ether, ether/pyridine mixtures, acetone or ethyl acetate, but was eventually eluted with the methanol/sulphuric acid (19:1) esterification mixture. The pigment was collected as a purple-red solution, intensely red fluorescent. A greyish non-fluorescent band was left on the column.

The acid/methanol solution was diluted with two-thirds of its volume of water, and the pigment was extracted with chloroform to form a purple hypophase with intense red fluorescence. The hypophase was then separated, washed several times with 2% sodium chloride, and finally with water. The solution was roughly dried by filtration through chloroform-soaked paper and evaporated to dryness on the water-bath. The pigment was redissolved in dry chloroform and the absorption spectrum determined spectrophotometrically (Unicam S.P. 500), and plotted as $E_{1\,cm}$ against wave-length. The maxima were:

I	II	III	IV	Sorêt band	
624	572	535·5	501	406	mμ

This suggested uroporphyrin and an authentic specimen of uroporphyrin I, when examined in chloroform in the same spectrophotometer, gave maxima at:

I	II	III	IV	Sorêt band	
624	570·5	535	501	406	mμ

These peaks show very good agreement, as may be seen in Fig. 1. A mixture of uroporphyrin I octamethyl ester and the *Duvaucelia* porphyrin ester were made in equal proportions as far as possible and examined in the spectrophotometer. (If two dissimilar substances are examined spectrophotometrically the peaks of the absorption curve are usually very rounded and the

curve presents an undulating instead of a sharp appearance.) The maxima in this case were:

I	II	III	IV	Sorêt band	
624	570·5	535	501	405	mμ

The peaks of the curve (Fig. 1) are very sharp and the curve suggests the presence of one pure substance only.

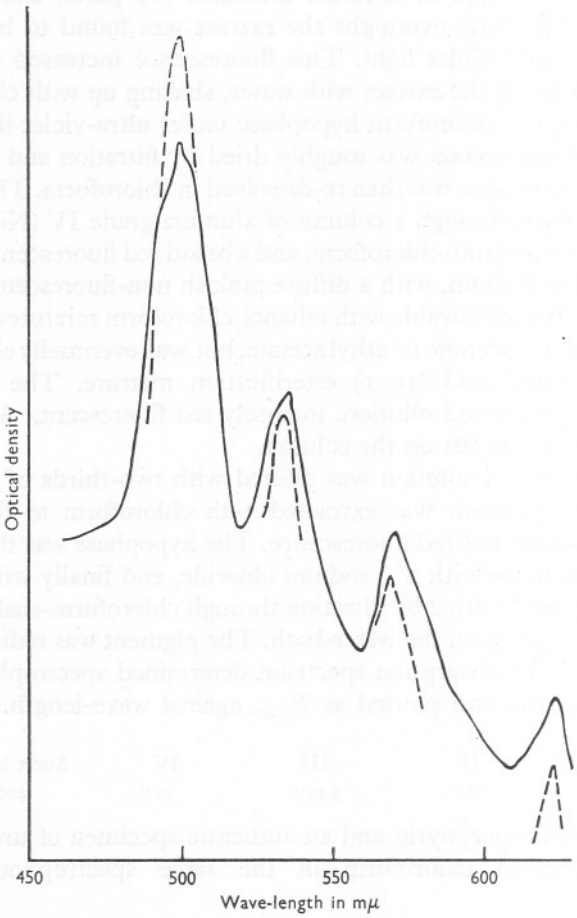

Fig. 1. Absorption spectra of *Duvaucelia* porphyrin in chloroform. ——, absorption spectrum of *Duvaucelia* porphyrin alone; - - - - -, absorption spectrum of a mixture of *Duvaucelia* porphyrin and authentic uroporphyrin I.

A little of the porphyrin ester solution from *Duvaucelia* was evaporated to dryness and hydrolysed by dissolving it in 7N-HCl and standing at room temperature for 36 h. At the end of this time the excess acid was removed by placing the dish in a vacuum desiccator over solid potassium hydroxide until

dry. The residue was dissolved in a little chloroform, spotted on a long paper (Kennedy, 1953*b*) and developed at 23° C with 2:6-lutidine/water (5:3) in an atmosphere of ammonia. The resulting chromatogram showed a single spot at R_F 0·15, indicating a porphyrin with eight carboxyl groups. This test was repeated using pure uroporphyrin I as a marker. With mixed spots only one large spot appeared at R_F 0·15 with a small spot above it at R_F 0·09 (the 'second spot' of uroporphyrin). In chromatograms with authentic uroporphyrin I as marker, parallel spots formed at R_F 0·15 and one small spot on the marker side at R_F 0·09.

These results together with the absorption spectrum confirm that the *Duvaucelia* porphyrin is uroporphyrin. The uroporphyrin was characterized as isomer I by the two-dimensional paper chromatogram technique of Falk & Benson (1953), and (at Sheffield) by its decarboxylation to coproporphyrin I identified by the Chu, Green & Chu (1951) technique.

The Porphyrin of Aplysia punctata

The upper integument was carefully removed from fifteen *Aplysia punctata*, all collected from Looe by Dr B. C. Abbott, to whom we are indebted for his kindness. The material was washed in several changes of fresh water to remove as much as possible of the 'aplysiopurpurin' pigment. The integuments were then dried roughly on filter-paper and extracts made in exactly the same way as for *Duvaucelia*. The final chloroform extract was evaporated to dryness and redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The material quickly resolved itself into a series of bands as follows, from the top downwards:

(1) Greyish.
(2) Brown-purple.
(3) Green.
(4) Blue.
(5) Purple (red fluorescent).
(6) Yellow (blue fluorescent).

It proved impossible to separate bands 5 and 6 from one another, and they were collected together. The other bands, although interesting, were discarded for the present, to be investigated in the future. The red fluorescent chloroform extract containing bands 5 and 6 was evaporated to dryness, to give a fatty residue. This was redissolved in dry chloroform and the absorption curve determined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt band
624	570	534	501	406 mμ

This strongly suggested uroporphyrin, an authentic specimen of which gave the maxima described under the *Duvaucelia* pigment.

Some of the *Aplysia* porphyrin ester was hydrolysed by standing in 7N-HCl for 48 h and the acid removed by standing over solid KOH *in vacuo* over-

night. The residue was dissolved in 2:6-lutidine and examined in the long-paper chromatograph in a 2:6-lutidine/water system in an atmosphere of ammonia at 23° C. Three papers were run, including the *Aplysia* porphyrin alone, and with authentic uroporphyrin I as marker in mixed and adjacent spots. On all papers two spots were obtained at R_F 0·09 and 0·15, indicating that the porphyrin of *Aplysia* was a uroporphyrin.

The Chu *et al.* (1951) technique, followed by that of Falk & Benson (1953) established the uroporphyrin as isomer I, confirmed (at Sheffield) by its conversion to coproporphyrin I.

The Porphyrins of Luidia ciliaris

The upper integument of the starfish *L. ciliaris* was extracted in the usual way with a methanol-sulphuric acid (19:1) mixture and the porphyrin brought into chloroform. After evaporation to dryness on the water-bath this extract was redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The column was monitored by ultra-violet light. A blue fluorescent band of fatty material passed rapidly down the column and was discarded. Two purplish bands, both red fluorescent, then appeared and passed very slowly down the column about 2 cm apart. These were eventually eluted with chloroform-methanol (100:3) (band 1) and chloroform-methanol (100:5) (band 2) respectively.

Band 1. This was identified as protoporphyrin by spectrophotometry in pyridine (including comparison with authentic protoporphyrin) and by conversion to mesoporphyrin.

Band 2. This band in chloroform solution was evaporated to dryness in portions. One of these was dissolved in pyridine and examined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt	
648	590	562	519	425	mμ

The curve obtained was clearly of the rhodo-type (that is, the optical density of the peaks of absorption decreased in the order III, IV, II and I). This fact, together with the positions of the maxima of absorption, strongly suggested chlorocruoroporphyrin. The oxime was prepared (at Sheffield) and a spectrophotometric examination in pyridine showed a characteristic shift of the absorption maxima towards the blue:

I	II	III	IV	
638	580	548	507	mμ

This is good evidence for the presence of a formyl (—CHO) group. Authentic chlorocruoroporphyrin, prepared from *Myxicola–Sabella* blood, gave an oxime with maxima at:

I	II	III	IV	
639	579	543	507	mμ

To determine the presence of vinyl groups, the porphyrin was treated with diazo-acetic ester followed by hydroxylamine. This produced a shift to the blue, but the rhodo-type spectrum was retained. The maxima in pyridine were:

I	II	III	IV	
645	586	557	516	mμ

This is consistent with the presence of one vinyl group, and is confirmatory evidence for the porphyrin being chlorocruoroporphyrin.

As an additional check on the identity of the main porphyrin of *Luidia*, the following experiments were carried out.

Fresh *Luidia* tissue was extracted by the ether : acetic acid procedure, and after the usual purification, the porphyrins were esterified by standing dissolved in methanol : HCl for 48 h at 0° C. The esters, after purification, were dissolved in dry chloroform and separated by chromatography on a column of alumina packed in a mixture of equal parts of chloroform and ether (Lemberg & Parker, 1952). The band of protoporphyrin dimethyl ester passed rapidly down the column well in advance of the main band of chlorocruoroporphyrin ester, and was discarded.

The chlorocruoroporphyrin band was collected, filtered, and the solvent removed *in vacuo* without heat. The residue was dissolved in glacial acetic acid saturated with sodium chloride and the chlorocruorohaematin prepared, the iron being introduced by the method of Paul (1950).

The chlorocruorohaematin was converted into the haemochromogen by the method of Warburg, Negelein & Haas (1930), viz.: the haematin was dissolved in aqueous pyridine (pyridine 1 ml. : water 3 ml.) and one-tenth of the volume of hydrazine hydrochloride solution added (hydrazine hydrochloride 0·7 g in 5 ml. water + 5 ml. of 2N-NaOH). The haemochromogen gave the following bands in the Hartridge Reversion Spectroscope:

I	II	Intensity
584·4	545·9 mμ	I > II

This result agrees well with that obtained for chlorocruorohaemochromogen by Lemberg & Falk (1951):

I	II	Intensity
583·1	545·1 mμ	not given

The haemochromogen from the 'haem *a*' obtained from ox-hearts by Rawlinson & Hale (1949) gave only *one* band in the visible spectrum at 587 mμ (Lemberg & Falk, 1951; Falk & Rimington, 1952; Rimington, Hale, Rawlinson, Lemberg & Falk, 1949).

These experiments distinguish clearly between the main porphyrin obtained from *Luidia* and the porphyrin *a* from haem *a* of heart muscle. In addition, the spectrum of the *Luidia* porphyrin—chlorocruoroporphyrin—is of the rhodo-type—intensities III, IV, II, I—whereas that of the porphyrin *a*

is of the oxorhodo-type—intensities III, II, IV, I. The porphyrin in *Luidia* occurs free, and not as a haem.

The Porphyrins of Astropecten irregularis

The integuments of fifty specimens of *Astropecten irregularis* were carefully stripped off and extracted with methanol/sulphuric acid overnight in the usual way. The pigments were taken into chloroform and the extract washed and evaporated by the conventional technique. The evaporated chloroform extract was redissolved in dry chloroform and chromatographed on a column of magnesia grade III (Nicholas, 1951), developing with chloroform with increasing concentrations of methanol, as for *Luidia*. The same type of chromatogram was obtained, and the two red fluorescent bands were shown to be protoporphyrin III type 9 and chlorocruoroporphyrin in the same way and by the same tests as were applied to the porphyrins of *L. ciliaris*. *Astropecten*, however, appeared to have much less porphyrin (and particularly protoporphyrin) in the integument than *Luidia*, although no quantitative investigation was carried out at this stage.

Discussion

This survey of a number of marine invertebrates from eight phyla shows that porphyrins are present in eight species for certain (with one doubtful species *Tethya aurantium*), and that these eight species belong to only three phyla—Annelida, Mollusca and Echinodermata. Porphyrins were not found in any of the coelenterates, crustaceans or tunicates examined, although MacMunn (1887) found haematoporphyrin in the madreporarian corals *Flabellum variabile* and *Fungia symmetrica*.

The finding of uroporphyrin in the upper soft integument of *Duvaucelia plebeia* (better known as *Tritonia*) and *Aplysia punctata* is of considerable interest in view of the presence of this pigment in some mollusc shells (Nicholas & Comfort, 1949). MacMunn (1887) found 'haematoporphyrin' in the black slug *Arion empiricorum* (a synonym introduced by Férussac, Férussac & Deshayes, 1819–51, to cover the numerous colour and pattern varieties of the slug more generally known as *A. ater*). Thus the only molluscs which have been shown definitely to contain a porphyrin in their soft parts are either species without shells (*Duvaucelia* and *Arion*) or species with uncalcified shells (*Aplysia*), whereas uroporphyrin, with traces of coproporphyrin, occurs in several of the shells of shell-bearing species. This suggests that in molluscs the porphyrin is normally laid down in the shell (an integumentary product), or failing that in the uncalcified integument. The former would be in keeping with the deposition of uroporphyrin I in the bones and teeth in congenital porphyria, and in the bones of the Pennsylvanian fox-squirrel *Sciurus niger* (Turner, 1937). Turner connected the formation of the

uroporphyrin I with the megaloblasts of the bone marrow, and postulated that in *Sciurus* there exists a unique persistence into the adult span of the normal method of foetal haemoglobin synthesis.

Protoporphyrin III type 9 occurs in the shells of the eggs of the hen and of a plover (Fischer & Kögl, 1924). Borst & Königsdorfer (1929), Fikentscher (1932), Fraenkel (1924) and Hammer (1930) found that small amounts of porphyrin occur in the bones of the human foetus, as well as in those of newborn babies and other newborn mammals, mainly in the centres of ossification. It is also interesting to recall the experiments of these workers with growing animals, in which injected uroporphyrin I and haematoporphyrin III type 9 were quickly deposited, the latter being required in large amounts before deposition takes place. Bingel (1937) showed that uroporphyrin III was also deposited in bones and teeth. Coproporphyrin I, mesoporphyrin III type 9 and protoporphyrin III type 9 appear to have a slight tendency to deposit in this way. The bones of the foetus do not become impregnated with porphyrins injected into the mother, according to Borst & Königsdorfer (1929) and others, but Kench, Langley & Wilkinson (1953) have shown that porphyrins are transmitted through the placenta but are rapidly excreted. This is surprising in view of the known affinity of one porphyrin at least—haematoporphyrin—for actively growing tissues (Figge, Weiland & Manganiello, 1948; Kennedy, 1952). The deposition of porphyrins in the asteroids is perhaps somewhat similar, although it is difficult to demonstrate whether the porphyrins are present in the spicules or not, owing to the difficulty of separating the spicules from the integument without destroying any porphyrin they might contain. The presence of free chlorocruoroporphyrin in *Luidia ciliaris* and also in *Astropecten irregularis* is of great interest, since this porphyrin has hitherto only been found to occur naturally in the form of its haemoglobin, chlorocruorin, in the blood of sabellid worms (Fox, 1926, 1949). Lemberg & Legge (1949) have suggested that 'since this pigment is found only in a small group of worms which live in the same type of environment as do others containing erythrocruorin with protohaem IX as prosthetic group, the peculiarity does not appear to be of adaptive importance, and may be an evolutionary relic'.

In the three species of echinoderms shown to contain porphyrins, *Asterias rubens* has only protoporphyrin (Kennedy & Vevers, 1953*b*) which has two vinyl groups, while *Luidia ciliaris* and *Astropecten irregularis* have chlorocruoroporphyrin, which has one vinyl and one formyl group. *Luidia ciliaris* and *Astropecten irregularis* also have protoporphyrin in the integument. Warburg (1932) considered that the presence of a carbonyl (=CO) group in a side chain (which later becomes a vinyl group) is a primitive characteristic. The occurrence of chlorocruoroporphyrin in *Luidia* and *Astropecten*, both phanerozonian asteroids, may therefore be regarded as an additional argument for classifying the Phanerozonia as less specialized than the Forcipulata such

as *Asterias rubens* (Grassé, 1948, p. 237). Apart from this, however, it would appear that the distribution of integumentary porphyrins in starfishes is not only random in relation to their taxonomic position but also in relation to their ecology and mode of life. Thus the presence of protoporphyrin in *A. rubens* and its absence in *Marthasterias glacialis* is surprising, for not only are these two starfishes classified in the same family (Asteriidae), but their larvae are almost identical in form and scarcely distinguishable (Mortensen, 1927), and the adults feed in the same way on the same type of food. In areas where their geographical ranges overlap, as they do off Plymouth, these two species may, in fact, be said to occupy the same ecological niche.

In the polychaetes the porphyrin is probably present in the viscera, but this is less certain as it is often difficult to separate integument from viscera in sufficient quantity for analysis. In *Chaetopterus*, in which the digestive tract was separated from the rest of the animal for extraction, the porphyrins certainly came from the viscera.

Summary

A survey of forty-eight species of British marine invertebrates has shown that porphyrins which have not previously been described in these animals are present in three representatives of the Annelida, two of the Mollusca and two of the Echinodermata. The occurrence of protoporphyrin in *Asterias rubens* was confirmed in a previous paper (Kennedy & Vevers, 1953*b*). A red-fluorescent extract was obtained from the sponge *Tethya aurantium*, but it contained too little material for further investigation. No trace of a porphyrin was found in the coelenterates, crustaceans and tunicates examined, although Moseley found 'polyperythrin' (which MacMunn later identified with the 'haematoporphyrin' of *Asterias* and *Arion*) in *Flabellum* and *Fungia*.

Chlorocruoroporphyrin was found free in the starfishes *Luidia* and *Astropecten*. Uroporphyrin was extracted from the soft dorsal integument of *Duvaucelia* (= *Tritonia*) *plebeia*, and from the integument of *Aplysia punctata*, observations of interest in view of the widespread occurrence of uroporphyrin in mollusc shells.

Coproporphyrin III was characterized from extracts of the viscera of *Myxicola infundibulum*, *Nereis diversicolor* and *Chaetopterus variopedatus*. Evidence of the occurrence of a penta-carboxylic porphyrin was obtained from paper chromatography of the extract from the gut of *Chaetopterus*.

These findings are discussed in comparison with the distribution of porphyrins in birds and mammals.

REFERENCES

BINGEL, A., 1937. Die Bedeutung der Porphyrine für die Pathogenese gewisser neurologischer Krankheitsbilder. *Z. ges. Neurol. Psychiat.*, Bd. 158, pp. 79–83.

BORST, M. & KÖNIGSDORFER, H., 1929. *Untersuchungen über Porphyrie, mit besonderer Berücksichtigung der porphyria congenita.* 279 pp. Leipzig.

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

FALK, J. E. & BENSON, A., 1953. Separation of uroporphyrin esters I and III by paper chromatography. *Biochem. J.*, Vol. 55, pp. 101–4.

FALK, J. E. & RIMINGTON, C., 1952. Attempted isolation of haem *a* and porphyrin *a* from heart muscle. *Biochem. J.*, Vol. 51, pp. 36–45.

FÉRUSSAC, J. B. L. d'A., Baron de, FÉRUSSAC, A. E. J. P. J. F. d'A. de & DESHAYES, G. P., 1819–51. *Histoire naturelle des mollusques terrestres et fluviatiles.* 4 Tomes and Atlas. Paris.

FIGGE, F. H. J., WEILAND, G. S. & MANGANIELLO, L. O. J., 1948. Cancer detection and therapy. Affinity of neoplastic, embryonic and traumatized tissues for porphyrins and metalloporphyrins. *Proc. Soc. exp. Biol., N.Y.*, Vol. 68, pp. 640–1.

FIKENTSCHER, R., 1932. Quantitative Porphyrin-Bestimmung durch Lumineszenzintensitätsmessung mit dem Stufenphotometer. *Biochem. Z.*, Bd. 249, pp. 257–69.

FISCHER, H. & KÖGL, F., 1924. Zur Kenntnis der natürlichen Porphyrine. IX. Über Ooporphyrin aus Kiebitzeierschalen und seine Beziehungen zum Blutfarbstoff. *Hoppe-Seyl. Z.*, Bd. 138, pp. 262–75.

FRAENKEL, E., 1924. Experimentelles über Hämatoporphyrie. *Virchows Arch.*, Bd. 248, pp. 125–136.

FOX, H. MUNRO, 1926. Chlorocruorin: a pigment allied to haemoglobin. *Proc. roy. Soc.*, B, Vol. 99, pp. 199–220.

—— 1949. On chlorocruorin and haemoglobin. *Proc. roy. Soc.*, B, Vol. 136, pp. 378–88.

GRASSÉ, P., 1948. [Ed.] *Traité sur la Zoologie*, T. 11. p. 1077. Paris.

HAMMER, H., 1930. Zur experimentellen Hämatoporphyrose der Knochen. *Virchows Arch.*, Bd. 277, pp. 159–73.

KENCH, J. E., LANGLEY, F. A. & WILKINSON, J. F., 1953. Biochemical and pathological studies of congenital porphyria. *Quart. J. Med.*, Vol. 22, pp. 285–94.

KENNEDY, G. Y., 1952. *British Empire Cancer Campaign, XIIIth Annual Report*, p. 201.

—— 1953*a*. A new apparatus for column chromatography. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 201–2.

—— 1953*b*. Partition paper chromatography of the porphyrins. A new apparatus. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KENNEDY, G. Y. & VEVERS, H. G., 1953*a*. Protoporphyrin in the integument of *Asterias rubens*. *Nature, Lond.*, Vol. 171, p. 81.

—— —— 1953*b*. The biology of *Asterias rubens* L. V. A porphyrin pigment in the integument. *J. Mar. biol. Ass. U.K.*, Vol. 32, pp. 235–47.

LEMBERG, R. & FALK, J. E., 1951. Comparison of haem *a* the dichroic haem of heart muscle and of porphyrin *a* with compounds of known structure. *Biochem. J.*, Vol. 49, pp. 674–83.

LEMBERG, R. & LEGGE, J. W., 1949. *Haematin Compounds and Bile Pigments.* New York.

LEMBERG, R. & PARKER, J., 1952. Porphyrins with formyl groups. II. Preparation of chlorocruoroporphyrin diformyldeuteroporphyrin. *Aust. J. exp. Biol. med. Sci.*, Vol. 30, pp. 163–70.

MACMUNN, C. A., 1887. On the presence of haematoporphyrin in the integument of certain invertebrates. *J. Physiol.*, Vol. 7, pp. 240–52.

MORTENSEN, T., 1927. *Echinoderms of the British Isles*. 471 pp. Oxford University Press.

NICHOLAS, R. E. H., 1951. Chromatographic methods for the separation and identification of porphyrins. *Biochem. J.*, Vol. 48, pp. 309–13.

NICHOLAS, R. E. H. & COMFORT, A., 1949. Acid soluble pigments of molluscan shells. 4. Identification of shell porphyrins with particular reference to conchoporphyrin. *Biochem. J.*, Vol. 45, pp. 208–10.

NICHOLAS, R. E. H. & RIMINGTON, C., 1949. Qualitative analysis of the porphyrins by partition chromatography. *Scand. J. clin. Lab. Invest.*, Vol. 1, pp. 12–18.

PAUL, K-G., 1950. The conversion of ferri-porphyrins (hemins) to their corresponding porphyrins by means of pyruvic acid. *Acta chem. Scand.*, Vol. 4, pp. 1221–32.

RAWLINSON, W. A. & HALE, J. H., 1949. Prosthetic groups of the cytochromes present in *Corynebacterium diphtheriae* with especial reference to cytochrome *a*. *Biochem. J.*, Vol. 45, pp. 247–55.

RIMINGTON, C., HALE, J. H., RAWLINSON, W. A., LEMBERG, R. & FALK, J. E., 1949. *1st Int. Congr. Biochem.* (Abstr.), p. 379.

TURNER, W. J., 1937. Studies on porphyria. I. Observations on the fox-squirrel, *Sciurus niger*. *J. biol. Chem.*, Vol. 118, pp. 519–30.

WARBURG, O., 1932. Das sauerstoffübertragende Ferment der Atmung. *Z. angew. Chem.*, Bd. 45, pp. 1–6.

WARBURG, O., NEGELEIN, E. & HAAS, E., 1930. Spirographishämin. *Biochem. Z.*, Vol. 227, pp. 171–83.

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

J. Mar. biol. Ass. U.K. (1954) 33, 677–679
Printed in Great Britain

PROTOSTYELA HETEROBRANCHIA N.GEN., N.SP., A STYELID ASCIDIAN FROM THE SCOTTISH WEST COAST

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(Text-fig. 1)

The ascidian described in this paper was collected on 22 April 1952 from the shore of the island of Luing, Argyll. It was taken, along with colonies of *Didemnum maculosum* (Milne Edwards), from the level of low water of spring tides, on a part of the shore which, although very sheltered from waves, is subject to fast tidal currents. Only a single zooid was found, but it differs so clearly from all known ascidians that it is being made the type of a new genus and new species. The following description applies to the zooid after it had been stored in 10% formalin for nearly two years.

Description

The body is about 2·0 mm long, ovoid, and very slightly depressed perhaps owing to contraction (Fig. 1 A). It is pale brown and has a smooth surface with no incrusting material. There is a narrow ventral area of attachment and a small basal extension of the test which spreads over the substratum and which in life was probably linked to other zooids. The test is thin and moderately tough, semi-transparent and marked with small brown spots. A circular area round each siphon is without pigment. Both siphons are round, without lobes, and are so short that they scarcely project from the surface of the body. The body-wall is thin and almost transparent and possesses very slender muscles which are visible only under high magnification. They form an open irregular mesh except on the siphons, which have radial muscles. There are about twelve simple oral tentacles of alternating sizes. The branchial sac (Fig. 1 C) is very characteristic. It possesses no folds but on each side are three narrow longitudinal bars, the dorsal one on the right side being very close to the dorsal lamina. No transverse bars are present. The stigmata, of which there are about sixteen rows, are transverse, instead of longitudinal as in almost all other ascidians. They are long rather wide rectangular openings and number about three in each transverse row. The dorsal lamina is an undivided membrane. Details of the small dorsal tubercle were not seen. A short curved oesophagus leads to the barrel-shaped stomach which has eight to ten complete longitudinal folds (Fig. 1 B). The stomach and intestine

lie to the left of the branchial sac. The intestine forms a narrow simple loop bending back close to the stomach. No pyloric caecum was seen but one may possibly be present. The short flattened rectum turns forward to end in the smooth-edged elliptical anus. On the right side of the body there are three gonads (Fig. 1D), which are attached to the body-wall and form a row parallel to the endostyle but a short distance from it. The posterior gonad (Fig. 1E) is hermaphrodite and consists of an undivided dorsal testis and a ventral ovary.

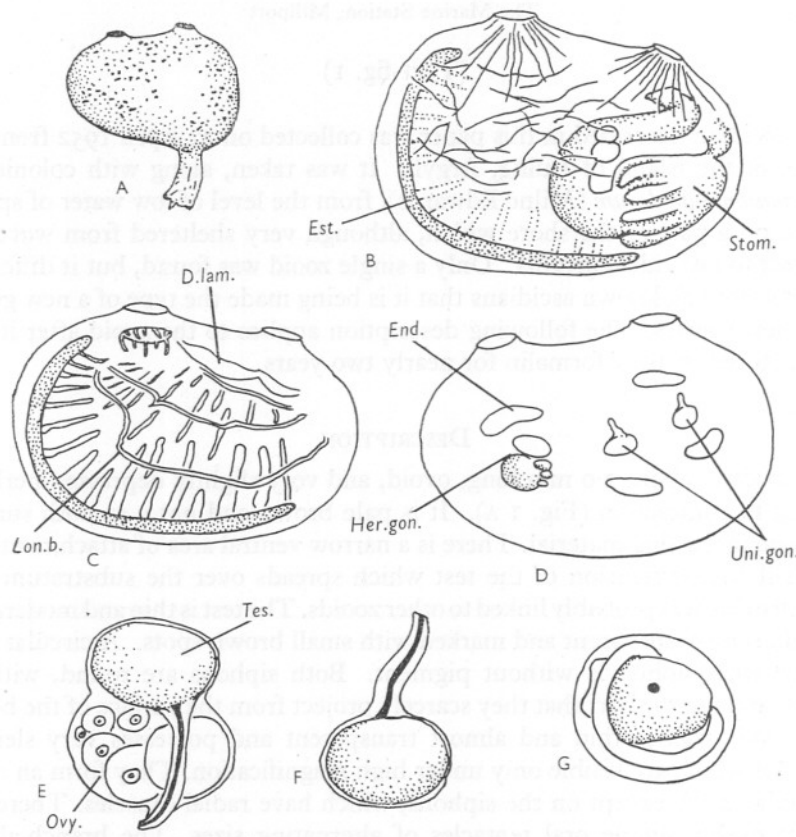

Fig. 1. *Protostyela heterobranchia*. A, zooid from right; B, zooid from left, with test removed; C, zooid from left, dissected to show right half of branchial sac; D, zooid from right to show gonads; E, hermaphrodite gonad; F, unisexual (male) gonad; G, larva. *D.lam.*, dorsal lamina; *End.*, endocarp; *Est.*, endostyle; *Her.gon.*, hermaphrodite gonad; *Lon.b.*, longitudinal bar; *Ovy.*, ovary; *Stom.*, stomach; *Tes.*, testis; *Uni.gon.*, unisexual (male) gonad.

The sperm duct passes down across the mesial surface of the ovary and opens immediately ventral to it. No oviduct was distinguished. In the two anterior gonads (Fig. 1F) only a simple testis was seen and these gonads are almost certainly unisexual. The sperm duct of each of these two male gonads is

directed dorsally. On the left side of the body there is also a hermaphrodite gonad like that on the right, but other gonads were not seen. A few ovoid endocarps are present on each side of the body.

Some developing eggs and one apparently nearly fully developed larva (Fig. 1G) were found in the atrial cavity, and the species is therefore viviparous. The developing eggs have a diameter of about 0·23 mm measured over the chorion. The trunk of the larva is about 0·22 mm long and has at its anterior end a triangular projecting structure which represents the three adhesive papillae. A single black sense organ is present. The tail of the larva was still coiled round the trunk and therefore could not be measured.

The new species, although represented by a single zooid, clearly belongs to the group of compound styelid ascidians sometimes recognized as the subfamily Polyzoinae (Hartmeyer, 1903). In many species of this group the colony consists of zooids closely united within a common test. In others, however, the zooids are widely separated and united only by a basal creeping stolon or sheet of test material, and this is apparently the condition in *Protostyela heterobranchia*.

In possessing transverse stigmata the genus differs from all known compound styelids except *Berrillia* (Brewin, 1952), but *Berrillia* is distinguished by having four longitudinal bars on each side of the branchial sac, and by the presence of gonads on the left side only.

Protostyela n.gen. may be defined as follows: a genus of the family Styelidae forming colonies of loosely united zooids; branchial sac without folds; stigmata transverse; hermaphrodite and unisexual gonads present in the same zooid.

The geographical distribution of *P. heterobranchia* is still unknown, but its apparent absence from the intensively collected areas of the Channel suggests that it may have a more northerly range. This possibility is strengthened by the presence of larvae in April long before high summer temperatures are reached.

Summary

Protostyela heterobranchia n.gen. n.sp. is a compound styelid ascidian from Scottish waters. The branchial sac has three longitudinal bars on each side and no folds, and the stigmata are transverse. Both hermaphrodite and unisexual (male) gonads are present.

REFERENCES

Brewin, B. I., 1952. Ascidians of New Zealand. Part VII. Ascidians from Otago coastal waters. Part II. *Trans. roy. Soc. N.Z.*, Vol. 79, pp. 452–8.

Hartmeyer, R., 1903. Die Ascidien der Arktis. In Römer, F., and Schaudinn, F., *Fauna Arctica*, Bd 3, Pt. 2, pp. 91–412. Jena.

J. Mar. biol. Ass. U.K. (1954) 33, 681–687
Printed in Great Britain

THE BREEDING AND DEVELOPMENT OF THE ASCIDIAN *PELONAIA CORRUGATA* FORBES AND GOODSIR

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(Text-figs. 1–3)

Pelonaia is a genus of the family Styelidae (subphylum Tunicata; class Ascidiacea) which is very distinct, anatomically, from all other ascidians. It appears, however, to be closer to *Styela* than to other genera of the family. The only known species, *Pelonaia corrugata* Forbes & Goodsir, has a mainly circumpolar arctic distribution and extends for a short distance southward into boreal waters. In British waters it occurs chiefly round the Scottish coast, and is not definitely known south of the Firth of Clyde and Cullercoats. Within its geographical range *Pelonaia* occurs only locally, being restricted to bottoms of sand or mud. The loose nature of the substratum has had a great influence on the form of the body, which is long, narrow and worm-like. *Pelonaia* is not attached to a solid object, but instead lives upright in the sand or mud, with the lower part of the body embedded and the upper part, which bears the siphons, projecting freely into the water. The shape and habitat are unlike those of most sand-dwelling ascidians which are usually globular and are often anchored in the surface layer of the sand by filaments of the test.

The development of *P. corrugata* was unknown and was interesting for two reasons: first, the species has a rather isolated position in the family Styelidae, and secondly, the specialized habitat of the adult might be expected to affect the method of development.

Material and Methods

Specimens were dredged from sandy mud at depths of 40–47 m, off Fairlie in the Firth of Clyde. Fertilized eggs were obtained in two ways: (1) by allowing animals kept in aquarium tanks to liberate eggs and sperm spontaneously, the eggs being removed to Petri dishes after fertilization; and (2) by taking eggs and sperm from the ducts of animals within a few hours of collection, and adding sperm from one individual to eggs from another. Developing eggs were kept in covered Petri dishes or watch-glasses containing freshly collected unfiltered sea water. The water, which was changed once daily, maintained a temperature of 8·0–10·0° C. A few embryos were fixed in Boveri's picro-acetic mixture, embedded in paraffin wax (m.p. 49° C.), and sectioned at 8μ.

Observations

The Breeding Season

Specimens were collected at about monthly intervals between August 1950 and February 1954. In most samples the population could be separated by inspection into two groups representing different age classes. There therefore appeared to be a limited breeding season. Fig. 1 shows the distribution by body volume of the specimens taken on 11 January 1954. Animals up to about 1·0 ml. were about 1 year old and those between 1·2 and 2·4 ml. about 2 years old. Only the animals in the older group had fully developed and functional

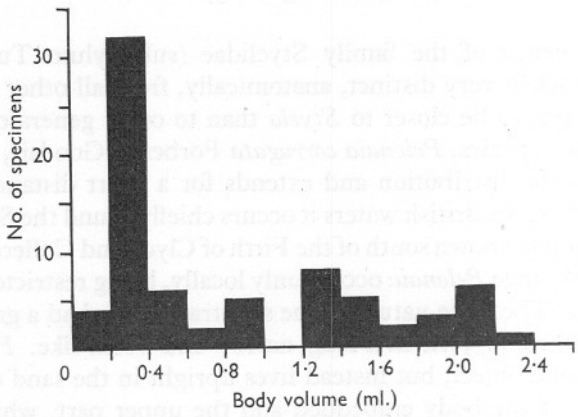

Fig. 1. Distribution by body volume of the specimens of *P. corrugata* collected on 11 Jan 1954

gonads at this time of year. The breeding season was found by examining the specimens in the monthly samples, and noting when the gonads started to empty and when they had completely emptied. In 1952 spawning had not yet started on 17 January, but was completed before 25 March. In 1953 spawning had just started on 12 January, and was finished by 26 January. In 1954 spawning started between 15 January and 22 January and ended about 15 February. The breeding season in this area is therefore confined to a period of 2–4 weeks in January or February. During breeding in 1954 the bottom temperature over the spawning animals was about 7·5° C., and animals kept in an aquarium tank spawned at a temperature of about 7·0° C. Thus *P. corrugata* breeds, in the Firth of Clyde, when the sea is almost at its coldest, a fact correlated with the mainly arctic distribution of the species. The southern limit of its world distribution coincides roughly with the February isotherm of 7·2° C. (Fig. 2); higher temperatures further south probably limit distribution by preventing breeding.

The ripe ovum (Fig. 3A) is spherical, bright orange in colour, and from 270 to 280μ in diameter. It is surrounded by a thin chorion forming a spherical transparent shell 300 to 320μ in diameter. The outer follicle cells, which are

rounded and have no vacuoles, cover the chorion. Inner follicle cells are inconspicuous. Much yolk is present and the egg has a high specific gravity. Consequently the eggs of *Pelonaia* drop to the bottom when shed into the

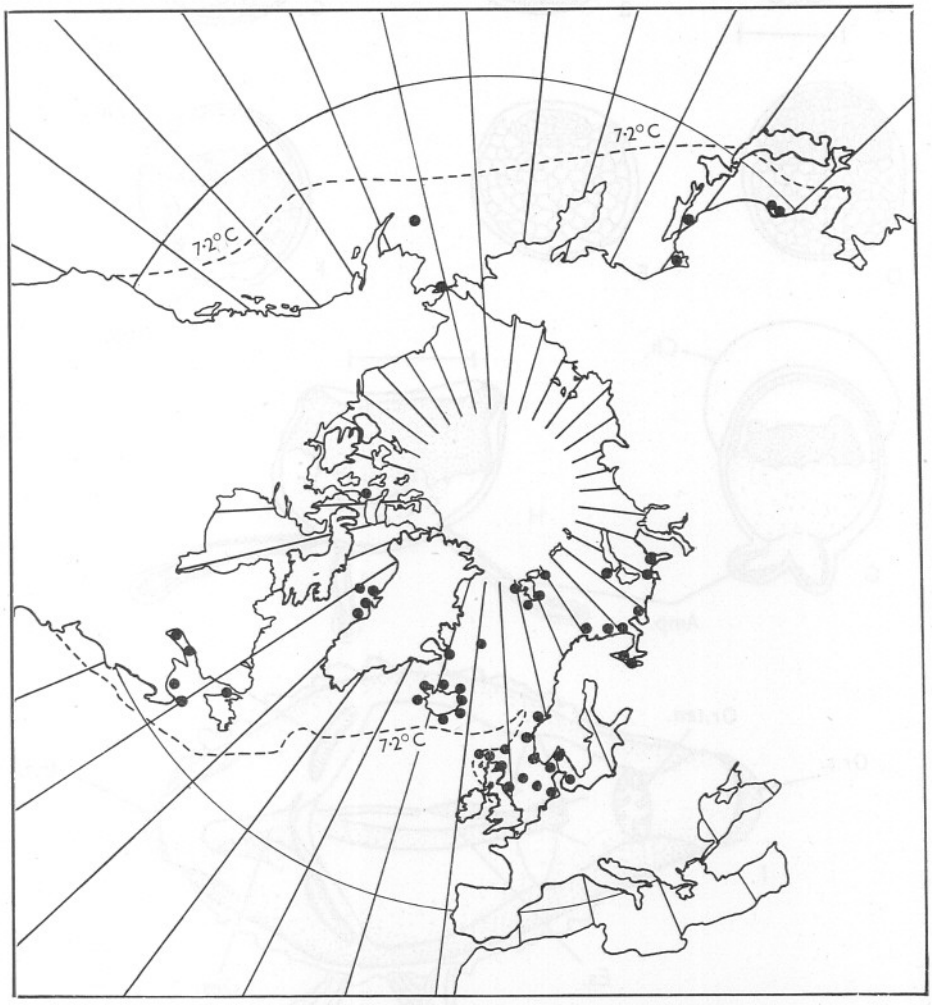

Fig. 2. Known geographical distribution of *P. corrugata*, showing also the February isotherm of 7·2° C.

water, instead of remaining suspended as do the eggs of many ascidians. This is important in connexion with the mode of development, as will be seen later.

The sperm has the shape common in ascidians. The narrow rod-like head about 12 μ long has, near one end, an asymmetrical protoplasmic bulge, and arising from this end, a tail about 62 μ long.

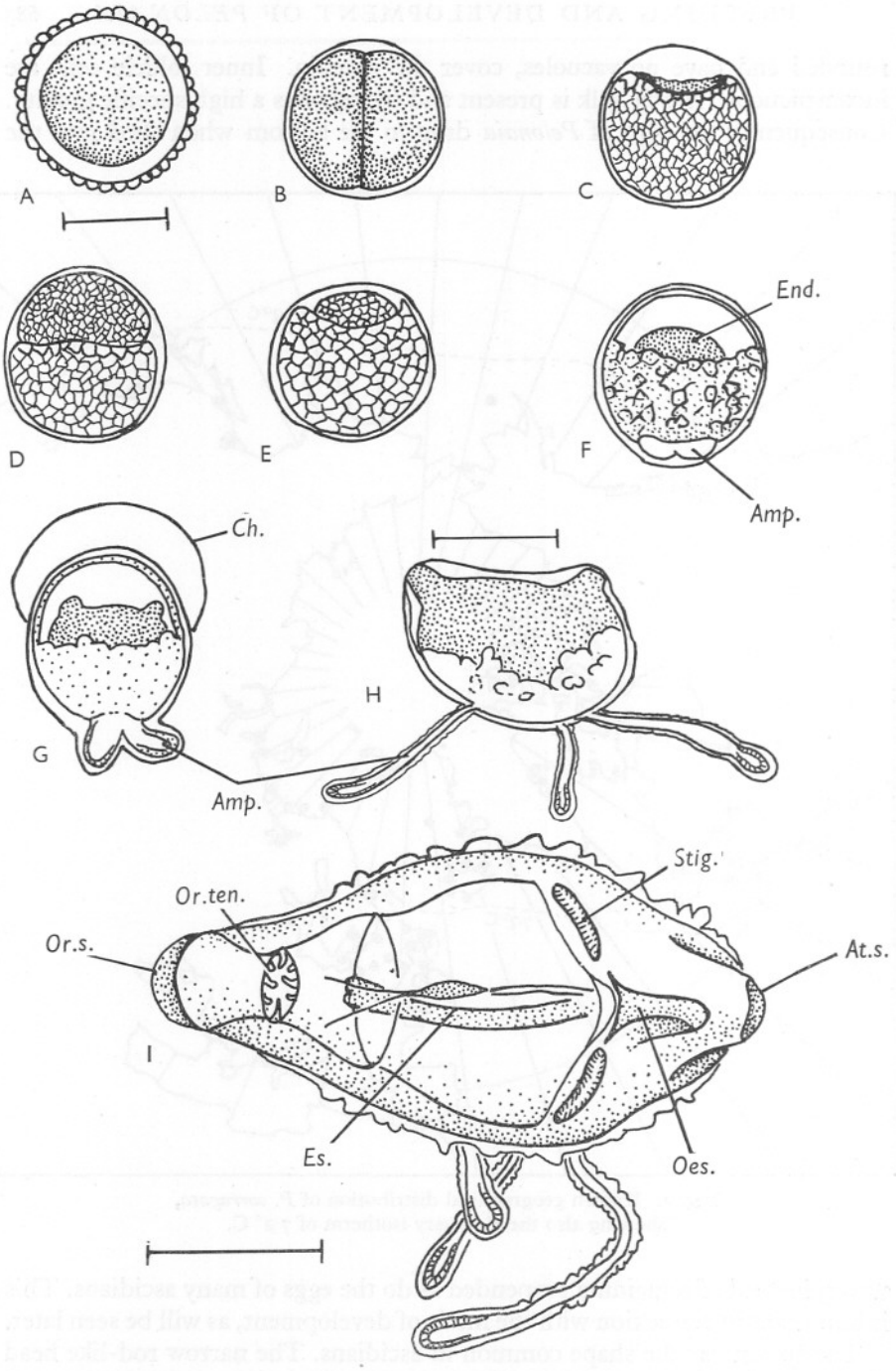

Fig. 3. Development of *P. corrugata*. A, unfertilized egg; B, two-cell stage, showing area of pale protoplasm in each cell; C, gastrula; D, two-hemisphere stage, the lower hemisphere consisting of large pale cells and the upper one of small dark cells; E, later two-hemisphere stage; F, embryo showing rudiments of two ampullae; G, rupture of chorion; H, embryo after attachment; I, young ascidian. D-H are lateral views and I a dorsal view. In B–G the outer follicle cells have been omitted. The scales represent 200 μ. *Amp.*, ampullae; *At.s.*, atrial siphon; *Ch.*, chorion; *End.*, endoderm; *Es.*, endostyle; *Oes.*, oesophagus; *Or.s.*, oral siphon; *Or.ten.*, oral tentacles; *Stig.*, stigma.

Embryonic Development

Artificial fertilization was attempted each month for over 2 years, but succeeded only in January 1953 and January and February 1954. At other times the sperm was almost completely inactive. Only a few, and usually less than 12, of the eggs taken from any animal became fertilized. There were therefore not enough embryos to allow fixation and sectioning of all the most important stages, and the following outline of development was obtained mainly from the study of live embryos.

Cleavage of the fertilized egg is symmetrical and is like that of other ascidians up to the time of gastrulation. The gastrula (Fig. 3C) is formed within about 12 hr. of fertilization at a temperature of 8·0–10·0° C. There is little localization of coloured protoplasm into presumptive areas, such as occurs in some styelid ascidians and occasionally amongst other families. In the 2- and 4-cell stages, however, each cell shows a pale area in the otherwise uniformly coloured protoplasm (Fig. 3B), and in the 16-cell stage there is a group of four dark cells. Later stages show no colour pattern. Following gastrulation the embryo rounds off and remains spherical for about a day. During the next 2 days the spherical stage is transformed into a two-hemisphere stage (Fig. 3D). In this, one half of the embryo consists of large pale orange cells and the other half of small dark orange cells. The hemisphere of large cells now slowly grows and begins to envelop the other hemisphere, which meanwhile shrinks (Fig. 3E). At the end of the process, usually 4–6 days after gastrulation is complete, the embryo has an internal ovoid mass of small dark cells surrounded by a sphere, part of which is still recognizable as the large pale cells of earlier stages. The internal dark mass is endoderm and the surrounding sphere ectoderm. The ectoderm at one point now differentiates and by rapid growth forms a pair of blister-like swellings, which are the rudiments of two ampullae (Fig. 3F). The whole development so far has taken place within the chorion, which however is now ruptured by the elongation of the ampullae and by the swelling due to the secretion of test over the whole ectoderm (Fig. 3G). The ampullae grow rapidly, and by secreting sticky test substance, fix the embryo to the substratum. The swollen ends of the ampullae have tall epidermal cells and are regions especially active in the secretion of test. In the culture vessels the embryos became attached to the glass by means of the ampullae, but in nature they would presumably be fixed to sand grains by the same mechanism. At this point in development the central endodermal mass shows the rudiments of the two siphons projecting outwards towards the enclosing ectoderm. The remaining central mass is the rudiment of the pharynx and gut. The orientation of the future ascidian can now be established: the ampullae arise from the ventral surface and the siphons mark the dorsal surface. In most specimens a third ampulla appears at this stage (Fig. 3H), but in some only two are present throughout development. The siphons are now completed by fusion of the

endodermal rudiments with the ectoderm, which also enters into their formation. The endostyle and dorsal lamina appear in the pharynx, and the oesophagus and other parts of the gut become visible. Branchial stigmata are not formed until some time later; in one individual the first pair appeared on the 20th day after fertilization, but in others not until the 40th day. The young ascidian (Fig. 3 I) now has its essential organization and is soon able to feed.

Discussion

A few attempts have been made to relate the geographical distribution of ascidians with sea temperatures (Hartmeyer, 1923–24; Thompson, 1930–34; D. & A. Carlisle, 1954) but very little is known of the exact way in which temperature limits the range. We do not know in any particular species, for instance, whether high summer temperature or low winter temperature is a limiting factor, and whether the boundaries of distribution are fixed by inability of the adult to survive, or to breed successfully, beyond the temperature limits. In *Pelonaia* it appears that the southern boundary is fixed by the failure of breeding in higher winter temperatures farther south. It would be interesting to know the breeding period in northern waters, and we would expect it to be shifted progressively towards the summer in the more northerly parts of the range. Although the limiting factor at the northern boundary of the range is unknown, it may well be a winter temperature too low for survival of the adult. If this is so *Pelonaia* illustrates the type 4 geographical zonation, as defined by Hutchins (1947); in this type of distribution both the poleward and the equatorward limits are set by the winter temperature.

The distribution of the species is further limited by the need of a substratum loose enough to allow the adult to be partially embedded, but not subject to shifting with the consequent danger of burial. Such a habitat, however, is local, and the species may require for survival not the wide dispersal of larvae but rather their retention in the area already inhabited by the adults. The pelagic larval stage which is normal in other styelids and indeed in most ascidians, has been eliminated in *Pelonaia*. Moreover, the egg is heavy, and probably sinks to the bottom instead of drifting in water currents. The young stages therefore develop and are established close to the breeding parent stock. In the elimination of a pelagic larval-stage *Pelonaia* presents a striking parallel with the sand-dwelling species of the Molgulidae. Berrill (1931) has shown that in this family the sand-flat habitat is common and is usually accompanied by anural development. He regards the suppression of the larval stage as correlated with the habitat although he does not consider 'that such a habitat is directly responsible for its origin, but that conceivably through a lessening of the rigour of natural selection, those species in which it does arise are allowed to survive, whereas among attached shore forms they are not'. But the occurrence of anural development in the genus *Pelonaia*, which although unrelated

occupies a similar habitat, suggests that this type of development is of positive survival value.

In this investigation I have not been able to make a detailed study of the embryology, but intend to do so in the future, as several very interesting problems are involved. First, there is the fate of the tissues which in urodele forms produce the tail. Secondly, the larval sense organs (ocellus and otolith) fail to develop, but we do not know whether the larval nervous system is totally suppressed, and if so, how the adult system arises. Thirdly, the stages intermediate between the gastrula and the hatching embryo are obscure, particularly the two-hemisphere stage which requires an explanation in terms of cell-lineage. Fourthly, what is the relation between those changes leading to the formation of the young ascidian stage and the changes which occur in the metamorphosing larva of urodele species?

I wish to thank the skipper and crew of M.F.V. *Calanus* for their help in collecting material, and my wife for her care of the cultures.

Summary

Pelonaia corrugata in the Firth of Clyde breeds for only 2–4 weeks in January or February. The species is oviparous. Early cleavage is similar to that of other ascidians, but after gastrulation the embryo passes through a two-hemisphere stage. One of these hemispheres is endodermal. From the other hemisphere, which slowly envelops the endoderm, two ampullae grow and fix the embryo to the substratum. The young ascidian develops directly from this attached embryo, and the development is therefore anural. Elimination of the pelagic larval stage is regarded as an adaptation to the sand-dwelling habit of the adult, as it prevents dispersal to unsuitable habitats.

REFERENCES

Berrill, N. J., 1931. Studies in tunicate development. Part 2. Abbreviation of development in the Molgulidae. *Phil. Trans.*, B, Vol. 219, pp. 281–346.

Carlisle, D. B. & Carlisle, A. I., 1954. Notes on the Didemnidae (Ascidiacea). 1. The presence of *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) in the Plymouth area. *J. Mar. biol. Ass. U.K.*, Vol. 33, pp. 21–5.

Hartmeyer, R., 1923–24. Ascidiacea. *Dan. Ingolf-Exped.*, Vol. 2, pt. 6, 368 pp.; pt. 7, 278 pp.

Hutchins, L. W., 1947. The bases for temperature zonation in geographical distribution. In Symposium on marine ecology. *Ecol. Monogr.*, Vol. 17, pp. 325–35.

Thompson, H., 1930–34. The Tunicata of the Scottish area. Parts I–III. *Sci. Invest. Fish. Bd Scot.*, 1930, No. 3, 45 pp.; 1931, No. 1, 46 pp.; 1932, No. 2, 42 pp.; 1934, No. 1, 44 pp.

J. Mar. biol. Ass. U.K. (1954) 33, 689–697
Printed in Great Britain

THYROIDECTOMY OF THE DOGFISH, *SCYLLIUM CANICULA* (L.), AND THE EFFECT OF DOGFISH THYROID UPON THE OXYGEN CONSUMPTION OF RATS

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(Text-fig. 1)

The function of the thyroid gland in fish is still obscure in spite of the numerous attempts to elucidate it by means of the administration of thyroid extracts, thyroxin, thyrotropin and antithyroid drugs, as well as by the carrying out of direct histological and cytological observations upon the gland at various seasons of the year and phases of the life cycle (Goldsmith, 1949; Hopper, 1952). The influence of this gland upon the metabolic rate of higher vertebrates is well established, but, despite the recent demonstration that the teleost thyroid contains a principle which raises the oxygen consumption of mammals (Smith & Brown, 1952), most attempts to influence the rate of oxygen consumption in fish themselves by the use of thyroid stimulants or depressants have been negative (Root & Etkin, 1937; Etkin, Root & Mofshin, 1940; Hasler & Meyer, 1942; Smith & Everett, 1943), the only positive result being that of Smith & Matthews (1947), who injected extracts of the Bermuda parrot fish into the Bermuda white grunt.

Studies of the growth and maturation of thyroid-treated fish have also been contradictory (Hoar, 1951; Hopper, 1952; Smith, Sladek and Kellner, 1953), and while chemical thyroidectomy appears to inhibit growth (Goldsmith, Nigrelli, Gordon, Charipper & Gordon, 1944; Nigrelli, Goldsmith & Charipper, 1946) and gametogenesis (Barrington & Matty, 1952), the possibility remains, as the latter authors point out, that these results may be due to some action of the drug which is not mediated by the thyroid gland.

Despite the fact that surgical removal of the glands has proved an invaluable procedure in endocrine research, thyroidectomy has not been carried out in teleosts, which have been mainly used in these studies, probably because the gland is usually diffuse and intimately related to the ventral aorta. Thus a lack of information regarding the influence of the absence of their own gland upon the oxygen consumption of fish remains a serious gap in our knowledge, and it has seemed essential to attempt to fill this by devising a procedure for surgical thyroidectomy in elasmobranchs in which group the distinct and encapsulate nature of the gland presents a more promising situation, as was realized by Vivien (1941). The present paper describes such a

procedure, and presents the results of observations upon the oxygen consumption of fish after thyroidectomy. For comparison with these results the influence of elasmobranch thyroid on the oxygen consumption of male white rats has also been studied.

Materials and Methods

Operative Technique

Dogfish of lengths ranging from 60 to 70 cm and of both sexes were used in all the operations, the animals being first anaesthetized by placing them in a 1·5% solution of urethane in sea water for 15 min. After washing in tap water they were placed ventral side uppermost in an operating cradle.The head was strapped down over the oro-nasal grooves on to the operating board and the lower jaw held back by retractors, thus exposing the floor of the mouth. A half-inch transverse incision was made through the mid-line in the fold of loose skin lying immediately behind the teeth of the lower jaw. After loosening the connective fascia between the coracomandibular and coracohyoids these muscles were held apart by small stainless steel retractors in order to expose the thyroid gland. This narrows anteriorly into a long 'thyroglossal cord' which terminates in the region of the symphysis of Meckel's cartilages. The anterior end of the 'thyroglossal cord' was first severed from its connective tissue attachment and the intact gland removed in an anterior-posterior direction, the region of the anterior attachment and also the region of the posterior attachment of the gland to the muscle lying dorsal to the coracohyoids being cauterized locally with an ophthalmic cautery in order to destroy any stray follicles that might have been left by the operation. The duration of the operation varied from 20 to 30 min; there was no appreciable haemorrhage, and the animals recovered consciousness almost immediately after being replaced in sea water. After some operations the sinus left by removal of the gland was packed with absorbent sterilized gelatin sponge. Nylon thread was used to stitch the skin incision, and the normal aseptic operating conditions were employed.

After a few days all the operated animals began to feed upon strips of fresh squid muscle. An autopsy was performed on every thyroidectomized fish after the completion of the respiration experiments; in no case was thyroid tissue identified macroscopically. The mortality rate was satisfactorily low, no deaths having occurred in the experiment, to be described later in detail, in which the oxygen consumption of operated animals was determined. Operated dogfish have, in fact, been kept alive for a period of at least 72 days after the complete removal of the gland.

A vertical approach through the throat was attempted in some preliminary experiments, but this method was abandoned when it was found to result in nearly a 100% mortality within a week or so.

Determination of Oxygen Consumption of Dogfish

As a result of the experience gained from some preliminary work carried out in the summer of 1952, using the Winkler technique for the determination of dissolved oxygen, it was decided to use the polarographic technique in the definitive experiments, in order to make possible the handling of a large number of fish. The respiration apparatus was of the continuous flow type similar in principle to that described by Hall (1929). Each dogfish was placed separately into a respiration chamber consisting of a bitumen-painted compressed asbestos pipe, 15 cm in diameter and 76 cm long. The ends of each chamber were made of Perspex plates which could be removed for inserting the fish, and through which it was possible to observe the movements of the animals in order to investigate the possibility of activity influencing the oxygen consumption during the metabolism experiments. No appreciable activity was in fact observed. A constant flow of water maintained at 400 ml./min was allowed to pass through the chamber containing the animal for 8 h. Four chambers were used simultaneously. The temperature of the tank of water surrounding the respiration chambers, which varied during the experiment from 16·2 to 17·6° C, was recorded daily.

As mentioned above, oxygen content of the water was measured by the polarographic method, the apparatus used being of the manually operated type with a dropping mercury electrode (Milton & Waters, 1949; Giguère & Lauzier, 1945). Samples of the inflow and outflow water, taken between 5 and 7 p.m. each day, were run directly into 20 ml. polarographic cells, and their oxygen contents measured at 16·5° C. (The sampling error of duplicate samples of water was always less than 1·0%.)

Determination of Oxygen Consumption of Rats

Twenty adult male albino rats were used in this study, their weights ranging from 185 to 260 g. Four rats were used as untreated controls, their oxygen consumption being measured every day for 6 days. Four more control rats were injected intra-peritoneally with 2·0 ml. of 0·9% sodium chloride in distilled water, whilst in a further group of eight each animal was given a single injection of 100 mg of mammalian thyroid powder. The remaining four were each given a single injection of 95 mg of dried dogfish thyroid. This dogfish thyroid was prepared by placing the fresh gland in acetone at 4° C and then washing several times in acetone to remove the fats. Finally the glands were washed in alcohol, dried in a desiccator and ground up into a fine powder.

The four rats which received saline injections were at a later date each given a single injection of a suspension of 100 mg dried trout muscle.

The oxygen consumption measurements were made using the method of Grad (1952), except that 9,000 ml. desiccators were found to be more convenient than the original stoppered jars as respiration chambers.

All determinations were made between 9.30 and 11.30 a.m. at a temperature of $28 \pm 0{\cdot}5^{\circ}$ C. During the experiment between the periods in which they were in the respiration chambers, the animals were kept supplied with abundant food and water.

Results

Oxygen Consumption of Thyroidectomized Dogfish

The oxygen consumption of thyroidectomized dogfish was measured individually on three male and three female animals from the 1st to the 42nd day after the operation, at 3-day intervals. Six animals, three male and three female, given sham operations consisting of the complete operative procedure except for the removal of the gland, were used as controls, their oxygen consumption being also measured at 3-day intervals. Both of these groups of fish were measured before the operations in order to determine the normal oxygen consumption of untreated fish. The results expressed as the mean oxygen consumption of the six fish in each of the two groups are given in Table I. They show that there is no significant decrease in the oxygen consumption of either group for a period of 42 days after the operation, nor is there any significant difference between the oxygen consumption of the two groups when these are compared every 3rd day.

Table I. The Effect of Thyroidectomy on Oxygen Consumption of *Scyllium canicula*

Six fish in each group (* Five fish only in group). In the first column a minus (−) sign indicates days before operation, a plus (+) sign days after operation.

	Thyroidectomy		Sham operation		
Days	Mean O_2 consumption (ml./g/h)	S.E.	Mean O_2 consumption (ml./g/h)	S.E.	't'
− 5	47·8*	3·02	48·0*	1·00	0·056
− 2	49·3	3·96	47·5	2·97	0·644
+ 1	48·3	3·33	42·5	1·52	1·446
+ 4	48·3	3·52	42·8	3·85	0·962
+ 7	45·8	2·70	43·7	1·92	0·578
+10	47·0	3·07	44·2	3·00	0·596
+13	44·2	3·44	47·2	3·76	0·537
+16	51·0	3·98	55·2	2·98	0·784
+19	51·6	3·54	52·0	3·50	0·073
+22	49·8	4·57	50·0	3·16	0·033
+26	49·3	3·86	45·8	2·38	0·704
+31	46·5	3·55	—	—	—
+36	47·7	2·84	—	—	—
+39	47·2	2·86	44·3	1·87	0·775
+42	44·2	3·73	—	—	—

The Effect of Dogfish Thyroid upon the Oxygen Consumption of Adult Rats

The results given in Fig. 1 are expressed as average percentage change in oxygen consumption. The values of oxygen consumption for the various test

series before injection gave a total mean of 1·37 ml./g/h (S.E. ± 0·039) which is seen to be in close agreement with the mean oxygen consumption of 1·33 ml./g/h (S.E. ± 0·039) determined on a completely separate series of twenty-four animals. The mammalian thyroid preparation caused a maximal increase in oxygen consumption of 26·1%, 5 days after the injection; whilst the dogfish thyroid preparation gave a maximal response of 24·8%, 4 days

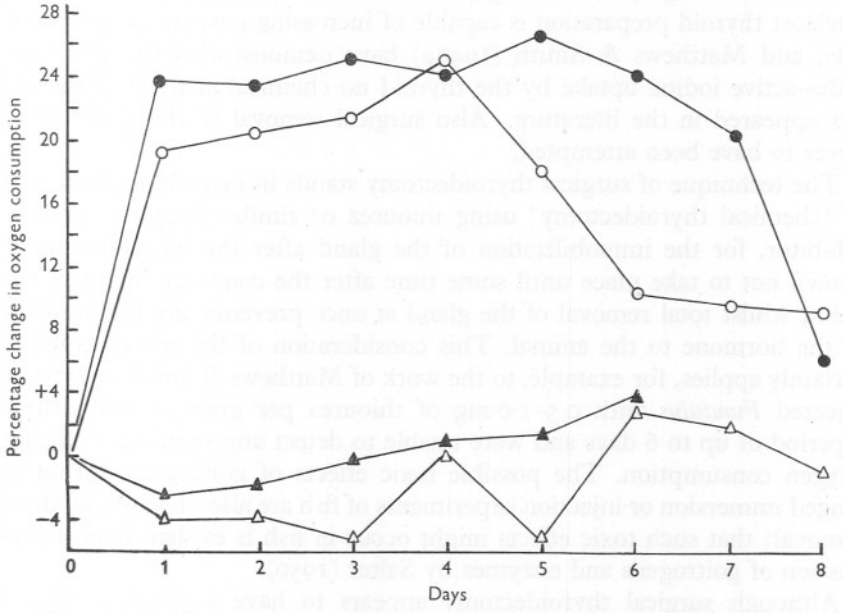

Fig. 1. Percentage change in oxygen consumption of rats injected intraperitoneally with thyroid preparations. △, normal controls; ▲, injected with 2·0 ml. 0·9% NaCl; ●, injected with 100 mg mammalian thyroid powder; ○, injected with 95 mg dogfish thyroid.

after the injection. Since the degree of response is about the same for approximately the same level of dosage it is probable that both preparations are of similar strength.

The saline-injected rats showed no increase in oxygen consumption either before or after injection with dried trout muscle.

Discussion

Gorbman, Lissitzky, Michel & Roche (1952), from radio-active iodine chromatographic studies on *Scyllium* thyroids, have shown that di-iodotyrosine and thyroxine are formed in the thyroid gland of these animals whilst the present experiments have demonstrated that dried dogfish thyroid gland when injected intraperitoneally into rats is capable of increasing their oxygen consumption. These investigations indicate clearly that there is present in the

dogfish thyroid a thyroxine-containing hormone similar to that found in mammals. Furthermore, as thyroidectomy of the dogfish has been shown above to cause no change in the oxygen consumption of the whole animal for a period of up to 42 days it may be concluded that although the thyroid hormone is present it has no influence over the immediate oxygen consumption of the dogfish. In the teleosts the evidence for a thyroxine-containing thyroid hormone is meagre, for although Smith & Brown (1952) have shown that a teleost thyroid preparation is capable of increasing oxygen consumption in rats, and Matthews & Smith (1947*a*) have demonstrated the presence of radio-active iodine uptake by the thyroid no chemical analysis of the gland has appeared in the literature. Also surgical removal of the gland appears never to have been attempted.

The technique of surgical thyroidectomy stands in marked contrast to that of 'chemical thyroidectomy' using thiourea or similar drugs as a thyroid-inhibitor, for the immobilization of the gland after the latter treatment is known not to take place until some time after the commencement of treatment, whilst total removal of the gland at once prevents any further supply of the hormone to the animal. This consideration of the use of goitrogens certainly applies, for example, to the work of Matthews & Smith (1947*b*) who injected *Fundulus* with 0·5–1·0 mg of thiourea per gram of fish daily for a period of up to 6 days and were unable to detect any significant change in oxygen consumption. The possible toxic effects of goitrogens during prolonged immersion or injection experiments of fish are also obviated by thyroid removal; that such toxic effects might occur in fish is evident from the discussion of goitrogens and enzymes by Salter (1950).

Although surgical thyroidectomy appears to have advantages over the chemical thyroidectomy approach for the investigation of thyroid function both suffer from the fact that the investigations so far reported, including the present work, may not have had the experiments prolonged for sufficient period for delayed effects on oxygen consumption to become evident. Thyroidectomy or goitrogen treatment of mammals results in a significant decline in oxygen consumption within some 5 days, but does not generally produce a maximal depression of basal metabolic rate until 2 or 3 weeks after the commencement of treatment (Siebert & Smith, 1930; Barrett & Gassner, 1951), and as the rate of oxidative metabolism in poikilotherms is known to be lower than that of homoiotherms the rate of response in the former might be expected to be substantially slower. Moreover, the fact that the temperature of poikilotherms is generally lower than that of homoiotherms might also effect the speed of response, for it has been demonstrated in fish that the thyroid gland shows cellular hypertrophy during treatment with thiourea more rapidly at high than at low temperatures (Fortune, 1953). Another possibility to be taken into account in evaluating the present results is the fact that the animal may be able to synthesize extra-thyroidally a similar

hormone to that produced by the thyroid. Preliminary experiments have shown the presence of protein-bound radio-active iodine in the plasma of dogfish which have been thyroidectomized for at least 6 weeks (Matty, unpublished). It might be supposed that if the extra-thyroidal hormone maintained the normal oxygen consumption level in thyroidectomized dogfish then treatment of such animals with anti-thyroid drugs for some period might prevent any production of the hormone and so cause a fall in the oxygen consumption, assuming, of course, that these drugs also inhibit the extra-thyroidal synthesis. Until the results of such further work are reported the possibility of extra-thyroidal hormone supply is not eliminated but, as in the higher vertebrates where the conditions of complete thyroxine deficiency after thyroidectomy is difficult to obtain (Leblond & Eartly, 1952), such investigations initially depend on surgical thyroidectomy.

Having established the presence of a thyroxine-containing hormone in dogfish which does not have any immediate effect on the oxygen consumption of the whole animal the search for identifying the functions of the thyroid must be directed elsewhere. According to Waring, Landgrebe & Bruce (1942), thyroids removed from the dogfish hypophysectomized a month previously afford no evidence for pituitary control of thyroid activity. There is some indication of a correlation between reproduction and thyroid activity in teleosts (Barrington & Matty, 1952; Robertson & Chaney, 1953; Scott, 1953), and there is evidence also that the activity of the gland is increased in elasmobranchs during the breeding season (Guariglia, 1937; Zenza, 1937). Some observations during the present work have been in conformity with these latter reports, but unfortunately there is as yet no direct experimental evidence for a relationship between the thyroid gland and the reproductive activity of elasmobranchs.

Another possible function of the thyroid is that it is concerned with the early stages of growth and maturation of fish. Experiments on teleosts have shown that on immersion in goitrogens growth is retarded (Hopper, 1952; Smith *et al.*, 1953), but as Smith *et al.* have pointed out the effect may be a non-physiological toxic one, and, as already mentioned, efforts to accelerate growth of teleosts by thyroxine, thyroid or thyrotropin treatment have given most equivocal results. The situation in elasmobranchs is even more obscure, for no studies on the possible relationship between the thyroid gland and growth in these animals seem to have been reported. It is, however, hoped that the use of surgical thyroidectomy, as described in the present work, will provide a useful basis for investigating some of these outstanding problems of thyroid relationships in the elasmobranchs.

My thanks are due to Prof. E. J. W. Barrington for his advice and encouragement, and to Mr A. Aitken, for the benefit of continued discussion throughout this work. The elasmobranch experiments were carried out at the Marine

Biological Laboratory, Plymouth, with financial support from the University of Nottingham, and I am indebted to the Director and his staff for their unfailing co-operation.

SUMMARY

Surgical thyroidectomy has been successfully performed on the dogfish, *Scyllium canicula*, and has been shown to have no signficant effect on the animal's oxygen consumption over a period of 6 weeks.

The injection of extracts of dogfish thyroid into adult male white rats results in an increase in the animal's oxygen consumption of the same order as that produced by mammalian thyroid preparations.

The significance of these results is discussed in the light of recent investigations of thyroid function in fish.

REFERENCES

BARRETT, H. W. & GASSNER, F. X., 1951. Effect of 5-halogeno-2-thiouracil and of 6-methyl-5-iodo-2-thiouracil on oxygen consumption in rats. *Endocrinology*, Vol. 48, pp. 197–204.

BARRINGTON, E. J. W. & MATTY, A. J., 1952. Influence of thiourea on reproduction in the minnow. *Nature, Lond.*, Vol. 170, pp. 105–6.

ETKIN, W., ROOT, R. W. & MOFSHIN, B. P., 1940. The effect of thyroid feeding and oxygen consumption of the goldfish. *Physiol. Zoöl.*, Vol. 13, pp. 415–29.

FORTUNE, P. Y., 1953. Thyroid activity in teleosts. *Nature, Lond.*, Vol. 171, p. 483.

GIGUÈRE, P. A. & LAUZIER, L., 1945. Le dosage polarographique de l'oxygene dissous dans l'eau de mer. *Canad. J. Res.*, Vol. 23, Sect. B, pp. 76–83.

GOLDSMITH, E. D., 1949. Phylogeny of the thyroid. *Ann. N.Y. Acad. Sci.*, Vol. 50, Art. 5, pp. 283–316.

GOLDSMITH, E. D., NIGRELLI, R. F., GORDON, A. S., CHARIPPER, H. A. & GORDON, M., 1944. Effect of thiourea upon fish development. *Endocrinology*, Vol. 35, pp. 132–4.

GORBMAN, A., LISSITZKY, S., MICHEL, R. & ROCHE, J., 1952. Thyroid metabolism of iodine in the shark *Scyliorhinus* (*Scyllium*) *canicula*. *Endocrinology*, Vol. 51, pp. 311–21.

GRAD, B., 1952. A simple method for the measurement of the oxygen consumption and heart rate of rats. *Endocrinology*, Vol. 50, pp. 94–9.

GUARIGLIA, G., 1937. Tiroide e ciclo sessuale nei selaci ovipari. *Boll. Soc. ital. Biol. sper.*, Vol. 15, pp. 690–2.

HALL, F. G., 1929. The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Amer. J. Physiol.*, Vol. 88, pp. 212–8.

HASLER, A. D. & MEYER, R. K., 1942. Respiratory response of normal and castrated goldfish to teleost and mammalian hormones. *J. exp. Zool.*, Vol. 91, pp. 391–403.

HOAR, W. S., 1951. Hormones in fish. *Univ. Toronto Stud. biol.*, Vol. 59, pp. 1–34. (Publication of the Ontario Fisheries Research Laboratories, No. 71).

HOPPER, A. F., 1952. Growth and maturation response of *Lebistes reticulatus* to treatment with mammalian thyroid powder and thiouracil. *J. exp. Zool.*, Vol. 119, p. 205–17.

LEBLOND, C. P. & EARTLY, H., 1952. An attempt to produce complete thyroxine deficiency in the rat. *Endocrinology*, Vol. 51, pp. 26–41.

MATTHEWS, S. A. & SMITH, D. C., 1947*a*. Concentration of I^{131} by the thyroid gland of the Bermuda parrot fish. *Anat. Rec.*, Vol. 99, p. 592. (Abstr. 77.)

—— —— 1947*b*. The effect of thiourea on the oxygen consumption of *Fundulus*. *Physiol. Zoöl.*, Vol. 20, pp. 161–4.

MILTON, R. F. & WATERS, W. A., 1949. *Methods of Quantitative Micro-analysis*. 599 pp. London: Arnold.

NIGRELLI, R. F., GOLDSMITH, E. D. & CHARIPPER, H. A., 1946. Effects of mammalian thyroid powder on growth and maturation of thiourea treated fish. *Anat. Rec.*, Vol. 94, p. 523. (Abstr. 173.)

ROBERTSON, O. H. & CHANEY, A. L., 1953. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: A comparative study of Lake Michigan and California sea-run trout. *Physiol. Zoöl.*, Vol. 26, pp. 328–40.

ROOT, R. W. & ETKIN, W., 1937. Effect of thyroxine on oxygen consumption of the toadfish. *Proc. Soc. exp. Biol., N.Y.*, Vol. 37, pp. 174–8.

SALTER, W. T., 1950. The control of thyroid activity. In *The Hormones*, Vol. 2, pp. 301–345. Ed. Pincus and Thiman, Academic Press, New York.

SCOTT, J. L., 1953. The effects of thiourea treatment upon the thyroid, pituitary and gonads of the zebra fish, *Brachydanio rerio*. *Zoologica, N.Y.*, Vol. 38, pp. 53–61.

SIEBERT, W. J. & SMITH, R. S., 1930. The effect of various anterior pituitary preparations upon basal metabolism in partially thyroidectomised and completely thyroidectomised guinea pigs. *Amer. J. Physiol.*, Vol. 95, pp. 396–402.

SMITH, D. C. & BROWN, F. C., 1952. The effect of parrot fish thyroid on the respiratory metabolism of the white rat. *Biol. Bull., Woods Hole.*, Vol. 102, pp. 278–85.

SMITH, D. C. & EVERETT, G. M., 1943. The effects of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish *Lebistes reticulatus*. *J. exp. Zool.*, Vol. 94, pp. 229–40.

SMITH, D. C. & MATTHEWS, S. A., 1947. The effect of extracts of the thyroid gland of the Bermuda parrot fish on the oxygen consumption of the Bermuda white grunts, (*Haemulou* sp.). *Anat. Rec.*, Vol. 99, p. 593. (Abstr. 78.)

SMITH, D. C., SLADEK, S. A. & KELLNER, A. W., 1953. The effect of mammalian thyroid extract on the growth rate and sexual differentiations in the fish *Lebistes reticulatus* treated with thiourea. *Physiol. Zoöl.*, Vol. 26, pp. 117–24.

VIVIEN, J., 1941. Contribution a l'étude de la physiologie hypophysaire dans ses relations avec l'appareil genital, la thyröide et les corps suprarénaux chez les poissons sélaciens et téléosteens. *Scylliorhinus canicula* et *Gobius paganellus*. *Bull. biol.*, T. 75, pp. 275–309.

WARING, H., LANDGREBE, F. W. & BRUCE, J. R., 1942. Chromatic behaviour of *Scyllium canicula*. *J. exp. Biol.*, Vol. 18, pp. 306–16.

ZENZA, P., 1937. Tiroide; maturita sessuale e gestazione in *Torpedo ocellata*. *Boll. Soc. ital. Biol. sper.*, Vol. 15, pp. 75–7.

J. Mar. biol. Ass. U.K. (1954) 33, 699–708
Printed in Great Britain

ON THE DIVERSE COLOURS OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1 and 2)

The variable colour of the polychaete *Nereis diversicolor* has been known since O. F. Müller described the species as 'Die bunte Nereide' in 1771. Most of the worms belonging to this species appear to be orange or brown in colour, though close examination reveals that some green pigment is invariably present. However, others may be found which are predominantly green in appearance, and a few which are completely green and appear to lack brown or orange pigments in the epidermis.

Several previous authors have given attention to this. Mendthal (1889) considered the green colour of some worms to be due to a diet of green algae. McIntosh (1910) rejected this idea, and suggested that the green colour was due to 'pale greenish ova'. However, it may be pointed out that males are also green, and in our experience the oocytes of this species are colourless or have only a pale straw colour. Various other writers have commented on the variability of the colour of this animal without contributing to the elucidation of the problem, Thomas (1930) alone has attempted an analysis of the different pigments. He concluded that the green pigment was probably a porphyrin, possibly a modified chlorophyll. Herpin (1923, 1925) and Dehorne (1925), as well as Thomas (1930) and Dales (1950), have drawn attention to the rather different appearance of the two sexes when mature. Hempelmann's paper (1939) on the chromatophores of *Platynereis dumerilii* has little bearing on the present problem.

Most of the chemical work described here has been done on worms from Plymouth in 1953 and 1954, but observations on variation of colour have been made mainly at Chalkwell, Essex, over the last five or six years, and some chemical analyses have also been made on worms from this locality.

Identification of the pigments

In one extract, twenty-five whole worms were placed, without grinding, in absolute methanol 19 parts and concentrated sulphuric acid 1 part, overnight in the ice chest. The following day the deep-green extract was filtered through glass wool into a separating funnel, and diluted with an equal volume of water.

The mixture was then extracted with one-third of the volume of chloroform. The chloroform hypophase was dark brown, and the aqueous epiphase was bright green. The hypophase was transferred to another funnel and washed with 2 % NaCl and then with water, forming 'chloroform extract A'. The aqueous epiphase was again extracted with chloroform, and this time the hypophase was bluish green and the epiphase pale green. The hypophase was removed and washed as before, filtered through chloroform-soaked paper, and evaporated to dryness *in vacuo*. A greenish residue remained which was used for subsequent tests; this was 'chloroform extract B'.

Chloroform Extract A

Extract A was evaporated to dryness *in vacuo* and redissolved in dry chloroform. The solution was chromatographed on ungraded Brockmann's alumina packed in chloroform, and then exhibited the following bands:

Band 1. A dark green band at the top of the column; non-fluorescent.

Band 2. A narrow yellow band; also non-fluorescent.

Band 3. A reddish brown band which travelled rapidly down the column and passed out. This was strongly red-fluorescent and gave the following spectrum (Hartridge Reversion spectroscope):

	I	II	III	IV
	660–680	600–620	590–565	520– mμ
Centres	670	610	579·5	(520)

Bands 1 and 2 could not be eluted by any of the usual solvents or combinations of solvents, and were finally eluted by ether-acetic acid 5:1 mixture, giving a green solution with no fluorescence and very indeterminate spectrum. The pigment had the extraordinarily high HCl-number of 36.

Band 3 was further chromatographed on MgO grade III (Nicholas, 1951), packed in chloroform, and displayed the following bands:

Band A2. A dark green band at the top; non-fluorescent.

Band B2. A brown band, also non-fluorescent, separated from the upper band by a gap.

Band C2. An apparently colourless wide band, red-fluorescent, passing down and out of the column very readily.

Band A2 (Fig. 1)

This band when cut off and eluted with methanol displayed the clear spectrum of biliverdin:

I	II	
639	390	mμ

and when acidified with HCl there was a shift of the bands to:

I	II	
678	375	mμ

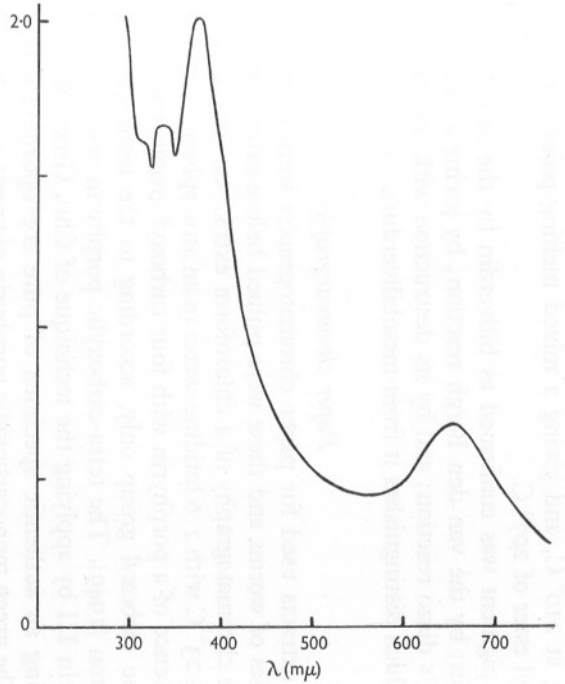

Fig. 1. Absorption curve of an acetic acid extract of the body-wall of entirely green worms. Vertical scale in arbitrary (Unicam spectrophotometer) units.

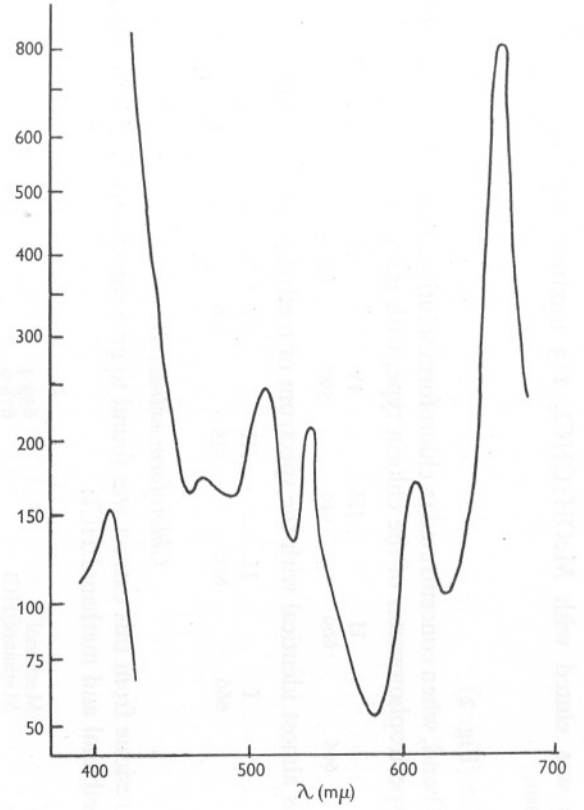

Fig. 2. Absorption curve of a chloroform extract of whole worms. Vertical scale in $E_{1cm.} \times 10^3$.

Band B2

This was eluted with MeOH:$CHCl_3$ 1:3 mixture, but gave no clear spectrum.

Band C2 (Fig. 2)

This band, when concentrated in chloroform solution, gave a clear spectrum in the spectrophotometer of the chlorin type, with maxima at

I	II	III	IV	V	Sorêt
666	609	540	507	(468)	414 mμ

which is almost identical with the spectrum of methyl phaeophorbide-*a*:

I	II	III	IV	Sorêt
666	610	535	506	412 mμ

Chloroform extract B

The residue from this extract was found to give the spectrum of biliverdin with methanol and methanol/HCl:

	I	II
Methanol	639·4	391·2 mμ
Methanol/HCl	678·6	375·4 mμ

The acid-number was 1.

The solution of the pigment in methanol/HCl was allowed to stand for 36 h and then extracted with chloroform in the usual way, followed by crystallization from chloroform-methanol. Fine blue-green needles were obtained, melting at 210° C, and giving a mixed melting-point with pure biliverdin dimethyl ester of 207° C.

The pigment was confirmed as biliverdin by the formation of zinc bilipurpurin; by the van den Bergh reaction; by giving a negative result with Ehrlich's diazo reaction; and by its destruction with concentrated sulphuric acid (which distinguishes it from mesobiliverdin).

Paper chromatography

The extracts used for paper chromatography were obtained from larger quantities of worms and these were pulped before extraction.

Paper chromatography of a chloroform extract on long papers (Kennedy, 1953) at 23° C with 2:6 lutidine-water in an atmosphere of ammonia revealed the presence of a porphyrin with four carboxyl groups and a green pigment with one carboxyl group only, according to the technique of Nicholas & Rimington (1949). The tetra-carboxylic porphyrin was shown to be coproporphyrin III by applying the technique of Chu, Green & Chu (1951), and employing an authentic specimen of pure coproporphyrin III tetramethyl ester. The green monocarboxylic porphyrin pigment gave the same spectrum as that obtained from the band C2 already mentioned. Since the extract

from which C2 was obtained was made by using methanol and concentrated sulphuric acid, the pigment of band C2 would be an ester. From its solubility in boiling absolute ethanol, and its acid-number of 16, the pigment appeared to be methyl phaeophorbide-*a* only. Hydrolysis of the ester and formation of derivatives—phylloerythrin, phaeoporphyrin-a_5 and phytorhodin-*g*—confirmed that this was so. Extraction of the ether solution of the pigment with 0·2% sodium bicarbonate was without result, proving the absence of phaeophorbide-*b* (Willstätter & Stoll, 1913). That the pigment is present in the worm as the free phaeophorbide-*a* is shown by the formation of the spot of R_F 0·94 on the long-paper chromatogram of worm extracts made in chloroform only without any esterification.

As compared with the quantity of biliverdin, phaeophorbide-*a* is present in small amounts and may indeed be restricted to the gut. Worms thus owe their green appearance to biliverdin, while the yellow, brown and orange pigments also present in the epidermis in most worms are carotenoids.

Distribution of the Pigments in the Body

In most worms biliverdin occurs in the form of minute granules 1–2 μ in diameter scattered through the epithelial cells immediately under the cuticle. The pigment is deposited mainly along the borders of the blood capillaries, and is therefore most dense on the dorsal side of the body and in the parapodia; but it also occurs at the bases of the parapodia and between the segments. The appearance of the worms suggests that there is less biliverdin in the most anterior part of the body on the dorsal side, and while this seems to be so, the darker appearance is due mainly to the much larger amounts of carotenoids in this region. This darker appearance of the anterior region of the body may have some significance in relation to survival of these worms which protrude from the burrows while browsing on the surface mud. Biliverdin does not usually occur in any quantity on the ventral side of the body except in the parapodia, but depositions are found in the pygidium, and under the cuticle lining the proboscis. However, the intestine and the coelomic cells are also usually faintly green and contain small quantities of similar granules of biliverdin, and in females which have wholly or partially spawned, the gut and the coelomic cells may present a vivid green appearance. All worms possess at least some biliverdin though its presence may be masked by yellow, orange or brown carotenoid pigments.

Some individuals may appear entirely green, biliverdin alone being represented in the epidermis. These worms are always either ripe males with free sperm in the coelom, ripe females, or females which have spawned. This does not mean that females with an orange appearance are not sexually mature or do not spawn. Such green individuals, whether male or female, owe their striking appearance not only to a disappearance of the orange and brown carotenoids from the skin, but also to an increase in the amount of biliverdin in the body.

On the other hand, slight variations in the 'greenness' of worms which are not breeding seem to be due to variations in the quantity of carotenoids, worms appearing greener when they have relatively small amounts, and more orange when they have relatively greater quantities of carotenoids. On the other hand, apart from the breeding worms, there seems to be little variation in the amount of biliverdin deposited in the epidermis throughout most of the year.

Seasonal Changes in Colour

In the Chalkwell population during the early spring of 1949, nearly all the worms assumed a predominantly green appearance by the end of February when the main spawning occurred (Dales, 1950). It was naturally concluded that this colour change was in some way related with the maturation of the gametes. This is now known to be only partly true, since subsequent experience has shown that the same population in other years, and populations which have been seen elsewhere at the time of spawning, may have only a relatively small number of green individuals. Other observers have also reported the rather variable occurrence of green specimens, which as a general rule constitute only a small proportion of any population. During the summer such green individuals are definitely rare. In the early spring of 1951, 1952, and 1954 there were few green worms at Chalkwell, but in the winter and spring of 1952–53 there was again a much larger proportion of worms with a green appearance, but by no means as many as in 1948–49.

Discussion

Breeding takes place in mid-February in the *Nereis diversicolor* population at Chalkwell, but only a very small proportion of the females spawn successfully owing to the relative scarcity of the males and the fact that spawning takes place only in the presence of the male. All males with mature sperm appear a bright green owing to the complete extraction of the orange and brown pigments and also to an increase in the relative amount of biliverdin, as already mentioned. This was shown to be so by the much greater amount of pigment which could be extracted per unit weight of such worms as compared with non-breeding animals. This increase in the total quantity of biliverdin is almost certainly associated with a reduction in the quantity of haemoglobin (in the sense of Keilin & Hartree, 1951). As the males reach maturity their tissues undergo phagocytosis, the body-wall muscles are eroded away, making the worm extremely fragile. As this process continues the amount of haemoglobin decreases; there is often barely enough blood to fill the dorsal vessel in the fully mature male. This suggests that the biliverdin is indeed derived from the worm's own haemoglobin.

On the other hand, females when apparently mature at the time of spawning

are more variable in appearance; some are predominantly green, but all at this time have quantities of orange or brown pigments as well as the biliverdin in the body-wall. Later, however, those females which have spawned lose their orange and brown pigments and become as green as mature males. Males soon die when they have emitted their sperm. Females that have spawned often live for several weeks after spawning. Females rarely spawn so completely that one or two oocytes are not left in the coelom enabling their sex to be determined with certainty, and all such females undergo a phagocytosis of the tissues during or after spawning, accompanied by a loss of carotenoids and a greatly increased destruction of the blood haemoglobin with a corresponding increase in the quantity of biliverdin in the body. In the female the process of phagocytosis seems to have got out of step with the spawning, and this rather anomalous situation may be related in some way to the loss of the epitokous phase in this species. In unspawned females the oocytes are eventually broken down by the coelomic cells, being converted into a milky mass which might be mistaken for sperm. Soon afterwards quantities of orange pigments appear in the body-wall. These worms live throughout the following summer and may double their length, but it seems unlikely that they have a second opportunity to spawn (see Dales, 1951). There is no doubt that the green female individuals are those which have spawned, even if an appreciable number of oocytes remains in the coelom. Spawning takes place in the burrows and the worms are remarkably sedentary, at least at this time of year. For the first 8 weeks at least the larvae develop in the parent burrow and are just visible to the naked eye when about 6 weeks old. Larvae are invariably found in burrows occupied by a spawned or partially spawned green female, or in empty burrows, but never in burrows occupied by orange worms in which the oocytes are being absorbed. The explanation of these colour differences may be simply a consequence of sexually mature worms ceasing to feed. Worms that spawn subsequently draw on their carotenoids and even on their muscular tissues and become green and fragile. Worms that do not spawn, on the other hand, have a large reserve supply of food in the form of their own oocytes, and when these are absorbed the worms become even more orange in appearance than before. If the carotenoids form part of food reserves, the occurrence of greater numbers of green individuals in winters with early cold periods (1948–49, 1952–53) could be due to the earlier depletion of these stores. On the other hand, the carotenoids may be utilized directly in the production of the gametes, but if this is so it is surprising that though the carotenoids disappear in the male at maturity, they may not disappear in the female until after spawning. Incidentally, the greater number of green individuals in the early spring of 1949 was certainly not due to a larger proportion of males or spawning females in that year. Counts to determine the sex ratio in early February 1949 showed that less than 10% of the total population were males, and this proportion has

not varied materially in subsequent years. The proportion of spawned females has also remained at approximately the same value.

Although it is difficult to observe or measure changes in the quantity of epidermal biliverdin in adults which already have variable amounts of green, orange and brown pigments, it was thought that some indication of whether the biliverdin was derived from ingested blood or not could be solved by rearing worms from the larval stage on different substances. Young worms were collected in large numbers from the burrows in early May when the average age was estimated at about 8 weeks. At this time there are about ten to twelve chaetigerous segments and they are completely colourless. These young worms were reared on (1) dried and powdered nettle leaves alone, (2) dried slaughterhouse blood alone, and (3) a dried concentrated aquarium fish-food ('Brosiam') containing negligible amounts of chlorophyll or haemoglobin. The young *Nereis* seemed to be sufficiently omnivorous to thrive well on each of these substances for at least long enough for the present purpose. The water in each culture dish was replaced daily with filtered sea water to ensure that the worms were in fact feeding on the diet provided. The haemoglobin appeared in the blood when the young worms were about 9 weeks old, and the other pigments appeared soon after. The orange and brown pigments first made their appearance as granules in stellate chromatophores concentrated in the anterior region and arranged as a double row down the body on the dorsal side. The biliverdin appeared at about the same time, and from the first was more uniformly scattered through the epidermal cells on the dorsal side. All the young worms were similarly pigmented after several weeks' growth on these different foods; those fed entirely on nettle leaves, and those on dried blood, had much the same quantity of epidermal biliverdin as those fed on the dried fish-food.

Thus there is little doubt that the biliverdin is formed by the breakdown of the haemoglobin of the blood. This takes place along the margins of the vessels, mainly between the capillaries on the dorsal side of the body around the proboscis and in the pygidium. Bloch-Raphaël (1939) concluded, in her review of the seat of haemoglobin synthesis and breakdown in polychaetes, that in nereids these processes probably take place in the body-wall and around the proboscis, and that the bile pigments are excreted into the gut. This view agrees with the present observations on *N. diversicolor*. The granules of biliverdin may gradually be removed from the epidermis by the coelomic cells and conveyed to the gut, the cells travelling down the septa and oblique muscles. Coelomic cells loaded with biliverdin, often in granules 4–5 μ across, can be seen in these positions in spawned females. When the rate of haemoglobin breakdown is increased as in ripe males and spawned females, the rate of elimination is not increased in proportion, so that the pigment accumulates in the body.

SUMMARY

The variable colour of *Nereis diversicolor* is due to variations in the proportion of green, orange and brown pigments. The orange and brown pigments are mainly carotenoids; the green colour is due to biliverdin.

Phaeophorbide-*a* and coproporphyrin III also occur, but both these pigments may be restricted to the gut wall; biliverdin occurs both in the wall of the gut, and in the epidermis and coelomic cells.

The biliverdin is formed by the breakdown of the haemoglobin of the blood.

Haemoglobin-breakdown takes place in the epidermis on the dorsal side of the body, in the epithelial tissue surrounding the proboscis and in the pygidium. Granules of biliverdin are probably removed by the coelomic cells and conveyed to the gut into which they are excreted.

In ripe males, and in females during and after spawning, phagocytosis of the tissues is accompanied by an increased haemoglobin-breakdown with a corresponding accumulation of biliverdin in the body. The green appearance is due not only to an increased amount of biliverdin, but also to a complete extraction of carotenoids from the body-wall.

REFERENCES

BLOCH-RAPHAËL, C., 1939. Localisation, formation et destruction de l'hémoglobine chez les Annélides Polychètes. *Ann. Inst. océanogr. Monaco*, T. 19, Fasc. 1, pp. 1–78.

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

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.

—— 1951. An annual history of a population of *Nereis diversicolor* O. F. Müller. *Biol. Bull., Woods Hole*, Vol. 101, pp. 131–7.

DEHORNE, A., 1925. Observations sur la biologie de *Nereis diversicolor*. *C.R. Acad. Sci., Paris*, T. 180, pp. 1441–3.

HEMPELMANN, F., 1939. Chromatophoren bei *Nereis*. *Z. wiss. Zool.*, Bd. 152, pp. 353–83.

HERPIN, R., 1923. Comparaison entre le comportement sexuel de quelques Néréidiens des côtes de la Manche. *C.R. Acad. Sci., Paris*, T. 176, pp. 536–9.

—— 1925. Recherches biologiques sur la reproduction et le developpement de quelques Annélides polychètes. *Bull. Soc. Sci. nat. Ouest.*, Ser. 4, T. 5, pp. 1–250.

KEILIN, D. & HARTREE, E. F., 1951. Relationship between haemoglobin and erythrocruorin. *Nature, Lond.*, Vol. 168, pp. 266–9.

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

MCINTOSH, W. C., 1910. *A Monograph of the British Marine Annelids*, Vol. 2, pt. 2, pp. 233–524. London: Ray Soc.

MENDTHAL, M., 1889. Untersuchungen über die Mollusken und Anneliden des Frischen Haffs. IV. Über die Geschlechtsverhältnisse der *Nereis diversicolor*. *Schr. phys.-ökon. Ges. Königsb.*, Bd. 30, pp. 27–42.

MÜLLER, O. F., 1771. *Von Würmen des süssen und salzigen Wassers*, 200 pp., 16 pls. Copenhagen.

NICHOLAS, R. E. H., 1951. Chromatographic methods for the separation and identification of the porphyrins. *Biochem. J.*, Vol. 48, pp. 309–13.

NICHOLAS, R. E. H. & RIMINGTON, C., 1949. Qualitative analysis of the porphyrins by partition chromatography. *Scand. J. clin. Lab. Invest.*, Vol. 1, pp. 12–18.

THOMAS, J. A., 1930. Sur les pigments vert et brun de *Nereis diversicolor* M. *Bull. Soc. zool. Fr.*, T. 55, pp. 97–103.

WILLSTÄTTER, R., & STOLL, A., 1913. *Untersuchungen über Chorophyll.* Berlin: Springer.

MATTHEWS, S. A. & SMITH, D. C., 1947*a*. Concentration of I^{131} by the thyroid gland of the Bermuda parrot fish. *Anat. Rec.*, Vol. 99, p. 592. (Abstr. 77.)

—— —— 1947*b*. The effect of thiourea on the oxygen consumption of *Fundulus*. *Physiol. Zoöl.*, Vol. 20, pp. 161–4.

MILTON, R. F. & WATERS, W. A., 1949. *Methods of Quantitative Micro-analysis*. 599 pp. London: Arnold.

NIGRELLI, R. F., GOLDSMITH, E. D. & CHARIPPER, H. A., 1946. Effects of mammalian thyroid powder on growth and maturation of thiourea treated fish. *Anat. Rec.*, Vol. 94, p. 523. (Abstr. 173.)

ROBERTSON, O. H. & CHANEY, A. L., 1953. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: A comparative study of Lake Michigan and California sea-run trout. *Physiol. Zoöl.*, Vol. 26, pp. 328–40.

ROOT, R. W. & ETKIN, W., 1937. Effect of thyroxine on oxygen consumption of the toadfish. *Proc. Soc. exp. Biol., N.Y.*, Vol. 37, pp. 174–8.

SALTER, W. T., 1950. The control of thyroid activity. In *The Hormones*, Vol. 2, pp. 301–345. Ed. Pincus and Thiman, Academic Press, New York.

SCOTT, J. L., 1953. The effects of thiourea treatment upon the thyroid, pituitary and gonads of the zebra fish, *Brachydanio rerio*. *Zoologica, N.Y.*, Vol. 38, pp. 53–61.

SIEBERT, W. J. & SMITH, R. S., 1930. The effect of various anterior pituitary preparations upon basal metabolism in partially thyroidectomised and completely thyroidectomised guinea pigs. *Amer. J. Physiol.*, Vol. 95, pp. 396–402.

SMITH, D. C. & BROWN, F. C., 1952. The effect of parrot fish thyroid on the respiratory metabolism of the white rat. *Biol. Bull., Woods Hole.*, Vol. 102, pp. 278–85.

SMITH, D. C. & EVERETT, G. M., 1943. The effects of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish *Lebistes reticulatus*. *J. exp. Zool.*, Vol. 94, pp. 229–40.

SMITH, D. C. & MATTHEWS, S. A., 1947. The effect of extracts of the thyroid gland of the Bermuda parrot fish on the oxygen consumption of the Bermuda white grunts, (*Haemulou* sp.). *Anat. Rec.*, Vol. 99, p. 593. (Abstr. 78.)

SMITH, D. C., SLADEK, S. A. & KELLNER, A. W., 1953. The effect of mammalian thyroid extract on the growth rate and sexual differentiations in the fish *Lebistes reticulatus* treated with thiourea. *Physiol. Zoöl.*, Vol. 26, pp. 117–24.

VIVIEN, J., 1941. Contribution a l'étude de la physiologie hypophysaire dans ses relations avec l'appareil genital, la thyröide et les corps suprarénaux chez les poissons sélaciens et téléosteens. *Scylliorhinus canicula* et *Gobius paganellus*. *Bull. biol.*, T. 75, pp. 275–309.

WARING, H., LANDGREBE, F. W. & BRUCE, J. R., 1942. Chromatic behaviour of *Scyllium canicula*. *J. exp. Biol.*, Vol. 18, pp. 306–16.

ZENZA, P., 1937. Tiroide; maturita sessuale e gestazione in *Torpedo ocellata*. *Boll. Soc. ital. Biol. sper.*, Vol. 15, pp. 75–7.

J. Mar. biol. Ass. U.K. (1954) 33, 699–708
Printed in Great Britain

ON THE DIVERSE COLOURS OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1 and 2)

The variable colour of the polychaete *Nereis diversicolor* has been known since O. F. Müller described the species as 'Die bunte Nereide' in 1771. Most of the worms belonging to this species appear to be orange or brown in colour, though close examination reveals that some green pigment is invariably present. However, others may be found which are predominantly green in appearance, and a few which are completely green and appear to lack brown or orange pigments in the epidermis.

Several previous authors have given attention to this. Mendthal (1889) considered the green colour of some worms to be due to a diet of green algae. McIntosh (1910) rejected this idea, and suggested that the green colour was due to 'pale greenish ova'. However, it may be pointed out that males are also green, and in our experience the oocytes of this species are colourless or have only a pale straw colour. Various other writers have commented on the variability of the colour of this animal without contributing to the elucidation of the problem, Thomas (1930) alone has attempted an analysis of the different pigments. He concluded that the green pigment was probably a porphyrin, possibly a modified chlorophyll. Herpin (1923, 1925) and Dehorne (1925), as well as Thomas (1930) and Dales (1950), have drawn attention to the rather different appearance of the two sexes when mature. Hempelmann's paper (1939) on the chromatophores of *Platynereis dumerilii* has little bearing on the present problem.

Most of the chemical work described here has been done on worms from Plymouth in 1953 and 1954, but observations on variation of colour have been made mainly at Chalkwell, Essex, over the last five or six years, and some chemical analyses have also been made on worms from this locality.

Identification of the pigments

In one extract, twenty-five whole worms were placed, without grinding, in absolute methanol 19 parts and concentrated sulphuric acid 1 part, overnight in the ice chest. The following day the deep-green extract was filtered through glass wool into a separating funnel, and diluted with an equal volume of water.

The mixture was then extracted with one-third of the volume of chloroform. The chloroform hypophase was dark brown, and the aqueous epiphase was bright green. The hypophase was transferred to another funnel and washed with 2 % NaCl and then with water, forming 'chloroform extract A'. The aqueous epiphase was again extracted with chloroform, and this time the hypophase was bluish green and the epiphase pale green. The hypophase was removed and washed as before, filtered through chloroform-soaked paper, and evaporated to dryness *in vacuo*. A greenish residue remained which was used for subsequent tests; this was 'chloroform extract B'.

Chloroform Extract A

Extract A was evaporated to dryness *in vacuo* and redissolved in dry chloroform. The solution was chromatographed on ungraded Brockmann's alumina packed in chloroform, and then exhibited the following bands:

Band 1. A dark green band at the top of the column; non-fluorescent.

Band 2. A narrow yellow band; also non-fluorescent.

Band 3. A reddish brown band which travelled rapidly down the column and passed out. This was strongly red-fluorescent and gave the following spectrum (Hartridge Reversion spectroscope):

	I	II	III	IV
	660–680	600–620	590–565	520– mμ
Centres	670	610	579·5	(520)

Bands 1 and 2 could not be eluted by any of the usual solvents or combinations of solvents, and were finally eluted by ether-acetic acid 5:1 mixture, giving a green solution with no fluorescence and very indeterminate spectrum. The pigment had the extraordinarily high HCl-number of 36.

Band 3 was further chromatographed on MgO grade III (Nicholas, 1951), packed in chloroform, and displayed the following bands:

Band A2. A dark green band at the top; non-fluorescent.

Band B2. A brown band, also non-fluorescent, separated from the upper band by a gap.

Band C2. An apparently colourless wide band, red-fluorescent, passing down and out of the column very readily.

Band A2 (Fig. 1)

This band when cut off and eluted with methanol displayed the clear spectrum of biliverdin:

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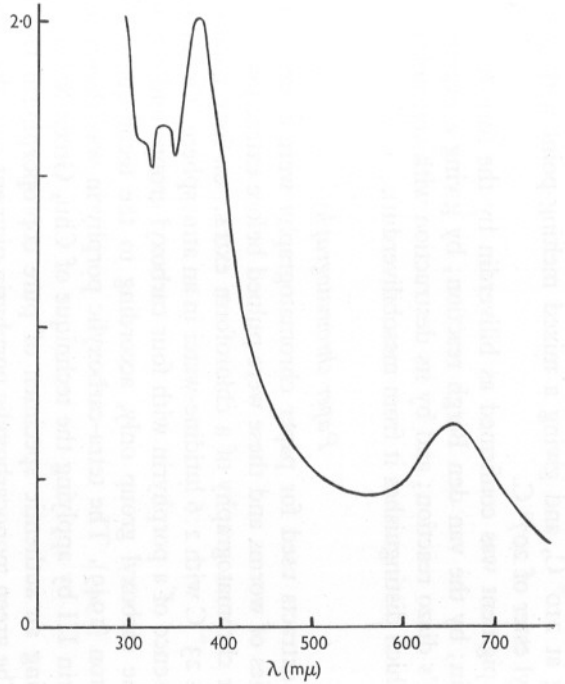

Fig. 1. Absorption curve of an acetic acid extract of the body-wall of entirely green worms. Vertical scale in arbitrary (Unicam spectrophotometer) units.

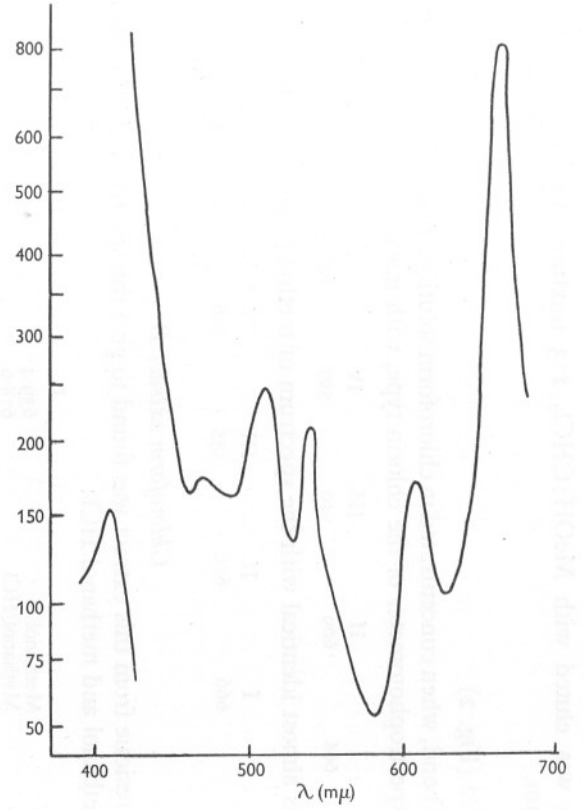

Fig. 2. Absorption curve of a chloroform extract of whole worms. Vertical scale in $E_{1cm.} \times 10^3$.

Band B2

This was eluted with MeOH:$CHCl_3$ 1:3 mixture, but gave no clear spectrum.

Band C2 (Fig. 2)

This band, when concentrated in chloroform solution, gave a clear spectrum in the spectrophotometer of the chlorin type, with maxima at

I	II	III	IV	V	Sorêt
666	609	540	507	(468)	414 mμ

which is almost identical with the spectrum of methyl phaeophorbide-*a*:

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The residue from this extract was found to give the spectrum of biliverdin with methanol and methanol/HCl:

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Methanol	639·4	391·2 mμ
Methanol/HCl	678·6	375·4 mμ

The acid-number was 1.

The solution of the pigment in methanol/HCl was allowed to stand for 36 h and then extracted with chloroform in the usual way, followed by crystallization from chloroform-methanol. Fine blue-green needles were obtained, melting at 210° C, and giving a mixed melting-point with pure biliverdin dimethyl ester of 207° C.

The pigment was confirmed as biliverdin by the formation of zinc bilipurpurin; by the van den Bergh reaction; by giving a negative result with Ehrlich's diazo reaction; and by its destruction with concentrated sulphuric acid (which distinguishes it from mesobiliverdin).

Paper chromatography

The extracts used for paper chromatography were obtained from larger quantities of worms and these were pulped before extraction.

Paper chromatography of a chloroform extract on long papers (Kennedy, 1953) at 23° C with 2:6 lutidine-water in an atmosphere of ammonia revealed the presence of a porphyrin with four carboxyl groups and a green pigment with one carboxyl group only, according to the technique of Nicholas & Rimington (1949). The tetra-carboxylic porphyrin was shown to be coproporphyrin III by applying the technique of Chu, Green & Chu (1951), and employing an authentic specimen of pure coproporphyrin III tetramethyl ester. The green monocarboxylic porphyrin pigment gave the same spectrum as that obtained from the band C2 already mentioned. Since the extract

from which C2 was obtained was made by using methanol and concentrated sulphuric acid, the pigment of band C2 would be an ester. From its solubility in boiling absolute ethanol, and its acid-number of 16, the pigment appeared to be methyl phaeophorbide-*a* only. Hydrolysis of the ester and formation of derivatives—phylloerythrin, phaeoporphyrin-a_5 and phytorhodin-*g*—confirmed that this was so. Extraction of the ether solution of the pigment with 0·2% sodium bicarbonate was without result, proving the absence of phaeophorbide-*b* (Willstätter & Stoll, 1913). That the pigment is present in the worm as the free phaeophorbide-*a* is shown by the formation of the spot of R_F 0·94 on the long-paper chromatogram of worm extracts made in chloroform only without any esterification.

As compared with the quantity of biliverdin, phaeophorbide-*a* is present in small amounts and may indeed be restricted to the gut. Worms thus owe their green appearance to biliverdin, while the yellow, brown and orange pigments also present in the epidermis in most worms are carotenoids.

Distribution of the Pigments in the Body

In most worms biliverdin occurs in the form of minute granules 1–2 μ in diameter scattered through the epithelial cells immediately under the cuticle. The pigment is deposited mainly along the borders of the blood capillaries, and is therefore most dense on the dorsal side of the body and in the parapodia; but it also occurs at the bases of the parapodia and between the segments. The appearance of the worms suggests that there is less biliverdin in the most anterior part of the body on the dorsal side, and while this seems to be so, the darker appearance is due mainly to the much larger amounts of carotenoids in this region. This darker appearance of the anterior region of the body may have some significance in relation to survival of these worms which protrude from the burrows while browsing on the surface mud. Biliverdin does not usually occur in any quantity on the ventral side of the body except in the parapodia, but depositions are found in the pygidium, and under the cuticle lining the proboscis. However, the intestine and the coelomic cells are also usually faintly green and contain small quantities of similar granules of biliverdin, and in females which have wholly or partially spawned, the gut and the coelomic cells may present a vivid green appearance. All worms possess at least some biliverdin though its presence may be masked by yellow, orange or brown carotenoid pigments.

Some individuals may appear entirely green, biliverdin alone being represented in the epidermis. These worms are always either ripe males with free sperm in the coelom, ripe females, or females which have spawned. This does not mean that females with an orange appearance are not sexually mature or do not spawn. Such green individuals, whether male or female, owe their striking appearance not only to a disappearance of the orange and brown carotenoids from the skin, but also to an increase in the amount of biliverdin in the body.

On the other hand, slight variations in the 'greenness' of worms which are not breeding seem to be due to variations in the quantity of carotenoids, worms appearing greener when they have relatively small amounts, and more orange when they have relatively greater quantities of carotenoids. On the other hand, apart from the breeding worms, there seems to be little variation in the amount of biliverdin deposited in the epidermis throughout most of the year.

Seasonal Changes in Colour

In the Chalkwell population during the early spring of 1949, nearly all the worms assumed a predominantly green appearance by the end of February when the main spawning occurred (Dales, 1950). It was naturally concluded that this colour change was in some way related with the maturation of the gametes. This is now known to be only partly true, since subsequent experience has shown that the same population in other years, and populations which have been seen elsewhere at the time of spawning, may have only a relatively small number of green individuals. Other observers have also reported the rather variable occurrence of green specimens, which as a general rule constitute only a small proportion of any population. During the summer such green individuals are definitely rare. In the early spring of 1951, 1952, and 1954 there were few green worms at Chalkwell, but in the winter and spring of 1952–53 there was again a much larger proportion of worms with a green appearance, but by no means as many as in 1948–49.

Discussion

Breeding takes place in mid-February in the *Nereis diversicolor* population at Chalkwell, but only a very small proportion of the females spawn successfully owing to the relative scarcity of the males and the fact that spawning takes place only in the presence of the male. All males with mature sperm appear a bright green owing to the complete extraction of the orange and brown pigments and also to an increase in the relative amount of biliverdin, as already mentioned. This was shown to be so by the much greater amount of pigment which could be extracted per unit weight of such worms as compared with non-breeding animals. This increase in the total quantity of biliverdin is almost certainly associated with a reduction in the quantity of haemoglobin (in the sense of Keilin & Hartree, 1951). As the males reach maturity their tissues undergo phagocytosis, the body-wall muscles are eroded away, making the worm extremely fragile. As this process continues the amount of haemoglobin decreases; there is often barely enough blood to fill the dorsal vessel in the fully mature male. This suggests that the biliverdin is indeed derived from the worm's own haemoglobin.

On the other hand, females when apparently mature at the time of spawning

are more variable in appearance; some are predominantly green, but all at this time have quantities of orange or brown pigments as well as the biliverdin in the body-wall. Later, however, those females which have spawned lose their orange and brown pigments and become as green as mature males. Males soon die when they have emitted their sperm. Females that have spawned often live for several weeks after spawning. Females rarely spawn so completely that one or two oocytes are not left in the coelom enabling their sex to be determined with certainty, and all such females undergo a phagocytosis of the tissues during or after spawning, accompanied by a loss of carotenoids and a greatly increased destruction of the blood haemoglobin with a corresponding increase in the quantity of biliverdin in the body. In the female the process of phagocytosis seems to have got out of step with the spawning, and this rather anomalous situation may be related in some way to the loss of the epitokous phase in this species. In unspawned females the oocytes are eventually broken down by the coelomic cells, being converted into a milky mass which might be mistaken for sperm. Soon afterwards quantities of orange pigments appear in the body-wall. These worms live throughout the following summer and may double their length, but it seems unlikely that they have a second opportunity to spawn (see Dales, 1951). There is no doubt that the green female individuals are those which have spawned, even if an appreciable number of oocytes remains in the coelom. Spawning takes place in the burrows and the worms are remarkably sedentary, at least at this time of year. For the first 8 weeks at least the larvae develop in the parent burrow and are just visible to the naked eye when about 6 weeks old. Larvae are invariably found in burrows occupied by a spawned or partially spawned green female, or in empty burrows, but never in burrows occupied by orange worms in which the oocytes are being absorbed. The explanation of these colour differences may be simply a consequence of sexually mature worms ceasing to feed. Worms that spawn subsequently draw on their carotenoids and even on their muscular tissues and become green and fragile. Worms that do not spawn, on the other hand, have a large reserve supply of food in the form of their own oocytes, and when these are absorbed the worms become even more orange in appearance than before. If the carotenoids form part of food reserves, the occurrence of greater numbers of green individuals in winters with early cold periods (1948–49, 1952–53) could be due to the earlier depletion of these stores. On the other hand, the carotenoids may be utilized directly in the production of the gametes, but if this is so it is surprising that though the carotenoids disappear in the male at maturity, they may not disappear in the female until after spawning. Incidentally, the greater number of green individuals in the early spring of 1949 was certainly not due to a larger proportion of males or spawning females in that year. Counts to determine the sex ratio in early February 1949 showed that less than 10% of the total population were males, and this proportion has

not varied materially in subsequent years. The proportion of spawned females has also remained at approximately the same value.

Although it is difficult to observe or measure changes in the quantity of epidermal biliverdin in adults which already have variable amounts of green, orange and brown pigments, it was thought that some indication of whether the biliverdin was derived from ingested blood or not could be solved by rearing worms from the larval stage on different substances. Young worms were collected in large numbers from the burrows in early May when the average age was estimated at about 8 weeks. At this time there are about ten to twelve chaetigerous segments and they are completely colourless. These young worms were reared on (1) dried and powdered nettle leaves alone, (2) dried slaughterhouse blood alone, and (3) a dried concentrated aquarium fish-food ('Brosiam') containing negligible amounts of chlorophyll or haemoglobin. The young *Nereis* seemed to be sufficiently omnivorous to thrive well on each of these substances for at least long enough for the present purpose. The water in each culture dish was replaced daily with filtered sea water to ensure that the worms were in fact feeding on the diet provided. The haemoglobin appeared in the blood when the young worms were about 9 weeks old, and the other pigments appeared soon after. The orange and brown pigments first made their appearance as granules in stellate chromatophores concentrated in the anterior region and arranged as a double row down the body on the dorsal side. The biliverdin appeared at about the same time, and from the first was more uniformly scattered through the epidermal cells on the dorsal side. All the young worms were similarly pigmented after several weeks' growth on these different foods; those fed entirely on nettle leaves, and those on dried blood, had much the same quantity of epidermal biliverdin as those fed on the dried fish-food.

Thus there is little doubt that the biliverdin is formed by the breakdown of the haemoglobin of the blood. This takes place along the margins of the vessels, mainly between the capillaries on the dorsal side of the body around the proboscis and in the pygidium. Bloch-Raphaël (1939) concluded, in her review of the seat of haemoglobin synthesis and breakdown in polychaetes, that in nereids these processes probably take place in the body-wall and around the proboscis, and that the bile pigments are excreted into the gut. This view agrees with the present observations on *N. diversicolor*. The granules of biliverdin may gradually be removed from the epidermis by the coelomic cells and conveyed to the gut, the cells travelling down the septa and oblique muscles. Coelomic cells loaded with biliverdin, often in granules 4–5 μ across, can be seen in these positions in spawned females. When the rate of haemoglobin breakdown is increased as in ripe males and spawned females, the rate of elimination is not increased in proportion, so that the pigment accumulates in the body.

SUMMARY

The variable colour of *Nereis diversicolor* is due to variations in the proportion of green, orange and brown pigments. The orange and brown pigments are mainly carotenoids; the green colour is due to biliverdin.

Phaeophorbide-*a* and coproporphyrin III also occur, but both these pigments may be restricted to the gut wall; biliverdin occurs both in the wall of the gut, and in the epidermis and coelomic cells.

The biliverdin is formed by the breakdown of the haemoglobin of the blood.

Haemoglobin-breakdown takes place in the epidermis on the dorsal side of the body, in the epithelial tissue surrounding the proboscis and in the pygidium. Granules of biliverdin are probably removed by the coelomic cells and conveyed to the gut into which they are excreted.

In ripe males, and in females during and after spawning, phagocytosis of the tissues is accompanied by an increased haemoglobin-breakdown with a corresponding accumulation of biliverdin in the body. The green appearance is due not only to an increased amount of biliverdin, but also to a complete extraction of carotenoids from the body-wall.

REFERENCES

BLOCH-RAPHAËL, C., 1939. Localisation, formation et destruction de l'hémoglobine chez les Annélides Polychètes. *Ann. Inst. océanogr. Monaco*, T. 19, Fasc. 1, pp. 1–78.

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

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.

—— 1951. An annual history of a population of *Nereis diversicolor* O. F. Müller. *Biol. Bull., Woods Hole*, Vol. 101, pp. 131–7.

DEHORNE, A., 1925. Observations sur la biologie de *Nereis diversicolor*. *C.R. Acad. Sci., Paris*, T. 180, pp. 1441–3.

HEMPELMANN, F., 1939. Chromatophoren bei *Nereis*. *Z. wiss. Zool.*, Bd. 152, pp. 353–83.

HERPIN, R., 1923. Comparaison entre le comportement sexuel de quelques Néréidiens des côtes de la Manche. *C.R. Acad. Sci., Paris*, T. 176, pp. 536–9.

—— 1925. Recherches biologiques sur la reproduction et le developpement de quelques Annélides polychètes. *Bull. Soc. Sci. nat. Ouest.*, Ser. 4, T. 5, pp. 1–250.

KEILIN, D. & HARTREE, E. F., 1951. Relationship between haemoglobin and erythrocruorin. *Nature, Lond.*, Vol. 168, pp. 266–9.

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

MCINTOSH, W. C., 1910. *A Monograph of the British Marine Annelids*, Vol. 2, pt. 2, pp. 233–524. London: Ray Soc.

MENDTHAL, M., 1889. Untersuchungen über die Mollusken und Anneliden des Frischen Haffs. IV. Über die Geschlechtsverhältnisse der *Nereis diversicolor*. *Schr. phys.-ökon. Ges. Königsb.*, Bd. 30, pp. 27–42.

MÜLLER, O. F., 1771. *Von Würmen des süssen und salzigen Wassers*, 200 pp., 16 pls. Copenhagen.

NICHOLAS, R. E. H., 1951. Chromatographic methods for the separation and identification of the porphyrins. *Biochem. J.*, Vol. 48, pp. 309–13.

NICHOLAS, R. E. H. & RIMINGTON, C., 1949. Qualitative analysis of the porphyrins by partition chromatography. *Scand. J. clin. Lab. Invest.*, Vol. 1, pp. 12–18.

THOMAS, J. A., 1930. Sur les pigments vert et brun de *Nereis diversicolor* M. *Bull. Soc. zool. Fr.*, T. 55, pp. 97–103.

WILLSTÄTTER, R., & STOLL, A., 1913. *Untersuchungen über Chorophyll.* Berlin: Springer.

J. Mar. biol. Ass. U.K. (1954) 33, 709–719
Printed in Great Britain

INNERVATION OF AN AMPHIPOD HEART

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(Text-figs. 1 and 2)

For investigation of the innervation of the heart in the Amphipoda, *Marinogammarus* marinus* has been chosen as it is among the largest species available at Plymouth. The smaller size of other species added greatly to the difficulties in making the preparations. Some observations were made with *Gammarus locusta*, but because of certain features of its anatomy referred to below it was found to be less suitable for this kind of study.

For staining of the nerves methylene blue was used. The immersion of the tissues into a weak solution of this dye (10–15 drops of 0·5% solution in distilled water to 100 ml. of sea water) gave more constant results than the injection of a stronger solution into the body of the animal. However, the staining of certain elements, such as the nerve cells in the ganglionic trunk, could be obtained with the latter method only. The individual reactions of the animals to the staining varies greatly: thus, when several specimens are injected with the same solution at the same time the nerves of some stain more or less well while for others the results are poor or often nil.

To expose the heart the ventral body wall of the animal should be cut along the middle line or, to avoid damage to the ventral neural cord, along the side of the latter. After the parts of the sectioned body wall have been pulled out and pinned to the paraffin plate, the digestive organs are removed, leaving *in situ* the two dorsal diverticula which are so linked with the posterior aorta that it is practically impossible to separate them from it. The gonads should also be removed. The digestive organs are connected with the pericardial diaphragm by strands of connective tissue, but there is no particular difficulty in severing these connexions and in obtaining preparations with the heart tube and the pericardial diaphragm in their normal positions. In *G. locusta*, however, this diaphragm seems to be a little weaker and is more easily damaged; the heart tube then becomes flaccid or even displaced, and subsequent manipulations are much more difficult.

Such preparations with the heart and the pericardium left *in situ* show many of the details described below, but for better observation of heart nerves the heart tube should be opened along its middle line. This operation can be done with a very fine scalpel and preferably after some staining has

* Separated from *Gammarus* by Sexton & Spooner (1940).

made the outlines of the tissue elements more distinguishable. After the heart has been opened its ganglionic trunk is more easily accessible for staining and observation, but even then, being situated on the outside of the dorsal heart wall, it is seen by transparency only. For its direct observation the heart has to be isolated and mounted with the dorsal side up. This is a difficult operation, and it is more expedient to perform it at one of the later stages, after the preparations have been stained and fixed and are already either in water or alcohol or xylol.

The fixation of the staining has not been quite satisfactory with the tissue of *Marinogammarus*. As is already known, the elements stained with methylene blue, to be well fixed with ammonium molybdate solution, must attain a certain intensity of colour. In gammarids some of the nerves do not reach this point, and although they show up distinctly before being fixed they turn pale or even become indistinguishable in the completed preparations. Neither was the double fixation, i.e. in ammonium picrate followed by ammonium molybdate, of much help. With such material the best practice is, after having examined the permanent preparations, to check repeatedly the dubious points on fresh preparations during the staining process before their fixation.

The Anatomy of the Heart

The heart of *Marinogammarus* has the shape of a tube extending from the posterior edge of the first thoracic segment (which enters into the composition of the cephalothorax) to the middle of the 7th thoracic segment. Its wall is made up of a layer of cross-striated muscle fibres turning in left-handed spirals surrounded by connective tissue fibres of longitudinal direction. Numerous rounded cells adhere to the inside of the muscle fibres without forming a continuous layer. The three pairs of ostial orifices piercing the lateral heart wall are situated in the 3rd, 4th and 5th thoracic segments. In front and backwards the heart-tube continues with the anterior and posterior aorta respectively (Fig. 1A).

Four pairs of arteries arise from the heart: the first, running in antero-lateral direction, starts near the origin of the anterior aorta, while three others originate on the ventral side of the heart in the 4th, 5th and 6th segments. The walls of the arteries are very thin and not often noticeable, but their valves sometimes stain more deeply than the muscles of the heart, and then stand out distinctly. Even the play of these valves opening at each systole can occasionally be seen. Klövekorn (1934), who found these vessels in *Gammarus pulex*, called them 'Darm-Leber-Arterien', since they run to the digestive organs. It can be added that in methylene-blue preparations some finer branches of these vessels can be seen to ramify in the pericardial septum.

The heart is suspended in the pericardial cavity by means of bands of connective tissue, the strongest of them stretching from the terga to the dorsal

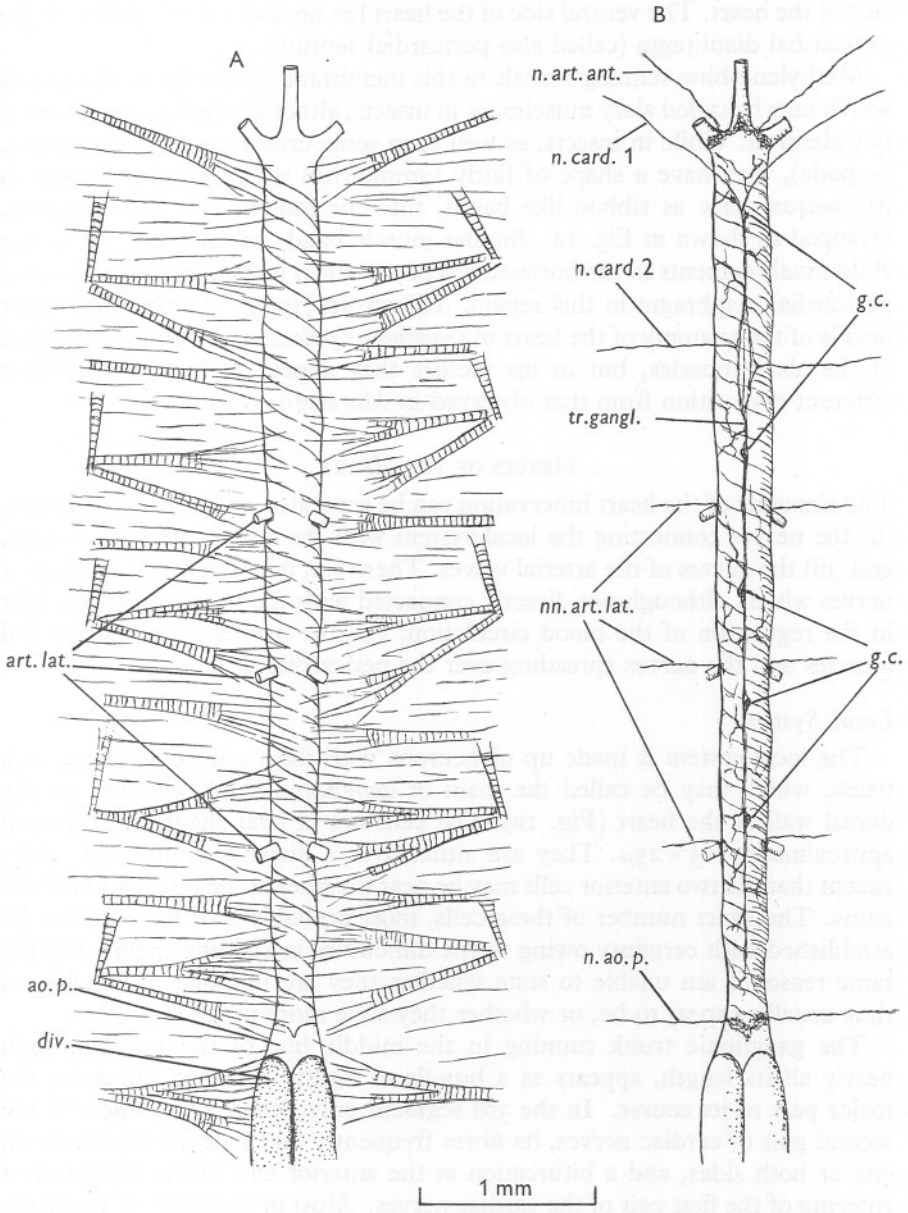

Fig. 1. *Marinogammarus marinus.* A: view of the heart from the ventral side with its arteries and alary muscles of the pericardium. *art.lat.* lateral arteries of the 4th–6th thoracic segments; *ao.p.* posterior aorta; *div.* coecal diverticula. B: view of the heart from the dorsal side. *tr.gangl.* ganglionic trunk; *g.c.* nerve cells; *n.card.*1, first cardiac nerve; *n.card.*2, second cardiac nerve; *n.art.ant.* nerve of arterial valves at the anterior end of the heart; *nn.art.lat.* nerves of the valves of the lateral arteries; *n.ao.p.* nerve of the valve of the posterior aorta. The ostia, situated on the lateral wall of the heart, cannot be seen in both figures.

side of the heart. The ventral side of the heart lies on and is connected with the pericardial diaphragm (called also pericardial septum).

Methylene-blue staining reveals in this membrane the presence of muscles which may be called alary muscles, as in insects, although their arrangement is not identical. While in insects, as well as in some crustaceans (Stomatopoda, Isopoda), they have a shape of fairly symmetrical triangles, they appear in *Marinogammarus* as ribbon-like bands, three or four in each half segment, arranged as shown in Fig. 1A. Similar muscle bands are also present in the abdominal segments in the horizontal septum which is the continuation of the pericardial diaphragm in this region. Klövekorn (1934), among many other details of the anatomy of the heart in *Gammarus pulex*, gives also a description of the alary muscles, but in his picture they appear to have a somewhat different disposition from that observed in *Marinogammarus*.

Nerves of the Heart

The elements of the heart innervation can be separated as (i) the local system, (ii) the nerves connecting the local system with the central nervous system, and (iii) the nerves of the arterial valves. There are, moreover, two systems of nerves which, although not directly connected with the heart itself, take part in the regulation of the blood circulation, viz. the nerves of the pericardial muscles and the nerves spreading over the pericardial wall.

Local System

The local system is made up of neurons with their cell bodies lying in a trunk, which may be called the main or ganglionic trunk, situated on the dorsal wall of the heart (Fig. 1B). The cells are of oval shape and measure approximately $15 \times 25\mu$. They are situated at a distance from each other, except that the two anterior cells may lie near together at the bifurcation of the trunk. The exact number of these cells, most probably 5 or 6, could not be established with certainty owing to the difficulties in staining them. For the same reason I am unable to state whether they are unipolar or bipolar, as they usually appear to be, or whether they have more processes.

The ganglionic trunk running in the middle line of the heart through nearly all its length, appears as a bundle of tightly collected fibres for the major part of its course. In the 3rd segment only, where it is joined by the second pair of cardiac nerves, its fibres frequently take a deviating course on one or both sides, and a bifurcation at the anterior end marks the point of entering of the first pair of the cardiac nerves. Most of the fibres of the trunk are the axons of its own nerve cells. They give off branches which, with their further divisions, supply the muscle fibres of the heart including those of the ostia but not those of the arterial valves. The finer ramifications are difficult to stain, but whenever the reaction is more favourable they appear, as in other crustaceans, abundantly distributed among the muscle fibres.

Nerves Connecting the Local System with the Central Nervous System

Two paired nerves which may be called the first and second cardiac nerves run from the neural cord to the ganglionic trunk of the heart (Fig. 1B, *n.card.* 1, *n.card.* 2). The fibres of the first of them are conveyed by a trunk emerging from the posterior part of the infraoesophageal ganglion (Fig. 2, *n.card.* 1). Not far from its origin it crosses a nerve trunk arising more anteriorly from the same ganglion and carrying fibres to the pericardial muscles and pericardial organs, and associates with it for a short distance (Fig. 2, *X*). As, besides, there are in this region anastomoses between various nerves, it cannot be excluded that in the cardiac nerve fibres of some other origin may also be present. From the point of junction mentioned above this nerve takes a more superficial course crossing the extensor muscles on their outer side near to their insertions. It then crosses on its inner side a thin oblique muscle (Fig. 2, *m*), inserting into the integument above the anterior end of the heart, and gives off a branch spreading over this muscle in a dense plexus. Passing on to the dorsal side of the heart the 1st cardiac nerve forms a Y-shaped figure with its fellow from the opposite side and with the ganglionic trunk.

The second cardiac nerve (Fig. 2, *n.card.* 2) runs to the heart with the dorsal branch of the segmental nerve originating from the connective linking the 1st and 2nd free thoracic ganglia (Fig. 2, *n.segm.*).This branch is a mixed nerve containing motor fibres passing to the dorsal layer of the extensor muscles, sensory fibres coming from the integument, fibres of the muscle receptors, and fibres to the pericardial organs (Fig. 2, on the right). The nerve running to the heart separates from other fibres at the lateral border of the median portion of the extensor muscles and passes on to the dorsal heart surface. It enters, often asymmetrically to that of the opposite side, the ganglionic trunk or one of its diverging branches, and bifurcates into branches running into opposite directions (Fig. 1B).

Each of the two cardiac nerves appears to consist of more than one fibre, but it is very difficult to ascertain their true number. Neither has it been possible to trace their course in the ganglionic trunk for a longer distance and find out their terminations. It may be assumed, with a great degree of probability, that they enter into relation with the cells of the local system.

Nerves of the Arterial Valves (Fig. 1B)

The nerves supplying the muscles of the arterial valves have no connexion with the elements of the systems described above. Two pairs of these valve nerves, viz. running to the anterior and posterior end of the heart, can be more easily noticed. The first of them approach the heart obliquely from the sides and, to judge from the direction of their course, come from the infraoesophageal ganglion, but could not be traced up to their origin. On reaching the valves the nerve fibres break up in a characteristic pattern of ramifications

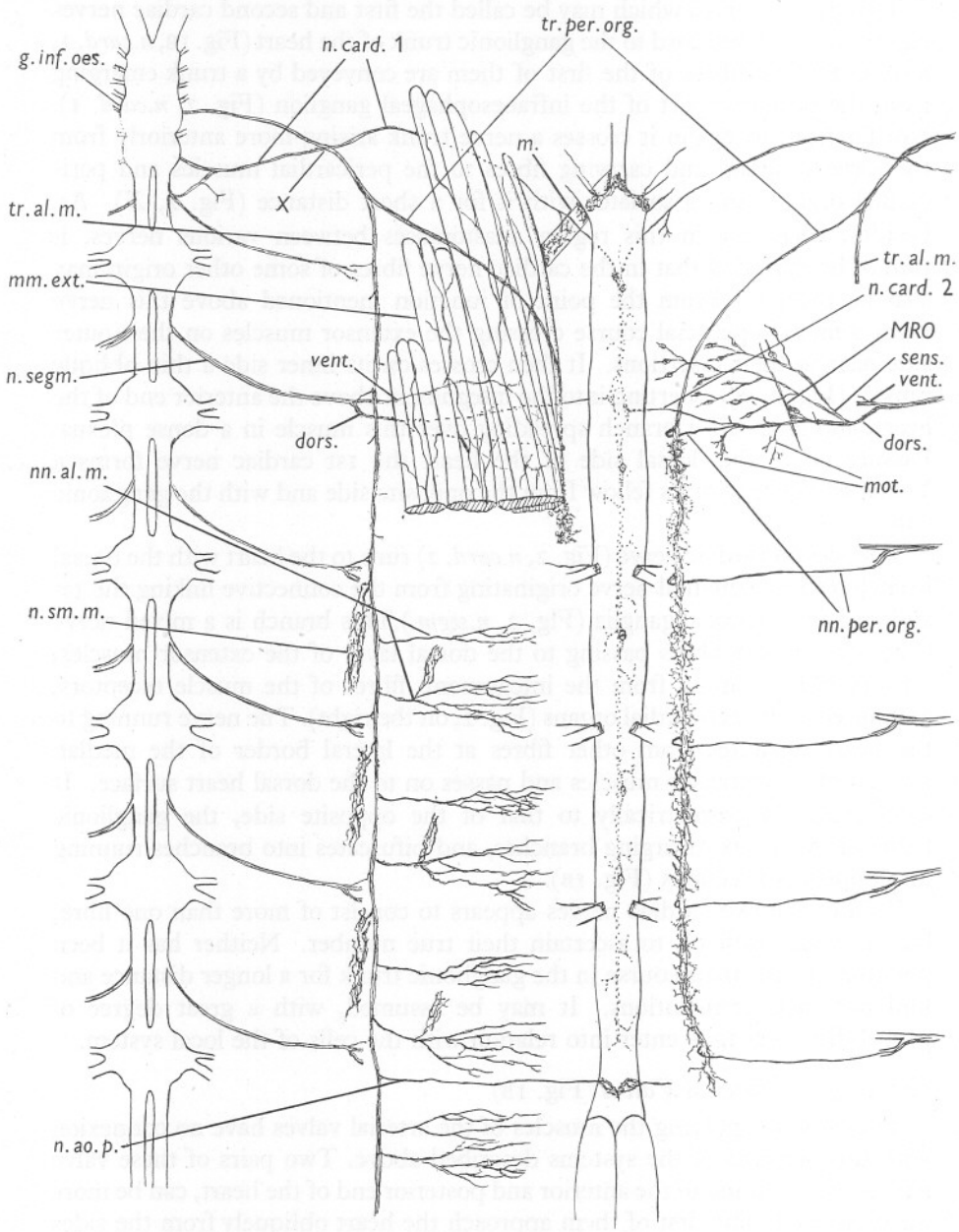

Fig. 2. See opposite page.

different from that observed on the heart muscles. Some branches pass on to the valve of the anterior aorta and probably are the only source of innervation of this valve, since no separate nerve could be noticed to run down the aorta.

The nerves of the valve of the posterior aorta run transversely on the ventral surface of the extensor muscles. They arise from the longitudinal trunk giving off fibres supplying the alary muscles of the pericardium (Fig. 2, *n.ao.p.*), but, as pointed out below, this does not necessarily imply that they have a common origin and are in functional relation with this system of nerves.

The nerves of the valves of the arteries in the 4th–6th segments are not easily noticeable. Tiny filaments of transverse direction pass on to these valves, but do not stain clearly. Moreover, very fine connective tissue fibres running also transversely in the pericardial septum and looking often very similar to the fine nerves, contribute to the difficulties of interpretation.

Nerves of the Alary Muscles

The nerves of the alary muscles of the pericardium are given off by a trunk running on each side through the thorax and the abdomen at the lateral edge of the extensor muscles (Fig. 2, *tr.al.m.*). This trunk takes origin in the infra-oesophageal ganglion, being in the first part of its course united into a common bundle with the trunk of the pericardial organs, as well as with some fibres supplying the ordinary muscles. When crossing the successive segmental nerves the trunk of the alary muscles is joined by fibres branching from these nerves, but whether these additional fibres take part in the innervation of the alary muscles, or travel in the trunk for a short distance only to leave it and supply the ordinary muscles, I cannot say. The branches innervating the alary muscles (Fig. 2, *nn.al.m.*) are of very fine calibre. There seem to be more

Fig. 2. *Marinogammarus marinus*. Nerve elements controlling the blood circulation as seen in an animal sectioned along the left side and opened so that the neural cord is viewed from the dorsal side and the heart from its ventral side (semi-diagrammatic). The nerves represented in one plane of the drawing are situated in the animal in various planes and those running transversely follow in their course the curvature of the body; the nerves of the dorsal heart wall, same as in Fig. 1B, but seen here by transparency are drawn in dotted lines. *mm.ext.* extensor muscles sectioned at the level of the 3rd thoracic segment (in numbering the segments the first of thoracic segments is considered as fused with the cephalothorax); *m.* small oblique muscle; *g.inf.oes.* infraoesophageal ganglion; *n.card.* 1, 1st cardiac nerve; *X*, anastomose between the first cardiac nerve and the trunk bifurcating into the trunk of the alary muscles (*tr.al.m.*) and the trunk of the pericardial organs (*tr.per.org.*); *n.segm.* nerve arising from the connective and running to the dorsal part of the 3rd thoracic segment; *vent.*, *dors.*, its ventral and dorsal branches; on the left side of the figure the ventral branch is seen ramifying on the ventral side of the extensor muscles; on the right side the dorsal branch is shown conveying: sensory nerves (*sens.*), motor nerves to the superficial layer of the extensor muscles (*mot.*), second cardiac nerve (*n.card.* 2), nerve to the pericardial organs (*nn.per.org.*), and nerves of the muscle receptor organs (*MRO*); *nn.al.m.* nerves of the alary muscles; *n.sm.m.* nerve of small muscles at the lateral edge of the pericardial diaphragm; *n.ao.p.* nerve of the valve of the posterior aorta.

than one of them in each half segment, but they do not stain well and even in good preparations are difficult to detect.

From the same trunk arise also branches supplying small segmentally arranged muscles lying close to the trunk (Fig. 2, *n.sm.m.*). These muscle slips of longitudinal and slightly oblique direction are situated at the lateral edge of the extensor muscles, but appear as independent muscle units. As pointed out below, they may possibly be in some functional relation to the pericardial septum.

Nerves of the Pericardial Wall (Pericardial Organs)

The nerve trunk of the pericardial organs, after separating from the trunk of the alary muscles, crosses the ventral surface of the extensor muscles obliquely, and in the region of the 3rd thoracic segment passes on to their median surface (Fig. 2, *tr.per.org.*). In this position it runs backwards as far as the 7th segment. As the median surface of the extensor muscles constitutes the lateral wall of the pericardial cavity, this trunk comes to lie on the pericardial wall for most of its course. During this course it becomes strengthened by fine fibres which arise from the dorsal branches of the segmental nerves and curve ventrally round the convexity of the extensor muscles to join the pericardial trunks (Fig. 2, *nn.per.org.*). The first of them is situated in the 3rd segment and that can be more easily noticed. Their probable number is five on each side, but owing to their situation, particularly unfavourable both for staining and observation, this number is not certain.

There is a clear difference in structure of the trunks of the pericardial organs and that of the ordinary nerves, and even between the portions of the pericardial trunks themselves. Before passing on to the pericardial wall they look quite like the ordinary nerves, and so do the branches coming from the segmental nerves. On the pericardial wall, however, the trunks become broader, show uneven outlines, and look as if they consist of very small granules. Closer scrutiny of the best preparations reveals that the nerve fibres here break up into very fine filaments and during the staining process these in turn disintegrate into granules. In most favourable cases strands of such fine neuropile-like nerve tissue may be seen branching from the main trunks and entangling in a plexus spreading over the lateral wall of the pericardium. The more frequently observed uneven appearance of the trunk outlines is due to the incomplete staining of the strands starting from the trunks. Occasionally some bodies can be noticed which might be interpreted as indistinctly stained nerve cells, but their nature has always been doubtful.

Owing to the size of the animals and to technical difficulties in obtaining nerve preparations the picture of the nerves in the pericardium is not so impressive as in large crustaceans, but there can hardly be any doubt that these elements belong to the same category as the pericardial organs of the Stomatopoda and Decapoda described previously (Alexandrowicz, 1953*a*, *b*).

Discussion

The innervation of the heart of *Marinogammarus* proves to have much in common with that of other Crustacea and in particular, owing to the shape and anatomy of the heart, with that of the Isopoda (Alexandrowicz, 1952). There is, however, a difference in the situation of the ganglionic trunk, in that in the Isopoda investigated (*Ligia oceanica*, *Mesidotea entomon*, *Idotea emarginata*) it lies on the inner surface of the heart, while in the Gammaridae it lies outside of it. The latter position is the same as in the Stomatopoda (Alexandrowicz, 1934), and, according to some observations I have recently made, in the Mysidacea too. Otherwise the ganglionic trunk appears to have a similar shape and structure as in isopods, i.e. it is composed of a few neurons of the local system and some fibres connecting this system with the neural cord. As in other crustaceans the local system should be regarded as an autonomous apparatus ruling the heart beat.

The situation of the heart in the Amphipoda extending up to the cephalothorax proved to be more favourable than in the Isopoda for the observation of the nerves connecting the heart with the neural cord, and gave the chance of establishing the origin of these nerves as from the infraoesophageal ganglion and the 1st free thoracic ganglion. There are, however, still some doubtful points. For instance, as the 1st thoracic segment is fused with the cephalothorax and its ganglion with the infraoesophageal ganglionic mass, the question remains whether the 1st cardiac nerve originates in the ganglion of the 1st thoracic segment, which judging from its course is more likely, or from one of the cephalic segments. If the former is true, the cardiac nerves, which presumably have a regulatory function on the heart rhythm, would originate in the two anterior thoracic segments (the term segment being used as equivalent to metameres).

As regards the innervation of the arterial valves a particular feature in gammarids would be the absence of the nerve running down the anterior aorta, which, known as nerve of Lemoine, was one time considered to be the main nerve of the heart of decapods, but was found in these animals as well as in stomatopods and isopods to supply only the valve of the anterior aorta (Alexandrowicz, 1932, 1934, 1952). In gammarids, unless this negative evidence is due to some particularly refractory behaviour of this nerve to staining with methylene blue, it appears to be wanting.

The question whether the innervation of the arterial valves is linked with that of the pericardial muscles in a way securing their synchronous action remains open. In decapods all these nerves, with exception of that supplying the valve of the anterior aorta, arise from a common bundle, whereas in stomatopods the two systems have independent courses; in isopods their relations have not been determined, and the fact that in *Marinogammarus* the nerve to the posterior aorta arises from the trunk of the alary muscles does not prove that the fibres of both have a common origin.

The assumption that the nerves of the valves and of the alary muscles are in functional relation is insecure until the role of the pericardial muscles is definitely established. The opinions of the writers who have studied this problem in crustaceans as well as in insects differ as to whether the function of these muscles may be passive or active, i.e. whether they simply strengthen the elastic resistance of the pericardial septum, or whether their contractions contribute to the movements of this septum. Even the two authors who described the alary muscles in amphipods, v. Haffner (1934) in *Phronima sedentaria*, and Klövekorn (1934) in *Gammarus pulex*, are not quite in accord. On this question the following remarks may be added. First, it seems rather unlikely that organs made up of cross-striated muscle tissue and supplied by a special system of nerves would function merely as elastic bands; and, secondly, that considering their possible action there should be taken into account not only their relation to the heart-beat, but their possible co-ordination with the movements of the animal. It is obvious that during the movements of the body the respective pressure in the large sinuses can vary widely and if, as is likely, such fluctuations of the blood pressure in the pericardial cavity would impair the heart action, the appropriate contraction of the alary muscles might regulate the position of the pericardial diaphragm and compensate for these changes. It is, moreover, possible that not only the alary muscles, but also those small muscle slips mentioned above as receiving their nerve supply from the trunk of the alary muscles, are active in the same way. They end, in fact, exactly at the level of the lateral attachment of the pericardial septum and it appears very likely that their contractions may have a pulling action on the latter. It should be mentioned that in *Phronima* v. Haffner has found small muscles attached to the lateral edge of the pericardium which, as he puts it, 'dienen...zur Befestigung des Pericardialseptums'.

The nerves expanding over the pericardial wall in neuropile-like plexuses show such a resemblance in their mode of termination to those found in stomatopods and decapods that their inclusion in that category of elements called pericardial organs may be considered as well founded. It is therefore highly probable that they have a neurosecretory function, releasing into pericardial cavity some hormone or hormones which influence the heart rhythm in a similar way as in those animals (Alexandrowicz & Carlisle, 1953). In the present description of these organs it has been pointed out that no uncontestable evidence of the presence of nerve cells in them could be obtained. If the assumption that they are missing is correct, all the fibres of the pericardial trunks would come from the cells situated in the central nervous system, and thus this arrangement in gammarids would resemble more that in decapods than in stomatopods, since in the latter most of the fibres of the pericardial organs originate in the nerve cells lying outside the ganglionic cord.

It is interesting to note that different elements of the systems described above, viz. the first of the cardiac nerves, the trunk of the alary muscles, and

the trunk of the pericardial organs, originate in the infraoesophageal ganglion. Hence it may be conjectured that the centre controlling the blood circulation could be localized in this part of the nervous system. Admittedly some of the nerves described, as the 2nd cardiac nerve and the additional nerves to the pericardial organs, start from the thoracic ganglia, but it is possible that the cell-bodies of their neurons are situated in the infraoesophageal ganglion while their axons run down the neural cord to leave it with the nerves arising farther backwards. This problem must await solution until by successful tracing of all the nerves in question their true origin and the connexions of their neurons can be established.

Summary

In the heart of *Marinogammarus marinus* three systems of nerve elements have been found, viz. (1) a local system consisting of a ganglionic trunk situated on the dorsal surface of the heart and sending branches to the muscle fibres, (2) two paired nerves, termed the 1st and 2nd cardiac nerves, connecting the local system with the central nervous system, and (3) nerves supplying the muscles of the arterial valves. It is assumed that the local system rules the beat of the heart and that the cardiac nerves have a regulating function.

Not directly connected with the heart, but having relation to the blood circulation are two sets of nerves: (1) nerves supplying the alary muscles, presumably regulating the pressure in the pericardial cavity, and (2) nerves spreading over the lateral wall of the pericardium in neuropile-like plexuses and supposed to have a neurosecretory function.

It is suggested that the centre controlling the blood circulation could be localized in the infraoesophageal ganglion.

References

Alexandrowicz, J. S., 1932. The innervation of the heart of the Crustacea. I. Decapoda. *Quart. J. micr. Sci.*, Vol. 75, pp. 181–249.

—— 1934. The innervation of the heart of the Crustacea. II. Stomatopoda. *Quart. J. micr. Sci.*, Vol. 76, pp. 511–48.

—— 1952. Innervation of the heart of *Ligia oceanica*. *J. Mar. biol. Ass. U.K.*, Vol. 31, pp. 85–96.

—— 1953*a*. Nervous organs in the pericardial cavity of the decapod Crustacea. *J. Mar. biol. Ass. U.K.*, Vol. 31, pp. 563–80.

—— 1953*b*. Notes on the nervous system in the Stomatopoda. II. The system of dorsal trunks. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 29–39.

Alexandrowicz, J. S. & Carlisle, D. B., 1953. Some experiments on the function of the pericardial organs in Crustacea. *J. Mar. biol. Ass. U.K.*, Vol. 32, pp. 175–92.

v. Haffner, K., 1934. Der Blutkreislauf von *Phronima sedentaria*, mit besonderer Berücksichtigung des lacunären Systems. *Z. wiss. Zool.*, Bd. 146, pp. 283–328.

Klövekorn, J., 1934. Das Organsystem der Blutbewegung bei *Gammarus pulex* L. *Z. wiss. Zool.*, Bd. 146, pp. 153–92.

Sexton, E. W. & Spooner, G. M., 1940. An account on *Marinogammarus* (Schellenberg) gen.nov. (Amphipoda), with a description of a new species, *M. pirloti*. *J. Mar. biol. Ass. U.K.*, Vol. 24, pp. 633–82.

J. Mar. biol. Ass. U.K. (1954) 33, 721–732
Printed in Great Britain

A MARINE FUNGUS *PLECTOSPIRA DUBIA* N.SP. [SAPROLEGNIACEAE], INFECTING CRUSTACEAN EGGS AND SMALL CRUSTACEA

By D. Atkins, D.Sc.

(Text-figs. 1–5)

Among eggs, cut from a recently dead *Pinnotheres pisum* on 11 June 1953 (spawned on 9 May), about six were infected with a fungus *Plectospira dubia* n.sp. not previously observed and quite distinct from both *Leptolegnia marina* (Atkins, 1929, 1954*a*) and *Pythium thalassium* (Atkins, 1954*b*) also parasitic in crustacean eggs. The parent showed no fungus in the gills (the body was not examined). The crab had been in Plymouth laboratory tank water of salinity of 36–37‰ with added townettings from 26 August 1952 to the time of death, for 3 months of the time within a *Mytilus edulis*. The eggs were kept in a finger bowl, as it was hoped they would hatch, but in little more than a week most of the eggs of the small egg-mass were infected. This occurred with temperatures between 14 and 15° C.

The same fungus developed independently in the eggs taken, in clean and healthy condition, from a recently dead *Gonoplax rhomboides* which had been in the laboratory for some time. This infection started in the form of a mycelium given over entirely to the production of gemmae; but mycelia giving rise to sporangia developed in other eggs of this egg-mass within a few days (see also p. 728).

The following crustacean eggs became infected by placing one to several infected eggs with them: *Typton spongicola*, *Crangon vulgaris*, *Leander serratus*, *Macropodia* sp. and *Portunus depurator*. All infected eggs had embryos in various stages of development, up to about two-thirds developed.

The fungus is of dense, but slow, growth. The first sign of infection in the egg was the development of a space between the inner and outer egg membranes, and in this space the young infecting hyphae first appeared (Fig. 1D). The space increased with the growth of the infection and this, together with some bulging of the sporangia beyond the egg, increased the size of infected eggs. The healthy egg of *Pinnotheres pisum* is about 0·33 mm in diameter, that of the infected egg about 0·5 mm. The egg of *Gonoplax rhomboides* increased with infection from the normal diameter of about 0·5 mm to 0·65 mm; that of *Portunus depurator* from about 0·32 mm to 0·42 mm; and that of *Crangon vulgaris* from 0·8 by 0·58 mm to 1·3 by 0·8 mm. The eggs of *Gonoplax rhomboides* with the gemmae stage of the fungus did not show increase in size.

Plectospira dubia while an active parasite—infecting hyphae entering eggs containing embryos with heart beating—does not appear to be as severely pathogenic as *Leptolegnia marina* (Atkins, 1929, 1954*a*). The hyphae seem to be first attracted toward the yolk remnant, but a few prezoeae and zoeae have become infected and also three *Evadne*: it is possible that the latter may have been moribund before infection.

The method of sprouting of the infecting spores has not been clearly seen, as they tend to collect in groups, which soon become coated with other organisms.

Mycelial characteristics

The hyphae are generally stout; the mycelium becomes irregularly branched; the ends of branches tend to be swollen. Young hyphae soon after entry attain a width of about 27μ, and the main hyphae of a young mycelium may be 50μ (Fig. 1C). The cytoplasm is hyaline, but with groups of minute shining granules, the groups more or less evenly spaced, as though collected round nuclei (Fig. 1A, B, D).

Asexual reproduction

Within the host the growth soon becomes irregular (Fig. 1A), the branches inflated and bulbous, up to about 100μ wide, acting as sporangia. So far as can be seen in such a restricted space there is no distinction between vegetative and reproductive hyphae, and eventually the whole of the mycelium is used up in spore-formation and the host is left a network of empty sporangia (Fig. 2A).

The inflated sporangia for the most part are within the host tissue, but characteristically bulge beyond the crustacean egg (Fig. 2B, C); occasionally much of a sporangium may be extra-matrical. In a covered dish from which there had been some slight evaporation with increased salinity the extra-matrical portions of sporangia were exceptionally inflated (Fig. 2D). The inflated sporangia with large central vacuole and widely spaced spore initials gives to an infected egg a coarsely vesicular appearance.

Sporangia are provided with one or more efferent hyphae, 50–400μ long, and 10–30μ wide (Fig. 3). When long the tubes are frequently corrugated and may be twisted (Fig. 3A). Sporangia quite often have paired efferent hyphae (Fig. 3D).

Spores are formed not only in the sporangia, but in the efferent hyphae, except for an apical portion of a length up to about 50μ, which remains hyaline. During spore formation the tip of the efferent hypha frequently enlarges and becomes flattened across the top and when the tip dissolves or gives way—it is not known which occurs—the aperture is then left with flared lips. Other shapes of aperture are shown in Fig. 3C. Occasionally the pore may be exceedingly small, so that zoospores in the second motile stage,

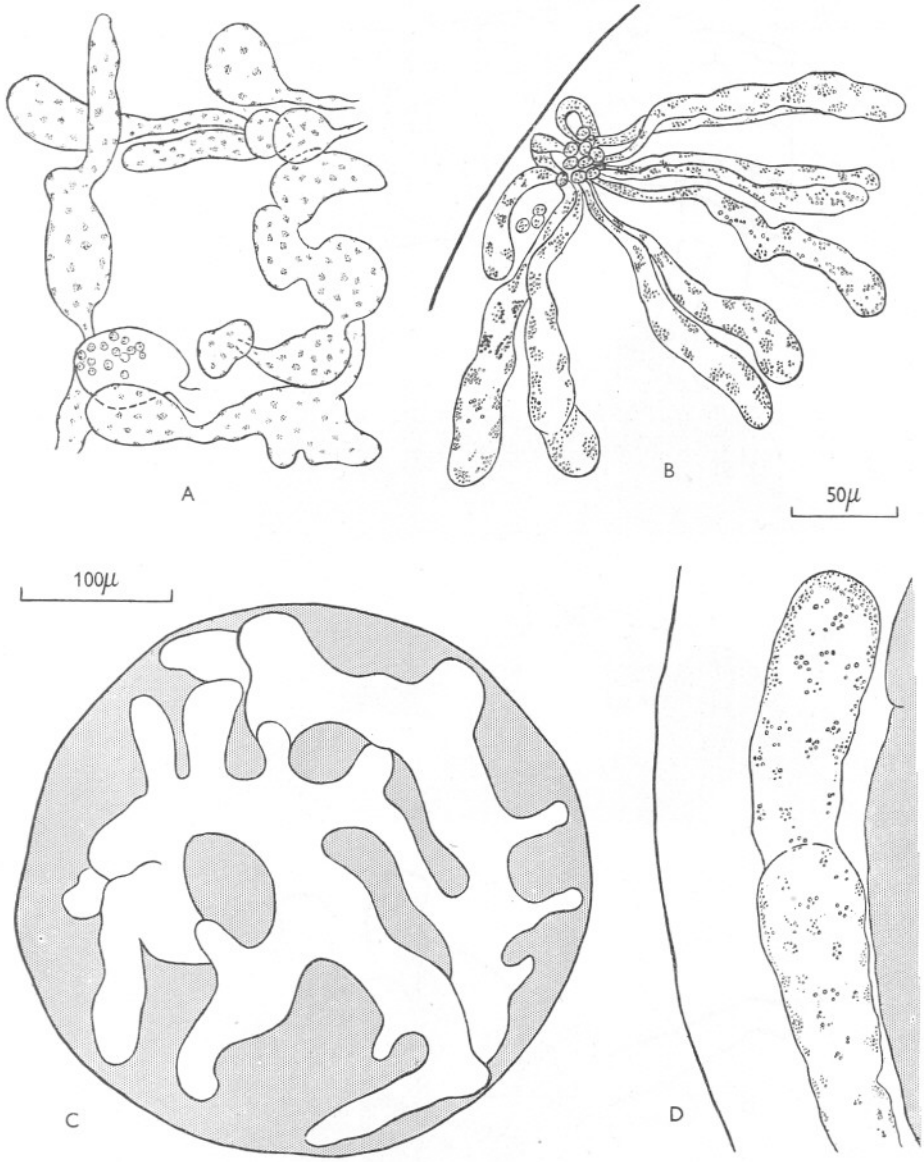

Fig. 1. *Plectospira dubia*. A, mycelium dissected from egg of *Macropodia*; B, group of young infecting hyphae below dorsal cuticle of *Evadne*; C, young mycelium in egg of *Pinnotheres pisum*; D, young hypha growing in space between outer egg membrane and embryo.

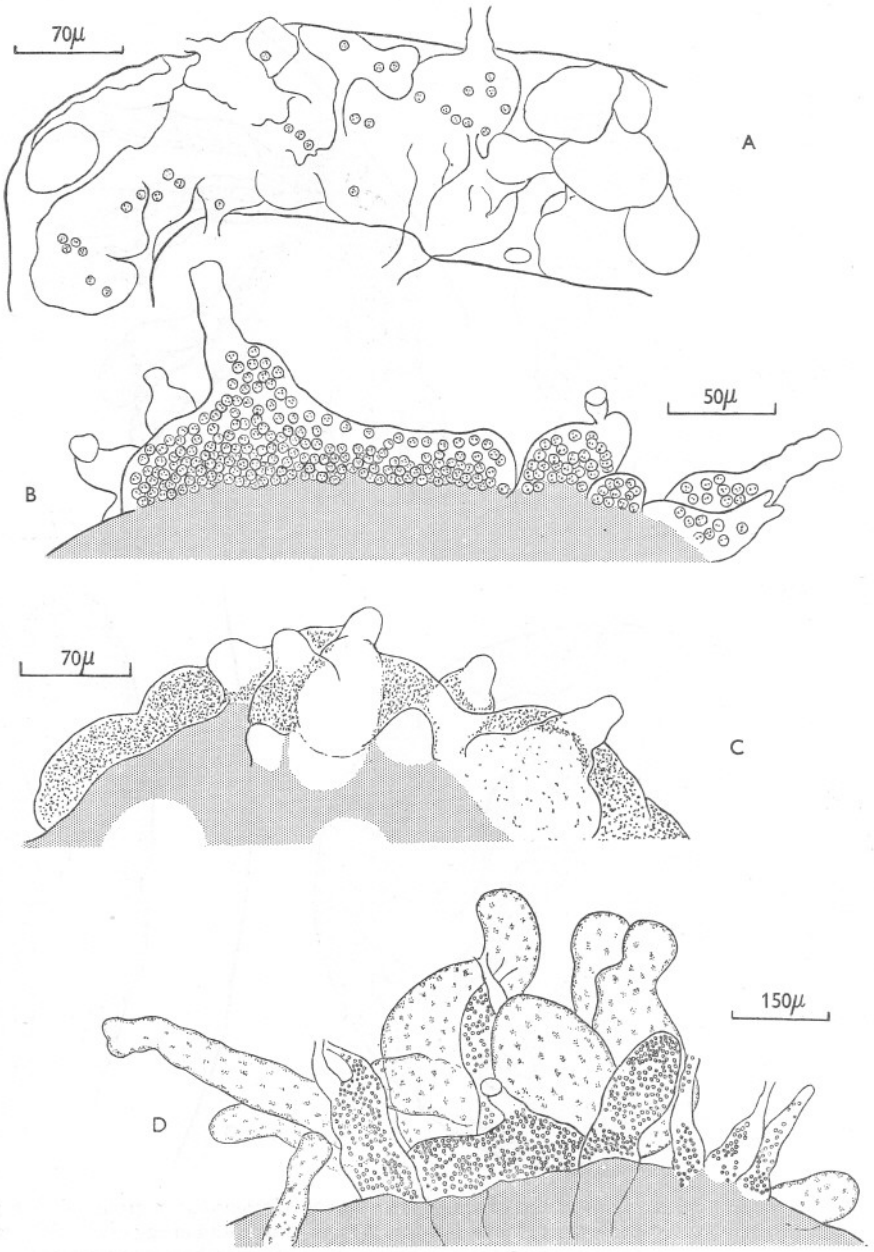

Fig. 2. *Plectospira dubia*. A, sporangia, empty except for a few encysted spores, within abdomen of a zoea; B, sporangia, containing encysted spores, projecting from egg of *Pinnotheres pisum*; C, young sporangia, with large vacuoles, projecting from egg of *P. pisum*: the growing efferent hyphae have clear tips; D, much inflated sporangia projecting from egg of *Crangon vulgaris*: taken from a dish in which there had been some increase in salinity.

in passing through are constricted and have a difficult and slow passage: one was observed to be pinched almost in two, the portion beyond the aperture rounding up as did the larger portion inside, so blocking the aperture. The tips of all the external hyphae of a sporangium do not always give way, as occurs also in *Plectospira myriandra* and *P. gemmifera* (Drechsler, 1927, 1929).

Pyriform flagellated zoospores are formed within the sporangium and efferent hyphae.

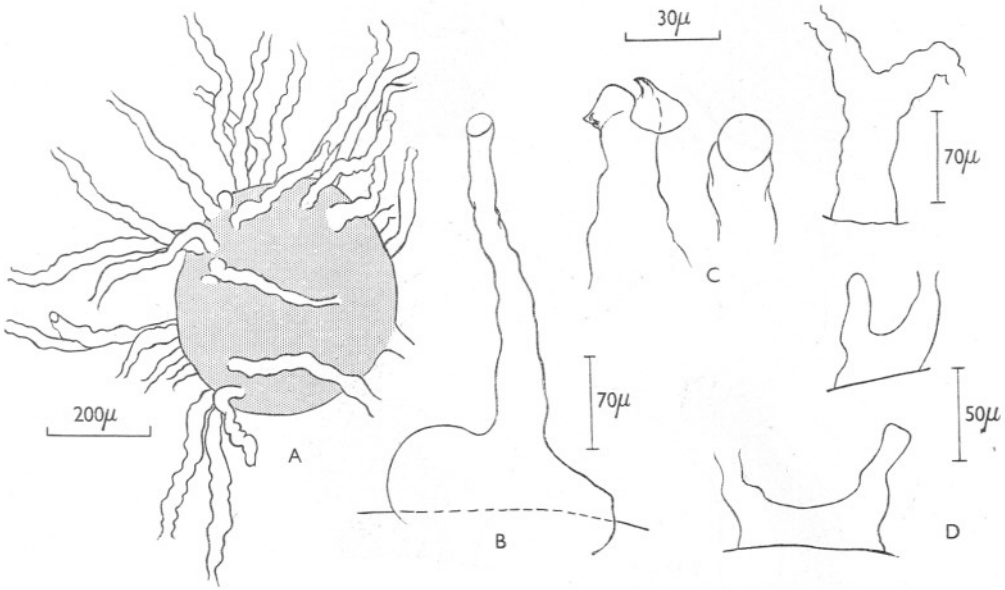

Fig. 3. *Plectospira dubia*. A, corrugated efferent hyphae on egg of *Pinnotheres pisum*; B, external portion of sporangium with a single efferent hypha; C, apertures of efferent hyphae. D, three external portions of sporangia with paired efferent hyphae, two of which have not functioned.

Only about nine instances of zoospore formation have been watched, for it occurs mostly at night, rarely during the day. Spore initials (Fig. 5*c*) are visible for some hours, for as many as five, before moving zoospores are formed. The active formation of zoospores is generally preceded by contraction of the contents of the sporangium. The degree of contraction varies, the contents rapidly withdrawing entirely from the efferent hyphae, and in extreme instances contracting from the wall of the sporangium. It would seem that contraction is greater if it occurs when the spore initials are not widely separated. In one sporangium, watched between 4 and 9.20 p.m. G.M.T. (Fig. 4), the sudden contraction was so great that the appearance of spore initials was lost, and the contents of the sporangium appeared darkly granular with a lighter edge. There followed a gradual expansion, small rounded

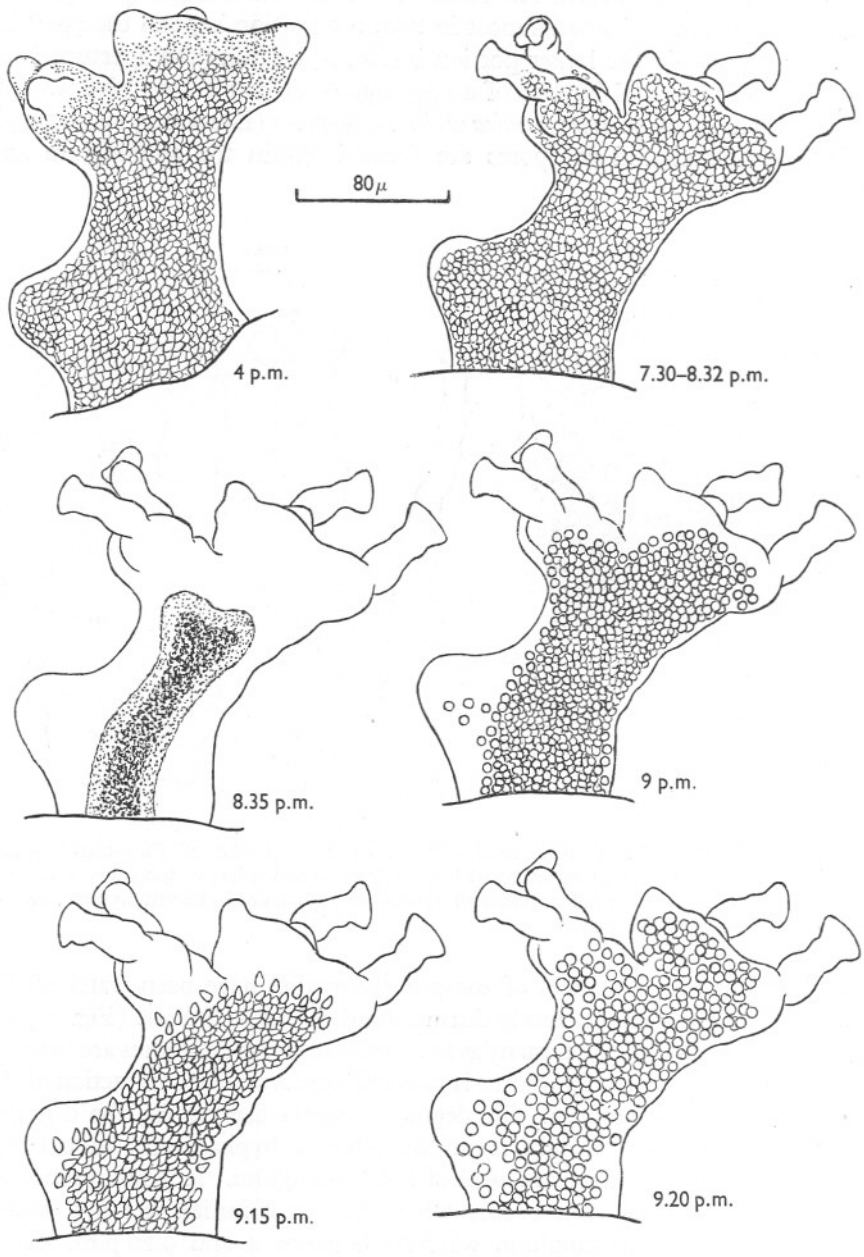

Fig. 4. *Plectospira dubia*. Formation of zoospores in external portion of a sporangium: for description see text pp. 725–7.

bodies appearing on the edge of the protoplasmic mass. Expansion continued and rounded spores became clearly visible; these gradually became pear-shaped, and the appearance was then of a moving and heaving mass of spores, which were evidently flagellated. On the outskirts of the main body single zoospores rocked gently. A high-power objective was lowered into the water to get a clearer view, but unfortunately it had an adverse effect, for the spores rapidly rounded up within the sporangium.

In another sporangium kept under observation at the same time, in a separate watch-glass, the spore initials were widely spaced before contraction, and although the contents withdrew from the two efferent hyphae of a length of about 150μ, there was little contraction in the sporangium itself: the spore initials remained visible. On expansion, the spores reached about half-way along the efferent hyphae. Moving zoospores were visible, but although a few spores moved to near the end of the efferent hyphae, none escaped; all encysted.

One day, from about 5 p.m. G.M.T., four efferent hyphae were kept under observation. In three the process of spore formation was as described, with moderate degree of contraction and expansion of contents of the sporangium and all spores encysted; none escaped. In the fourth, on expansion, a single string of zoospores, diamond-shaped and attached end to end by delicate strands of protoplasm, passed up the efferent hypha. At intervals eight feebly rocking pyriform zoospores slowly emerged from the aperture, swam away slowly at first, then with more speed. No doubt these zoospores were biflagellate, as usual in many members of the Saprolegniacea, but it was not possible to determine this, as it had been found that lowering a high-power objective into the water caused encystment. The remaining spores encysted in the sporangium and did not begin to emerge from the efferent hypha in the second motile stage until about 8 p.m. G.M.T. next day.

It is apparently during the formation of the pyriform zoospores that the tips of the efferent hyphae give way, but it is not always possible to be sure that a pore is actually present unless zoospores escape.

The pyriform zoospores are only feebly active within the sporangium, and that usually for not more than 5–10 min, or less. The time they remain encysted varies: sporangia left with newly encysted spores overnight may be found with empty cysts the next morning, or spores may still be present 20 hours later.

The fact that many crustacean eggs contained mycelia crowded with encysted spores confirms that the behaviour of the pyriform zoospores is as observed in the few instances recorded.

Zoospores in the first motile stage are about 10μ long and when encysted are 7–8μ in diameter.

Owing to the large central vacuole of the sporangium, the generally wide spacing of the spore initials and the length of clear protoplasm at the end of

the efferent hyphae, there is sufficient space for at least some of the pyriform zoospores to round up; these seem to collect round the walls, leaving a central space through which the more internally situated zoospores can pass on their way to the exterior. When crowded the encysted spores are angular.

Zoospores have not been seen actually emerging from cysts within the sporangium, but were seen escaping singly at intervals through the apertures of the efferent hyphae at all times of the day. They are rather long and thin, distinctly grooved longitudinally, with two long, laterally inserted, flagella, one directed anteriorly and one posteriorly: the flagella work with a looping motion. Zoospores in the second motile stage are 11–12μ long: when encysted they are 6–7μ in diameter. Time normally spent in the second swimming stage is unknown. They have been seen to encyst within a few minutes of emerging from the sporangium, but this is unusual and probably due to unfavourable conditions on a slide. These zoospores swim actively within the sporangium and efferent hyphae seeking a way of escape, for the tips of all efferent hyphae do not give way; on emergence they swim rapidly away.

The walls of the sporangia and efferent hyphae are persistent and do not disintegrate or rupture irregularly as do the sporangia of *Thaustotheca* and some species of *Dictyuchus* for the liberation of the laterally biflagellate zoospores (Coker & Matthews, 1937, pp. 50, 52).

The thin-walled empty cysts are visible in the sporangia and basal parts of efferent hyphae after the escape of the zoospores: they are round or angular according to the degree of crowding.

Proliferation

Very occasionally proliferation occurs, a new sporangium growing up within the old one (Fig. 5A, B), as occasionally in *Leptolegnia* and typically in *Saprolegnia*. The new growth may extend beyond the old (Fig. 5B), or may not reach the end of the old efferent hyphae, when it may send out short exit tubes; in the example figured there were eight (Fig. 5A).

Gemmae

The first appearance of *Plectospira dubia* in eggs (with embryos two-thirds developed) removed from a recently dead *Gonoplax rhomboides* occurred a week after their removal, and was under a form quite different from that previously seen: it was distinctive because of spherical bodies of varying size, showing white by reflected and dark by transmitted light. The plants under this form did not entirely fill the egg, and infected eggs were no larger than healthy ones, but the magenta colour from the small yolk remnant was diffused throughout the egg. Young plants dissected out from the egg had the appearance shown in Fig. 5D. The cytoplasm was finely and densely granular; the mycelium was producing rounded bodies up to about 100μ in diameter,

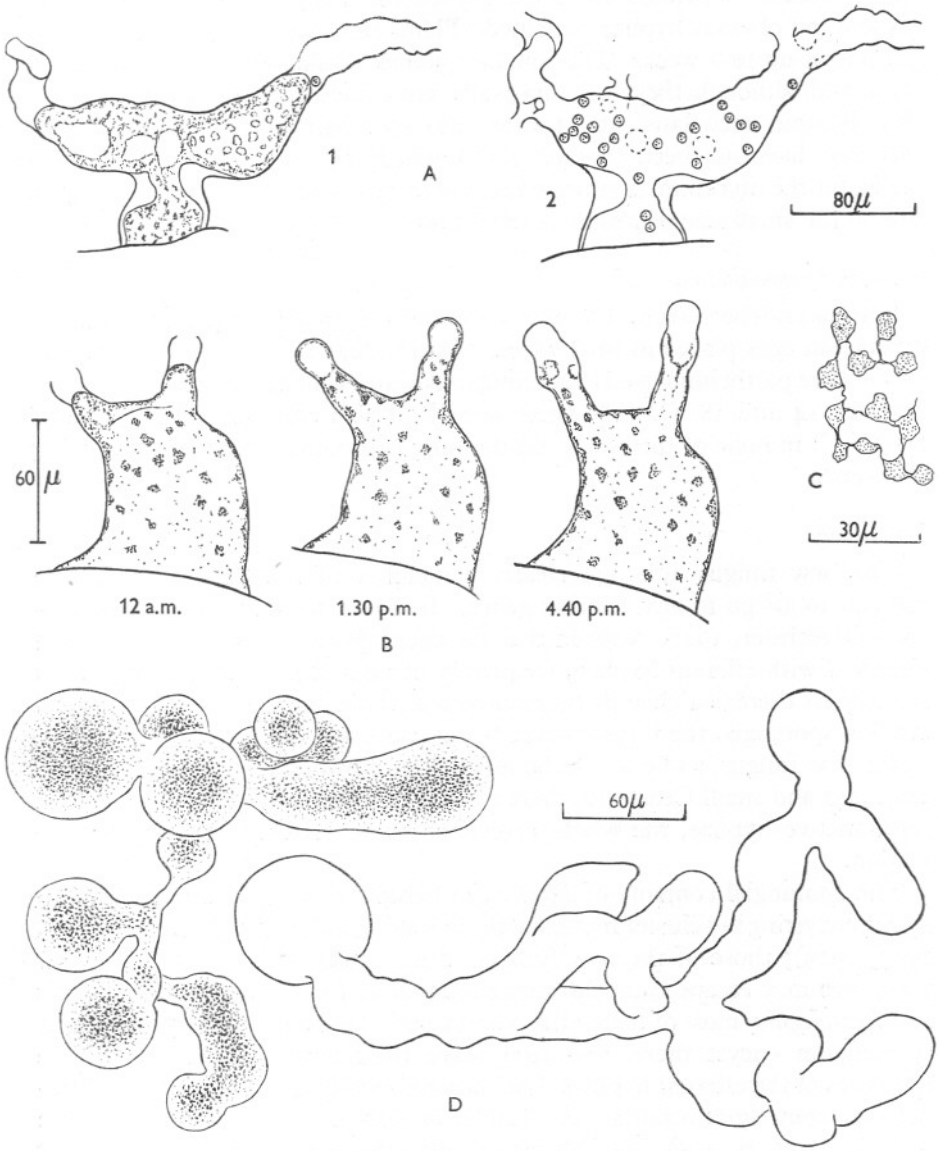

Fig. 5. *Plectospira dubia*. A and B, two instances of proliferation: in A 1 the protoplasm is much vacuolated; in A 2 the apertures of four efferent hyphae on the under surface are indicated by broken lines; C, spore initials in an efferent hypha. D, mycelia producing gemmae, dissected from egg of *Gonoplax rhomboides*: contents indicated in mycelium on left.

which became separated from the mycelium. When many gemmae were present, no obvious hyphae remained. Plants remained in this condition for periods of up to 2 weeks. The rounded bodies would appear to be a resting stage and, although they had thin walls, are evidently gemmae. Eventually they became vacuolated and turned into sporangia with efferent hyphae. Gemmae have not been seen on plants which had already produced sporangia, for the mycelium is entirely used up in zoospore formation: this may be due to the small size of plants in small hosts.

Sexual reproduction

This has not been seen. For over 3 months in 1953 the fungus was grown in crustacean eggs placed in small bowls of sea water of salinity 36–37‰; some bowls were partly immersed in running tank water which kept the temperature between 14 and 18° C, and others were kept at a constant temperature of 15–16° C: in none of the bowls did the fungus produce sexual organs during this time.

Taxonomy

The new fungus although clearly a member of the Saprolegniaceae is difficult to assign to any existing genus. It would seem to resemble *Plectospira* (Drechsler, 1927, 1929) in that the sporangia are composed of inflated elements with efferent hyphae, frequently of uneven diameter, but while in *Plectospira* there is a clear distinction between slender vegetative hyphae and swollen sporangia, the former never being converted directly into sporangia, in the new fungus, so far as can be seen under crowded conditions in crustacean eggs and small Crustacea, there is no distinction between vegetative and reproductive hyphae, the whole mycelium being used up in zoospore formation.

The sporangial contents of *Plectospira* behave as in *Achlya* and *Aphanomyces*, encysting in a cluster immediately on leaving the sporangium (Drechsler, 1927, 1929); those of the new fungus do not. Although a few of the first zoospores may escape from the sporangium after the manner of *Saprolegnia* and *Leptolegnia*, most of them after a short period of feeble activity within the sporangium encyst there, and later leave their cysts and escape by the apertures of the efferent hyphae. The sporangia would seem to be of the false-net type sometimes occurring in *Achlya*, in that the sporangial wall persists (Couch, 1931, p. 225). In *Dictyuchus*, although the first zoospores encyst within the sporangium, the secondary ones either escape from their cysts directly through the wall of the sporangium, or else in the false-net group the wall of the sporangium quickly disappears.

In both *Saprolegnia* and *Achlya*, according to Coker (1923, p. 9), 'it frequently happens that the discharge of the spores is only partial, a few or even a good many spores being left in the sporangium. These retained spores may emerge

from their cysts, as normally, for a second swimming stage, moving about within the sporangium until they find their way out by its mouth, if they ever do.' This partial retention of the pyriform zoospores in *Saprolegnia* and *Achlya* is carried much further in the new fungus and is the normal occurrence; moreover the latter differs from both *Saprolegnia* and *Achlya* in that the sporangia have no definite shape, but are lobulate inflated segments.

Rather than create a new genus for this marine fungus, while the sexual organs are unknown, it is proposed to place it provisionally in the genus *Plectospira* with the specific name *dubia*.

Diagnosis

Mycelio plerumque intramatricali, rudi, composito de hyphis irregularibus latitudine (50–100 μ); sporangiis irregulariter inflatis, subinde proliferosis, depletis per hyphas efferentes, longitudine variabiles et saepe latitudine irregulares; sporangii pariete persistenti; zoosporis primas et secundas capsulas formantibus, primo tamen statu natanti plerumque suppresso; zoosporis plerumque capsulas formantibus intra sporangium postquam paulisper infirmiter nataverunt, postea valide natantibus ad secundum statum; sporis tum 6–8 μ latitudine cum in capsulas inclusa sunt; gemmis numerosis, sphaeralis, ad 100 μ latitudine; partibus sexualibus ignotis.

Mari incolens; parasitica in ovis crustacearum et in parvis crustaceis.

Type material has been deposited in the British Museum (Natural History).

This work was made possible by the kindness of the Board of Studies in Zoology of London University in granting me the use of the London University Table at the Laboratory of the Marine Biological Association at Plymouth, and was done while working on the biology of *Pinnotheres*. I am indebted to Dr B. Barnes for reading the manuscript. Dr J. Morton has most kindly turned the diagnosis into Latin.

Summary

A marine fungus, *Plectospira dubia* n.sp., has been found infecting crustacean eggs and small Crustacea in the Plymouth Laboratory. It has the following characteristics: mycelium mostly intra-matrical, coarse, hyphae 50–100μ in diameter. Sporangia irregularly inflated, emptying by efferent hyphae of variable length and frequently of irregular diameter; sometimes proliferous; wall persistent. Zoospores dicystic and dimorphic, but the first swimming stage largely suppressed, the first zoospores mostly encysting within the sporangium after a short period of feeble activity, then swimming in the second stage. The first zoospores pyriform and flagellate (probably biflagellate): in the second motile stage laterally grooved with two laterally inserted flagella. First zoospores about 10μ long, when encysted 7–8μ in diameter; in the second motile stage 11–12μ long, when encysted 6–7μ in diameter. Gemmae numerous, spherical, up to 100μ in diameter. Sexual organs so far unknown.

REFERENCES

ATKINS, D., 1929. On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. *J. Mar. biol. Ass. U.K.*, Vol. 16, pp. 203–19.

—— 1954*a*. Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n.sp., infecting certain invertebrates. *J. Mar. biol. Ass. U.K.*, Vol. 33, pp. 613–25.

—— 1954*b*. *Pythium thalassium* n.sp. infecting the egg-mass of the pea-crab *Pinnotheres pisum*. *Trans. Brit. mycol. Soc.* vol. 37, pt. 4. (In the Press.)

COKER, W. C., 1923. *The Saprolegniaceae, with Notes on other Water Molds*. University of North Carolina Press.

COKER, W. C. & MATTHEWS, V. D., 1937. Saprolegniales. *N. Amer. Flora*, Vol. 2, pt. 1.

COUCH, J. N., 1931. Observations on some species of water molds connecting *Achlya* and *Dictyuchus*. *J. Elisha Mitchell sci. Soc.*, Vol. 46, pp. 225–9.

DRECHSLER, C., 1927. Two water molds causing tomato rootlet injury. *J. agric. Res.*, Vol. 34, pp. 287–96.

—— 1929. The beet water mold and several related root parasites. *J. agric. Res.*, Vol. 38, pp. 309–61.

J. Mar. biol. Ass. U.K. (1954) 33, 733–738
Printed in Great Britain

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

II. INFLUENCE OF PERFUSION PRESSURE ON URINE VOLUME

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(Text-figs. 1–4)

In a previous publication (Brull, Nizet & Verney, 1953) it was shown that the kidneys of *Lophius*, perfused with heparinized blood, secrete urine which is similar to bladder urine. One of the conclusions was that 'changes in perfusion pressure up to 100–150 mm of water, raised the urine flow; above 150 mm there was no effect'. The object of the experiments described below was to investigate the relationship between perfusion pressure and urine flow, especially below the critical level of 150 mm water.

The experiments were carried out in July–August in the Plymouth Laboratory, where we were provided with a large supply of live *Lophius*, for which we are much indebted to the Director and Staff of the Laboratory.

Methods

We improved our perfusion technique by the use of a pump made of plexiglass, and a small rotating oxygenator built by one of us (Y. Cuypers) for this purpose.

The pump and oxygenator were started working before the kidneys were connected, so as to oxygenate the blood. A closed circulation circuit was made with plastic tubes, from the reservoir to the kidneys and from the kidneys to the pump. To prevent twisting of the vessels, the kidneys were laid on a thin layer of perforated wood on to which the cannulae could be tied down.

The venous flow was measured directly with a burette and stop-watch. The experiments were carried out at room temperature.

Results

Experiment 9

Two kidneys, from the same *Lophius* weighing 4·8 kg, were perfused by the same pool of blood, kidney A at a pressure of 80 mm water, and kidney B at 260 mm throughout the experiment (Table I).

The blood flow in kidney B was much higher than in A; but the figure was missed by accident. In this pair of kidneys, the urine flow was no better at 260 mm than at 80 mm; we are very likely above the critical maximum level of perfusion. The secretion needs to be studied while starting at lower perfusion pressures.

TABLE I. PERFUSION EXPERIMENT 9

	Kidney A (weight 9·5 g)				Kidney B (weight 9·5 g)			
		Urine				Urine		
Time (min)	Blood flow (ml./g/min)	No.	Vol. (ml.)	Flow (ml./g/h)	Blood flow (ml./g/min)	No.	Vol. (ml.)	Flow (ml./g/h)
0	Perfusion started							
30	—	—	—	—	Perfusion started			
45	Pressure regulated at 80 mm				Pressure regulated at 260 mm			
71	—	First drop in 3 min			—	First drop in 3 min		
130	—	1	1·0	0·10	—	—	—	—
145	—	—	—	—	—	1	1·0	0·09
245	1·3	2	2·0	0·13	—	2	1·7	0·10

Both ureteral cannulae contain 0·5 ml. extra urine.

TABLE II. PERFUSION EXPERIMENT 13

	Kidney A				Kidney B			
Time (min)	Perfusion pressure (mm blood)	Blood flow (ml./g/min)	Urine no.	Urine flow (ml./g/h)	Perfusion pressure (mm blood)	Blood flow (ml./g/min)	Urine no.	Urine flow (ml./g/h)
0	80	Perfusion started						
15	—	—	First drop					
45	—	—	—	—	25	Perfusion started		
60	80	0·45	—	—	25	0·10	—	—
74	—	—	1	0·18	—	—	—	—
100	80→70	—	—	—	25→70	—	—	—
107	70	0·40	—	—	—	0·70	—	—
120	—	—	—	—	—	—	First drop	
140	—	—	2	0·16	—	—	—	—
145	70→45	—	—	—	—	—	—	—
150	—	0·30	—	—	—	—	—	—
200	45→75	0·24	3	0·12	—	—	1	0·80
295	75	—	4	0·11	70	—	2	0·10

Experiment 13

Perfusion with the same pool of blood was carried out on two kidneys: kidney A weighing 11 g, from a fish of 6·4 kg; kidney B weighing 6 g, from a fish of 3·5 kg (Table II). At the first three stages of perfusion pressure, 80, 70 and 45, the urine flow of kidney A is parallel to the pressure: 0·18, 0·16 and 0·12. After 3 h 20 min of perfusion, the kidney no longer responds to a rise of pressure from 45 up to 75. Perfusion of kidney B at the low pressure of 25 gives no urine; at 70 mm secretion starts with an average output of 0·09.

Exps. 9 and 13 confirm our previous results, namely that the kidney is sensitive to changes in pressure below about 100 mm of water. We therefore decided to investigate this in detail by recording the number of drops of urine as a function of perfusion pressure.

Experiments 21–23

In these three experiments (see Fig. 1) two kidneys from the same fish were simultaneously perfused by the same pool of blood at rising or decreasing pressures. The arrow on the tracings indicates whether the perfusion is started at low or high pressure. Urine drops were recorded and the urine flows are translated into ml per gram of kidney per minute.

At each rise of pressure the reaction of the kidney is usually slow, and a certain time may elapse before the urine flow reaches its maximum, as shown where some curves slope up at the beginning and down later when the pressure is lowered (22 B).

In Exp. 21, we succeeded in perfusing one kidney only; during the first 2 h of perfusion, the pressure was progressively lowered (see curve $21B_1$), while during the next 2 h, the perfusion was carried out at rising pressures ($21B_2$).

The most striking facts shown by the slope of the six curves would seem to be the following. (1) Below about 25–30 mm pressure, no urine is secreted. (2) Between 30 mm and about 200–250 mm, the volume of urine secreted per minute follows the perfusion pressure in a regular way, the response following an exponential curve. (3) Above perfusion pressures of about 200–250, the urine flow is no longer influenced by further rises of pressure; this last finding confirms the results of the first publication on the same subject (Brull *et al.*, 1953).

The *blood flow* through the kidneys is of the same magnitude as in the experiments previously published (Brull *et al.*, 1953), in which arterialized blood was used. The results are shown in Figs. 2–4. In kidney A, Exp. 23, and at low pressure, we found the curve had the same tendency as reported before to change its slope (Fig. 4).

Discussion

In our opinion, one striking fact appears from these results: below a critical perfusion pressure varying from about 100 up to 225 mm from one kidney to the other, the urine flow responds to the pressure in the form of an exponential curve. We have plotted the figures of urine flow against the blood flow, but have not reproduced them here, because these curves are similar to those shown in Fig. 1. It would be interesting to find out which of the two factors is the determining one: pressure or blood flow.

In Exp. 21 (Fig. 2), there are two perfusion phases: we started with a pressure of 190 mm, decreased it to 75 mm, and raised it again to 220 mm;

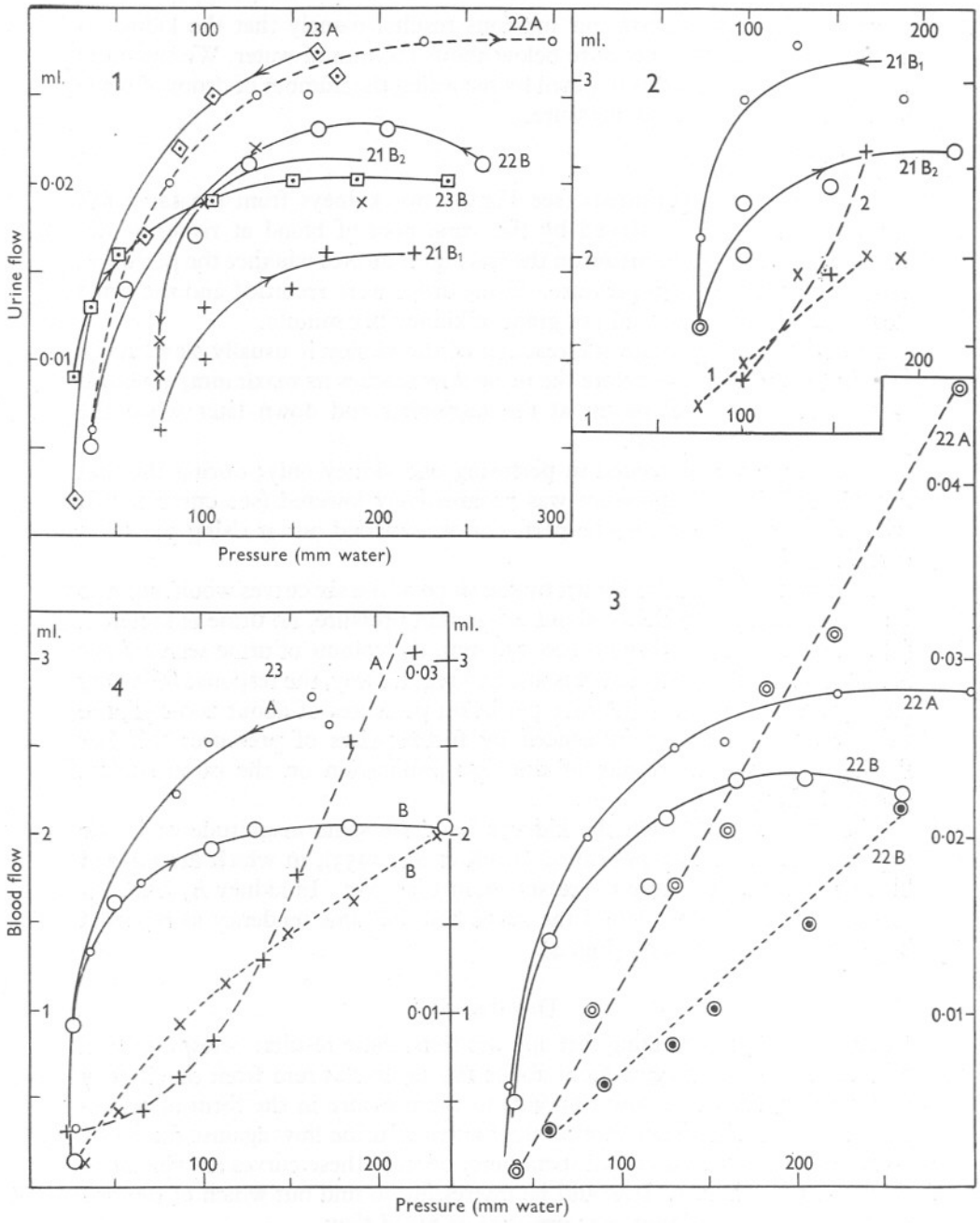

Fig. 1. Urine flow (in ml./g/min) plotted against perfusion pressure (in mm water) in five kidneys, two in Exp. 22, two in Exp. 23, one in Exp. 21 at two stages. The arrows indicate whether the perfusion started at high or at low pressure.

Figs. 2–4. Renal blood flows (dotted lines) and urine flows (plain lines) in three experiments.

during the first stage, the curve of urine flow runs higher than during phase 2, whereas there is no similar difference to be seen in the blood flow.

In Exp. 22 (Fig. 3), two kidneys were perfused at the same ranges of pressure, kidney A starting from a low level, kidney B from a high level; the first kidney produces more urine, and its blood flows are higher. Exp. 23 (Fig. 4) was carried out under similar conditions: here, kidney A, with higher urine flows, has higher blood flows at the beginning and lower ones at the end.

Owing to the inconsistency of these results, we cannot conclude that blood flow may be more determinant than perfusion pressure. The question remains open.

The question arises whether the water is secreted through a purely physical force or through an active process. A detailed cataphoretic analysis of plasma proteins has been published in a previous paper (Brull & Nizet, 1953) and gave 39 g/l. of total proteins with 6·7 albumin and 93·3% globulin. This might mean a colloid osmotic pressure of about 6·5 mm of mercury or 85 mm of water. Since urine is secreted at pressures of 30 and 40 mm of water, this must be done against the osmotic pressure of the proteins, through an active process.

On the other hand, Bieter (1931) found that the kidney of *Opsanus tau*, also aglomerular, secretes urine at a pressure higher than that in the dorsal aorta. Yet this finding is irrelevant when *Lophius* is considered, since Nizet (in press) has shown that China ink injected into the arterial blood does not reach the tubules, going no farther than the capsulae. The blood supply to the tubules is conveyed solely by the renal portal veins.

Thus, admittedly, the tubules of *Lophius*, which have lost their glomeruli during the first stage of life, secrete water like a salivary gland. Have glomular kidneys lost such a property? This question will be worth discussing elsewhere, in the light of research done by one of us on the kidney of the dog.

Let us consider for a moment the *oxygen consumption* of the *Lophius* kidney. We already know (Brull *et al.*, 1953) that perfusion with oxygenated blood does not provide more, or more concentrated, urine than venous blood as it is collected from the fish. In the present experiments, our attempts to measure the degree of oxygenation of the blood have shown that the venous blood which normally irrigates the kidney contains no 'measurable' amount of oxygen. If an increased flow of arterialized blood acted merely by way of increasing the oxygen supply, it would be difficult to explain how a similar increase of venous blood supply would produce a result of the same magnitude.

It seems that the oxygen requirement of the *Lophius* kidney is very small. Its significance requires further study. Still, there are very active enzymic systems in such kidneys, considering they concentrate Mg about 50 times (with venous blood as well).

SUMMARY

The urine secretion of the kidneys of *Lophius piscatorius* perfused with heparinized *Lophius* blood is very sensitive to perfusion pressure below a critical level, above which it becomes insensitive. The response of the urine flow to pressure takes the form of an exponential curve.

The blood flow through the kidneys, while rising slowly at pressures of about 20–30 mm of water, responds arithmetically to pressure above such levels.

At present it is impossible to make out whether pressure or blood flow has the greatest influence on secretion.

Water secretion in the aglomerular kidney is an active process. The oxygen consumption of the *Lophius* kidney is unmeasurably low, yet remains a possible factor in secretion.

REFERENCES

BIETER, R. W., 1931. The secretion pressure of the aglomerular kidney. *Amer. J. Physiol.*, Vol. 97, pp. 66–8.

BRULL, L., NIZET, E. & VERNEY, E. B., 1953. Blood perfusion of the kidney of *Lophius piscatorius* L. *J. Mar. biol. Ass. U.K.*, Vol. 32, pp. 329–36.

BRULL, L. & NIZET, E., 1953. Blood and urine constituents of *Lophius piscatorius* L. *J. Mar. biol. Ass. U.K.*, Vol. 32, pp. 321–8.

NIZET, E., (In the Press). Quelques aspects de l'anatomie du rein du *Lophius piscatorius*. *Pubbl. Staz. zool. Napoli.*

J. Mar. biol. Ass. U.K. (1954) 33, 739–747
Printed in Great Britain

NOTES ON THE GROWTH AND BIOLOGY OF THE PRAWN *SPIRONTOCARIS LILLJEBORGII* (DANIELSSEN)

By R. B. Pike

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(Text-figs. 1–3)

The biology of the spirontocarids appears to have attracted little attention and the larvae of the European species are imperfectly known.

While working on the prawn *Pandalus bonnieri* Caullery (Pike, 1952), a considerable number of *Spirontocaris lilljeborgii* (Danielssen) were obtained on the same ground. This spirontocarid was infected by the bopyrid parasite, *Phryxus abdominalis* Kröyer. Collections of the prawn were made so that its biology, as well as that of the bopyrid, could be studied.

The author expresses indebtedness to colleagues at the Marine Station for assistance in preparing this paper, and to the Skipper and the crew of M.V. *Calanus* for their unfailing help in collecting material; to Miss E. R. Wallace for assisting in measuring specimens and to Mr J. N. Thomson for cutting and staining the sections.

Nomenclature

Most European authors appear to have regarded *Spirontocaris lilljeborgii* as a distinct species from *S. spinus* (Sowerby). Appellöf (1906) does point out that although the two forms are usually distinct on the Scandinavian coast, intermediate forms have been obtained off Iceland. Kemp (1910) reviewed the situation while dealing with the Decapoda Natantia of Ireland and decided to call *S. lilljeborgii* a variety of *S. spinus* (*S. spinus* var. *Lilljeborgi* (Danielssen)). Holthuis (1947) has since reviewed the known species of Hippolytidae and has divided the genus *Spirontocaris* into further genera and given this variety specific status.

Collection of Material

All the prawns were collected from the Cumbrae Deep ground in the Firth of Clyde, Scotland. This ground is situated off the Isle of Bute (National Grid Reference 26/1159–1162), at a depth of 70–90 m. The gear used for collecting the adults was an 8 ft agassiz trawl having a net mesh of 1·3 cm bar; this was found to retain prawns of above 7 mm carapace length. A one-metre modified agassiz, working 15 cm above the bottom and fitted with a mesh of 1 cm bar was used for younger prawns, and the same frame fitted with a one-metre

stramin net was used for the collection of larvae and early post-larval forms. It was difficult to obtain adequate samples of all size groups in a single day, especially in the summer when the finer mesh nets had to be used for the smaller size groups. Frequently, 2 days were required to obtain an adequate sample, and in each case the earlier date for sampling is given; the second sample was obtained within 7 days of the first sample.

Measurement of Material

The standard measurement made was the 'length of the carapace', being the distance from the posterior margin of the eye socket to the dorsal posterior margin of the carapace. All early post-larval prawns were measured under the binocular microscope. The larger specimens were measured with fine draughtsman's calipers to the nearest 1 mm below and the data recorded in 1 mm groups.

Table I

Carapace length (mm)	Total length (mm)			No. of eggs
	Juveniles	Males	Females	
1	—	—	—	—
2	11·8	—	—	—
3	13·6	—	—	—
4	—	20·0	18·5	—
5	—	23·5	22·6	—
6	—	26·2	28·1	—
7	—	33·8	33·5	130
8	—	36·5	37·1	164
9	—	40·3	42·3	256
10	—	—	44·9	430
11	—	—	48·0	516
12	—	—	50·2	687
13	—	—	52·3	577

At the same time total lengths, tip of rostrum to the tip of telson were measured, and the arithmetic mean taken for thirty specimens in each mm size group. These means are shown in Table 1, together with the mean number of newly laid eggs taken from five individuals in each available mm size group.

Geographical Distribution

S. lilljeborgii is common in the Skagerrak and along the south and west coasts of Norway and has been recorded from Iceland (Stephensen, 1939, p. 17). It is not recorded by the same author (1928, p. 13) for the Faeroes, and he is doubtful of his East Greenland record (1912, p. 510). This doubt is also shared by Heegaard (1941, p. 42). Southward, *S. lilljeborgii* extends from the Irish Sea into the English Channel on the west and to the Norfolk coast on the east. *S. spinus*, however, is obtained from the Kattegat and along the whole west coast of Norway. It is recorded in small numbers from the Faeroes area,

occurs on both the east and west coast of Iceland, and Greenland and as far north as Spitzbergen, Franz Joseph Land and Ellesmere Island. Its southern range is rather uncertain. According to Kemp (1910) only one rather atypical form was obtained from Irish waters. Dr N. S. Jones from the Marine Station, Port Erin, Isle of Man, informs me that he has obtained typical *S. spinus* from the Isle of Man, although *S. lilljeborgii* is more usually obtained. Thus in the British Isles the southern limit for *S. spinus* appears to be the Irish Sea on the west and possibly the Norfolk coast on the east.

Occurrence in the Clyde

As already stated, *S. lilljeborgii* occurs along with *Pandalus bonnieri* on a soft muddy substratum, inhabited by a community similar to that associated with *P. borealis* (Kröyer), as described by Hjort & Ruud (1938). I have already detailed (Pike, 1952) the main animal communities of this ground, and further details can be obtained from Allen (1953). Heegaard (1941) notes that *Spirontocaris spinus* in Greenland prefers shallow water (12–50 m) which is considerably shallower than its habitat in Spitzbergen. He suggests that this is possibly because *Balanus porcatus* da Costa, among which it hides, often occurs at Spitzbergen at greater depths than off the east coast of Greenland, and in both places *Spirontocaris spinus* is always found in association with this barnacle. As *S. lilljeborgii* does not occur in Greenland no similar observations were made for that species. *Balanus porcatus* is generally distributed within the Clyde, but does not occur in any number on the ground from which the specimens of *Spirontocaris lilljeborgii* have been obtained for this work, and no association with *Balanus* has been observed.

Migration

The adults were extremely difficult to obtain during May and June, that is, just after the breeding season when they are moulting. It is thought that an inshore migration takes place at this time and that the prawns do not return again in numbers to the deeper water until July.

Breeding

In the Clyde the ovaries of the female prawns begin to mature at the end of August (Fig. 1, A, 1) when the developing eggs can be seen through the cuticle of the thorax as sage green masses. Egg-laying begins late in November, and is about complete in a month's time. Incubation of the eggs lasts 3 months.

My records for 1951 show that on 21 November out of 100 females of *Spirontocaris lilljeborgii* captured, two were in berry. By 7 January 1952, nine out of 200 females carried eggs showing eye pigmentation, and on 19 February four out of 205 females had hatched their eggs. These dates and figures suggest

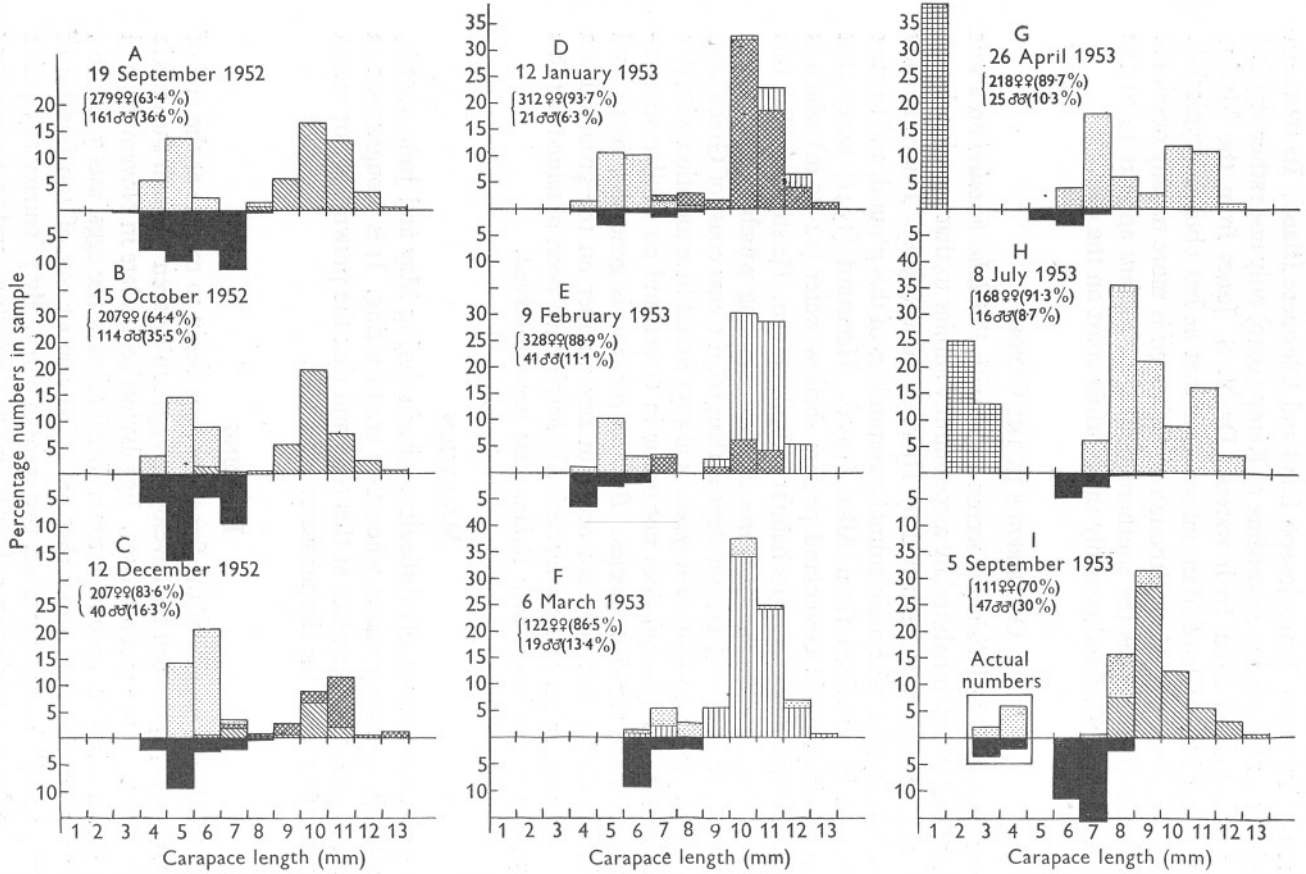

Fig. 1. Breeding in *Spirontocaris lilljeborgii*. ■, males (below the datum line); females: ▯, resting stage; ▨, ovaries green; ▩, carrying eggs; ▥, eggs with eye pigment; ▦, early post-larvae.

a total incubation period of 90 days. Egg-laying takes place about the time when the bottom sea temperature is highest (surface *c.* 9·6° C, bottom *c.* 11·5° C), and hatching when it is lowest (surface *c.* 7·1° C, bottom *c.* 6·5° C). Eye pigmentation first becomes visible in the egg about the 47th day after egg-laying. This is in keeping with the observations of Höglund (1942) who found that in *Leander squilla* (L.), kept in aquaria, eye pigmentation became visible half-way through egg development. The egg of *L. squilla* measures *c.* 0·45 × 0·59 mm whereas that of *Spirontocaris lilljeborgii* is much larger, *c.* 1·0 × 0·9 mm. There appears, however, to be little difference either in the time taken for the eggs to develop or in the number of larval stages in these two prawns.

Rate of Growth

The data obtained on growth are shown in graphic form in Figs. 1 and 2 and summarized in Table II. In preparing Fig. 2 the smallest and largest size has been shown for the age groups on the dates given in Fig. 1 and the lines that appeared to fit these drawn to show the general trend. The third year (II-group) is doubtful for both males and females, as the numbers are very small, and the difference in size might be explained by differential growths within the sexes during the second year. It did not seem advisable, on the data available, to work out the arithmetic means. The larvae of *S. lilljeborgii* were first recorded in 1953 on 5 March which is somewhat later than in 1952. In both cases hatching appeared to be complete in about a month. Records show that the larvae take 4–5 weeks to pass through the larval stages and become bottom living. They grow quickly and reach a carapace length of from 2–3 mm by early July (Fig. 1, H). Growth continues to be rapid until September when at a length of from 3 to 4 mm (Fig. 1, I) the males can be distinguished from the females by the shape of their pleopods. At this time the ratio of males to females is approximately 1 : 1.

By December the males have reached a carapace length of 4–6 cm and the females from 5 to 7 cm. From now onwards the females grow much faster than the males, as will be seen in Table I and Fig. 2. During the winter months growth almost ceases for both sexes.

The usual life span for males appears to be 18 months, with a possible maximum of 30 months. With the females 24 months is the usual life span, although a few appear to live for 36 months.

The majority of the first-year males are sexually mature by the first autumn and probably fertilize the females, while only the largest of this 0-group females are able to breed. (Fig. 1, B, C). By December of their first year the largest of the 0-group males have reached the same size as the smallest of the I-group males of the previous year. After the I-group male population has fertilized the females they appear to die (Fig. 1, C). After December it was difficult to obtain any males and it was not until September

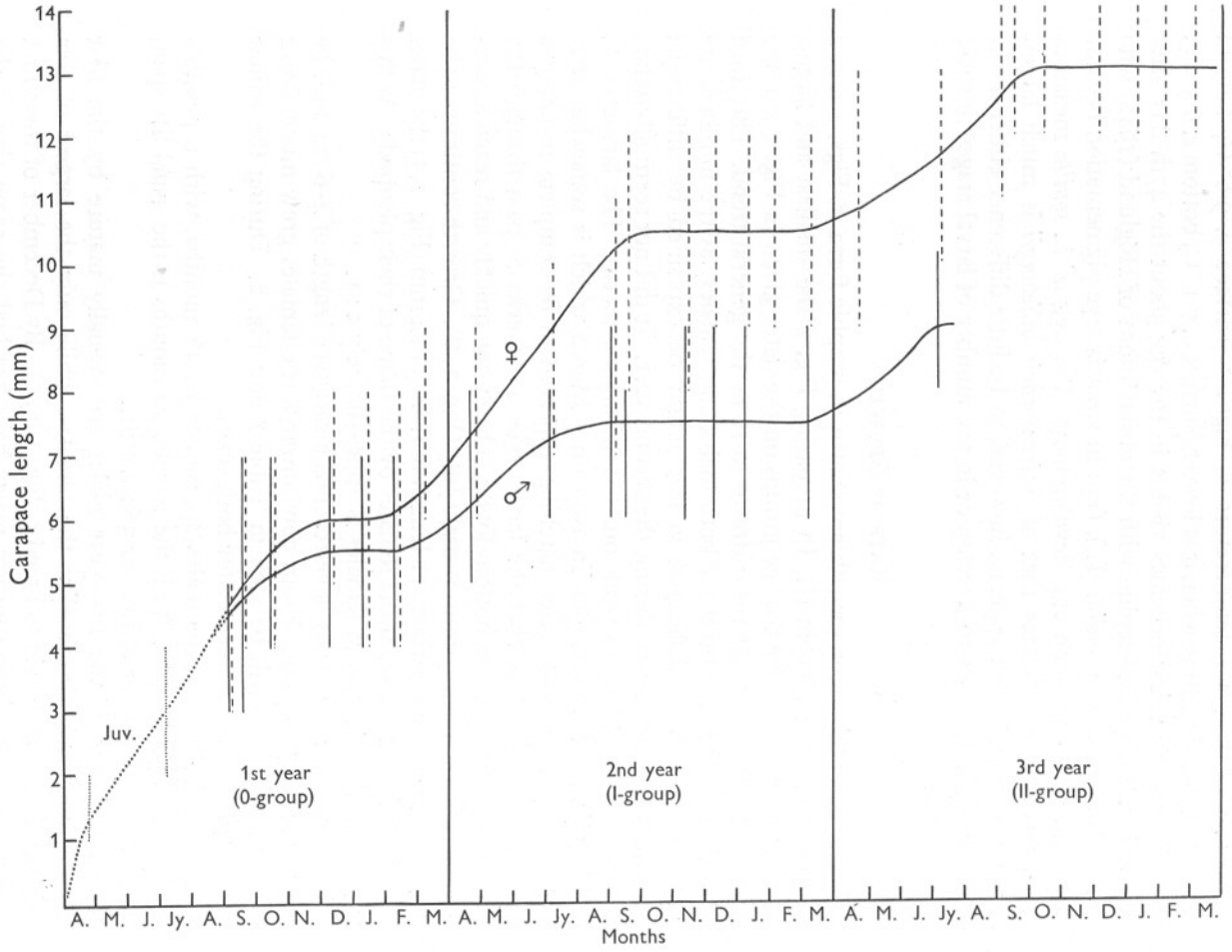

Fig. 2. Growth in *Spirontocaris lilljeborgii*.

following that a number of males were again collected (Fig. 1, 1). This same scarcity of males has been noted over 2 years of sampling, and on no occasion has the collection of the second year (I-group) male population exceeded 30 % of the total female population. I have pointed out above that the ratio of males to females for the 0-group in September and October is roughly 1 : 1, and although later sampling may be inadequate the ratio is probably less than

TABLE II. GROWTH OF *SPIRONTOCARIS LILLJEBORGII*

	0-group		I-group		II-group	
Month of year	Age (months)	Carapace length (mm)	Age (months)	Carapace length (mm)	Age (months)	Carapace length (mm)
26 Apr. (G)	0–1	1·1–1·9	12–13	♂ 5·1–7·9 ♀ 6·1–8·9	24–25	♂ Absent ♀ 9·1–12·9
8 July (H)	1–4	2·1–3·9	13–16	♂ 6·1–7·9 ♀ 7·1–9·9	25–28	♂ 8·1–9·9 ♀ 10·1–12·9
5 Sept. (I)	4–5	3·1–4·9	16–18	♂ 6·1–8·9 ♀ 7·1–10·9	28–30	♂ Absent ♀ 11·1–13·9
19 Sept. (A)	4–5	♂ 3·1–6·9 ♀ 4·1–6·9	16–18	♂ 6·1–8·9 ♀ 8·1–11·9	10–18	♂ Absent ♀ 12·1–13·9
15 Oct. (B)	6–7	♂ 4·1–6·9 ♀ 4·1–6·9	18–19	♂ 6·1–7·9 ♀ 8·1–11·9	30–31	♂ Absent ♀ 12·1–13·9
12 Dec. (C)	7–9	♂ 4·1–6·9 ♀ 5·1–7·9	19–21	♂ 6·1–8·9 ♀ 9·1–11·9	31–33	♂ Absent ♀ 12·1–13·9
12 Jan. (D)	9–10	♂ 4·1–6·9 ♀ 4·1–7·9	21–22	♂ 6·1–8·9 ♀ 9·1–11·9	33–34	♂ Absent ♀ 12·1–13·9
9 Feb. (E)	10–11	♂ 4·1–6·9 ♀ 4·1–7·9	22–23	♂ Absent ♀ 9·1–11·9	34–35	♂ Absent ♀ 12·1–13·9
6 Mar. (F)	11–12	♂ 5·1–7·9 ♀ 6·1–8·9	23–24	♂ 7·1–8·9 ♀ 9·1–11·9	35–36	♂ Absent ♀ 12·1–13·9

1 : 2. It looks therefore as though some of the males die in their first year after fertilizing the females. To try and find out if this was the true ratio of males for the I-group for September 1953 (Fig. 1, 1) sampling was carried out over 3 days with the 1-m agassiz using the 1 cm bar mesh, so that no small males should escape. By this means it was found that the ratio of males to females was just under 1 : 2. Such a method of collecting was too slow for general use, and the fine mesh was used for not more than half the collecting time.

SEX CHANGES

No change over from the male sex to the female sex has been observed. To check this point dissections of both sexes have been made and serial sections cut. The vas deferens of the male is a very thick and tightly coiled tube, and if any protandry occurred one would expect some trace of the vas deferens to remain after the change in sex had taken place, but this was not seen. The male pleopods are quite distinctive and intermediate forms should be easily seen during the examination of the male appendages. Lastly, serial sections through the male testis showed no trace of oocytes in the testicular tissue.

Secondary Sexual Characters

Höglund (1942) has given details of the breeding-dress in some Swedish eucyphideans (Caridea), and has described and figured the 1st pair of female pleopods for *S. lilljeborgii* during the breeding season, and during the rest of

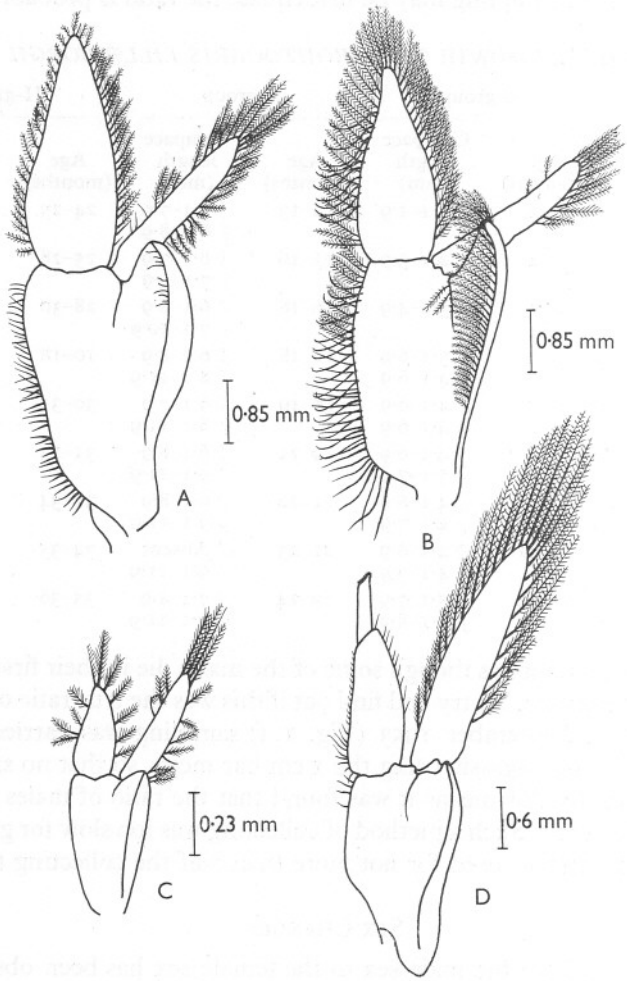

Fig. 3. The right pleopod of *Spirontocaris lilljeborgii*. A, female during the non-breeding season; B, female during the breeding season; C, juvenile; D, adult male.

the year. I agree entirely with his findings, and for completeness have made drawings of these, from my material, together with the undifferentiated pleopods of the juvenile and also those of the adult male prawn (Fig. 3).

SUMMARY

Spirontocaris lilljeborgii is a dioecious species and no protandrous hermaphrodites have been seen.

The majority of males become sexually mature in the first year (7–9 months). Some of these may die after fertilizing the females and the majority die after fertilizing the females for a second year (18 months old).

Only a small proportion of the females breed in their first year, all breed during their second year and a few appear to live for a third year.

A first-year female lays from 130 to 160 eggs, and the usual output of a second-year female is from 450 to 650 eggs.

Egg laying begins at the end of November and hatching is complete by the end of March.

The total incubation period is about 90 days. Eye pigmentation first appears at about half the incubation period (47 days).

Juveniles can be distinguished as males and females by the shape of their pleopods within 4–6 months of hatching.

REFERENCES

ALLEN, J. A., 1953. Observations on the epifauna of the deep-water muds of the Clyde Sea Area, with special reference to *Chlamys septemradiata* (Müller). *J. Anim. Ecol.*, Vol. 22, pp. 240–60.

APPELLÖF, A., 1906. Die Dekapoden Crustacean. *Meeresfauna, Bergen*, Heft 2 and 3, pp. 133–233.

HEEGAARD, P. E., 1941. The Zoology of East Greenland. Decapod Crustaceans. *Medd. Grønland*, Bd. 121, No. 6, 72 pp.

HJORT, J. & RUUD, J., 1938. Deep-sea prawn fisheries and their problems. *Hvalråd. Skrift.*, No. 17, 144 pp.

HÖGLUND, H., 1943. On the biology and larval development of *Leander squilla* (L.) forma *typica* de Man. *Svenska hydrogr.-biol. Komm. Skr.*, N.S. (Biol.), Bd. 2, Nr. 6, 43 pp.

HOLTHUIS, L. B., 1947. The Decapoda of the Siboga Expedition. Pt. IX. The Hippolytidae and Rhynchocinåtidae collected by the Siboga and Snellius expeditions, with remarks on other species. *Siboga Exped.*, Monogr. 39, a^8, 100 pp.

KEMP, S., 1910. The Decapoda Natantia of the coast of Ireland. *Sci. Invest. Fish. Br. Ire.*, 1908, No. 1, 190 pp.

PIKE, R. B., 1952. Notes on the growth and biology of the prawn *Pandalus bonnieri* Caullery. *J. Mar. biol. Ass. U.K.*, Vol. 31, pp. 259–67.

STEPHENSEN, K., 1912. Report on the Malocostraca, Pycnogonida and some Entomostraca collected by the Danmark Expedition to N.E. Greenland. *Medd. Grønland*, Bd. 45, No. 11, p. 503–630.

—— 1928. Marine Crustacea Decapoda. *Zoology of the Faroes*, Vol. 2, pt. 1, 24 pp. Copenhagen.

—— 1939. Crustacea Decapoda. *The Zoology of Iceland*, Vol. 3, pt. 25, 31 pp. Copenhagen.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Enzymic Oxidation of Amines in Decapods

By H. Blaschko and J. M. Himms

J. exp. Biol., Vol. 31, 1954, pp. 1–7

The occurrence of the enzyme amine oxidase in the tissues of *Eusepia officinalis* has already been previously reported. In the present work, a survey was made of the enzymic activity in the organs of two decapod cephalopods, *E. officinalis* and *Loligo forbesii*. In all organs of the alimentary tract, including the salivary glands, the liver and the pancreas, high enzymic activity was found. The enzyme was also present in nervous tissue, in the gonads, the renal appendages, the hearts, the pericardial glands and in other tissues. It was not found in muscle and skin.

The enzyme oxidized tyramine and related compounds, e.g. *p*-hydroxyphenylethanolamine (synthetic 'octopamine'), tryptamine and 5-hydroxytryptamine ('enteramine' or 'serotonin') and many aliphatic amines.

The liver of *Loligo* also contains the enzyme D-amino acid oxidase; this enzyme is already known to occur in *Octopus vulgaris* and in *Eusepia officinalis*.

H.B.

Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). III. On the Activity of the Moult-accelerating Principle when administered by the Oral Route. IV. On the Site of Origin of the Moult-accelerating Principle—Experimental Evidence. V. The Ovarian inhibiting Hormone and the Hormonal Inhibition of Sex-reversal. VI. Notes on the Structure of the Neurosecretory System of the Eye-stalk

By D. B. Carlisle

Pubbl. Staz. zool. Napoli, Vol. 24, 1953, pp. 279–85, 285–92, 355–72 and 435–47

The moult-accelerating principle of the eye-stalk of *Lysmata* is active when administered orally as well as upon injection; a far greater dose is required when the material is taken orally. It is present in extracts of both parts of the X organ, but was not found in extracts of sinus gland, of the retina and lamina ganglionaris or of the medullae interna and externa. Outside the eye-stalk it was found in extracts of the cerebral and thoracic ganglia. The hormone is probably a product of neurosecretory cells.

Eye-stalk ablation leads to an increase in ovarian growth and to an increase in the rate of reversal of sex from male to female in this protandrous hermaphrodite. Extracts of whole eye-stalks, of sinus glands or of the ganglionic part of X organs, when injected into intact animals intramuscularly, inhibit ovarian growth and depress the rate of sex reversal. Extracts of other tissues within the eye-stalk do not have this effect. The principle responsible for these effects is not active orally. Probably a single hormone is responsible for both effects—inhibition of ovarian growth and inhibition of sex reversal. This principle is presumably formed in the cells of the ganglionic part of the X organ and thence passed via the fibres of the sinus gland tract to the sinus gland where it is stored until finally released into the blood-stream. It is not the same as the moult-inhibiting or as the moult-accelerating hormone. The topography and anatomy of the neurosecretory structures which produce, transport and store these various hormones are described, and the relationship of their structure to the secretion of hormones discussed. A method is described by which it has been possible to observe the passage of neurosecretory material along the axons of the X organ connective; the entire axoplasm appears to be flowing along the axon. D.B.C.

On the Relationship between Mammary, Sweat, and Sebaceous Glands

By **D. B. Carlisle**

Quart. J. micr. Sci., Vol. 95, 1954, pp. 79–83

As a result of an investigation into the development of the mammae of male rabbits responding to regular injections of oestrogen and progesterone, it is suggested that the phylogenetic origin of mammary tissue lies in proliferated sebaceous glands. D.B.C.

Seismic Prospecting in the English Channel and its Geological Interpretation

By **M. N. Hill and W. B. R. King**

Quart. J. geol. Soc. Lond., Vol. 109, 1953, pp. 1–20

Recent geological information obtained by core sampling in the English Channel off the South Devon coast is set forth. This information has been combined with the results obtained from a seismic survey along a line southwards from Plymouth.

The evidence from these two sources points to the existence of a trough filled with New Red Sandstone deposits which extends from within a few

miles of the English coastline to a point south of the middle of the Channel. The thickness of these deposits reaches approximately 3000 ft.

The line of reefs formed of metamorphic rocks extending from Bolt Tail to the west of the Eddystone projects through the New Red Sandstone formations, which appear to be swamping an irregular topography.

Southwards from the Eddystone the breccias and sandstones of the Permian and Trias are followed by Keuper Marl and a small thickness of Lower Jurassic formations. These in turn are covered unconformably by a few hundred feet of Chalk. M.N.H.

The Atrial Nervous System of Amphioxus (*Branchiostoma*)

By W. Holmes

Quart. J. micr. Sci., Vol. 94, 1953, pp. 523–35

An interconnected system of neurons lies throughout the lining of the atrial cavity of *Branchiostoma*. Since the neurons lie in or immediately below the atrial epithelium, and are not confined to the gut wall, it is suggested that the homology with the craniate autonomic (enteric) system, proposed by Boeke, gives an incomplete interpretation of the system.

The atrial nervous system is connected with the central nervous system through the dorsal spinal roots, and contains receptor and effector elements. It is particularly dense in the walls of Lankester's atrio-coelomic 'funnels' and round the inner surface of the atriopore. The distribution of smooth muscle in the gut wall is discussed. Since the contents of the atrial cavity are a constantly refreshed sample of the external environment, and since the outer surface of the animal is so sparsely innervated, it is suggested that the atrial nervous system may play an important part in determining behaviour. W.H.

Contraction and Relaxation in the Adductor Muscles of *Pecten maximus*

By J. Lowy

J. Physiol., Vol. 124, 1954, pp. 100–5

The phenomena of contraction and relaxation in the adductor muscles of *Pecten maximus* have been studied by simultaneously recording muscle potentials and movement. The results confirm and extend the findings obtained in similar experiments with the adductor muscles of *Mytilus edulis*. Thus it was shown that in *Pecten* slow phasic contractions are accompanied by smooth muscle action-potentials whose number and amplitude depends on the extent of contraction, and that the maintenance of tension is associated

with continuous electrical activity in the smooth muscle. The striated muscle works by large synchronous contractions. As in the case of the *Mytilus* adductor, a state of contraction in the smooth *Pecten* muscle can be terminated by nervous stimulation.

It is concluded that tonic contraction in lamellibranch smooth muscles is a tetanic phenomenon and that the slow speed of these muscles adequately accounts for their capacity to maintain a state of tension for long periods of time without appreciable fatigue. J.L.

Facilitation in Sea Anemones. III. Quick Responses to Single Stimuli in *Metridium senile*

By D. M. Ross

J. exp. Biol., Vol. 29, 1952, pp. 235–54

This paper describes further work on anemone extracts which in earlier experiments had the effect of enabling *Metridium* to respond to single stimuli. (In untreated animals a single stimulus elicits no response; it sets up facilitation and enables the animal to respond to subsequent stimuli.) The new information can be summarized as follows. (1) Extracts are most effective in causing responses to single stimuli from 15 to 30 sec after introduction, which is too soon for any material to reach the neuromuscular junctions where facilitation is set up. (2) There is no difference in the potency of extracts from stimulated and unstimulated animals, so the active substance in the extracts can have no relation to neuromuscular activity in the animals. (3) Other treatments have similar effects, big changes in pH, K ions, bile salts and saponin, but they have no obvious neuromuscular significance, suggesting that the effect is not due to any specific neuromuscular action.

The general conclusion is that these are not direct facilitating effects but arise from sensory excitation set up by the treatments. Yet there is a singular absence of spontaneous contractions and post-stimulus contractions, suggesting differences between this kind of excitation and the impulses set up by electrical stimulation. D.M.R.

Researches on Phoronidea of the Gullmar Fiord Area (West Coast of Sweden)

By Lars Silén

Ark. Zool., Ser. 2, Bd. 4, 1952, pp. 95–140

Four species of *Phoronis* were treated, viz. *P. pallida* (Schneider) (previously unknown in the adult form), *P. mülleri* Selys-Longchamps, *P. hippocrepia* Str. Wright, and *P. ovalis* Str. Wright. Material of *P. hippocrepia* collected

during a stay at the Plymouth Laboratory in 1949 was used for comparison. The anatomy and histology of the different organs, except the nervous system, were investigated. The four species proved to represent as many distinct types. However, it was considered premature to erect separate genera for them as the phoronid species known are sufficiently few to be easily handled, and too few to allow of more than a very dim idea of the phylogenetic lines within the group. L.S.

On the Nervous System of *Phoronis*

By Lars Silén

Ark. Zool., Ser. 2, Bd. 6, 1954, pp. 1–40.

Investigations were made on six species. *P. hippocrepia*, represented by material collected by the author at Plymouth in 1949, proved to be the form most suited for studying the histology in general of the nervous system. Fixation in Bouin's fluid and staining according to Bodian gave fairly selective results. Certain experiments were performed on living material. The nervous system is situated in the epidermis. Interior organs are innervated by fibres perforating the basal membrane. The chief constituent is a nerve net, typically developed in the metasoma, concentrated into a nervous ring (including the 'ganglion') at the border between pro- and metasoma. The components are described. There are two, one, or no decussating giant fibres ('lateral nerves') in different species. A paired lophophoral sense organ is described. The organization of the nervous system in *Phoronis* is easy to interpret from the functional, but difficult to interpret from the phylogenetic point of view. L.S.

BOOK REVIEWS

A Treasury of New Zealand Fishes

By David H. Graham, F.R.M.S., F.Z.S.

Published by A. H. and A. W. Reed, Wellington, N.Z. Pp. 1–404. Price 63*s*. 1953

In this book on New Zealand fishes the author claims to be catering for a very wide public including senior boys and girls at school, school teachers, university lecturers, sporting and commercial fishermen and fisheries naturalists, both amateur and professional. In so doing he provides a wealth of information, much of it anecdotal and not always as accurate in fact or precise in expression as it might be. The otter trawl is not a 'method of trawling'—it is a fishing implement. Sixty fathoms of wire are not needed to reach the bottom in 20 fathoms of water, though this may well be the best length of warp to use for effective fishing in such depth. It is really too much of a simplification to say that the swim bladder 'can be filled with air and gases by the fish relaxing the muscles surrounding it'. Such inaccuracies are numerous and detract greatly from the usefulness of the book.

Part I, consisting of nine introductory chapters, gives a brief résumé of fish anatomy and biology and of fishery economics. Part II extends to 127 chapters, each chapter, with few exceptions, being devoted to a single species of fish. No taxonomic descriptions are given, but each species is illustrated by either a photograph or a black and white drawing. Both photographs and drawings are of quite a high standard and will prove useful in making rough identifications; but the absence of detailed descriptions leaves the book unsuitable for arriving at precise determinations. In short, the reviewer agrees wholeheartedly with the publishers' statement that Mr Graham's work is pre-eminently a fireside book. As such, it is rather highly priced at three guineas.

G. A. Steven

The Sea Angler's Fishes

By Michael Kennedy

Published by Hutchinson & Co. (Publishers) Ltd., London. Pp. 1–524, with 13 colour plates and numerous black and white illustrations. Price 50*s*. 1954

This book has been written for the benefit of sea anglers, commercial fishermen and others concerned with the British sea-fish trade, all of whom will find it to be a mine of valuable information. Even professional fisheries biologists will discover much to interest them within its pages.

A great deal is known about many of our sea fishes but the information is mostly stored away in technical journals that are not readily available to the general public. Mr Kennedy has endeavoured to fill this gap by summarizing existing knowledge concerning the food, migrations, spawning habits, growth rates and other characteristics of the salt-water fishes of the greatest general interest. Detailed descriptions of the 120 or so species dealt with are also given, together with illustrations of most of them. To the subject-matter abstracted from technical and other printed journals the author has added considerable additional information acquired at first hand by himself or at second hand from those of his many friends who are well versed in the lore of the sea.

Short appendixes on sea angling and commercial fishing methods might well have been omitted, both subjects having been already adequately dealt with in numerous readily available publications. The reviewer would also have preferred the combined index-glossary to have been printed separately—but this, perhaps, is only a personal predilection.

No criticism of this well-written and well-produced book is implied in the further suggestion that a small handbook covering identifications of all British sea fishes—e.g. Jenkins's *Fishes of the British Isles*—would be a useful companion volume. Keen sea anglers are sure to encounter species other than those dealt with by Mr Kennedy. G. A. STEVEN

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1953–54

The Council have to record with very deep regret the death on 31 December 1953 of Dr G. P. Bidder, a Governor of the Association for nearly fifty years, and one of its greatest benefactors and most valued counsellors. An obituary notice will appear in the Journal of the Association.

During the year the Earl of Verulam has been elected a Vice-President.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was nineteen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Council record with very great pleasure that Her Majesty the Queen has conferred the honour of knighthood on our President, Professor James Gray, C.B.E., M.C., F.R.S.

The Plymouth Laboratory

The museum on the top floor of the main building has been partitioned on the north side to form two research rooms, one of which will be used for sterilizing glassware for the culture of micro-organisms. The histological workroom has been improved by the addition of a new bench, microscope storage cupboard and electrical embedding oven. All this work has been done by direct labour.

The Council are pleased to report that H.M. Treasury have sanctioned a Capital Grant from the Development Fund to meet the cost of an extension to the library and the construction of a small building for the preparation and storage of specimens for the Sales Department.

The Aquarium

All the large tanks on the north side of the aquarium have been emptied and cleaned; some of the rockwork has been re-set and new rockwork provided where needed. The conger eels, dogfishes and nursehounds, which for many years had occupied the largest tank, have been transferred to the second largest, and their place has been taken by some of the most active fishes such as sea-bream, pollack, and large wrasses. This has greatly increased the attractiveness of our biggest tank, formerly occupied by so many normally

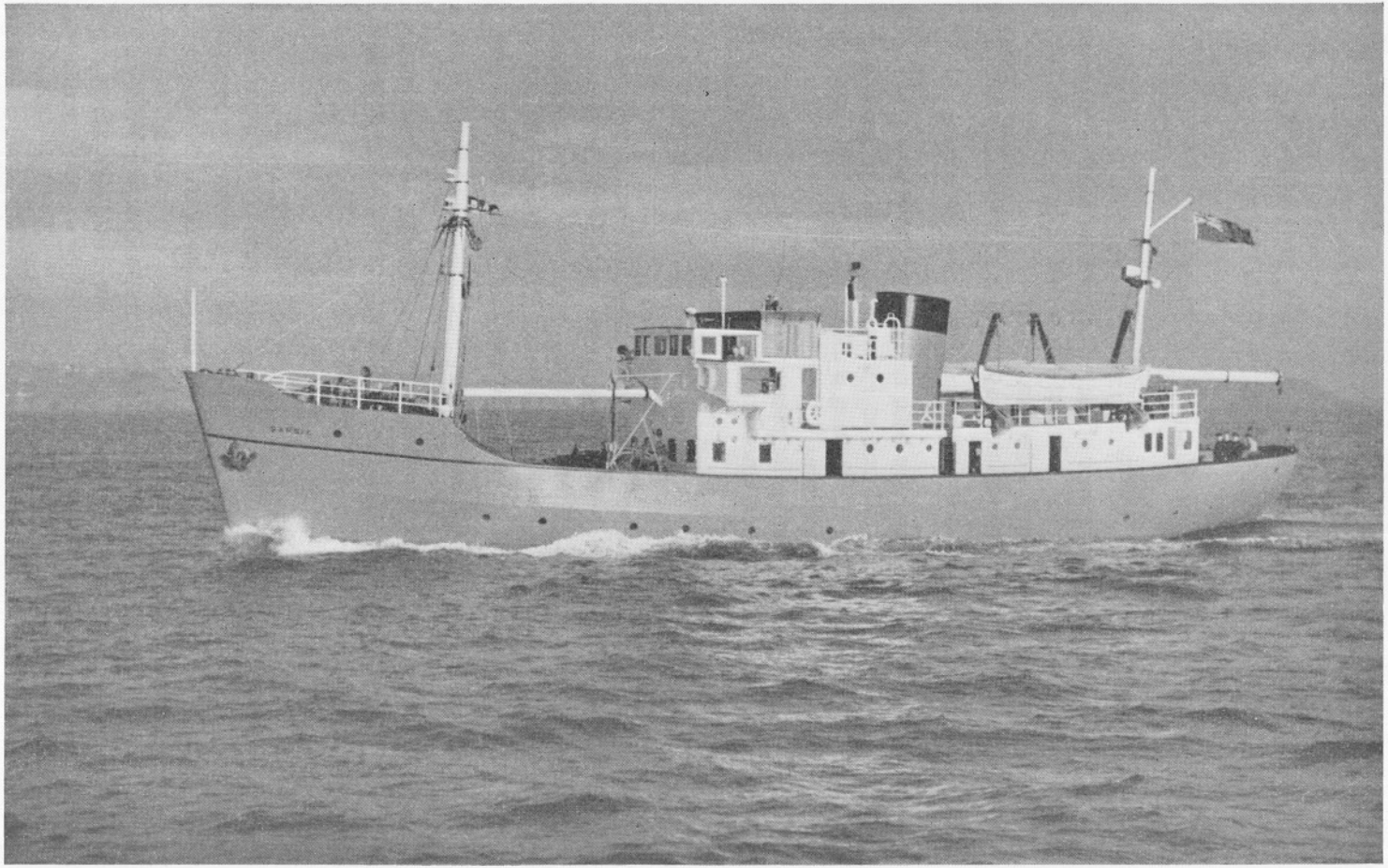

Photo *D. P. Wilson*

Research Vessel *Sarsia*

inactive species. There have been some other rearrangements in the aquarium designed to give most room to the more active fishes, with beneficial results to their health. The aquarium has continued to be very popular, especially with summer visitors.

Towards the end of the year the appearance of the Aquarium was greatly improved by the removal of the central tanks and screen, and the erection of a new screen above the tanks on the south side. As a result there is now a large dry hall giving excellent views of the tanks on either side and with an increased floor space for the visiting public.

Dr D. P. Wilson has published in the *Journal*, Vol. 32, no. 1, a further set of observations on the habits of some of the fishes. These include the first detailed account of the method by which the electric ray (*Torpedo*) captures and kills its prey, and an account of the previously unknown burrowing habits of the red band-fish (*Cepola rubescens*).

Research Ships

The *Sabella* was working regularly until the late summer, when she was off-surveyed at Plymouth on 10 and 11 September 1953 and returned to the Admiralty. The seven years' good service that she has given while on charter have made possible many valuable contributions to the science of the sea from the Plymouth laboratory.

During the year the *Sula* completed her Lloyds full special survey of hull and machinery. In order to keep the vessel fully classified considerable overhaul was necessary in the gear box and electrical system. Both the *Sula* and the *Gammarus* are in good condition and apart from time required for survey and routine maintenance have been in continuous and active service during the year.

The Council wish to record their thanks to Captain W. J. Creese and the crew of the *Sula* for carrying out so successfully the extra demands made upon them during the three months when a second vessel was not available.

R.V. *Sarsia*

The new research ship, built by Messrs Philip and Son Ltd., Dartmouth, and designed by Messrs Graham and Woolnough of Liverpool, has been named *Sarsia* and was launched by Mrs James Gray at the firm's Noss yard on Tuesday, 14 April 1953. Trials were carried out at the end of October and the vessel was taken over by the Association on 2 November 1953. Her principal dimensions are: length o.a. 128 ft.; length b.p. 115 ft.; main breadth to outside of plank 28 ft.; mean draft about 9 ft. 3 in.—giving a gross tonnage of 318·93 (95·02 tons registered).

The propulsive machinery consists of a main engine—National R4 AUM6 diesel—developing 330 b.h.p. at 486 r.p.m. with a propeller speed of 162 r.p.m. obtained through oil operated reverse-reduction gear. Maximum speed

is just over 10 knots, giving an economic speed of about 9 knots at 130–140 r.p.m. (propeller). An auxiliary low-speed drive is also provided by a 20 h.p. variable speed electric motor geared to the propeller shaft through the main gear box. This motor provides the very slow speeds required for plankton work and can serve also as emergency propulsion at speeds up to about 4 knots in the event of adjustments to the main engine becoming necessary. The vessel's normal endurance range is 3000 miles.

There are two laboratories—a larger laboratory on the main deck forward under the wheelhouse and chartroom, and a smaller laboratory on the main deck abaft the engine-room casing. Both laboratories are provided with direct and alternating current at 220 V. Direct current supply varying from 6 to 72 V. can also be tapped from a battery of accumulators housed in a special accumulator compartment. Standard racks for carrying electronic equipment are fitted in both laboratories. In the forward laboratory there is a large gimbal table and a fitted dark room opens off the after laboratory.

Navigational aids with useful scientific applications consist of:

Decca Navigator type 5 with lane identification.
Decca Radar type 12 with 12 in. p.p.i.
Kelvin and Hughes Echo Sounder type M.S. 21*b* with range 0–720 ft. and 0–720 fm.
Kelvin and Hughes Echo Sounder type M.S. XXIe with a normal range of 0–2250 fm.
Marconi type 848 Recording Echometer with range of 0–140 fm.
Redifon Radio Telephone.

A three-drum trawl winch carries 500 fm. of $2\frac{1}{2}$ in. trawl warp on each of the two starboard drums and 1800 fm. of $1\frac{3}{8}$ in. wire on the port drum. A main hydrographical winch situated forward on the port-side under the forecastle deck carries 5000 m. of 4 mm. (diam.) wire on a single drum. A smaller two-drum winch on the after-end of the boat deck carries 500 m. of 4 mm. wire on one drum and 300 m. of 7 mm. wire on the other drum. All winches are hydraulically operated by Vickers-Armstrong's V.S.G. installations.

Two refrigerated cold chambers—one wet and one dry—are provided for scientific purposes, in addition to one domestic refrigerated store.

The ship's company totals sixteen, and there is cabin accommodation for four scientists in one double-berth and two single-berth cabins.

The Council wish to record their appreciation of the very valuable assistance given by Dr G. A. Steven during the building of this ship. They wish also to thank Captain C. A. Hoodless, D.S.C., and Chief Engineer, Mr R. S. Harrison, D.S.M., who were always in close consultation and latterly spent much of their time at Dartmouth, and Mr F. J. Warren who gave much help where electrical knowledge was required.

The Staff

Dr H. G. Vevers, M.B.E., has been promoted to the grade of Principal Scientific Officer.

Dr J. Lowy left the staff at the end of September on his appointment as Lecturer in Experimental Zoology at Queen's University, Belfast.

Dr J. A. C. Nicol has been awarded a Guggenheim Fellowship and he left Plymouth in September 1953 to work for several months at the Scripps Institution of Oceanography at La Jolla.

Mr N. A. Holme spent October and November 1953 in India, where he investigated a problem of underwater damage by boring molluscs at the request of a British firm of Civil Engineers.

Dr B. C. Abbott attended the International Physiological Congress in Montreal in September 1953.

Dr H. G. Vevers attended the meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1953.

Mr D. B. Carlisle attended the International Symposium on Neurosecretion held in Naples in May 1953.

The Council have pleasure in recording the award by Her Majesty the Queen of Coronation Medals to Mr F. S. Russell and Mr W. H. Searle.

Mr F. S. Russell, F.R.S., was elected President of the Devonshire Association for the Advancement of Science, Literature, and Arts for the year 1953–54 and delivered an address on the English Channel at the meeting held in Plymouth in June 1953.

Occupation of Tables

The following one hundred and forty workers have occupied tables at the Plymouth laboratory during the year:

E. ADAMS, Plymouth (Library).
W. H. L. ALLSOPP, British Guiana (General).
Señora A. ALVARIÑO, Madrid (Plankton and fouling organisms).
D. C. ARNOLD, Oxford (Biology of Carapidae).
Miss D. E. ASHHURST, Oxford (General).
Dr DAPHNE ATKINS, London (Ciliary feeding mechanisms).
Dr J. R. BAKER, Oxford (Decapod crustaceans and Lucernarians).
Miss D. BALLANTINE, Development Commission (Taxonomy and culture of marine flagellates).
Dr R. H. BHATTACHARYYA, Calcutta (General).
Dr C. BLIDING, Borås, Sweden (Marine algae).
B. BOCZAROW, Plymouth (Library).
A. D. BONEY, Plymouth (Biology of *Pygmaea*).
E. R. BRAITHWAITE, Plymouth (Library).
Prof. & Mme L. BRULL, Liége (Physiology of kidney in *Lophius*).
Prof. R. BUCHSBAUM, Pittsburgh, Pa. (General).
W. BURKE, London (Histological methods).
Dr R. W. BUTCHER, Burnham-on-Crouch (Culture of small algae).

Dr P. C. CALDWELL, London (Giant nerve fibres in *Loligo*).
P. CHILDS, Plymouth (Comparative anatomy of biliary tract).
Dr P. N. J. CHIPPERFIELD, Brixham (Library).
B. CLARKE, Leeds (Structure of flagella).
Miss E. CLAY, Brixham (Library).
J. C. CLEVERLY, Cambridge (Geology of the English Channel).
R. D. COHEN, Plymouth (Library).
J. A. COLE, Cambridge (Geology of the English Channel).
D. J. COLLINS, Plymouth (Library).
C. A. COSWAY, Torquay (Library).
C. B. COWEY, Oxford (*Nitzschia*).
R. I. CURRIE, National Institute of Oceanography (Oceanography).
Dr D. H. CUSHING, Lowestoft (Development of pilchard eggs).
Dr Y. CUYPERS, Liége (Physiology of kidney in *Lophius*).
Dr R. PHILLIPS DALES, London (Biology of nereids).
Dr D. DAVENPORT, Santa Barbara, Cal. (Physiology of commensalism).
A. A. DAY, Cambridge (Geology of the English Channel).
Dr D. DE GIUSTI, Wayne Univ., Detroit (Acanthocephalan parasites of *Gobius*).
Miss J. M. DODWELL, Birmingham (General).
Miss J. DONOVAN, Cambridge (Nerve net of sea anemones).
Mlle L. DUBOIS, Liége (Physiology of kidney in *Lophius*).
C. EDWARDS, Belfast (Ecology of *Marinogammarus*).
Prof. REGINA CUBILLOS DE ETCHEVERRY, Valparaiso (Chemistry of sea water).
D. ETHERINGTON, London (Parasites of *Stolonica*).
M. A. FAITHFULL, London (Shore ecology).
Dr and Mrs J. BRUCE FALLS, Toronto (General ecology).
Major G. B. O. FARRINGTON, Plymouth (Library).
Dr MARIA FELINSKA, Buckden, Huntingdon (Ciliates).
Miss V. E. FORD, Brighton (Development of *Fucus* eggs).
Prof. BJÖRN FÖYN, Oslo (Colour pattern in *Pomatoceros*).
Cdr R. H. C. FRAMPTON, R.N. (Rtd.), Plymouth (Library).
Dr VERA FRETTER, London (Ion exchange in osmoregulation).
Mrs E. GIBBON, Yelverton (Library).
Dr D. R. GLASSON, Plymouth (Library).
Prof. A. GRAHAM, Reading (Prosobranch gastropods).
Surg.-Cdr P. H. K. GRAY, R.N., Plymouth (Library).
Miss S. G. GREENING, Birmingham (Physiology of *Arenicola*).
D. N. F. HALL, Colonial Fisheries (Plankton).
Miss M. F. HALL, Oxford (Behaviour of *Spinachia*).
Prof. B. HANSTRÖM, Lund (Neurosecretory cells in polychaetes).
Dr T. J. HART, National Institute of Oceanography (Plankton).
B. T. HEPPER, Conway (*Mytilicola intestinalis*).
Dr M. N. HILL, Cambridge (Geology of the English Channel).
Prof. A. L. HODGKIN, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).
L'Abbé H. HOESTLANDT, Lille (Colour variation in *Sphaeroma serratum*).
Dr W. R. HOWELLS, Plymouth (Library).
O. D. HUNT, Newton Ferrers (Library).
R. F. HUTTON, Miami Univ. (Fulbright Scholar) (Trematode parasites).
L. A. J. JACKMAN, Paignton (Library).
F. J. JEFFERY, Plymouth (Library).
Dr C. H. JELLARD, Plymouth (Bacteriological tests).

Dr A. Bohus Jensen, Copenhagen (Fertilization processes in *Ciona*).
D. Jones, Teddington (Opacity of sea water).
J. D. Jones, Bangor (Library).
L. W. G. Jones, Brixham (Library).
Dr G. Y. Kennedy, Sheffield (Porphyrins in marine animals).
Miss J. Kerslake, London (Systematics of *Ectocarpus*).
Dr B. H. Ketchum, Woods Hole (General).
Dr R. D. Keynes, Cambridge (Giant nerve fibres in *Loligo*).
M. C. Kingwell, South Brent (Library).
Sir Francis Knowles, Bart., Marlborough (Neurosecretion in crustaceans).
Dr F. Koczy, Gothenburg (General).
M. E. Korn, London (Holothurians).
Miss L. Lake, London (Ecology of buoys in Plymouth Sound).
D. H. Leaback, London (Cephalopods).
Dr M. V. Lebour, Plymouth (Decapod crustaceans).
J. Llewellyn, Birmingham (Fish parasites).
P. A. Longton, Plymouth (Library).
Prof. O. E. Lowenstein, Birmingham (Sense physiology in elasmobranchs and rockling).
Dr A. G. Lowndes, Plymouth (Bottom sediments).
Dr G. A. C. Lynch, Plymouth (Library).
G. O. Mackie, Oxford (Nervous system of coelenterates).
Prof. R. Magalef, Barcelona (General).
Prof. I. Manton, Leeds (Structure of flagella).
P. T. Marshall, Cambridge (General).
Mlle M. M. Martin, Paris (Marine animal painting).
Mrs J. B. Matthews, St Merryn (Library).
Prof. S. A. Matthews, Williamstown, Mass. (Endocrinology of fish).
Dr A. J. Matty, Nottingham (Endocrinology of elasmobranchs).
R. B. Mayne, Plymouth (Library).
D. A. McGill, Bucknell, Pa. (Fulbright Scholar) (Iodine metabolism in *Ciona*).
Dr J. E. Morton, London (Morphology of molluscs and ascidians).
Dr R. H. Murray, Birmingham (Sense physiology in elasmobranchs).
Dr Wheeler J. North, Cambridge and La Jolla (Light sensitivity of *Metridium*).
Mrs Maj Östbring, Lund (Chromatophores in polychaetes).
Miss G. Owen, Worthing (Coelenterates).
J. S. Owen, Sudan (Library).
Dr R. W. Owen, Leeds (Fish parasites).
Dr C. F. A. Pantin, F.R.S., Cambridge (Nerve net and light sensitivity of sea anemones).
A. H. Papworth, Northampton (Plankton).
Dr L. M. Passano, Washington University, Washington (Nerve net of sea anemones).
A. R. Paul, Eastbourne (Skeletal fibres in *Chalina*).
Dr H. H. Poole, Dublin (Photoelectric measurements of submarine illumination).
P. E. Potter, Bristol (Plant pigments).
Miss B. Rickard, Plymouth (Library).
Miss E. A. Robson, Cambridge (Nerve net of sea anemones).
Miss L. M. Rood, Teddington (Opacity of sea water).
D. P. Sharman, Birmingham (General).
R. J. Snell, Ruislip (Library).
Dr A. J. Southward, D.S.I.R. (Shore ecology).

Miss F. A. STANBURY, Plymouth (*Cladophora*).
Prof. J. H. STARLING, Lexington, Virginia (General).
OVE SUNDENE, Oslo (Algae).
J. C. SWALLOW, Cambridge (Geology of the English Channel).
F. H. TALBOT, Durham (Otoliths in *Raia*).
Dr J. T. TEMPLE, London (Nervous system of *Hemimysis*).
H. WILSON THOMAS, Plymstock (Library).
I. M. THOMAS, Adelaide (Iodine uptake in lower chordates).
Dr G. THORSON, Copenhagen (*Scaphander*).
Prof. E. B. VERNEY, F.R.S., Cambridge (Physiology of kidney in *Lophius*).
I. VIGELAND, Oslo (Bryozoans).
J. M. WARREN, Uganda (General).
Dr G. P. WELLS, London (Sedentary polychaetes).
Dr J. H. WELSH, Harvard (Acetylcholin bioassay; coelenterate toxin).
Miss M. R. WHEELER, Oxford (General).
Miss M. WHITEAR, London (Histology of fish skin).
J. H. WICKSTEAD, Colonial Fisheries (Plankton).
M. S. WILLS, Teddington (Opacity of sea water).
Mlle L. WILSENS, Liége (Physiology of kidney in *Lophius*).
H. V. WYATT, Plymouth (*Calyptraea chinensis*).
Prof. J. Z. YOUNG, F.R.S., London (Optic nerve in cephalopods).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr Hiroshi Tamiya, Tokio; Dr F. D. Ommanney, Singapore; Dr G. Rollefsen, Norway; Prof. Buzzati-Traverso, Pavia; Dr K. Pampapathi-Rao, Rajahmundry; Dr S. Jones, Calcutta; Prof. T. Katsuki, Japan; W. L. Necker, Chicago; Dr Fr. Hustedt, Germany; J. M. Thomson, Cronulla; Dr and Mrs G. Hoecker, Chile; Dr Björn Holmgren, Chile; Miss D. L. Ray, Seattle; Dr Dorothy Bliss, Harvard; Prof. H. R. Dew, Sydney; Miss L. E. Foster, San Francisco; P. B. N. Jackson, Northern Rhodesia; Prof. Tohru Uchida, Japan; Dr W. Schmitz, Germany; T. E. Allfree, Nairobi; Dr J. F. G. Wheeler, Zanzibar; Dr G. E. Maul, Madeira; Dr E. Steemann Nielsen, Copenhagen; Dr Warren Weaver and Dr J. George Harrar, Rockefeller Foundation, New York; K. Baalsrud, Oslo; Miss E. C. Pope, Sydney.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by thirty-nine students from the following Universities and University Colleges: Oxford, Cambridge, Edinburgh, London, Durham, Sheffield, Nottingham, Newcastle, Southampton, Aberystwyth, Cardiff, Exeter, and the Regent Street Polytechnic.

Also during the Easter Vacation Mr I. F. Thomas brought a party of four boys from Oundle School. In June Mr R. W. Webster brought fourteen students from Regent Street Polytechnic.

Dr G. E. Newell, Dr J. E. Forrest, and Dr J. E. Morton conducted a course in September for nineteen students from Queen Mary College.

A meeting of the British Phycological Society was held at the Plymouth laboratory during the last week of September at which the attendance was forty-two.

A special meeting was held in December at the Plymouth laboratory to discuss the present position regarding the possibilities of mass culture of marine unicellular algae. Dr F. N. Woodward, Director of the Institute of Seaweed Research, took the chair, and twenty-seven attended the meeting, including representatives of fourteen laboratories.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

In conjunction with Dr H. H. Poole, who again visited the laboratory, Dr W. R. G. Atkins has worked on a method for measuring the extinction coefficient of light in the sea using two submerged photometers and thus avoiding certain errors inherent in a comparison with a cell in air.

They have done further work on the scattering of light in sea water, studying in particular the effect of the colour of the light and of filtering the water with collodion membranes of different average pore diameters; a triple filtration ending up with a filter 0·1 μ A.P.D. gave water with slightly less scattering than a fairly good sample of distilled water. Differences can be detected from sample to sample.

Dr Atkins, Mr F. J. Warren and Miss P. G. Jenkins have studied the depth of visibility of a 20 cm. Secchi disk in relation to the quantity of chlorophyll in the upper 20 m. of sea water at station E 1 for two years. The relation is an inverse one; the results are often surprisingly concordant, but the admixture with deeper water, when the autumnal cooling leads to an isothermal column, introduces a discrepancy as one would expect.

The very clear water found in June was identical in salinity with the less clear May water, but coincided with the summer minimum of chlorophyll. The phytoplankton scatters light and reduces visual range, while offering little obstruction when diffuse light is measured as it falls on a horizontal plate.

Miss Jenkins has continued the work on the phytoplankton at station E 1 by means of spectrophotometric determinations of chlorophyll and the identification of the organisms by obtaining cultures from nine depths every month, or fortnightly at important times. This essential link between the living organisms and the effects due to them has heretofore been lacking at station E 1. Especially with the nanoplankton the changes may be very rapid and cells may accumulate near the bottom.

Mr F. A. J. Armstrong has continued the regular cruises to station E 1 and analyses of water samples for phosphate, total phosphorus and silicate. These cruises have been more frequent in 1953 than earlier years, and samples have

been taken at extra depths. The results of the analyses for the four years 1950 to 1953 have been cast into a graphical form for publication in the *Journal*. A set of samples taken for Dr Cooper by U.S.S. *Rehoboth* has also been analysed for phosphate and silicate. Mr Armstrong has assisted Dr Wilson with the chemical and bacteriological part of his experiments on biological differences between sea waters.

Dr H. W. Harvey has examined a wide range of experimental data bearing upon the factors which affect the growth rate of phytoplankton, which embraced a voluminous literature dealing with algal metabolism. It was hoped to find possible reasons for many apparently anomalous observations which might help in designing future experiments.

Dr Harvey has rewritten much of his book, *Recent Advances in the Chemistry and Biology of Sea Water*, with the aim of presenting the inter-relations between composition of the water, plant production and the animal community, which are becoming clearer through investigations made in several countries since the first edition was published seven years ago. These embrace work done in the Plymouth laboratory on the quantities and forms in which phosphorus compounds occur in the water, plants and animals, their change with the seasons and from place to place—an inquiry which is now also being pursued in the tropics and eastern Atlantic and by the Ice Patrol in the Arctic in order to differentiate water masses. A reason for correlating the many and diffuse observations, necessary for rewriting a general account of changes taking place in the sea, is to foresee better what future lines of research are likely to prove most productive. Some preliminary work has been done on methods of estimating zooplankton populations below unit area of the sea in terms of weight of organic matter or value as food for other animals; it is hoped to continue this in collaboration with other members of the staff.

In reports since the war the search for an understanding of the relative richness in the 1920's of the English Channel in plant nutrients and associated rich production of animals has been described. Dr L. H. N. Cooper has now been able to erect a generalized hypothesis relating conditions in the English Channel to the degree of coldness of Arctic winters—the hypothesis of upward displacement.

In part, Atlantic deep water is formed by cooling of saline water derived from the North Atlantic Drift system. Only around southern Greenland and probably in the Norwegian Sea can this water be formed in bulk. In the first two decades of this century a succession of cold Arctic winters probably led to considerable formation of deep water which could be accommodated in other parts of the Atlantic only by upward displacement of the water already there. This should have led to nutrient enrichment of the upper waters by physical processes which may operate always. The Arctic winters of 1918, 1920 and 1921 were exceptionally cold and should have given rise to exceptional upward displacement. Consequently, surface enrichment with nutrients should

have reached its maximum in 1921. Since then there has been a long succession of mild winters, presumably reducing (*a*) the amount of deep water formed, (*b*) the extent of upward displacement, and (*c*) surface enrichment with nutrients of the eastern North Atlantic.

The impoverishment of the English Channel which set in after 1930 may have been initiated by exceptional cascading in that year due to a local cold winter and strong east winds. There is, however, an inconvenient phase difference of one year. All the work on the subject since 1946 has now fallen into place.

During 1953 the generous co-operation of U.S. Navy Hydrographic Office enabled U.S.S. *Rehoboth* to obtain essential information from fifteen deep stations in the Bay of Biscay and more is to come from deep stations worked west of Ireland. From these it is hoped to establish the southward course of Arctic water during mild winters. A number of corollaries which follow from the hypothesis of upward displacement are being investigated.

In 1945 Dr Cooper co-operated with Major Grange Moore of the Army Photographic Research Unit who was then evolving his brightness profile method of assessing beach gradients by aerial photography. Extinction coefficients of sea water in tubes determined by the Pulfrich photometer were notably greater than by the brightness profile method. Later it was found that the Pulfrich method also gave much higher values than those based on underwater measurements of vertical illumination at sea. An explanation can now be based on the work of Poole and Atkins on forward scattering of light and on the geometry of the light paths in the several methods.

Dr D. P. Wilson's and Mr F. A. J. Armstrong's experiments on biological differences between natural sea waters were continued during the 1953 breeding season of *Echinus esculentus*. Again Clyde water was compared with water from the English Channel at the international hydrographical station E 1. This year the two water masses were closely similar in their effect on the structure and health of the *Echinus* larvae reared in them, but the larvae were slightly better formed and a little healthier in the Clyde than in the E 1 water. Various experiments were made in an attempt to narrow the field within which must be sought an explanation of the marked differences previously observed. The 1952 experiment with active carbon was repeated, for it had been found that the carbon then used had contained a significant amount of copper. A copper-free carbon was obtained for comparison, with some differences in the results. A partially successful attempt was made to filter the water free from bacteria at the time of collection. An account of these and other tests is being prepared for early publication in the *Journal*.

During 1953, Mr P. G. Corbin has observed no change from the prevailing low level of macroplankton production off Plymouth as sampled by half-hour hauls with the 2 m. stramin ring trawl.

The monograph on the Medusae of the British Isles by Mr F. S. Russell has now been published by the Cambridge University Press.

The collection of species-pure cultures of marine phytoplankton organisms has been maintained by Dr Mary Parke throughout the year and a number of new forms have been isolated. Work is continuing on new members of the Chrysophyceae with three flagella.

During the year cultures for research purposes have been sent abroad and also to institutions in this country. A great number have also been produced for the use of research workers in the laboratory.

Miss D. Ballantine, on a Development Commission Research Grant, has been carrying out experiments in a constant temperature room on the growth of flagellates, estimated by increase in numbers, under various light intensities using fluorescent lamps. Growth curves for one organism are strictly comparable from one set of experiments to the next, but results obtained using different organisms vary widely.

Miss Ballantine has also continued her work on the descriptions and life histories of two undescribed species of *Gymnodinium* from the culture collection.

Macro-Fauna and Flora

The results of Dr D. P. Wilson's experiments in 1952 on the settlement of *Ophelia bicornis* larvae have been published in Vol. 32, No. 1, of the *Journal*. It was in 1952 that it was first clearly demonstrated that an attractive factor can be transferred from fresh Bullhill Bank sand to a neutral acid-cleaned sand. During the summer of 1953 many experiments were made with the object of obtaining further information about the nature of this attractive factor. It is expected that when analysis of the results is completed it will become clear that living micro-organisms play an indispensable role in making a sand attractive to the larvae of *O. bicornis*.

Mr G. M. Spooner has continued systematic and faunistic work on isopods and amphipods. An additional species of *Sphaeroma*, newly recognized on the Continent, proves to be prevalent in Britain. It has to be split off from the *S. serratum* of British authors. It has a preference for lowered salinity, with an optimum between that of *S. serratum* (marine) and *S. rugicauda* (mid-estuarine). To it belong the '*serratum*' of the Tamar Estuary.

The amphipods are being examined with a view to a revised list for the Plymouth Area, a considerable reference collection having been built up, from which the existing museum collection can be supplemented. Some quantitative counts are being made of amphipod populations of sessile epiphyte growths on rocks hand-collected by Mr G. R. Forster beyond low-water level.

Assistance has been given in determining critical species in collections from east Scotland and the Channel Islands.

Further collections of *Jassa* have been examined, including some from east Scotland and South Africa. The identity of the form in this country recently recognized as *J. valida* Dana with the native South African species has been confirmed. Material is now being assembled for writing up.

Additional information has been obtained by Mr Spooner on the occurrence of commensalism in the polynoid genus *Harmothoë*, the existing published records for the Plymouth area being unexpectedly incomplete. The most plentiful species is *H. spinifera*, which can be found not only in shale crevices intertidally, associated with *Amphitrite gracilis* (as found recently by Dr D. Davenport), but occurring freely in Plymouth Sound as a commensal of *Polycirrus caliendrum* inhabiting cavities in loose stone and clinkers. Normally the dredged material is not split open and this association has remained unnoticed hitherto.

The better-known *Harmothoë lunulata*, found in the tubes of various burrowing invertebrates, has so far produced three populations of more or less distinct colour pattern: (*a*) with *Amphitrite johnstoni* as host, in the Yealm Estuary; (*b*) smaller specimens with host *Acrocnida* and larger with *Leptosynapta*, at Salcombe; (*c*) with *Arenicola marina*, at Salcombe. The last is easily recognized by its dark colour and is var. *arenicolae* St Joseph. Small individuals of this species can be obtained from Plymouth Sound, but it is not clear whether these are commonly free-living: one has been found associating with *Thalassema neptuni*.

Dr H. G. Vevers has continued his studies on the biology of the echinoderms, and in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, has published a paper in Vol. 32, No. 1, of the *Journal*, on the isolation and estimation of protoporphyrin in the integument of *Asterias rubens*. The relative amounts of this pigment in different colour types of *A. rubens* were determined by a fluorimetric method, and were found to decrease rapidly in the series: violet-brown, dark-brown, red and pale. Neither a porphyrin nor a porphyrinogen was found in the soft parts of the queen scallop (*Chlamys opercularis*) or mussels (*Mytilus edulis*) which form the main food of *Asterias rubens* in the Plymouth area. The source of the integumentary porphyrin is still uncertain, but it is suggested that it may be derived from the chlorophyll pigments found in the digestive caeca. Further work is now being done on the occurrence of porphyrins in other marine invertebrates.

Dr Vevers is also continuing his work on the carotenoid pigments of marine invertebrates, with a view to tracing their origin and function, and has started an investigation of some algal pigments. He is also designing new improvements to the lighting system of his underwater photographic apparatus, and it should be possible to test these at sea during the coming year.

Mr N. A. Holme has completed a paper on the biomass of the bottom fauna at Plymouth, which has been published in Vol. 32, No. 1, of the *Journal*. The

mean dry weight of living matter at twenty stations was 11·2 g./m.^{2}. Much of this weight (64·4%) was made up of relatively large individuals weighing more than 0·2 g. dry. Polychaetes, lamellibranchs and echinoderms together made up about 80% of the total dry weight.

An attempt was made to compare the 1950 populations with those found by E. Ford in 1922–23. There seems to have been an appreciable overall decline in numbers of lamellibranchs, the group studied in most detail, since the previous survey. The lamellibranchs *Abra alba* and *Cultellus pellucidus* showed a considerable drop in numbers, as did the echinoderm *Echinocardium cordatum*. The decline in numbers may be due to the change in the hydrographic conditions in the Plymouth area which occurred in the early 1930's.

Work has now been completed on the ecology of the three species of *Ensis* found in British waters, a report of which has been published in Vol. 33, No. 1, of the *Journal*. Three ecological factors seem to be mainly responsible for the distribution of each species: wave-action, soil-grade, and depth. Tolerance of wave-action is dependent on the stability of a sandy beach; *Ensis siliqua* is much more tolerant of wave-exposure than either *E. ensis* or *E. arcuatus*. Where wave-action on a beach is too great, a species may be restricted to shallow water offshore. All three species have distinct preferences for soil of a particular grade. *E. arcuatus* occurs in soils typically containing nearly 30% over 1 mm. particle size, while the other species occur in soils where there is only 2 or 3% over this size. All species occur in a fairly wide range of grades, so that *E. arcuatus* may sometimes be found in the same place as either or both the other species. The three species occur on the shore and in shallow water offshore, *E. siliqua*, alone, having a definite lower depth limit of *c.* 20 m.

Some differences in ecology were noted on the south side of the English Channel compared with south-west England. It is possible that separate populations with slightly different habitat preferences occur.

Mr D. B. Carlisle has continued his studies on the Plymouth and Naples tunicates and contributed papers on this subject to the *Journal*, to the *Pubbl. Staz. Zool. Napoli*, and to the *Proc. Zool. Soc. London*.

Mr G. R. Forster has completed two short papers on the *anchor* dredge and on peritrophic membranes in the *Caridea*; these have been published in Vol. 32, No. 2, of the *Journal*.

During the summer he continued his investigation of sublittoral rocky shores by diving, whenever possible. Six dives have been made off Stoke Point, in which the sub-*Laminaria* zone has been reached, at a depth of approximately 10 fathoms below L.W.S.T. The occurrence and distribution of the more conspicuous forms have been observed down to a maximum depth of 14 fathoms.

The sublittoral gulley in Wembury Bay surveyed by Kitching, Macan & Gilson (*J. Mar. biol. Ass. U.K.*, Vol. 19, No. 2) in diving helmets in 1931 has been re-examined by Mr Forster. The most successful collecting method has

been to split off slabs of the slate rock with a lead hammer and cold-chisel. Many small Crustacea have been taken in this way, as they remain clinging to the sponges and other sessile forms.

Early in the year, Mr P. G. Corbin obtained several artificial fertilizations of the eggs of the Atlantic smooth sand-eel, *Gymnammodytes semisquamatus*, from which larvae were successfully hatched. Earlier work by Einarsson had shown that one of the post-larval sand-eels commonly occurring in this area belonged to the above species, although formerly it had long been attributed to the greater sand-eel, *Ammodytes lanceolatus*. The larvae from the artificial fertilization provided independent confirmation of Einarsson's identification. There remains the interesting anomaly that in this area the young stages of the commonest species, the lesser sand-eel, *A. tobianus*, have not yet been seen. The young stages previously attributed to *A. tobianus* were shown by Einarsson to belong to *A. lanceolatus*.

Mr Corbin has been re-examining Saville-Kent's long overlooked notice of a fourth species of sucker fish, *Lepadogaster couchii*, in the British fauna. His account was preliminary and incomplete, but it leaves no doubt that he referred to a species which occurs in much the same region of the shore as the commoner Cornish sucker, *L. gouani*, but in the rather different habitat afforded by the cover of bushy weeds, particularly the *Cystoseira* spp., in preference to the under side of stones where *Lepadogaster gouani* is commonly found breeding early in the year. Although the species resembles the off-shore *L. bimaculatus* with which it was earlier confused, it has several readily distinguishing characters in addition to an entirely different habit and habitat.

Mr Corbin has continued his observations on the Lucernariidae, in particular by fortnightly or monthly searches at Wembury and Looe, which are providing data on seasonal abundance of the species.

No further work on the large mid-water net was undertaken this year by Mr Corbin and Dr Steven after the trials made in the summer of 1952 aboard R.R.S. *Discovery II*. It was considered that the work could not profitably be advanced until the facilities of the new ship were available.

For forecasting purposes Dr G. A. Steven has continued his regular sampling of landings from the Newlyn (Cornwall) spring mackerel fishery. Returns for the 1953 season were in encouraging agreement with his prediction of a moderately successful fishery. Unfortunately, the prospects for the 1954 season are rather less favourable than those for the previous two years.

Dr Steven is also collaborating with Mr L. A. J. Jackman of Paignton, Devon, in preparing a report on the part that appears to be played by temperature in regulating the arrival and departure of mackerel in inshore localities in Torbay. Dates of first arrival have been noted over the 9-year period 1945–53 and departure dates from 1941 to 1952 inclusive. The records reveal strikingly close correlations with sea surface temperatures. With only one exception

mackerel made their first appearance when the sea temperature was within the range 11·1–11·6° C. In six different years the temperature was exactly 11·6° C. Over the 12-year period of departure records (with the exception of 1945) mackerel left the area when the temperatures were between 12·2 and 13·9° C. with a mean value of 12·9° C., the departure dates ranging from 24 September in 1952 to 11 November in 1949.

The trawling survey of the grounds in the vicinity of Plymouth, mentioned in last year's report, is nearing completion. Present catches continue to be better, on the whole, than those of the 1913–14 period and poorer than those of the 1920's.

Careful records of young fish catches in standard hauls with the 2 m. stramin ring trawl at a fixed position have been made from 1924 onwards. No comparable records exist for trawled catches of the fish after they have become large enough to be captured by this method. Dr Steven has, therefore, begun to record total catches of demersal fish from standard trawl hauls at international station L4 off Plymouth as nearly as possible at weekly intervals. After some years the data so collected should be of great interest and value.

Experiments are also being carried out by Dr Steven with the preparation of an artificial bait for crab-pots. The bait is easily made, easily stored, and retains its properties for a year or more. If it proves sufficiently attractive to crabs, lobsters and prawns it should be useful to fishermen at times when natural bait is scarce. A sample of the artificial bait kept for four years was still wholesome but had lost its power to attract prawns.

The compilation of a preliminary Check-List of the British Marine Algae has been completed by Dr Mary Parke, and the list was published in Vol. 32, No. 2, of the *Journal*. The separate collections of marine algae in the laboratory's museum have now been incorporated into a single catalogued collection. This has involved considerable checking of old specimens and remounting for better preservation. Miss D. Ballantine has given much assistance in this work, which is now more than half finished.

To fill gaps in the collection many specimens from the Devon and Cornwall coasts have been added, and it is hoped that eventually the Herbarium will be representative of all British coasts.

A close watch is being kept for new occurrences or increasing numbers of more southern forms along our coasts, and some new records have in fact already been made.

Physiology of Marine Organisms

The majority of marine animals, perhaps all, have some means of controlling the emission of light. Among recognized mechanisms are: nervous control of secretion; regulation of intracellular oxidation; muscular contraction of reservoirs containing luminescent material; and muscular control of shutters,

screens, etc., for exposing or covering light organs. Detailed information has now been obtained by Dr J. A. C. Nicol for two such mechanisms in polychaetes, viz. nervous control of secretion (expulsion of luminescent material) in *Chaetopterus*, and regulation of intracellular oxidation in Polynoinae. The course of fatigue of the luminescent response in *Chaetopterus* presents certain interesting features, since the intensity of response which can be evoked by successive stimuli progressively declines to a low level long before the exhaustion of luminescent material. There is good reason for believing that the expulsion of luminescent material is effected by protoplasmic contraction of the cell periphery. Quantitative recording has permitted an analysis of the factors involved in fatigue, which resolve themselves into: reduction of luminescent material present; fatigue of contractile mechanism under rapid stimulation; decreased efficiency of the contractile mechanism, with diminution in volume of intracellular contents. This last factor is mainly responsible for the decline in intensity of successive, discrete responses.

Further studies have now clarified many features of the luminescent responses of polynoids. The normal luminescent response of an elytrum is a series of flashes which can be evoked by a single electrical shock. This rhythmic flashing is controlled by a peripheral ganglion in the elytrum, from which nerves radiate to the light cells; preparations lacking this ganglion give only a single flash to each stimulus. Consecutive flashes, following each other at suitable intervals, show an increase in intensity. This is in part due to summation when the frequency is high enough to permit fusion, but is to a great extent attributable to facilitation. This process, lasting as long as 4 min., occurs peripherally at the neuroglandular junction or in the photocytes. The time relations of the flashes have been determined from oscillographic records, and the course of fatigue in the rhythmicity of the underlying neural mechanism investigated. This experimental work, together with further observations on the effects of various salt solutions and biochemical agents on luminescence, has been published in Vol. 33, No. 1, of the *Journal*.

Dr Nicol has also written up his experiments, carried out in collaboration with Dr D. Whitteridge at Oxford, on conduction in the giant axon of *Myxicola*. This work deals with the course and speed of conduction in the giant nerve fibre of *Myxicola*, and the relation of conduction velocity to axon diameter. An abstract of the work appeared in Vol. 29, No. 1, of the *Journal*, and it is being published in full in *Physiologia Comparata et Oecologia*.

Dr J. S. Alexandrowicz has continued his studies of the muscle receptors in crustaceans. In *Squilla mantis* the presence of these organs in twelve segments has been established. The two nerve cells and the muscle fibres in each half segment differ in their structure, and in this and many other respects the receptors in stomatopods resemble those in decapods, having however certain peculiar features of their own. The results are being published in *Pubblicazioni della Stazione Zoologica Napoli*.

In *Leander serratus* muscle receptors have been found in five abdominal segments and in the thorax. The latter show an unusual arrangement in that four nerve cells are connected with one thin muscle bundle. The receptor elements of another category, called N-cells, have also been observed in *Leander* and found to be comparatively very large in this species.

The occurrence of muscle receptor organs has also been established in amphipods (*Marinogammarus marinus*), isopods (*Ligia oceanica* and *Idotea emarginata*), and mysids (*Praunus flexuosus*). In all these species the receptors are situated at the level of the extensor muscles on their dorsal surface, or near to it, and appear to be of essentially similar structure to those of decapods and stomatopods.

Dr Alexandrowicz has also investigated the innervation of the heart in the amphipod *Marinogammarus marinus*. Several systems of nerve elements have been found supplying the heart, viz. a ganglionic trunk situated on the dorsal heart wall, two pairs of nerves coming from the central nervous system and nerves for the arterial valves. There are, in addition, special nerves supplying the pericardial muscles and two trunks running in the pericardial wall, presumably belonging to the category of the pericardial organs.

Dr J. S. Alexandrowicz and Mr D. B. Carlisle have published in Vol. 32, No. 1, of the *Journal* an account of the experiments showing the effects of the extracts of the pericardial organs of decapods and stomatopods on the heart rhythm of these animals. Further experiments are being made in order to determine the nature of the active principle in these organs. The evidence at present suggests that this is not adrenalin, noradrenalin, tyramine, acetylcholine (though this is probably present in extracts), nor 5-hydroxytryptamine, but in some of its properties it is very like this last.

Mr D. B. Carlisle has continued his investigations into the endocrinology of moulting and sex in prawns and crabs. The moult-accelerating hormone is produced by the nerve-cells of the ganglionic portion of the *X* organ of the eyestalk and passed within the axons to the distal part of the *X* organ where it is stored until released. Reversal of sex and ovarian development in the Mediterranean prawn *Lysmata* have been shown to be under hormonal control; probably the same hormone inhibits both (*Pubbl. Staz. Zool. Napoli*, Vol. 24). No evidence has been found for the existence of the eyestalk moult-inhibiting hormone in *Leander*, nor in *Carcinus* in the moulting season, in which species it is known to exist outside this season. The structure and relationships of the endocrine structures of the decapod eyestalk have been investigated comparatively in collaboration with Dr L. M. Passano and a brief account published in *Nature*. A preliminary account of these organs in *Lysmata* appeared in *C.R. Acad. Sci. Paris*, and a fuller account has now been published in *Pubbl. Staz. Zool. Napoli*. A letter to *Nature* by Mr Carlisle and Sir Francis Knowles has proposed a simplification in the nomenclature of certain nervous and endocrine structures of crustaceans—the 'neurohaemal' organs.

Dr B. C. Abbott has studied the concentration by marine organisms of certain radioactive isotopes resulting from uranium fission processes. Attention has been mainly directed to the uptake of yttrium by cultures of *Nitzschia closterium* forma *minutissima* under various conditions of growth, illumination and aeration. The exchange of caesium and potassium between the external medium and living cells has also been studied in these unicellular algae and also in invertebrate muscle tissue.

Iodine accumulation in the ascidian *Ciona* has been investigated by Mr D. A. McGill, a Fulbright Scholar, in collaboration with Dr Abbott using the radioisotope ^{131}I; radio-autographs of sectioned material served to indicate the localization in organically bound form within the endostyle.

A preliminary comparison of the mechanical properties of a number of isolated lamellibranch muscles has been made by Dr Abbott in collaboration with Dr Lowy; the results have been published in the *Journal of Physiology*. The muscles used have varied in speed from the fast striated *Pecten* adductor to the very slow posterior adductor in *Mytilus*. It has been shown that when isolated in good condition these muscles are as excitable as vertebrate muscle: they all conform to A. V. Hill's characteristic force-velocity relationship for isotonic shortening and exert isometric tensions of about 2·5 kg./cm.^{2}.

Measurements with a very sensitive piezo electric recorder of the early tension changes after stimulation showed no sign of latency relaxation. Thermal changes during activity are still being studied.

Investigations of the contractile mechanism of lamellibranch muscle has been continued by Dr J. Lowy. The spontaneous electrical and mechanical activity in the smooth and striated parts of *Pecten*'s adductor muscle was recorded simultaneously and continuously for long periods with the intact animal in water. It was found that slow phasic contractions are accompanied by smooth muscle action-potentials whose number and amplitude depend on the extent of contraction and that the maintenance of a state of tension is associated with continuous electrical activity in the smooth muscle. If the nerves leading to this muscle are cut it relaxes and electrical activity ceases completely. The similarity between the contractile mechanism of the *Pecten* smooth muscle and that of the wholly smooth adductors of *Mytilus edulis*, *Mya arenaria* and *Lutraria lutraria* is pointed out, and it is concluded that tonic as well as phasic contraction in smooth lamellibranch muscle is due to nervous excitation.

Observations of spontaneous relaxation and experiments in which relaxation was brought about by reflex stimulation show that the termination of a state of contraction in the smooth *Pecten* muscle is under nervous control of an inhibitory nature. This finding confirms the results of experiments with preparations of the posterior adductor of *Mytilus* and suggests that lamellibranch smooth muscles may be regulated in a manner similar to that found in other invertebrate muscles which do not give a triggered type of response.

On the other hand, it is shown that the *Pecten* striated muscle works by large synchronous contractions and possibly here the elementary reaction is the all-or-none response of whole motor units.

In the light of the results of the comparative observations made with Dr Abbott on isolated lamellibranch muscles, and considering the evidence from experiments with intact lamellibranch adductor muscles, there is now good reason to believe that tonic contraction in lamellibranch smooth muscles is a tetanic phenomenon and that the slow speed of these muscles adequately accounts for their capacity to maintain a state of tension for long periods of time without appreciable fatigue.

An account of the mechanism of contraction and relaxation in the adductor muscle of *Pecten maximus* will appear in the *Journal of Physiology*.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume 32, No. 1, of the *Journal* was published in June 1953, and No. 2 in October 1953.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

ATKINS, W. R. G. & JENKINS, PAMELA G., 1953. Reduction in light accompanying exceptionally heavy rain. *Nature, Lond.*, Vol. 172, p. 79.

BACCI, GUIDO & LA GRECA, MARCELLO, 1953. Genetic and morphological evidence for subspecific differences between Naples and Plymouth populations of *Ophryotrocha puerilis*. *Nature, Lond.*, Vol. 171, p. 1115.

BIEBL, RICHARD, 1953. Über die Seewasserresistenz von Meerstrandpflanzen. *Photographie und Forschung*, Vol. 5, pp. 174–80.

BLASCHKO, H. & HIMMS, JEAN M., 1954. Enzymic oxidation of amines in decapods. *J. Exp. Biol.*, Vol. 31, pp. 1–7.

BODEN, BRIAN P., 1954. The Euphausiid crustaceans of Southern African Waters. *Trans. Roy. Soc. S. Afr.*, Vol. 34, pp. 181–243.

BRIGHTWELL, L. R., 1953. Further notes on the hermit crab *Eupagurus bernhardus* and associated animals. *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 61–64.

BULJAN, M., 1951. A modification of Teorell's method for determining small quantities of ammonia. *Arhiv za Kemiju*, Vol. 23, pp. 119–22.

CARLISLE, DAVID B., 1953. The larva and adult of *Polycitor crystallinus* Renier (Ascidiacea, Polycitoridae). *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 259–65.

CARLISLE, DAVID B., 1953. Note préliminaire sur la structure du système neurosécréteur du pédoncule oculaire de *Lysmata seticaudata* Risso (Crustacea). *C.R. Acad. Sci. Paris*, T. 236, pp. 2541–42.

CARLISLE, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). III. On the activity of the moult-accelerating principle when administered by the oral route (p. 279). IV. On the site of origin of the moult-accelerating principle —experimental evidence (p. 284). *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 278–91.

CARLISLE, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 355–72.

CARLISLE, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VI. Notes on the structure of the neurosecretory system of the eyestalk. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 434–46.

CARLISLE, D. B., 1953. Origin of the pituitary body of chordates. *Nature, Lond.*, Vol. 172, p. 1098.

CARLISLE, D. B. & DOHRN, P. F. R., 1953. Sulla presenza di un ormone d' accrescimento in un crostaceo decapode, la *Lysmata seticaudata* Risso. (On the presence of a growth hormone in a decapod crustacean, *Lysmata seticaudata* Risso). *Ric. sci.*, suppl., 1953, pp. 95–100.

CARLISLE, DAVID B. & KNOWLES, SIR FRANCIS, Bart., 1953. Neurohaemal Organs in Crustaceans. *Nature, Lond.*, Vol. 172, pp. 404–05.

CARLISLE, D. B. & PASSANO, L. M., 1953. The X-organ of Crustacea. *Nature, Lond.*, Vol. 171, pp. 1070–71.

CHAPMAN, GARTH, 1953. Studies of the mesogloea of coelenterates. I. Histology and chemical properties. *Q. Journ. Micr. Sci.*, Vol. 94, pp. 155–76.

CHAPMAN, GARTH, 1953. Studies on the mesogloea of Coelenterates. II. Physical properties. *J. Exp. Biol.*, Vol. 30, pp. 440–51.

ETHERINGTON, D., 1953. On a sporozoon in the coelomic corpuscles of *Phascolosoma minutum* Keferstein (Sipunculoidea). *Parasitology*, Vol. 43, pp. 160–69.

HART, T. J., 1953. Plankton of the Benguela Current. *Nature, Lond.*, Vol. 171, pp. 631–34.

HILL, M. N. & KING, W. B. R., 1953. Seismic prospecting in the English Channel and its geological interpretation. *Quart. J. Geol. Soc. Lond.*, Vol. 109, pp. 1–20.

HOLMES, WILLIAM, 1953. The atrial nervous system of Amphioxus (*Branchiostoma*). *Quart. J. Micr. Sci.*, Vol. 94, pp. 523–535.

JAMES, W. WARWICK, 1953. The succession of teeth in elasmobranchs. *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 419–42.

JONES, F. R. HARDEN, 1951. The swimbladder and the vertical movements of teleostean fishes. *J. Exp. Biol.*, Vol. 28, pp. 553–66.

KAMPA, ELIZABETH M., 1953. New forms of visual purple from the retinas of certain marine fishes: a re-examination. *J. Physiol.*, Vol. 119, pp. 400–09.

KERSLAKE, JOY, 1953. Occurrence of *Asparagopsis armata* Harv. on the coast of Devon. *Nature, Lond.*, Vol. 172, p. 874.

LORCH, I. J., DANIELLI, J. F. & HÖRSTADIUS, S., 1953. The effect of enucleation on the development of sea urchin eggs. I. Enucleation of one cell at the 2, 4 or 8 cell stage. *Exp. Cell. Res.*, Vol. 4, pp. 253–62.

LOWENSTEIN, OTTO, 1953. Effect of galvanic polarization on the impulse discharge from the horizontal ampulla of the isolated elasmobranch labyrinth. *Nature, Lond.*, Vol. 172, pp. 549–50.

LOWNDES, A. G., 1953. The densities of some common Echinodermata from Plymouth. *Ann. Mag. Nat. Hist.*, 12th ser., Vol. 6, pp. 623–24.

LOWNDES, A. G., 1953. The densities of some common aquatic Mollusca from Plymouth. *Ann. Mag. Nat. Hist.*, Ser. 12, Vol. 6, pp. 950–952.

LOWY, J., 1953. Contraction and relaxation in the adductor muscles of *Mytilus edulis*. *J. Physiol.*, Vol. 120, pp. 129–40.

ROBERTSON, JAMES D., 1953. Further studies on ionic regulation in marine invertebrates. *J. Exp. Biol.*, Vol. 30, pp. 277–96.

RUSSELL, FREDERICK STRATTEN, 1953. *The Medusae of the British Isles*. Cambridge. (E. T. Browne Monograph of the Marine Biological Association of the United Kingdom.)

RUSSELL, F. S., 1953. The English Channel. *Trans. Devon. Assoc.*, Vol. 85, pp. 1–17.

SILÉN, LARS, 1954. On the nervous system of *Phoronis*. *Ark. Zool.*, Ser. 2, Bd. 6, pp. 1–40.

TRUEMAN, E. R., 1953. Observations on certain mechanical properties of the ligament of *Pecten*. *J. Exp. Biol.*, Vol. 30, pp. 453–467.

WESTBLAD, EINAR, 1953. *Boreohydra simplex* Westblad, a 'bipolar' Hydroid. *Ark. f. Zool.*, Vol. 4, pp. 351–54.

WESTBLAD, EINAR, 1953. Marine Macrostomida (Turbellaria) from Scandinavia and England. *Ark. f. Zool.*, Vol. 4, pp. 391–408.

WESTBLAD, EINAR, 1953. New Turbellaria parasites in echinoderms. *Ark. f. Zool.*, Vol. 5, pp. 269–88.

WILSON, DOUGLAS P., 1953. Electronic-flash photomicrography. *Discovery*, Vol. 14, pp. 347–349.

Membership of the Association

The total number of members on 31 March 1954 was 770, being 61 more than on 31 March 1953; of these the number of life members was 94 and of annual members 676. The number of Associate members is six.

During the year Prof. Louis Fage has been elected an Honorary Member.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Capital Grant. The Council wish to record their thanks to the Development Commissioners for the very substantial Capital Grant to meet the cost of construction of the new research vessel *Sarsia*. This is the first vessel to be owned by the Association which has been designed and built especially for marine biological research.

Private Income. The Council gratefully acknowledges the following generous grants received during the year:

From the Fishmongers' Company (£300), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Nottingham (£10. 10*s*.), Southampton (£15. 15*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), Hull (£10. 10*s*.), Reading (£21), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1954–55:

President

Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of IVEAGH, C.B., C.M.G.
Sir NICHOLAS E. WATERHOUSE, K.B.E.
Col. Sir EDWARD T. PEEL, K.B.E., D.S.O., M.C.
Vice-Admiral Sir JOHN A. EDGELL, K.B.E., C.B., F.R.S.
Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S.
Sir EDWARD J. SALISBURY, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir AUBREY C. H. SMITH, K.B.E., C.B., M.V.O.
A. T. A. DOBSON, C.B., C.V.O., C.B.E.
Major E. G. CHRISTIE-MILLER
MORLEY H. NEALE, C.B.E.
The Earl of VERULAM

COUNCIL

To retire in 1955

Prof. H. GRAHAM CANNON, Sc.D., F.R.S.
O. D. HUNT
Prof. O. E. LOWENSTEIN, D.Sc.
G. P. WELLS, Sc.D.
R. S. WIMPENNY

To retire in 1956

J. N. CARRUTHERS, D.Sc.
Prof. H. MUNRO FOX, F.R.S.
Prof. A. L. HODGKIN, F.R.S.
Prof. J. E. SMITH, Sc.D.
Prof. V. C. WYNNE-EDWARDS

To retire in 1957

Miss ANNA M. BIDDER, Ph.D.
D. J. CRISP, Ph.D.
Prof. J. E. HARRIS, Ph.D.
C. E. LUCAS, D.Sc.
Prof. C. M. YONGE, D.Sc., F.R.S.

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2

Secretary

F. S. RUSSELL, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. WALL (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
S. SMITH, Ph.D. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
H. W. PARKER, D.Sc. (Zoological Society)
Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S. (Royal Society)

BALANCE SHEET 1953–54

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1954

	£	£	£	£
CAPITAL RESERVE ACCOUNT:			**FIXED ASSETS:**	
As at 31st March 1953	111,099		At valuations as estimated by the Director at 31st March 1954:	
Add: Grants received and receivable during the year from the Development Fund in respect of cost to date of R.V. 'Sarsia'	59,348	170,447	Boats and Equipment:	
SURPLUS ACCOUNT:			M.F.V. 'Sula'	11,000
As at 31st March 1953	9,781		Motor Boat 'Gammarus'	200
Add: Excess of Income over Expenditure for the year	1,703			11,200
	11,484		Laboratory apparatus, equipment and machinery at cost	10,300
Less: Buildings Reconstruction Fund Expenditure transferred ...	32	11,452	Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost	20,200
		181,899	R.V. 'Sarsia' at cost	137,758
				179,458
AQUARIUM SINKING FUND:			**GENERAL FUND INVESTMENT** at book value	
As at 31st March 1953	279		£352. 2*s.* 3*d.* 2½% Treasury Stock	232
Add: Donations for Rebuilding Aquarium Tanks	26		(Market Value £228; last year £208)	
Bank Deposit Interest credited during year	2	307	**E. T. BROWNE BEQUEST FUND INVESTMENT,** at cost	
E. T. BROWNE BEQUEST FUNDS:			£4000. 15*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ...	3,912
	£		(Market value £3,441 last year £3,221)	
Library Fund as at 31st March 1953	1,260		**COMPOSITION FEES FUND INVESTMENT,** at cost:	
Add: Interest on Investments	36		£18. 8*s.* 6*d.* 2½% Treasury Stock	16
		1,296	£1165. 15*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ...	1,077
Special Apparatus Fund as at 31st March 1953 ...	2,714		(Market value £1,013 last year £874)	1,093
Add: Interest on Investments	81		**CURRENT ASSETS:**	
		2,795	**STOCKS ON HAND,** as valued by the Director:	
		4,091		£
Less: Scientific Publications Fund as at 31st March 1953	2,290		Specimens	600
Add: Bank Deposit Interest	38		Chemicals	250
	2,328		Journals	400
	£		Nets, gear and general boats' equipment	100
Less: Payment towards Publication of Monograph on Medusae of the British Isles	1,984			1,350
Deficiency on Realization of Investments ...	365		**SUNDRY DEBTORS:**	
	2,349		Sales of Specimens, Journals, Nets and Hydrographical Gear	862
		21	Recoverable Expenditure	194
		4,070		1,056
ROYAL SOCIETY GRANT—PUBLICATION OF MONOGRAPH ON MEDUSAE OF THE BRITISH ISLES:			**PREPAYMENTS**	312
As at 31st March 1953	766		**BALANCE AT BANK AND CASH IN HAND:**	
Less: Payment to Cambridge University Press	2,750		Coutts & Co., London	
	1,984		Current Account	1,812
Less: Paid out of E. T. Browne Bequest—Scientific Publications Fund	1,984	—	Deposit Accounts	3,243
			Lloyds Bank Ltd, Plymouth	1,766
A. R. T. MOMBER BEQUEST FUND:			Cash in Hand	103
Received during the year	938			6,924
Add: Bank Deposit Interest credited	4	942		9,642
COMPOSITION FEES FUND:				
As at 31st March 1953	1,014			
Add: Fees Received during year	79	1,093		

BUILDINGS RECONSTRUCTION FUND:			
As at 31st March 1953		1,298	
Add: Surplus Account—expenditure transferred		32	
		1,330	
Less: Expenditure during year	8		
Transfer to Radioactive Substances Laboratory Building Fund	186		
		194	
			1,136
WAR DAMAGE COMPENSATION (PART II):			
Received during year:			
Compensation	764		
Interest (Gross)	240		
		1,004	
Less: Refunded in respect of personal equipment		47	
			957
RADIOACTIVE SUBSTANCES LABORATORY BUILDING FUND:			
Transfered from Buildings Reconstruction Fund		186	
Less: Adverse Balance at 31st March 1953	183		
Expenditure during year	3		
		186	
			—
RADIOACTIVE SUBSTANCES RESEARCH FUND:			
As at 31st March 1953		584	
Add: Grants Received from the Development Fund		1,360	
		1,944	
Less: Expenditure during year		1,289	
			655
RESEARCH FUND—MISS D. BALLANTINE:			
As at 31st March 1953		87	
Add: Grants Received from the Development Fund		589	
		676	
Less: Expenditure during year		571	
			105
CURRENT LIABILITIES:			
Accrued Expenses		1,408	
Subscriptions and Grants Received in advance		57	
Equipment and Apparatus—R.V. 'Sarsia'		1,708	
			3,173
Note: Capital commitments outstanding amount to £18,072 (1953—£60,670) in respect of the Library extension and New Dogfish store of which £16,365 will be recoverable under a Development Fund Grant			
			£194,337

O. D. HUNT
ALASTAIR GRAHAM
Members of the Council

£194,337

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1954, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
24 May 1954

PRICE WATERHOUSE & CO.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1954

	£	£
SALARIES, including Pay Additions and Association's contributions to Superannuation Scheme and National Insurance		23,784
LABORATORY AND BOATS' CREWS' WAGES, including Pay Additions, National Insurance, contributions to Superannuation Scheme, War Bonus and Employers' Liability Insurance		19,316
UPKEEP OF LIBRARY		723
SCIENTIFIC PUBLICATIONS, *less* Sales		1,748
UPKEEP OF LABORATORIES AND AQUARIUM:		
Buildings and Machinery	442	
Electricity, Gas, Coal and Water	932	
Chemicals and Apparatus	1,277	
Insurances, Ground Rent and Rent of Stores	164	
Travelling Expenses	670	
Audit Fee	63	
Stationery, Postages, Telephone, Carriage and Sundries	958	
Specimens	195	
		4,701
MAINTENANCE AND HIRE OF BOATS:		
Petrol, Oil, Paraffin, etc.	1,091	
Maintenance of and Repairs to Nets, Gear and Apparatus	2,784	
Boat Hire, Collecting Expenses and Upkeep of Truck	240	
Insurances	1,347	
Hire of 'Decca' Navigator—R.V. 'Sarsia'	166	
Hire of R.V. 'Sabella'	629	
		6,257
ENTERTAINMENT EXPENSES		55
BALANCE, being excess of income over expenditure for the year		1,703
		£58,287

Note: No depreciation is provided on fixed assets.

	£	£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture and Fisheries grant from Development Fund		51,353	
Fishmongers' Company		400	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of: London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £21, Sheffield £10. 10*s*., Imperial College £10 and Ministry of Works £104)		1,235	
			52,988
SUBSCRIPTIONS (excluding those received in advance)			648
SALES:			
Specimens		2,438	
Fish		447	
	£		
Nets, Gear and Hydrographical Apparatus	694		
Less: Cost of Materials	351		
		343	
			3,228
INTEREST ON INVESTMENTS			41
INTEREST ON BANK DEPOSITS LESS CHARGES			65
SALE OF DR M. V. LEBOUR'S BOOK			8
SALE OF 'PLYMOUTH MARINE FAUNA'			—
AQUARIUM:			
Admission Fees		1,648	
Sale of Guides		103	
		1,751	
Less: Maintenance of Building	194		
Printing Guides and Tickets	11		
Advertising	51		
Food	83		
Wages	103		
		442	
			1,309
			£58,287

LIST OF GOVERNORS, FOUNDERS, MEMBERS, HONORARY AND ASSOCIATE MEMBERS

1954

GOVERNORS

The British Association for the Advancement of Science, *Burlington House, W.* 1
The University of Oxford
The University of Cambridge
The Worshipful Company of Clothworkers, 48 *Fenchurch Street, E.C.* 3
The Worshipful Company of Fishmongers, *London Bridge, E.C.* 4
The Prime Warden. (**Council,** 1886→)
Edwards, Harrison S., *Westhumble Lacey, nr Dorking, Surrey.* (**Council,** 1950→)
Christie-Miller, Major E. G., 38 *Hyde Park Street, W.* 2. (**Council,** 1941→; **Hon. Treasurer,** 1941→; **Vice-President,** 1951→)
The Zoological Society of London, *Regent's Park, N.W.* 8
The Royal Society, *Burlington House, Piccadilly, W.* 1
Ministry of Agriculture and Fisheries, 3 *Whitehall Place, S.W.* 1
Bayly, Robert (the late). (**Council,** 1896–1901)
Bayly, John (the late)
Browne, E. T. (the late). (**Council,** 1913–19; 1920–37)
Thomasson, J. P. (the late). (**Council,** 1896–1903)
Bidder, G. P., Sc.D. (the late). (**Council,** 1899–1953; **President,** 1939–45; **Vice-President,** 1948–53)
The Lord Moyne, P.C., D.S.O. (the late). (**Vice-President,** 1929; 1939–45; **President,** 1930–39)
Allen, E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary.) (**Council,** 1895–1942; **Secretary,** 1895–1936; **Hon.Governor,** 1937–42)

FOUNDERS

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1884 Bulteel, Thos. (the late)
1884 Burdett-Coutts, W. L. A. Bartlett (the late)
1884 Crisp, Sir Frank, Bart. (the late). (**Council,** 1884–92; **Hon. Treasurer,** 1884–88)
1884 Daubeny, Captain Giles A. (the late)
1884 Eddy, J. Ray (the late)
1884 Gassiott, John P. (the late)
1884 Lankester, Sir E. Ray, K.C.B., F.R.S. (the late). (**Hon. Secretary,** 1884–90; **President,** 1891–1929)
1884 Lord Masham (the late)
1884 Moseley, Prof. H. N., F.R.S. (the late). (**Chairman of Council,** 1884–88)

1884 Lord Avebury, F.R.S. (the late). (**Vice-President**, 1884–1913)
1884 Poulton, Prof. Sir Edward B., F.R.S. (the late). (**Council**, 1888–94)
1884 Romanes, Prof. G. J., LL.D., F.R.S. (the late) (**Council**, 1884–91)
1884 Worthington, James (the late)
1885 The 15th Earl of Derby (the late)
1887 Weldon, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890–1901; representing British Association, 1901–5)
1888 Bury, Henry, *The Gate House*, 17 *Alumdale Road, Bournemouth West*
1888 The Worshipful Company of Drapers, *Drapers' Hall, E.C.* 2
1889 The Worshipful Company of Grocers, *Grocers' Hall, Princes Street, E.C.* 2
1889 Thompson, Sir Henry, Bart. (the late). (**Vice-President**, 1890–1903)
1889 Lord Revelstoke (the late)
1890 Riches, T. H. (the late). (**Council**, 1920–25)
1892 Browne, Mrs E. T. (the late)
1898 Worth, R. Hansford, M.Inst.C.E. (the late)
1899 The Earl of Iveagh, C.B., C.M.G., 11 *St James's Square, S.W.* 1. (**Vice-President**, 1929→)
1902 Gurney, Robert, D.Sc. (the late). (**Council**, 1932–5)
1904 Shaw, Joseph, K.C. (the late)
1909 Harding, Colonel W. (the late)
1910 Murray, Sir John, K.C.B., F.R.S. (the late). (**Council**, 1896–99; **Vice-President**, 1900–13)
1912 Swithinbank, H. (the late)
1913 Shearer, Dr Cresswell, F.R.S. (the late)
1913 Heron-Allen, E., F.R.S. (the late)
1918 Evans, George (the late). (**Hon. Treasurer**, 1915–31; **Vice-President** 1925–33)
1920 McClean, Capt. W. N., 39 *Phillimore Gardens, W.* 8
1920 Lord Buckland of Bwlch (the late)
1920 Llewellyn, Sir D. R. (the late)
1921 Harmer, F. W. (the late)
1924 The MacFisheries, Ltd., *Ocean House, Pudding Lane, E.C.* 3
1924 Lady Murray (the late)
1925 The Institution of Civil Engineers, *Great George Street, Westminster, S.W.* 1
1925 Discovery Committee
1927 Bidder, Miss Anna M., Ph.D., 4 *Hills Avenue, Cambridge* (**Council**, 1948–51, 1954→)
1933 Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel and Co., Ltd. P.O. Box* 331, *Alexandria, Egypt*. (**Vice-President**, 1936→)
1938 Buchanan, Dr Florence (the late)
1945 Brown, Arthur W. W. (the late)

MEMBERS

* Life Members

1949 Abbott, B. C., Ph.D., A.Inst.P., *The Laboratory, Citadel Hill, Plymouth, Devon*
1945 Aberdeen University Library, *The University, Aberdeen*
1934 Adam, Mrs K. M. G., 84 *Lasswade Road, Edinburgh* 9
1951 Adams, E., 2 *Woodford Crescent, Marsh Mills, Plympton, Devon*

1940 Adrian, Prof. E. D., O.M., M.D., D.Sc., LL.D., P.R.S., *St Chad's, 48 Grange Road, Cambridge*
1947 Affleck, R. J., 1 *Helmsdale Road, London, S.W.* 16
1949 Aleem, A. A., Ph.D., *Faculty of Science, Farouk I University, Moharram Bey, Alexandria, Egypt*
1950 Alexandrowicz, J. S., Ph.D., M.D., *The Laboratory, Citadel Hill, Plymouth, Devon*
1954 Allen, G. L., *The Nook, 87a Bury Old Road, Sedgley Park, Prestwich, Manchester*
1951 Allen, J. A., *Dept. of Zoology, The University, Glasgow, W.* 2
1952 Allen, Miss J. M., *Tenements Farm, Chipperfield, Herts*
1949 Allen, Mrs M. E., 82 *Oaks Avenue, Worcester Park, Surrey*
1953 Alvariño, Señora A., *Doce de Octubre* 11–1 °C, *Madrid, Spain*
1927 Amirthalingam, C., Ph.D., 2 *Dickmans Path, Colombo, Ceylon*
1950 Arnold, D. C., *Fair Acre, Abbotskerswell, Newton Abbot, Devon*
1944 Ashby, D. G., 195 *Chesterton Road, Cambridge*
1954 Ashhurst, Miss D. E., *St Hugh's College, Oxford*
*1929 Atkins, Miss D., D.Sc., *The Laboratory, Citadel Hill, Plymouth, Devon*
*1939 Atkins, W. R. G., C.B.E., O.B.E., Sc.D., F.R.I.C., F.Inst.P., F.R.S., *The Old Vicarage, Antony, Torpoint, Cornwall*
*1910 Atkinson, G. T., *Gresham House, Esplanade, Lowestoft, Suffolk*
1951 Atlantic Biological Station, *St Andrews, N.B., Canada*

1948 Baal, H. J., 3 *Bel Royal Villas, Jersey, C.I.*
1950 Baerends, Prof. G. P., *Zoölogisch Laboratorium, Rijksstraatweg* 78, *Haren (Gron.), Holland*
1949 Bagenal, T., *Marine Station, Millport, Isle of Cumbrae, Scotland*
*1952 Baily, Joshua L. Jr., 4435 *Ampudia Street, San Diego* 3, *California, U.S.A.*
1950 Bainbridge, R., Ph.D., 43 *Strathmore Avenue, Hull*
1953 Bainbridge, V., *West African Fisheries Research Institute, Freetown, Sierra Leone*
*1920 Baker, J. R., D.Sc., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford*
1936 Baldwin, Prof. E., Ph.D., *Dept. of Biochemistry, University College, Gower Street, London, W.C.* 1 (**Council**, 1946–48)
1950 Ballantine, Miss D., *The Laboratory, Citadel Hill, Plymouth, Devon*
1951 Bangor, Marine Biological Station, *University College of North Wales, Bangor, Caern*
1953 Barker, J. A., 27 *Finchley Way, Finchley, London, N.* 3
1949 Barnard, E. E. P., 7 *Webster Gardens, Ealing, London, W.* 5
1939 Barnes, H., Ph.D., F.R.I.C., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1954 Barnes, M. McC., *Mandeville, Rosebank Crescent, Pennsylvania, Exeter, Devon*
1953 Barns, H. N., *Spearwood, Combpyne, near Axminster, Devon*
1939 Barrington, Prof. E. J. W., D.Sc., *Dept. of Zoology, The University, Nottingham*
1951 Barron, H., 65 *Sumerton Road, Belfast, N. Ireland*
1946 Barter, W. Y., 29 *Sea View Avenue, Plymouth, Devon*
1939 Bassindale, R., *Dept. of Zoology, The University, Bristol*
1946 Batham, Miss E. J., Ph.D., *Portobello Marine Biological Station, via Port Chalmers, Otago, New Zealand*
1950 Baughman, J. L., *Las Olas Oceanographic Foundation, Rockport, Texas, U.S.A.*

1939 Baxter, E. W., *Biology Dept., Medical School, Guy's Hospital, London, S.E.* 1
*1929 Baylis, L. E., Ph.D., *Dept. of Physiology, University College, Gower Street, London, W.C.* 1
1934 Beadle, L. C., *Dept. of Biology, University College of East Africa, P.O. Box* 262, *Kampala, Uganda*
1928 Beer, Sir Gavin de, Kt., D.Sc., F.R.S., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1950 Bell, Mrs E. B., *Solva, Glanford Road, Brigg, Lincs*
1954 Berner, L. D. Jr., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1947 Berrill, Prof. N. J., F.R.S., *Dept. of Zoology, McGill University, Montreal, Canada*
1947 Best, A. C. G., 6 *Station Road, Loudwater, High Wycombe, Bucks*
1948 Betts, Slade, 100 *Avondale Road, Bromley, Kent*
1953 Bhattacharyya, Dr R. N., 44/*B Kalighat Road, Calcutta-26, India*
1953 Bhimachar, B. S., D.Sc., F.N.I., *Central Marine Fisheries Research Station, West Hill, Calicut* 5, *South India*
1903 Bidder, Col. H. F., *The Malting House, Nettlebed, near Henley-on-Thames, Oxon*
*1945 Bingley, F. J., *Flatford Mill Field Centre, East Bergholt, near Colchester, Essex*
1925 Birkbeck College, *The Library, Malet Street, London, W.C.* 1
1951 Birkett, L., *Fisheries Laboratory, Lowestoft, Suffolk*
1947 Bishop, M. W. H., *Meadow Farm, Waterbeach, Cambs*
1945 Black, J. A., *Ash House, Caton, near Lancaster, Lancs*
1951 Blackburn, M., *C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*
1930 Blaschko, Dr H., *Department of Pharmacology, South Parks Road, Oxford*
1952 Blaxter, J. H. S., *Pathside, Frithesden Copse, Berkhamsted, Herts*
1910 Bloomer, H. H., *Longdown, Sunnydale Road, Swanage, Dorset*
1953 Boalch, G. T., *Westfield, Beer, near Seaton, Devon*
1953 Boczarow, B., *Greenbank Hospital, Plymouth, Devon*
1951 Boden, B. P., Ph.D., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1936 Bogue, Prof. J. Yule, D.Sc., *Heyscroft, Hartley Road, Altrincham, Cheshire*
1932 Bolitho, Capt. R. J. B., *Gorey, Jersey, C.I.*
1945 Boney, A. D., 1 *Whiteford Road, Mannamead, Plymouth, Devon*
1954 Bonham, Dr K., *Applied Fisheries Laboratory, University of Washington, Seattle, Washington, U.S.A.*
*1933 Boschma, Prof. Dr H., *Rijksmuseum van Natuurlijke Historie, Leiden, Holland*
1947 Bossanyi, J., *Dove Marine Laboratory, Cullercoats, Northumberland*
1954 Bowers, A. B., *Marine Biological Station, Port Erin, Isle of Man*
*1954 Bradshaw, J. S., *P.O. Box* 891, *Del Mar, California, U.S.A.*
1949 Braithwaite, E. R., *Research Dept,. Acheson Colloids Ltd., Prince Rock, Plymouth, Devon*
1940 Brambell, Prof. F. W. Rogers, D.Sc., F.R.S., *Dept. of Zoology, University College of North Wales, Bangor, Caern.* (**Council** 1944–47, 1948–51)
1954 Brehaut, R. N., *La Canurie, Collings Road, St Peter Port, Guernsey, C.I.*
1924 Brightwell, L. R., 1 *Edith Avenue, Peacehaven, Sussex*
1933 Bristol University, *Dept. of Zoology, Bristol*
*1941 British Celanese Ltd., *Celanese House, Hanover Square, London, W.* 1
1948 British Cod Liver Oils (Hull and Grimsby) Ltd., *P.O. Box No.* 18, *Hull*

1939 British Ropes Ltd., *Western Avenue, Cardiff*
*1946 Brock, Mrs C. H., Ph.D., *Dept. of Agriculture, Downing Street, Cambridge*
1953 Brockman, Miss P. E., *Pathology Dept., South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon*
1946 Brough, Prof. James, D.Sc., *Dept. of Zoology and Comparative Anatomy, University College, Newport Road, Cardiff*
1928 Brown, Miss E. M., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingston-on Thames, Surrey*
1936 Brown, Herbert H., O.B.E., Ph.D., *Fisheries Division, F.A.O. of U.N., Viale delle Terme di Caracalla, Rome, Italy*
*1954 Brull, Prof. L., *Institut de Clinique et de Policlinique Médicales, Hôpital de Baviere, Université de Liége, Liége, Belgium*
1953 Buchsbaum, Prof. R., Ph.D., *Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh* 13, *Pa., U.S.A.*
1953 Buckee, R. E., 32 *Weston Drive, Stanmore, Mddx*
*1925 Bull, Herbert O., D.Sc., *Dove Marine Laboratory, Cullercoats, Northumberland*
1951 Burgess, G. H. O., *Dept. of Zoology, University College, Hull*
1954 Burley, Miss E. A. M., *Avondale,* 2 *Narborough Road South, Leicester*
1948 Burrows, Mrs E. M., Ph.D., *Hartley Botanical Laboratories, The University, Liverpool* 3
1950 Bursa, A., *c/o J. Kordowski,* 206 *W.* 109 *Street, New York* 25, *U.S.A.*
1947 Burton, Miss J. M., *Pinecroft, Ockham Road North, East Horsley, Surrey*
1930 Burton, M., D.Sc., *British Museum (Natural History), Cromwell Road, London, S.W.* 7. (**Council**, 1936–39)
1947 Burton, R. F., *Pinecroft, Ockham Road North, East Horsley, Surrey*
1949 Bush, Prof. S. F., D.Phil., *Dept. of Zoology, University of Natal, Pietermaritzburg, S. Africa*
1949 Butcher, A. W., *Three Salmons Hotel, Usk, Mon*
1950 Butler, Mrs B. R. R., *c/o* 23 *Russell Avenue, Hartley, Plymouth, Devon*

1949 Cameron, H. D., 5 *Compton Park Road, Mannamead, Plymouth, Devon*
*1950 Cameron, Prof. W. M., *Institute of Oceanography, University of British Columbia, Vancouver, Canada*
1920 Cannon, Prof. H. Graham, Sc.D., F.R.S., *Dept. of Zoology, Victoria University, Manchester.* (**Council**, 1927–30, 1932–34, 1937–41, 1942–45, 1952→)
1950 Cant, Miss M. A., 8 *Clyde Road, Sutton, Surrey*
1954 Canterbury Museum, *Rolleston Avenue, Christchurch, New Zealand*
1950 Capstick, C. K., 24 *Kennersdene, Tynemouth, Northumberland*
1953 Carr-Saunders, E. M., *Bridge House, St John's Vale, Keswick, Cumb*
1927 Carruthers, J. N., D.Sc., F.Inst.P., *National Institute of Oceanography, Wormley, near Godalming, Surrey.* (**Council**, 1948–51, 1953→)
1923 Carter, G. S., Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
*1931 Cattell, Dr McKeen, *Cornell University Medical College,* 477 *First Avenue, New York City, U.S.A.*
*1948 Cattley, J. G., *Fisheries Laboratory, Lowestoft, Suffolk*
1949 Centre de Recherches et D'Etudes Océanographiques, 1 *rue Victor Cousin, Paris (Ve), France*
*1949 Chang, H. W., *Zoological Institute, Academia Sinica,* 320 *Yo Yang Road, Shanghai, China*
*1952 Chaplin, Viscount, *Wadstray House, Blackawton, near Totnes, Devon*
*1949 Chapman, Garth, Ph.D., *Nunns, Coxtie Green, Brentwood, Essex*

1951 Chappell, R. J., *Kenilworth, Paul Hill, Newlyn, Penzance, Cornwall*
1936 Charterhouse School, *Biological Dept., Godalming, Surrey*
1947 Cheng, Prof. Chung, Ph.D., *Dept. of Oceanography, National Amoy University, Amoy, China*
*1947 Chidambaram, K., *Fisheries Biological Station, West Hill Post, South Malabar, India*
1952 Childs, P., F.R.C.S., 8 *The Crescent, Plymouth, Devon*
1953 Chile: Dr Parmenio Yañez Andrade, Director, *Marine Biological Station of the University of Chile, Casilla* 13-*D, Viña del Mar, Chile.*
1946 Chipperfield, Philip N. J., Ph.D., *Hillside, Manor Road, Brixham, Devon*
1951 Christie, I. G., 7 *Quarry Park Road, Cheam, Surrey*
1942 Christie-Miller, Major E. G., 38 *Hyde Park Street, London, W.* 2. (**Council,** 1941→; **Hon. Treasurer,** 1941→; **Vice-President,** 1951→)
1952 Clark, J. T., 12 *Hazel Avenue, Torquay, Devon*
1952 Clark, R. B., *Dept. of Zoology, The University, Glasgow, W.* 2
1951 Clarke, Mrs J., 32 *Boolarong Road, Pymble, Sydney, N.S.W., Australia*
1949 Clarke, K. U., *Dept. of Zoology, The University, Nottingham*
1944 Clarke, Robert H., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1951 Claxton, A. J., 26 *Broad Street, King's Lynn, Norfolk*
1951 Clay, Miss E., *Green Hollow, Gattery Lane, Brixham, Devon*
1936 Clothier, Peter, *Hill Close, Street, Somerset*
1939 Clowes, A. J., *Division of Fisheries, Beach Road, Sea Point, Cape Town, S. Africa*
*1886 Coates and Co. (Plymouth) Ltd., *Black Friars Distillery, Southside Street, Plymouth, Devon*
*1945 Cobham, Lt.-Cdr. A. J., R.N., *Noel Cottage, Castle Street, Porchester, Hants.*
*1925 Cockshott, Lt.-Col. A. M., R.A.S.C., *Cotteswold Naturalists' Field Club, City Library, Gloucester*
1953 Cohen, R. D., 2 *St Lawrence Road, Plymouth, Devon*
1933 Cole, H. A., D.Sc., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
*1948 Collier, Albert, *c/o Fish and Wildlife Service, Ft. Crockett, Galveston, Texas, U.S.A.*
*1885 Collier and Co., 53 *Southside Street, Plymouth, Devon*
1952 Collinge, C. F., 25 *Elmstead Avenue, Chislehurst, Kent*
1954 Collins, D. J., 48 *Belgrave Road, Mutley, Plymouth, Devon*
1950 Collins, William N., 603 *Thatcher Avenue, River Forest, Illinois, U.S.A.*
1947 Collis, Miss M. M., 27 *Mowbray Road, Cambridge*
1930 Colman, J. S., *Marine Biological Station, Port Erin, Isle of Man.* (**Council,** 1951–54)
1947 Cook, Miss P. M., 51 *Runnymeade Crescent, Streatham, London, S.W.* 16
1940 Cook, R. H., *Moor Close, Melbourn, Cambridge*
*1933 Cooper, L. H. N., D.Sc., F.R.I.C., *The Laboratory, Citadel Hill, Plymouth, Devon*
1937 Corbin, P. G., *The Laboratory, Citadel Hill, Plymouth, Devon*
1937 Corbin, Mrs P. G., Ph.D., *Dostabrook, Horrabridge, Devon*
1954 Corcoran, E. F., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1946 Corlett, John, M.Sc., *Fisheries Laboratory, Lowestoft, Suffolk*
1937 Cosway, C. A., *South Devon Technical College, Teignmouth Road, Torquay, Devon*
1941 Cott, H. B., D.Sc., *University Museum of Zoology, Cambridge*

1948 Council for Promotion of Field Studies, *Dale Fort Field Centre, Haverfordwest, Pembs*
1952 Cowper, T. R., *C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australia*
1936 Crawford, G. I., 18 *East Drive, Carshalton Beeches, Surrey*
1952 Crawshaw, K. Ridgway, *Heron Cottage, White Notley, Witham, Essex*
1953 Creek, Miss Gwen (Mrs J. M. Grant), 9 *Caergog Terrace, Aberystwyth, Cardigan*
*1928 Crew, Prof. F. A. E., M.D., D.Sc., F.R.S., *Usher Institute, Warrenden Park Road, Edinburgh*, 9
*1952 Crisp, D. J., Ph.D., *Bryn Hydd, Holyhead Road, Bangor, Caern.* (**Council**, 1954→)
1929 Crofts, Miss D. R., D.Sc., *Deerbank, Noisey Wood, Billericay, Essex*
1954 Croghan, P. C., *Dept. of Zoology, Downing Street, Cambridge*
1951 Currie, R. I., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1954 Curtis, A. S. G., 17 *Parkhill Road, London, N.W.* 3
1950 Cushing, D. H., D.Phil., *Fisheries Laboratory, Lowestoft, Suffolk*
*1930 Cuthbertson, Norman, 101 *Johnstone Avenue, Dartmouth, Nova Scotia, Canada*

1952 Dale, E., 13 *North Street, Royton, Oldham, Lancs*
1922 Dale, Sir Henry H., O.M., G.B.E., F.R.C.P., LL.D., F.R.S., *The Wellcome Trust*, 28 *Portman Square, London, W.* 1. (**Council**, 1922–28)
*1948 Dales, R. Philips, Ph.D., 67 *Westmoreland Avenue, Squirrels Heath, Essex*
1947 Dall, William, *Grace Street, Corinda, S.W.* 4, *Brisbane, Queensland, Australia*
*1919 Damant, Capt. G. C. C., C.B.E., R.N., *Thursford, Cambridge Road, East Cowes, I. of W.* (**Council**, 1928–31, 1937–40)
1939 Danielli, Prof. J. F., D.Sc., *Dept. of Zoology, King's College, Strand, London, W.C.* 2. (**Council**, 1944–45)
1947 Danmarks Akvarium, *Charlottenlund, Denmark.*
1929 Darby, Dr H. H., *Carnegie Institution of Washington*, 5241 *Broad Branch Road, N.W., Washington* 15, *D.C., U.S.A.*
1954 Dare, P. J., 30 *Aylesbury Crescent, Whitleigh, Plymouth, Devon*
1948 Dartmouth: The Royal Naval College, *Dartmouth, Devon*
1952 Davenport, Demorest, Ph.D., *Dept. of Biology, University of California, Santa Barbara College, Santa Barbara, California, U.S.A.*
1920 Davidson, Dr W. Cameron, *Avonleigh, Acadia Road, Torquay, Devon*
1954 Davies, Miss P. A., *Midland Bank House, Fore Street, Kingsbridge, S. Devon*
1931 Dawes, B., D.Sc., *Dept. of Zoology, King's College, Strand, London, W.C.* 2
1944 Day, Prof. J. H., D.F.C., *Dept. of Zoology, University, Rondebosch, Cape Town, S. Africa*
1948 Day, Lionel E., 24 *Inverness Avenue, Westcliff-on-Sea, Essex*
1938 Deacon, G. E. R., C.B.E. D.Sc., F.R.S., *Flitwick House, Milford, Surrey.* (**Council**, 1946–49, 1950–53)
1952 Deevey, Miss Georgiana B., Ph.D., *The Bingham Oceanographic Laboratory, Yale University, New Haven, Conn., U.S.A.*
1952 Delaware: Dept. of Biological Sciences, University of Delaware, *Newark, Delaware, U.S.A.*
1939 Dennell, Ralph, D.Sc., *Dept. of Zoology, The University, Manchester*, 13
1954 Deshpande, R. D., *Zoology Dept., The University, Southampton*

*1915 Dick, G. W., J.P., 500 *Manning Road, Durban, Natal, S. Africa*
1944 Digby, P. S. B., *Dept. of Biology, St Thomas's Hospital Medical School, Westminster, London, S.W.* 1
1948 Dodd, J. M., Ph.D., *Gatty Marine Laboratory, The University, St Andrews, Fife*
1954 Dominion Museum, *Wellington, New Zealand*
1953 Dorsett, D. A., 68 *Slewins Lane, Hornchurch, Essex*
1949 Douglas, Leslie, 11 *Harbour View, Seahouses, Northumberland*
1940 Dowson, Capt. W. B., *Roskellan, Mawnan, near Falmouth, Cornwall*
1954 Dowson, Mrs L. R. M., 27 *Pemberton Gardens, London, N.* 19
1952 Droop, M. R., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1946 Duly, S. J., 68 *Richmond Hill Court, Richmond, Surrey*
1950 Duncan, Miss N., *Royal Holloway College, Englefield Green, Surrey*
1939 Dundee University College Library, *Dundee, Forfar.*
1949 Dussart, B. H., 13 *Quai de Rives, Thonon (Haute-Savoie), France*

*1934 Eales, Miss N. B., D.Sc., *Zoology Dept., The University, Reading*
1945 Edgell, Vice-Admiral Sir John A., K.B.E., C.B., F.R.S., 4 *Royal Avenue, Worcester Park, Surrey.* (**Council**, 1945–48, **Vice-President**, 1948→)
1951 Edwards, C., 45 *Queensbury Park, Rosetta, Belfast, N. Ireland*
1927 Eggleton, P., D.Sc., *Dept. of Physiology, The University, Edinburgh*
1928 Egypt: Coastguard and Fisheries Service, *Alexandria, Egypt*
1948 Elgood, J. H., 27 *Knightscroft Avenue, Rustington, Sussex*
1950 Elliott, Mrs J. H., 39 *Woodhaw, Egham, Surrey*
1953 El Maghraby, A. M., *Queen Mary College, Mile End Road, London, E.* 1
*1929 Elmhirst, L. K., *Dartington Hall, Dartington, near Totnes, Devon*
1951 Etherington, D., 1 *St John's Gardens, London, W.* 11
*1923 Evans, W. Edgar, 38 *Morningside Park, Edinburgh*
1942 Ewer, D. W., *Dept. of Zoology, Natal University College, P.O. Box* 375, *Pietermaritzburg, Natal, S. Africa*

*1929 Faouzi, Dr Hussein, *Faculty of Science (Dept. of Zoology), Farouk I University, Moharram Bey, Alexandria, Egypt*
1953 Farrington, Major G. B. O., 4*b Balmoral Place, The Hoe, Plymouth, Devon*
1948 Faulkner, I. J., Ph.D., *I.C.I. Billingham Division, Billingham, E. Durham*
1950 Felinska, Mrs Maria, Dr Phil., *Diddington, near Buckden, Hunts*
*1933 Fellowes, Miss Rosalind, 23 *The Cloisters, Windsor Castle, Berks*
1950 Fisher, L. R., Ph.D., *Green Bower, Beech Lane, Lower Earley, near Reading, Bucks*
1954 The Fishing News, 68 *Victoria Street, London, S.W.* 1
1928 Ford, E., *Marine Station, Millport, Isle of Cumbrae, Scotland.* (**Council**, 1950–53)
1935 Ford, E. B., D.Sc., F.R.S., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford*
1953 Ford, D. F., 71 *Brooklyn Road, South Norwood, London, S.E.* 25
1952 Ford, Miss V. E., 43 *Woodbourne Avenue, Brighton, Sussex*
1939 Forrest, J. E., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1950 Forster, G. R., *The Laboratory, Citadel Hill, Plymouth, Devon*
1949 Fountain, H. C., *The Store Cottage, Polbathic, Torpoint, Cornwall.*
1954 Fox, Prof. D. L., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*

1912 Fox, Prof. H. Munro, F.R.S., 2 *Westbourne Terrace Road, London, W.* 2 (**Council**, 1928–30, 1931–34, 1944–47, 1953→)
1942 Foxon, G. E. H., *Dept. of Biology, Guy's Hospital Medical School, London Bridge, London, S.E.* 1
1953 Föyn, Prof. Björn, *Universitetets Biologiske Stasjon, Drøbak, Norway*
1950 Frampton, Cdr R. H. C. F., R.N. (Rtd.), *Ministry of Agriculture and Fisheries, Citadel Hill, Plymouth, Devon.*
1924 Fraser, Miss E. A., D.Sc., *Orchard Cottage, Abinger Common, Dorking, Surrey*
1935 Fraser, F. C., D.Sc., *British Museum (Natural History), Cromwell Road, London, S.W.* 7. (**Council**, 1950–53)
*1935 Fraser, James H., D.Sc., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1952 Freeman, R. J. H., 43 *Sherwood Road, Croydon, Surrey*
*1939 Fretter, Miss Vera, D.Sc., *Dept. of Zoology, The University, Reading*
1953 Frings, Prof. H., *Dept. of Zoology and Entomology, The Pennsylvania State College, Pennsylvania, U.S.A.*
1949 Fuller, A. S., 30 *Staines Avenue, Cheam, Surrey*
1948 Furness, W. J., *Inglewood, Abbey Park Road, Grimsby, Lincs*

1941 Gardiner, Mrs A. C., *United Nations Korean Reconstruction Agency, P.O. Box* 20, *Grand Central Station, New York*, 17, *N.Y., U.S.A.*
*1928 Gates, Prof. R. R., D.Sc., LL.D., F.R.S., *Biological Laboratories, Harvard University, Cambridge* 38, *Mass., U.S.A.*
1948 Gatty Marine Laboratory (The Principal), *The University, St Andrews, Fife*
1950 Gauld, D. T., Ph.D., *Dept. of Zoology, University College, Achimota, Gold Coast*
1953 Gibbon, Mrs E., *Glenview, Clearbrook, Yelverton, Devon*
1935 Gilson, H. Cary, *Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside, Westmorland.* (**Council**, 1940–43, 1947–50, 1951–54)
1951 Glaister, Mrs K., 12 *Grey Close, London, N.W.* 11
1945 Glasgow University, *Zoology Dept., Glasgow, W.* 2
1951 Glasson, D. R., Ph.D., 34 *Craven Avenue, St Judes, Plymouth, Devon*
1946 Glover, R. S., *Oceanographical Laboratory*, 78 *Craighall Road, Edinburgh*, 6
1951 Gokhale, S. V., Ph.D., *c/o The Directorate of Marine Products, Jamnagar (Saurashtra), India*
1951 Gold Coast, University College of, *P.O. Box* 4, *Achimota, Gold Coast*
1954 Goldberg, Dr E. D., *Chemistry Division, Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1951 Goodbody, I. M., *Natural History Dept., Marischal College, Aberdeen*
1954 Goodier, R., *Deanwater Lodge, Woodford, near Bramhall, Cheshire*
1954 Gooding, R. U., *St John's College, Cambridge*
1950 Goodley, E. F. W., 2 *Thickthorn Lane, Laleham, Staines, Mddx*
1939 Goodrich, Dr Helen Pixell, 12 *Park Town, Oxford*
1939 Gordon, Miss Isabella, D.Sc., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1943 Gourock Ropework Co., Ltd., 92 *Bay Street, Port Glasgow, Renfrew*
1931 Graham, Prof. Alastair, D.Sc., *Dept. of Zoology, The University, Reading.* (**Council**, 1951–54)
1949 Graham, F. G., *Trethake, Plymstock Road, Oreston, near Plymouth, Devon*
1931 Graham, Michael, C.M.G., O.B.E., *Fisheries Laboratory, Lowestoft, Suffolk.* (**Council**, 1931–32, 1933–36, 1943–46, 1949–52)

1912 Gray, Prof. Sir James, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S., *Dept. of Zoology, Downing Street, Cambridge.* (**Council**, 1920–24, 1928→; representing Cambridge University, 1928–45; **President**, 1945→)
1943 Great Grimsby Coal, Salt and Tanning Co., *Fish Dock Road, Grimsby, Lincs.*
1949 Green, J., 61 *Ruskin Road, Crewe, Cheshire*
1950 Greene, Miss S. M., *Dalegarth, Milner Road, Ansdell, Lytham-St-Annes, Lancs*
1950 Greenfield, Leonard J., *Marine Laboratory, University of Miami, Coral Gables* 34, *Florida, U.S.A.*
*1953 Griffiths, Major R. J., *Rosslyn, Edward Road, Biggin Hill, Kent*
1948 Grigg, Miss Ursula M., *Dove Marine Laboratory, Cullercoats, Northumberland*
1948 Grove, A. V., 86 *Gower Road, Sketty, Swansea, Glam.*
1947 Guiler, E. R., *Dept. of Zoology, University of Tasmania, Hobart, Tasmania*
1947 Gundry, Joseph, and Co., *Bridport, Dorset*
1953 Gunn, Miss B. M., *Merry Ways, Chalk Lane, Ashtead, Surrey*

1951 Hague, Miss K. M., 14 *Ferndale Terrace, Dartmouth, S. Devon*
1946 Haifa: Sea Fisheries Research Station, *P.O. Box* 50, *Haifa, Palestine*
1950 Haines, Surg.-Lieut. D. O., R.N., 217 *Cliffe Road, Strood, Rochester, Kent*
1952 Hall, D. N. F., *Fisheries Research Station, P.O. Box* 749, *Singapore,* 1
1950 Hall, Major H. W., O.B.E., M.C., *Downton Fields, Hordle, Lymington, Hants*
1949 Hall, Miss N. F., *Tyne Brand Products Ltd., North Shields, Northumberland*
1952 Hamburg: Institut für Küsten- und Binnenfischerei, *Neurewall* 72, *Hamburg* 36, *Germany*
*1946 Hamond, Richard, *Morston, Holt, Norfolk*
1954 Hampton, Capt. T. A., *Boat Cottage, Warfleet Creek, Dartmouth, Devon*
1953 Hancock, D. A., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
1954 Hannaford, Miss J. E., 17 *Lorrimore Avenue, Stoke, Plymouth, Devon*
1954 Harding, J. P., Ph.D., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1923 Hardy, Prof. A. C., D.Sc., F.R.S., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford.* (**Council**, 1938–41, 1942→; representing Oxford University, 1946→)
1950 Harley, Miss M. B., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1932 Harris, Prof. J. E., Ph.D., *Dept. of Zoology, The University, Bristol.* (**Council**, 1946–49, 1950–53, 1954→)
1946 Harris, T. R., 31 *All Saints Road, Wyke Regis, Weymouth, Dorset*
1939 Harrison, R. J., D.Sc., M.R.C.S., L.R.C.P., *Vinicombe, The Woodlands, Farnborough, Kent*
1929 Hart, T. J., D.Sc., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1934 Hartley, The Rev. P. H. T., *Badingham Rectory, Woodbridge, Suffolk*
1924 Harvey, H. W., Sc.D., F.R.S., *The Laboratory, Citadel Hill, Plymouth, Devon*
1933 Harvey, Prof. L. A., *Dept. of Zoology, University College of the South West, Exeter, Devon.* (**Council**, 1940–43)
1951 Harvey, Mrs M. S. J., *Rowhorne House, Whitestone, Exeter, Devon*
1954 Hasegawa, Yoshio, *Hokkaido Regional Fisheries Research Laboratory, Yoichi, Hokkaido, Japan*
1950 Haswell, G. A., 23 *Russell Avenue, Hartley, Plymouth, Devon*

1954 Hatley, Miss J., 1659 *Pershore Road, Cotteridge, Birmingham,* 30
1952 Hawes, F. A. B., *c/o Grindlay's Bank,* 54 *Parliament Street, London, S.W.* 1
1939 Hayes, Dr F. R., *Dalhousie University, Halifax, N.S., Canada*
1939 Hayes, Mrs F. R., *Dalhousie University, Halifax, N.S., Canada*
1950 Hazevoet, A. C., *Vogelenzangstraat* 19–*I, Amsterdam West, Holland*
1949 Heaysman, Miss J. E. M., 69 *Brook Road, Merstham, Surrey*
1954 Hedgpeth, Dr Joel W., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1948 Hedley, Ronald H., *Remora, Quarry House Lane, Crossgate Moor, Durham City*
1931 Henderson, G. T. D., D.S.C., Ph.D., *Oceanographic Laboratory,* 78 *Craighall Road, Edinburgh* 6
1953 Henderson, Mrs W. M., 37 *Moor Lane, St Budeaux, Plymouth, Devon*
1925 Hentschel, C. C., 7 *Dudley Court, Upper Berkeley Street, London, W.* 1
1952 Hepper, B. T., *Elmfield, Combe-in-Teignhead, Newton Abbot, Devon*
1950 Herring Industry Board, 1 *Glenfinlas Street, Edinburgh,* 3
1939 Hewer, H. R., *Assistant Professor, Department of Zoology, Imperial College of Science, London, S.W.* 7
1926 Hickling, C. F., C.M.G., Sc.D., *Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W.* 1. (**Council**, 1947–50)
1951 Hidaka, Prof. K., *Geophysical Institute, Tokyo University, Tokyo, Japan.*
1926 Hill, Prof. A. V., C.H., O.B.E., Sc.D., F.R.S., 16 *Bishopswood Road, Highgate, London, N.* 6. (**Council**, 1925–29, 1930–33, 1934–37, 1938–41, 1942→; representing Royal Society, 1944→; **Vice-President**, 1948→)
*1949 Hill, D. K., *Postgraduate Medical School of London, Ducane Road, London, W.* 12
1947 Hill, M. N., Ph.D., 6 *St Eligius Street, Cambridge*
*1921 Hindle, E., Sc.D., F.R.S., 51 *Warwick Avenue, London, W.* 9. (**Council**, 1946–49; representing British Association, 1950→)
1937 Hinton, M. A. C., F.R.S., *Glaister, Wrington, near Bristol, Somerset*
1926 Hobson, Prof. A. D., *King's College, Newcastle-upon-Tyne* 1, *Northumberland*
1954 Hobson, G. E., 139 *Thornbury Road, Isleworth, Mddx*
1948 Hockley, A. R., *Dept. of Zoology, The University, Southampton*
1939 Hodgkin, Prof. A. L., F.R.S., *Physiological Laboratory, Cambridge* (**Council**, 1951–52, 1953→)
*1953 Hoestlandt, Prof. H., D.Sc., 13 *rue de Toul, Lille* (*Nord*), *France*
1952 Holden, M. J., *W.A.F.R.I., Birnin Kebbi, Sokoto Province, N. Nigeria*
1954 Holland: *The Director, Koninklijk Zoologisch Genootschap, Natura Artis Magistra, Amsterdam*
1948 Holme, N. A., *The Laboratory, Citadel Hill, Plymouth, Devon*
1939 Holmes, W., D.Phil., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford*
1948 Holsgrove, H. E., 121 *Eden Bridge Road, Bush Hill Park, Enfield, Mddx*
1950 Honeker, C., 83 *Marshall Road, Rainham, Kent*
1951 Hornby, Miss C. E., 53 *Langley Drive, Wanstead, London, E.* 11
1933 Horne, F. R., *National Institute of Agricultural Botany, Huntingdon Road, Cambridge*
1950 Horridge, G. A., *St John's College, Cambridge*
1952 Horvath, C., 8649 *Clifton Way, Beverley Hills, California, U.S.A.*
1950 Howard, G. H., 14 *Westlands Avenue, Bargate, Grimsby, Lincs.*
1948 Howe, Surg.-Cdr. (D) D.C., R.N., *H.M.S. 'Terror', R.N. Base, Singapore*
1950 Howells, D. V., 42 *Radstock Road, Reading, Berks*

1953 Howells, Dr W. R., 60 *James Street, Llanelly, Carms*
1932 Howes, N. H., *Dept. of Zoology, University College, Gower Street, London, W.C.* 1
1952 Hoyle, G., *Dept. of Zoology, The University, Glasgow, W.* 2
1953 Hull: The Librarian, *The University College, Hull*
1928 Hunt, O. D., *Corrofell, Newton Ferrers, Devon.* (**Council**, 1944–47, 1948–51, 1952→)
1947 Hunter, W. Russell, *Dept. of Zoology, The University, Glasgow, W.* 2
*1947 Hurrell, H. G., J.P., *Moorgate, Wrangaton, Devon*
1939 Hurst, C. P., *Landulph Rectory, Saltash, Cornwall*
*1920 Hutton, J. Arthur, *Woodlands, Alderley Edge, Manchester*
*1952 Hutton, Robert F., 86 *N. Main Street, Red Lion, Pennsylvania, U.S.A.*

1946 Iceland: *Atvinnudeild Háskólans (Fiskideild), Reykjavik, Iceland*
1945 Imperial Chemical Industries, Ltd., *Nobel House*, 2 *Buckingham Gate, London, S.W.* 1
1954 Indonesia: The Professor of Zoology, *University of Indonesia, Djalan Taman Sari* 64, *Bandung, Java*
1952 Inglis, W. G., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1953 Institute of Seaweed Research, *Inveresk, Midlothian.*

1952 Jackman, L. A. J., 44 *Old Torquay Road, Preston, Paignton, Devon*
1954 Jager, J. C., *van Reesstraat* 122, *The Hague, Holland*
1953 Jagger, J. R., 5 *Duckworth Street, Stoke, Devonport, Devon*
1952 Jean, Prof. Yves, *School of Fisheries, Ste. Anne de la Pocatier, Kam., P.Q., Canada*
1945 Jefferies, H. S., *The Mill, Avoncliffe, Bradford-on-Avon, Wilts*
1952 Jeffery, F. J., *Plymouth College, Plymouth, Devon*
1953 Jellard, Dr C. H., B.M., *Public Health Laboratory, South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon*
1935 Jenkin, Miss P. M., *Dept. of Zoology, The University, Bristol*
1934 Jepps, Miss M. W., D.Sc., 61 *Chesterton Road, Cambridge*
1949 Jeremy, W. H. R., 6 *Elm Grove Road, Exeter, Devon*
1937 Jersey: Conservateur honoraire du Musée de la Société Jersiaise
*1924 Jesus College, *Oxford*
*1947 John, C. C., D.Sc., *Dept. of Marine Biology and Fisheries, Beach P.O., Trivandrum, S. India*
*1947 Johnson, D. S., 10 *St John's Road, Cambridge*
1944 Johnson, Dr F. R., *Osu Fisheries Station, P.O. Box* 630, *Accra, Gold Coast Colony*
*1948 Jones, C. Burdon, Ph.D., *Dept. of Zoology, University College of N. Wales, Bangor, Caern*
*1950 Jones, E. Idris, M.D., 6 *Kewferry Road, Northwood, Mddx*
*1949 Jones, E. W. Knight, Ph.D., *Marine Biological Station, University College of N. Wales, Bangor, Caern*
1949 Jones, F. R. Harden, Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
1952 Jones, T. Howell, *Mydrim House, Llewellyn Street, Tucynon, Aberdare, Glam*
1948 Jones, J. D., *Nantyci, Llysonnen Road, Carmarthen, S. Wales*
1946 Jones, L. W. G., *Cumber House, Manor Road, Brixham, Devon*
1946 Jones, N. S., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1953 Jones, P. G. W., *Woodberry, Tolmers Road, Cuffley, Herts*

1949 Jones, R. J., 14 *Canning Road, Croydon, Surrey*
1936 Jones, Rodney R. M., *Tros-yr-Afon, Penmon, Anglesey*
1946 Jones, Prof. R. V., C.B., C.B.E., D.Phil., F.Inst.P., *Dept. of Natural Philosophy, Marischal College, Aberdeen*
1950 Jones, W. C., *Dept. of Zoology, University College of N. Wales, Bangor, Caern.*
1951 Jones, W. E., *Marine Biological Station, University College of N. Wales, Bangor, Caern.*
1947 Jörgensen, C. Barker, *Slettevej* 8, *Copenhagen Söborg, Denmark*
1952 Joysey, Mrs V. C., 26 *Bury Road, Epping, Essex*
1923 Judge, J. J., *Virginia House, Palace Street, Plymouth, Devon*
1950 Juniper, A. J., *The Polytechnic, Regent Street, London, W.* 1

1952 Kabata, Z., 26 *Orchard Road, Aberdeen*
1954 Kain, Miss J. M., 11 *Acol Road, London, N.W.* 6
1951 Kampa, E. M., Ph.D. (Mrs B. P. Boden), *Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.*
1954 Karnatak University: *Karnatak University Library, Dharwar, (State Bombay), India*
1948 Katterns, L. B., 115 *Feltham Hill Road, Ashford, Mddx*
1945 Katz, Max, Ph.D., *c/o Prof. R. E. Dimick, Dept. of Fish and Game Management, Oregon State College, Corvallis, Oregon, U.S.A.*
1951 Kay, Miss E. A., *Box* 522, *Kolva, Kanai, Territory of Hawaii, U.S.A.*
1940 Keilin, Prof. D., Sc.D., F.R.S., *Molteno Institute, Cambridge.* (**Council**, 1940–43)
1950 Keir, Ronald S., *Newfoundland Fisheries Research Station, Water Street East, St Johns, Newfoundland*
1946 Kelley, D. F., 17 *Bainbridge Avenue, Hartley, Plymouth, Devon*
1954 Kelly, A. L., 352 *Nautilus, La Jolla, California, U.S.A.*
1952 Kemp, Miss J. R., *North Riding Training College, Filey Road, Scarborough, N. Yorks*
1949 Kennedy, G. Y., Ph.D., F.R.I.C., *Dept. of Cancer Research, University Field Laboratory, Blackbrook Road, Sheffield* 10
1946 Kenya: The Game Warden, *Game Department, P.O. Box* 241, *Nairobi*
*1952 Kerkut, G., *Pembroke College, Cambridge*
1951 Kerslake, Miss J., 17 *Hardman Road, Kingston-upon-Thames, Surrey*
*1954 Kesteven, Dr G. L., *c/o Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy*
1950 Keynes, R. D., Ph.D., *Physiological Laboratory, Cambridge*
1949 Kimmins, B. J., *P.O. Box* 603, *Addis Ababa, Ethopia*
1928 King, Mrs A. Redman, *Mixton House, Lerryn, Lostwithiel, Cornwall*
1947 Kingsbridge Modern Secondary School, *Kingsbridge, Devon*
1952 King's College: *Dept. of Zoology, Newcastle upon Tyne,* 1
1949 Kingston Technical College, *Kingston Hall Road, Kingston-upon-Thames, Surrey*
1954 Kingwell, M. C., *Beechfield, South Brent, S. Devon*
1927 Kirtisinghe, P., *Dept. of Zoology, University of Ceylon, Colombo* 3, *Ceylon*
1950 Kisch, B. S., C.I.E., 38 *Boulevard Thiers, St Jean-de-Luz, B.P., France*
1930 Kitching, J. A., O.B.E., Ph.D., *Dept. of Zoology, The University, Bristol*
1939 Knight, Miss Margery, D.Sc., *University Hall for Woman Students, Holly Road, Fairfield, Liverpool.* (**Council**, 1943–46)
1945 Knowles, Sir Francis G. W., Bt., D.Phil., *Marlborough College, Marlborough, Wilts*

1950 Korn, M. E., 62 *Aberdare Gardens, Hampstead, London, N.W.* 6
1950 Kott, Miss P., *C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*
1949 Kristensen, Dr Ingvar, *Zoölogisch Station, den Helder, Holland*

1954 Lacey, A. J., 24 *The Gardens, Monkseaton, Northumberland*
1952 Lake, Miss L., *Grove House, Roehampton Lane, London, S.W.* 15
1953 Landa, Antonio, *P.O. Box* 3665, *Balboa, Canal Zone, S. America*
1950 La Rochelle: *Station Océanographique, Pavillon du Port-Le Gabut, La Rochelle (Charente-Maritime), France*
1950 Lasker, Reuben, *Dept. of Biological Sciences, Stanford University, Stanford, California, U.S.A.*
*1925 Lebour, Miss M. V., D.Sc., *Kean Hill, Cawsand, near Plymouth, Devon*
1947 Leehane, J. D. B., 14 *Wyndham Street East, Plymouth, Devon*
1935 LeMare, D. W., *Fisheries Dept., Federation of Malaya and Singapore, Penang, Malaya*
1951 Lewis, J. B., *c/o Dept. of Zoology, McGill University, Montreal, Quebec, Canada*
1952 Lewis, J. R., *Dept. of Zoology, University College of Wales, Aberystwyth, Cardigan*
1951 Lightowlers, F., 109 *Bradford Road, Brighouse, Yorkshire*
1932 Lisbon: Instituto de Biologia Maritima, *Cais do Sodré, Lisbon, Portugal*
1952 Lister-Bradney, J., Ph.D., 25 *Palmerston Road, Westcliff-on-Sea, Essex*
1949 Lodge, Miss S. M., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1950 Longhurst, A. R., *Landford Cottage, Landford, Wilts*
1953 Longton, P. A., 37 *Headland Park, North Hill, Plymouth, Devon*
1954 Lorraine, N. S. R., M.D., Ch.B., D.P.H., F.R.S.E., *Public Health Department, London Road, Hadleigh, Essex*
1948 Lovegrove, T., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1953 Lowe, Mrs E. K., *Highfield Lodge, Highfield Road, Carnforth, Lancs*
1950 Lowenstein, Prof. O. E., D.Sc., Ph.D., *Dept. of Zoology, The University, Birmingham*, 15. (**Council**, 1952→)
1926 Lowndes, A. G., Sc.D., F.R.I.C., *c/o The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Lowy, J., Ph.D., *Dept. of Zoology, Queen's University, Belfast, N. Ireland*
1931 Lucas, C. E., D.Sc., *Marine Laboratory, Wood Street, Torry, Aberdeen.* (**Council**, 1949–52, 1954→)
1930 Lumley, Adrian, *Beechcroft, Udney Park Road, Teddington, Mddx*
1953 Lynch, Dr G. A. C., *Beeches, Crapstone, Yelverton, Devon*
1938 Lysaght, Miss A. M., Ph.D., 6 *Cumberland Gardens, London, W.C.* 1

1953 Macaulay, J. H., *c/o John Leckie Ltd.*, 77 *Wellington Street, West Toronto, Ontario, Canada*
1938 MacDonald, R., 112 *Antrim Road, Belfast, N. Ireland*
1935 Mackenzie, Col. W., O.B.E., *c/o Messrs Peel & Co., Ltd., P.O. Box* 331, *Alexandria, Egypt*
1929 Mackinnon, Prof. D. L., D.Sc., 44 *St Leonard's Terrace, Chelsea, London, S.W.* 3. (Council, 1938–42)
1937 Mackintosh, N. A., C.B.E., D.Sc., 7 *Hinde House, Hinde Street, London, W.* 1. (**Council**, 1946–49, 1951–54)
1947 Macnae, William, *Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa*
*1925 Magdalen College, *Oxford*

1951 Malkin, J. J. S., 33 *Elton Parade, Darlington*
1948 Mansfield, A. W., *Dept. of Zoology, McGill University, Quebec, Canada*
1952 Mansfield, Lt.-Col. K. F., *Luckwell Bridge, Wheddon Cross, Minehead, Som*
*1928 Manton, S. M., Sc.D., F.R.S. (Mrs J. P. Harding), 18 *Ennerdale Road, Richmond, Surrey*
1953 Manwaring, Miss R. A., *Dept. of Zoology, University College, Exeter, Devon*
1948 Marcotte, Alexandre, D.Sc., *Marine Biological Station, Grand River, Gaspé Co., P.Q., Canada*
1951 Marshall, C. J., M.D., F.R.C.S., *Penhaven, The Drive, Mevagissey, Cornwall*
1949 Marshall, Douglas, M.P., *Lanwithan House, Lostwithiel, Cornwall*
1951 Marshall, K. E., 126 *Park Road, Peterborough, Northants*
*1947 Marshall, Miss S. M., D.Sc., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1953 Marshall, P. T., *The Priory, Kingswear, Devon*
1951 Mason, J., 270 *Ashton Road East, Hailsworth, Manchester*
1939 Matthews, L. Harrison, Sc.D., F.R.S., *Zoological Society of London, Regent's Park, London, N.W.* 8. (**Council**, 1944–47, 1949–52)
*1947 Matthews, Mrs J. B. (Miss G. C. Evans), *Trevorrick Gate, St Merryn, Cornwall*
1937 Mayne, Cyril F., O.B.E., F.R.C.S., *c/o Barclays Bank Ltd., Plymouth, Devon*
1953 Mayne, R. B., *Martins Meadow, Yelverton, Devon*
1949 McCarthy, Miss D. F., 70 *Halton Terrace, Kensington Park, Adelaide, S. Australia*
1951 McCarton, Miss L., 89 *Eglantine Avenue, Malone Road, Belfast, N. Ireland*
1910 McClean, Capt. W. N., 39 *Phillimore Gardens, London, W.* 8
*1929 McEwen, Mrs Lawrence, *Isle of Muck, Isle of Eigg, Inverness-shire*
*1952 McGill, D. A., *Dept. of Zoology, Columbia University, New York* 27, *New York, U.S.A.*
1954 McGowan, J. A., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1948 McIntyre, A. D., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1950 McIntyre, J. M., *Horsley Hall, Eccleshall, Staffs*
1953 Menker, Donald F., *Marine Laboratory, University of Miami, Coral Gables, Florida, U.S.A.*
*1948 Menon, M. Devidas, *Parvathi Nivas, Diwans Road, Ernakulam, United States of Cochin and Travancore, S. India*
1954 Menzies, Dr R. J., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1953 Mercer, B. V., *Blakewell House, Painswick, Glos*
1953 Meredith, Miss H. M., 7 *The Lynchets, Lewes, Sussex*
1954 Merrett, M. G., 22 *Fullerton Road, Milehouse, Plymouth, Devon*
1950 Messenger, K. G., *Uppingham School, Rutland*
1951 Messent, R. K., 13 *Sedberg Street, Preston, Lancs*
1939 Metropolitan Water Board, 177 *Rosebery Avenue, London, E.C.* 1
1950 Mexico: Department of Physiology, National University, *Apartado Postal* 13699, *Mexico, D.F.*
1947 Miami University Marine Laboratory, *Coral Gables, Florida, U.S.A.*
1951 Mij tot Exploitatie van Kooksovengassen (Mekog), *Ijmuiden, Holland*
1923 Milford Haven Trawler Owners Association Ltd., *Milford Haven, Pembs*

1949 Millar, R. H., Ph.D., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1946 Miller, Cyril J., 42 *Westbourne Road, Peverell, Plymouth, Devon*
1949 Millott, Prof. N., Ph.D., *Dept. of Zoology, University College of West Indies, Mona, Liguanea, Jamaica, B.W.I.*
1953 Mills, D. H., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6
1949 Mistakidis, M. N., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
1947 Mitra, G. N., *Assistant Director of Fisheries, Cuttack (Orissa), India*
1952 Mohamed, Prof. Abdel Fattah, Ph.D., *Oceanography Dept., Faculty of Science, Alexandria University, Maharram Bey, Alexandria, Egypt*
1951 Mohr, J. L., Ph.D., *Dept. of Zoology, University of Southern California, Los Angeles* 7, *Calif., U.S.A.*
1952 Morgan, Mrs E. M., 11 *Oak Hill Park, Hampstead, London, N.W.* 3
1940 Moore, Hilary B., Ph.D., *Marine Laboratory, Miami University, Coral Gables, Florida, U.S.A.*
1949 Morton, J. E., Ph.D., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1953 Moss, Miss M. P., *Lady Margaret Hall, Oxford*
1931 Mount Desert Island Biological Laboratory, *Salisbury Cove, Maine, U.S.A.*
1938 Mowbray, Louis L., *Curator, Bermuda Government Aquarium, Flatts, Bermuda*
1942 Moynahan, Dr E. J., *Wayside, Jordans, near Beaconsfield, Bucks.*
1953 Muller, R. L., 38 *Brendon Street, London, W.* 1
1952 Munn, R. B., 11002 *East Saragossa Street, Whittier, Calif., U.S.A.*

1952 Naylor, E., *Marine Biological Station, Port Erin, Isle of Man*
1933 Neale, Morley H., C.B.E., *Chaffcombe House, Chard, Somerset.* (**Council** 1934–36, 1939–42, 1943–46, 1947–50; **Vice-President**, 1951→)
1934 Neale and West, *Trawler Owners, Wharf Street, Cardiff*
1939 Needham, Joseph, Sc.D., F.R.S., *Gonville and Caius College, Cambridge*
1947 Newell, G. E., Ph.D., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1953 Newell, B. S., *I.M.F.R.O., P.O. Box* 754, *Zanzibar*
1951 Newfoundland Fisheries Research Station, *Water Street East, St John's, Newfoundland*
1947 Newton, Prof. Lily, Ph.D., *Dept. of Botany, University College of Wales, Aberystwyth, Cardigan.* (**Council**, 1947–50)
1930 Nicholls, A. G., Ph.D., *C.S.I.R.O., Division of Fisheries, Stowell, Hobart, Tasmania*
1950 Nicol, J. A. C., D.Phil., *The Laboratory, Citadel Hill, Plymouth, Devon*
1947 Nisbet, R. H., 8 *Lichfield Road, Woodford Green, Essex*
1948 Nobbs, Miss G. E. D., 4 *Clifton Estate, Plympton, Devon*
1953 Nordli, O., *Statens Fiskarfagskule, Florø, Norway*
1949 Norris, E., *Biology Dept., Kingston Technical College, Kingston Hall Road, Kingston-upon-Thames, Surrey*
1951 North Carolina, University of, *Fisheries Research Laboratory, Morehead City, N. Carolina, U.S.A.*
1950 North, J., 316 *Lee High Road, Lewisham, London, S.E.* 13
1953 North, L. G., 7 *Belsize Square, Hampstead, London, N.W.* 3
1948 Nunn, S. M., *The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Nurse, F. R. (Mrs R. M. Allison), *Biology Dept., Canterbury University College, Christchurch, New Zealand*

1934 Oakley, Prof. C. L., 14 *Cardigan Road, Headingley, Leeds* 6
1954 O'hEocha, C., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1947 Oldfield, Mrs E., *County Technical College, Guildford, Surrey*
1930 Olive, G. W., C.B.E., *Dauntsey's School, West Lavington, near Devizes, Wilts*
1938 Oliver, James H., Ph.D., 16 *Southwark Street, London, S.E.* 1
1953 Oliver, Miss M., *Somerville College, Oxford*
1937 Omer-Cooper, J., *Zoological Dept., Rhodes University College, Grahamstown, S. Africa*
1939 Ommanney, F. D., Ph.D., *Fisheries Research Station, P.O. Box* 749, *Singapore*
1949 Orkin, P. A., *Natural History Dept., The University, Aberdeen*
1947 Orr, A. P., D.Sc., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1945 Otago University Library, *Dunedin, N.I., New Zealand*
*1928 Otter, G. W., *Southwood, Broadstone, Dorset*
1950 Owen, G., *Dept. of Zoology, The University, Glasgow, W.* 2
1953 Owen, Miss G., *Rustington, Flat* 2, *St Wilfred's Road, Worthing*
1949 Owrie, Mrs Harding B., *Dept. of Zoology, Natural Science Building, University of Michigan, Ann Arbor, Mich., U.S.A.*
1951 Oxford: Department of Zoology and Comparative Anatomy, *University Museum, Oxford*

1951 Pacific Biological Station, *Nanaimo, B.C., Canada*
1954 Packham, J. R., *Rylstone,* 8 *Highview Avenue, South Patcham, Brighton,* 6
1953 Pampapathi-Rao, K., Ph.D., *Dept. of Zoology, Erskine College of Natural Sciences, Andhra University, Waltair, India*
*1939 Panikkar, N. K., D.Sc., *Central Marine Fisheries Station, Mandapam Camp P.O., S. India*
*1923 Pantin, C. F. A., Sc.D., F.R.S., *Trinity College, Cambridge.* (**Council,** 1930–32, 1933–36, 1937–40, 1941–44, 1945–49, 1951–53; representing Cambridge University, 1946–49, 1951–53)
1951 Papworth, A. H., *The Terrace, Brixworth, Northampton*
1941 Parke, Miss M. W., D.Sc., *The Laboratory, Citadel Hill, Plymouth, Devon*
1927 Parker, The Hon. John H., *Pound House, Buckland Monachorum, Devon*
1954 Parker, R. H., *Scripps Institution of Oceanography,* 616 *Bonair Street, La Jolla, California, U.S.A.*
1954 Parrish, B. B., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1937 Parry, D. A., Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
1950 Parry, Miss G., *Dept. of Zoology, Downing Street, Cambridge*
*1920 Pass, A. Douglas, *Wooton Fitzpaine, Charmouth, Dorset*
1946 Paul, Mrs Vidya Vati, Ph.D., 116-*D, Kamlanagar, Delhi*-6, *India*
1952 Paul, A. R., 22 *Cooper's Hill, Willingdon, Eastbourne, Sussex*
1925 Pawlyn Bros., *Mevagissey, Cornwall*
1925 Pawlyn, T. A., *Mevagissey, Cornwall*
1933 Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel & Co., Ltd., P.O. Box* 331, *Alexandria, Egypt.* (**Vice-President,** 1936→)
1948 Peiping, Institute of Zoology, National Academy of Peiping, 58 *Museum Road, Peiping* 7, *China*
1953 Pelly, Miss J., *Lady Margaret Hall, Oxford*
1939 Pennel, V., 28 *Huntingdon Road, Cambridge*
1925 Pentelow, F. T. K., *Old Slade Lane, Iver, Bucks*
1929 Percival, Prof. E., *Canterbury University College, Christchurch, New Zealand*

1947 Perkins, E. J., *Cross Keys, Post Office, near Hereford*
1936 Perkins, F., *Red Hand Compositions Co.*, 2 *The Firs, York Road, Ripon, Yorks*
1951 Perry, Lt.-Cdr. W. J., U.S.N., *Office of Naval Research*, 34 *Grosvenor Square, London, W.* 1
1950 Phillipson, J., Ph.D., *Dept. of Zoology, Science Laboratories, South Road, Durham*
1934 Picken, L. E. R., Ph.D., *Trinity College, Cambridge*
1940 Pike, R. B., Ph.D., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1953 Pittam, M. D., *Magdalen College, Oxford*
1906 Plymouth Corporation (Museum Committee), *Tavistock Road, Plymouth, Devon*
1910 Plymouth Education Authority, *Cobourg Street, Plymouth, Devon*
1906 Plymouth, Port of, Incorporated Chamber of Commerce, *Plymouth, Devon*
1924 Plymouth Proprietary Library, 1 *Alton Terrace, North Hill, Plymouth, Devon*
1929 Plymouth Public Library, *Tavistock Road, Plymouth, Devon*
1948 Pollard, D. G., 60 *Alcester Street, Stoke, Plymouth, Devon*
1950 Polytechnic, *The Library, Regent Street, London, W.* 1
1947 Portsmouth Grammar School, *Biology Department, Portsmouth, Hants*
1929 Portsmouth Municipal College, *Portsmouth, Hants*
1950 Posner, Gerald S., *The Bingham Oceanographic Laboratory, Box* 2025, *Yale Station, New Haven, Conn., U.S.A.*
1953 Pounce, A. G., *Laurel Villa, Meopham, near Gravesend, Kent*
1950 Powell, H. T., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1950 Powell, R. R., 170 *Streatfield Road, Kenton, Mddx*
1954 Pratt, Dr Ivan, *Dept. of Zoology, Oregon State College, Corvallis, Oregon, U.S.A.*
1951 Pringle, I. G., St C., *Preston Hall, Uppingham, Rutland*
1939 Pringle, J. W. S., M.B.E., F.R.S., *Dept. of Zoology, Downing Street, Cambridge*
1954 Pritchard, Dr D. W., *Chesapeak Bay Institute, The Johns Hopkins University, Box* 426*A*, *RFD* 2, *Annapolis, Maryland, U.S.A.*
*1947 Pryor, Thomas, D.S.O., *Westbourne House, Saltash, Cornwall*
1939 Pumphrey, Prof. R. J., Ph.D., F.R.S., *Dept. of Zoology, The University, Liverpool*
*1939 Purchon, Prof. R. D., Ph.D., *Dept. of Zoology, University of Malaya, Singapore* 10

1953 Quebec: Station de Biologie Marine, *Grande Rivière, Gaspé, P.Q., Canada*
1936 Queen Mary College, *Dept. of Zoology, Mile End Road, London, E.* 1
1954 Queensland Museum, *Brisbane, Queensland, Australia*
1939 Queen's University, *Belfast, N. Ireland*
1947 Queckett Microscopical Club (W. Burrells, Hon. Sec.), 52 *Draycott Avenue, Northwick Park, Harrow, Mddx*
1932 Quick, H. E., F.R.C.S., *Craythorne, Shinfield Road, Reading, Berks*
1952 Quiroga, Dr M., *Constitucion* 36, *Veracruz, Ver., Mexico*

1954 Radford, Miss M., 27 *Thornhill Road, Mannamead, Plymouth, Devon*
1932 Ramalho, Dr A., *Instituto de Biologia Maritima, Cais do Sodré, Lisbon, Portugal*
1949 Rasmussen, E., *The Zoological Laboratory, Landbohøjskolen, Bülowsvej, Copenhagen V, Denmark*
1939 Rawlinson, Miss Ruth, 23*a Holbrook Road, Stoneygate, Leicester*

1947 Raymont, Prof. J. E. G., *Dept. of Zoology, The University, Southampton*
*1933 Rayner, G. W., *c/o C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*
1949 Rees, C. B., D.Sc., *Oceanographic Laboratory, 78 Craighall Road, Edinburgh* 6
*1936 Rees, W. J., D.Sc., 37 *Kings Road, Richmond, Surrey*
1951 Reish, Donald J., *Allan Hancock Foundation, University of Southern California, Los Angeles, California, U.S.A.*
1935 Renouf, Prof. L. P. W., *University College, Cork, Eire*
1953 Research Organization of Ships' Compositions Manufacturers, Ltd., *Grosvenor Gardens House, Grosvenor Gardens, London, S.W.* 1
1940 Richards, P. B., *c/o Lloyds Bank Ltd., Truro, Cornwall*
1951 Rickard, Miss B., 68 *Furze Hill Road, Mutley, Plymouth, Devon*
1946 Rifaat, Ahmed, *Alexandria Institute of Hydrobiology and Fisheries, Kayed Bey, Alexandria, Egypt*
1951 Rigby, Miss J. E., 184 *Walm Lane, London, N.W.* 2
1952 Rijal, Z., *c/o Government Boys' Secondary School, P.O. Box* 873, *Zanzibar*
1953 Riksmuseum (The Director), *Evertebratavd., Stockholm, Sweden*
*1919 Ritchie, Prof. A. D., *Old College, The University, Edinburgh.* (**Council**, 1929–32, 1934–37)
1944 Ritchie, Prof. James, C.B.E., D.Sc., 31 *Mortonhall Road, Edinburgh.* (**Council**, 1944–47)
1953 Robertson, J. D., Ph.D., F.R.S.E., *Zoology Dept., The University, Glasgow, W.*2
1948 Robinson, G. A., *Oceanographic Laboratory, 78 Craighall Road, Edinburgh* 6
1949 Rodhe, Dr W., *Institute of Limnology, Uppsala, Sweden*
1940 Rogers, Dr T. Howard, *Naval Research Establishment, Halifax, Nova Scotia, Canada*
1952 Rosa, Horatio, *F.A.O. of the United Nations, Western South American Regional Office, Huérfanos* 786–*7th floor, P.O. Box No.* 10095, *Santiago de Chile*
1939 Ross, D. M., Ph.D., *Dept. of Zoology, University College, Gower Street, London, W.C.* 1
1939 Ross, Miss Sybil S., 158 *Craiglea Drive, Edinburgh* 10
*1931 Rothschild, Rt Hon. Lord, G.M., Ph.D., F.R.S., *Merton Hall, Cambridge.* (**Council**, 1939–42)
*1932 Rothschild, The Hon. Miriam (The Hon. Mrs George Lane), *The Cottage, Ashton Wold, Peterborough, Northants.* (**Council**, 1942–45)
1951 Rowe, Rev. A. F. C., *The Vicarage, Kingskerswell, Newton Abbot, Devon*
1945 Rowett, Miss H. G. Q., *Plymouth Technical College, Tavistock Road, Plymouth, Devon*
1952 Royal Holloway College, *Englefield Green, Surrey*
1924 Rudge, Chas. H., *Trevalgan, Feliskirk Lane, Marazion, Cornwall*
1927 Russell, F. S., D.S.C., D.F.C., F.R.S., *The Laboratory, Citadel Hill, Plymouth, Devon.* (**Council**, 1945→; **Secretary**, 1945→)
1951 Russell, S. M. M., *Dept. of Zoology, Birkbeck College, Malet Street, London, W.C.* 1
1954 Ryland, J. S., 182 *Manor Road North, Thames Ditton, Surrey*

1946 Sagay, A. A. E., *Warri College, Warri, Nigeria*
1927 St Aubyn, Capt. The Hon. Lionel, M.V.O., *Saltram, Plympton, Devon*
1953 St Aubrey, Miss S. N. D., *St Martin's Vicarage, Vicar's Road, Gospel Oak, London, N.W.* 5

*1951 Salisbury, A. E., *Ormonde House*, 51 *Amersham Hills*, *High Wycombe*, *Bucks*
*1934 Salisbury, Sir Edward J., Kt., C.B.E., D.Sc., Sec.R.S., 49 *Kew Green*, *Kew Gardens*, *Surrey*. (**Council**, 1934–37, 1940–43; **Vice-President**, 1948→)
1950 Samuel, J. H., 68 *Conway Road*, *Cardiff*
1946 Sartory, P. K., 107 *Rivermeads Avenue*, *Twickenham*, *Mddx*
1911 Saunders, J. T., *University College*, *Ibadan*, *Nigeria*
1914 Savage, R. E., O.B.E., *Monfa*, *Pentywyn Road*, *Deganwy*, *Caern*
1946 Saville, Alan, *Marine Laboratory*, *Wood Street*, *Torry*, *Aberdeen*
*1945 Saville, Tom C., 31 *Mawton Crescent*, *Wollaton Park*, *Nottingham*
*1921 Scott, Peter M., M.B.E., D.S.C., *New Grounds*, *Slimbridge*, *Glos*
1953 Scott, A., 2 *North Street*, *Strichen*, *Aberdeenshire*
1953 Seaford College, *Larrington Park*, *Petworth*, *Sussex*
1950 Segrove, F., Ph.D., *Dept. of Zoology*, *The University*, *Sheffield*, 10
1928 Sen, B., *Vivekanada Laboratory*, *Baghbazar*, *Calcutta*, *India*
1922 Sewell, Lt.-Col. R. B. Seymour, C.I.E., Sc.D., F.R.S., *Dept. of Zoology*, *Downing Street*, *Cambridge*. (**Council**, 1935–38)
1950 Sharman, D. P., 69 *Wentworth Road*, *Harborne*, *Birmingham*, 17
1953 Sharman, Mrs D. P., 69 *Wentworth Road*, *Harborne*, *Birmingham*, 17
1947 Sharman, Miss M., 42 *Winchester Drive*, *Wallasey*, *Cheshire*
1953 Shaw, G. W., *West Buckland School*, *Barnstaple*, *N. Devon*
1933 Sheffield, *The Library*, *The University*, *Sheffield* 10
1952 Shelbourne, J. E., *Fisheries Laboratory*, *Lowestoft*, *Suffolk*
1953 Sherbourne, D. H., 85 *Harrowdene Road*, *Wembley*, *Mddx*
1935 Siam: Department of Fisheries, *Bangkok*, *Siam*
1940 Simpson, A. C., *Fisheries Laboratory*, *Lowestoft*, *Suffolk*
1953 Sims, Rudolph W., Jr., *Marine Laboratory*, *Box* 488, *Coral Gables* 46, *Florida*, *U.S.A.*
1946 Sinclair, Horace, *Southsea Aquarium*, *South Parade*, *Southsea*, *Hants*
1954 Skinner, J. G. 112 *Gurney Court Road*, *St Albans*, *Herts*
*1884 Skinners, The Worshipful Company of, *Skinners' Hall*, *Dowgate Hill*, *London*, *E.C.* 4
1948 Slack, H. D., Ph.D., *Dept. of Zoology*, *The University*, *Glasgow*, *W.* 2
1952 Slinn, D. J., *Marine Biological Station*, *Port Erin*, *Isle of Man*
1953 Smaridge, Mrs M. W., 116 *Pine Gardens*, *Eastcote*, *Ruislip*, *Mddx*
1949 Smith, Major A. A. Dorrien, D.S.O., *Tresco Abbey*, *Isles of Scilly*, *Cornwall*
1948 Smith, Admiral Sir Aubrey, K.B.E., C.B., M.V.O., *Hay's Wharf and Dock*, *Southwark*, *London*, *S.E.* 1. (**Council**, representing Fishmongers' Company, 1937–46, **Vice-President**, 1948→)
1932 Smith, B. Webster, 87 *Northumberland Road*, *New Barnet*, *Herts*
1949 Smith, F. F., *Brentwood*, *Fords Avenue*, *Healing*, *Lincs*
1929 Smith, F. G. W., *Zoology Dept.*, *Miami University*, *Coral Gables* 34, *Florida*, *U.S.A.*
1932 Smith, Prof. J. E., Sc.D., 149 *Huntingdon Road*, *Cambridge*. (**Council**, 1943–46, 1948–51, 1953→; representing Cambridge University, 1949–51)
1954 Smith, R. I., Ph.D., *Dept. of Zoology*, *University of California*, *Berkeley* 4, *Calif.*, *U.S.A.*
1939 Smith, S., Ph.D., *St Catharine's College*, *Cambridge*. (**Council**, representing Cambridge University, 1953→)
1953 Snell, R. J., 63 *Shaldon Drive*, *South Ruislip*, *Mddx*
1929 South Africa, Fisheries Division of, *Beach Road*, *Sea Point*, *Cape Town*, *S. Africa*

1943 Southampton, The University
1945 Sparrow, B. W., *Corrofell, Newton Ferrers, Devon*
1934 Spaul, Prof. E. A., D.Sc., Ph.D., *Dept. of Zoology, The University, Leeds*
1951 Spencer, C. P., Ph.D., *Marine Biological Station, University College of N. Wales, Bangor, Caern.*
1930 Spooner, G. M., M.B.E., *The Laboratory, Citadel Hill, Plymouth, Devon*
1938 Spooner, Mrs G. M., Ph.D., *Fiveoaks, Crapstone, Yelverton, Devon*
*1939 Sproston, Miss Nora G., *c/o The Director, Zoological Survey of India, Japakusum House, 34 Chittarajan Avenue, Calcutta 12, India*
1939 Stanbury, Miss F. A., *Biology Dept., Plymouth Technical College, Tavistock Road, Plymouth, Devon*
1954 Stanley, T. P. G., *Thorn, Wembury, near Plymouth, Devon*
1953 Steel, J. H., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1925 Stephen, A. C., D.Sc., *Royal Scottish Museum, Edinburgh*
1951 Stephenson, R. M., *Somewhere, St Mary's, Isles of Scilly, Cornwall*
1930 Stephenson, Prof. T. A., D.Sc., F.R.S., *Dept. of Zoology, University College of Wales, Cambrian Street, Aberystwyth, Cardigan.* (**Council**, 1941–44)
*1949 Stephenson, W., Ph.D., *Dept. of Zoology, University of Queensland, Brisbane, Australia*
1951 Steven, D. H., *Willowby House, Yelverton, Devon*
1950 Steven, G. A., D.Sc., F.R.S.E., *Willowby House, Yelverton, Devon*
1930 Steven, Mrs G. A., *Willowby House, Yelverton, Devon*
1935 Stewart-Evison, Chas., *Manor East Lodge, Sidbury, Sidmouth, Devon*
1947 Stobart, T. R., *Countryman Films Ltd., 18 Soho Square, London, W. 1*
1952 Stock, W. O., 24 *Hanham Road, Kingswood, near Bristol*
*1934 Stoneman, Eric, *Zoology Dept., The University, Reading*
1950 Street, P. A. R., 6 *Bellfield Avenue, Harrow Weald, Mddx*
1936 Stubbings, H. G., Ph.D., 13 *Third Avenue, Denvilles, Havant, Hants*
1935 Sturdy, Mrs R. S., *Woodpiece, Apsley Guise, Bletchley, Bucks*
1946 Subrahmanyan, R., Ph.D., 52 *Choolai High Road, Madras 7, India*
1952 Surtees, S. R., *Ministry of Agriculture, Infestation Control Division, 29 Rodney Street, Liverpool, 1*
1950 Sutcliffe, W. H., Jr., Ph.D., *Bermuda Biological Station, St George's West, Bermuda*
1949 Sutton, Miss M. F., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingston-on-Thames, Surrey*
1952 Symons, H. W., *The Old Forge, Cowbeach, Hailsham, Sussex*

1948 Tait, R. V., 62 *Nightingale Road, Rickmansworth, Herts*
1953 Talbot, F. H., *East African Marine Fisheries Research Organization, P.O. Box 668, Zanzibar*
1932 Taylor, Prof. Sir Geoffrey I., D.Sc., F.R.S., *Farmfield, Huntingdon Road, Cambridge.* (**Council**, 1932–35)
*1922 Taylor, Joseph A., 22 *Eaton Place, Brighton 7, Sussex*
1954 Tebble, N., *British Museum (Natural History), Cromwell Road, London, S.W.7*
1942 Templeman, Dr Wilfred, Director, *Newfoundland Fisheries Research Station, St John's, Newfoundland*
1948 Tham, Ah Kow, *c/o Fisheries Dept., 4th Floor, Fullerton Building, Singapore*
1953 Thomas, H. Wilson, M.R.C.S., L.R.C.P., *Furzehatt, Plymstock, near Plymouth, Devon*
1939 Thomas, I. M., *Dept. of Zoology, The University, Adelaide, S. Australia*

1950 Thomas, Miss J. G., Ph.D., *Royal Holloway College, Englefield Green, Surrey*
1947 Thompson, J. A., *c/o G. & F. Henderson, Oathill Farm, Enstone, Oxon*
1952 Thorson, Gunnar, D.Phil. *Universitetets Zoologiske Museum, Krystalgade, Copenhagen K, Denmark*
1953 Tobias, Miss M. A., 4*a Allan Way, North Acton, London, W.* 3
1903 Torquay Natural History Society, *The Museum, Torquay, Devon*
1939 Totton, A. K., M.C., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1936 Tremblay, Jean-Louis, D.Sc., *Station Biologique du Saint Laurent, Faculté des Sciences, Boulevard de l'Entente, Quebec, Canada*
*1946 Tripathi, Y. R., *c/o S. R. Tripathi, Esq., Narsinghgarh, C.I., India*
1938 Trout, Geoffrey C., *Fisheries Laboratory, Lowestoft, Suffolk*
1936 Truelove, P. H., F.R.I.C., *Red Hand Compositions Co.*, 2 *The Firs, York Road, Ripon, Yorks*
1946 Trumble, Prof. H. C., *Waite Institute, Private Mail Bag, Adelaide, S. Australia*
1948 Trythall, Major E. R., *Houndiscombe Place*, 60 *North Road, Plymouth, Devon*
1947 Tuck, A. W., *Upriver, Potter Heigham, Norfolk*
1943 Tucker, Denys W., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1954 Tucker, H. S., *Roseland, Menheniot, Liskeard, Cornwall*
1939 Tutin, Prof. T. G., *Botany Dept., University College, Leicester*

1950 Umpelby, G., 1 *Togo Terrace, The Hoe, Plymouth, Devon*
1951 Upton, Miss B. C., 21 *Buxton Avenue, Caversham, Reading, Berks*

1948 Vane, F. R., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6
*1951 Verulam, The Earl of, J.P., *Gorhambury, St Albans, Herts.* (**Vice-President**, 1953→)
*1946 Vevers, H. G., M.B.E., D.Phil., *The Laboratory, Citadel Hill, Plymouth, Devon*
1951 Victoria: Provincial Department of Fisheries, *Parliament Buildings, Victoria, B.C., Canada*
1949 Vinall, H. F., 93 *Lady Margaret Road, London, N.* 19
1954 Vinden, J. S., 79 *Ryland Road, Edgbaston, Birmingham*, 15
1951 Vladykov, V. D., Ph.D., *Dept. of Fisheries*, 150 *Rue St Paul, Quebec, Canada*

1952 Wagstaff, Miss M., 31 *Guildford Road, Horsham, Surrey*
*1948 Wake, Lieut. (E) K.D.O., R.N., 95 *Constable Road, Ipswich, Suffolk*
1951 Wakeham, A. W., 72 *London Road, Long Sutton, Spalding, Lincs.*
1924 Wales, National Museum of, *Cardiff*
1941 Wales: Dept. of Zoology, University College of Wales, *Cambrian Street, Aberystwyth, Cardigan*
1951 Walford, Dr Lionel A., *U.S. Fish and Wildlife Service, Washington* 25, *D.C., U.S.A.*
1954 Walkden, R., 76 *Dominion Street, Walney Island, Barrow-in-Furness, Lancs*
1949 Walne, P. R., *Fisheries Experiment Station, Castle Bank, Conway, Caern.*
1951 Ward, D. W., *Orchard Cot, Gordon Road, Whitstable, Kent*
1952 Wardropper, A. L., 24 *Rupert Street, Whitburn, Co. Durham*
1948 Warren, F. J., *The Laboratory, Citadel Hill, Plymouth, Devon*
1925 Waterhouse, Sir Nicholas, K.B.E., 3 *Frederick's Place, Old Jewry, London, E.C.* 2. (**Vice-President**, 1925→)

1945 Waters, C. A., *E. Wood Ltd., Talbot Works, Stanstead Abbots, Ware, Herts*
1923 Watson, Prof. D. M. S., F.R.S., 12 *Preston Way, Harrow, Mddx.* (**Council**, 1924–28, 1929–32, 1934–37)
1951 Watts, J. C. D., *West African Fisheries Research Institute, Freetown, Sierra Leone*
1952 Watts, P., Dr, 38 *Thornton Hill, Exeter, Devon*
1950 Waugh, G. Duncan, *Fisheries Laboratory, Burnham-on-Crouch, Essex*
*1938 Webb, D. A., Ph.D., *Trinity College, Dublin, Eire*
1951 Webber, S. T., *Anchorage, Richmond Walk, Devonport, Devon*
1951 Webster, R. W., *Dept. of Chemistry and Biology, The Polytechnic, Regent Street, London, W.* 1
1948 Weiss, Dr R. J., *rue Neuve* 33, *Esch-sur-Alsette, Luxembourg*
*1919 Wells, G. P., Sc.D., 7 *Buckland Crescent, London, N.W.* 3. (**Council**, 1935–38, 1946–48, 1952→)
1934 Went, A. E. J., D.Sc., *Dept. of Agriculture, Fisheries Branch,* 3 *Kildare Place, Dublin, Eire*
1950 West Indies, *University College of, Mona, Liguanea, Jamaica, B.W.I.*
1946 Wharton, R. H., *Birkrigg, Noggarth Road, Roughlee, near Burnley, Lancs*
1954 Wheeler, Miss M. R., *St Anne's College, Oxford*
1953 Whiffen, S. W., O.B.E., *Seaward, Fowey, Cornwall*
1948 White, K. G., *The College, Cheltenham, Glos*
1934 White, Miss Kathleen M., *The University, Reading, Berks*
1934 White, P. H. F., *Science Schools, Harrow School, Mddx*
1947 Whitear, Miss M., *Zoology Dept., University College, Gower Street, London, W.C.* 1
1954 Wickstead, J., *Fisheries Research Station, P.O. Box* 749, *Singapore* 1
1949 Wieczorek, K., *Hertford Grammar School, Hertford, Herts*
1950 Wieser, W., *Zoologiska Institution, Lunds University, Lund, Sweden*
1954 Wilkes, K. W., *Science School, Harrow School, Harrow, Mddx*
1951 Wilkins, G. L., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1940 Willcocks, T., *Beechfield, Cawsand, near Plymouth, Devon*
1950 Williams, F., *P.O. Box* 754, *Zanzibar, East Africa*
1948 Williams, J. E. Miles, *Talgarth,* 25 *Hillview Road, Hucclecote, near Gloucester*
*1951 Williamson, D. I., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1948 Wilmott, C. C., 42 *Chard Road, St Budeaux, Plymouth, Devon*
1948 Wilson, Arthur, 92 *Crescent Road, Ramsgate, Kent*
1927 Wilson, D. P., D.Sc., F.R.P.S., *The Laboratory, Citadel Hill, Plymouth, Devon*
1930 Wilson, Mrs D. P., Ph.D., 49 *Torland Road, Hartley, Plymouth, Devon*
1928 Wimpenny, R. S., *Fisheries Laboratory, Lowestoft, Suffolk.* (**Council**, 1946–49, 1952→)
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THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. XXVII (p. 761) and Vol. XXXI (p. 193) of this *Journal*.

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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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All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

PAGE

Vera Fretter and Alastair Graham. Observations on the opisthobranch mollusc *Acteon tornatilis* (L.) 565

Marie V. Lebour. The position of *Pontophilus echinulatus* (M.Sars) in the Crangonidae . 587

L. R. Fisher, S. K. Kon and S. Y. Thompson. Vitamin A and carotenoids in certain invertebrates. II. Studies of seasonal variations in some marine Crustacea . . . 589

D. Atkins. Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n.sp., infecting certain invertebrates 613

D. Atkins. Leg disposition in the brachyuran megalopa when swimming 627

J. N. Carruthers. A penetrometer for use on water-covered beaches 637

W. S. Preddy. The mixing and movement of water in the estuary of the Thames . . 645

G. Y. Kennedy and H. G. Vevers. The occurrence of porphyrins in certain marine invertebrates 663

R. H. Millar. *Protostyela heterobranchia* n.gen., n.sp., a styelid ascidian from the Scottish west coast 677

R. H. Millar. The breeding and development of the ascidian *Pelonaia corrugata* Forbes and Goodsir 681

A. J. Matty. Thyroidectomy of the dogfish, *Scyllium canicula* (L.), and the effect of dogfish thyroid upon the oxygen consumption of rats 689

R. Phillips Dales and G. Y. Kennedy. On the diverse colours of *Nereis diversicolor* . 699

J. S. Alexandrowicz. Innervation of an amphipod heart 709

D. Atkins. A marine fungus *Plectospira dubia* n.sp. [Saprolegniaceae], infecting crustacean eggs and small Crustacea 721

L. Brull and Y. Cuypers. Blood perfusion of the kidney of *Lophius piscatorius* L. II. Influence of perfusion pressure on urine volume 733

R. B. Pike. Notes on the growth and biology of the prawn *Spirontocaris lilljeborgii* (Danielssen) 739

Abstracts of Memoirs. Recording work done at the Plymouth Laboratory . . . 749

Book Reviews 755

Marine Biological Association of the United Kingdom. Report of the Council for 1953–54 757

List of Governors, Founders, Members, Honorary and Associate Members . . 783

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22
CANADA AND INDIA: MACMILLAN

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)