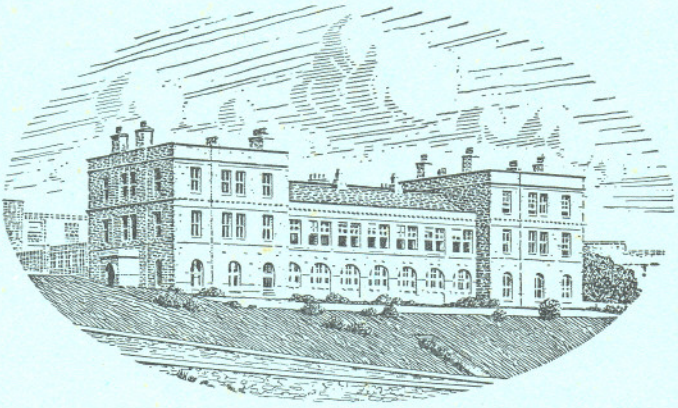
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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THE PROBOSCIS APPARATUS OF *ARENICOLA*

By G. P. Wells

Department of Zoology, University College, London

(Plates I–IV and Text-figs. 1–14)

CONTENTS

INTRODUCTION

The anterior end of *Arenicola* contains an elaborately organized apparatus which has hitherto escaped accurate description, although it is of evident importance in the processes of extrusion and withdrawal of the proboscis, and exhibits striking variations within the genus as at present constituted. The 'proboscis apparatus', as it will be termed, includes the following components: (i) the proboscis itself and the first part of the oesophagus, together constituting the anterior portion of the gut; and (ii) the retractor sheath and gular membrane ('first septum' or 'first diaphragm' of previous authors), both of which, in the writer's view, are derived from the first septum. Although of diverse morphological origin, these components are built together into a functionally unified whole. The purpose of the following paper is to give an account of the anatomy of the apparatus on which later studies of its mode of action can be based. The three species to be described, *A. ecaudata* Johnston, *A. claparedii* Levinsen and *A. marina* L., are chosen to represent the three sections into which the genus naturally falls. It will be shown that the proboscis apparatus undergoes characteristic structural modifications in each of the three, although its basal plan is always the same. So great are the divergences that probably each of them extrudes its proboscis in a different way.

A revision of the external characters, body wall and parapodia of the same three species was recently published (Wells, 1950). The material and anatomical methods described in that paper apply also to the present one, except that I have received some excellent additional material of *A. claparedii* from Departure Bay, Nanaimo, by the kindness of Dr Peter Ford, of the Department of Zoology, University of British Columbia.

The chief point of difficulty in studying the anatomy of the proboscis apparatus is the diversity of positions which it can assume. The proboscis may be wholly or partly extruded, or withdrawn. When withdrawn its arrangement, and that of the associated structures, can vary greatly according to the configuration of the anterior end as a whole. Most of the anatomical descriptions refer to worms killed in one or other of two attitudes, termed 'relaxed' and 'distended'. Details of the methods of killing are given in the earlier paper. A relaxed worm is in a tolerably normal attitude, with the proboscis partly or wholly withdrawn. A distended worm has been narcotized and its body cavity blown up with fixative at the moment of killing. Its proboscis is extruded and all the parts are stretched to an abnormal degree, but the result is useful for the elucidation of many of the finer anatomical details. Except where otherwise stated, the descriptions refer to relaxed worms.

General Account of the Proboscis Apparatus

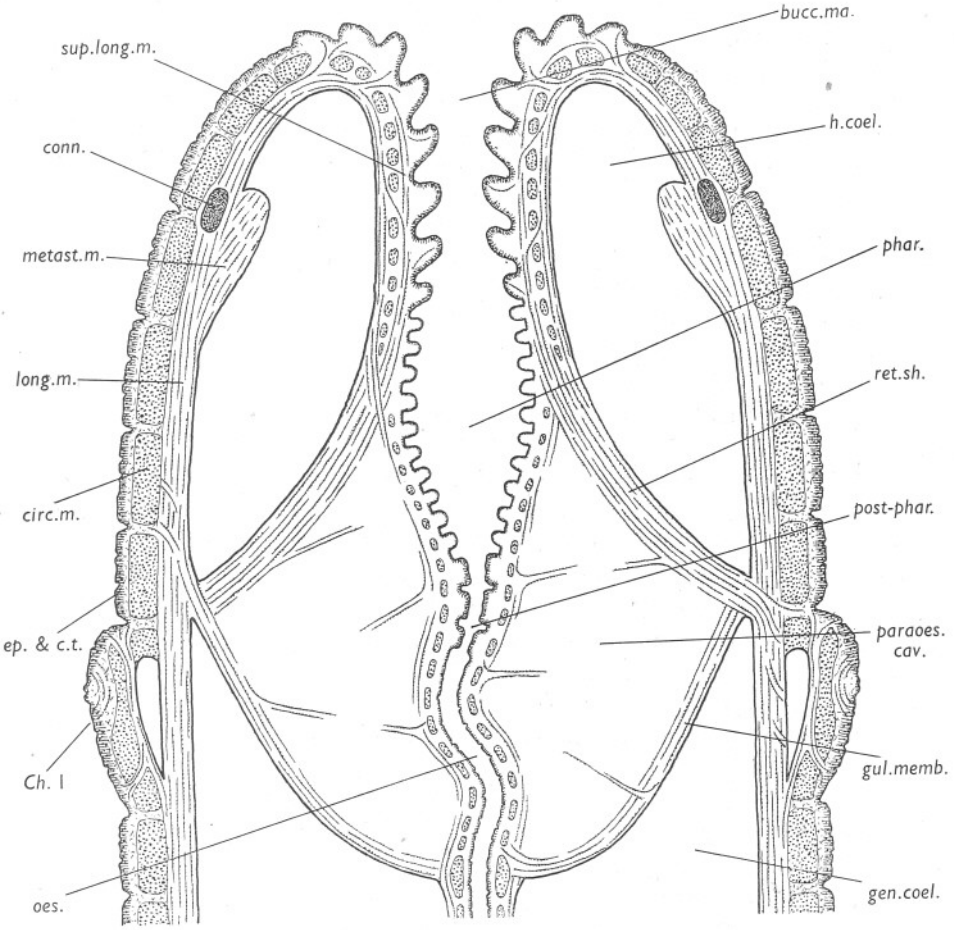
The three species to be considered represent divergent specializations of a common pattern. An account will first be given of the general plan of the proboscis apparatus, and afterwards the modifications shown by the three species will be described. The general account applies to all of the species, except where otherwise stated.

The simplest way to see the plan of the proboscis apparatus is to bisect the anterior end horizontally with a razor blade and examine it with a dissecting microscope (Pl. II, fig. 3). Sagittal sections are often confusing because of certain elaborations which appear in the median plane. A generalized horizontal section, with the proboscis withdrawn, is shown in Text-fig. 1, and described in the following paragraphs.

The 'head' of the worm is the bluntly conical region extending forwards from the anterior margin of the first chaetigerous annulus (*Ch.* I). Its body wall consists of the following layers: (i) epidermis (*ep.*); (ii) sub-epithelial connective tissue (*c.t.*); (iii) circular muscle (*circ.m.*); (iv) a layer of intermuscular connective tissue; (v) longitudinal muscle (*long.m.*); (vi) coelomic epithelium. The ventral nerve cord passes forward on to the head and divides into two connectives (*conn.*) which run upwards and forwards to the brain. The position of the nerve cord and connectives relative to the layers of the body wall varies from species to species. The connectives are accompanied by the metastomial muscles (*metast.m.*), which are derived from the longitudinal layer and pass obliquely upwards to meet each other immediately

ventral to the brain. The structure of the body wall of the head was described in fuller detail elsewhere (Wells, 1950).

The proboscis is the eversible part of the gut, and consists of: (i) a buccal mass, with large, prominent papillae whose form varies greatly from species to species (*bucc.ma.*); (ii) a pharynx, with finer, more closely set papillae



Text-fig. 1. Generalized horizontal section through the anterior end of *Arenicola*. Lettering as on p. 28.

(*phar.*); (iii) a short post-pharynx with rather larger papillae (*post-phar.*). The post-pharynx is the extreme limit of that part of the gut which is turned inside-out during extrusion. It is followed by the more or less laterally compressed oesophagus (*oes.*).

The body wall continues into the proboscis at the mouth, and the walls of the proboscis and oesophagus are composed of layers having fundamentally

the same arrangement as those of the body wall. There are, however, local specializations, of which the most striking is the presence in the buccal mass and the oral end of the pharynx of a superficial longitudinal muscle layer, lying between the circular muscle and the epithelium (*sup.long.m.*).

The individual proboscis papillae appear at first sight to be hollow outpushings of the gut wall. They are, however, more correctly described as thickenings of the wall which have become hollowed by the great development of the interstitial spaces between the cells (Pl. IV, figs. 1, 2). They are bounded, on the surface towards the gut, by the epithelium and a thin layer of underlying connective tissue; they contain blood vessels and muscle fibres, the latter partly derived from the main muscle layers of the gut and partly intrinsic to the individual papillae. Because of these muscle fibres, and the extensive interstitial spaces, they have considerable powers of movement. Ideally, the spaces are cut off from the true coelom by the lining of the latter cavity, but the actual relationships vary from species to species, and from region to region of the proboscis.

The pharyngeal papillae vary somewhat in size, small ones being scattered about among their larger neighbours. This fact suggests that new papillae are intercalated all over the surface of the pharynx as the worm grows. The buccal papillae are of very uniform size, except that those at the oral and pharyngeal ends of the buccal mass are rather smaller than those in the middle. The growth of the buccal mass seems therefore to occur by increase in size of the individual papillae, and not by the intercalation of new ones among the old. The buccal mass and pharynx are separated by a more or less extensive transition zone bearing papillae of the pharyngeal type intermixed with others of the buccal type; the latter decrease in size from the buccal to the pharyngeal end of the transition zone and are finally no larger than the pharyngeal papillae, into which they merge (Pl. I, fig. 1). The appearances could be due to a continual conversion of pharynx into buccal mass, taking place at the boundary between them.

The gut is suspended from the body wall of the head by two transverse sheets of tissue, the retractor sheath (*ret.sh.*) and the gular membrane (*gul.memb.*). The latter is the 'first diaphragm' or 'first septum' of Ashworth (1904, 1912), but for reasons explained below I have substituted a name carrying no implication of serial homology.

The retractor sheath consists of coelomic epithelium, connective tissue and a single muscle layer in that order, the muscle layer lying on its hinder face. My sections—the thinnest of which are 15 μ, as they were made for fine anatomy rather than histology—also show a delicate endothelial layer on the hind face of the muscle in *ecaudata*, but not with any certainty in *claparedii* or *marina*. The muscle fibres run radially from the body wall to the gut, which they reach about half-way along the pharynx. Most of them then pass through the circular muscle layer of the gut to constitute the superficial

longitudinal layer of the proboscis; the details of this region are, however, complicated and will be returned to in the detailed descriptions of the three species.

The gular membrane consists of the same layers as the retractor sheath, but in the reverse order—i.e. with the muscle layer in front of the connective tissue and epithelium. It varies greatly from species to species, both in general form and in the massiveness of its musculature. The latter runs radially in *claparedii* and *marina*, but is elaborated into a radial layer lying anterior to a circular one in *ecaudata*.

The retractor sheath and gular membrane are inserted together on the body wall, just in front of the first chaetigerous annulus. Their relations at this point can be imitated by tightly clasping one's hands and then extending the fingers. The palms of the hands represent the two sheets, and the fingers are the columns into which their radial muscle layers continue peripherally. The columns decussate and immediately enter the body wall. At first sight they appear to join the longitudinal layer, but serial sections show that a large part, at least, of their fibres make their way through to the connective tissue around and between the circular muscle bundles.

The horizontal section shows three large cavities between the gut and the body wall. These are the general coelome behind the gular membrane (*gen.coel.*), the head coelome in front of the retractor sheath (*h.coel.*), and the paraoesophageal cavity between the two sheets (*paraoes.cav.*). The paraoesophageal cavity contains a loose network of blood vessels lying freely within it, and is crossed by numerous very fine strands of muscle, the radial strands, which detach themselves from the retractor sheath and gular membrane and run to the wall of the gut.

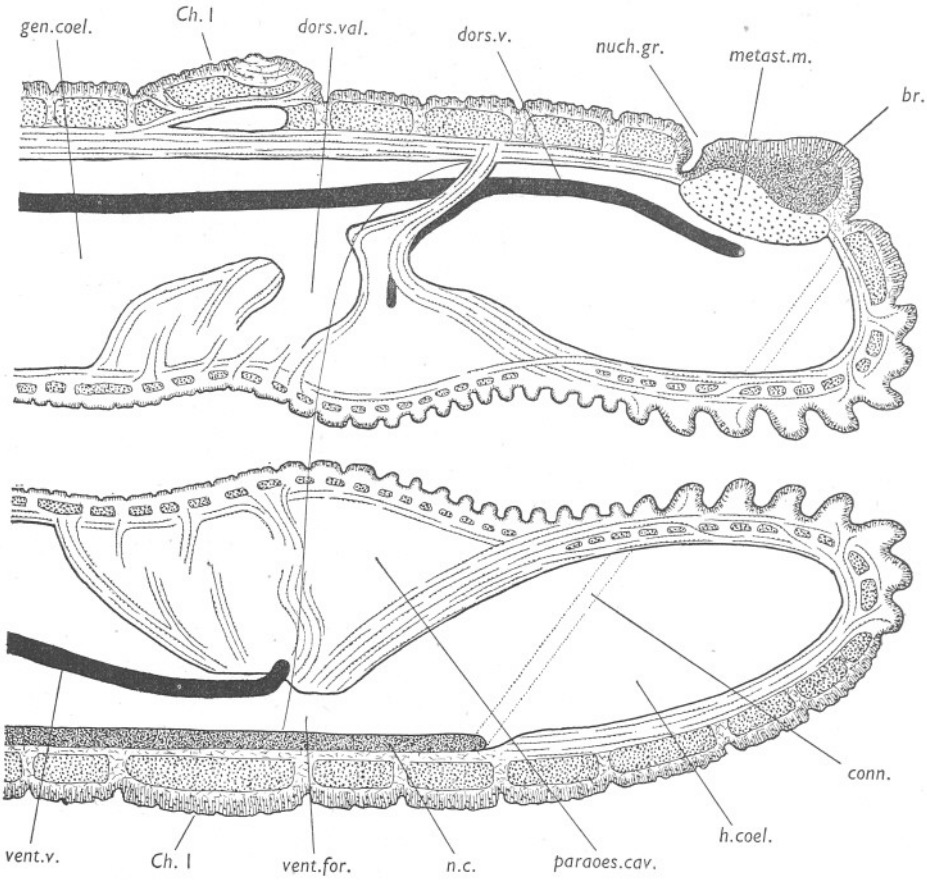
The gular membrane and retractor sheath are imperforate, and therefore isolate the paraoesophageal cavity completely from the other cavities, except at certain special points as described below. The head coelome is separated from the general coelome by the peripheral muscle columns of the two sheets as they cross from their decussation to the body wall—the bases of the fingers, in the analogy of the clasped hands—but this barrier is never complete. Fluid can flow between head coelome and general coelome to an extent which varies, not only according to specific differences in form, but also according to the degree of contraction of the muscle columns.

On examining sagittal sections, or a sagittally dissected worm, certain additional points can be seen. A generalized sagittal section is shown in Text-fig. 2. The position of the central nervous system is given by the ventral nerve cord (*n.c.*), the brain (*br.*), and the dotted lines indicating the course of the connectives (*conn.*).

The position of the insertion of the retractor sheath and gular membrane on the body wall is shown as a fine ruled line. Ventrally and laterally, it is placed at or near the front border of the first chaetigerous annulus, but as the mid-

dorsal line is approached it curves forwards to form, with its fellow of the opposite side, an anteriorly directed V, the apex lying about half-way between the first chaetigerous annulus (*Ch.* I) and the nuchal groove (*nuch.gr.*). The line varies slightly in position from species to species.

The insertion of the retractor sheath and gular membrane is interrupted ventrally, in such a way as to leave a ventral foramen (*vent.for.*) through



Text-fig. 2. Generalized median sagittal section through the anterior end of *Arenicola*. Lettering as on p. 28.

which the general coelome and head coelome communicate. This foramen is bounded laterally by the insertions of the two sheets, and could presumably be closed by contraction of their muscle columns. Its roof is the floor of the paraoesophageal cavity, which is thin and membranous in the mid-ventral line, the muscle columns having diverged to reach the body wall lateral to the foramen.

The ventral vessel (*vent.v.*) ends anteriorly by passing upwards through the floor of the paraoesophageal cavity in the region of the ventral foramen, and breaking up into branches which join the vascular network lying in that cavity.

The radial muscle strands which cross the paraoesophageal cavity are especially well developed in the median plane. In particular, one or more thickened strands run from the lateral wall of the ventral foramen to the gut, which they reach just behind the post-pharynx. These strands may be regarded as a ventral accessory retractor of the proboscis.

Mid-dorsally, the gular membrane is thrown into a vertically descending fold, the dorsal valve (*dors.val.*). This fold is narrow from side to side, so that it has the general form, in horizontal section, of a flattened tube. The muscular layer of its lateral walls passes down as a series of strands to reach the gut just behind the post-pharynx; they probably act collectively as a dorsal accessory retractor of the proboscis. The membranous layer of the fold is continuous in its dorsal part but becomes perforated more ventrally, as it nears the gut. Body fluid can therefore make its way down the fold from the general coelome into the paraoesophageal cavity—a fact which explains the presence in the latter of coelomic corpuscles and developing germ cells identical with those found in the general coelome—but, owing to the lateral flattening of the tube, the passage of fluid in the reverse direction is presumably difficult.

The dorsal vessel (*dors.v.*) passes forwards through the insertion of the retractor sheath and gular membrane. As it does so, it gives off one or more branches which run downwards and backwards into the paraoesophageal cavity to join its vascular network.

Serial Homology of the Proboscis Apparatus

Ashworth's well known monograph of *A. marina* contains the following passage (Ashworth, 1904, p. 19):

> 'The coelom is spacious and continuous from one end of the animal to the other. In front it is sub-divided transversely by three fenestrated septa, or diaphragms. The first of these is placed at the level of the anterior edge of the first chaetigerous annulus.... The second and third diaphragms mark the posterior limits of the second and third chaetigerous segments.'

The first of these diaphragms is our gular membrane. Ashworth evidently regarded all of the diaphragms as serially homologous with each other and as representing true septa (see also his pp. 22–4).

Ashworth's knowledge of the anatomical relationships inside the head was not very exact. In particular, he was unaware of the existence of the paraoesophageal cavity as a definitely walled-off space. He described the first diaphragm incorrectly as fenestrated, or 'perforated by numerous, rounded,

usually oval apertures', as the third is, and he drew the retractor sheath, as it runs forwards from its decussation with the gular membrane, in the form of a series of separate slips instead of a continuous sheet (see his plate III, fig. 23). Moreover, he said nothing about the serial homology of the retractor sheath. As this structure meets the body wall just behind the gular membrane and the gut wall in front of the membrane, its derivation presents a difficult morphological problem if the gular membrane is in fact a septum.

Lillie (1905) published an account of the development of *A. cristata* Stimpson, a species which closely resembles *A. marina*. His main interest was in the nephridia, and he apparently relied for the rest of the adult anatomy on the works of Gamble & Ashworth (1898, 1900), on which Ashworth's monograph of 1904 is largely based. Lillie believed, contrary to Ashworth, that the first diaphragm has a different origin from the other two, and that none of them represent the primitive septa. His views on the second and third are irrelevant to the present discussion. With regard to the first diaphragm, or gular membrane, he wrote as follows (his p. 353):

> 'The most anterior septum occupies from the first a position immediately behind the line of insertion of the proboscideal retractor muscles (plate 24, fig. 35). As growth proceeds, the relations of these two originally independent structures become closer, and at the stage represented in fig. 36—in which twelve somites have been laid down—the two have become intimately associated with one another, though still distinguishable on close examination. This association of proboscideal muscles and first septum becomes in later stages more complete, and both eventually enter into the formation of the muscular first diaphragm. The posterior peritoneal wall of the adult diaphragm may be regarded as representing the original first septum.'

Lillie gave no information about the origin of the retractor sheath, whose first appearance in his account is as quoted above. Moreover, I have failed to find conclusive evidence for his interpretation in his published illustrations: this point is returned to below.

Ashworth afterwards accepted Lillie's views on the nature of the first diaphragm (Ashworth, 1912, pp. 61–2).

My own preference is for a third hypothesis, which may be stated as follows. The first chaetigerous segment was originally bounded anteriorly by a septum inserted on the body wall at the front border of the first chaetigerous annulus. This septum would consist of two layers of coelomic epithelium separated by connective tissue with muscle fibres and blood vessels in it. Let us assume that a cleft—the paraoesophageal cavity—has appeared in the middle layer of the septum, splitting it into two sheets. The anterior sheet becomes the retractor sheath, and the posterior sheet the gular membrane. If this be true, the paraoesophageal cavity is not coelomic.

This hypothesis has the advantage over its predecessors of finding a place for the retractor sheath in the segmental scheme. It explains why the sheath and the gular membrane are inserted at the same level on the body wall. The

forward displacement of their plane of insertion in the mid-dorsal region supports the hypothesis, for a tendency to forward movement of the dorsal part of the septal insertions relative to the external annulation is visible in other segments of *Arenicola*. Moreover, the retractor sheath and gular membrane are so intimately related at certain points, notably in the median plane, that it is impossible to tell where one leaves off and the other begins. This might perhaps be due to the blending of two originally distinct structures, as Lillie suggested, but it is more simply explained if the sheath and membrane are in fact one structure.

The paraoesophageal cavity differs from the coelome in being crossed by the numerous radial muscle strands, and also in containing a network of vessels, lying freely in its cavity, to which both the dorsal and ventral vessels contribute. In other segments the dorsal and ventral vessels have no direct communication with each other except by means of commissural loops running in the septa,[1] and if the paraoesophageal cavity represents a cleft within a septum, its vascular network can be derived from the commissural loop. The presence or absence of an epithelial lining to the cavity cannot be taken as evidence, for on the one hand, the coelomic epithelium can break down and disappear locally (e.g. in the buccal region of *claparedii* and *marina*), while on the other, many non-coelomic cavities, such as blood vessels, may acquire endothelial linings. The following point is, however, suggestive. My sections of *claparedii* show conspicuous brown 'chlorogogenous' cells on the ventral vessel. These cells, which are presumably derived from the coelomic epithelium, continue forwards exactly to the point of entry of the ventral vessel into the paraoesophageal cavity, where they end abruptly.

Although the hypothesis is in good agreement with the adult anatomy, it is rather in the early development that conclusive proof of its truth should be sought. Here I have no direct experience; but I have tried without success to find decisive evidence in Lillie's figures in favour either of his interpretation or of mine. The passage quoted above refers to his figs. 35 and 36, which respectively show 8- and 12-somite larvae. Unfortunately, they are both sagittal sections and, because of the dorsal valve and the other complications which appear in the median plane, neither of them shows the relations of the retractor sheath and gular membrane at all clearly. If we turn to his figures of earlier stages, we find sections of 5- and 6-somite larvae in his figs. 7 and 8, plate 22. These are again sagittal, and their dorsal sides are difficult to understand, but their ventral sides are suggestive. In the 5-somite larva ('swarming stage') the mouth is still closed. The future proboscis appears to

[1] These loops can be seen very clearly in the hinder branchiate segments of *ecaudata*, by isolating a single segment and examining in end view. The loops supply the gills and give off branches to the main longitudinal vessels of the body wall. The loops often persist even where the septa have disappeared, and are variously modified in the different regions of the body. That of the (vanished) septum vii forms the so-called ventricles. I hope to describe the loops in detail in a later work.

be connected to the body wall by a thin membranous septum only. In the 6-somite larva ('beginning of crawling stage') the mouth is open and the proboscis has either just become, or is about to become, eversible (see his p. 347). The septum has now thickened into a broad wedge with its apex on the body wall and its base on the gut. This looks like the separation of a single septum into two sheets, rather than the merging of two originally distinct structures.

By the kindness of Dr Helen Pixell Goodrich, I have been allowed to use some horizontal sections of a 1·5 mm. *A. cristata* post-larva from Woods Hole, made by the late Prof. Goodrich. The tail is already nearly complete in this animal. A section of the anterior half, passing just ventral to the mouth, is shown in Pl. II, fig. 4. The metamerically arranged septa are clearly visible. If the convention is adopted that the septum at the anterior boundary of the *n*th chaetigerous segment is called the *n*th septum, then those marked *D.* II and *D.* III, which will become the 'second and third diaphragms' of the adult, are in reality the third and fourth septa. The second, marked *D.vest.*, will become a vestigial diaphragm which can be clearly seen in the adult, connecting the corresponding segmental vessels to the body wall. One segment farther forwards, the future retractor sheath and gular membrane meet the body wall, and the suggestion seems irresistible that they both represent the first septum. Conclusive proof could perhaps be found in horizontal sections of earlier stages, with 5 or 6 somites. There is at least a possibility that the first septum would be seen in the act of division in such material.

Modifications of the Proboscis Apparatus in *Arenicola ecaudata* Johnston, *A. claparedii* Levinsen, and *A. marina* L.

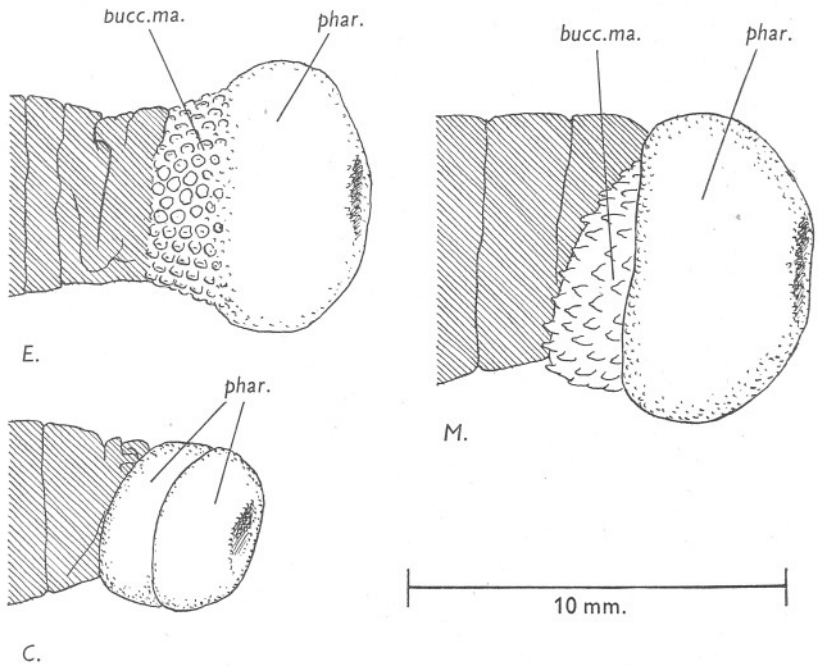
The description of the proboscis apparatus in the three species follows a comparative plan, those parts of the apparatus which show important modifications being taken in order.

The Proboscis

The proboscis varies from species to species in general form and also in the form of the papillae.

The extruded proboscis of *ecaudata* generally has the form of a very flat cone, rather like a loudspeaker cone, with a slightly domed top—the cone being the buccal mass and the top the pharynx. The animals drawn in Text-figs. 3*E.* and 12*E.* were artificially distended after narcosis and show an extreme degree of inflation which is seldom or never attained in the living animal. The pharyngeal papillae are conical. Those of the buccal mass appear, when the proboscis is fully distended, as rounded or polygonal raised areas with flat tops. In the partly distended or withdrawn organ the centres of the tops sink down so that the buccal papillae are sucker-shaped (Text-fig. 4*E.*;

Pl. I, fig. 1; Pl. II, fig. 1). The arrangement of their musculature indicates that a sucker action is of real importance in their normal functioning, for there are strands running down from the tops to the deeper layers of the gut wall, which could cause depression, and others running across on the deep surfaces of the tops, which could cause raising and flattening (Pl. IV, fig. 1). The contrast between the sucker-like buccal papillae of *ecaudata* and the tooth-like ones of the other two species is very great. The transition zone between buccal mass and pharynx, in which papillae of the two types are intermingled, is broad in *ecaudata*.

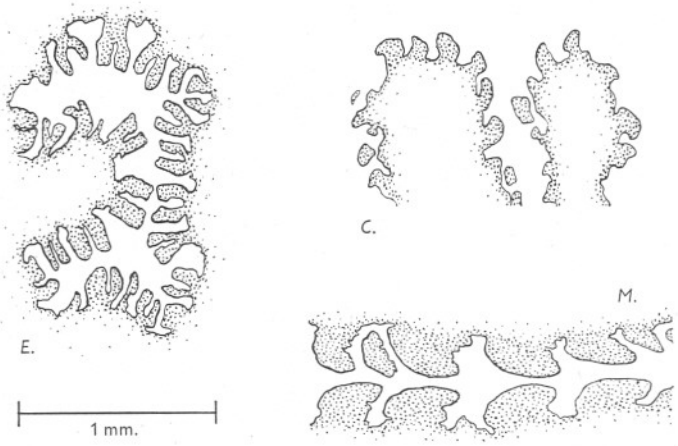


Text-fig. 3. Lateral views of the anterior ends of distended specimens of *ecaudata* (*E.*), *claparedii* (Neapolitan specimen) (*C.*) and *marina* (*M.*). Other lettering as on p. 28.

The pharynx of *claparedii* is relatively more extensive than that of *ecaudata*, and is blown out, at the moment of full extrusion, into a rounded, bubble-like form, which overlaps and conceals the buccal mass. The drawing of Text-fig. 3*C.* was made from an artificially distended specimen, but the form of the pharynx resembles that normally seen in the living worm. The expanded pharynx is characterized by a 'waist-line' which corresponds to the attachment of the retractor sheath muscles on its inner surface. The pharyngeal papillae are more spherical than those of *ecaudata*. The buccal papillae are stout conical teeth, with their apices directed towards the mouth (Text-

fig. 4*C*.).[1] There is a broad transition zone between buccal mass and pharynx, as in *ecaudata* (Pl. I, fig. 2).

The proboscis of *marina* resembles that of *claparedii* in its pharnyx which assumes a rounded form at full extrusion, and in its conical buccal papillae directed towards the mouth (Pl. I, fig. 3; Pl. II, fig. 2; Pl. IV, fig. 2). Its pharynx differs from that of *claparedii* in lacking the 'waist-line' and also in being slightly less extensive, so that it overlaps the buccal mass less completely at the moment of full extrusion. On the other hand, the buccal



Text-fig. 4. Camera lucida outlines of microtome sections, to show the form of the buccal papillae. *E.*, *ecaudata*: transverse section through withdrawn buccal mass. *C.*, *claparedii* (Neapolitan specimen): horizontal section through partly extruded buccal mass. *M.*, *marina*: sagittal section through withdrawn buccal mass, the oral end to the right.

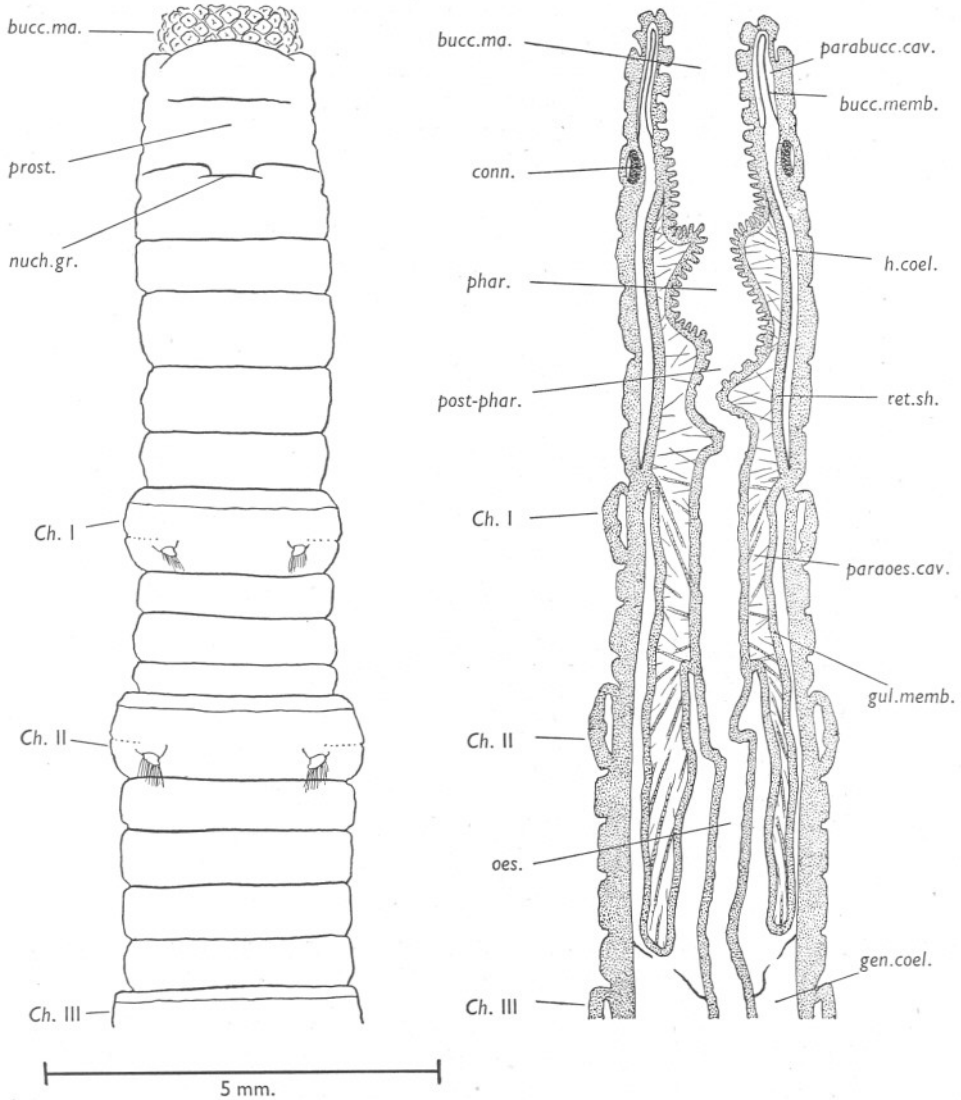
papillae of *marina* are larger and more powerful-looking than those of *claparedii*. I have suggested elsewhere that the buccal mass of *marina* is used as a rasp in burrowing (Wells, 1948). The boundary between buccal mass and pharynx is relatively abrupt in *marina*; the transition zone exists, but is much narrower than in the other species.

The Buccal Peritoneum

As already noted, the proboscis papillae are hollowed out by the great development of their interstitial spaces. The relations between these spaces and the main body cavities are of evident functional interest. In the part of the gut which traverses the paraoesophageal cavity, the interstitial spaces open freely into that cavity; this is to be expected if it is itself an enormous inter-

[1] The buccal teeth are more rounded in my Neapolitan specimens than in my Canadian ones. This may be due to the fact that the Neapolitan specimens are much smaller; but Ashworth (1912) has enumerated several other differences between Neapolitan and Pacific members of this species. The statements in my text about the form of the normally extruded proboscis in *claparedii* are based on worms watched at Naples in 1949. I have not seen living Canadian specimens.

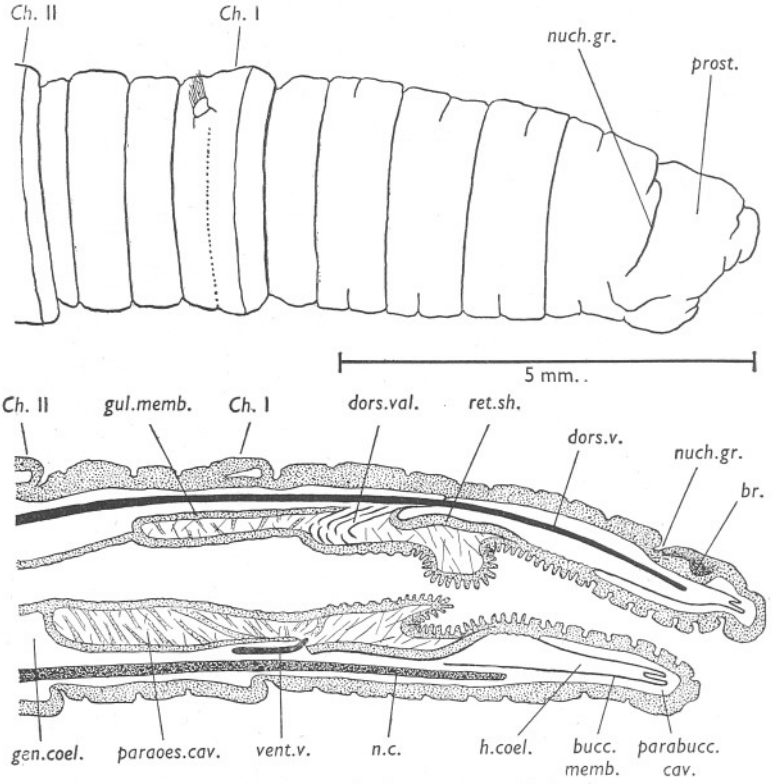
stitial cleft. The relations of the spaces in the buccal papillae to the head coelome vary from species to species, as follows.



Text-fig. 5. Horizontal section through the anterior end of *ecaudata*, and outline of the worm in dorsal aspect. Lettering as on p. 28. (In Text-figs. 5–10, the section was drawn first, from microtome sections or dissections of relaxed specimens, and the surface drawing was then fitted to the same outline.)

The buccal region of *ecaudata* is characterized by the presence of a stout membrane, the buccal membrane (Text-figs. 5, 6; Pl. IV, fig. 3; *bucc.memb.*).

The membrane consists of two very thin epithelial layers with dense connective tissue between them, and separates the head coelome from a smaller cavity, the parabuccal cavity (*parabucc.cav.*). There is apparently no peritoneal lining between the parabuccal cavity and the tissues of the buccal mass, whose interstitial spaces open freely into the parabuccal cavity. The simplest explanation of these facts is to suppose that the buccal membrane represents the peritoneal lining of the head coelome which has separated away from the



Text-fig. 6. Median sagittal section through the anterior end of *ecaudata*, and outline of the worm in lateral aspect. Lettering as on p. 28.

other tissues of the buccal mass—a modification which would obviously facilitate the movements of the papillae and of the buccal mass as a whole. On this interpretation, the parabuccal cavity is not coelomic, but an extension of the interstitial spaces. The epithelial layer on that face of the buccal membrane which abuts on the head coelome is the true coelomic epithelium and the other is comparable to the endothelial linings of blood vessels and other non-coelomic cavities.

The buccal membrane meets the gut at the junction of buccal mass and pharynx, and the body wall along the line of the brain and connective nerves.

Mid-ventrally, it extends backwards for a short distance over the ventral nerve cord and then ends abruptly, in such a way as to leave a narrow passage through which the parabuccal cavity communicates with the head coelome (Text-fig. 6).

There is no buccal membrane in *claparedii* or *marina*, and the state of affairs in these species can be derived from that in *ecaudata* by supposing that it has simply disappeared, throwing head coelome and parabuccal cavity together to form a single space. There is no barrier at all between the interstitial spaces of the buccal papillae and the head coelome (Pl. IV, fig. 4). The disappearance of the buccal peritoneum presumably increases the mobility of the papillae in these two species, since they can be inflated or deflated by varying the pressure in the head coelome.

The Gular Membrane

This structure varies very greatly from species to species, in general form and in the strength of its musculature.

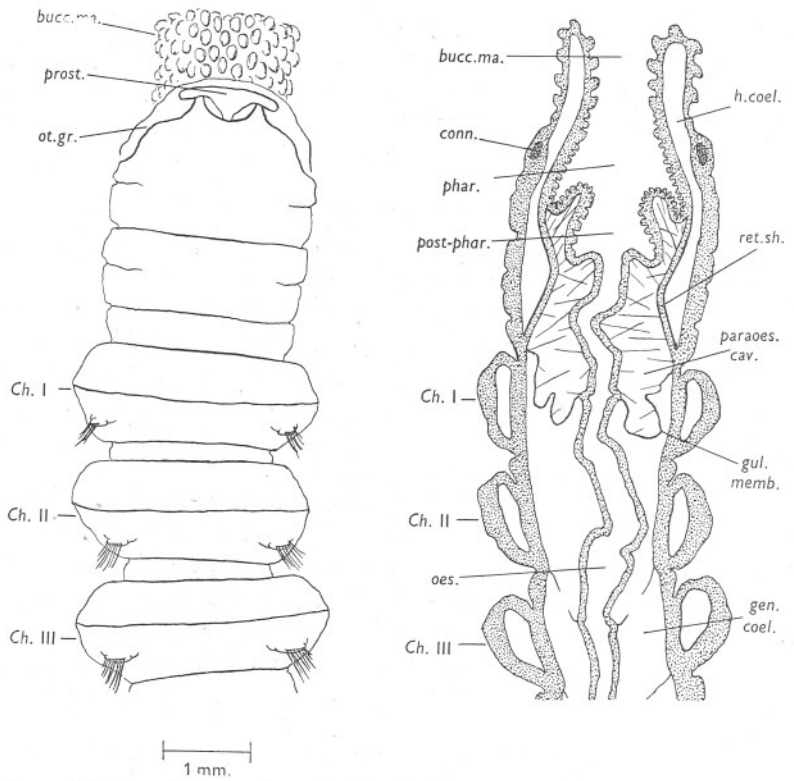
In *ecaudata*, the gular membrane reaches its highest degree of development (Text-figs. 5, 6). It is inserted on the oesophagus between the first and second chaetigerous annuli, but it continues farther backwards in the form of two capacious pouches—the septal pouches, in the terminology of Ashworth (1912)—which lie lateral and ventral to the oesophagus and reach nearly or quite as far as the third chaetigerous annulus. Sometimes they even pass beyond this annulus, their tips protruding backwards through the large clefts which exist on each side of the 'second diaphragm'. The musculature of the gular membrane of *ecaudata* differs from that of the other two species in having two layers, a circular lying behind and outside a radial, and each of these layers taken by itself is more massive than the whole musculature of the membrane in *marina* or *claparedii*. There is little doubt that contraction of the gular membrane plays a major part in proboscis extrusion in *ecaudata*.

The dorsal valve of *ecaudata* is narrow and its terminal perforations are not very large, but the muscle strands in its wall are numerous and well developed. On the ventral side, a pair of stout columns of muscle detach themselves from the lateral walls of the ventral foramen, just beside the point of penetration of the ventral vessel into the paraoesophageal cavity, and cross the cavity to reach the ventral side of the oesophagus, a little way behind the post-pharynx; these are the ventral accessory retractors, and are considerably more massive than those of the other species.

The gular membrane of *claparedii* shows the most striking contrast to that of *ecaudata* (Text-figs. 7, 8). It is extremely delicate and transparent, and generally thrown into loose folds. Its musculature consists of a very thin layer of radial fibres. The structure as a whole is evidently too delicate to

be of any importance in proboscis activity; it seems, indeed, to be a vestigial organ. There are no septal pouches.

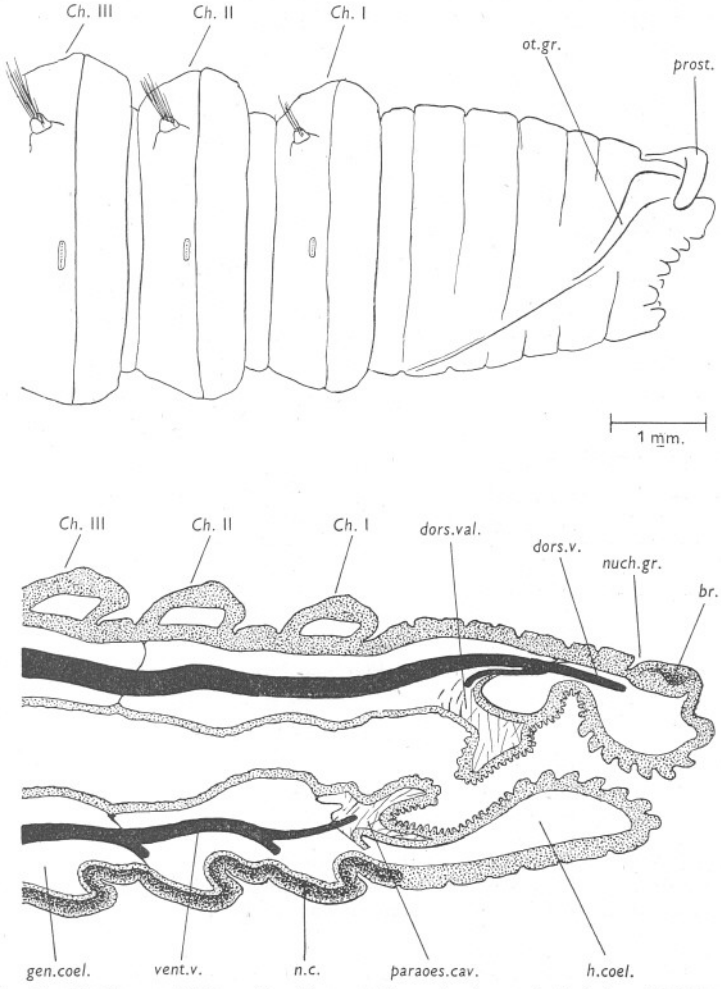
Along the mid-dorsal line, the gular membrane of *claparedii* adheres to the gut wall (contrast Text-figs. 6 and 8). The dorsal valve is widely perforated. These two features together suggest that body fluid driven forwards from the hinder segments could enter the paraoesophageal cavity more readily in *claparedii* than in *ecaudata*.



Text-fig. 7. Horizontal section through the anterior end of *claparedii* (Neapolitan specimen) and outline of the worm in dorsal aspect. Lettering as on p. 28.

The gular membrane of *marina* is intermediate in its degree of development between those of the other two species. It has the general form of a rather flat cone with the apex directed backwards (in the relaxed worm), and it continues posteriorly into a pair of small septal pouches below the oesophagus (Text-figs. 9, 10). At the point where it meets the oesophagus, the circular muscle layer of the latter forms a conspicuous sphincter. The musculature of the membrane is radial only, but considerably more powerful than that of *claparedii*.

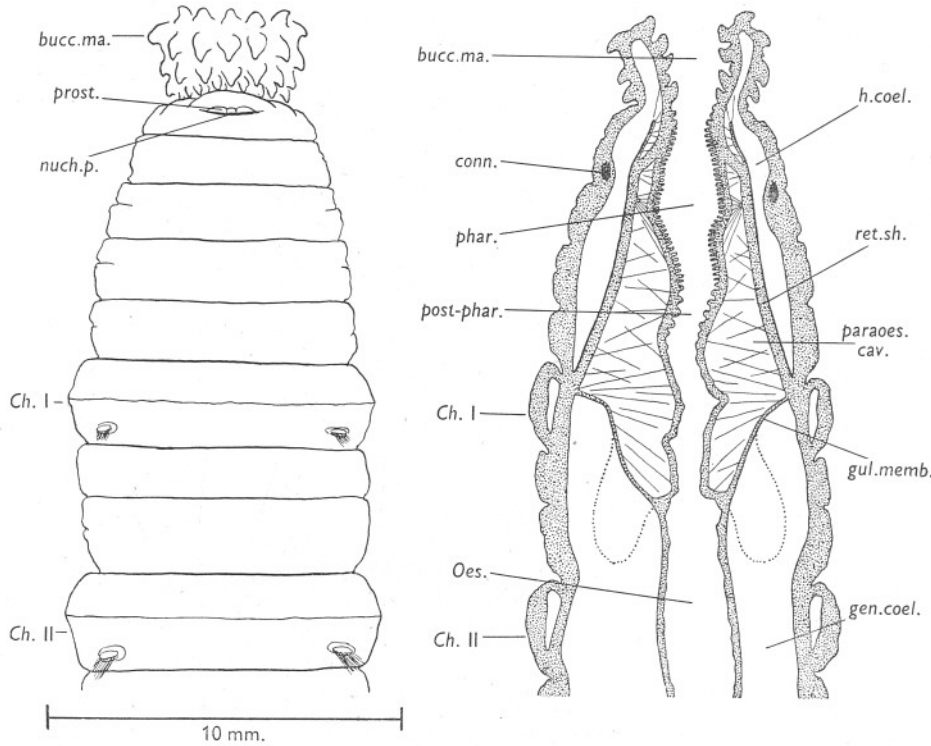
The gular membrane of *marina* is bound closely to the oesophagus by radial strands in the mid-dorsal line. The dorsal valve is more widely perforated than in *ecaudata*. Forwardly moving fluid in the general coelome could easily enter the paraoesophageal cavity through the dorsal valve.



Text-fig. 8. Median sagittal section through the anterior end of *claparedii* (Neapolitan specimen) and outline of the worm in lateral aspect. Lettering as on p. 28.

There is also a second entry point on the ventral side in *marina*. As the ventral vessel runs forwards to its point of entry into the paraoesophageal cavity, it is arched over by the floor of the latter (Text-fig. 10). The arch has, in transverse section, roughly the form of a V with the apex upwards. The lateral walls of the V contain numerous muscle strands running to the mid-

ventral line of the oesophagus, and their membranous part, though continuous below, is freely perforated between the strands near the apex of the V. The arch may be termed the ventral valve (Pl. III, *vent.val.*). Fluid can easily flow through the perforations from the general coelome into the paraoesophageal cavity, but a high pressure in the latter would tend to press together the imperforate ventral parts of the side walls, and this would obstruct a reverse flow.

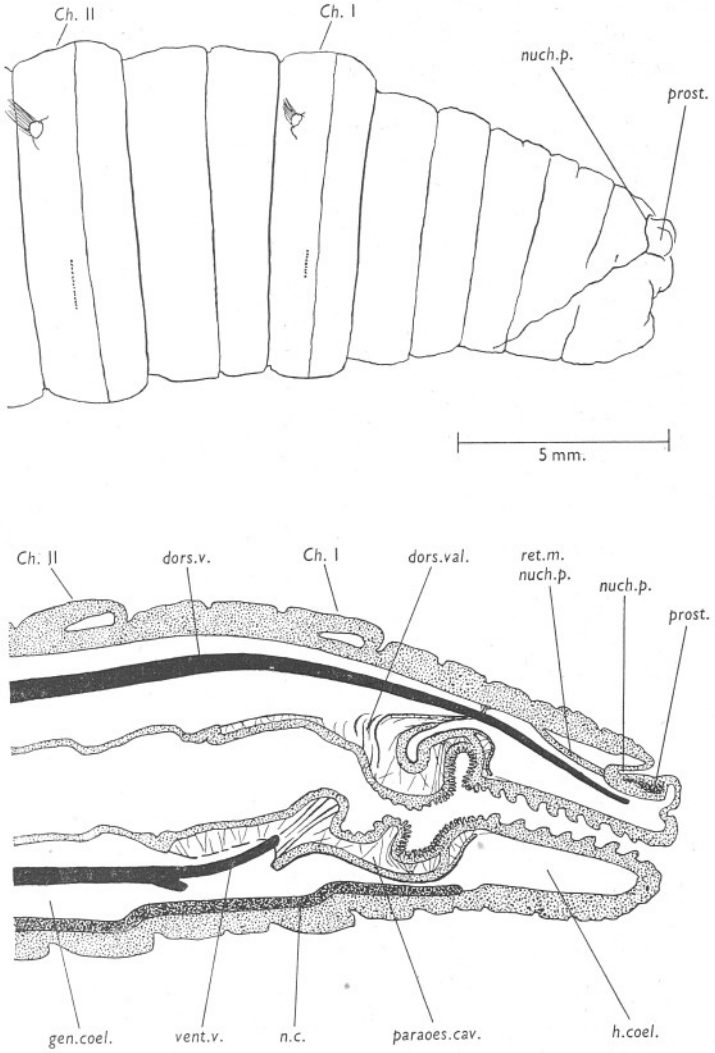


Text-fig. 9. Horizontal section through the anterior end of *marina*, and outline of the worm in dorsal aspect. The dotted lines give the position of the septal pouches, which lie ventral to the plane of the section. Lettering as on p. 28.

The Insertion of the Gular Membrane and Retractor Sheath on the Body Wall

The general form of the line of insertion of these two sheets on the body wall is always the same (Text-fig. 2), but its position varies slightly from species to species. In the mid-dorsal line it is farthest forward in *claparedii* and farthest back in *ecaudata* (Text-figs. 6, 8 and 10). Ventrally, immediately beside the ventral foramen, the sheets meet the body wall at the anterior boundary of the first chaetigerous annulus in *ecaudata*, and slightly in front of it in *claparedii*. In *marina* the gular membrane meets the ventral body wall

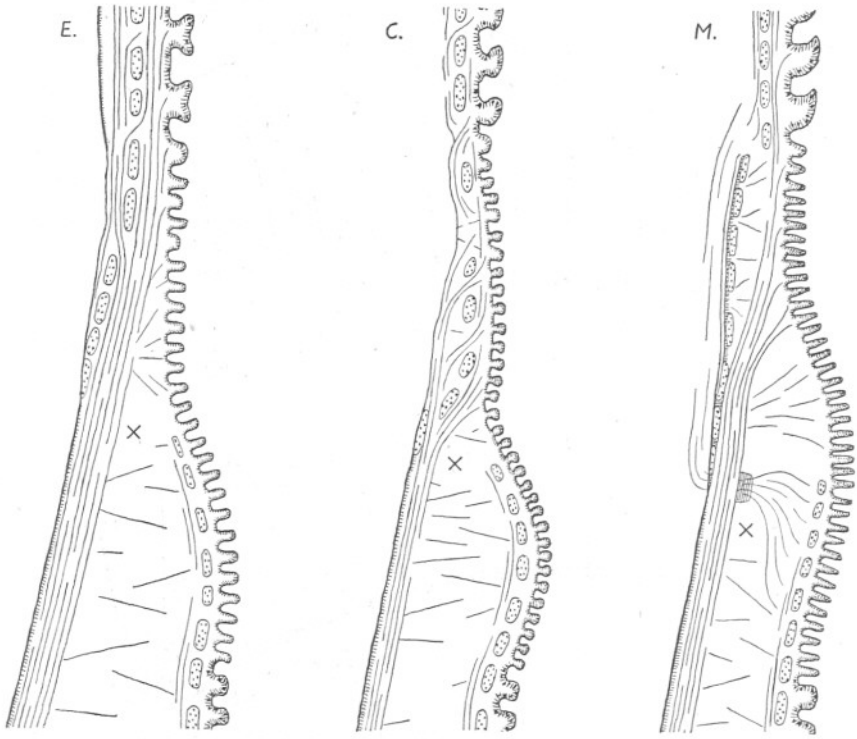
just behind the anterior margin of the first chaetigerous annulus, while the retractor sheath runs back to the hinder half of the annulus next behind the first chaetigerous annulus.



Text-fig. 10. Median sagittal section through the anterior end of *marina*, and outline of the worm in lateral aspect. Lettering as on p. 28.

The extent to which the head coelome and general coelome can communicate between the body wall and the decussation line of the two sheets is also variable. There is always a ventral foramen, and in *ecaudata* this is the only communication. In *claparedii* there are also two large foramina on each side,

at the levels of the notopodium and neuropodium. In *marina* there are numerous fine openings round the whole periphery, as can be clearly seen in a well distended specimen. The extent to which these various openings allow fluid to flow between the cavities must be greatly decreased, in any species, if the muscle columns of the two sheets contract in such a way as to pull their decussation against the body wall.



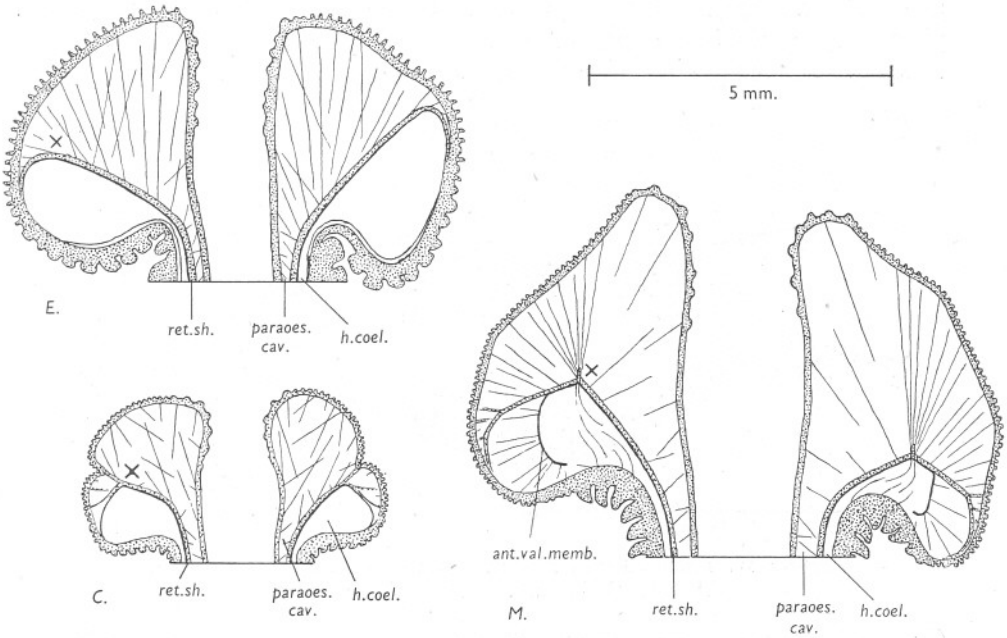
Text-fig. 11. Diagrams of longitudinal sections through the junction of retractor sheath and pharynx in *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). Proboscis withdrawn. The point of junction is marked *X*. Compare Text-fig. 1, p. 3.

The Union of Retractor Sheath and Pharynx

The essential features of this important region are drawn diagrammatically in Text-fig. 11 for *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). The following points are common to all three drawings. The aboral end of the buccal mass is shown above, its interstitial spaces being separated from the head coelome by the buccal membrane in *ecaudata* but not in the other species; then follow the pharynx and the oral end of the post-pharynx below. At the lower end of the drawing, the retractor sheath is seen approaching the gut; it consists of coelomic epithelium on the left, connective tissue (cross-shaded) and muscle. The oral end of the paraoesophageal cavity lies between retractor sheath and

gut. At the point *X*, in each drawing, circular muscle appears on the outside of the retractor sheath. This may be regarded as the true point of arrival of the sheath on the gut wall, from whose circular layer the fibres in question are presumably derived.

In *ecaudata* the retractor sheath musculature is separated from the gut lining for some distance beyond *X* by a forward continuation of the para-oesophageal cavity, crossed by numerous radial strands, which extends nearly to the buccal mass. The tissues of the junction of pharynx and buccal mass are compactly put together, and there is little or no possibility of a forward



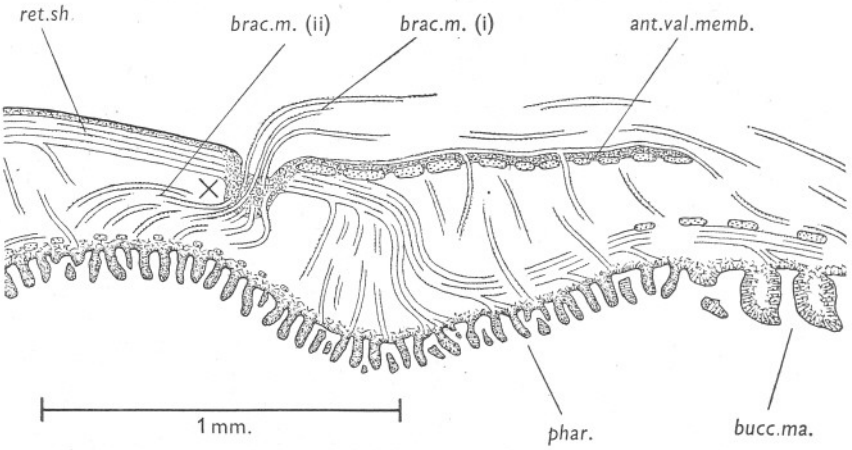
Text-fig. 12. Sections through the extruded proboscis of *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). The position of *X* is the same as in Text-fig. 11. Lettering as on p. 28.

flow of fluid out of the oral end of the paraoesophageal cavity. The positions assumed by the various components in the extruded proboscis are shown in Text-fig. 12*E*.

In *claparedii* the bulk of the retractor sheath musculature crosses to the gut lining immediately oral to *X* (Text-fig. 11*C*.). This is the cause of the 'waist-line' seen in the distended pharynx (Text-figs. 3*C*., 12*C*.). The muscle columns constitute a nearly, but not quite, complete partition at this level. On its oral side the tissues of the pharynx are very loose, with extensive interstitial spaces; they are cut off from the head coelome by a delicate but imperforate membrane, presumably peritoneal, which is bound to the gut lining by radial strands, and extends forwards as far as the junction of

pharynx and buccal mass. Here the tissues are tightly bound together, so there is little or no possibility of a forward escape of fluid from the paraoesophageal cavity.

It is in *marina* that the most remarkable specializations of this region are found. The breakdown of the buccal peritoneum extends backwards as far as *X*, and the wall of the oral half of the pharynx is greatly modified to form a structure which will be termed the anterior valve (Text-fig. 13). The most characteristic feature of the valve is the anterior valve membrane (*ant.val.memb.*): this is a continuous membrane encircling the gut and ending anteriorly in a sharp but rather irregular boundary at the level where the pharynx meets the buccal mass. It consists of the following layers: (i) a very thin layer of longitudinal muscle, on the side towards the head coelome;

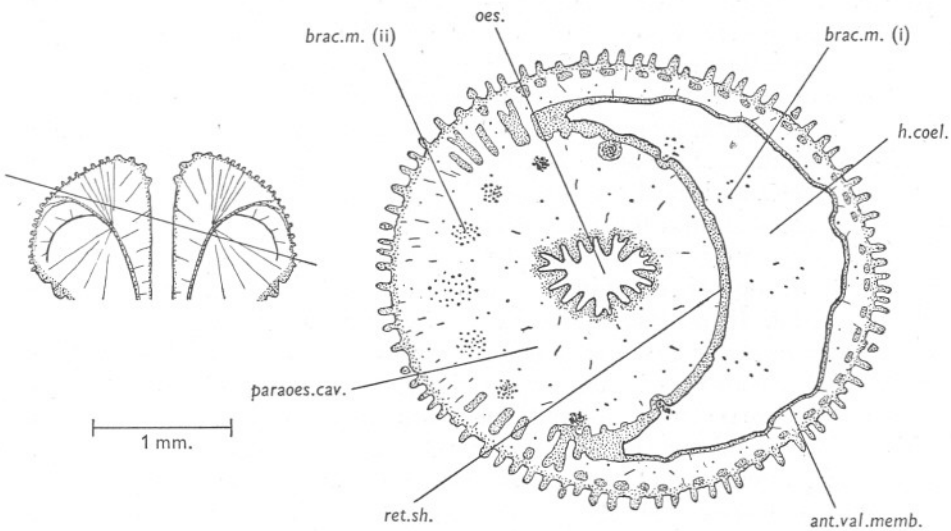


Text-fig. 13. Longitudinal section through the anterior valve of *marina*. Proboscis withdrawn. Lettering as on p. 28.

(ii) connective tissue; (iii) circular muscle, on the side towards the gut lining. The anterior valve membrane is evidently a part of the gut wall which has split away from the rest. Beneath it there lies a clear space crossed by radial strands; then come the remaining layers of the gut wall (more of the circular muscle, then superficial longitudinal muscle, connective tissue and epithelium). The retractor sheath muscles cross this space (to become the superficial longitudinal layer) as a series of discrete, parallel columns. Fluid can easily flow forwards from the oral end of the paraoesophageal cavity, passing between these columns and under the anterior valve membrane, to reach the head coelome. The valve membrane presumably blocks any reverse flow, at least when the proboscis is withdrawn (Pl. IV, fig. 5).

The muscles of the main (deep) longitudinal layer of the buccal mass run backwards towards the valve as a series of roughly parallel strands. Some of them enter the longitudinal layer of the valve membrane at its free margin

(often accompanied by strands of connective tissue) and doubtless serve to hold it in position. Others run outside the membrane—i.e. over its coelomic face—and converge, like pencils of rays in an optical diagram, to focus on certain points which are evenly spaced round the periphery of the valve at its level of junction with the retractor sheath; these strands will be termed the oral bracer muscles (Text-figs. 12–14, *brac.m.* (i)). At the points on which these strands are focused, the retractor sheath muscles are separated by dense plugs of connective tissue, which are generally pulled rather inwards towards the gut lining. One of the plugs is shown in section in Text-fig. 13. A second series of muscle strands, more numerous than the first, radiates from each



Text-fig. 14. Slightly oblique transverse section through the extruded pharynx of *marina*, to show the relations of the bracer muscles. Lettering as on p. 28.

plug to the wall of the aboral half of the pharynx; these will be termed the aboral bracer muscles (*brac.m.* (ii)). Some of the aboral bracer muscles are direct continuations of the oral ones, but others arise in the plug. The main function of these remarkable muscle strands is probably the holding of the parts in position when the proboscis is extruded (Text-figs. 12, *M.*; 14).

The configuration of the anterior valve in the distended proboscis is shown in Text-fig. 12, *M.* and Pl. III. There are wide gaps between the parallel retractor muscle columns as they cross from the base of the anterior valve membrane to the gut lining, and also between the margin of the valve membrane and the buccal mass. No obstacle is presented to a flow of fluid from paraoesophageal cavity to head coelome—and possibly none to a reverse flow in the fully distended proboscis.

Functional Evolution in the Proboscis Apparatus

The structural differences between the species are great. The following discussion attempts to explain them in terms of function.

We may assume (i) that the retractor sheath is relaxed at the moment of extrusion, and (ii) that the proboscis is driven out, largely at least, by a forward flow of body fluid. Such a flow could be produced in two ways. The gular membrane could contract, pressing on the fluid in the paraoesophageal cavity—and the body wall of the hinder segments could contract, pressing on the fluid in the general coelome. Contraction of the gular membrane would cause a forward flow in the paraoesophageal cavity, while that of the body wall would have several effects. Thus the coelomic fluid could displace the gular membrane forwards, or enter the paraoesophageal cavity through the dorsal valve (and the ventral valve, in *marina*), or enter the head coelome through the ventral foramen and such other openings as exist between the decussation line and the body wall. We may note in passing that the structure of the 'second and third diaphragms' is such that they would not seriously obstruct a flow of fluid along the body.

The anatomy of *ecaudata* strongly suggests that in this species the gular membrane is the main agent in extrusion. Its capacity is great and its musculature is strong. Its contraction would cause a forward movement of the point of union of retractor sheath and pharynx, for the tissues at this point are compactly put together and would block the passage of fluid. The proboscis would therefore be pushed towards the mouth. The body wall could reinforce this action by raising the pressure in the general coelome. A swift and forcible injection of fluid from the general coelome into the paraoesophageal cavity through the dorsal valve seems unlikely to occur, because the extrusion of the proboscis is a reversible process, and there is no means by which the injected fluid could rapidly escape again in *ecaudata*. Such assistance as the hinder body wall may give is more probably by the application of pressure to the outside of the gular membrane. The function of the dorsal valve may be analogous to that of a trickle charger—to allow the entry of fluid into the paraoesophageal cavity as a replacement for any which has leaked away through the surrounding membranes during normal proboscis activity.

Other factors may play a part in extrusion. For example, contraction of the longitudinal muscles of the body wall of the head would tend to roll the buccal mass forwards and outwards, owing to the incompressibility of the fluid in the head coelome, if there were no simultaneous backward flow through the ventral foramen. It nevertheless seems, from the anatomical relationships, that a forward movement of fluid produced by contraction of the gular membrane musculature is of major importance in *ecaudata*.

The other two species differ from *ecaudata* in the following important respects: (i) they distend the proboscis into a fuller, more spherical form;

(ii) their buccal papillae are teeth, not suckers; (iii) their gular membranes are less extensive and less powerful; (iv) the only communication between the general coelome and head coelome in *ecaudata* is through the ventral foramen, but the other two species have additional communicating channels (through the dorsal, ventral and anterior valves in *marina*; through the large openings between decussation line and body wall at the levels of the notopodia and neuropodia in *claparedii*); (v) they have lost the buccal peritoneum. The third of these differences suggests that the main responsibility for propulsion has shifted from the gular membrane to the body wall; this conclusion seems certain for *claparedii* and probable for *marina*. The fourth suggests that the point at which the raised pressure is brought to bear has also shifted. In *ecaudata* we supposed a forward movement of fluid in the paraoesophageal cavity, blocked by and therefore displacing the union of retractor sheath and pharynx. In *marina* the development of the anterior valve completely alters the picture; it seems likely that the forwardly moving body fluid would flow through the valve and press on the advancing tip of the buccal mass. In *claparedii* a rise in the pressure could cause a forward displacement of the delicate gular membrane or a flow through the openings between decussation line and body wall; it could therefore be brought to bear both on the pharynx and on the buccal mass. The fifth difference allows the body fluid to press unhindered into the central cavities of the buccal teeth.

These considerations suggest a co-ordinated evolutionary picture. The supersession of the gular membrane by the body wall means that the proboscis machine is now driven by a motor of much greater power and capacity. The shift in the point at which the fluid pressure operates means that the buccal mass can be used more effectively as a rasp.

The above remarks are, however, speculative. Our knowledge of the movements which the living proboscis performs is still very incomplete, and any theories of its mechanism are therefore insecure. There are differences of habitat between the species: *marina* and *claparedii* are chiefly found on sandy and muddy beaches, while *ecaudata* lives among stones and rocks; but whether there are corresponding differences in the uses to which the proboscis is put remains to be seen. Perhaps, when fuller information about the living worms is available, we shall understand why one species has suckers, and another teeth, on the buccal mass, and this may give us the key to their internal modifications.

Summary

The anatomy of the structures responsible for proboscis activity is described in detail in *Arenicola ecaudata* Johnston, *A. claparedii* Levinsen and *A. marina* L.

The proboscis apparatus includes the following components: the proboscis itself (i.e. the eversible part of the gut), the first part of the oesophagus, the

retractor sheath, and the gular membrane ('first septum' or 'first diaphragm' of previous authors). The retractor sheath and gular membrane are two muscular sheets inserted on the body wall at the same level; the retractor sheath runs to the proboscis and the gular membrane to the oesophagus (Text-figs. 1, 2; pp. 3, 6). They divide the space between gut and body wall into three distinct cavities, which communicate with each other at certain special points only, as described in the text. These cavities are the head coelome, in front of the retractor sheath, the paraoesophageal cavity between the two sheets, and the general coelome behind the gular membrane. The paraoesophageal cavity is crossed by numerous fine muscle strands, running from the two sheets to the gut.

The suggestion is made that the retractor sheath and gular membrane are both derived from the first septum. A cleft has appeared in the septum, splitting it into the two sheets; the cleft becomes the paraoesophageal cavity.

The papillae of the buccal mass (the oral end of the proboscis) are sucker-shaped in *ecaudata* but conical teeth in *claparedii* and *marina*. The papillae are hollow, due to the great development of their interstitial spaces. In *claparedii* and *marina*, but not in *ecaudata*, the buccal peritoneum has broken down, so that coelomic fluid in the head coelome can directly enter and distend the papillae.

The gular membrane of *ecaudata* is very extensive and thrown backwards into a pair of large septal pouches latero-ventral to the oesophagus (Text-figs. 5, 6; pp. 13, 14). Its musculature is powerful and consists of two layers, radial and circular; in the other two species the musculature is radial only. Appearances suggest that the contraction of the gular membrane plays a major part in proboscis extrusion in *ecaudata*. Such contraction would cause a forward movement of fluid in the paraoesophageal cavity; this would press against and displace the junction of retractor sheath and proboscis and so drive the proboscis forwards.

The gular membrane of *claparedii* is extremely delicate and lacks septal pouches; it appears to be a vestigial organ (Text-figs. 7, 8; pp. 16, 17). Proboscis extrusion in this species is probably due to a forward movement of fluid driven by the muscles of the body wall. This fluid could displace the gular membrane and so cause a forward pressure on the junction of retractor sheath and proboscis. It could also flow into the head coelome, through five wide openings between the body wall and the junction of gular membrane and retractor sheath, and so bring pressure to bear on the teeth of the emerging buccal mass.

The gular membrane of *marina* is intermediate in strength between that of *ecaudata* and that of *claparedii*; it has a pair of small septal pouches (Text-figs. 9, 10; pp. 18, 19). The proboscis is probably driven out partly by the gular membrane and partly by the body wall. Fluid can pass from the general coelome into the paraoesophageal cavity through two openings in the median

plane, the dorsal and ventral valves. The junction of retractor sheath and proboscis is elaborately modified in *marina* to form the anterior valve, through which fluid can flow from the paraoesophageal cavity into the head coelome (Text-fig. 13, p. 22). These modifications probably mean that the main pressure of the forwardly moving body fluid is brought to bear on the teeth of the advancing buccal mass.

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EXPLANATION OF PLATES

PLATE I

Lateral views of the anterior ends of three artificially distended specimens, to show how the form of the proboscis differs from species to species.

Fig. 1. *Arenicola ecaudata*. The hollow to the left is due to caving-in of part of the pharynx after preservation. The mouth is just beyond the left-hand boundary. The buccal mass and transition zone are fully exposed.

Fig. 2. *A. claparedii* (Canadian specimen). The buccal mass is concealed by the pharynx, but the transition zone is clearly seen on the right of the latter.

Fig. 3. *A. marina*. Part of the buccal mass is seen ventrally, in a rather abnormal position due to the distension.

PLATE II

Figs. 1, 2. Lateral views of the anterior ends of relaxed specimens of *A. ecaudata* (fig. 1) and *A. marina* (fig. 2). The buccal mass is partly extruded in each. Note the contrast in form of the buccal papillae.

Fig. 3. Dorsal half of a horizontally bisected specimen of *A. marina*. Proboscis withdrawn. Compare Text-figs. 1 and 9, pp. 3 and 18.

Fig. 4. Horizontal section through the anterior segments of a 1·5 mm. post-larval *A. cristata*. Lettering as on p. 28.

PLATE III

Dissection from the right side of a distended specimen of *Arenicola marina* (same specimen as in Pl. I, fig. 3). An explanatory drawing is placed below the photograph. The dissection has been carried nearly, but not quite, to the median plane. The anterior part of the oesophagus and the tip of the dorsal valve have been removed altogether, to show the

internal structure of the proboscis; then follows an area over which the right lateral wall of the oesophagus has been removed (cross shading in the drawing). The ventral edge of the left septal pouch is visible in the angle between ventral vessel, ventral mesentery and gular membrane (dark stippling in the drawing). Lettering as below. Compare Text-figs. 10 and 12, *M.*, pp. 19 and 21.

PLATE IV

Figs. 1, 2. Longitudinal sections through single buccal papillae of *A. ecaudata* (fig. 1) and *marina* (fig. 2). Both worms are relaxed, with the proboscis withdrawn. The buccal papilla of *ecaudata* is sucker-shaped, with the top depressed; its internal musculature is clearly shown. That of *marina* is tooth-shaped (oral end to the left); a small blood vessel is entering the base of the papilla and there is another in the tip of the 'tooth'.

Figs. 3, 4. Transverse sections including the ventral body wall (below) and the ventral wall of the buccal mass (above) in *ecaudata* (fig. 3) and *marina* (fig. 4). The two sections are as nearly as possible from the same position, i.e. just in front of the bifurcation of the ventral nerve cord. The sucker-shape of the buccal papillae of *ecaudata* is again obvious. The two folds of the buccal membrane of *ecaudata* are seen as thick, rather wavy lines crossing the middle of fig. 3; the narrow space between them is the head coelome (compare Text-figs. 5 and 6, pp. 13, 14). In *marina*, there is no barrier between the head coelome and the interstitial cavities of the buccal papillae.

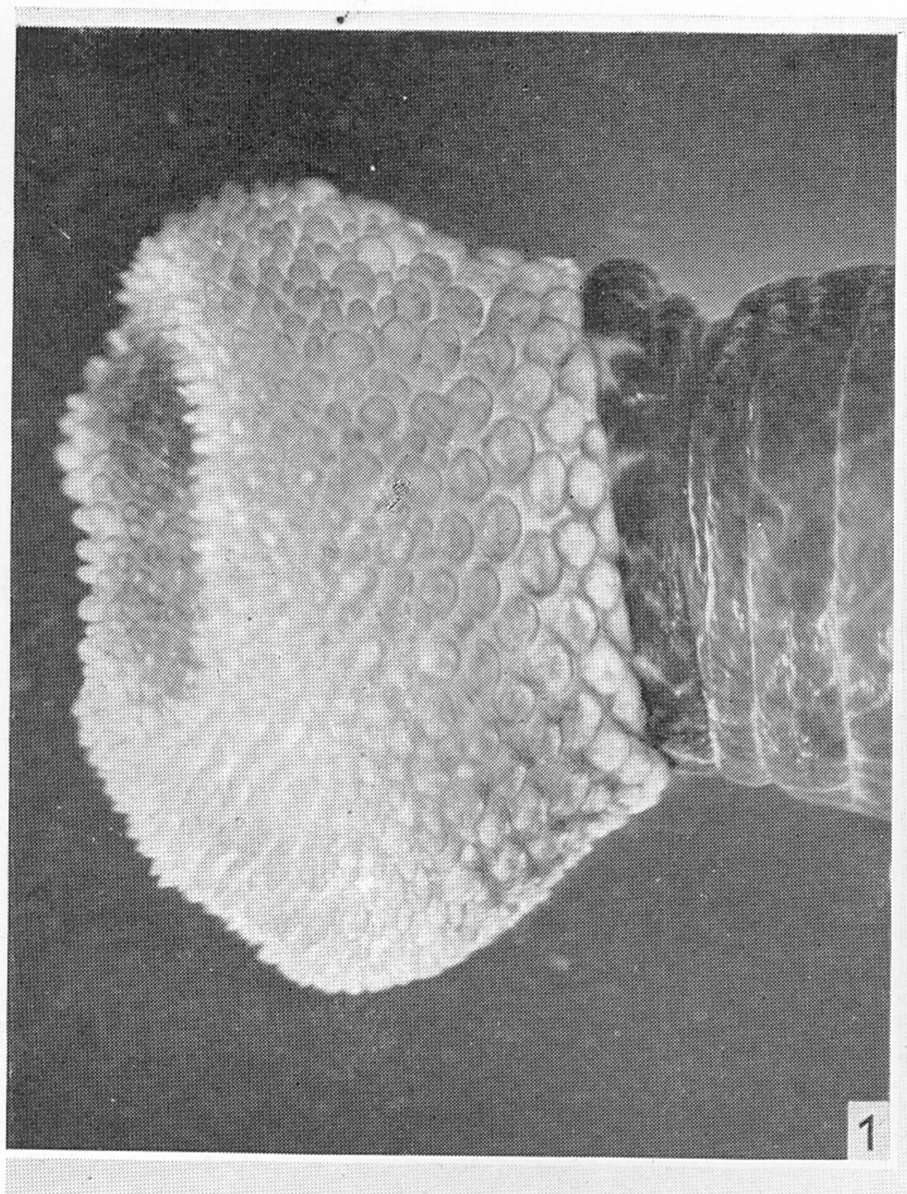
Fig. 5. Longitudinal section through the anterior valve of *marina*. Relaxed specimen; proboscis withdrawn; oral end to the right. The section corresponds to Text-fig. 13, p. 22, except that it does not happen to pass through a bracer muscle perforation. The pale grey object on the upper margin of the photograph, about half-way along, is a longitudinally-running blood vessel; a group of bracer muscle strands can be seen just below it.

Pl. II, fig. 3, was taken by the late Mr F. J. Pittock, and is reproduced from the *Journal of Experimental Biology*, Vol. 14, 1937, by kind permission of the Company of Biologists Ltd.

The other photographs were taken for this work by the following: Pl. I, by Miss J. Hubbard; Pl. II, figs. 1 and 2, and Pl. III, by Mr J. Armstrong; Pl. II, fig. 4, and Pl. IV, by Mr W. Brackenbury.

LIST OF ABBREVIATIONS USED IN THE TEXT-FIGURES AND PLATES

ant.val.memb.	anterior valve membrane	*metast.m.*	metastomial muscle
br.	brain	*n.c.*	nerve cord
brac.m. (i)	oral bracer muscles	*nuch.gr.*	nuchal groove
brac.m. (ii)	aboral bracer muscles	*nuch.p.*	nuchal pouch
bucc.ma.	buccal mass	*oes.*	oesophagus
bucc.memb.	buccal membrane	*ot.gr.*	otic groove
Ch.	chaetigerous annulus	*parabucc.cav.*	parabuccal cavity
circ.m.	circular muscle	*paraoes.cav.*	paraoesophageal cavity
conn.	connective nerve	*phar.*	pharynx
D.	diaphragm	*post-phar.*	post-pharynx
D.vest.	diaphragm which becomes vestigial	*prost.*	prostomium
		ret.m.nuch.p.	retractor muscle of the nuchal pouch
dors.mes.	dorsal mesentery		
dors.v.	dorsal vessel	*ret.sh.*	retractor sheath
dors.val.	dorsal valve	*sup.long.m.*	superficial longitudinal muscle
ep. & c.t.	epithelium and connective tissue	*vent.for.*	ventral foramen
		vent.mes.	ventral mesentery
gen.coel.	general coelome	*vent.v.*	ventral vessel
gul.memb.	gular membrane	*vent.val.*	ventral valve
h.coel.	head coelome		
long.m.	longitudinal muscle		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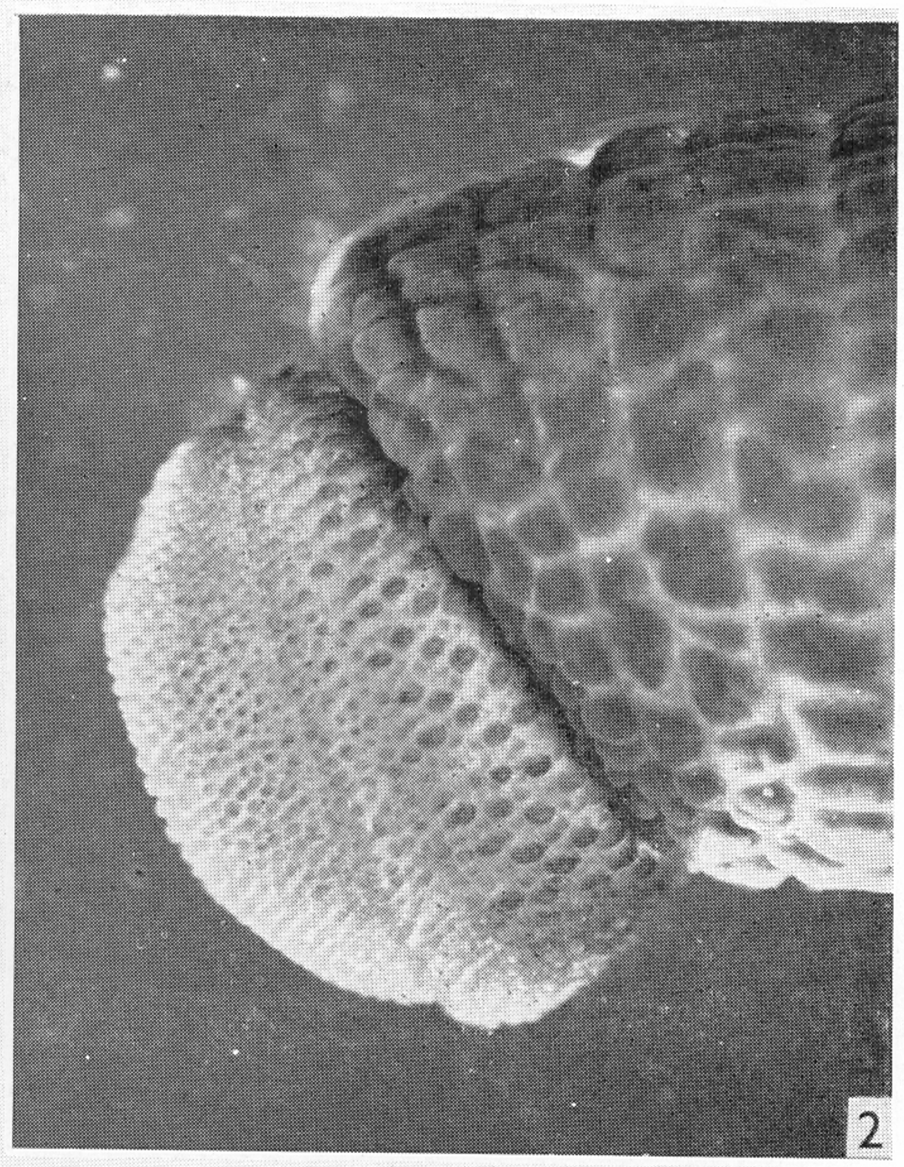
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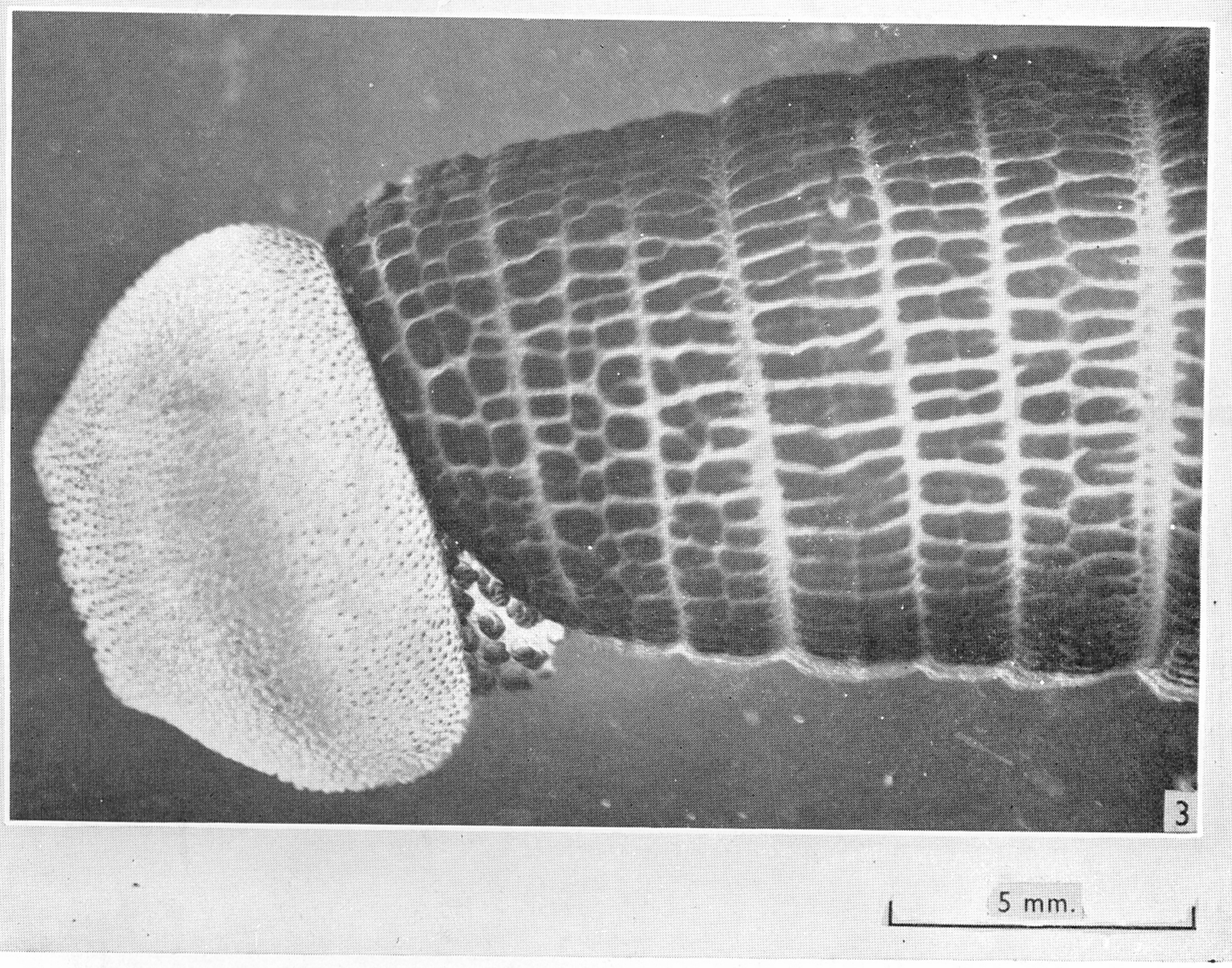
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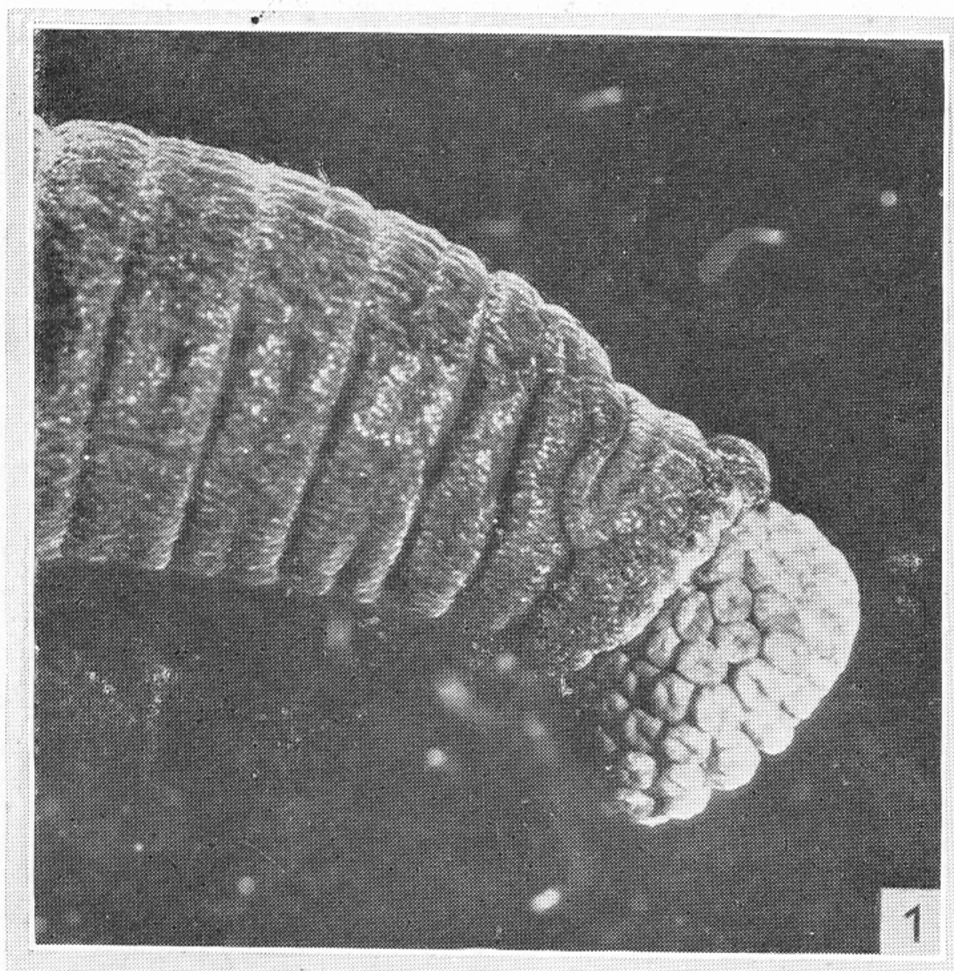

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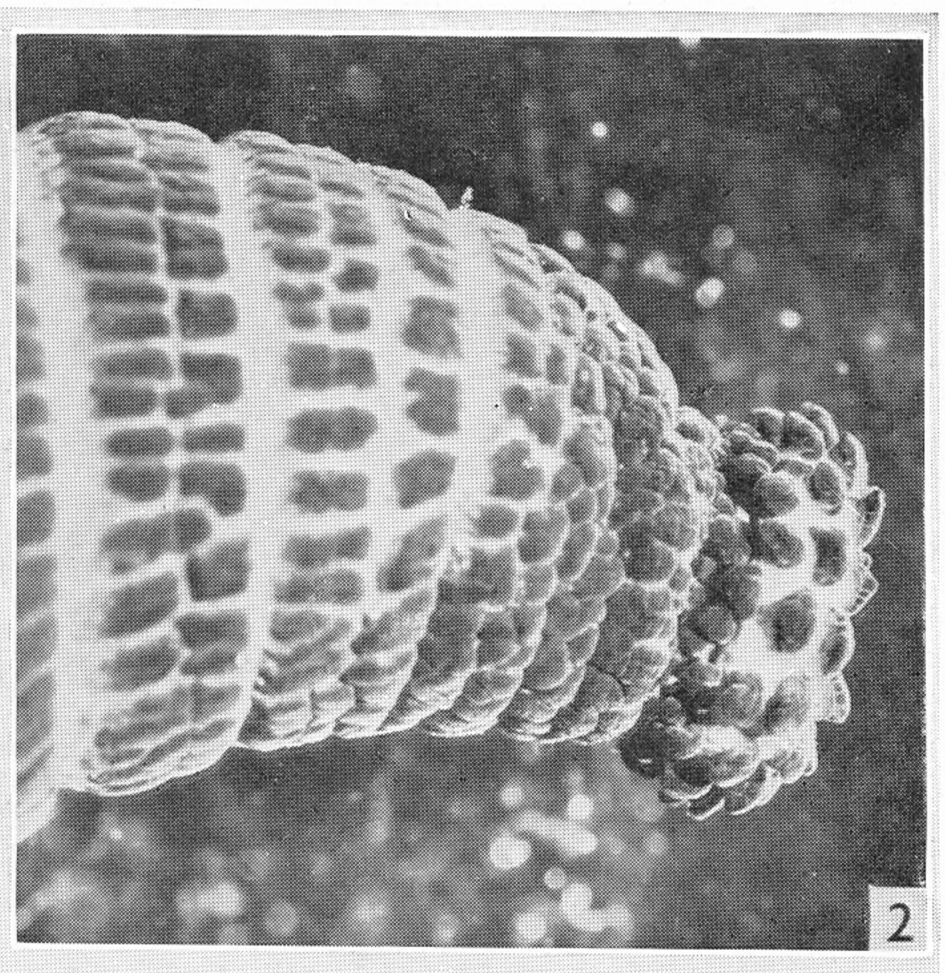

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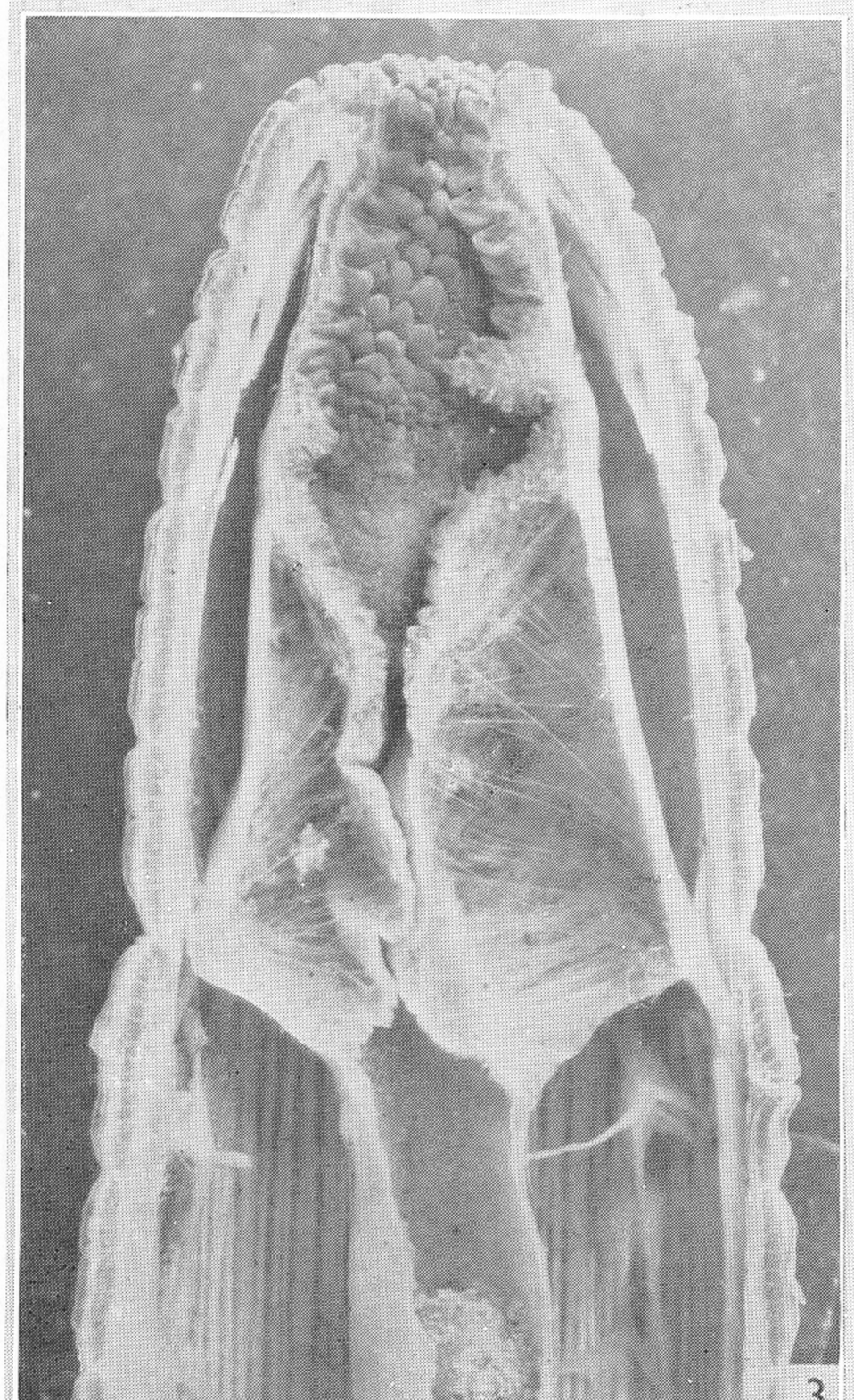

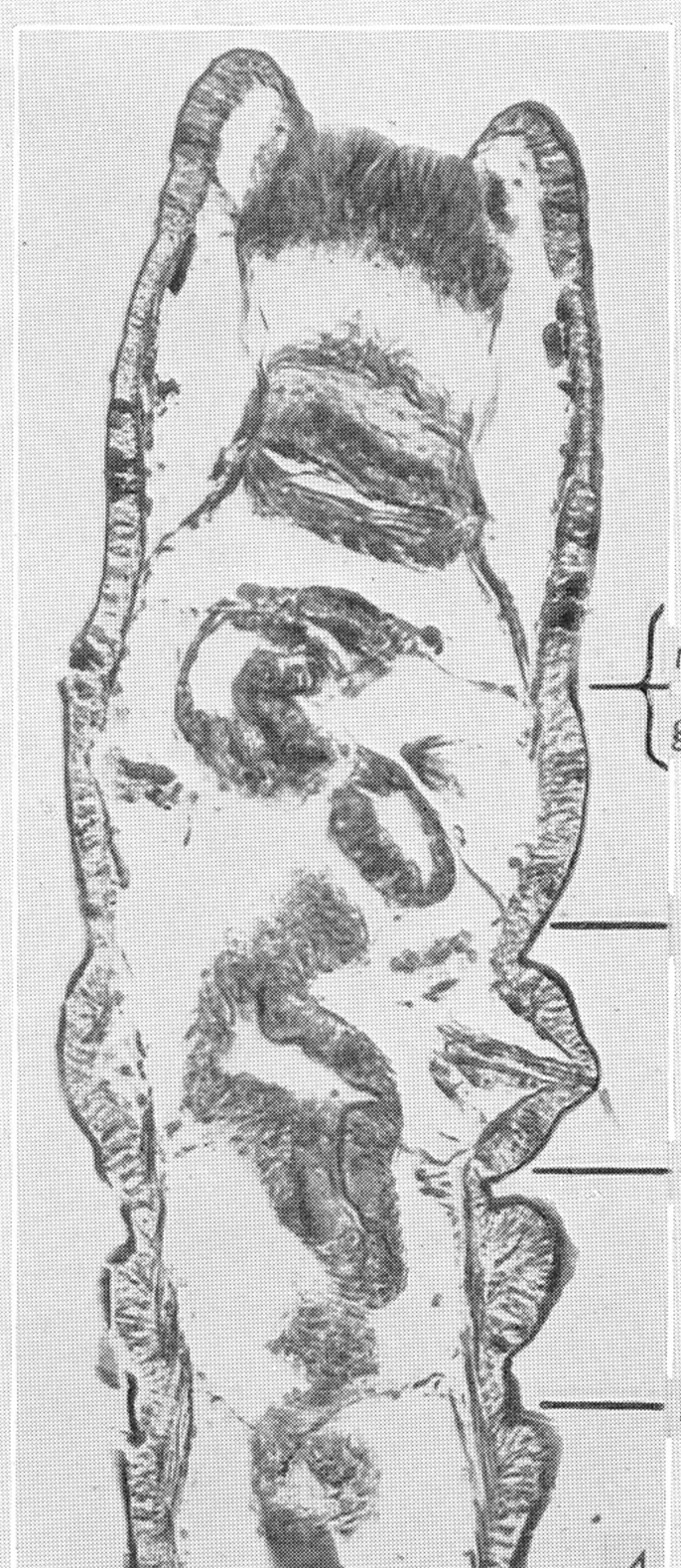

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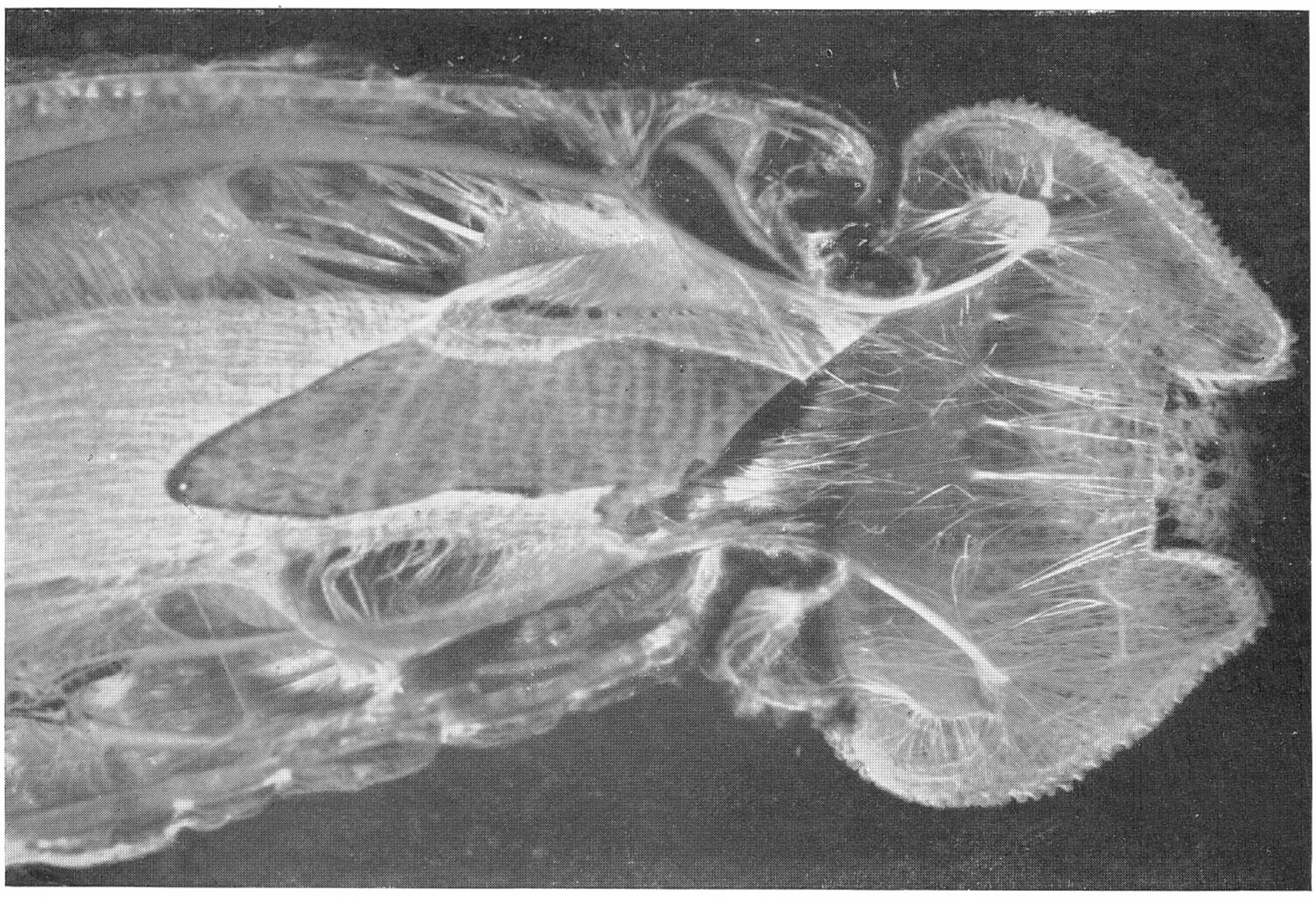

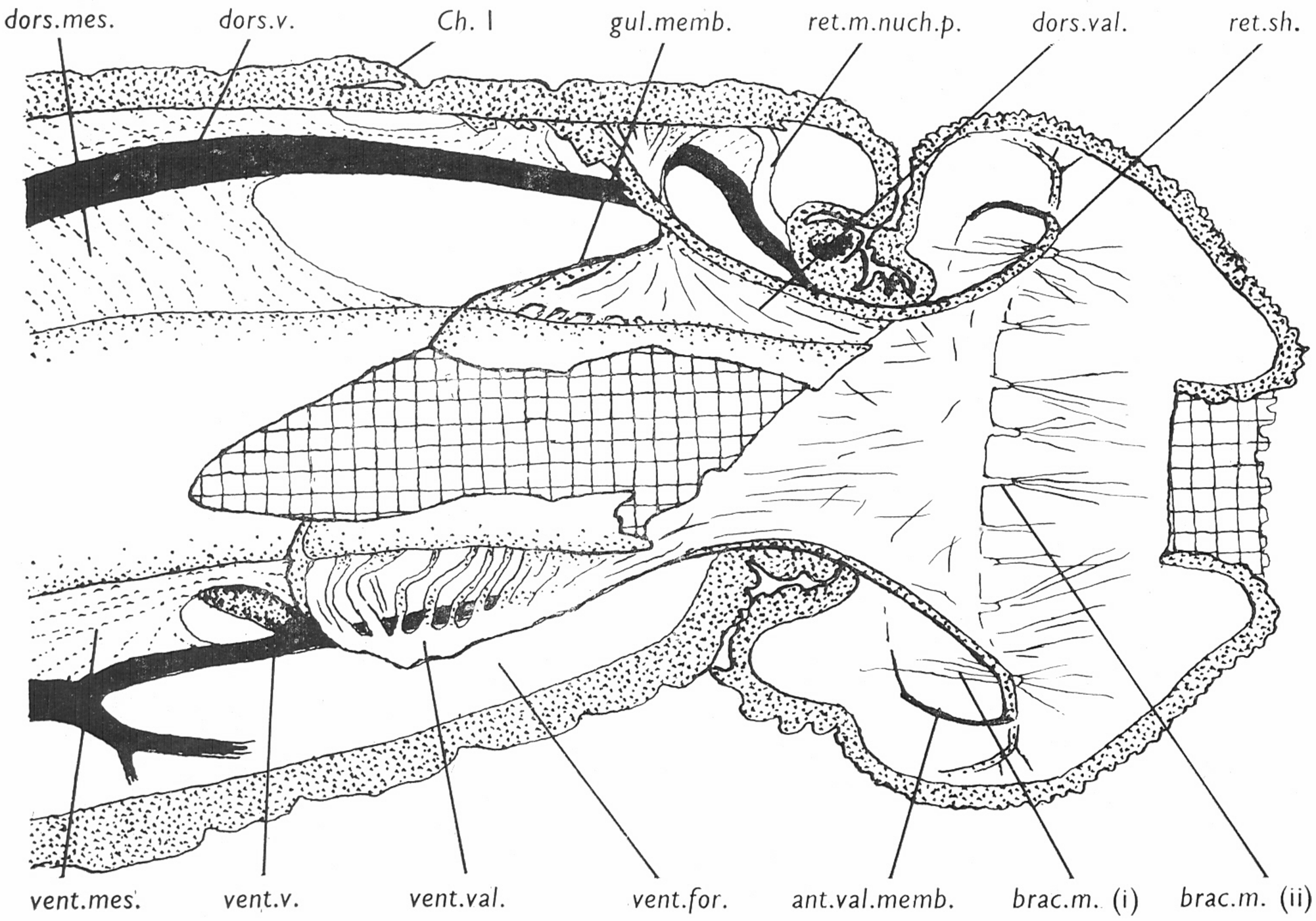
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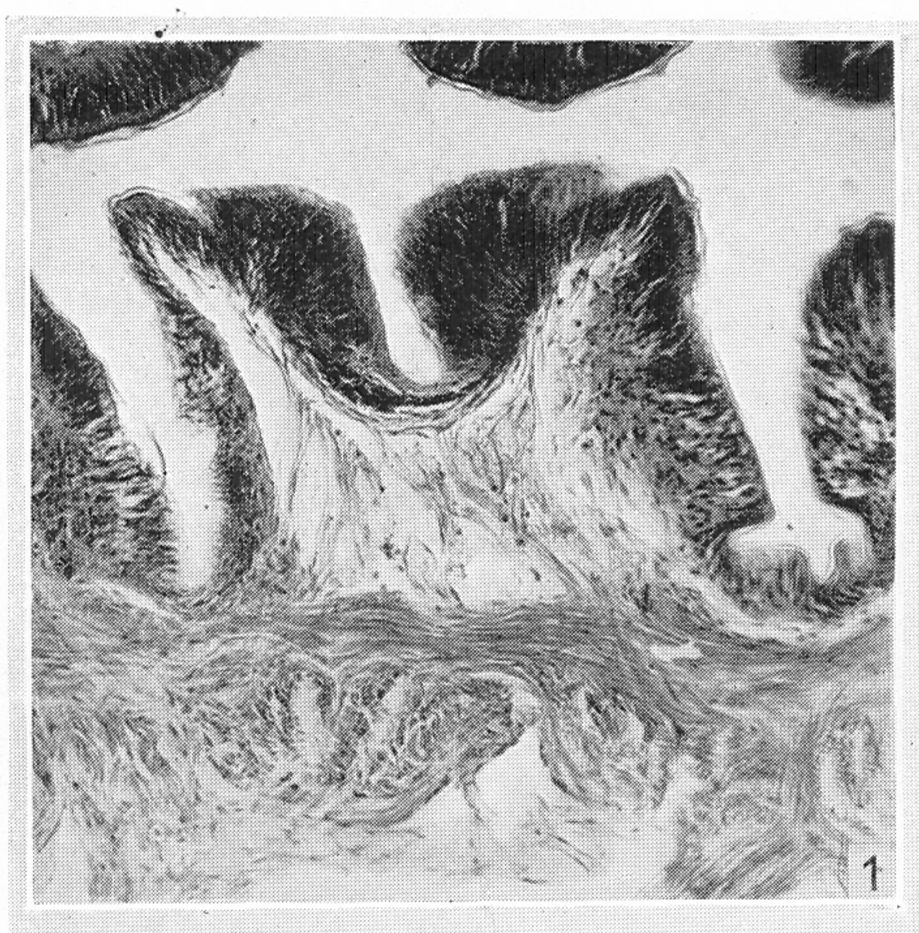

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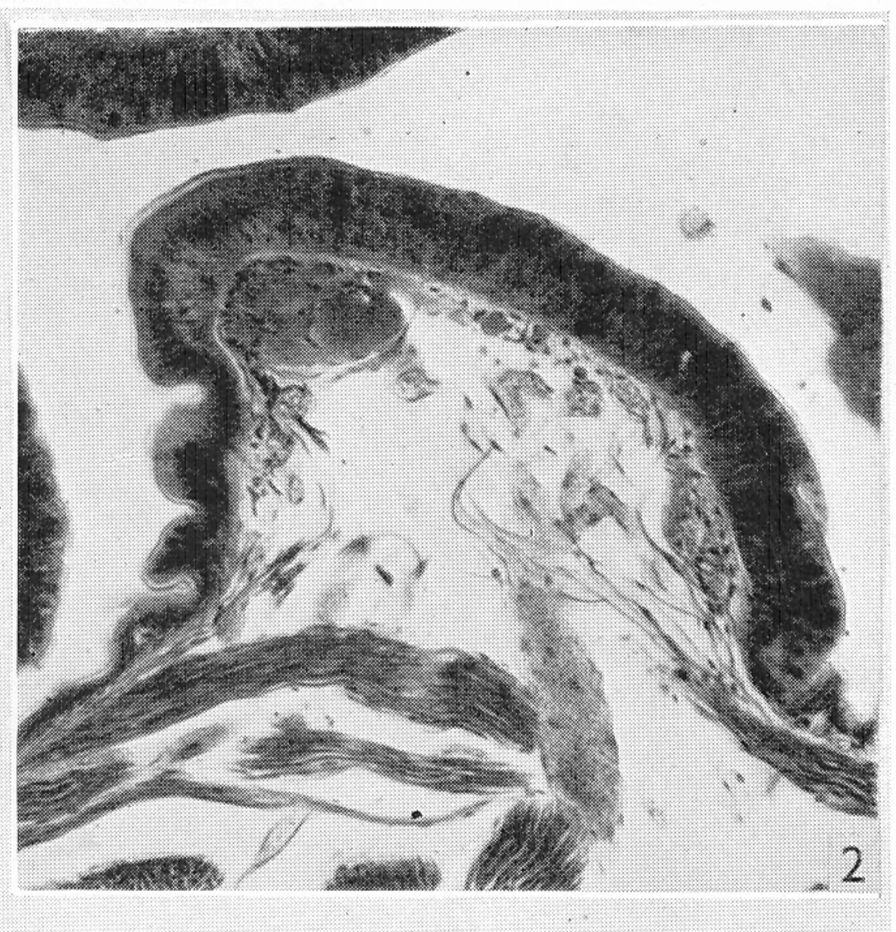

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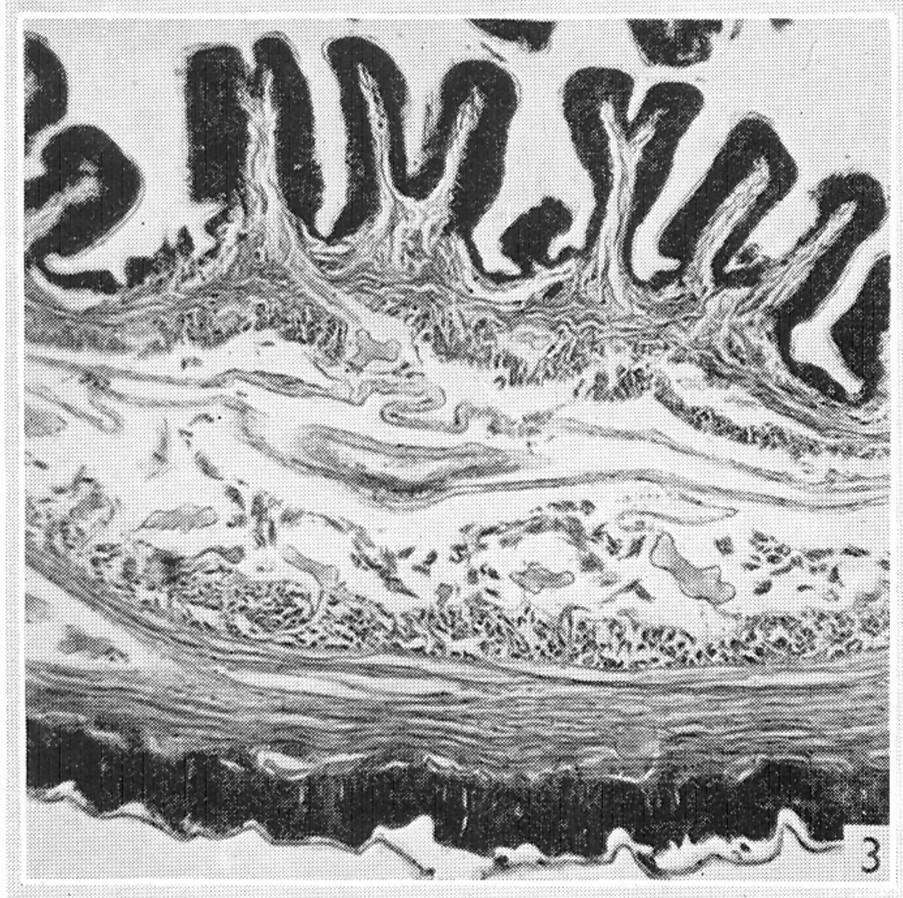

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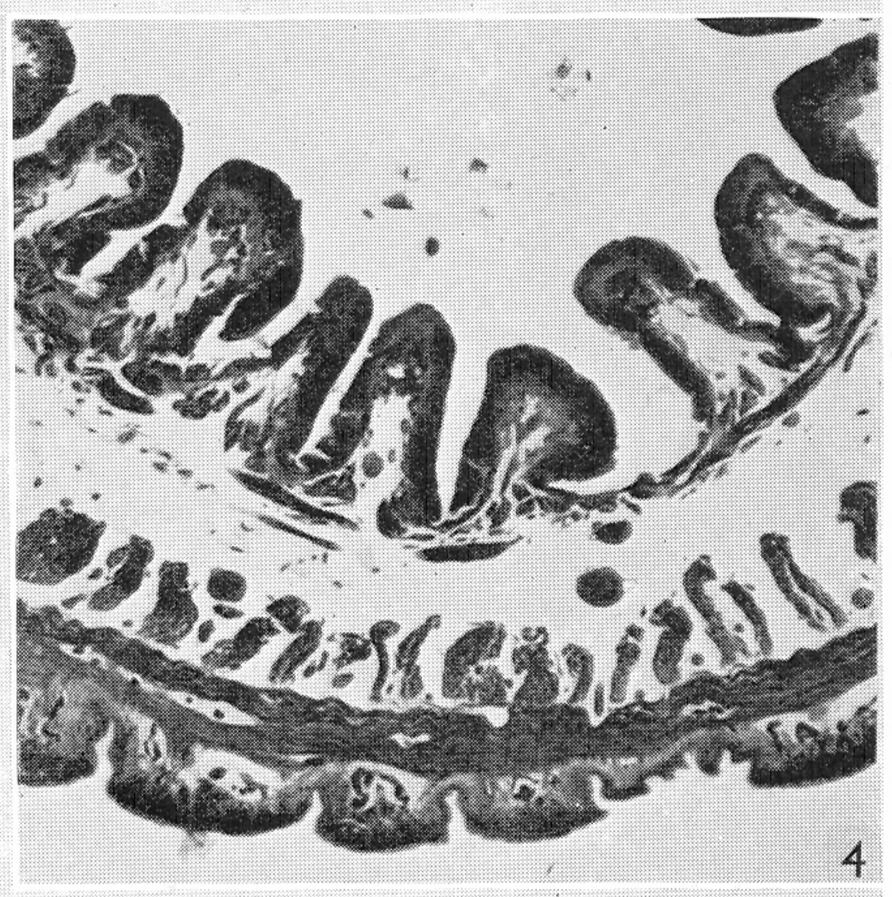

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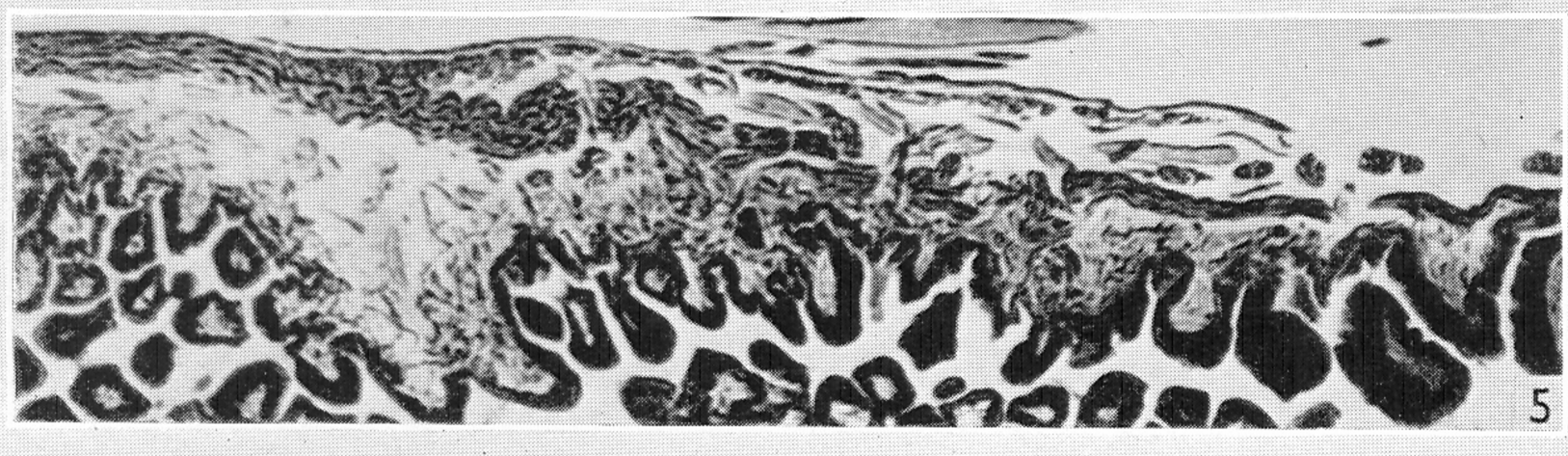

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VARIATIONS IN CHEMICAL COMPOSITION DURING THE DEVELOPMENT OF *HIMANTHALIA ELONGATA* (L.) S. F. GRAY

By Betty Moss
King's College, Newcastle-upon-Tyne

(Text-figs. 1, 2)

Himanthalia elongata (=*H. lorea* (L.) Lyngb.), although a common brown seaweed on parts of the British coast, does not occur in sufficient quantities to render it of commercial importance. As a result, the work of Colin & Ricard (1930) appears to be the only investigation on the chemical composition of this species. Their material was collected in August 1929, but they give no indication of the size or condition of the plants which were analysed.

Seasonal variations in the chemical composition of other brown seaweeds have been studied by various workers (e.g. Lapicque, 1919; Lunde, 1940; Black, 1948*a*, *b*, 1949, 1950*a*). Also Black (1950*b*) has considered the influence of habitat and depth of immersion on the composition of three species of *Laminaria*. In these investigations no indication is given as to whether young or old plants, or sterile or fertile plants, were analysed.

After it had been shown that marked changes in chemical composition were associated with the development of the fertile receptacles in *Fucus vesiculosus* (Moss, 1950), it appeared that different growth stages of the same species collected in any one season might show variations as great as the seasonal ranges which had been reported for other species. *Himanthalia elongata* collected from two habitats, one on the west coast of Scotland and the other on the north-east coast of England, was selected for this study.

Collection of Material

One set of material was obtained from Clachan Sound, where a narrow arm of the Atlantic Ocean separates the Island of Seil from the mainland of Argyllshire. The water here is shallow, with a swift current running through, and *Himanthalia* is abundant growing amongst *Laminaria digitata* and up into the succeeding zone of *Fucus serratus*.

Collections were made on three successive days in April 1949, at low-water spring tides, when the plants were never exposed completely. Fig. 1 shows the stages into which they were sorted for chemical analysis:

Stage 1. Young sporelings up to $\frac{1}{2}$ in. in length.

Stage 2. Young sporelings more than $\frac{1}{2}$ in. in length but still with a tubular structure and no flattening of the apex.

Stage 3. Young plants with flattened apices.
Stage 4. Fully grown vegetative plants with the typical button form.
Stage 5. Buttons with young thongs (i.e. fertile receptacles) up to 10 in. in length.
Stage 6. Buttons with thongs 5–7 ft. in length.
Stage 7. Old buttons with spent receptacles which persist several months after gamete extrusion.

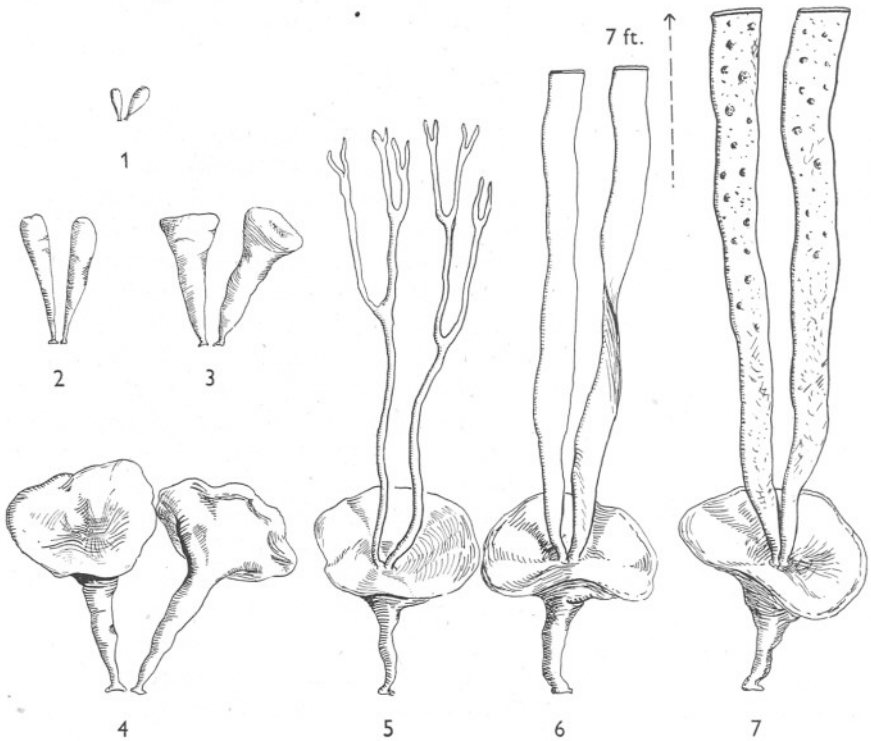

Fig. 1. Stages in the growth of *Himanthalia elongata* which were used in chemical analysis.

In the latter three stages, 5, 6 and 7, the vegetative buttons were cut from the fertile thongs and each part analysed separately, so that variations in chemical composition throughout the development of both the vegetative and fertile tissues could be studied independently.

The following April a similar series of samples were collected from St Mary's Island, Northumberland, where *Himanthalia* grows on exposed ledges, and not in association with *Laminaria* and *Fucus* as in Clachan Sound. Collections were made as the tide receded, and the plants were sorted into similar stages for analysis. However, similar developmental stages of these plants from St Mary's Island were much smaller in size than comparable stages from Clachan Sound.

The analytical methods used are those developed by the Institute of Seaweed Research (Black, 1948*a*; Cameron, Ross & Percival, 1948).

Results

Dry Weight. The percentages of dry matter recorded in Fig. 2A show that an increase occurs from the sporelings to the mature plants from both habitats, but the plants from St Mary's Island always give higher values. A marked increase in dry weight occurs in the old thongs when they become hard and leathery after gamete extrusion. Such an increase was not observed in *Fucus vesiculosus* (Moss, 1950), where the receptacles disintegrate rapidly.

Mineral Ash. Fig. 2B shows the percentage total mineral ash on the anhydrous basis in plants from both habitats, while Fig. 2C gives the water soluble and water insoluble constituents of the ash in plants from Clachan Sound only.

The higher percentage dry matter of plants from St Mary's Island is associated with a lower ash content. The young sporelings from Clachan Sound have an extremely high percentage of mineral ash, 64 %, calculated on the anhydrous basis. This appears to be a higher value than any recorded for a brown seaweed. Black (1948*a*) drew attention to the high percentage of ash, 55 %, in stipes of *Saccorhiza polyschides* (Lightf.) Batt. (= *S. bulbosa* La Pyl.) collected in July 1946. The percentage dry weight of this particular sample was lower than at any other season. In a similar manner, the highest percentage ash is found in the sporelings of *Himanthalia* when their percentage dry matter is lowest.

Crude Proteins. As Fig. 2D shows, there is a gradual increase in crude proteins during the development of the vegetative buttons. The young thongs give high values, but after gamete extrusion there is a marked decrease, until the protein content of the old thongs approximates to that of the senescent buttons.

It is interesting to note that the range in crude proteins from 6·8 to 14·3 % of the dry matter of plants from Clachan Sound is identical with the seasonal range which has been published for *Laminaria digitata* collected from the same habitat (Black 1948*a*).

Mannitol and Laminarin. Fig. 2E shows the percentage mannitol, calculated on the anhydrous basis, in plants from both habitats. There is a marked decrease in the mannitol content of the vegetative buttons as the young thongs begin to grow.

Laminarin was present in very small quantities (less than 2 %) in all samples. The low values of both mannitol and laminarin may be related to the time of the year when the plants were collected, for it has been shown

(Black, 1948*a*, *b*; 1949) that in both *Laminaria* and *Fucus* these substances are lowest during the early part of the year.

Alginic Acid. Variations in the percentage alginic acid calculated on the anhydrous basis are shown in Fig. 2F. This substance is very low in the young

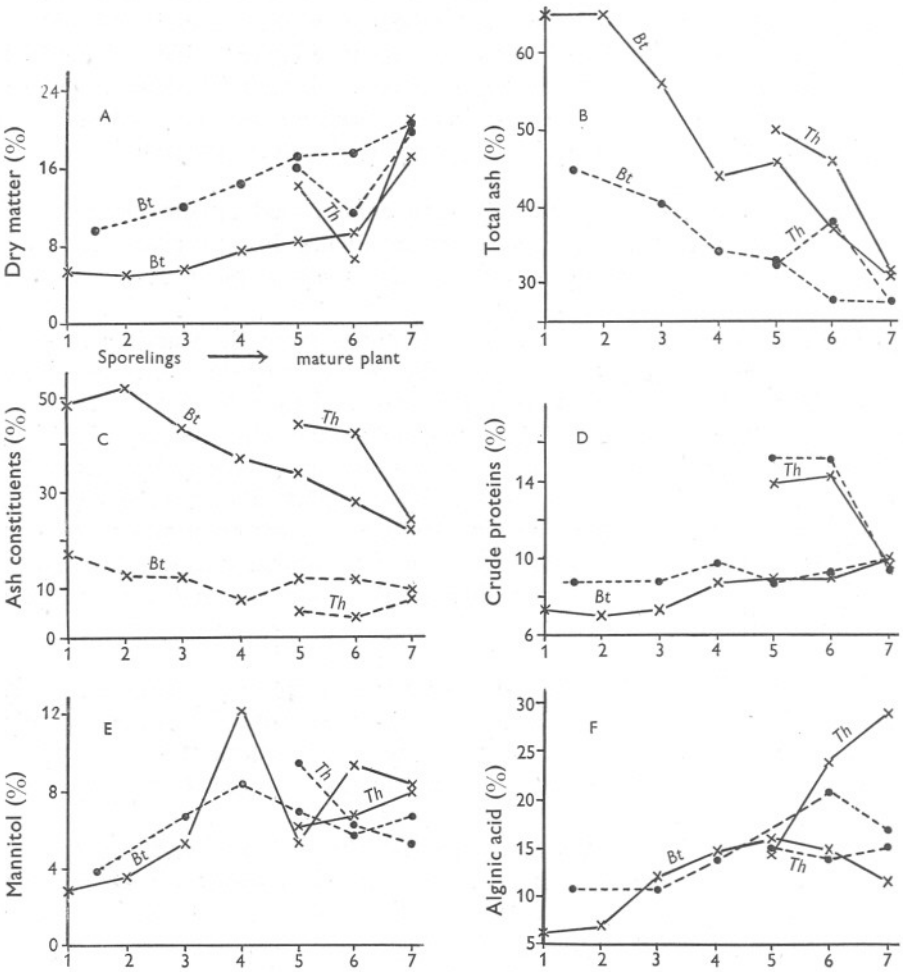

Fig. 2. Variation in chemical composition during development of *Himanthalia elongata*, expressed as percentages of weight. A, dry weight; B, total mineral ash (dry basis); C, water soluble (×——×) and water insoluble (× - - - - ×) constituents of the mineral ash (dry basis), of plants from Clachan Sound; D, crude proteins (dry basis); E, mannitol (dry basis); F, alginic acid (dry basis). *Bt*, buttons; *Th*, thongs; ×——×, plants from Clachan Sound; •- - - -•, plants from St Mary's Island.

sporelings, especially in those from Clachan Sound. The high value in the very long thongs from this habitat was not recorded in the shorter thongs from St Mary's Island.

Discussion

The young sporelings of *Himanthalia elongata*, with their very low percentage of dry matter, are also characterized by a very high mineral ash and low organic content. These young plants are growing rapidly, and as differentiation proceeds so does the percentage of organic constituents increase. The completion of vegetative activity is then followed by the rapid development of the fertile tissues or thongs. Apart from the noted decrease in mannitol, the growth of the thongs does not influence to any great extent the chemical composition of the buttons, suggesting that there is little or no storage of materials for translocation to the developing thongs.

The young thongs, being lower in mineral ash content and very much higher in proteins, mannitol and alginic acid, have a form of metabolism, as shown by their chemical composition, which differs considerably from that of young vegetative tissues developing at the same season.

While the curves obtained for plants from St Mary's Island follow the same trends as those of plants from Clachan Sound, nevertheless they do show higher percentage dry weights and lower mineral ash contents.

These analyses have shown that during the development of plants of *H. elongata* there may be a range in dry weight of 15 %, in mineral ash of 35 %, in proteins of 7 % and in alginic acid of 20 %. This emphasizes that in *Himanthalia*, as possibly in other brown seaweeds, the stage of development of the plant to be analysed is a major factor to be considered in relation to any variations in chemical composition.

Summary

Chemical analyses have been made of different stages in the growth of *Himanthalia elongata*, which have been collected at the same season from two habitats.

During the development of the vegetative buttons increases in the percentage dry weights, mannitol, crude proteins and alginic acid are found, while mineral ash decreases. The young fertile tissues or thongs are rich in proteins and alginic acid compared with the buttons which produce them.

Smaller plants from St Mary's Island have higher percentage dry weights and lower ash content, while their thongs do not show the high alginic acid content found in larger plants of a similar developmental stage from Clachan Sound.

The range in chemical constituents shown in different growth stages of the same species, collected at the same time from the same habitat, stresses the importance of describing the plant which is analysed when the effects of seasonal or other factors upon the chemical composition of brown seaweeds are being considered.

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THE BIOLOGY OF *ASTERIAS RUBENS* L. IV. VARIATION IN THE SEX RATIO

By H. G. Vevers, M.A., D.Phil.

Zoologist at the Plymouth Laboratory

(Text-fig. 1)

There are few records in the literature of the relative proportions of the sexes in natural populations of echinoderms, and the samples studied have mostly been small. Selenka (1867) has stated that in Holothurians in general males are rarer than females, and Becher (1907) found that males were very rare in *Rhabdomolgus ruber*. Koehler (1907) noted that in the ophiuroid *Ophiacantha vivipara* there were more females than males.

Among the echinoids records of sex ratios are more numerous, but the samples used were mostly taken over a short period of the year. In a group of *Centechinus setosus* from Suez there were 400 males and 370 females, and in two samples of *Strongylocentrotus lividus* from Alexandria there were 291 males : 259 females and 238 males : 266 females (Fox, 1924). In a sample of 358 *Echinocardium cordatum* from Port Erin, Moore (1935) found 181 males : 177 females. Ikeda (1931) has recorded a sex ratio approaching equality (100 : 101·8) in the Japanese echinoid *Temnopleurus toreumaticus*. The most valuable contribution to these records is the work of Neefs (1938) on *Strongylocentrotus lividus* from Roscoff. She found that the sex ratio in this species varied from month to month. The percentages of females in her monthly samples, taken over more than one year, were: January, 26·5 and 1; February, 44; March, 45·5; April, 47 and 57; May, 40 and 50; June, 41 and 53; July, 46; August, 46 and 51; September, 62 and 56; October, 62; November, 53, 50·5 and 51; December, 41 and 45. These figures show clearly a preponderance of females in September, October and November. The reason for these fluctuations is not clear although it is possible that they may be connected with the hermaphroditism found in this species (Neefs, 1937).

Pelseneer (1926, p. 157) found that in a sample of 400 *Asterias rubens* taken at Wimereux in summer 1924 there were 184 males and 216 females.

Sex Ratios in Medium and Large Starfishes

In a recent study of large numbers of *Asterias rubens* from four populations in the Plymouth area samples taken throughout the year have yielded further data on the sex ratio in this species. An account of the growth, reproduction and situation of these populations has already been given (Vevers, 1949), and additional material has been collected since then.

The percentage of females in each population, as given in Table I, has been calculated from the total number of medium and large starfishes of each sex caught by otter trawl on the grounds at all times of the year, during the period 1947–50.

It has already been shown that the mean body size of *A. rubens* caught by the otter trawl is larger at E1 and in the Outer Grounds than it is in the Rame-Eddystone Grounds and Plymouth Sound (Vevers, 1949). It will be seen from Table I that populations with larger starfishes have an excess of females over males (Outer Grounds and E1 area). In populations with medium-sized starfishes, as in Plymouth Sound and the Rame-Eddystone Grounds, the sexes are almost equal in number with a slight preponderance of females.

TABLE I

	Mean radius (cm.)	Percentage of females in population	No. of samples	Total in all samples
Rame mud (Agassiz trawl)	3·11	45·6	2	187
Plymouth Sound	9·40	53·6	5	266
Rame-Eddystone Grounds	10·48	51·3	23	1066
Outer Grounds	15·47	55·1	32	2337
E1 area	19·61	70·3	6	336

TABLE II. ANALYSIS OF HAULS OF *ASTERIAS RUBENS* ON RAME MUD IN 1949

(Measurements in centimetres.)

		Males			Females			Percentage of females in sample
Date	Total in catch	No.	Range	Mean	No.	Range	Mean	
12. v. 49	103	57	1–5·5	3·15	46	1–6·0	3·20	44·7
1. vi. 49	84	45	2–6·0	3·09	39	1–6·0	3·01	46·4

SEX RATIO IN SMALL-SIZED STARFISHES

It is not possible to obtain representative samples of small-sized starfishes (radius less than 6 cm.) with the otter trawl, but on three occasions they have been caught in sufficiently large numbers by Agassiz trawl. All these catches were taken on or near the Rame mud area, south-west of Plymouth Sound. The first catch of ninety-five starfishes taken in April 1948 was not sexed, but the other two catches have been satisfactorily sexed and their radius lengths recorded (Table II).

These analyses show that in this population of small starfishes the males tend to be more numerous than the females.

SEASONAL VARIATIONS IN THE SEX RATIO

As the number of samples is relatively large the percentage of females in each catch from the Outer Grounds and Rame-Eddystone populations can be separated, according to season of capture, into six equal (two-monthly)

periods covering the year (Table III and Fig. 1). It is then found that in these large and medium-sized starfishes the proportion of females is below 50% during the winter months (November–February), but rises to 55% and even more during the spring and summer.

TABLE III. PERCENTAGE OF FEMALES IN THE OUTER GROUNDS AND RAME-EDDYSTONE GROUNDS POPULATIONS AT DIFFERENT TIMES OF THE YEAR

	Outer Grounds	Rame-Eddystone Grounds
January–February	48·3	46·6
March–April	57·1	54·6
May–June	57·1	54·9
July–August	53·9	54·8
September–October	51·9	52·5
November–December	49·4	47·6

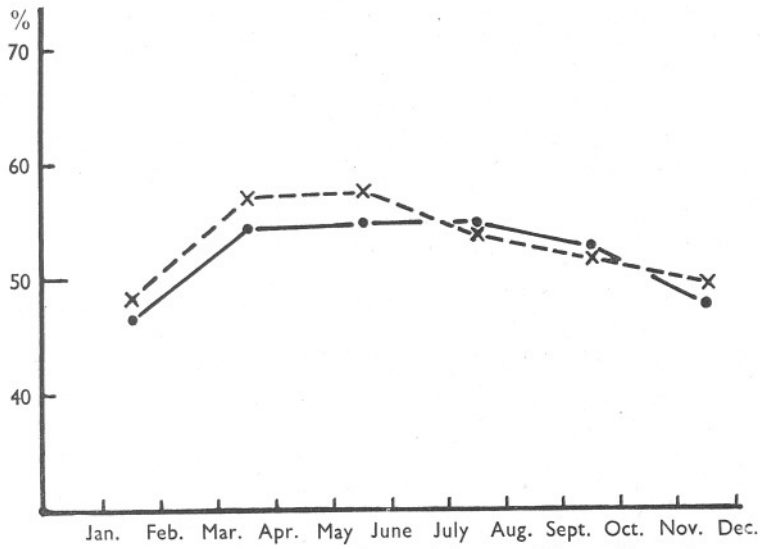

Fig. 1. Percentage of females in two populations at different times of year. ● Rame-Eddystone Grounds; × Outer Grounds.

There are insufficient data for this analysis to be carried out on the samples from the E1 area, Plymouth Sound and Rame Mud populations.

The curves in Fig. 1 are similar, with that for the Outer Grounds showing a higher percentage of females, at any rate during the spring and early summer.

DISCUSSION

Pelseneer (1926) has shown that in animals in general the males are more numerous at birth or metamorphosis while females are more numerous among the adults of a species. He considered that males were probably a little weaker in constitution and died earlier in life than females. He gave examples

which showed that, in some molluscs, insects, fishes, birds and mammals the male is more liable to parasitization than the female.

From the present data on *Asterias rubens* it is apparent that in starfishes over about 6 cm. radius the population samples tend to have relatively more females as the mean body size of the individuals increases.

Seasonal variation in the sex ratio of a population of animals may be associated with selective swarming of one sex, with protandrous hermaphroditism of the type found in *Asterina gibbosa*, with parthenogenesis as in aphids, or with a greater mortality rate in one sex. In *Asterias rubens* there is no evidence of hermaphroditism as a generally occurring phenomenon. Over 4000 specimens of this species have been examined to determine body size and the state of gonad development, and there has been no instance of a gonad showing traces of a change in the nature of its sexual elements.

Kirk (1912) has described a sample of thirty specimens of the crinoid *Actinometra japonica* from one locality in which there were no males, and the females all contained nearly ripe eggs. He considered that this was a case of swarming due to sexual activity, and that it might account for the large isolated masses of the fossil *Uintacrinus* which are often found, pointing out that such a large crinoid with an elaborate system of plankton-gathering arms could not normally live and feed in such dense masses.

At first sight the seasonal change in sex ratio in *Asterias rubens* might appear to be due to a selective swarming of females on the breeding grounds. However, if this happened, and the lacking males still survived, it is difficult to account for their absence from trawl hauls taken over such large uniform areas. It is scarcely possible that large and medium-sized males could hide on an even, flat sandy bottom of the type found on the Outer Grounds, the source of all the larger catches. Nor is there any evidence for long distance movements of starfishes.

There is, however, a possibility of a differential mortality, due to an inherent weakness in the male or to infection by parasites. Although there is no direct evidence of a difference in viability of adult males and females, it has been observed that when a large catch of *A. rubens* (taken by otter trawl) is brought into the laboratory the mortality during the first 2 days of captivity is invariably higher in the males than in the females. This suggests that the males may be more susceptible to the bruising of the ciliated epithelium and other organs which is inevitably suffered in the cod end of the otter trawl.

It is also possible that a differential rate of mortality between the sexes is related to the occurrence in the testes only of infestations of the ciliate parasite *Orchitophrya stellarum* (Vevers, 1951). Not only does this parasite cause complete atrophy of the testes, but it has also been observed that many infected males show very weak carotenoid pigmentation of the aboral integument and a general flabbiness of the body which lacks the fresh turgid appearance of a healthy specimen. It is considered that these infestations may

materially affect the mortality rate of the males. It is noteworthy that in the Outer Grounds the larger percentage of females in the population is accompanied by a very heavy parasitic infection of the males in that area. In the Rame-Eddystone Grounds, on the other hand, where the sex ratio is nearer equality, the gonad parasite has not been recorded. In the Plymouth Sound population the percentage of females was slightly higher than in the Rame-Eddystone Grounds, and this may similarly be associated with the presence of the parasite in the former population, causing greater mortality of males, thus giving an increase in the percentage of females. The parasite has not been recorded in the E 1 area, but the few samples from that locality were all taken too late in the season for the presence of the parasite to be expected. Nor has the parasite been recorded in the Rame Mud population of small starfishes.

The parasite only occurs in the testes during January–April with an occasional occurrence in May. It is reasonable to suppose that, if the parasite *Orchitophrya* has an adverse effect on the mortality rate of the male starfishes, the losses due to it should occur around that time, i.e. spring and early summer. It is, in fact, considered that the rise in the percentage of females which occurs at that time is due to a differential mortality caused partly by the weakening effects of the parasite *Orchitophrya* which only attacks medium- and large starfishes with well developed gonads, and partly by a general weaker constitution and shorter life span of the males. These factors would tend to reduce the percentage of males in a population of large and medium sized starfishes. This decline in the numbers of males in a population would be cumulative if it were not made good by the growth of smaller males, which reach a size large enough to be taken by the trawl and are thus included in the population samples, or by the gradual immigration from poorly fed populations of small starfishes such as that on the Rame Mud which have an excess of males.

Summary

The sex ratio of *Asterias rubens* in five populations near Plymouth has been obtained from observations on over 4000 specimens. The overall percentage of females in samples of large and medium-sized starfishes was 51·3 in the Rame-Eddystone Grounds, 55·1 in the Outer Grounds and 70·3 in the E 1 area; in the isolated Plymouth Sound population the percentage was 53·6. In the Rame Mud population of small starfishes (taken only by Agassiz trawl) the percentage of females was 45·6.

On the Outer Grounds and Rame-Eddystone Grounds the percentage of females was found to vary according to the time of year, being highest in March–June and lowest in January–February.

In a general discussion of these results it is considered that they are probably not related to any change of sex in the individuals, as no trace of hermaphroditism has been found in many thousands of gonads examined. It is also

unlikely that there is any degree of differential sexual swarming. It is, however, considered possible that the rise in the percentage of females is due to a greater mortality rate in the males. There is indirect evidence that males are in general less resistant to injury than females, and it is known that they are liable to gonad parasitization by *Orchitophrya*. It is suggested that a greater male mortality rate among medium and large starfishes may be due to both these factors, and that the resulting deficiency in males is made good by recruitment of small starfishes among which males preponderate, either from the same grounds or from neighbouring grounds.

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THE ANNUAL GROWTH AND REPRODUCTIVE CYCLE IN FOUR ASCIDIANS

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(Text-figs. 1–11)

This study was undertaken to increase our very incomplete knowledge of the annual cycle in ascidians.

Berrill (1950) states that 'the age of ascidians, with one exception, is practically impossible to estimate, unless a certain inhabited area is followed closely through seasons and years'. In the present work the method has been to follow certain aspects of the ascidian population of a chosen area for a period of nearly two years. The area was the Old Dock in Ardrossan Harbour, Ayrshire, and the ascidian population contained four species: *Diplosoma listerianum* (Milne Edwards), *Ciona intestinalis* (Linnaeus), *Ascidiella aspersa* (Müller) and *Botryllus schlosseri* (Pallas). *Diplosoma* belongs to the order Enterogona, suborder Aplousobranchiata; *Ciona* and *Ascidiella* to the order Enterogona, suborder Phlebobranchiata; and *Botryllus* to the order Pleurogona, suborder Stolidobranchiata. Thus each of the three main suborders is represented, and the four ascidians studied are amongst the commonest British species.

There are three main aspects of the annual cycle: (i) growth, (ii) number and life-span of generations in the year, (iii) sexual reproduction.

Growth. In the simple ascidians *Ciona* and *Ascidiella* increase in length of the body suffices as a measure of growth. In the compound forms *Diplosoma* and *Botryllus*, however, the case is complicated by the existence of budding. This may involve rapid and almost continual production of new individuals which replace, or live along with, old ones in the colony. It is therefore difficult to find a significant measure of growth in these compound forms. The most convenient measurement, however, is area, since during growth the colonies of both *Diplosoma* and *Botryllus* increase in surface area but scarcely at all in thickness.

Number and life-span of generations in the year. The appearance of a new generation and the disappearance of an old one can be detected by inspection of the samples. It is difficult to see the small newly settled larvae, however, and the first evidence of a new generation is usually the presence of small individuals or colonies which have been established for a short but unknown time.

Sexual reproduction. Faunistic lists and papers sometimes give notes on the breeding season of various species, but these are usually generalized

conclusions based on observations made over a number of years. Also they fail to define the term 'breeding season', which might include a variety of conditions and times from the onset of gonadial development to the settlement of larvae. The period when an ascidian carries eggs and sperm is often considerably longer than the period of their liberation and development in nature. This latter period depends on internal and external limitations on spawning and successful development.

In the present study the progress of breeding was followed by noting the periods when animals carried eggs and sperm, developing eggs, or larvae, and by finding the period during which larvae had settled.

The quantity of eggs and sperm in the ducts can be readily seen in *Ciona* and *Ascidiella*. *Diplosoma* and *Botryllus*, however, do not have long genital ducts in which eggs and sperm are stored, and in these animals the reproductive condition was assessed by the state of development of gonad, and by the presence or absence of developing eggs and larvae in the zooids or the colony.

The Area Studied

The Old Dock, Ardrossan, is a semi-tidal sea-water dock, the water-level in which is controlled by sea gates. The amount of interchange of water between the dock and the sea is uncertain and variable, and depends partly on the traffic of ships through the gates. The predicted depth at H.W.O.S.T. is 19 ft. and the area of the dock is 4·23 acres. The walls, which are of stone, are vertical and form the substratum to which *Ciona* and *Ascidiella* are attached. *Diplosoma* and *Botryllus* are generally fixed to the test of the simple ascidians.

Methods

Samples were collected by scraping the walls of the dock at intervals throughout 1950 and 1951.

The size of samples varied with the availability of material, which depended partly on the time of year and partly on the water-level in the dock. The number of specimens measured (N) is shown on histograms of size distribution where percentages are used. The animals were narcotized with menthol, fixed in the expanded state in strong formalin and stored in weak formalin. Measurements of length in *Ciona* and *Ascidiella* were made to the nearest 1 mm. The colonies of *Diplosoma* and *Botryllus* were carefully removed from the substratum (generally the test of *Ciona* or *Ascidiella*), placed flat on the glass negative-carrier of a photographic enlarger, covered with a glass slide, and inserted in the enlarger. The outline of the projected image was traced on squared paper and the area of the colony calculated from the outline tracing.

Specimens of *Ciona* and *Ascidiella* were dissected and the genital ducts examined for eggs and sperm. A number of zooids were removed from each

colony of *Botryllus* and examined for the presence of gonad, developing eggs and larvae. In the colonies of *Diplosoma* zooids were examined for eggs, and the common test for developing eggs and larvae. In each sample of *Botryllus* and *Diplosoma* about twenty colonies were thus examined. Pieces of several colonies of the compound forms, in each sample taken during 1950, were sectioned to confirm the results of dissection.

Ascidiella aspersa (Müller)

Growth, number of generations in the year, and life-span.

The length of the specimens was taken as a measure of their size. In Fig. 1 a series of histograms shows the distribution by size of *A. aspersa* in the samples taken in 1950 (left) and 1951 (right).

The sample of 24 January 1950 showed two populations, one whose body length had the mode about 25 mm., and the other with the mode about 50 mm. The population of small animals represented rather more than 80% of the sample and consisted of animals that settled as larvae in the previous summer (1949). The remaining population, of large specimens, was rather less than 20% of the sample and represented animals that settled as larvae in the summer of 1948.

Between January and April there appears to have been some growth of the smaller animals of the 1949 group, but less amongst the larger ones. During this period the percentage of the total sample represented by the 1948 group gradually decreased until in April it accounted for only about 2% of the total sample. Thus, although a large part of the 1948 group had perhaps died before the first sample was taken in 1950, it was in the months from January to April that this group virtually disappeared.

Growth for the 1950 season became important between 21 April and 24 May and continued at a high rate until about the middle of June. From then until the middle of July growth was considerably slower, at least amongst the larger individuals.

The sample of 14 July gave the first evidence of the new generation, and from this time until about the end of September growth of animals of this 1950 group continued. During the same period there was no appreciable growth in specimens of the 1949 group, and after the end of September there appeared to be no further growth in either the 1949 or the 1950 group.

On 27 November about 20% of the sample belonged to the 1950 group and about 80% to the 1949 group.

It is evident that in 1950 only one new generation of *A. aspersa* was produced, and that this was established mainly in July but possibly also somewhat earlier and later.

The samples of 11 January and 13 April 1951 are too small to be very useful but tend to confirm the results of 1950, that at this early period of the year

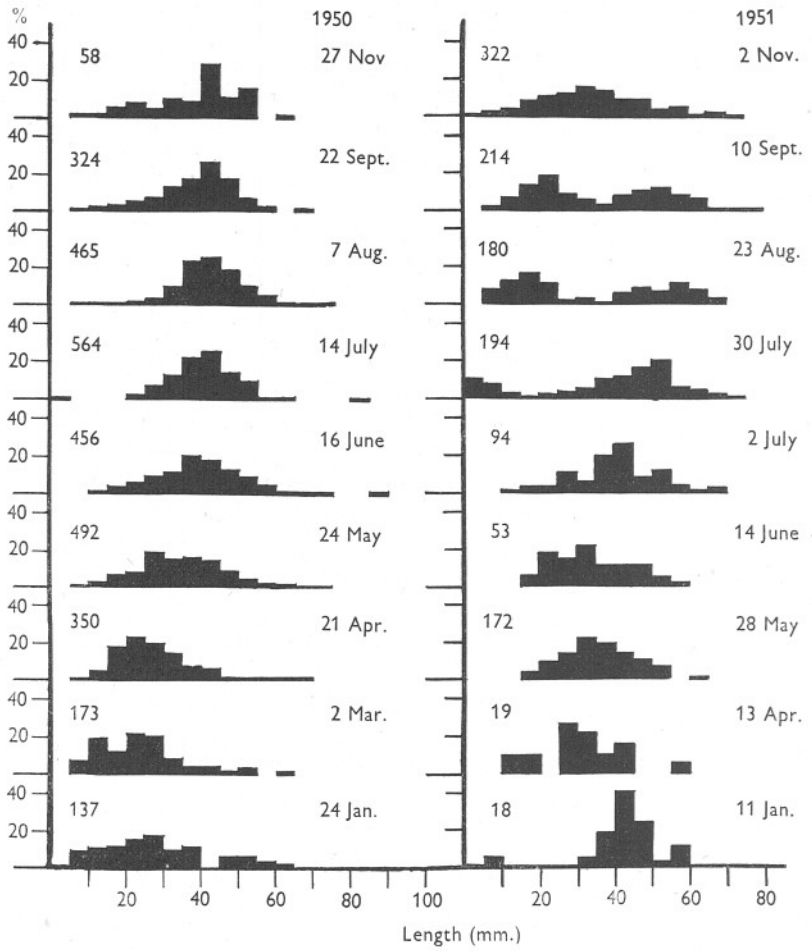

Fig. 1. Size-frequency histograms (in percentages) of *Ascidiella aspersa* in 1950 and 1951. The figures on the left of each histogram denote the number of specimens in the sample.

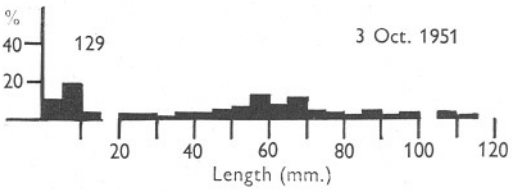

Fig. 2. Size-frequency histogram of *A. aspersa* on 3 October 1951, from Loch Sween, Argyll; as in Fig. 1.

the population represents two year-groups. The animals of these two groups had settled as larvae in the summers of 1949 and 1950 respectively. The samples also suggest that many of the larger individuals (i.e. of the 1949 group) died between 11 January and 13 April.

The figures for the rest of 1951 follow the same general pattern of growth and settlement that was found in 1950. In 1951, however, no growth was apparent until after 14 June, whereas in 1950 the animals had grown considerably before 24 May. On 2 July no newly settled individuals were found. They were present on 30 July, however, but in the sample of 23 August and in all later samples none was found. The period of larval settlement in 1951 therefore started between 2 July and 30 July and finished between 30 July and 23 August.

Animals of the new, 1951, group continued to grow from the end of July until some time between 10 September and 2 November, but during this period the animals of the old, 1950, group made no measurable growth. Only one new generation was established in 1951.

A single sample of *A. aspersa* was taken from Loch Sween, Argyll, on 3 October 1951 to provide a comparison with the autumn samples from Ardrossan. Fig. 2 shows the size distribution of the specimens. It appears that here also the population represents two clearly separate age-groups, the smaller animals, up to about 15 mm. long, belonging to the 1951 settlement and the larger ones belonging to the 1950 settlement. The size distribution of this sample suggests that the general pattern of growth and reproduction may be similar over much of the west coast of Scotland. The main differences in the sample from Loch Sween, as compared with autumn samples from Ardrossan, are: (i) the smaller size of the individuals of the 1951 group, and (ii) the larger size of those of the 1950 group.

These features may result from a later breeding season and more favourable conditions for growth in Loch Sween.

It may be deduced, from the samples of these two years, that the life-span is of the order of 18 months, extending approximately from the middle of one summer until the winter of the following year. *A. aspersa* in this area is essentially an annual, a conclusion which Hŭus (1937) also reached for this species on the Norwegian coast.

Sexual maturity

Fig. 3 shows, for each of the size-groups, the percentage of sexually mature animals in the samples of 1950. The upper half (*S.*) refers to sperm, and the lower half (*E.*) to eggs, in the genital ducts. Sexual maturity is here taken to mean the presence of sperm or eggs in the genital ducts, irrespective of the quantity. The figures show two features:

(1) *A. aspersa* is hermaphrodite, but is slightly protandrous, as the sperm duct starts to fill before the oviduct. The 20–25 mm. group shows this

protandrous tendency most clearly. This finding contradicts Herdman's (1899) general statement that *Ascidia* is protogynous.

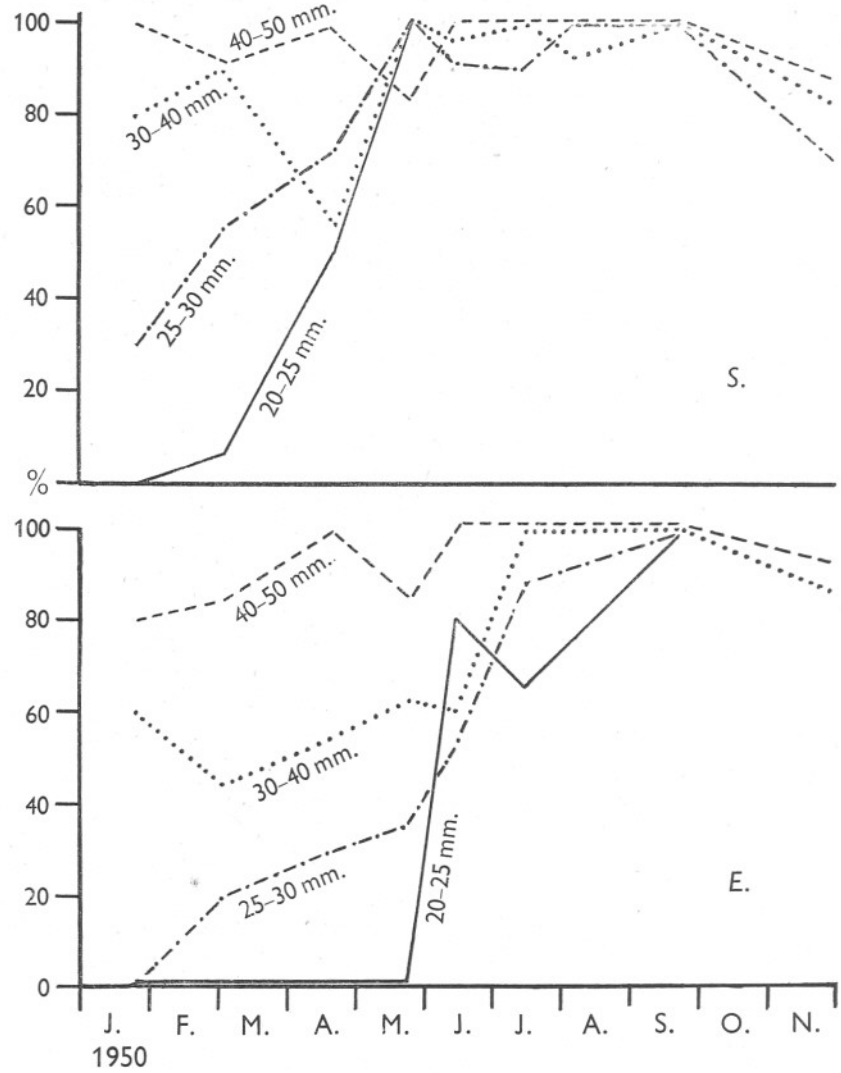

Fig. 3. Relations between the time of year and the percentage of specimens of *A. aspera* with sperm (*S.*) and eggs (*E.*) in the ducts, for each of the size groups 20–25, 25–30, 30–40 and 40–50 mm., during 1950.

(2) Sexual maturity depends primarily on the size of the animal. When an individual reaches a certain critical size sperm begins to appear in the sperm duct and at a slightly larger size eggs pass into the oviduct. The critical size, however, changes during the year. Thus practically all individuals over

40 mm. in length are mature as male and female throughout the year. In the 30–40 mm. group over 80% are mature as males during the whole year, but from January until June only about 40–60% of this group are mature as females. In the 25–30 mm. group there is a steady rise in the proportion with sperm, from January to May, when all have become mature; there is a similar but more gradual rise in the proportion with eggs. The 20–25 mm. group shows the sharpest rise in the percentage mature, both as male and as female, between January and June. In June sperm generally starts to fill the sperm duct when the animal is slightly under 25 mm. long, and eggs pass into the oviduct when the animal is rather less than 30 mm. long. This difference of about 5 mm. in the critical body length for male and female maturity is supported by observations made on a sample of *A. aspersa* taken on 20 August 1948 from a tufnol plate suspended in the waters of Loch Sween, Argyll. Fig. 4 shows, for this sample, the relation between the body length and the presence or absence of sperm and eggs in the ducts. Incidentally, the animals in Loch Sween became mature at a smaller body size than did those in any of the samples from Ardrossan. This may have resulted from environmental differences like those which appear to have influenced the Ardrossan population during the course of the season.

The time of settlement, growth rate, critical body length and life-span were such that a sample taken at Ardrossan at any time of year contained a large proportion of mature individuals. In the sample of 27 November 1950, for example, most of the 1949 group carried eggs and sperm, and the larger members of the 1950 group also did so. Of this population, however, the 1949 group died before spawning again, in 1951. Its place was taken by the 1950 group, which spawned in the summer of 1951. In the area studied, therefore, it appears that a given year group of *A. aspersa* has only one spawning season, and that is in the year after it settled as larvae.

Ciona intestinalis (Linnaeus)

Growth, number of generations in the year, and life-span

Ciona is more difficult to measure than *A. aspersa* because it is more contractile and because its body form varies more according to its contact with the substratum. It was therefore essential to narcotize the animals thoroughly before fixing and measuring them. Another difficulty was the limited supply of animals necessitating small samples.

Fig. 5 is a series of histograms showing the distribution by size of *Ciona* taken at intervals throughout 1950 and part of 1951. The samples of January, March and April 1950 showed two populations, but these were not clearly distinguishable. The numerically larger population had a small body length, and most specimens were between 10 and 30 mm. long. These animals had settled as larvae in 1949. The other population had a body length ranging

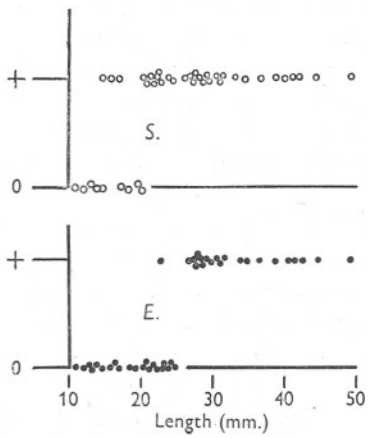

Fig. 4.

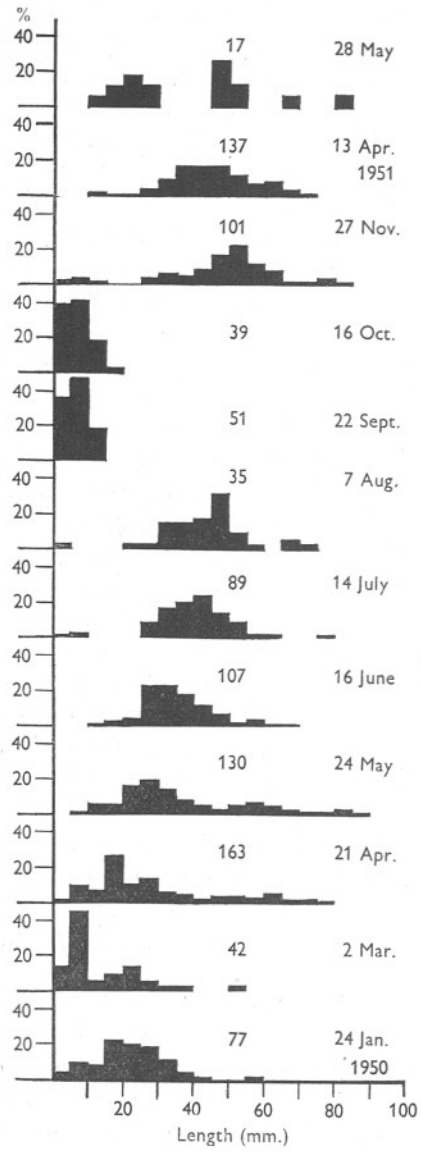

Fig. 5.

Fig. 4. Relation between the body length and the presence or absence of sperm and eggs in the ducts of *A. aspersa*, in a sample from Loch Sween, Argyll, taken on 20 August 1948. *S.* sperm in duct; *E.* eggs in duct.

Fig. 5. Size-frequency histograms (in percentages) of *Ciona intestinalis* in 1950 and early 1951. The figures denote number of specimens in the sample.

approximately from 50 to 70 mm. These animals had settled as larvae in 1948. The 1949 group tended to be bimodal, suggesting that during the previous year settlement may have been more intense at two periods separated by an interval when fewer larvae settled. Little or no growth was evident between January and 21 April, but during May, June and July the animals of the 1949 group grew considerably. By the end of May the bimodal tendency could no longer be detected in the 1949 group, the smaller individuals having grown faster than the larger ones. The animals of the 1948 group made little, if any, growth between May and July, as most of them had already reached their maximum size. There are, unfortunately, gaps in the records of the 1949 group during September and October, as continued sampling had reduced the stock. It was not until November that numbers of this population were found in a different part of the dock. These gaps in the records, however, are not serious, since it is obvious from the histograms for August and November that we are dealing with the same year-group. Between August and November there appears to have been little growth within the 1949 group.

It is more difficult to assess the proportion of the 1948 and 1949 groups in each sample of *Ciona* than in the samples of *Ascidiella*, and to say when the 1948 group died out. Most of the 1948 group had apparently disappeared before the first sample was taken in 1950, but a few were certainly still present in the sample of 24 May.

The first evidence of the 1950 settlement was in the sample of 14 July, in which a few young specimens were found. As one of these was already 7 mm. in length, settlement of larvae must have started a few weeks before this sample was taken. It was difficult to find numbers of the 1950 group sufficient to give a clear idea of their growth rate. This may have been due partly to depleted breeding stock and partly to an unsuccessful breeding season. The similarity between the histograms for 22 September and 16 October suggests that growth of the members of the 1950 group did not continue after the end of September.

Only one generation of *Ciona* was produced in 1950 in the area studied. Settlement of larvae appears to have started in July and continued through August and probably into September, as at the end of this month specimens of only 2 mm. length were still being found. The presence of individuals in the 0–5 mm. group during October and November does not necessarily indicate further settlement. As growth had already stopped for the year individuals which settled in September could still be in the 0–5 mm. group throughout the winter. This suggestion is supported by the presence of specimens only 3 and 4 mm. long in the samples of January and March 1950.

In 1951 samples were obtained up to 28 May after which the population was apparently so depleted that only occasional specimens were taken. These

few samples, however, tend to confirm two features of the 1950 sampling: (i) growth of the animals of the recent year-group (in this case the 1950 group) started before the end of May; and (ii) most of the remaining animals of the previous year-group (in this case the 1949 group) died before the end of May.

The general pattern of growth, reproduction and replacement of generations in *Ciona* was very like that found in *Ascidiella*. Individuals settled in the summer, grew until autumn, resumed growth in the following spring, spawned in the summer of that year, and died in the following winter. They are therefore essentially annuals, although they may be 18 months old or somewhat more when they die.

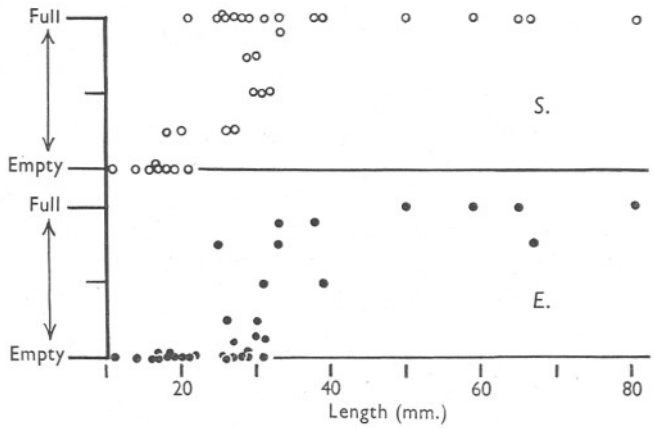

Fig. 6. Relation between the body length and the quantity of sperm (*S.*) and eggs (*E.*) in the ducts of *C. intestinalis* in the sample taken on 24 May 1950.

Orton (1914, 1920) found that in the Plymouth area *Ciona* breeds from April to November, producing two or three generations in the year. Hŭus (1937) states that on the Norwegian coast *Ciona* is sexually mature within its first year of life and dies off after breeding, having a life of about one year. In Naples, Lo Bianco (1909) records *Ciona* as breeding throughout the year. Rünnstrom (1936) found that *Ciona* has three physiological races which breed within different temperature ranges. On the west coast of Norway he found *Ciona* to spawn from May to August, after which the old generation dies off. It is replaced by the new one which becomes sexually mature by the end of August but does not, apparently, produce a further generation: eggs and sperm may be shed from October to December but fail to develop.

The population of *Ciona* studied in Ardrossan Old Dock resembles the animals of the Norwegian west coast in breeding behaviour and the annual cycle of generations, although differing somewhat in the timing of events and speed of processes.

Sexual maturity

Fig. 6 shows the relation between the length of the body and the quantity of eggs and sperm in the genital ducts of a number of specimens of *Ciona* from the sample of 24 May 1950. The remaining samples were not examined in detail, but the same general relations were found.

Ciona, like *Ascidiella*, is a slightly protandrous hermaphrodite. Sperm starts to enter the sperm duct at a critical body length and eggs appear in the oviduct later, when the body length is somewhat greater. Fig. 6 suggests that the critical body length for the appearance of sperm in the duct is about 25 mm. and of eggs in the oviduct about 30 mm., in the sample examined.

Diplosoma listerianum (Milne Edwards)

Growth

The area of the colonies was measured as described on p. 42. In Fig. 7 a series of histograms shows the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

All colonies of the sample of January 1950 originated from larvae that settled in 1949. Most of the colonies of this sample were less than 50 mm.^{2} in area and the largest was 170 mm.^{2}. Growth in 1950 started between 2 March and 21 April and continued until about the end of May. Maximum size appears to have been reached by 16 June, when a number of colonies were between 1000 and 1500 mm.^{2} in area.

In the sample of 14 July a few colonies showed degeneration of the zooids, but it was in the sample of 7 August that this process first became widespread. Food was not seen in the gut of zooids in August, and this is taken as another sign of lowered vitality. By 22 September 1950 most of the 1949 generation of colonies had died and many of those remaining were degenerate.

New colonies of the 1950 generation were first observed in the sample of 14 July but were few. The new colonies were more common on 7 August. On 22 September most of the sample, and on 27 November all of it, consisted of the new, 1950, generation.

Appreciable growth of the newly established colonies took place before 22 September.

The pattern of growth and replacement of generations in 1951 was in general similar to that observed in 1950. In 1951, however, spring growth up to 28 May was much less than spring growth during a corresponding period of 1950 (up to 24 May). A similar feature was observed in the growth of *Botryllus* (see p. 58). In the autumn of 1951 colonies of the new generation continued to grow after 10 September, as shown in Fig. 7, bottom right.

Sexual reproduction

Asexual reproduction by budding leads to an increase in the size of the colony and not to the founding of new colonies, which is the function of

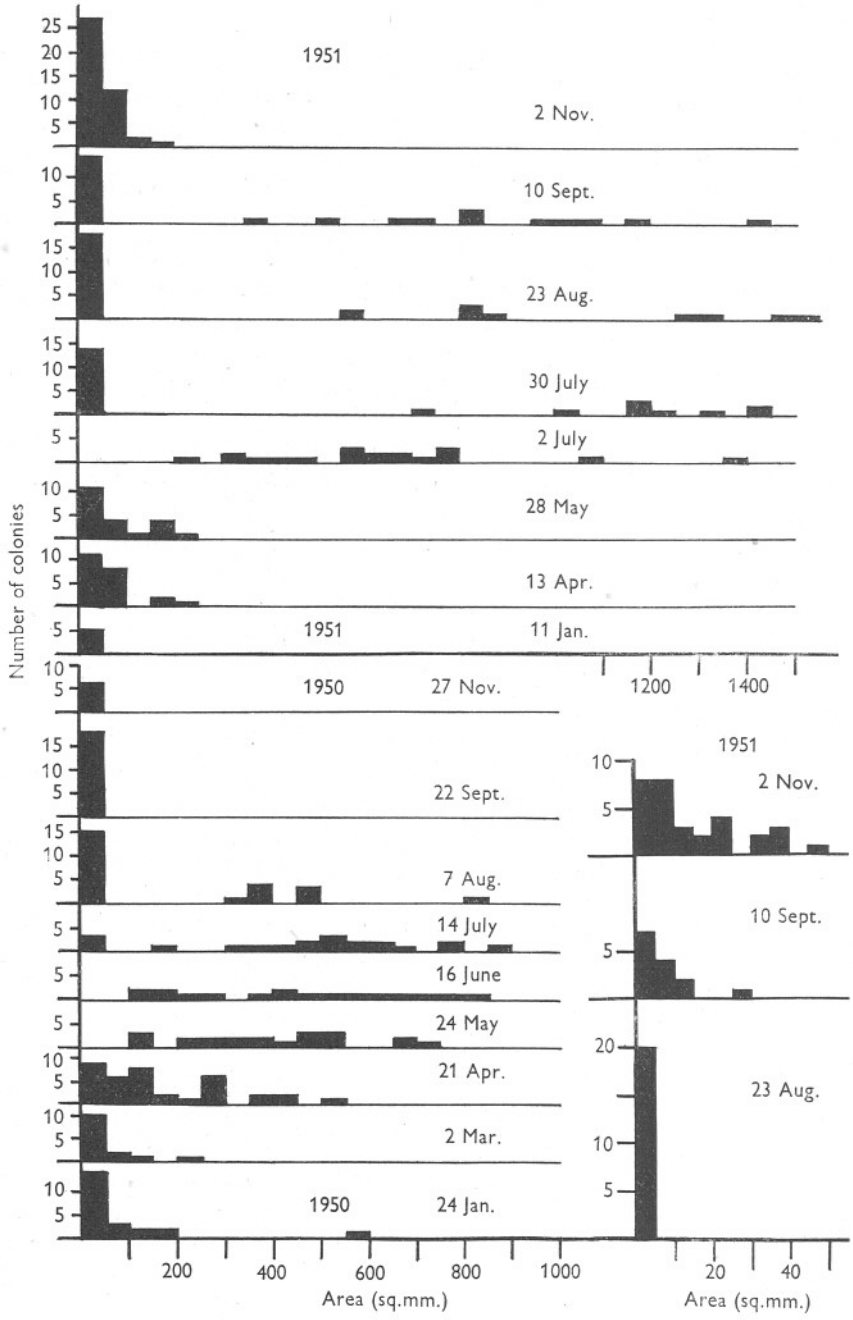

Fig. 7. Size-frequency histograms (by area) of *Diplosoma listerianum* in 1950 and 1951. The smallest specimens for three successive dates are further analysed in the inset at right bottom.

sexual reproduction. During sexual reproduction budding was reduced but it continued long after the onset of gonadial development and egg production. It only ceased, presumably, when there was serious competition for raw materials between the sexual and the asexual processes. Huxley (1921) and Berrill (1935) have drawn attention to this competition in colonial ascidians.

The ovary (Fig. 8, *Ov.*) of *Diplosoma* lies in the lower part of the abdomen, and shows only a few large eggs at any time. As the season advances the eggs enlarge and pass singly from the abdomen down into the basal layer of test. Lahille (1890) states that the egg is fertilized only after breaking through the body wall and coming to lie in the common test, but Berrill (1950) maintains that it is fertilized before leaving the abdomen. Development to the larval stage certainly takes place within the common test.

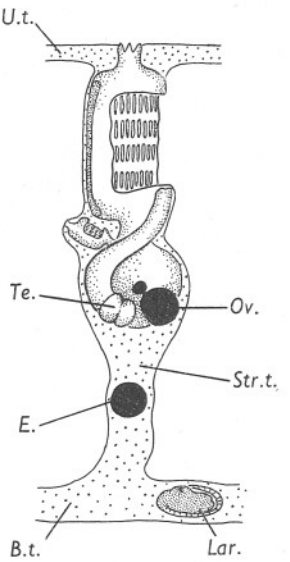

Fig. 8. Zooid of *D. listerianum* within the common test of the colony. *B.t.* basal layer of common test; *E.* egg; *Lar.* larva; *Ov.* ovary; *Str. t.* strand of common test securing zooid to basal layer; *Te.* testis; *U.t.* upper layer of common test.

Fig. 9 summarizes the conditions found in the samples of *Diplosoma* throughout 1950, and records the percentage of colonies with: (A) neither eggs nor larvae in zooids or test; (B) eggs in zooids but not in test; (C) eggs in zooids and eggs or larvae in test; (D) eggs or larvae in test but no eggs in zooids; (E) larvae.

The graphs indicate the progress of breeding activity.

January to March was a period of sexual inactivity, during which colonies of the 1949 generation had eggs neither in zooids nor in test. Some colonies, however, showed eggs or larvae, or the degenerate remains of these, in zooids or test. These eggs and larvae appear to be residual products which failed to develop completely and escape during the previous year. They may be regarded as abnormal, as they were probably held within the incompletely disintegrated basal layer of colonies of the 1948 generation. This condition was more common in 1951 (see p. 55).

Between the March and April samples there had been an increase in the proportion of colonies with eggs in the ovaries, and this period marks the onset of sexual activity for the year. During May there was a sharp rise in the percentage of colonies with eggs in the common test as well as in the zooids, indicating a large-scale passage of eggs from the zooids down into the common test. It was on 24 May that the first developing embryos were seen. All

colonies examined in the middle of June had eggs in the zooids and also eggs or larvae in the test. Egg production started to diminish in July, as indicated by a slight drop in the percentage of colonies with eggs in zooids as well as test. Between mid-July and early August the ovary became inactive and the production of eggs ceased. In the July and August samples all colonies examined had larvae, and some had developing eggs, in the test.

The first signs of exhaustion resulting from breeding activity were seen in July, when a few colonies were found with degenerating zooids; in August this condition was common.

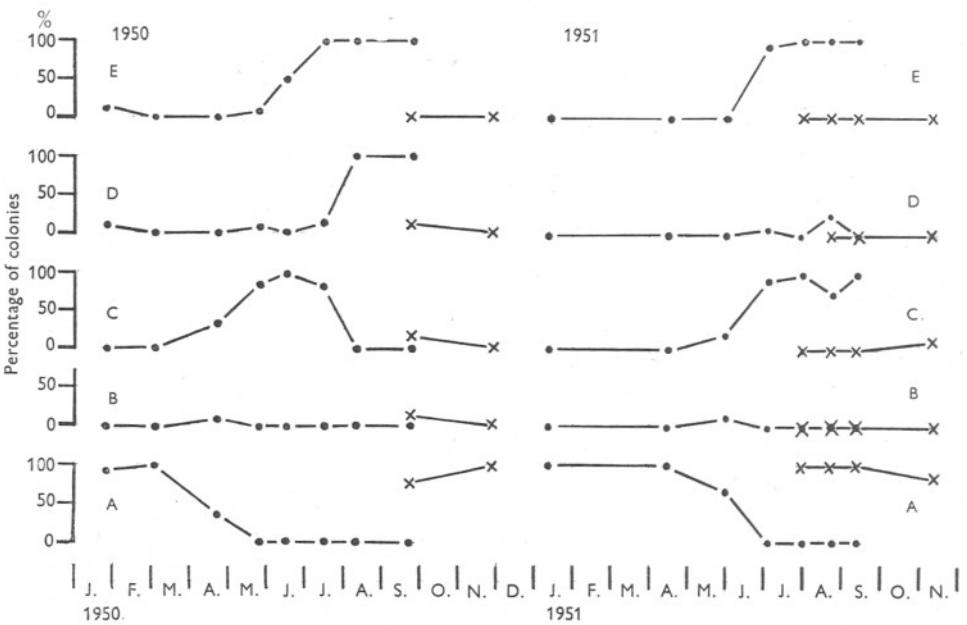

Fig. 9. The breeding activity of *D. listerianum* in 1950 (left) and 1951 (right). A, colonies with no eggs and no larvae in zooids or test; B, colonies with eggs in zooids but not in test; C, colonies with eggs in zooids and eggs or larvae in test; D, colonies with eggs or larvae in test but no eggs in zooids; E, colonies with larvae.

Larval settlement was indicated by the presence of young colonies in the samples of 14 July, 7 August and 22 September. The settling period was not determined more precisely and may not have extended into the second half of September. It is perhaps significant that the settling period coincided with the period during which the old colonies disappeared, and it may be that the larvae are liberated only with the disintegration of the breeding colonies. Berrill (1950), on the other hand, states that the active tadpoles escape from the test matrix to the common cloacal cavities of the colony and thence to the exterior. Many of the Ardrossan colonies examined, however, showed areas of degenerating zooids below which larvae were still retained in the

common test. The existence of many composite colonies (see below) in November 1951 also indicates prolonged retention of larvae in the test, even after the disappearance of the parent zooids.

The young colonies grew and, as the September sample showed, some of the zooids produced eggs. Developing larvae were also found in a few young colonies in September. That some of these young colonies may have given rise to a second generation is suggested by the presence of a few two-zooid colonies in late November. It is possible, however, that these small colonies in November came from late larvae of the old colonies.

A study of the breeding activities of *Diplosoma* during 1951 confirmed the general picture gained in 1950, as seen from the right-hand part of Fig. 9. It appears that the onset of breeding was later in 1951 than in 1950. This difference is shown in the later disappearance of the non-breeding condition (A), the later appearance of eggs in the zooids (B), the later rise in the percentage of colonies with eggs in both zooids and test (C), and the later transition of colonies to the final breeding stage (with eggs or larvae in the test only, D). By comparing the dates in 1950 and 1951 on which the same percentage of the samples was in a particular reproductive state, we can estimate the lateness of the 1951 season. The lateness was of the order of 6 weeks.

One other feature of the 1951 graphs deserves comment: this is the much smaller percentage of colonies during August with eggs or larvae in the test only, compared with the 1950 figures. The lower percentage in 1951 was due to the later degeneration of zooids, because as long as the zooids remain healthy some at least continue to produce eggs.

The delayed decay of the old colonies in the autumn of 1951 gave rise to another interesting condition: this was the formation of composite colonies. A composite colony consists of the basal layer of test of an old colony with its larvae still enclosed, and zooids of the new generation derived from these larvae which have metamorphosed *in situ*. No zooids of the old generation remain.

Later breeding in 1951 may have resulted from later growth, which was observed to occur, but more probably some environmental factor, such as lower water temperature, retarded both activities.

Number of generations

In the area studied *Diplosoma* produced, both in 1950 and 1951, one principal generation and possibly a minor one in 1950 late in the season. Orton (1914) states that, in the Plymouth area, the species passes through 'at least two crops in a year' and three or more in favourable seasons. Once he got larvae from a colony aged not more than 3 weeks and 5 days.

The rate of development of the colonies and the time at which larvae are liberated are the factors determining the number of generations per year.

These factors are greatly influenced by local and seasonal conditions. Berrill's (1950) statement that 'breeding occurs throughout the year' is too general, although no doubt true in specially favourable conditions.

Botryllus schlosseri (Pallas)

Growth

The colonies of *Botryllus* were removed from the substratum and their area measured by the method described on p. 42. Fig. 10 gives a series of histograms showing the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

In January 1950 the sample of *Botryllus* represented a single year-group, consisting of colonies that had been established in 1949. Many colonies were under 25 mm.^{2} in area and had only one system of zooids. The few large colonies in this sample were between 200 and 300 mm.^{2} in area and these had ten to twelve systems of zooids. The onset of growth for the 1950 season occurred between 2 March and 21 April. In the April sample most specimens lay within the 25–30 mm.^{2} group and these colonies had two or three systems of zooids. Large colonies between 500 and 750 mm.^{2} had fifteen to twenty systems. Growth continued during May and by 16 June maximum size had, in general, been attained. Only two colonies in the June sample had as few as six systems of zooids and the majority had fifteen to twenty or more systems. One or two of the large colonies showed some degeneration in June, the first to be observed in 1950.

The sample of 14 July showed two important features: (1) the first new colonies resulting from larval settlement, and (2) considerable degeneration in the large colonies.

The new generation was represented by two small colonies, each of a few zooids arranged in one system.

By 7 August the change of generations was almost complete, only one colony of forty-three examined belonging certainly to the 1949 generation. The remainder of this sample consisted of small colonies, mostly of one system, but a few of two, three, or four systems. Two single zooids were found, recently metamorphosed from settled larvae. On 22 September no colonies of the 1949 generation were found.

A more detailed comparison of the samples of 7 August, 22 September, and 27 November 1950 shows that the young colonies grew during late summer and autumn (Fig. 10, inset). Table 1 suggests that this growth took place without an increase in the number of systems per colony.

It can be seen by inspection of the histograms for 1951 that they agree fairly well with those for 1950. In the sample of January 1951 all colonies belonged to the 1950 generation. Growth had begun by 13 April and continued until June. Early in July degeneration of colonies had started but was most noticeable at the end of that month. The first new colonies, of the 1951

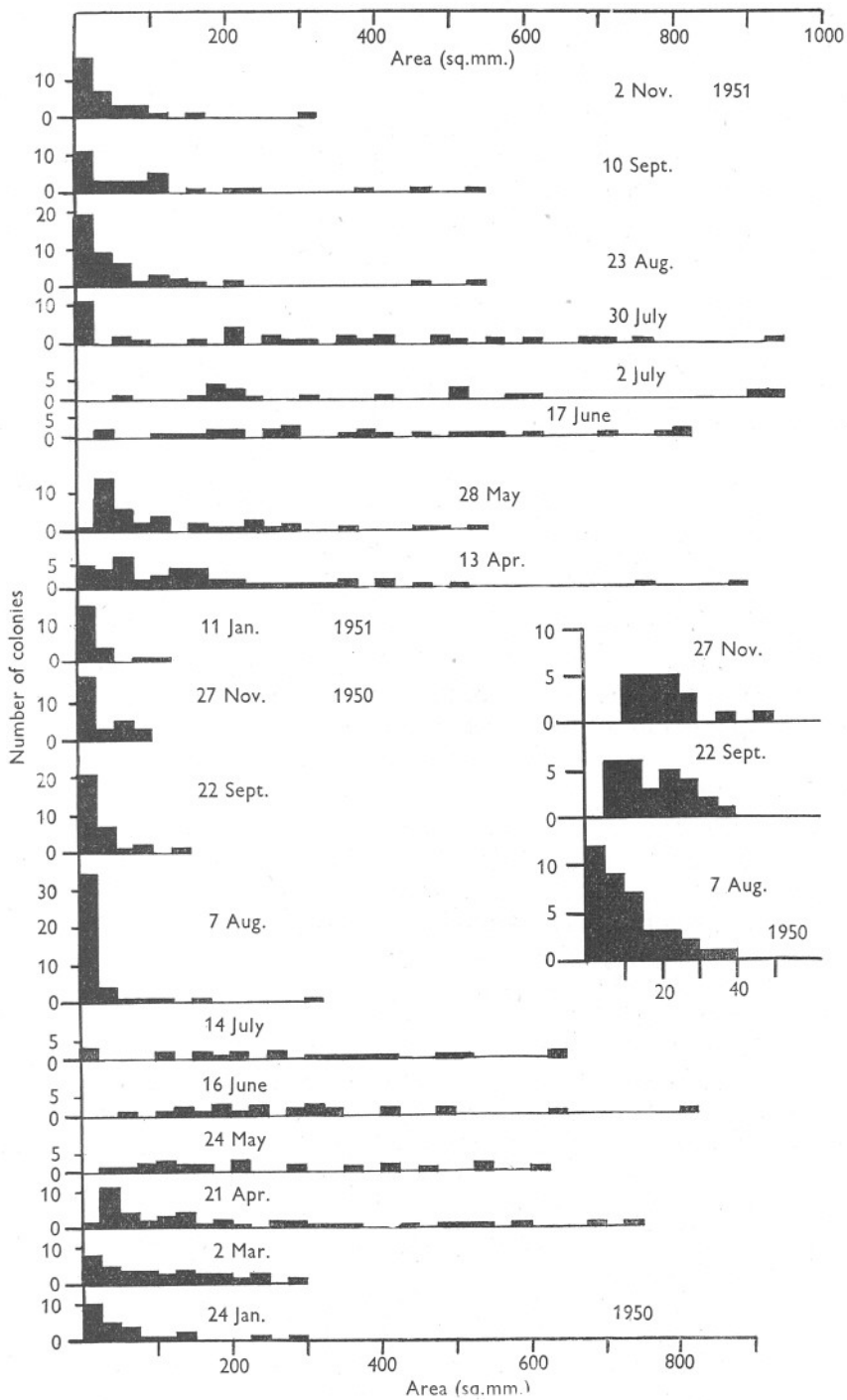

Fig. 10. Size-frequency histograms (by area) of *Botryllus schlosseri* in 1950 and 1951. The smallest specimens for three successive dates in 1950 are further analysed in the inset on the right.

generation, were seen at the end of July, and by 23 August most of the sample consisted of this new generation.

Although the general pattern of growth was similar in the two years, the onset of spring growth was later in 1951 than in 1950. This can be seen by comparing the histograms for approximately corresponding dates in the two years, viz. 21 April 1950 and 13 April 1951; 24 May 1950 and 28 May 1951. A similar retardation of growth was noticed in *Diplosoma*.

TABLE I

No. of systems per colony	No. of colonies			
	7. viii. 50	22. ix. 50	27. xi. 50	11. i. 51
A few zooids	2	0	0	0
1	24	20	15	16
2	9	5	3	1
3	4	2	4	2
4	1	2	5	0

Number of generations

The population of *Botryllus* passed through one generation in each of the years studied, and it seems unlikely that even the earliest new colonies were able to produce many larvae that settled to form a second new generation. Orton's (1914) findings that *Botryllus*, like *Diplosoma*, has at least two crops in the year applies to the Plymouth area.

Sexual reproduction

Berrill (1941, 1947, 1950) has described the process of bud formation and gonad development in *Botryllus* and *Botrylloides*. He has shown that the sex of the gonad and the state of development that it attains depend on the size of the bud bearing the gonad. Berrill (1935) also found that sexual and asexual reproduction take place simultaneously, but that budding may be relatively subdued during sexual reproduction.

The samples of *Botryllus* were examined, and Fig. 11 records the percentage of colonies in each sample during 1950 and 1951 with: (A) neither eggs nor larvae, (B) gonads, (C) large or developing eggs, (D) larvae.

Gonads were visible in a low percentage of colonies in January and March, and sections showed small developing ovaries and testes in some zooids and buds. No larvae were present in these months and only a few large eggs were found in one colony in March. Gonadial development was rapid during March and early April, but still there were only a few large eggs, and no larvae. The percentage of colonies with visible gonads was highest in May and June, dropped sharply to the level of July, and by late September had returned to the winter level. The percentage of colonies whose zooids had large or developing eggs followed a rather similar curve, but reached a high value for a shorter period.

Larvae were first found in the sample of 24 May, when some colonies had fully developed larvae and others had tailed but still incomplete larvae. The percentage of colonies bearing larvae continued to rise in June and reached a maximum in July, after which there was a rapid decrease. A few larvae were still found until the end of November. The first new colonies of the year appeared in the sample of 14 July and their number increased in August, but there was no evidence of very recent settlement in the sample of 22 September.

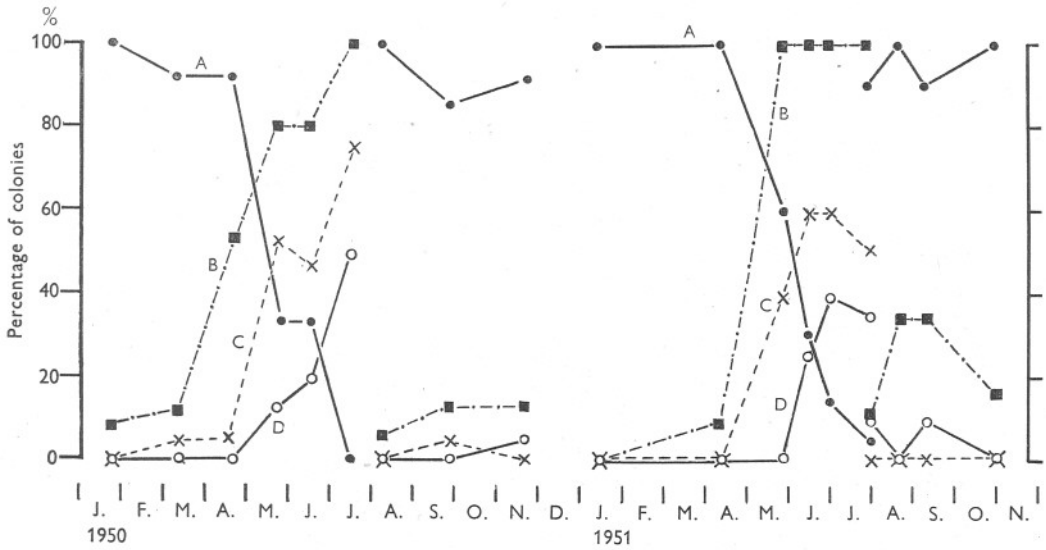

Fig. 11. The breeding activity of *B. schlosseri* in 1950 (left) and 1951 (right). A, colonies with no developing eggs and no larvae; B, colonies with visible gonad; C, colonies with developing eggs; D, colonies with larvae.

In 1951 the cycle of breeding in *Botryllus* was similar to that in 1950, but was later in most of its stages. This appears from a comparison of the graphs, for the two years, of the percentage of colonies with (1) gonad, (2) developing eggs, and (3) larvae. The period of larval settlement, however, was rather similar, starting some time between 2 July and 30 July, and extending certainly until the end of August and perhaps into September.

In both years the proportion of *Botryllus* colonies with larvae reached, at its maximum, only about 30–40% of the total examined. This is in marked contrast to the condition in *Diplosoma*, where 100% of the colonies examined in July and August had larvae. The probable explanation of the difference lies in the fact that the larvae of *Botryllus* develop in the atrial cavity of the zooid, from which they escape to the exterior when fully developed. The larvae of *Diplosoma*, on the other hand, completing their development embedded in the basal test of the colony, certainly escape less easily, and perhaps only with the dissolution of the parent colony.

Budding

Asexual reproduction by budding may, in general, occur at any season and a high proportion of all colonies examined had buds. The process appeared to be less active, however, in August and September, both in the old generation and in the newly established colonies. In *Botryllus*, as in *Diplosoma*, exhaustion follows the intense sexual activity of the summer, and reduced asexual reproduction is here the prelude to the death of the old colony. It is surprising that budding activity was not intense in the young colonies of August and September.

Summary

The populations of *Ciona intestinalis*, *Ascidiella aspersa*, *Diplosoma listerianum* and *Botryllus schlosseri* in the Old Dock of Ardrossan Harbour, Ayrshire, were studied in 1950 and 1951. Samples taken at intervals of about 4–7 weeks were used to follow growth, the progress of breeding, and the number and duration of generations.

In each species fastest growth was in May, June and early July. There was no growth in winter.

In all the species studied, except *Ciona intestinalis* for which sufficient data were not obtained, growth was several weeks later in starting in 1951 as compared with 1950.

Each species behaved as an annual, which settled as a larva, grew to sexual maturity, bred, and died, within a period of 12–18 months.

Breeding in each species was confined to a few months in the summer, and took place in the year following that in which the animals settled as larvae. *Diplosoma* may possibly also have bred at the end of its first summer.

In *Diplosoma* and *Botryllus* similar stages in the breeding cycle were several weeks later in 1951 than in 1950.

In *Ascidiella* and *Ciona* critical body sizes were found at which eggs and sperm began to fill the genital ducts. These species are protandrous; after the sperm starts to fill the sperm duct the body grows about 5 mm. before eggs pass into the oviduct.

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ON AMPULLARY TISSUE IN THE LARVA OF *POLYCLINUM AURANTIUM* MILNE EDWARDS

By D. B. Carlisle, M.A.

From the Plymouth Laboratory

(Text-fig. 1)

The larva of the compound ascidian *Polyclinum aurantium* Milne Edwards (1842) has been described by Berrill (1950), and his figures and description indicate a dissociation between the ampullae and epidermal vesicles. A re-examination of the larvae of this form shows that this is a false disjunction. This is most evident in the younger stages. Berrill describes (p. 46) a ring of eight ampullae, in the larva of *Polyclinum*, surrounding the anterior tip of the trunk, and 'numerous epidermal vesicles growing out or detached from two long strands reflected posteriorly' (p. 89).

The young larva, at the time when the yolk sac is incomplete ventrally and the atrial invaginations are just beginning (Fig. 1 *a*), already possesses fully formed sense organs and rudimentary suckers. In the epidermis there are two well-defined lateral ridges starting dorsally about one-third from the anterior end and running forward on either side of the suckers. They then run on each side of the mid-ventral line back to the base of the tail. Anteriorly these ridges swell, between and beside the suckers, to form ampullary lobe rudiments. There is no trace here of a ring of ampullae surrounding the anterior tip.

At a somewhat later stage (Fig. 1 *b*, *c*), when the larva is nearly fully formed, about four 'ampullae' have been developed on each side of the suckers by outgrowths from the ridges. Farther back the lateral ampullary ridges have budded off vesicles which remain attached to the ridges by thinner or thicker necks. In the intermediate region, dorsally and ventrally, between the ampullae and the vesicles are outgrowths which might receive either name—ampullae or vesicles. They are intermediate in position and size, and may be regarded as small ampullae or large vesicles. There is an even gradation from ampullae to vesicles. The arrangement at this stage is a pair of lateral ampullary ridges which start dorsally on either side of the branchial siphon, run forward and turn in a ventral direction beside the suckers; they then turn to run backwards on each side of the mid-ventral line and end with a dorsally reflected hook beside the base of the tail. These ridges bear outgrowths on short stalks, which, in the more posterior parts of the ridges are small and known as vesicles, but in the more anterior portions are large and known as ampullae. There is no morphological difference between them.

In the fully developed larva the lateral ampullary ridges are less obvious—perhaps more of their tissue has entered into the formation of vesicles and

ampullae; some of the vesicles seem to have severed their attachment to the ridge by an attenuation of the stalk, and the difference in size between the vesicles and ampullae is more pronounced, but the gradation between them is never quite obliterated.

I wish to thank the Director of the Plymouth Laboratory for facilities for research, and Miss P. Kott for her co-operation.

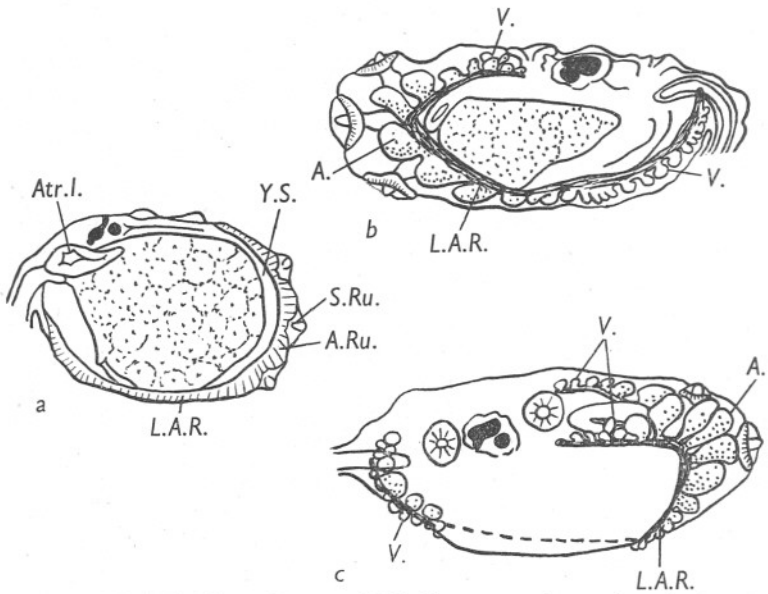

Fig. 1. Camera lucida drawings of larvae of *Polyclinum aurantium* to show the lateral ampullary ridges. *a*, a larva at the stage when the yolk sac is incomplete ventrally, from the right side; *b*, a nearly fully formed larva, from the left side; *c*, a larva at the same stage as *b*, a dorso-lateral view from the right side. *A.* ampulla; *A.Ru.* rudiment of ampulla; *Atr.I.* atrial invagination; *L.A.R.* lateral ampullary ridge; *S.Ru.* rudiment of sucker; *V.* vesicle; *Y.S.* yolk sac.

Summary

The morphology of the ampullary lobes and epidermal vesicles of the larva of *Polyclinum aurantium* M. Ed. (Tunicata) is described anew and the two structures are shown to be outgrowths, differing only in size and antero-posterior position, of two lateral ampullary ridges.

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OBSERVATIONS ON COMPOUND ASCIDIANS OF THE PLYMOUTH AREA, WITH DESCRIPTIONS OF TWO NEW SPECIES

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From the Plymouth Laboratory.

(Text-figs. 1–3)

There is still often confusion as to the identity of species of compound ascidians. This is due sometimes to a lack of published observations on the group, and sometimes to seasonal variations from the described condition. An attempt has been made to follow the growth and variation of the species in the Plymouth area throughout the year. During the course of these investigations all the species of the sub-order Aplousobranchia formerly recorded from this area were encountered, with the one exception of *Distaplia garstangi* Berrill. In addition, two new species were taken: a species of *Polycitor*, the first record of the genus in these waters, and a species of *Lissoclinum*.

L. argyllense Millar, recently described from the west coast of Scotland, was also taken from the Mewstone Ledge and from Salcombe.

No general attempt has been made to give synonymy or literature already adequately supplied by Berrill (1950) in his recent Ray Society Monograph on the Tunicata.

Conversations with Dr R. H. Millar of the Scottish Marine Biological Station at Millport, and with Mr D. B. Carlisle at Plymouth, have been most helpful and stimulating. My grateful thanks are also due to Mr F. S. Russell, Director of the Plymouth Laboratory, for his constant encouragement and advice.

Archidistoma aggregatum Garstang

Distribution. Previously taken from Salcombe and Plymouth Sound, it also occurs plentifully on the Mewstone Ledge, and so is probably present at other places around the coast.

The Colony. Berrill (1948, 1950) has described zooids 'a few millimetres long'. In June–July, when the colonies are well developed and may form carpets over the rock, the zooids extend up to 5–6 mm. in length. Clumps of two to six zooids extend from the sand-covered basal ramifying stolonial processes; the distal part of the vertical cylindrical clumps, which houses the

thorax of the zooid is, however, always quite free of sand and gives the colony its typical appearance (Fig. 1 C).

Growth of the colonies takes place especially from February until maturity of the zooids in August. There is a proliferation of the posterior abdomen into the basal stolonial connexions and subsequent swelling and development of new upright clumps of zooids in the colony (Fig. 1 A, B), or addition of zooids to any already existing clump. This is similar to the asexual type of budding found in *Morchellium* and takes place not only when accompanied by a resorption of the thorax in October and November, as Berrill has suggested.

Since the development of the zooids comprising a lobe of the colony may thus occur in the basal test and the subsequent vertical system arise from this, it is unlikely that single zooids arise from the basal test and later join together (Garstang, 1891), despite the sand embedded in the test between the zooids. This sand inside the colony is very sparse and is not an unusual feature in compound ascidians. The sand-free thoracic part of the test, alone, shows any tendency to independence of the zooids. Therefore it seems possible that there is no justification for even a subgeneric group Eudistoma. The larva of a species of the genus *Eudistoma* (Berrill, 1947*b*) is identical with the larva in this species.

Polycitor searli **sp.nov.**

This species is named after Mr William Searle, who has for 56 years been fisherman and collector for the Marine Biological Association at Plymouth.

Distribution. It has been taken as yet only from Duke Rock and New Grounds in Plymouth Sound.

The Colony. The colonies are inconspicuous, of 4–6 mm. height (Fig. 1 D). They occur in rock crevasses in water of 4–5 fm. The posterior part of the colony is sand-covered. Anteriorly the test is clear and glassy and the line between the sandy and clear part of the test is very abrupt.

From 4 to 12 zooids occur in each colony and open on the surface with separate 6-lobed branchial and atrial openings. The shape of the colony varies considerably from rather narrow cylindrical specimens to a more squat circular form, and occasionally there is a constriction where the sand-covered part changes to the smooth transparent test.

The Zooids. The zooids (Fig. 1 E) vary in colour from white to red and orange, and each occupies the whole length of the colony. The atrial apertures are all directed toward the centre, and there is no common cloaca, as in a clump of *Archidistoma*. Both the atrial and branchial apertures are six-lobed. There are four rows of stigmata with six to eight stigmata in each row. The oesophagus is long and narrow, opening into an elliptical stomach (Fig. 1 F) in the posterior part of the abdomen. The stomach is smooth-walled externally but internally has four folds as in *Clavelina*. The mid-intestine after running posteriorly and dorsally opens into the enlargement of the posterior intestine. Gonads are enclosed posterior to the stomach in the alimentary loop. There

are about ten pear-shaped testicular lobes, and a 3–4-celled ovary to the right of the testes. The testes do not extend posteriorly to the alimentary loop as in *P. vitreus* (Sars).

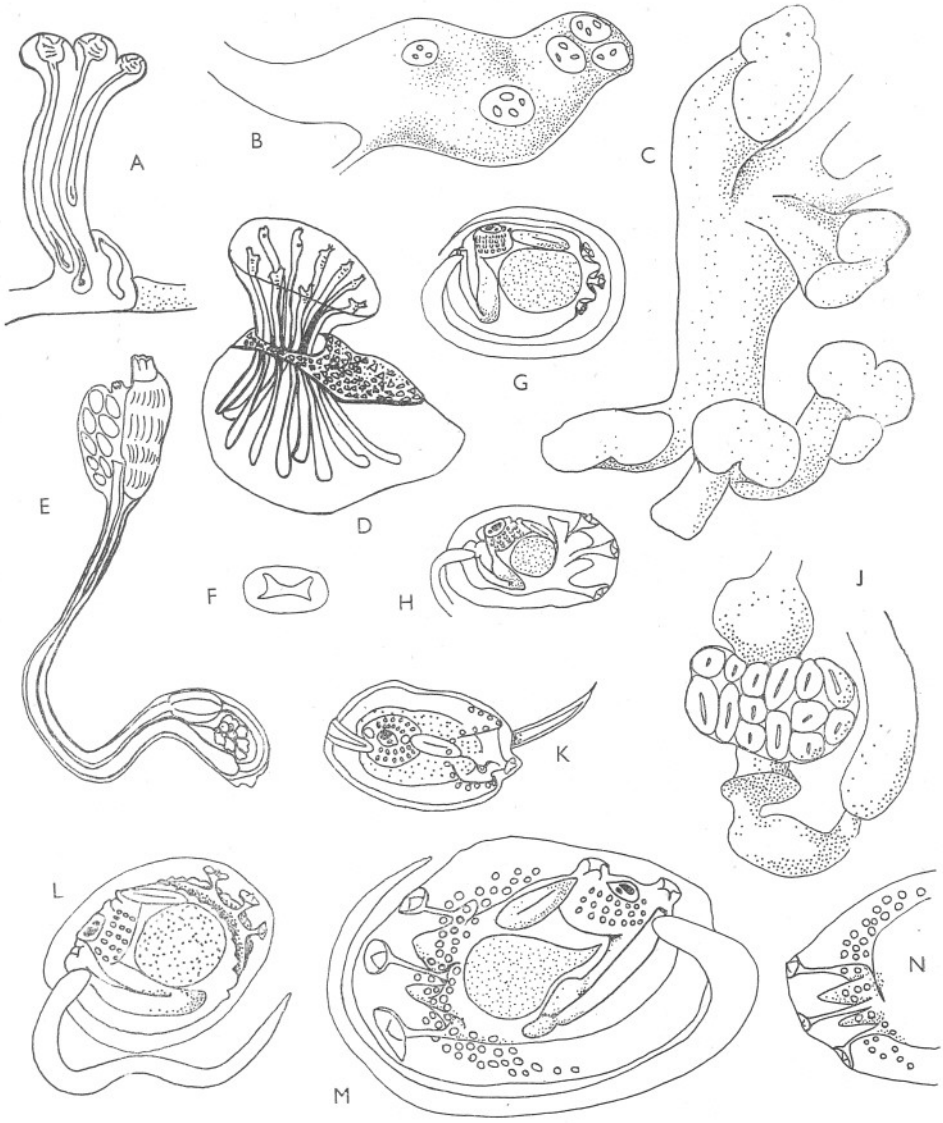

Fig. 1. A–C, *Archidistoma aggregatum*: A, section through a clump of zooids showing formation of a new zooid, ×4; B, zooids developing in a basal stolon, ×4; C, part of a mature colony, ×6. D–H, *Polycitor searli* n.sp.: D, colony, ×6; E, zooid, ×16; F, transverse ection through stomach; G, young larva, ×60; H, older larva, ×60. J–L, *Sidnyum turbinatum*: J, alimentary canal, ×50; K, larva, dorsal view, ×60; L, young larva, ×60. M, N, *Sidnyum elegans*: M, larva, ×60; N, anterior portion of older larva, ×60.

The Larvae. Larvae (Fig. 1 G, H) and developing embryos appear in a brood pouch at the side of the branchial sac in June, and are present throughout July and August. The brood pouch is formed by an enlargement of the distal part of the oviduct. The larvae are small, 0·36 mm. in length without the tail. There are four rows of stigmata, a 3-celled ocellus and an otolith, three suckers and the anterior part of the larva is separated off into a horn, bearing an ampulla between each two suckers. This is similar to the condition to be described for *Aplidium proliferum* (p. 74) and *Sidnyum elegans* (p. 70). In earlier larvae the yolk material projects farther forward and the ampullae are not differentiated. The tail is twice the length of the body.

Discussion. This is the first record of a species of *Polycitor* from British waters. The lack of knowledge on its distribution is possibly, as with others, due to its small size and inconspicuous appearance. Due to the delicate and glassy nature of the test the colony is practically invisible to the naked eye, especially if the colony has been damaged and the zooids, as often happens, have been retracted into the basal part of the test. The species differs from others of the genus by the small size and delicacy of the colony and zooids. *P. vitreus* (Sars) and *P. crystallinus* Ren. are, however, the only species to have been described with any certainty. Berrill (1948) has described the larva of *P. vitreus*, but apart from the length of the tail and the number of rows of stigmata it is not similar to the larva of this present species. Nor is the brood pouch of *P. searli* constricted off in the same way as the brood pouch of *P. vitreus* (Sars).

The type specimen is in the British Museum (Natural History), South Kensington.

Distaplia rosea Della Valle

This is a common species which is extremely variable in shape and colour, ranging from pink to purple and brown. Colonies may be sessile on a spreading base or stalked. Sometimes the stalks are branched, and several stalked colonies may arise from a common base. The larvae appear in August, two to four in each brood sac. The ampullae associated with the suckers vary in each larva, and occasionally are reduced to the condition described by Berrill for *D. garstangi*. No specimens of *D. garstangi*, as characterized by a smooth stomach, were taken; but since one of Berrill's (1947*a*) distinguishing characters was the shape of the colony and all specimens of *D. rosea* are not squat as he described but often vase-shaped (cf. *D. garstangi*), it is possible that the two may be synonymous.

Sidnyum turbinatum Fleming *nec* Savigny

This name was first given to a species of compound ascidian by Savigny (1816). Savigny describes his species, however, as having six branchial lobes. Milne Edwards, in 1845, described a genus *Parascidia* with eight lobes to distinguish it from *Amaroucium*; and in 1863 Alder included, in this genus

Parascidia, the species *Sidnyum turbinatum* (Fleming, 1828; Forbes & Hanley, 1848) under the names *Parascidia flemingii* and *P. forbesii*. Thus Fleming was the first to describe a species with eight branchial lobes, and the features of the species *Sidnyum turbinatum* as it is known to-day, but the name was actually preoccupied by Savigny whose definition does not distinguish the genus from *Amaroucium*, and certainly does not include the species Fleming and Forbes described under the same name. Therefore the genus is correctly *Parascidia* Milne Edwards, and the specific name *flemingii* Alder. Since, however, the genus is known widely to-day as *Sidnyum*, that generic name is proposed as a *nomen conservandum*: *Sidnyum* Fleming. The specific name *turbinatum* applies automatically to the type species of *Sidnyum* and would therefore be retained. (For other synonymy see Alder & Hancock, 1912, pp. 19, 20, under *Parascidia forbesii* and *P. flemingii*; and Berrill, 1950.)

The Colony. The colony varies considerably in different environments. The typical condition on the shore, where it is one of the highest ascidians in the intertidal area, is that of clumps of conical colonies each containing two to four systems of about six to ten orange-coloured zooids. Colonies are joined by basal stolons and fixed in small niches in the rocks. These increase in size from February to August, when they reach a height of about 20 mm. and measure 10 to 15 mm. across the top. In August the larvae appear, developing in a brood pouch in the distal end of the oviduct, and the posterior abdomen is cut off from the thorax which is then resorbed.

Other colonies dredged from deeper waters on the Mewstone Ledge (10–15 fm.) have a more fleshy test. Zooids in these specimens vary considerably in size within a colony and vary in colour from white to orange. Large colonies also reach a maximum size (in August) of 40 mm. in height and with a corresponding increase in the number of systems, but the smaller colonies have the same dimensions as those found in the intertidal area. It may have been these larger colonies (which have the same dimensions as *S. elegans*) which led Berrill (1950) to confuse the specimens with the latter species and to describe their larvae as of *S. elegans*. The larva of *S. elegans*, as described below, is entirely different.

The Zooids. It is the zooid which, with the larvae, distinguishes the species from *S. elegans*. The branchial siphon is 8-lobed and there are occasionally four red pigment spots around the siphon, as in *Morchellium argus*. The branchial sac has from seven to nine rows of stigmata, with nine to ten stigmata in each row. The stomach has from twelve to fifteen folds, in all degrees of proliferation, but never acquiring the mulberry-like form of *Morchellium*, and occasionally showing only complete folds. The proliferations occur first on the ventral and right sides of the stomach and anteriorly. There is a prepyloric expansion of the oesophagus, a posterior stomach, and oval mid-intestine, decreasing in diameter before expansion into the posterior intestine: these features of the alimentary canal are found in all species of

the genera *Aplidium*, *Morchellium* and *Sidnyum*, although they are not evident unless the gut is empty (Fig. 1 J).

The Larva. The young larva has the developing ectodermal ampullae supported on two ridges anteriorly, extending ventrally to either side of the tail and continuous dorsally; the three suckers are borne on a ridge between the developing vesicles and the tail coils in a groove to the left of the suckers, between these and the epidermal vesicles on the left side (Fig. 1 K, L). Later the tail is coiled around the body more loosely, the epidermal vesicles extend away from the body of the larvae, lose their connexions, and the whole larva acquires the characteristic appearance (cf. Berrill, 1950: *Sidnyum elegans* and *S. turbinatum* larvae). In the fully developed larva of 0·5–0·6 mm. there are thirteen to eighteen epidermal vesicles, and the row of vesicles from each side of the body meet dorsally. The anterior horn increases in length and in its degree of separation from the body as the larva develops. There is no specific distinction, therefore, in the two larvae described by Berrill (1950); he has described two different stages of the same species.

Sidnyum elegans (Giard)

Distribution. There are new records from Salcombe and Wembury on the shore, and Mewstone Ledge from 10 to 15 fm.

The Colony. Colonies are rounded, fleshy, from 20 to 60 mm. across, and about 20 mm. high. The larger colonies are sessile, and it is only the smaller ones which have a short, sand-covered, stalk. Berrill (1950) describes the colony as 'rose with milky patches'. The milky patches are due to the test and to the branchial and atrial siphons which are white; the rose colour is due to the branchial sac. The zooids are arranged along long common cloacal canals which, when several meet, form a common cloaca. In smaller colonies the systems are oval or elongate. The zooids are, however, closely packed in the test, and are much more dense than in *S. turbinatum*, therefore the systems are often hard to distinguish.

The Zooid. The zooid is larger than that of *S. turbinatum*. There are ten to twelve rows of stigmata with ten to fourteen stigmata in each row. The stomach is deeper, with eighteen to twenty-two folds which are only rarely broken or proliferated. The posterior abdomen varies immensely in length, as is usual with all species of this family.

The Larva. Larvae are present in the brood pouch (the distal part of the oviduct) from August to November, and therefore this is one of the longest breeding of the compound ascidians (excluding Didemnidae). The size is large (Fig. 1 M, N), almost 0·8 mm. in length when fully developed. The larva is very similar to that of *Aplidium nordmanni*, but slightly smaller. There are three suckers and between them two ampullary cones, convex dorsally where they embrace the base of the suckers. Later there is an anterior horn which separates slightly at its dorsal extremity and supports the suckers and ampul-

lary cones. The anterior horn extends varying distances dorsal to the origin of the most dorsal sucker. Sometimes there are extra ampullary cones between either the first and second or second and third cones. Plentiful epidermal vesicles which have lost their attachment with the rest of the larva are present anteriorly and arise from the ridge either side of the curled tail in the earlier embryo. These extend no farther forward than the base of the ampullary cones, unlike *A. proliferum*, where they occur around and anterior to the cones.

Morchellium argus (Milne Edwards)

The Colony. This is a common form in this area, and the appearance of the colony varies in the extreme throughout the year. Colonies described by Berrill (1950) represent only the mature forms found from the end of July to September. The thorax then resorbs and the colonies are left as flattened and rounded basal portions containing the posterior abdomina. New colonies develop in February (Fig. 2A) and consist of a central corm with a single system of about eight zooids and basal spreading stolons. The posterior abdomen strobilates into these spreading stolons which increase in size and cover a greater area; new zooids arise from the strobilated posterior abdomina and new heads appear in the spreading stolons (Fig. 2B). Strobilation of the posterior abdomen continues, and in May and June the colonies consist mainly of extremely swollen basal test (Fig. 2C) with a few rounded systems protruding. The colonies now appear as closely packed, pinkish, and very opaque, rounded masses covering large areas. In between each rounded mass, but hidden by them, there remain the connecting basal strands, themselves much swollen. Then in July the spread of the colonies stops, but they increase in height due to a growth of the zooids. There are now zooid-filled corms with sand-covered stalks which may reach a height of 150 mm. in favourable areas (e.g. Salcombe), but are usually only 2 to 3 cm. high. Larvae are then found in the brood pouches and later the thorax is resorbed and the colonies consist again of strobilated posterior abdomina. The process of asexual budding from the strobilated posterior abdomen is, therefore, in this species most exceptionally active throughout the year.

The Zooid (cf. Berrill, 1950, p. 95). The adult characters of the zooid are most consistent in this species and the stomach is quite distinctive.

The Larva. Berrill (1950) has described a larva for this species, but it is apparently undeveloped or an aberrant form, as none of the many specimens examined by me showed the same characters.

The larva (Fig. 2E) has four rows of stigmata in the branchial sac, an otolith and an ocellus, and a large yolk sac. Anteriorly, there is a horn bearing three suckers with two anterior ampullae between them (as in *Sidnyum elegans*). There are sixteen pairs of epidermal vesicles on either side of the body, arranged in a double row. In the younger larva each pair of epidermal vesicles arise from a single stalk on the anterior ridges on either side of the

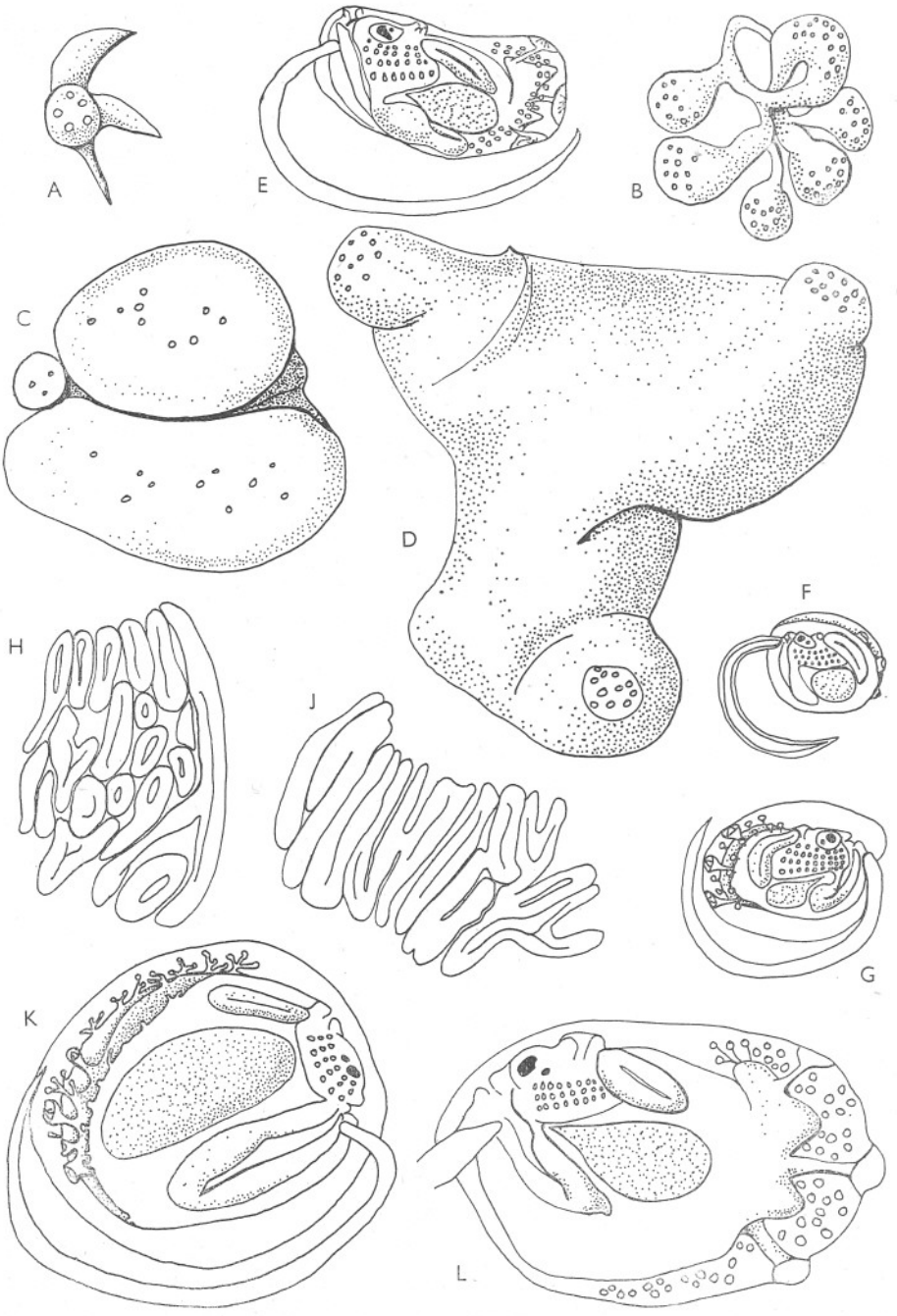

Fig. 2. A–E, *Morchellium argus*: A, young colony, February, × 1·2; B, zooids developing in spreading stolons, March and April, × 1·2; C, swollen basal stolons from which develop upright systems, May and June, × 4; D, upright systems developing, × 4; E, larva, × 60. F, G, *Aplidium pallidum*: F, young larva, × 60; G, older larva, × 60. H–L, *Aplidium proliferum*: H, '*proliferum*'-type stomach (D. B. Carlisle del.), × 100; J, '*nordmanni*'-type stomach, from the same colony as H (D. B. Carlisle del.), × 100; K, f. *nordmanni*, young larva, × 60; L, f. *nordmanni*, older larva, × 60.

coiled tail and the ridge bearing suckers and ampullae (as in *S. turbinatum*). The left and right rows of epidermal vesicles meet dorsally and lose their connexion with the body of the larva. The specimen Berrill has described is perhaps a young larva, since the suckers tend to develop more at the dorsal and ventral side of the cup which may account for the 2-lobed condition in Berrill's figure (1950, p. 96).

Aplidium pallidum (Verrill)

The Colony and the Zooid. The colony and the zooid are characteristic throughout the year.

The Larva. The larva 'is distinctive, partly because of its small size but more because of the absence of both ampullae and vesicles. Also notable is the more or less vertical position of the endostyle' (Berrill, 1950, p. 99). This is true of all the younger larvae found in the brood pouch (Fig. 2E), but in more fully developed forms (Fig. 2G), when the tail is less tightly curled around the larva, a single row of six to eight epidermal vesicles develop on each side of the body, as in *S. turbinatum* and *Morchellium argus*. These vesicles, however, unlike the larvae of *Sidnyum* and *Morchellium* maintain for longer their connexion with the rest of the larva. The larva is, as Berrill remarks, very small, which is to be expected since the zooids of this species are also small.

Aplidium proliferum (Milne Edwards)

There has been considerable confusion between this species, *Aplidium nordmanni* and *A. densum*. Thompson (1934) and Berrill (1950) have suggested synonymy between at least two of the species. The main differences for distinction exist by definition in the number of rows of stigmata in the branchial sac and the number and condition of folds in the stomach. It has been found, however, as will be shown in the discussion below, that there is no constancy, in any species, of any one of these characters; that the size of the zooids and the size and colour of the colony is the only dependable character for separation and that there are intermediate forms between these. And indeed *A. nordmanni* and *A. proliferum* were originally separated by Milne Edwards (1841) solely on the appearance of the colony. The proliferations of the stomach in the latter species, which have since been the distinguishing character, is not described (Milne Edwards, 1841, p. 287): 'l'estomac...est marqué d'une serie de plis verticaux'. It is proposed, therefore, that the species of *A. nordmanni* and *A. proliferum* Milne Edwards are forms of the same species, and that they should be known as *A. proliferum* f. *nordmanni* and *A. proliferum* f. *typicum* respectively, and that *A. densum* (Giard) is synonymous with the latter.

Aplidium proliferum (Milne Edwards) f. *nordmanni* Milne Edwards

The Colony. The colonies are thick (usually about 20 mm.) and sessile. Each colony has about three systems. The common cloaca of each system is not always central. Around the walls of the colony the test is red, but around the zooids the test is transparent and white, allowing the red of the branchial sac to show through it. The branchial and atrial siphons are white. The colony is thus in some ways similar to that of *Sidnyum elegans*, but the zooids are considerably larger and more sparsely distributed in the test.

The Zooid. Rows of branchial stigmata vary from nine to twelve with twelve to sixteen stigmata in each row. The stomach is large, varies in depth, and the number of folds (even within one colony) varies from twenty to thirty. Similarly, in their condition, the folds (again from zooids on the one colony) vary from complete unbroken through branching to completely broken up, when the stomach is given a 'mulberry' appearance (Fig. 2H, J). In a colony from Salcombe the zooids are slightly smaller, but the test is red on the outside and colourless around the systems of which there are many, and the colony is flat and investing. This, then, suggests the condition of the *typicum* form and may be considered intermediate between the two, but the numbers of folds and branchial rows conform with the *nordmanni* form.

The Larva. The fully formed larva (Fig. 2L) is the largest known and measures 1 mm. without the tail. There is an anterior horn as in *S. elegans*. It is distinguishable from the latter species only by extension farther anteriorly of the epidermal vesicles. Larvae are present in the distal part of the oviduct in August. In the younger larvae (Fig. 2K) the epidermal vesicles usually develop from the branched stalks on the anterior ridges either side of the coiled tail, as they do in all other species with epidermal vesicles. Here, as in *S. elegans*, however, there are many vesicles to each stalk, while in *Morchellium argus* there are two vesicles from the one basal stalk, and in *Aplidium pallidum* and *Sidnyum turbinatum*, a single vesicle only is borne on each stalk. In *Aplidium proliferum*, in addition to the lateral vesicles, there are similar vesicles borne on the anterior ampullae in the median line between the suckers, and these are usually very much better developed and persist longer than the lateral vesicles which sometimes do not appear to be present. The median ampullary lobes seem to be reduced in size as the larva grows older; this is also shown by Milne Edwards (1841) in his account of the development of *Amaroucium proliferum*. Therefore, it seems unlikely that the median ampullary lobes serve the same functions as the lateral paired ampullary lobes in other larvae, in consolidating the animal's hold on the substratum.

Aplidium proliferum (Milne Edwards) f. *typicum* nom.nov.

The Colony. Usually flattened ellipsoidal forms with yellowish zooids in a transparent test. The zooids are much smaller than those of *nordmanni* form,

the systems are less distinct and the zooids more closely packed in the test.

The Zooid. The branchial sac has from eight to eleven rows of stigmata with ten to sixteen stigmata in each row, and from twenty to twenty-five stomach folds. These figures cover the conditions found for *A. densum* (Giard).

The Larva. The larva is similar in every respect to the larva of the *nordmanni* form.

Didemnum maculosum (Milne Edwards)

Nothing further can be said on the synonymy of this species with Michaelsen's *D. helgolandicum.* Several authors (Hartmeyer, 1923; Millar, 1949) have pointed out its similarity, but unfortunately Hartmeyer died before he had completed a comparison of the two species.

Spicules. These are from 10 to 30 μ and are as figured by Hartmeyer (1923, Taf. I, fig. 19). They are indeed similar to those found in all the didemnid and tridemnid species of this area (except *D. gelatinosum*), and are of geometrical design. They are spherical, and as the diameter of each plane through the sphere increases, so the number of points of the star increases. Therefore, looking into any aspect of the sphere, there are 1, 4, 8 (in the greatest diameter) points. In larger spicules 1, 6 and 9 points, and in smaller spicules simply 4 and 8 points (Fig. 3A).

The density of the spicules varies, but there is never at any time the same density as in *Tridemnum alleni.* They are present mainly in the surface layer of test and in the layer of test separating the thorax from the abdomen of each zooid.

The Colony. Zooids are not arranged in well defined systems, but occur sometimes in rows, or irregularly scattered in the test opening into an extensive common cloaca. The surface test is therefore joined to the basal test by the zooids and by test material around the zooids.

The Zooid. The orientation of the thorax may be in a vertical position, at right angles to the surface, or at an angle to the surface. This latter condition is associated with a greater density of spicules, and colonies of this nature are often confused with *T. alleni.* There are six branchial lobes; the ventral three lobes tend to be longer than the three dorsal lobes, especially when the thorax is inclined to the surface. The endostyle is uppermost and the ventral lobes need this extra length to turn dorsally to the surface past the thickness of the endostyle. There are four rows of branchial stigmata with six stigmata in each row. The atrial siphon is a simple opening into the common cloaca. There is a lateral spicule-forming organ either side of the thorax. The abdomen is usually smaller than the thorax. The testis has seven coils.

The Larva. The larva has four paired anterior ampullae and two suckers. In the branchial sac there are four rows of stigmata by which the larva is distinguished from that of *T. alleni.* The anterior ampullae develop from the two ridges either side of the coiled tail and show a tendency to divide so that

occasionally five are found on one side of the body. In several colonies from Duke Rock the larvae have only three paired anterior ampullae. The larvae are present in the cloacal cavity and are found from March to August.

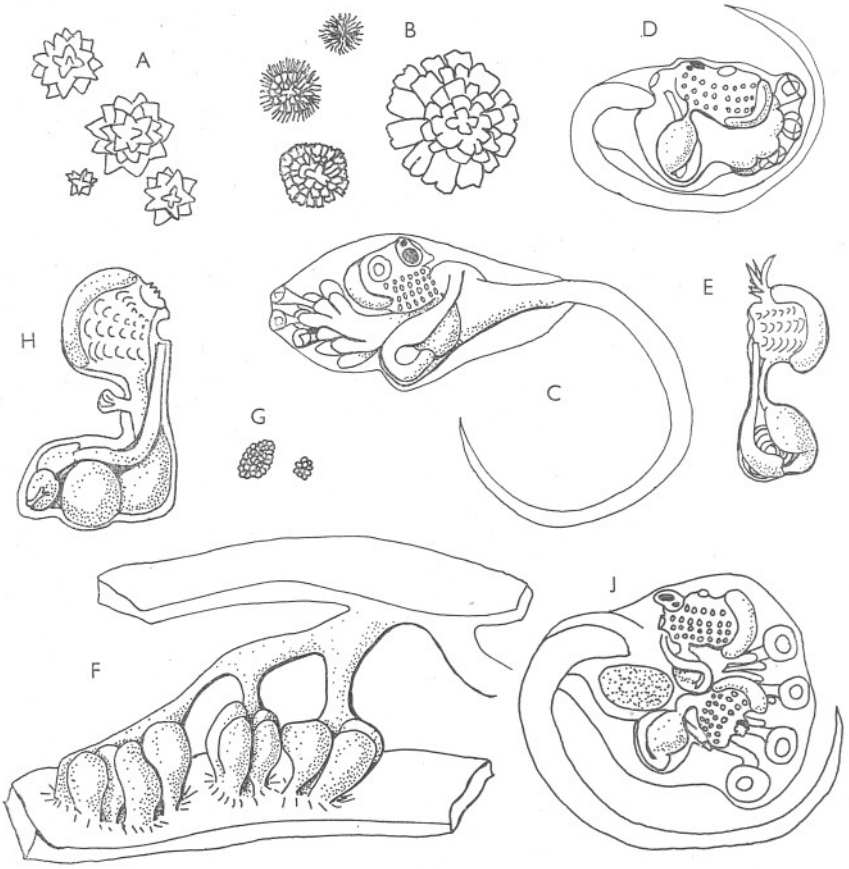

Fig. 3. A, *Didemnum maculosum*: spicules, ×400. B, C, *Didemnum gelatinosum*: B, spicules, ×400; C, larva, ×60. D, *Tridemnum tenerum*: larva, ×60. E, *Tridemnum alleni*: zooid, ×60. F–J, *Lissoclinum cupuliferum* n.sp.: F, portion of a colony showing strands of test containing zooids at the surface, ×30; G, spicules, ×400; H, zooid, ×30; J, larva, ×60.

Didemnum gelatinosum Milne Edwards

To the synonymy of this species must be added: 1912 *Leptoclinum punctatum* Alder & Hancock, Ray Society, Tunicata, VIII, Vol. 3, p. 49, and all synonyms given there, a conclusion based on the similarity of the described *L. punctatum* larva and development to those observed in the present species. The larval form of *Didemnum gelatinosum* Alder & Hancock (1912, p. 32) also conforms with the description given below.

Distribution. *D. gelatinosum* is found commonly in the intertidal zone in this area. The habitat, therefore, given by Berrill (1950) is not exclusive.

The Colony. The test is firm and gelatinous and the zooids are found in rows along an extensive common cloaca.

Spicules. These are present occasionally around the zooids, especially at the edges of the colony. The upper layer of test contains no spicules and when present they occur only in the upper part of the basal test around the base of the thorax. They are star-shaped, spherical and twice the size of the spicules of *D. maculosum* and vary from 20 to 60 μ. The rays of the larger spicules are indented, and increase in width toward the edge. The smaller ones consist of radiating spines and there are all degrees of mixing between the two kinds of ray (Fig. 3B).

The Zooid. The thorax is smaller than the abdomen and vertical to the surface of the colony. There are six branchial lobes which are small but the siphon is long. The atrial opening has no lips and the branchial sac has four rows of stigmata with six stigmata in each row. There are lateral organs opposite the second and third rows of stigmata. The abdomen is large, considerably larger than the thorax. The testis is a single lobe with the vas deferens wound eight times around it.

The Larva. These are found in the colony from March to August. They are similar to the larva of *D. maculosum.* There are, however, three suckers and usually five paired anterior ampullae, and the larvae of *D. maculosum* are smaller by about one-quarter of their length. Just as the number of anterior ampullae in *D. maculosum* is occasionally reduced to three pairs, so in *D. gelatinosum* it is rarely reduced to four pairs. Berrill (1950, p. 123) has shown a larva from a degenerating colony with four pairs of ampullae only.

Tridemnum tenerum (Verrill)

The Colony. The zooids are arranged in systems of double rows or irregular circles. The colonies are usually grey.

Spicules. The spicules are never as dense as those in *T. alleni*, or even *Didemnum maculosum*, and quite often are absent altogether. The colonies with spicules are but rarely encountered on the west coast of Scotland, and possibly the number of colonies with spicules decreases towards the north. Spicules in specimens from this area are identical with those of *Tridemnum alleni* and *Didemnum maculosum* (Fig. 3A).

The Zooid. The posteriorly directed atrial siphon is a unique and constant character. The thorax is larger than the abdomen and has eight to ten stigmata in each of the three rows, and a lateral spicule organ. There are six branchial lobes, longer than those of *D. gelatinosum.* The testis has twelve coils of the vas deferens.

The Larva. This is of the same size or larger than that of *D. gelatinosum* and has three suckers and four ampullae on each side. There are, however,

several features which distinguish it from all other didemnid larvae (Fig. 3 D). The thorax is wide with three rows of stigmata and about eight stigmata in each row, and occupies the greater part of the body. The atrial siphon is well developed in the larva, and takes the adult form before the ampullae are fully differentiated from the ridges on either side of the coiled tail. The thorax occupies such a large part of the larva that the ampullae do not attain the same length as in other species. Two or three eggs develop from the ovary at the same time so that there are immense numbers of eggs accumulated in the abdomen, where they develop into larvae.

Tridemnum alleni Berrill

Distribution. This species has been taken only from the Mewstone Ledge in the Plymouth area.

The Colony. Colonies are small, brilliant white. The species is in many ways similar to *Didemnum maculosum*, the arrangement of zooids in the colony is similar and on the surface of the colony there are sometimes spicule-filled cones covering the branchial opening. These also occur in *D. maculosum*.

Spicules. These are of the same types and size as found in *D. maculosum*, but more densely packed.

The Zooid. The branchial siphon has six lobes. Berrill (1947*a*) describes this species with eight branchial lobes, but although many zooids were examined no specimens with eight lobes were seen. As found to a smaller extent in *D. maculosum*, the three most ventral siphonal lobes are larger than the dorsal lobes. The difference in size of these lobes is often so great that the inclination of the thorax to the surface of the colony does not explain the condition (Fig. 3 E). The thorax is larger than the abdomen, but altogether the zooid is the smallest didemnid zooid known.

The Larva. The larva has two suckers and four pairs of anterior ampullae. The ampullae in older larva are long and extend forwards from a much reduced base, unlike the larva of *Tridemnum tenerum* but similar to *Didemnum gelatinosum* and *D. maculosum*. The larva is about half the size of that of *Tridemnum tenerum*, and slightly smaller than that of *D. maculosum*, but the main difference here is in the number of rows of stigmata.

The whole species is most markedly similar to *D. maculosum* except in the reduced size of the zooid, which may be due to the reduction of size of colony perhaps affected by the hydroids and weed to which it is attached; and the three rows of stigmata in the branchial sac may, in turn, be a result of reduction in size of the zooid.

Lissoclinum argyllense Millar

Distribution. This species was described from the west coast of Scotland (Millar, 1950), and has since been taken in the Plymouth area at Salcombe and from the Mewstone Ledge. Thus its distribution may be expected to extend down the west coast of England, in the Irish Sea and the English Channel.

The Colony, Zooids and Spicules are as described by Millar (1950). No *larvae* were present in the colonies in March or in April.

Lissoclinum cupuliferum **sp.nov.**

Distribution. One large colony has been taken from the Mewstone Ledge in 15 fm. The size of the colony, the constancy of zooids and larvae found therein, and the unique character of all these, seem to justify the description of a new species although this was the only colony taken.

The Colony. The colony is greyish white, spicule-filled and where the spicules are most dense the white colour predominates. It is thick, investing. The surface and basal test, containing the greatest density of zooids, are continuous and are joined to one another by strands of test material which branch toward the surface, and enclose, at the surface, groups of four to six zooids very close together. The cavity between the basal and surface test is therefore extensive and crossed only by these strands, a typical arrangement in *Diplosoma* and *Lissoclinum*. The zooids in each clump opening at the surface of the colony are, however, held closer together in their strand of test than in *Diplosoma*, and the test is altogether tougher so that the individual zooids are not visible until, with difficulty, they are separated from the test and from one another (Fig. 3F).

Spicules. These are present in the surface and basal test and in the parts of the connective strands immediately surrounding the zooids. They are absent from the area immediately surrounding the apertures. Spicules are spherical to oval with a number of rounded protuberances so that they appear 'mulberry' like (Fig. 3G).

The Zooid. There are six small branchial lobes and four rows of stigmata in the branchial sac each with eight to ten stigmata (Fig. 3H). The abdomen is greater than the thorax. The atrial aperture is a large and simple opening into the common cloaca. The oesophagus is long with one or two buds arising from it. There is a large spherical stomach followed by posterior stomach, mid-intestine and posterior intestine as in *Diplosoma listerianum*. There is a large one-egg ovary in mature forms. So far the zooid is similar to that of *D. listerianum*, but whereas in the latter species the testis has two distinct lobes which lie against the body wall, this species has a single testis conical in cross-section, which is grooved by the vas deferens as it curves around it posteriorly and enters the testis by two branches on the left side of the larva. This grooved condition of the testis is common in *Lissocliinum. L. argyllense* has a vas deferens which follows the same course but the testis is not grooved. The condition here is obviously related to that in *Diplosoma listerianum*, where the lobes have become separated.

The Larva. This provides the greatest distinguishing character between this species and *D. listerianum*. The larva (Fig. 3J) is large (0·6 mm.), and exceeded in size only by the larva of *Distaplia*, *Aplidium proliferum*, *Sidnyum*

elegans and *Polyclinum*. There are usually four suckers, but this number may be increased to five or six by the subdivision of one or more of the original four. The stalks of the suckers are bent at right angles at their distal end so that the suckers themselves face to the right. There are six anterior ampullae, three on each side. As in the larva of *Diplosoma*, however, budding is precocious, and the blastozooid is as well formed as the oozoid long before the larva is free swimming. The blastozooid forms on the right of the suckers: hence some explanation of their turning to the right. *Diplosoma* larvae are considerably smaller (0·4 mm. without the tail) than the larvae of this species.

The type specimen is in the British Museum (Natural History).

Diplosoma listerianum (Milne Edwards)

The Colony. The common test is always without spicules and is of a transparent greyish colour. The greatest variation lies in the density of zooids in the test. These are found in the branching strands of test connecting the basal to surface test, as in *Lissoclinum*, but the zooids are never arranged in such large or dense clumps. The number of zooids in a strand of test at the surface varies from one to three.

The Zooid. This is typical and invariable, being recognizable by its size and large branchial sac. The branchial siphon, however, has six lobes, not eight as in Berrill's figure (1950, p. 126, Fig. 38).

Taxonomic Characters in the Didemnid Species

In the accompanying classificatory key for the Didemnidae of the Plymouth area an attempt is made to eliminate some of the confusion existing in this family. Close resemblances may be observed between certain species of different genera, especially between the genera *Lissoclinum* and *Diplosoma*, which here are separated only by the presence or absence of spicules. This character is variable in other genera (*Tridemnum tenerum*, *Didemnum gelatinosum*) and hardly of sufficient weight to separate a genus. Other Pacific species of *Lissoclinum*, however, have more than two lobes in the testis and therefore, until relationships between these species and their east Atlantic counterparts have been compared, a union of the two genera is inadvisable. Resemblance between *Tridemnum alleni* and *Didemnum maculosum* is also very close.

Didemnidae

A. Zooids embedded in strands of test between surface and basal layers. Vas deferens not coiled.

I. With spicules. *Lissoclinum*

i. Testis with single lobe, ungrooved. Larva with three suckers. *L. argyllense* Millar

ii. Testis with single lobe, grooved. Larva with four suckers and precocious buds. *L. cupuliferum* n.sp.

II. Without spicules. *Diplosoma*
Testis two-lobed. Larva with three suckers and precocious buds.
D. listerianum (M. Edw.)

B. Zooids embedded in the basal surface test and not clumped together by strands. Vas deferens coiled.

I. With four rows of stigmata. *Didemnum*

i. Spicules usually absent. Larva with three suckers and five pairs of ampullae. Six stigmata in each row. Larva large. No atrial siphon.
D. gelatinosum M. Edw.

ii. Spicules present. Larva with two suckers and four pairs of ampullae. Larva small. Colony large. *D. maculosum* (M. Edw.)

II. With three rows of stigmata. *Tridemnum*

i. Spicules sometimes absent. Larva with three suckers and four pairs of ampullae. Eight to ten stigmata in each row. Larva large. Atrial siphon present. *T. tenerum* (Verrill)

ii. Spicules present. Larva with two suckers and four pairs of ampullae. Larva small. Colony small. *T. alleni* Berrill

Notes on the Larvae of Compound Ascidians

It has been generally assumed that the epidermal vesicles and the ampullae of ascidian larvae are of homologous origin and function. During the present study, however, it has been observed that the anterior ampullae are of two different types—(i) the most common, with a lateral origin from two anterior ridges either side of the coiled tail in the earlier embryo, and (ii) others which arise later in the embryonic life from the median line between the suckers. The ampullae with a lateral origin persist and assist the young individual to fix itself to the substratum. The ampullae of median origin are reduced and have disappeared entirely when the larva completes its free-swimming life. The lateral ampullae occur in *Archidistoma*, *Polyclinum* and in all Didemnidae. In *Sidnyum*, *Morchellium* and *Aplidium pallidum* the lateral ridges give rise to epidermal vesicles which, to a greater or less extent, finally lose their attachment to the bulk of the larvae and undergo all degrees of multiplication from the simple row of vesicles on each side in *Aplidium pallidum* and *Sidnyum turbinatum* to a double row in *Morchellium argus* and many rows in *Sidnyum elegans*. In *Polyclinum* Mr David Carlisle, of the Plymouth Laboratory, has observed that the posterior epidermal vesicles, supposedly borne on a posteriorly directed strand (Berrill, 1950), actually arise, as do these anterior epidermal vesicles, from a continuation backwards of the anterior ridges which embrace the coiled tail (Carlisle, 1952). He has also observed that there is a continuous gradation in these larvae from the anterior ampullae which are reduced in size dorsally, and ventrally and posteriorly become vesicles attached by thin stalks to the ridges. Therefore the homology of these structures is obvious. In *Polycitor*, *Distaplia*, *Aplidium punctum*, *A. proliferum* and *Sidnyum elegans* there are median ampullae which arise between

the suckers and are reduced in size as the larva grows. The presence of these is not exclusive to the presence also of the lateral outgrowths which also occur in *Sidnyum elegans*, but which are transitory and less well developed in *Aplidium proliferum*. In the latter species there is an interesting condition in which epidermal vesicles also arise from the median ampullae. Assumptions on ancestry may be made from these observations: the larva of *A. proliferum* is perhaps in this respect primitive, if the whole anterior part of the ancestral larva is capable of producing vesicles or ampullae which in smaller larvae are repressed completely or limited to the lateral ridges. This argument is prejudiced by the fact that species, in other ways considered primitive, lack this median growth of vesicles, although they may (e.g. *Polycitor*) have median ampullae whose function may have been to bear the vesicles. The fact that the median ampullae are transitory lends weight to this argument. No taxonomic importance can be given to the vesicles, however, since their condition and presence is so obviously affected by the size of the larva.

Summary

Seasonal variations in the form of the colony of aplousobranch ascidians in the Plymouth area are dealt with. Some of their larvae which were found to differ from existing descriptions have been re-described. Certain relationships between the larvae are discussed, especially in regard to anterior ampullae and epidermal vesicles.

A new species of *Polycitor*—*P. searli*—and a new species of *Lissoclinum*—*L. cupuliferum*—together with their larval forms, are described.

The species of the family Didemnidae and their relationships are discussed.

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INNERVATION OF THE HEART OF *LIGIA OCEANICA*

By J. S. Alexandrowicz

From the Plymouth Laboratory

(Plate I and Text-figs. 1–3)

During my stay at the Plymouth Laboratory in 1931 I had an opportunity of investigating the innervation of the heart of *Ligia oceanica*, and of making some experiments on the function of the ganglionic trunk which constitutes the main element of its innervation. In a short note describing these experiments (Alexandrowicz, 1931) it was stated that a description of the arrangement of the nerve elements would appear in a short time. However, on later examination of the preparations it seemed to be desirable to verify some of the findings, but the same animal was no longer available. Another isopod, *Mesidotea entomon*, with which I tried to continue the work during my visit to the Marine Biological Laboratory in Hel, proved to be much less suitable for this kind of investigation despite its larger size. Later events, unfavourable for scientific work, caused the loss of all my preparations and a further delay. It is only now, thanks to the chance of working again at the Plymouth Laboratory, that I have been able to resume my investigations.

I wish to record my gratitude to Mr F. S. Russell, F.R.S., for his kind help in preparing the manuscript.

Method

The observations recorded below were made on preparations stained with methylene blue, either by injection or by immersion of the tissues, or by a combination of the two methods (for details see Alexandrowicz, 1932, 1951). None of the methods or the various modifications, such as changing the concentration of the solutions, the temperature, or the pH have given fully satisfactory results since certain nerve elements, e.g. the ganglion cells in the main trunk, stain but rarely, while others, such as the nerves running alongside the aorta, stain fairly well in the part of their course near the heart but not at all at their origin from the ganglia in the anterior region of the animal. The latter phenomenon may be partly due to the effect of the secretion of the digestive glands which hinders the staining. It is therefore advisable when dissecting the animals to remove these glands as quickly as possible and to wash the tissues thoroughly with sea water. It is, however, difficult to prevent the organs in the anterior region of the animal from being contaminated by the secretion of the glands.

The heart can be exposed either from the ventral or from the dorsal side.

The first way is much the easier. It is sufficient to cut out the dorsal body wall and fix it to a paraffin plate with the ventral side turned upwards: the heart then appears in view partly covered by gonads which should be removed. In order to expose the nerve elements situated on the inner surface of the heart tube the ventral wall of the latter must be cut through along its median line. This should be done under a dissecting microscope with fine scissors, and it is not as difficult as it might appear owing to the fact that the walls of the heart are attached to the surrounding organs in such a way that they do not collapse. It is also very convenient both for staining and for observation that the ventral wall of the heart remains wide open after it is split, leaving the inside of the heart clearly visible. In *Mesidotea entomon* the heart tube is flaccid and it is much more difficult to split it and observe the inside.

A serious inconvenience to observation of the heart nerves in *Ligia* is due to chromatophores containing a pigment which does not dissolve in alcohol, so that the pigmented terga must be removed. This is the most difficult manipulation in the whole later procedure. It can be done best after the fixation when the preparations are being washed in water. The pigmented cells may also be present in the pericardial diaphragm and it is not possible to remove them at this spot. It is therefore preferable to choose animals which are less pigmented.

Access to the heart from the dorsal side is much more difficult, since it is necessary to cut out the terga one after another and to remove the dorsal muscles covering the heart. This is a tiresome operation, but this method exposes the dorsal surface of the heart and gives a view of its nerves from an additional aspect.

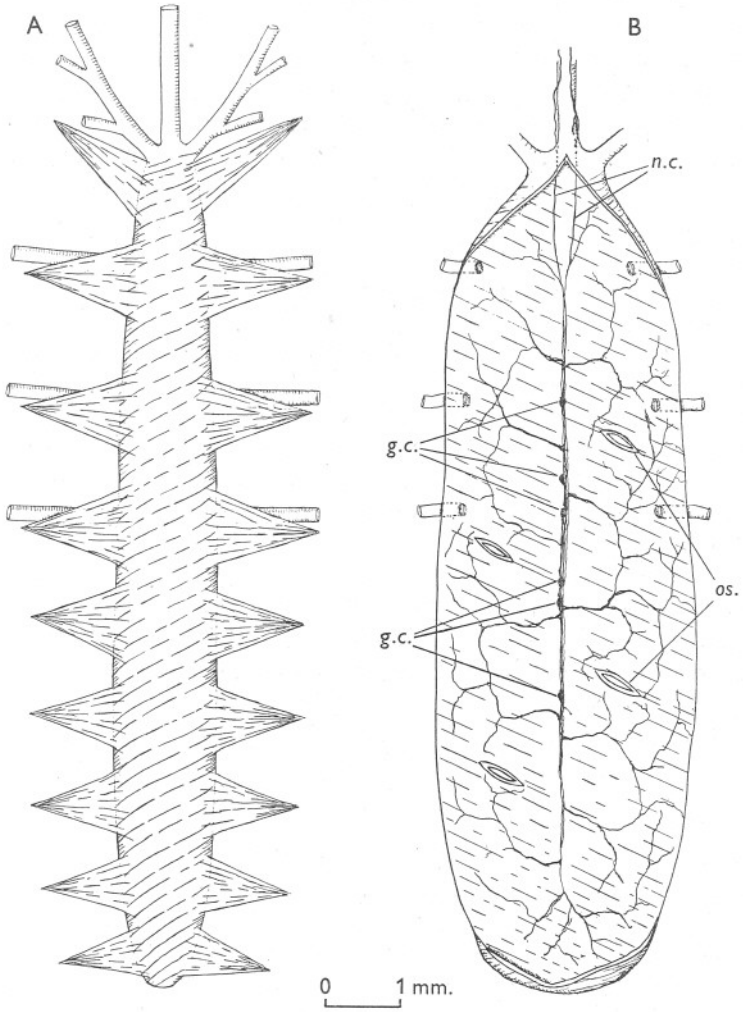
Observations

On the Anatomy of the Heart of Ligia oceanica

The heart of *L. oceanica* is shaped like a tube which in the largest specimens is about 15 mm. long. It is composed of a single layer of muscle fibres turning in right-handed spirals and of a thin coat of connective tissue (Text-fig. 1A; Pl. I, figs. 1, 3). On the inner side of the muscle layer there is a plexus of strands of connective tissue elements composed mostly of cells with highly refringent inclusions, presumably of fatty material (Pl. I, fig. 2).

The heart wall is pierced by four ostia situated alternately on either side as shown in Text-fig. 1B. Delage (1881) and Gordon Hewitt (1907) state that there are two only, but I have always found four of them, and they appear so distinctly in preparations in which the heart wall has been stretched (Pl. I, fig. 3) that there can be no doubt on this point. It may be mentioned that on the muscle fibres bordering the ostial orifices some cells can be observed staining deeply with methylene blue. These are mesenchymal elements differing in appearance from those arranged in the plexiform strands on the inside of the heart.

Nine arteries arise from the heart. One called the aorta runs forward in the mid-line, the others are four paired arteries situated in the anterior half of the heart as shown in Text-fig. 1 A.



Text-fig. 1. *Ligia oceanica*. A, view of the heart from the ventral side with its arteries and alary muscles; B, view of the heart cut in the mid-line of the ventral wall and stretched with the inner surface turned upwards. *g.c.*, ganglion cells in the main trunk (ganglionic trunk); *n.c.*, nerves connecting the local system with the central nervous system; *os.*, ostia.

The ventral wall of the heart is connected with the pericardial diaphragm, a horizontal septum reinforced by muscles of triangular shape which may be called 'alary muscles' as in insects. Connective tissue fibres attached to the dorsal and lateral walls suspend the heart tube in the pericardial sinus, thus

acting as the 'ligaments of the heart', a name given to similar elements in decapods.

As far as could be ascertained the presence of the alary muscles has not been mentioned in works on the anatomy of Isopoda, but on whole mounted preparations they can be clearly seen (Pl. 1, fig. 1). There are nine pairs of these muscles situated as shown in Text-fig. 1A, the first four a little more widely spaced from each other than the remainder. At the apex of each triangle the converging muscle fibres pass into a slender tendon attached to chitinous ridges of the anterior borders of the terga. Towards the basis of the triangle the muscle fibres pass into the connective tissue of the pericardial diaphragm.

Nerves of the Heart

As in other groups of crustaceans in which the innervation of the heart has been investigated (Alexandrowicz, 1932, 1934) there are in the Isopoda three systems of nerve elements connected with the heart, viz. (i) a local nervous system, (ii) nerves connecting the local system with the central nervous system, and (iii) nerves of the arterial valves (Text-fig. 1B). There are, moreover, nerve elements which, though not connected directly with the heart itself, have certain relations with its function, viz. the nerves of the alary muscles and the nerves of the pericardium and the heart ligaments.

Local Nervous System

The local nervous system consists of neurons, the cell bodies of which lie in a nervous trunk situated on the inner surface of the dorsal wall and which may therefore be called the ganglionic trunk of the heart. It runs in the midline for a great part of the length of the heart tube, being thickest along the middle region and tapering towards both ends. The whole of it is shown in Text-fig. 1B, and parts of it in Pl. I, figs. 1–4.

Ganglion Cells

I have already given brief mention of the presence of ganglion cells in the heart of *L. oceanica* in my paper of 1931. In 1934, S. Suzuki stated that in the heart of *L. exotica* six nerve cells are present, but as they were found on sections stained with non-specific methods this writer could not observe much of the innervation of the heart in this species as well as in two other isopods (*Tylos granulatus* and *Porcellio scaber*) investigated later (Suzuki, 1935; Tanita, 1939).

In methylene-blue preparations the nerve cells rarely stain and it is surprising to see no trace of them even in many preparations in which the nerve trunk and its branches are very well stained, although this whole system apart from a few elements of different origin is derived from the processes of these cells. They are six in all, situated at various distances from one another as shown in Text-fig. 1B. In methylene-blue preparations they appear in varying

numbers and only exceptionally are all of them seen together; therefore, in order to be certain that this number is correct I verified it on serial sections stained with ordinary methods.

Very few details of the cells can be observed, not only because they are visible merely after prolonged staining when the nerve elements begin to deteriorate, but also because of their situation in the trunk among the nerve fibres as seen in Pl. I, fig. 2. Such a picture as in Pl. I, fig. 4, showing a cell not covered by the fibres and not obscured by surrounding connective tissue, is very seldom met with. When a cell is only faintly stained a largish nucleus may be seen and fine dark blue granules in the cytoplasm. Sometimes the presence of a cell can only be recognized by these characteristic granulations. In a few cases fine nerve fibres have been observed in the vicinity of a cell, suggesting the presence of a basketwork around it, but this is much less distinct than in the ganglion cells found in other groups of crustaceans.

The processes of the cells stain badly, not only the shorter ones but also the axons at their origins. This feature, not uncommon in other nerve elements in arthropods, impedes the direct observation of the course taken by the axons belonging to each neuron of the ganglionic trunk; some evidence, however, may be gained from the composition of this trunk. It contains several fibres of stout calibre and a few thinner fibres, and it can be stated that in the middle portion of the trunk the stouter are six in number. This points to the conclusion that the axons of all the nerve cells are present here and it can also be assumed that the axons of the anterior cells are directed backwards and that those of the posterior cells run in the opposite direction.

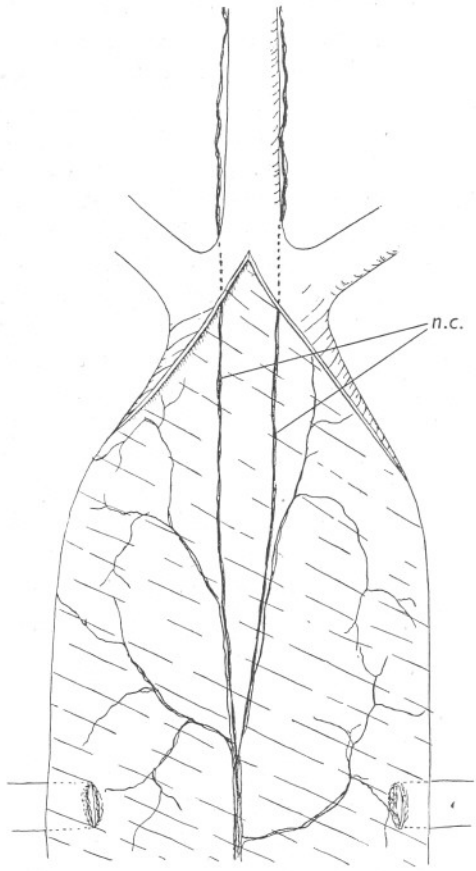
The ganglionic trunk gives off branches which pass on to the walls of the heart. Two of the branches originating in the bifurcation of the anterior end of the trunk take a symmetrical course, diverging obliquely from one another. These are the branches which are joined by the nerves from the central nervous system. The branches arising from the trunk distribute their fibres over the whole heart as shown in Text-fig. 1 B and Pl. I, fig. 1, and are interconnected by anastomoses. No special endings on the muscles can be seen.

It can be noticed that the branches springing from the main trunk are composed of more than one fibre each (Pl. I, fig. 4) and that these fibres originate from different elements of the trunk. It has not been possible to trace them further, but it may be inferred that in the same portion of the heart the muscles are innervated by more than one neuron of the local system and the presence of the anastomoses between the branches affords supporting evidence for this assumption.

Nerves Connecting the Local System with the Central Nervous System

The connexion of the local system with the central nervous system is established by a pair of nerves running alongside the aorta. They associate with the fibres innervating the valves of the aorta and this makes it difficult

to trace them, but on approaching the heart they separate from other fibres and, piercing the dorsal wall of the heart near its anterior end, pass on to its inner surface (Text-fig. 2). Meeting here the two anterior branches of the ganglionic trunk they run along with them into the trunk itself. These nerves appear to be composed of more than one fibre each and at some places three



Text-fig. 2. Anterior part of the heart of *Ligia oceanica* from a preparation stretched as in Text-fig. 1B. *n.c.*, nerves connecting the local system with the central nervous system.

of them could be seen, but owing to the size of the nerve elements in *Ligia* more exact observations are difficult. The further course of these fibres in the ganglionic trunk and their connexions could not be discerned. It may be inferred only that the finer fibres seen at some places in the trunk belong to these nerves. I was also unsuccessful in my endeavours to establish the origin of these elements. As the nerves accompanying the aorta can be followed up into the first thoracic segment and even beyond its anterior border it seems probable that they arise from the suboesophageal ganglion,

but, as I have said before, the staining of the nerves in that region has always been unsatisfactory. It is also not improbable that some fibres join these nerves during their course along the aorta, but no precise evidence could be established supporting this contention.

Suzuki believed that two pairs of nerves pass on to the dorsal wall of the heart from the abdominal ganglia and, moreover, reported the presence of small nerve cells on these fibres. I could not find such nerves in methylene-blue preparations, unless they are those which terminate on the pericardium and on the ligaments of the heart. As for the cells observed by Suzuki they were probably nerve cells of the muscle receptors, since these organs are also present in *Ligia* and are situated, as in decapods and stomatopods, on the dorsal muscles of the thoracic and abdominal segments at the place corresponding to that in which lie the cells seen by Suzuki.

Nerves of the Arterial Valves

All the arterial vessels arising from the heart are provided with valves the muscles of which receive their innervation from a special system of nerves.

The nerves for the valves of the aorta run down this vessel and originate somewhere in the most anterior ganglia of the central nervous system. Approaching the heart they divide into several branches, giving off numerous ramifications expanding on the valves which are apparently complicated in structure, having two main and two additional flaps.

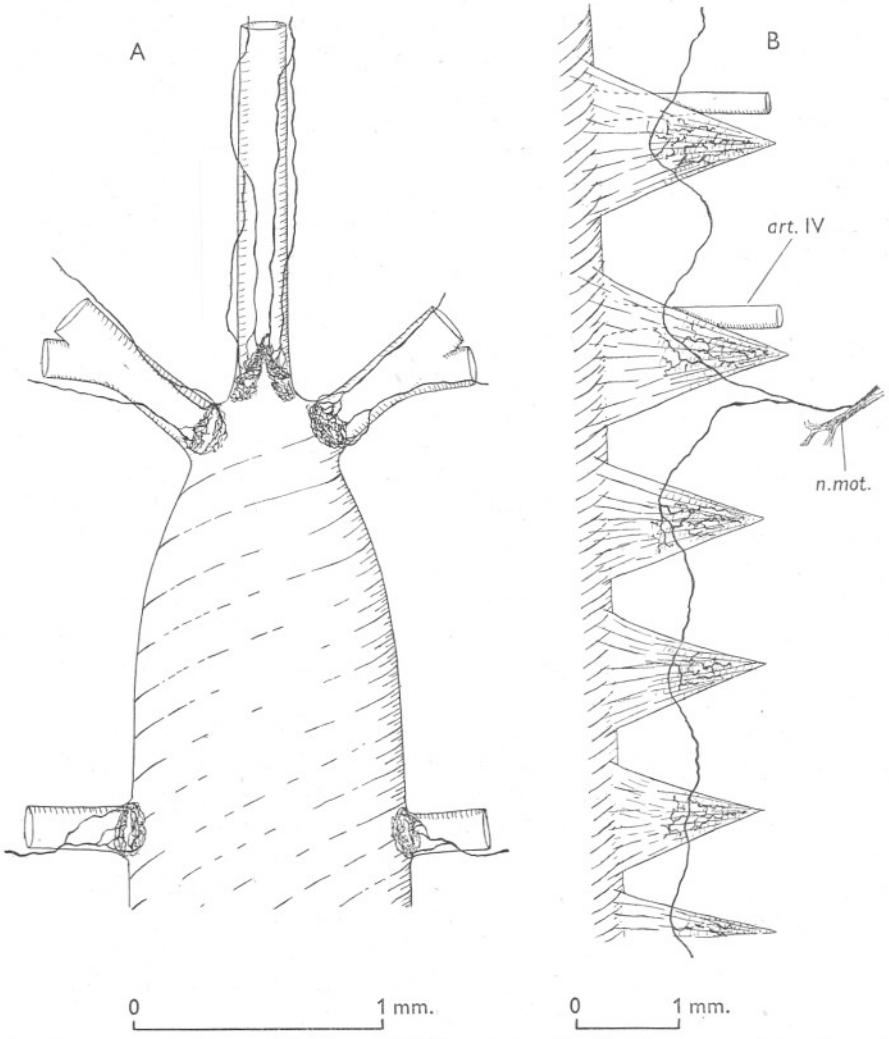
The valves of the first pair of lateral arteries receive their innervation from two fibres. One of them runs along the anterior wall of this vessel having an antero-lateral course, the other having a more transverse direction and, near the valve, lying close to the posterior wall of the artery (Text-fig. 3 A). Each of them associates more proximally with branches of motor nerves. The nerves for the valves of the following three pairs of arteries run from the ventral ganglionic cord with the motor fibres supplying the dorsal muscles of their respective segments. Owing to the different appearance of the nerves of the valves it may be assumed that they are of some special kind and that, apart from taking the same route as the motor fibres, they have nothing in common with the latter.

The distribution of the nerve fibres in the valves gives a characteristic picture of very abundant, densely arranged, tortuous ramifications. They are confined to the valves and when even nothing but the nerves are stained the outlines of the valves are well defined by their endings.

Nerve of the Alary Muscles

The nerve supplying the alary muscles runs from the central nervous system with the motor nerve of the dorsal muscles and, although its proximal course and its origin could not be traced, it may be assumed as for the nerves of the valves that it does not belong to the same category as the motor nerves

of the ordinary muscles. It comes near the heart behind the fourth (last) pair of arteries and bifurcates here into branches running in opposite directions (Text-fig. 3B). They have a characteristic sinuous course passing across the



Text-fig. 3. A, anterior part of the heart of *Ligia oceanica*, showing the nerves of the valves of the aorta and of the two first pairs of arteries; B, innervation of the alary muscles. *n.mot.*, motor nerve from which the nerve of the alary muscles is separating; *art.* IV, artery of the fourth pair.

dorsal sides of the alary muscles and sending to them short fibres ramifying in a pattern differing from that seen in ordinary muscles. The posterior branch is easily followed in its course to all posterior alary muscles. As to the anterior

branch I am not quite certain whether it supplies all the four anterior muscles. This uncertainty is to a great extent due to the presence in this region of the nerves of the arterial valves which complicate the picture. I tried to find out if the innervation of the valves is not in some way linked with that of the alary muscles, but no convincing evidence of such a connexion could be obtained.

Comparison of the Innervation of the Heart in Isopoda with that in other Crustacea

On comparison of the innervation of the heart of isopods with that of the two groups of crustaceans in which it is better known, i.e. in decapods and stomatopods, it becomes obvious that in all these animals it is built up on the same lines: there is everywhere a local nervous system consisting of neurons the cell bodies of which are situated in the heart itself and the processes of which spread over all its muscle fibres. The number of these neurons (six) in *Ligia oceanica* is smaller than in other species investigated (sixteen in *Astacus fluviatilis*, nine in other Macrura, in Anomura and Brachyura, sixteen in Stomatopoda). The nerves connecting the local neurons with the central nervous system show fewer details in *Ligia* than in the other Crustacea. In the latter these nerves, called 'dorsal heart nerves', have been found to contain two kinds of fibres one of which is represented by three thicker elements. As mentioned before, it was neither possible to state whether in *Ligia* they are of two sorts, nor what might be their number.

The innervation of the arterial valves has much in common in all these crustaceans, being in all of them independent of the local system, and in all animals the valves of the paired arteries have a segmental innervation, whereas the valves of the anterior aorta receive their supply from a nerve or nerves running along this vessel.

This nerve running down the aorta was the first to be discovered to have a relation with the heart in crustaceans. As it had been known for many years by the name of *nervus cardiacus*, I left this term when describing the innervation of the heart in decapods, adding only the word 'anterior' although it proved to end in the valve and not to pass farther into the heart. It may now seem inconsistent not to use the same term for the homologous elements in the Isopoda. The reason is that in the latter there are other nerves following the same route and which deserve even more the name 'anterior nerves' of the heart since they pass into its ganglionic trunk. They are most probably homologous with those nerves which in decapods and stomatopods were described as *nervi cardiaci dorsales*, but again, because of their topography, the latter term does not fit them. As for the term 'regulator nerves', also suggested by me previously, it may be objected that it is not advisable to derive a name of an organ from a function which is only probable. There is an obvious need of a unified nomenclature and for the adoption of terms which could be applied generally, taking into account the fact that homologous elements may have different topographical arrangements in different animals.

In both the Isopoda and the Stomatopoda the nerves of the arteries in any one segment are independent of those of the neighbouring segments. In *Ligia* even the valves of the same pair of arteries have completely separate nerves, whilst in *Squilla* they are connected by anastomoses.

The presence of the alary muscles both in isopods and stomatopods and their obviously similar function furthering the circulation in the big blood sinuses would suggest that there should be common features in their nerve supply. As to stomatopods, it has been stated that their alary muscles are innervated by branches belonging to the system of median connectives generally called 'unpaired nerve' (Alexandrowicz, 1952). Such median connectives are present in Isopoda too, but as yet I have been unable to find out their peripheral expansion and to state whether the origin of the nerves of the alary muscles can be traced to them. This problem, as well as that of the innervation of the pericardium and the heart ligaments, might perhaps be solved by further investigations.

As regards the function of the nerve elements in the heart of *Ligia* it could be shown in the experiments previously recorded that the action of the heart is ruled by the local nervous system, since by severing this trunk different rhythms of pulsations were produced in different parts of the heart. As to the nerves connecting the heart with the central nervous system, it may be supposed that they have a regulating function carrying accelerating and inhibiting impulses.

Summary

In the heart of *Ligia oceanica* (Crustacea, Isopoda) three systems of nerve elements have been distinguished: (i) a local system, (ii) nerves connecting the local system with the central nervous system, and (iii) nerves of the arterial valves.

The local nervous system of the heart is made up of six neurons which form a ganglionic trunk situated on the inner surface of the dorsal heart wall. The branches arising from this trunk expand over all the muscle fibres of the heart.

The nerves connecting the heart with the central nervous system run down the aorta and, piercing the heart wall, join the ganglionic trunk. The same route along the aorta is taken by the nerves of its valves. The valves of the four paired arteries are supplied by segmental nerves. The nerves of arterial valves have no connexion with the local nervous system of the heart.

It has been found that the pericardial diaphragm is strengthened by nine pairs of triangular 'alary' muscles. They have a particular innervation of their own, belonging neither to any of the systems of heart nerves nor to the category of motor nerves of the ordinary muscles.

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EXPLANATION OF PLATE I

All photomicrographs have been made from preparations of the heart of *Ligia oceanica* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol dammar. The heart wall is shown from its inner surface.

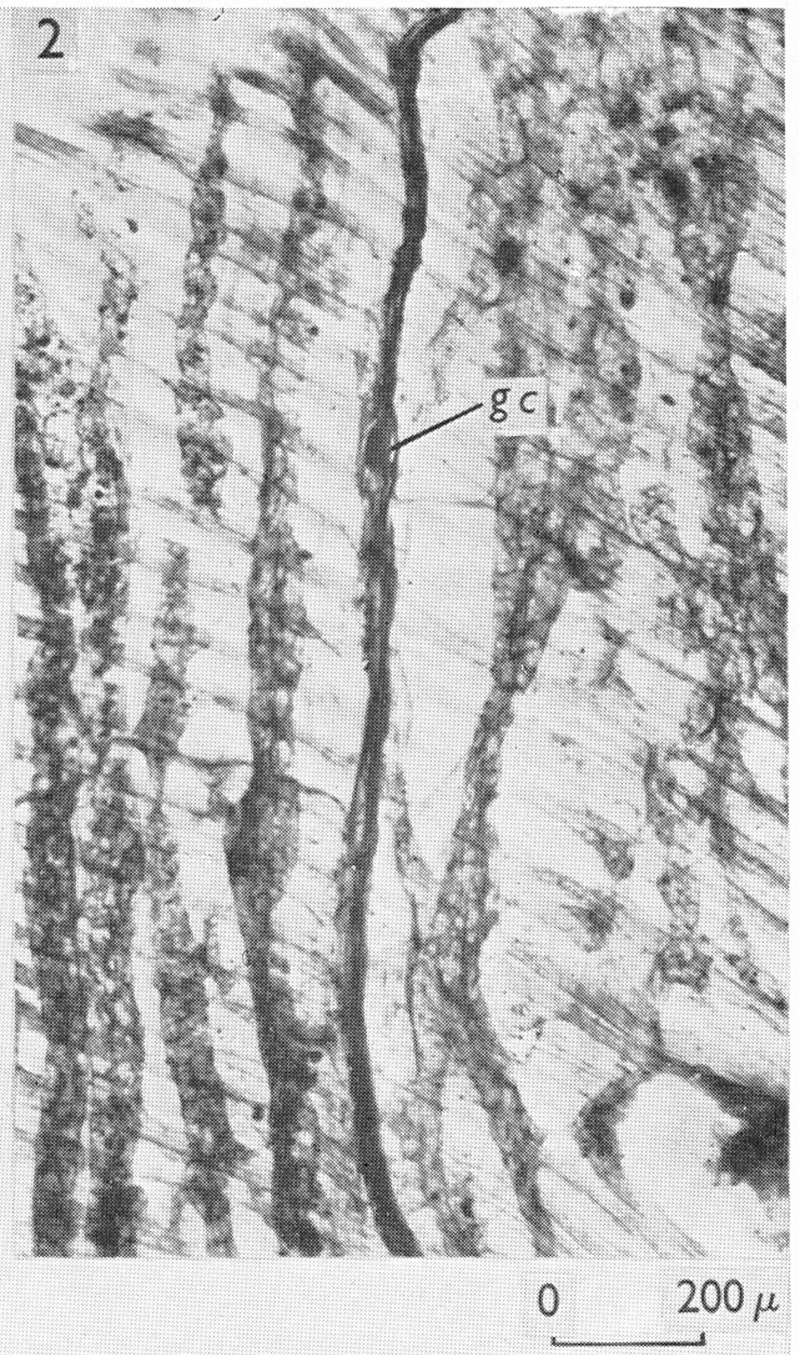
Fig. 1. Part of the heart with the ganglionic trunk and its branches. Two alary muscles are seen on the left side. The dark spots on the left side of the figure are the pigmented cells.

Fig. 2. The ganglionic trunk and strands of connective tissue elements. *g.c.*, ganglion cell.

Fig. 3. The ganglionic trunk and the muscle layer of the heart with an ostium. The fibres crossing the ostial orifice belong to one of the ligaments of the heart and are situated on the exterior side of the heart wall.

Fig. 4. Nerve cell of the ganglionic trunk. The nerve branching from the trunk has two roots proving its composition of two fibres.


1
0 500 μ


2
gc
0 200 μ

3
0 200 μ

4
0 50 μ

ON THE USE OF ANTIBIOTICS FOR ISOLATING BACTERIA-FREE CULTURES OF MARINE PHYTOPLANKTON ORGANISMS

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(Text-fig. 1)

In the course of some studies on the kinetics of growth and the biochemical activities of a marine diatom it became desirable to obtain bacteria-free cultures. The classical method of obtaining such pure algal cultures involves either repeatedly washing single cells in sterile medium or obtaining discrete bacteria-free algal colonies by growth on a solid medium. Both these methods have been widely applied to fresh-water species by Pringsheim (1946) and others, whilst Chu (1946) has used both methods with the marine diatom *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima*.

The bacteria associated with algae (especially diatoms) are normally very tenaciously attached, and some (e.g. the blue-green algae) may penetrate the gelatinous sheaths. This characteristic of the bacteria may make a washing procedure impractical. Chu found it necessary to make a minimum of six initial subcultures in sterile medium under conditions that favoured algal rather than bacterial growth (low organic content of medium, high light intensity and low temperature) before selecting single cells for washing in sterile medium. It seems probable, therefore, that the washing technique will remove free-living but not attached bacteria (cf. the results of Zobell & Allen (1935), who showed that more than half the bacteria in the sea will attach themselves to solid surfaces so tenaciously as to resist being washed off with running water). The plating technique does not seem to be widely applicable to marine species. Some thirty species have been tried during the present studies including representatives of the groups Cryptophyceae, Chrysophyceae, Chlorophyceae, Dinophyceae and Bacillariophyceae. Only two of the Chlorophyceae (*Chlorella* sp. and *Chlamydomonas* sp.) and one Bacillariophyceae (*Nitzschia closterium* f. *minutissima*) grew on Erdschreiber medium solidified with 1·5% agar. Numerous workers have investigated chemical and physical treatments in the hope of finding one that is bactericidal and which is tolerated by algae. Zobell has attempted to use heat, ultraviolet irradiation and various chemical bactericidal compounds (Zobell & Long, 1938). Of the latter acriflavine proved to be bacteriostatic in concentrations that did not

greatly interfere with the growth of diatoms. Similarly, Gerloff, Fitzgerald & Skoog (1950) have used ultraviolet irradiation to obtain bacteria-free cultures of the blue-green algae. Both these methods, however, depend upon a subsequent extinction dilution procedure.

The use of antibiotics seemed to offer a possible method. Fish (1950) employing exposure to massive doses of penicillin made some preliminary experiments with a green flagellate and an *Enteromorpha* culture. He was able to show that pre-treatment of the algae in 5000 units/ml. penicillin followed by subculture into a peptone broth (at a resultant concentration of 500 units of penicillin per ml.) prevented the growth of the bacterial flora for periods of up to 7 days and that the motility of the flagellate in Erdschreiber medium was apparently not affected by the presence of 1000 units/ml. of penicillin. In addition, by holding zoospores of *Enteromorpha* in a similar massive concentration of penicillin he was able to obtain a culture with no free-living bacteria in the medium. The flagellate was not subcultured in the absence of penicillin and subsequently tested for sterility nor was the algal material of the *Enteromorpha* submitted to sterility tests. In principle, however, the method seemed to offer a possible technique and these notes report some further work which has been directed towards extending the previous studies.

This work has been done in the laboratories of the Marine Biological Association at Plymouth. I wish to record my thanks to Mr F. S. Russell, F.R.S., for making freely available all the facilities of the laboratory, to Dr H. W. Harvey, F.R.S. and Dr Mary Parke for their continued interest and encouragement and for many helpful discussions and to the latter for supplying the cultures of phytoplankton organisms.

Experimental

Materials

Penicillin was obtained in a sterile condition as the crystalline sodium salt of 90% purity. Powdered sterile streptomycin (calcium chloride) was used. Solutions of required strength were made up in sterile water with aseptic precautions immediately before use.

Bacteriological Methods

Several media have been used for sterility testing. Their composition is detailed below.

Peptone sea water: 0·5% bacto-peptone, 0·01% ferric phosphate dissolved in 75% sea water.

Peptone sea water agar: as for peptone sea water plus 1·5% powdered agar.

Casein sea water agar: 0·05% bacto-peptone, 0·05% soluble casein, 0·05% soluble starch, 0·1% (v/v) glycerol, 0·02% dipotassium hydrogen phosphate, 1·5% agar dissolved in 75% sea water.

The media were made up in aged filtered sea water diluted to 75% with distilled water and were sterilized by autoclaving for 30 min. at 5 lb. pressure.

Plate cultures were inoculated by pouring, care being taken to cool the agar to 42° C. before addition. All cultures were incubated at 22° C.

Phytoplankton Cultures

Throughout these studies, normal bacteriological techniques were used to prevent contamination. Organisms were cultured in either plugged 100 ml. conical Pyrex flasks or plugged 25 × 150 mm. rimless Pyrex tubes.

The *Nitzschia closterium* forma *minutissima* used in these studies was a subculture from Allen's original strain which had been maintained in Miquel medium. The other organisms were obtained by subculture from stocks maintained in normal Erdschreiber medium.

During these experiments the *Nitzschia* was maintained in sterile enriched sea water. This was prepared by diluting aged filtered sea water to 75% with distilled water and enriching with 30 mg. nitrogen (as sodium nitrate) and 20 μg. manganese (as manganese sulphate) per litre. This was sterilized by autoclaving for 30 min. at 5 lb. pressure. After cooling it was further enriched with 3 mg. phosphorus (as disodium hydrogen phosphate), 8 mg. silica (as sodium silicate), and 100 μg. iron (as ferric citrate: Rhoda, 1948) per litre. The latter additions were made aseptically from sterile solutions of the required strength.

Other organisms were cultured in sterile Erdschreiber medium prepared by enriching 75% sea water with 0·03% sodium nitrate and, after sterilizing as above, enriching aseptically with 0·003% disodium hydrogen phosphate and 5% (v/v) soil extract. The latter was prepared by extracting soil with an equal weight of water by heating in the autoclave for 30 min. at 10 lb. pressure. After clarifying the extract by centrifuging it was finally sterilized by autoclaving for 15 min. at 30 lb.

The Observations

At low concentrations penicillin is essentially bacteriostatic towards those bacteria which depend, for growth, upon the assimilation of glutamic acid rather than upon its synthesis. Such organisms tend to be Gram positive whereas the majority of marine bacteria are Gram negative (Zobell, 1946). The technique of exposure for short periods to massive doses of antibiotic seemed to presuppose that under these conditions it may exert some bactericidal effect. There is some evidence that penicillin exerts a killing effect and may disorganize the ribonucleic acid metabolism of the cell (for references on mode of action of penicillin see review by Peck & Lyons, 1951), but unfortunately the sterility tests used by Fish were conducted in the presence of a concentration of antibiotic that might, in any case, be expected to be bacteriostatic. Information was therefore required on the persistence of the

antibiotic effect of penicillin (or its breakdown products) in sea water. Varying amounts of penicillin were added to sterile peptone sea water and stored in the light at room temperature. Inoculation of these with 1 ml. of a crude diatom culture at intervals showed that after 16 days' storage the inhibitory effect on bacterial growth of the original additions of 100 units/ml. was still very considerable.

Bearing these results in mind the effect of various concentrations of penicillin on the growth in peptone sea water of the bacterial flora associated with a crude diatom culture was investigated. The results shown in Table I are typical of those obtained.

TABLE I. EFFECT OF PENICILLIN ON THE GROWTH IN PEPTONE SEA WATER OF THE BACTERIAL FLORA ASSOCIATED WITH A DIATOM CULTURE

Concentration of penicillin (units/ml.)	Days incubation before growth first visible (turbidity)
Nil	2
5	3
10	4
50	8
100	>21
500	>21
1000	>21

To tubes of peptone sea water were added various concentrations of penicillin. Tubes inoculated with 1 ml. of a crude culture of *Nitzschia closterium*. Observed daily and day when growth first seen in each tube (turbidity) noted.

Concentrations of 100 units/ml. and over inhibited bacterial growth in this medium for over 3 weeks. The correspondence between these results and those obtained by Fish on a sample of sea water suggests that pre-treatment for a limited period in high concentrations of penicillin has little additional effect and that the inhibition he observed is explicable in terms of the bacteriostatic effect of the penicillin present during his sterility tests.

The results with the higher penicillin concentrations offered some hope that the prolonged bacteriostatic effect would prevent survival of the bacterial flora without adversely affecting the diatoms. Samples of a vigorously growing culture of *Nitzschia closterium* forma *minutissima* were dispensed in plugged sterile tubes and amounts of penicillin added to give resultant concentrations of 50, 100, 500, 1000, 5000, and 10,000 units/ml. The cultures were kept in a north window. In those containing 5000 and 10,000 units/ml. diatom growth was obviously inhibited, the cells settling and finally loosing all colour. The diatoms in the lower concentrations grew well and showed no obvious difference from a control culture and were, after 16 days, still viable on subculturing to penicillin-free media. At the same time as this diatom viability test, one ml. samples of all tubes showing diatom growth were inoculated into (*a*) peptone sea water, and (*b*) peptone sea water agar. The results of this test

are shown in Table II and indicate the essentially bacteriostatic action of the penicillin. Thus, though 100 units penicillin per ml. is sufficient to inhibit all growth in peptone sea water for over 21 days, some bacterial cells are still viable and will grow on subculture to penicillin-free peptone sea water. The negative results obtained in peptone sea water on inoculation with samples from the diatom culture which had contained 1000 units/ml. might be due to sufficient residual antibiotic activity having been carried over with the inoculum. On the other hand, on reinoculation of this tube some growth was obtained. Moreover, these penicillin concentrations yielded positive results when samples were used to inoculate peptone sea water agar. The same

TABLE II. EFFECT OF PENICILLIN ON THE SURVIVAL OF THE BACTERIAL FLORA ASSOCIATED WITH A GROWING CULTURE OF *NITZSCHIA CLOSTERIUM*

Penicillin concentration in diatom culture (units/ml.)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+ (Heavy)
100	+	+ (Heavy)
500	+	+ (Slight)
1000	−	+ (Slight)

1 ml. quantities of a diatom culture which had grown for 16 days in the presence of stated amounts of penicillin inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water agar (P.S.W.A.).

dilution factor was involved in this case and it seems most probable that the prolonged bacteriostatic effect of the penicillin is sufficient to prevent survival of the majority of the bacterial flora which will grow easily in peptone sea water. Some cells of the flora survive and continue to grow on transference to a penicillin free media. These 'resistant cells' seem to be culturally different from the bulk of the flora in that they will not easily grow in peptone sea water but do so in an identical medium solidified with agar.

A similar series of experiments with streptomycin showed that this antibiotic was not as effective a bacteriostatic agent for the bacterial flora associated with a diatom culture as penicillin (see Tables III, IV and Fig. 1). It was, moreover, more inhibitory to diatom growth, 500 units/ml. producing a marked inhibition. A comparison of the gross cultural characteristics of the 'streptomycin resistant' bacteria on peptone sea water agar showed a marked predominance of orange- or brown-pigmented colonies. In contrast, these organisms were sparsely distributed amongst the colonies that survived prolonged penicillin exposure. This suggested that the two antibiotics might be active against different sections of the bacterial flora and the possibility of the use of mixtures of the two was therefore investigated. A vigorously growing culture of *Nitzschia closterium* forma *minutissima* was dispensed in

TABLE III. EFFECT OF STREPTOMYCIN ON THE GROWTH IN PEPTONE SEA WATER OF THE BACTERIAL FLORA ASSOCIATED WITH A DIATOM CULTURE

Concentration of streptomycin (units/ml.)	Days incubation before growth first visible (turbidity)
Nil	2
5	4
10	4
50	5
100	7
500	10
1000	11

To tubes of peptone sea water were added various concentrations of streptomycin. Tubes inoculated with 1 ml. of a crude culture of *Nitzschia closterium*. Observed daily and day when growth first seen in each tube (turbidity) noted.

TABLE IV. EFFECT OF STREPTOMYCIN ON THE SURVIVAL OF THE BACTERIAL FLORA ASSOCIATED WITH A GROWING CULTURE OF *NITZSCHIA CLOSTERIUM*

Streptomycin concentration in diatom culture (units/ml.)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+
100	+	+
500	+	+
1000	+	+

1 ml. quantities of the diatom culture which had grown for 16 days in the presence of stated amounts of streptomycin inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water agar (P.S.W.A.).

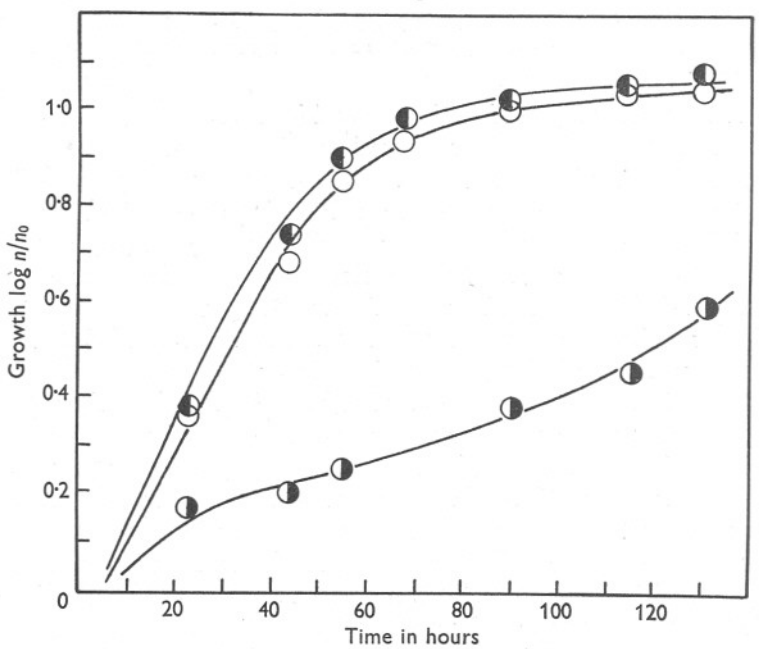

Fig. 1. Growth of *Nitzschia* in enriched sea water (for details see text) with additions of antibiotics. Growth plotted as log n/n_0, where n_0 = number of cells per ml. at zero time and n = the number at time t. —○—○—, control (no antibiotic added); —◐—◐—, 500 units/ml. penicillin; —◑—◑—, 500 units/ml. streptomycin.

plugged sterile tubes and penicillin and streptomycin added to give resultant concentrations of 50, 100 and 500 units/ml. of both. The tubes were illuminated in a north window for 16 days during which time diatom growth occurred (somewhat inhibited in the highest concentration), all concentrations finally yielding viable diatom cells on subculture to antibiotic-free medium. At the same time 1 ml. quantities of the culture were tested for bacterial growth by inoculation into (*a*) peptone sea water, and (*b*) peptone sea water agar. The results are shown in Table V.

TABLE V. EFFECT OF PENICILLIN AND STREPTOMYCIN ON THE SURVIVAL OF THE BACTERIAL FLORA ASSOCIATED WITH A GROWING CULTURE OF *NITZSCHIA CLOSTERIUM*

Antibiotic concentration (units/ml. of both)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+ (Slight)
100	+	–
500	–	–

1 ml. quantities of a diatom culture which had grown in the presence of stated amounts of both penicillin and streptomycin for 16 days inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water agar (P.S.W.A.).

The diatom cells grown in the presence of 500 units/ml. of both streptomycin and penicillin showed no bacterial growth after inoculation into both testing media. On reinoculation of these tests with 1 ml. of a crude diatom culture, only slight growth was obtained in peptone sea water and none on peptone sea water agar, and it therefore seems probable that the negative results obtained were due to residual antibiotic activity being carried over with the inoculum. Considerable numbers of diatom cells were still viable and good growth was obtained on inoculation into sterile enriched sea water without additions of the antibiotics. On repeating the same procedure with other samples of the same crude diatom culture, treatment with 500 units/ml. of the two antibiotics did not always yield completely negative sterility tests of the type shown in Table V. Regardless of this, the bacteriostatic effect of the antibiotics is always very considerable and providing a vigorous diatom growth has occurred the ratio of bacteria : diatoms will always have increased tremendously in favour of the diatoms. It has invariably been found possible to obtain a bacteria-free subculture of the diatom by inoculating numerous samples of sterile enriched sea water with very small inocula from the antibiotic treated culture (e.g. loop inocula of approximate volume 0·006 ml.). 1 ml. samples from a proportion of such subcultures normally prove to be bacteria-free. One such strain of *Nitzschia* has been maintained for several months by serial subculture in sterile medium. Regular sterility tests show it to be still bacteria-free.

A preliminary investigation has been made of the possibility of applying

the technique to other phytoplankton cultures. Of numerous organisms tested only one Chrysophyceae (*Chromulina pleiades* Parke), one Dinophyceae (*Peridinium trochoideum* (Stein) Lemm.) and three Chlorophyceae (*Chlorella* sp., *Chlamydomonas* sp., and *Stichococcus* sp.) would grow in the presence of 500 units/ml. of penicillin and streptomycin. By dropping the streptomycin concentration to 100 units/ml. the growth of two other Chrysophyceae (*Isochrysis galbiana* Parke, and Flagellate 25 Plymouth), *Prorocentrum micans* Ehrenb. and *Coccolithus huxleyi* (Lohm.) Kamptner occurred. No members of the Cryptophyceae tested showed any growth except *Hemiselmis rufescens* Parke, which gave variable results. A feature of these latter investigations was the reluctance to grow on subculture from the antibiotic-treated culture to ordinary Erdschreiber of those members of the Dinophyceae and Chrysophyceae which had produced good growth in the presence of the antibiotics.

Discussion

The preliminary investigations reported in this note indicate that the use of penicillin and streptomycin for obtaining bacteria-free cultures of diatoms depends upon their selective bacteriostatic action. It seems possible that the high concentrations used do exert some bactericidal effect, but some of the bacterial flora survives long exposure under these conditions. Success, therefore, depends upon some form of extinction dilution procedure, though under suitable conditions this can be reduced to the use of small inocula for subculture from the antibiotic-treated media.

It must be emphasized that sterility tests can give misleading results if there is a chance of sufficient antibiotic activity being carried over with the inocula, and in view of this such tests are best carried out on samples of subsequent subcultures in sterile antibiotic-free medium. In any case it is desirable that several successive subcultures in the absence of antibiotics should prove to be sterile before the culture is considered to be bacteria free. In addition, experience has shown the desirability of extensive sterility testing on a variety of media. Cultures have frequently been obtained which yielded no bacterial growth on peptone sea water agar but did in peptone sea water and vice versa. The casein medium has sometimes shown the presence of bacteria (and actinomycetes) which did not grow on either peptone sea water or peptone sea water agar. The latter medium (Zobell, 1941) seems, however, to satisfy the requirements of most marine heterotrophes and the use of both liquid and solid media for sterility testing appears more important than the use of media of widely differing nutrient composition.

The antibiotics used are not effective against moulds and actinomycetes and their addition in the quantities used causes an enhanced growth of such organisms. It is essential that the crude phytoplankton cultures used should be free of such organisms, otherwise the culture will be heavily contaminated

with mycelial growth. Vigorously growing algal cultures are, however, normally only slightly contaminated with such organisms and a mould-free algal culture can normally be obtained by the usual washing procedure prior to antibiotic treatment. It is of interest that very heavy mycelial growth which occurred in some flagellate cultures treated with antibiotics did not cause any marked inhibition of algal growth.

Throughout these studies it has been obvious that organisms which best survived the antibiotic treatment were those of a robust nature (e.g. *Nitzschia* and members of the Chlorophyceae). In general these organisms are known to be non-exacting in their nutritional requirements and will grow in Miquel Sea Water. Organisms which need soil extract for continued growth are the most affected by the antibiotics. The organisms of this class, which will tolerate the action of the antibiotics, frequently do not grow on subsequent subculture to sterile antibiotic-free media. In such it is possible that the antibiotics have directly affected the algae. It seems more probable, however, that such effects are due to an indirect action. If the more nutritionally exacting algae are dependent upon symbiotic bacteria for essential nutrients or growth factors, then control of the bacterial flora may ultimately limit algal growth. Such considerations make the direct application of the technique to a wide variety of phytoplankton organisms difficult. For any organisms with complex nutritional requirements, media other than Erdschreiber may have to be used in the absence of the symbiotic bacteria.

Summary

To develop a technique for the isolation of bacteria-free algal cultures, the use of penicillin and streptomycin for controlling bacterial growth has been investigated.

By using the selective bacteriostatic properties of the antibiotics it has been found possible to increase the ratio algal cells : bacteria markedly in favour of the algae. From suitably treated cultures it has been found possible to obtain subcultures of *Nitzschia closterium* forma *minutissima* and two members of the Chlorophyceae which produce no bacterial growth on a variety of media.

A preliminary investigation of the application of the technique to other algal cultures has been made. The possibilities and difficulties of this are discussed.

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UNDERWATER OBSERVATIONS ON THE SWIMMING OF MARINE ZOOPLANKTON

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(Text-figs. 1–4)

Many experiments have been made in the laboratory by different workers to study the swimming behaviour of plankton animals in relation to light and other factors. At the present time the special apparatus described by Hardy & Bainbridge (1951) is being used for a study of vertical migration, and other experimental work on the inter-relationships of zooplankton and phytoplankton (Bainbridge, 1949) is also in progress. Both this type of work and current theories (see Cushing, 1951) on the migrations of zooplankton make it of some consequence to learn the actual nature of the movements of these animals in the sea itself; and it is of special importance to compare their swimming behaviour in the different types of apparatus with their behaviour in the sea.

Dammant (1921), whilst hanging below a ship during salvage operations, had already made interesting observations on mackerel feeding upon members of the plankton and shown that a diver could see quite small plankton animals under natural conditions; and Beebe (1935), making descents in the bathysphere, had frequently remarked on the visibility of plankton, although his primary aim was the observation of larger forms such as fish.

Simple diving helmets as commonly used in warmer seas are not suitable for sustained observations in colder waters: a more protective and elaborate diving suit is required. Professor Hardy, with whom I was working under a Leverhulme Research Grant, consequently approached Messrs Siebe, Gorman and Company, and their assistance was most generously and freely given. They provided training in the use of various types of diving gear and made a loan for the greater part of the summer of that type of gear which was finally decided to be most convenient for the work.

I am deeply indebted to Messrs Siebe, Gorman and Co., whose provision of training and loan of equipment made the work possible; to Professor A. C. Hardy, who gave every encouragement and who made the time and facilities available; and to Muriel C. Morris, and the staff and boat crews of the Millport Marine Station upon whom my safety depended.

Equipment and Methods

The gear chosen was a modified compressed-air breathing apparatus together with a two-piece frog suit. The breathing apparatus had the normal face piece and a demand valve on the harness, but had a rubber air hose connected to a reducer valve on large (165 cu.ft.) compressed-air cylinders on the shore. The hose could be employed as a life-line and the standard cylinders were of greater capacity and were more easily replaced when used than the small cylinders usually carried on the diver's back. The hose allowed of quite sufficient movement for the type of observing desired and, with practice, swimming with foot flippers was possible, while more weights and the use of heavy boots allowed for walking on the bottom.

The work was performed at the Millport Marine Station and tours of observation of three kinds were tried. First, the observer simply walked down the shore to a suitable depth. This involved moving amongst rocks and in the seaweed zone and stirring up a good deal of detritus, with the result that viewing was sometimes made rather difficult. The site chosen for this method was sheltered and still. Secondly, the observer went down a ladder from Keppel Pier directly into deeper water with a hard shingly bottom. The water here was generally clearer but was without tidal movement only for very limited times during the day, whereas at the first site it was still for much longer. Thirdly, he was lowered to a suitable depth in a bosun's chair over the side of the research ship '*Calanus*', which was either anchored or drifting over much deeper water. All these methods could be attempted only when weather and tidal conditions were exactly right and this naturally greatly limited the scope of the work. A fairly clear sky, with sun if possible, little wind and slack water were found to be most convenient. Thick woollen clothing allowed tours of up to 40 min. duration in water of 6·5° C. without very great discomfort.

Under suitable conditions it was found easy to see and recognize many copepods. *Calanus finmarchicus* was visible up to 4 or 5 ft. away in clear water, while amongst the smaller forms those more opaque, such as *Temora* and *Centropages* spp., were visible up to a foot away or even more. The clearest views could be seen by looking about 10° either to the left or to the right of the bright patch formed by the sun on the surface of the water. In this manner a sort of dark ground illumination is obtained and even the most transparent forms stand out very clearly. By crouching or standing it is possible to follow vertical movements of about 10 ft. in extent while with the flippers greater distances are feasible, although the observer's movement is then more erratic and actual distances less easy to judge with accuracy. Chains of diatoms such as *Thalassiosira* and *Skeletonema* spp. and even individuals of larger species, such as *Coscinodiscus*, were frequently visible as occasionally were such flagellates as *Noctiluca* and *Phaeocystis*. Need for sufficient sunlight

naturally limits good viewing to the more surface waters and observations were not generally made at a greater depth than 20 ft. Altogether twelve descents were made over a period of about 5 weeks.

Observations

In the Clyde Sea area the spring brood of *Calanus* is often to be found in large numbers in the surface layers throughout the 24 hr. and is therefore particularly suitable for investigation. It does not, of course, follow that the behaviour of this brood, under these conditions of lighting, represents the normal behaviour of the copepod which is at other times of the year found in much deeper layers (at least 50 m. down); but it may be allowed as an indication of the probable type of behaviour at these greater depths, where observation is not yet possible.

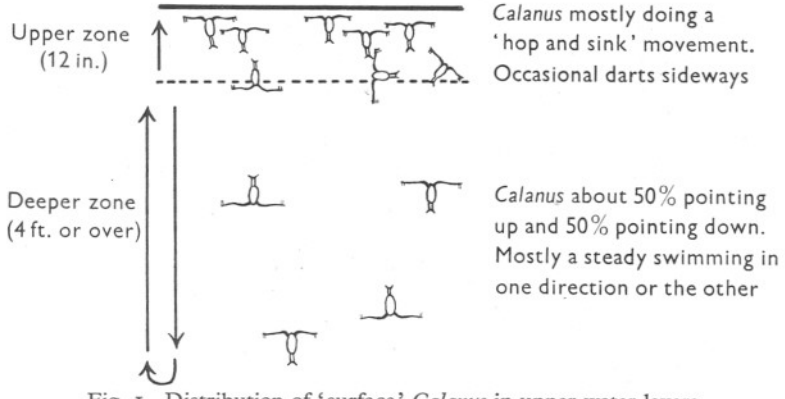

Fig. 1. Distribution of 'surface' *Calanus* in upper water layers.

Two zones of differing behaviour are clearly recognizable in the spring *Calanus* (Fig. 1). In the upper 12 in. of water there is a high concentration of animals (up to as many as 10/sq.ft. of surface) and there is a continuous gentle sinking and swimming up again vertically within this zone. Occasionally violent oblique or horizontal darts of several feet may occur, especially when two or more animals come close to each other; and a good deal of horizontal movement may result from a sort of bouncing on the underside of the surface film. The *Calanus* in this zone are often aggregated into groups of a dozen or so which sometimes swim round and round each other like a group of mayflies. Both in these little swarms and on a larger scale, like most other plankton animals, they are extremely patchy in distribution. Frequently, after the tedious and complex preparations for diving, one finds that there is no zooplankton in the area at all and on other occasions there may be relatively few swarms, or shoals, with large stretches of empty water between them. This patchiness was amply confirmed by the variable results of hauls with plankton nets.

Below the upper zone of 12 in. is a second one of indeterminate depth. Animals are scarcer in this zone and are more uniformly distributed. They appear in about equal numbers swimming either vertically upwards or downwards and seem to be going to or from the upper layer. I was able to follow (separately) two or three individuals which left the upper layer, turning over and swimming vertically and steadily downwards for 5 or 6 ft., where they again turned upright and hung motionless for a short time before swimming steadily up again to the surface layer. Others swam up and down, presumably to and from greater depths, but I was only able to follow the few individuals making more shallow excursions. It may be that this deeper zone is one of migration between two more concentrated zones, the one at the surface and the other either just above the bottom or at some optimum level about 50 to 60 m. down. Tow-nettings on these occasions certainly show animals in large numbers both at the surface and much deeper. It is reasonable to suppose that the whole population may be uniform and that there is a constant interchange between what are generally termed the 'surface' and 'deep' forms.

Fig. 2. Two types of swimming movement by *Calanus*.

An outstanding feature of all the populations observed was that, at any one time, as high a proportion as 50% would be quite motionless, most of these remaining so for long periods and many so delicately balanced as not to be even sinking in the water. On some occasions, especially when the sky was overcast, the whole population would be hanging motionless or drifting passively.

A clear horizontal migration by *Calanus* was seen only once. This occurred when there were very few specimens in view and the two seen, in the words of my diary, 'were just under the surface film and were moving horizontally by a series of oblique leaps and looping movements. They did not go deeper than about six inches. Horizontal progress was quite rapid and there were only very short motionless pauses' (Fig. 2). On the same occasion I watched another *Calanus* swim up about 3 ft., to the surface, in a rapid spiralling movement at an angle of about 30° to the vertical.

Observations on animals other than *Calanus* were only incidental, but the following points may be of interest.

Many smaller copepods were frequently seen in the upper 2 or 3 in. of water. These seemed to swim in an entirely random fashion with very little vertical and a good deal of horizontal movement.

Sagitta was common, generally exhibiting a very rapid vibration of the whole body, but showing little forward movement. I was able to make a record, on a piece of ground perspex, of the orientation of a specimen at 5 sec. intervals. This is reproduced in Fig. 3 and shows that, over a period of 1 min., the anterior end was generally pointing upwards but rarely vertically so.

Fig. 3. Orientation of *Sagitta* sp. at 5 sec. intervals.

Coelenterates and ctenophores were almost always present and their swimming movements and the effect on these of such crude stimuli as touch were easily observed. A small gadoid associated with a medusa (probably *Cyanea*) was watched for some time and the fish was seen generally above the bell in an almost vertical position. It repeatedly sank down towards the bell and then swam up again (Fig. 4). The coelenterate was gently pulsating and the pair gradually drifted obliquely upwards. The fish showed no alarm and made no attempt to conceal itself under the bell during observation.

On another occasion large shoals of saith about 2 in. long were watched. The most striking feature about them was the constant very vigorous movement of the whole body. They again appeared undisturbed by observation unless I waved my arms in the midst of a shoal.

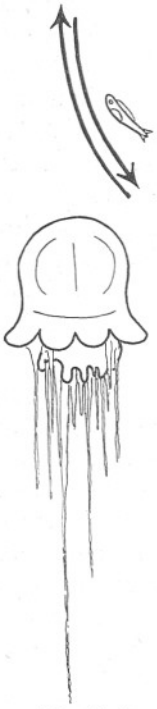

Fig. 4. Detail of swimming movement of small fish associated with a coelenterate.

Discussion

It can be said with fair confidence that few of the animals observed were disturbed in any apparent way by the presence of the diver. Those which did show a reaction to his presence were the larger bottom-living fish and crustacea. The type of breathing set chosen shares, with all but those utilizing oxygen and absorbent salts, the disadvantage of releasing used air into the water. This, however, escapes behind the diver and only when he is breathing out. It is quite simple to regulate breathing so that, during crucial minutes, there is no air escaping. When it does so it causes no noticeable movement of

the water and animals do not appear to react to it. The observations on the planktonic forms are therefore believed to be valid as records of their natural behaviour under the general environmental conditions mentioned. *Calanus* in particular showed a great preponderance of vertical movements in its swimming and it seems reasonable to deduce from this that the diurnal vertical migrations that it performs at other times of the year are a direct and straight swimming up or down through the water column, and not, as has been suggested by some authors, the result of random movements. A random type of swimming is, however, sometimes indulged in, particularly when near the surface film and when the population density is high. Those smaller copepods which are more often permanently at the surface seem more prone to swim in a random fashion. This behaviour may be a function of their very high population density.

However scientific and detached an account of the movement of these animals may be, it would be wrong not to make mention of the extraordinary beauty both of these forms and of their underwater surroundings. Experience of its richness and its strangeness would in itself be more than adequate reward for the discomfort and effort of diving.

Summary

Attempts to make underwater observations on the movement of plankton animals using a modified frogman apparatus are described.

With practice, observation of even the smallest planktonic animals, under suitable conditions, is perfectly feasible.

Most data were obtained about individuals of the spring brood of *Calanus* inhabiting the surface layers. This population occupied two zones—an upper 12 in. where movement was generally a 'hop and sink' one and occasionally random, and a deeper zone where individuals were found swimming in equal numbers vertically upwards and downwards.

Calanus is often to be found in small swarms or clusters of a dozen or so individuals.

Smaller copepods and younger stages in the upper layers frequently indulge in a random motion.

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STUDIES ON *CHAETOPTERUS VARIOPEDATUS* (RENIER). III. FACTORS AFFECTING THE LIGHT RESPONSE

By J. A. Colin Nicol

Zoologist at the Plymouth Laboratory

(Text-figs. 1–26)

Luminescence in *Chaetopterus* is an extracellular phenomenon and is due to the secretion of photogenic material by certain glandular cells in definitely circumscribed regions of the body. These gland cells are eosinophilic elements scattered singly or massed together in dense aggregations in certain regions. They are particularly abundant in the epithelium covering the distal surface of posterior notopodia, and in two glandular areas on the dorsal surface of the aliform notopodia. These two regions also display the brightest luminescence (Nicol, 1952*a*).

The gland cells producing the photogenic material are under nervous control in *Chaetopterus*, and secrete only as the result of stimulation. A preliminary investigation of light production in this animal has established certain facts about the nature of nervous regulation (Nicol, 1952*b*). With electrical stimulation it has been found that a single shock, above threshold strength, will induce secretion and luminescence, and that the amount of secretion, and hence luminescence, is augmented by increasing the number of stimuli. The luminescent powers of the animal are soon fatigued or exhausted, however, under repetitive stimulation, and after a few shocks the light tends to diminish. Moreover, it was also noticed that at low frequencies of stimulation the light response was confined to the region directly stimulated, but as the frequency was raised the response showed a tendency to spread to other parts of the body. This effect was most pronounced in the posterior region of *Chaetopterus*.

The present study is concerned with analysing in greater detail the factors concerned with regulation of the magnitude of the luminescent response. In particular, attention has been concentrated on the effect of altering the frequency and number of impulses. The regions found most favourable for study were the aliform notopodial light glands and the notopodia of the posterior region, and quantitative investigations were confined to these structures.

I am indebted to Dr W. R. G. Atkins, F.R.S., for the loan of a galvanometer and camera used in this investigation, and to Mr F. J. Warren for technical assistance. Part of the expenses incurred in this research was defrayed by a grant-in-aid of scientific investigations from the Royal Society.

Material and Methods

The methods of recording luminescence have already been described (Nicol, 1952*b*), and involve the employment of a multiplier photocell, and a sensitive galvanometer or oscilloscope. More use of the latter instrument was made in the present investigation. Electrical stimuli consisted of condenser discharges from an electronic stimulator, unless otherwise stated, and were delivered through platinum electrodes laid on the surface of the animal.

Observations

Luminescence in the Aliform Notopodia

The experiments described in this section consisted of stimulating the light glands on the aliform notopodia, and of recording the light which resulted. The effects of altering the frequency and number of stimuli were explored.

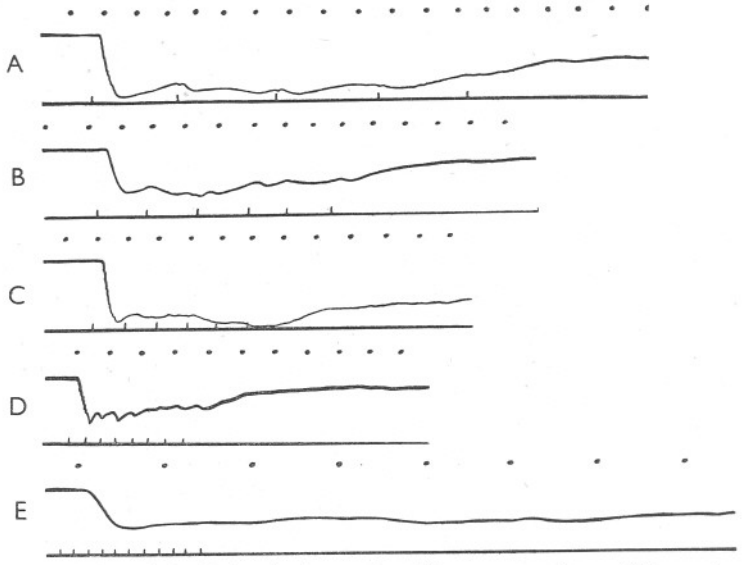


Fig. 1. Stimulation of the light glands on the aliform notopodia at different frequencies. Each record was obtained from a different specimen. A, stimulation 1 per 30 sec.; B, 1 per 15 sec.; C, 1 per 10 sec.; D, 1 per 5 sec.; E, 3 per 5 sec. Time scale above each record, 1 per 10 sec. Stimuli shown on bottom lines. In A–D separate inflexions can be seen, in relation to the separate stimuli. In E, however, the response curve becomes smoothed out. Oscilloscope records.

Following a suggestion made by Professor A. V. Hill, F.R.S., I have prepared a series of records to permit a comparison of the general character of the luminescent response with records of muscular contraction (Fig. 1). Stimuli were delivered at low frequencies of 2–36 per min., and the responses were photographed at low camera speeds. At the lower frequencies (Fig. 1 A–D)

a definite inflexion can be seen corresponding to each stimulus. These inflexions result from the discharge (secretion) of photogenic material. Above a rate of 30 per min. the separate inflexions tend to become smoothed out (Fig. 1 E) and a response curve somewhat resembling a curve of tetanic contraction is produced. At the low frequencies employed in this series of experiments the response to the first stimulus is maximal, and subsequent responses do not exceed the initial response. The rate of decay of luminescence, however, is very slow, and successive stimuli tend to maintain the initial level of light intensity. The reduced response to the second and succeeding stimuli is due to some kind of fatigue, and it is not possible to demonstrate summation at these low frequencies (below 1 per sec.).

In a previous communication (Nicol, 1952*b*) some observations were presented to show that by increasing the number of stimuli the height of the resultant light response was increased in consequence. This effect has been explored further to determine the nature of the summation process involved and the relative roles of frequency and number of impulses in determining the magnitude of the response. A very extensive series of experiments was carried out on the light glands of the aliform notopodia, and over 150 animals were used.

In order to have comparable records showing the differential effects of varying the experimental conditions, say the frequency of stimulation, it is necessary to be able to make repeated observations on the same animal. Fatiguing of the light response is very marked in *Chaetopterus*, however, and recovery has not been observed in any period of time that would be advantageous for experimentation. The experiments to be described consisted of stimulating an animal in a given way, allowing a rest period of 5 min. in which decay of luminescence could occur, and then stimulating once more at a higher rate or for a longer period. Periods of stimulation were kept as brief as the experiment would allow. These experiments have been carried out on the assumption that the response to a second stimulation, which duplicates the first stimulation, may be equal to the first response or less than the first response, but will not be greater. Furthermore, if fatigue should occur, it is possible that any augmented response, resulting from increasing some characteristics of the stimulation, may be sufficient to offset the fatigue effect, and reveal itself as an increment over the first response.

In general, it has been found that when a specimen is subjected to successive periods of identical stimulation, and the conditions of the experiment are maintained constant, the succeeding responses fall below the level of the initial response.

Effect of Increasing the Frequency of Stimulation

To determine any possible effect dependent upon frequency of stimulation, specimens were excited electrically for two periods, once at a low frequency,

and again after a period of rest, at a higher frequency. To eliminate the influence of varying the number of impulses, the duration of the stimulation period was regulated so that the same number of impulses (within 10%) was administered on each occasion. Impulse duration and voltage were kept constant. Frequencies which were used ranged from 1 to 33 per sec., and the number of stimuli varied from three to twenty-one in the different experiments.

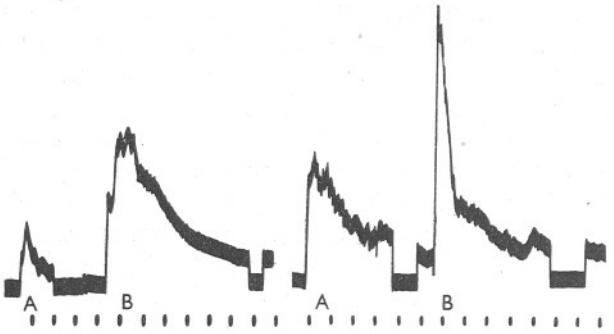


Fig. 2 (*left*). Stimulation of the light glands on the aliform notopodia. A, response to a single electrical stimulus; B, twenty-nine stimuli at 1 per sec.

Fig. 3 (*right*). A record showing the effect of stimulating the light glands at different frequencies. A, $4\frac{1}{2}$ sec. burst at 2 per sec.; B, 1 sec. burst at 9 per sec. Time scale below both figures, 1 per min.

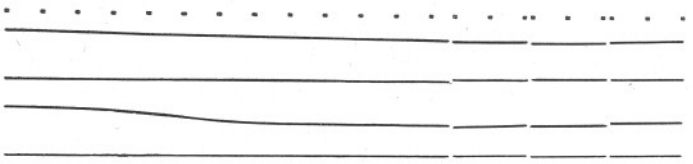


Fig. 4. Oscilloscope records of light produced by stimulation of the light glands on the aliform notopodia at different frequencies. Top record, 2 sec. burst at 5·5 per sec. Bottom record, 0·5 sec. burst at 23·4 per sec. Time scale, 1 per 2 sec. Interruptions in the records are intervals of 20 sec.

A number of definite and clear-cut positive results was obtained in which the second burst of stimuli, at a higher frequency, resulted in a much larger response than that evoked by the previous stimulation at a lower rate. The increment lay both in the initial response peak, and in the total amount of light produced. A typical record, obtained by photographing a galvanometer deflexion, is shown in Fig. 3. In this experiment the first stimulation consisted of a 4·5 sec. burst at 2 per sec., the second stimulation of a 1 sec. burst at 9 per sec. The peak intensity of light resulting from the higher frequency is about twice as great as that evoked by the lower frequency, but the total light is about the same in both. In other records initial and total light showed a pronounced increase (Figs. 4, 5).

Effect of Increasing the Number of Stimuli

By increasing the number of stimuli it is possible to increase the amount of light produced, but the amplitude of the effect varies with the specimen. An augmentation of the light response on increasing the number of stimuli first becomes apparent at a frequency of about one per sec. (Fig. 2).

Increasing the number of stimuli has the same kind of augmentative effect as raising the frequency, but the effect is more pronounced and is more easily

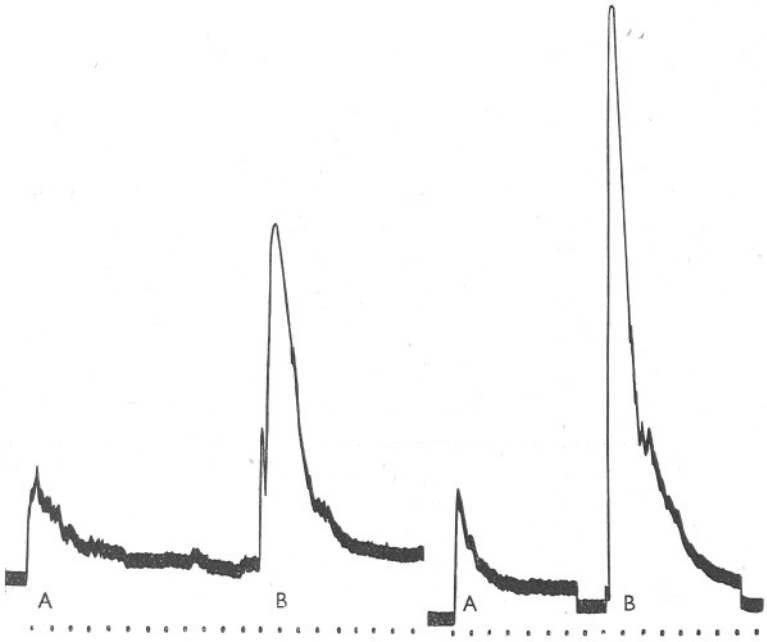


Fig. 5 (*left*). Galvanometer records of light produced by glands of the aliform notopodia. A, twenty shocks at 5·5 per sec. for 3·6 sec.; B, nineteen shocks at 32·2 per sec. for 0·6 sec.

Fig. 6 (*right*). A record of light produced by glands of the aliform notopodia after dissection and removal of the nerve cord. A, a burst of eighteen stimuli at 1·3 per sec. for 14 sec.; B, eighteen stimuli at 9 per sec. for 2 sec.

The upper half of the second response curve in both records (B) has been extrapolated on the basis of visual readings of galvanometer deflexions. Time scale, 1 per min.

elicited. The height of the response, that is the initial intensity, and the total light emitted, that is the amount of secretion, are both increased by prolonging the duration of stimulation (Fig. 7).

It is possible to consider two mechanisms that could be operating in these responses, one a simple effect of summation in the effector, and the other a facilitatory effect induced by the build-up of an excitatory state under prolonged rapid stimulation. Facilitation, if present, could be peripheral or central, since the preparation consisted of the whole animal. The data presented above are not adequate to allow a choice among these alternatives, and

towards this end the experiments described in the following four sections were carried out. These experiments consisted of testing the effects of various anaesthetics on the luminescent response; stimulating the nerve cord in contradistinction to the peripheral field; stimulating pieces of glandular tissue which were severed from the nerve cord; and comparing the effects of one versus two electrical stimuli. The results may be anticipated by stating that they favour the concept of a peripheral contractile mechanism controlling secretion, and capable of summation under repeated stimulation.

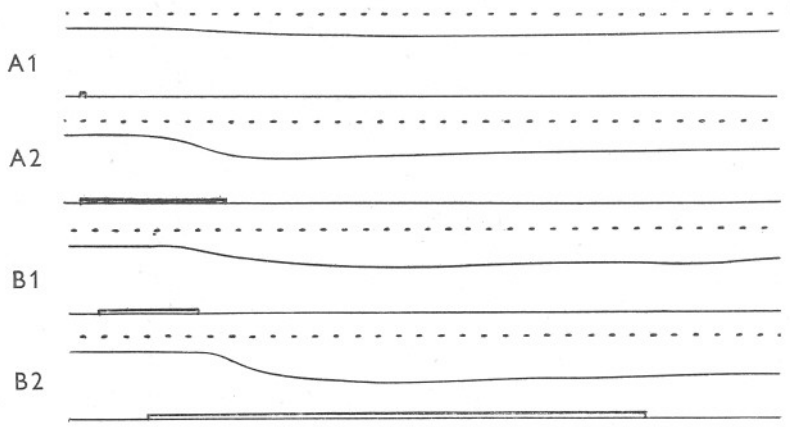


Fig. 7. Effect of number of impulses on light production in *Chaetopterus*. Oscilloscope records of luminescence in the aliform notopodia. A1 and A2 from the same animal, stimulated at a frequency of 25 per sec. A1, burst of 0·2 sec. duration; A2, burst of 5 sec. duration. B1 and B2 from another animal, stimulated at 2·2 per sec. B1, duration 4 sec.; B2, duration 22 sec. Stimulation represented by horizontal lines at bottom of records; time scale above each record 1 per sec.

Effects of Anaesthetics on the Luminescent Response

Several anaesthetics, that are known blocking agents of nervous tissue, were employed in an attempt to prevent nervous transmission without halting the activity of the peripheral effector concerned with the luminescent response. The anaesthetics employed were chloretone (acetone chloroform), MS. 222 (tricaine methansulphonate : Rothlin, 1932), cocaine, stovaine, procaine, eucaine, ether, and isotonic magnesium chloride ($MgCl_2.6H_2O$, 7·3%).

Stimulation of the Anaesthetized Gland Cells

In the following experiments the animals were anaesthetized for varying periods of time, and the light glands were then stimulated directly by placing a pair of platinum electrodes on them. The animals were then placed in running sea water for several hours to wash out the anaesthetic, following which they were stimulated as before. Stimulation consisted of 10 or 20 sec. bursts at 5 per sec.

Chloretone (0·1%). Specimens were immersed in this anaesthetic for 5–30 min. After 10 min. the anaesthetic caused a marked diminution in the amount of light produced, which was only about a third of that given off by the same animal free of anaesthetic (Fig. 8).

MS. 222. Animals immersed in this anaesthetic for 10–30 min. showed greatly reduced luminescence under electrical stimulation when compared with the same individuals free of anaesthetic (Fig. 9). The results were similar to those obtained with chloretone.

Stovaine (0·5%) and *eucaine* (0·5%). These two drugs were tried at several concentrations and for various periods to determine effective dosages and action times. Concentrations of 0·5% acting for 15 or 30 min. proved effective in greatly reducing luminescence while still permitting subsequent recovery (Figs. 10, 11). A peculiarity of many of the records obtained is the occurrence of a small primary peak before the luminescence reaches its maximum in specimens from which the anaesthetic has been washed out.[1]

Cocaine. Solutions of cocaine hydrochloride were used at concentrations of 0·5% (pH 7·6) and 1% (pH 7·3). After acting for 30–60 min., these solutions greatly reduced the luminescence produced by electrical stimulation (Fig. 12).

Procaine at concentrations 0·5–2%, acting up to 30 min., failed to significantly reduce the amount of luminescence. Solutions of 4% were then made up, and the pH was returned to 8·0 with NaOH. Procaine, at this concentration, greatly reduced luminescence in 30 min. (Fig. 13).

Ether (0·1%) failed to significantly reduce luminescence in 1 hr.

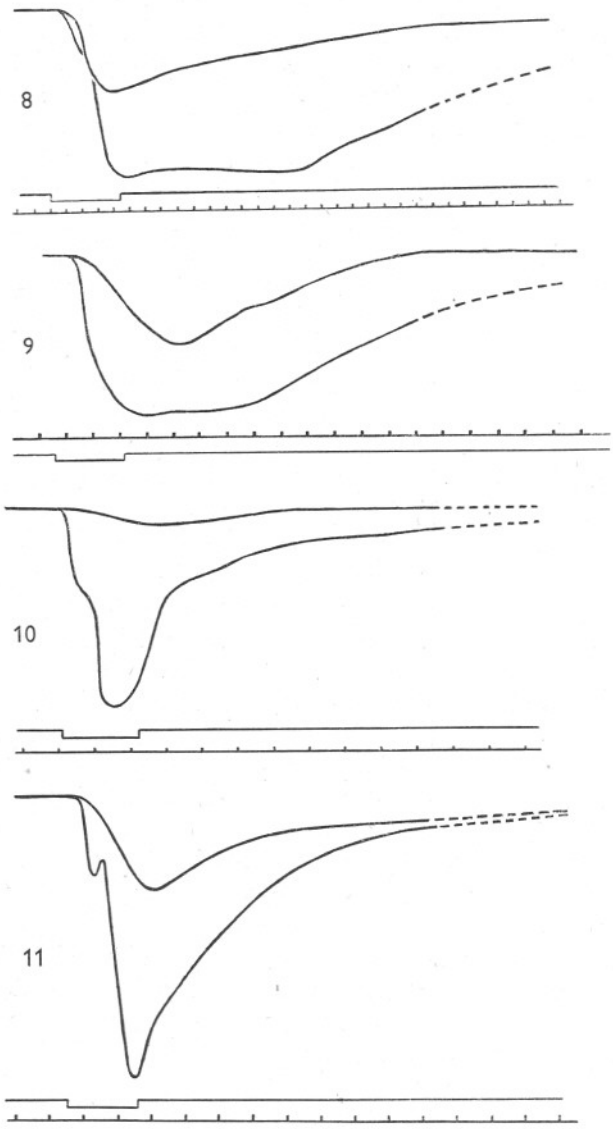
Isotonic *magnesium chloride* (pH 8·2). After immersion for 15–30 min. in this solution the animals showed greatly reduced luminescence to electrical stimulation (Fig. 14).

The noteworthy feature of all these results (except with ether) is that the anaesthetic greatly diminishes but fails to completely abolish the luminescent response. The drugs greatly reduce the intensity of light, and appear to restrict its appearance to a narrowly confined region immediately underneath the electrodes. The responses under anaesthesia are interpreted as suggesting that nervous transmission has been blocked, but that the luminescent effector is still responding directly to electrical stimulation.

Stimulation of the Nerve Cord in Anaesthetized Animals

Evidence for this viewpoint has been sought by stimulating the nerve cord directly. Specimens were anaesthetized as before, using MS. 222 for 30 min. They were then pinned out on a platform with ventral side uppermost and with the dorsal light glands in the aliform notopodia exposed through an aperture situated above a mirror. Fine silver electrodes arranged to give

[1] This effect, also seen in other records, is due to quantitative differences in the secretion produced by the two luminescent glands of the aliform notopodia.



Figs. 8–11. Luminescent responses of specimens when anaesthetized, and after recovery from anaesthesia. The upper curve in each record is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Stimulation, indicated as a horizontal interval, consisted of a 10 sec. burst at 5 per sec. Fig. 8, chloretone 0·1 % for 10 min. Fig. 9, MS. 222 1/2000 for 30 min. Fig. 10, stovaine 0·5 % for 30 min. Fig. 11, eucaine 0·5 % for 30 min. Time scale in Fig. 8, 1 per 2 sec.; time scales in Figs. 9–11, 1 per 5 sec.

localized stimulation were then inserted into the nerve cord in the mid-ventral surface of segment XII, and the preparation was subjected to prolonged repeated stimulation. It was found that normal animals responded to

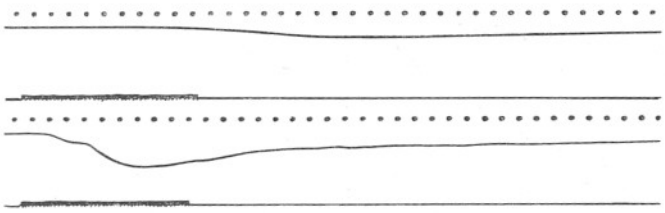
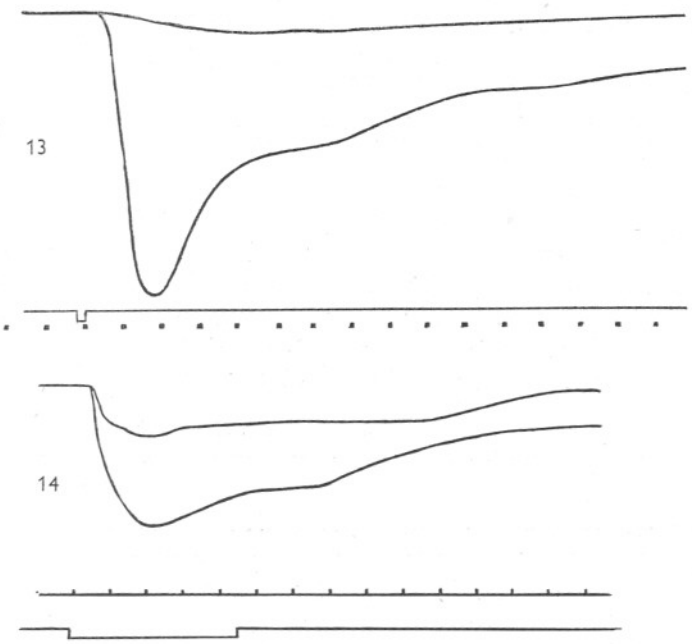


Fig. 12. Luminescent response of a specimen narcotized with cocaine hydrochloride (0·5 %) for 52 min. The upper curve is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Stimulation, 10 sec. burst at 5 per sec. Time scale above, 1 per sec.



Figs. 13, 14. Luminescent responses of specimens when anaesthetized, and after recovery from anaesthesia. The upper curve in each record is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Fig. 13 (*above*), procaine hydrochloride 4 % for 28 min. Stimulation, 1 sec. burst at 5 per sec. Fig. 14 (*below*), isotonic $MgCl_2$ for 15 min. Stimulation, 22 sec. burst at 5 per sec. Time scale in both records, 1 per 5 sec.

stimulation of the nerve cord by a bright flash along the aliform notopodia and by the release of luminous secretion in the light glands at the bases of those structures. After anaesthetization, however, repeated stimulation failed to produce any light.

This is strong evidence that the anaesthetics employed do actually stop nervous transmission, while allowing the peripheral effectors to respond directly to electrical stimulation. If the assumption be made that the anaesthetics have little or no effect on the activity of the glandular effectors, certain conclusions can be drawn about the effect of electrical stimulation of the light glands on the aliform notopodia as carried out in this investigation. Direct stimulation of the effector cells apparently produces much less light than stimulation of the nerves supplying them. This may be due to the effectors having a higher threshold than the nerve fibres so that, with a given voltage, fewer glandular cells would be affected than nerve fibres. Or it may result from the spatial organization of the nerve fibres themselves such that very localized stimulation of peripheral nerves initiates impulses that spread through a widely distributed network (not necessarily non-synaptic), and thereby reach the entire glandular area. In either event it follows that electrical stimulation of the peripheral light gland causes excitation of the nerve fibres supplying the glandular cells as well as direct excitation of the effector cells. But the major part of the light is due to indirect stimulation of the light cells via the nerve fibres, and only a small fraction is ascribed to direct stimulation of the effector cells themselves.

The Effect of Raising the Voltage in Anaesthetized Specimens

If the slight luminescence of anaesthetized specimens is due to a higher threshold in the responding structure or to the small field directly affected it is to be expected that a greater response could be secured by raising the voltage. With this in mind experiments were carried out as follows. Animals were anaesthetized in isotonic $MgCl_2$ and the light glands in the aliform

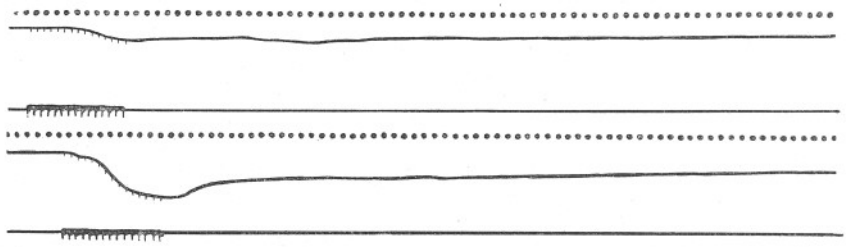


Fig. 15. Records of light produced by the photogenic glands of segment XII, demonstrating the effect of raising the stimulation-voltage in anaesthetized animals. The preparation was narcotized with isotonic $MgCl_2$ for 28 min., and it was then stimulated at 9V. (*upper record*), and 49V. (*lower record*). Stimulation consisted of a burst of 15 pulses at 84 per min. Time scale above each record, 1 per sec.

notopodia were then stimulated at different voltages (charging voltages of the condenser, 9V.–210V.). A relay-operated stimulator activated by an electronic device was employed for these experiments. Luminescent responses at low voltages were small, but were greatly increased by raising the voltage (Fig. 15).

Moreover, by raising the voltage it was possible to secure responses equal in magnitude to those obtained from normal animals. These results favour the interpretation that in anaesthetized animals the gland cells are being directly stimulated and that raising the voltage overcomes the higher threshold of these elements, or brings more into activity.

The Effect of Prolonging the Period of Stimulation in Anaesthetized Specimens

The action of narcotics in blocking nervous transmission while still allowing the glandular cells to react presented the possibility of testing the responses of the effectors to direct stimulation for varying periods. Animals were narcotized with isotonic $MgCl_2$ (pH 8·2) and MS. 222 (1/2000), and the light glands of the aliform notopodia were then stimulated through electrodes placed directly upon them. Stimulation consisted of short bursts at 5 per sec.,

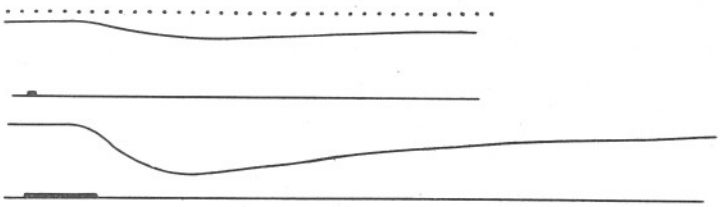


Fig. 16. Response of the light glands of the aliform notopodia in a specimen narcotized with MS. 222 (1/2000) for 11 min. The light glands were stimulated directly. Upper curve, response to a 1 sec. burst at 5 per sec.; lower curve, response to a 10 sec. burst at 5 per sec. Time scale above, 1 per 2 sec.

first for 1 sec., then again for 10 sec. Considerably increased amplification was used in recording the responses. In these preparations it was found that the second period of more prolonged stimulation produced a much greater luminescent response (Fig. 16).

It is emphasized that the luminescent responses recorded from these narcotized animals were very weak, and were only a small fraction of those obtained from normal animals. The explanation is advanced that the small response is due to the excitation of only a small number of gland cells under direct stimulation. The fact that the same kind of augmented response can be obtained both in narcotized and in untreated animals by increasing the number of stimuli suggests that the processes involved are taking place peripherally, in the effector cells, and not in efferent nervous pathways.

Light Produced by Stimulation of the Nerve Cord in Normal Animals

For comparison with the effects of peripheral stimulation some experiments have been carried out in which the nerve cord has been stimulated and the resultant luminescent response recorded. The arrangement was similar to that described above, p. 119. The animal was spread out with the dorsal side downwards: the basal light glands of the aliform notopodia

overlay an aperture which was focused on a photocell lying below. Fine silver wire electrodes, giving localized stimulation, were inserted into the nerve cord on the median ventral surface, and stimuli were applied. Periods of stimulation were regulated so that the effects of short and prolonged bursts could be compared.

These experiments gave results similar to those obtained by stimulating peripherally, with the electrodes placed on the surface of the light glands. Two or three stimuli were delivered at a rate of 3 per sec., the response was recorded, and the light was allowed to fade; then a burst of thirty to forty stimuli at the same rate was applied, and a record was made of the response. In the majority of specimens (four out of five) the response to the second prolonged burst was appreciably greater than the previous response to fewer stimuli; in one specimen it was about the same (Fig. 17).

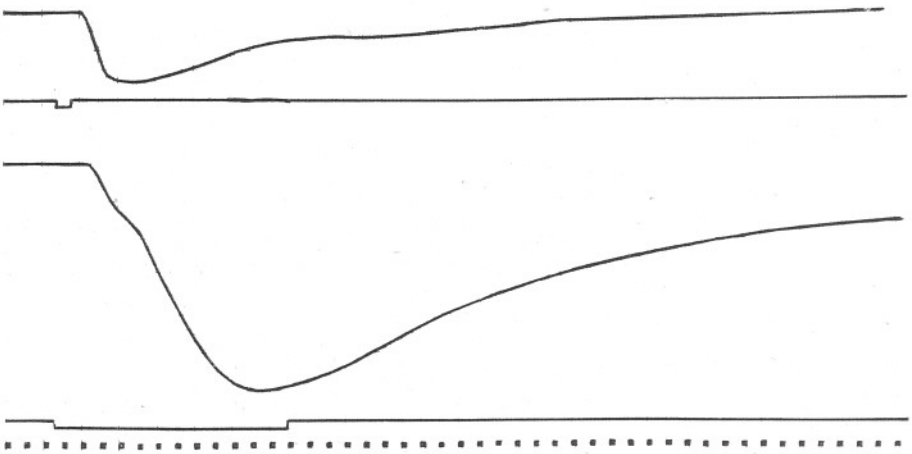


Fig. 17. Light produced by the aliform notopodia as the result of localized electrical stimulation of the nerve cord in segment XII. Upper tracing, three stimuli; lower tracing, thirty-eight stimuli. Frequency of stimulation, 3·3 per sec. Periods of stimulation are shown as horizontal intervals below each record. Time scale below, 1 per sec.

This is considered to be significant in view of the onset of fatigue or exhaustion previously discussed. The salient point that emerges from these results is that an augmentation of the luminescent response can be obtained by prolonging the period of nervous stimulation, that is, an increase in the number of nerve impulses brings about a greater response and produces more light. This result, read in conjunction with the previous conclusions relating to peripheral stimulation, lends weight to the viewpoint that when stimuli are delivered to the periphery they excite nerve fibres which in turn affect the glandular cells; reasons are presented above for considering that direct stimulation of the effector cells is a minor consequence. This point is of some importance in the present investigation since the animal

does not lend itself readily to stimulation through the nerve cord, with simultaneous recording of the luminescent response, and the majority of quantitative results were obtained by applying stimuli through electrodes resting on the basal light glands of the aliform notopodia.

The Responses of Specimens from which the Nerve Cord had been Removed

The reverse experiment to the preceding one has been carried out, namely stimulation of the peripheral light glands without involving the central nervous system (nerve cord). In the usual experimental arrangement, with electrodes resting on the dorsal surface of the animal, it is likely that solely efferent paths are involved rather than the nerve cord; but excitation of the latter, and consequent central regulation of the response, are not excluded. The following experiment was undertaken to resolve this difficulty, and the results indicate that the events responsible for augmentation of the light response on increasing the duration or frequency of stimulation occur at the periphery.

The worms used in these experiments were first anaesthetized by immersion in isotonic $MgCl_2$ for 15 min. The anterior regions were cut off at the junction between segments XII and XIII, and the ventral surface was removed from these anterior fragments. This operation removed the nerve cord (central nervous system) and left pieces which consisted of dorsal body wall and gut, and which contained the light glands of segment XII. The experimental material was afterwards washed in running sea water for several hours. These pieces were then stimulated either at two different frequencies or with short and long bursts of stimuli.

This experimental material showed the same responses as intact worms containing central nervous system. The response to a second period of stimulation at a higher frequency was significantly greater than after the first burst of stimuli at a lower frequency (Fig. 6). The response was also augmented by increasing the number of stimuli (Fig. 18). Both the maximal height of response and the total light produced were greatly increased by stimulating at a higher frequency or for a longer period. In addition, the rate of rise of light intensity showed a pronounced increment. It follows that the augmentatory effect, whatever its nature, that is produced by more stimuli or faster rates of stimulation, can be a peripheral affair, and experimentally is not dependent on the central nervous system and centrally located nerve cells.

Histological sections of the aliform notopodia, impregnated with silver (Holmes's method and Bodian's activated protargol), have revealed some details of innervation. The nerve cord, situated mid-ventrally, gives off small nerves which proceed peripherally underneath the epidermis. The nerve fibres in these nerves are very small and it has not been possible to distinguish terminal details of peripheral innervation, but the multiplicity of fibres and

their close proximity to the glandular photogenic cells form a structural basis for the physiological mechanisms reported above.

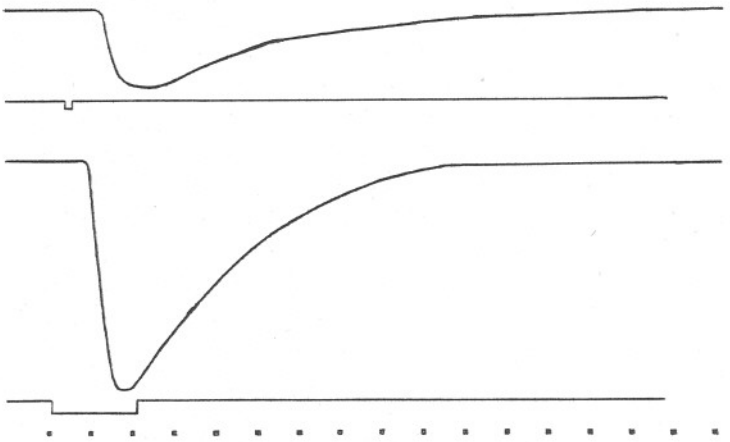


Fig. 18. Record of light produced by the light glands in the aliform notopodia of a preparation from which the nerve cord has been removed. Upper record, three stimuli; lower record, 10 sec. burst (fifty stimuli). Frequency of stimulation, 5 per sec. The tracing below each record indicates the period of stimulation. Time scale below, 1 per 5 sec.

Comparison of the Effects of One versus Two Stimuli

In order to secure critical data on whether the augmentation of the light response is due to a process of summation in the effectors (luminescent gland cells), or to facilitation at the neuro-effector junction, specimens have been stimulated with a single stimulus and with a pair of stimuli, and the responses compared. Two sets of experiments were carried out as follows.

In the first set of experiments each animal was stimulated twice. It was stimulated by a single impulse; then, after allowing a period for decay, two electrical stimuli were applied. The two impulses were separated by an interval of 0·25 sec. In these experiments all the results obtained were negative. Of seventeen specimens examined a few gave about the same amount of light with two stimuli as with one; the majority, however, gave off even less light following the second period of stimulation (Fig. 19). It was concluded that a fatigue effect was operating here, in that the first stimulus had partially exhausted the light glands; any augmentative effect due to two stimuli would be masked by the previous partial evacuation of the light glands.

A second approach to the problem was essayed by stimulating separate specimens either with a single stimulus, or with two stimuli (interval 0·25 sec.). Forty-six animals were used, half of which were stimulated with a single impulse, and half with two stimuli. Since the experiments lasted several hours and there was the possibility of an alteration in the experimental conditions during that time, the animals were divided into small groups. In

each group several animals were stimulated with one stimulus, and a corresponding number with two stimuli. The mean response of each group was determined, and from these values weighted means were calculated for the whole assemblage. (Measurements of response refer to displacement of the response curves.)

Group	Mean responses of each group (in arbitrary units)	
	one stimulus	two stimuli
1	10·0	5·2
2	2·9	4·3
3	11·4	13·7
4	10·3	12·0
5	9·6	5·4
6	10·2	7·7
Weighted means	8·3	7·8

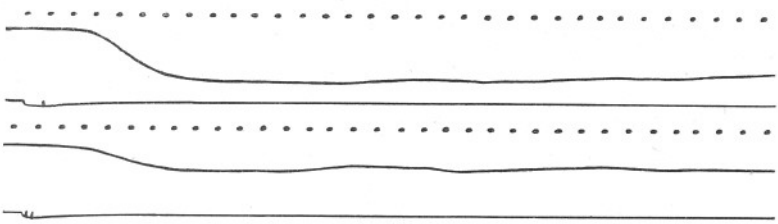


Fig. 19. Records of light produced by the photogenic glands on the aliform notopodia. *Upper record*, the response to a single stimulus; *lower record*, the response of the same specimen to two stimuli (interval of 0·25 sec.). Stimulation indicated below each record. Time scale above, 1 per sec.

To reduce the possibility that some partly exhausted specimens were vitiating the results, a comparison was made of the twenty maximal responses. Means for these were 14·2 units for one stimulus (ten animals) and 12·5 units for two stimuli (ten animals).

In these experiments two stimuli produced rather less light than a single impulse. The actual fact that the mean response was slightly less for two stimuli than for one, however, is certainly a statistical accident. If two stimuli were to give a disproportionate response, equal to many times that produced by a single impulse, then it would be expected that it would be revealed by the technique employed, and might be sufficient even to offset partial exhaustion resulting from a previous stimulus. Other records demonstrate, moreover, that a series of repeated stimuli do give a significantly greater response than one or a few stimuli (Figs. 2, 7, 15, 16).

Facilitation due to build-up of an excitatory state seems to be ruled out by these results, but they can be explained by summation in a contractile tissue in the light glands. They suggest a mechanism in which the first stimulus brings about a large response (or contraction) and subsequent stimuli are responsible for only small additional increments. This explanation of peripheral summation is in line with the results of experiments reported above (p. 123),

in which augmentation of the light response was obtained by stimulation of the photogenic glands in narcotized animals, presumably due to direct excitation of the effector-gland cells. The subject is discussed more fully in a later section.

Rate of Rise of Light Intensity and Temporal Characteristics of the Luminescent Response

The augmented responses observed in some of the experiments described above obviously invite comparison with results obtained in investigations of neuromuscular functioning. From experiments on crab muscle Pantin (1936) was able to show that as the frequency of electrical stimulation was raised, there was a corresponding increase both in the maximal tension developed and in the rate of rise of tension. These studies in neuromuscular functioning have prompted further analyses of the light response of *Chaetopterus*.

Measurements made on those records that show an increased response to a higher frequency of stimulation, or to more prolonged stimulation, also reveal a corresponding increase in the rate at which the luminescent response develops (Figs. 4, 7). Data from five experiments in which the frequency of stimulation was raised are presented below.

Specimen	Frequency of stimulation (per sec.)	Rate of rise per sec. (mm.)
1	1·3	4·0
	9·0	8·9
2	1·3	1·7
	9·0	2·8
3	1·3	1·3
	9·0	3·8
4	2·0	3·0
	9·0	4·9
5	5·5	2·4
	32·2	4·9

Mean increment at lower rate, 2·5 mm./sec.
Mean increment at higher rate, 5·1 mm./sec.

In these experiments the frequency of stimulation was raised 1/2–7 times and the rate of rise of light intensity increased about twofold at the higher frequencies. The rate of rise of light intensity is also significantly increased by increasing the number of stimuli (Fig. 7). An increased response, therefore, reveals itself in three ways: by a rise in peak intensity, by an increase in the rate of rise of light intensity, and by an increase in the total amount of light.

Detailed analyses of a large number of response curves have yielded information about certain temporal features of the light response and the rate of increment of light intensity. The following data were extracted from each record: the rate of rise of the response curve, the time taken to reach maximal height, the maximal height of the response, and the rate of decay for a period

extending up to 30 sec. after the beginning of the response. Due to the great variation between individual specimens, it has been necessary to reduce the records to a comparable basis, and this was accomplished by expressing all intensities as a percentage of the maximal response, set at 100. Fig. 20 shows a representative plot for a series of nineteen specimens. In this figure two curves represent records which showed maximal and minimal rates of increment, and a third curve represents mean values for all specimens.

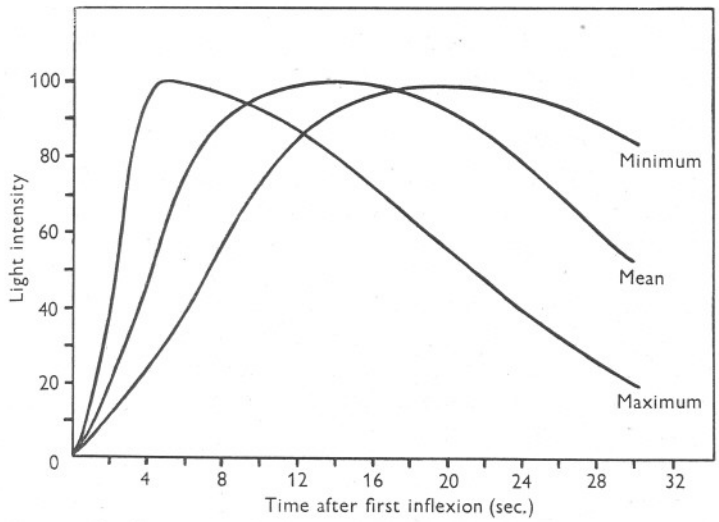


Fig. 20. Curves showing temporal characteristics of the luminescent response evoked by electrical stimulation (2·3 sec. burst at a frequency of 6·2 per sec.). Maximal and minimal curves are selected records showing fastest and slowest rates of rise of light intensity. The mean curve is based on the average for all records (19). All data have been replotted as a percentage of maximal response (= 100).

Mean values obtained in this series of experiments were:

Time to reach maximal height, 14·2 sec.;
Rate of rise, 13·1/100 per sec.;
Height at first $\frac{1}{2}$ min., 53·8/100.

These figures illustrate the levels of magnitude involved, but absolute values will depend on the conditions of stimulation.

The relationship between the rate of rise of light intensity and the maximal intensity of response was considered in the following manner. Records were obtained of sixty-eight animals which were stimulated at two different frequencies, first at 2 per sec., and again at 25 per sec. The absolute increment (h_1) 5 sec. after the beginning of the response was measured, and this was expressed as a fraction of the maximal response (h_2), viz.

$$\frac{h_1}{5} \times \frac{1}{h_2} = r, \quad \text{the fractional increment per sec.}$$

A mean value for r was obtained by pooling measurements from all records. The absolute rate of increment is sometimes greater at higher frequencies, yet, when expressed as fractional increments of the total response, the rates of rise at low and high frequencies show no significant difference, i.e. $r_2 = r_m = r_{25}$, where r_2 = fractional increment at a frequency of 2 per sec., r_{25} = fractional increment at a frequency of 25 per sec., and r_m = the mean fractional increment of all records irrespective of the frequency of stimulation. This result indicates that the rate of rise of light intensity (i.e. the rate of cellular secretion), and the maximal intensity reached (i.e. the maximal output of secretion) are related to one another.

Effect of Drugs on the Neuro-glandular Junction

The results just described reveal the characteristics of certain excitatory events taking place in the light glands. It has also been shown that acetylcholine is an effective agent in causing luminescence in this animal, and acts at the neuro-glandular boundary (Nicol, 1952*b*). In conventional terminology, the nerve fibres to the light glands are therefore cholinergic, and acetylcholine becomes implicated as a chemical transmitter in the light response. In view of these facts it seemed of value to test the effects of atropine and curare, which separately antagonize the action of acetylcholine in different neuro-effector systems of vertebrates.

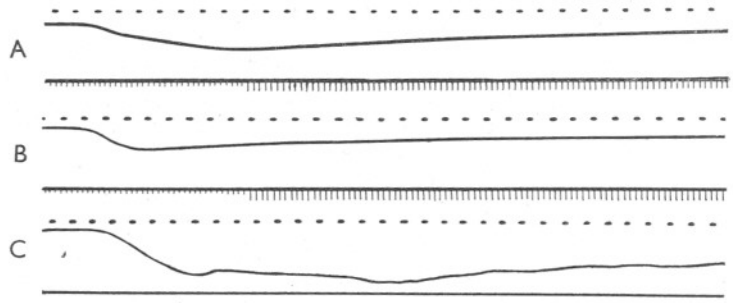


Fig. 21. Records of the light produced by animals treated with atropine or curare and subjected to electrical stimulation (10 sec. burst at 4 per sec.). A, specimen treated with atropine, 1/10,000, for ½ hr., and then stimulated; B, same specimen stimulated after washing out atropine for ½ hr.; C, specimen treated with *d*-tubocurarine, 1/10,000, for ½ hr., and then stimulated. Neither atropine nor *d*-tubocurarine had any effect in abolishing the light response. Time scale, 1 per sec.

Atropine. This drug was applied in concentrations of 1/10,000 and 1/1000 for 30 min., and records of the luminescent response to electrical stimulation were obtained. The drug was then washed out for 30 min., and stimulation was repeated. The drug had no apparent effect in reducing the amplitude of the response (Fig. 21).

d-*tubocurarine.* At a concentration of 1/10,000, *d*-tubocurarine failed to

diminish or abolish the luminescent response evoked by electrical stimulation (Fig. 21).

Eserine. Some previous experiments with eserine failed to reveal any excitatory effect of this drug when used in conjunction with acetylcholine. However, little reliance is placed on this negative result, since the light produced by acetylcholine itself is usually very faint and shows much variation from one specimen to another. The effect of eserine has been further explored in a quantitative manner by stimulating specimens electrically to determine the normal level of response, and then stimulating again after the application of eserine to discover any possible augmentative effect. Such an experiment suffers from the same defects as those mentioned previously in describing the effects of increasing the frequency or duration of stimulation, namely fatigue of the light glands. Despite this latter factor an increase in response after eserinization might still be encountered, and such a result may be considered as positive.

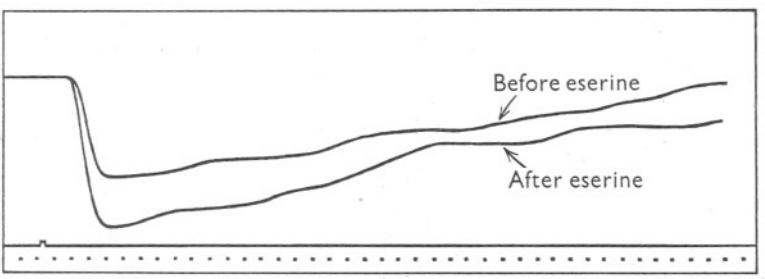


Fig. 22. The effect of eserine on light production in *Chaetopterus*. The upper tracing shows the response to electrical stimulation before eserine, the lower tracing after the application of eserine (1/10,000, 45 min.). Electrical stimulation, in both instances, was a 0·2 sec. burst at 33 per sec. Lower tracing: stimulation, and time scale, 1 per sec.

Specimens of *Chaetopterus* were treated with eserine (physostigmine salicylate) 1/10,000 for 45–60 min., and the light produced before and after eserinization was recorded and compared. Of twelve animals examined, three gave positive results in that a brighter response followed the application of eserine (Fig. 22).

Acetylcholine. It was considered possible that the luminescent responses to electrical stimulation might be affected by acetylcholine, and experiments along this line were carried out as follows. A record was first obtained of the light resulting from a short burst of electrical stimuli. The animals were then immersed in 1/10,000 acetylcholine for 15 min., and stimulation, as before, was repeated. In all instances (five specimens) the light resulting from the second period of stimulation was less than that obtained previously. There was, consequently, no evidence that acetylcholine applied externally can increase the luminescent response that is evoked by stimulation of the nerves.

The negative results with atropine and *d*-tubocurarine do not directly

provide any information about the nature of neuro-glandular transmission occurring in the light glands. However, other cases are known among invertebrates in which cholinergic systems are insensitive to these depressive drugs. Presumably, the receptor surfaces involved in neuro-muscular transmission are not affected by these substances, and the reactant systems consequently do not readily lend themselves to classification as muscarinic or nicotinic. The results obtained with eserine are interesting and point towards acetylcholine being involved as a chemical mediator in the light glands.

Transmission Through the Nerve Cord

In a previous study it has been shown that when the frequency of electrical stimulation is raised above 5 per sec., the luminous response spreads and affects more distant segments. The transmission of excitation involved in this process occurs through the nerve cord (Nicol, 1952*b*). The passage of excitation from one body region to another is rather irregular in occurrence. For example, it is only occasionally that light appears in the middle and posterior regions of the body when the anterior region is stimulated. In order to obtain repeatable quantitative information, therefore, experiments were confined to the posterior region.

The posterior regions of a series of specimens were stimulated at various frequencies in the dark, and the number of posterior segments that lighted was noted. The electrodes were placed on the first few segments (I–III) of the posterior region, and the voltage output of the stimulator was kept constant. The effect of increasing either the number or frequency of stimuli was investigated. A diagrammatic representation of the results is shown in Fig. 23.

Increase in frequency of stimulation. There is no spread of excitation at low frequencies. Stimulation at 1–2 per sec. results in light confined to the notopodia in the neighbourhood of the electrodes. On raising the frequency to higher rates, excitation spreads to involve more distantly located segments, and the whole posterior region finally lightens. The critical frequency is about 6 per sec.

Increase in duration of stimulation. Prolonged stimulation at low frequencies (1–2 per sec.) does not widen the response area, but at higher frequencies (above 5 per sec.) the spatial character of the response depends upon the duration of stimulation as well as the frequency (Fig. 23). Two protocols illustrate the results obtained.

Specimen 1. Electrodes on segments I–II of the posterior region.
Stimulation: 2 per sec.
Time: 10 sec. Segments I–II responded.
22 sec. Segments I–IV responded.
22 sec. Segments I–IV responded.
Stimulation: 25 per sec.
Time: 20 sec. Bright luminescence from all segments of the posterior region.

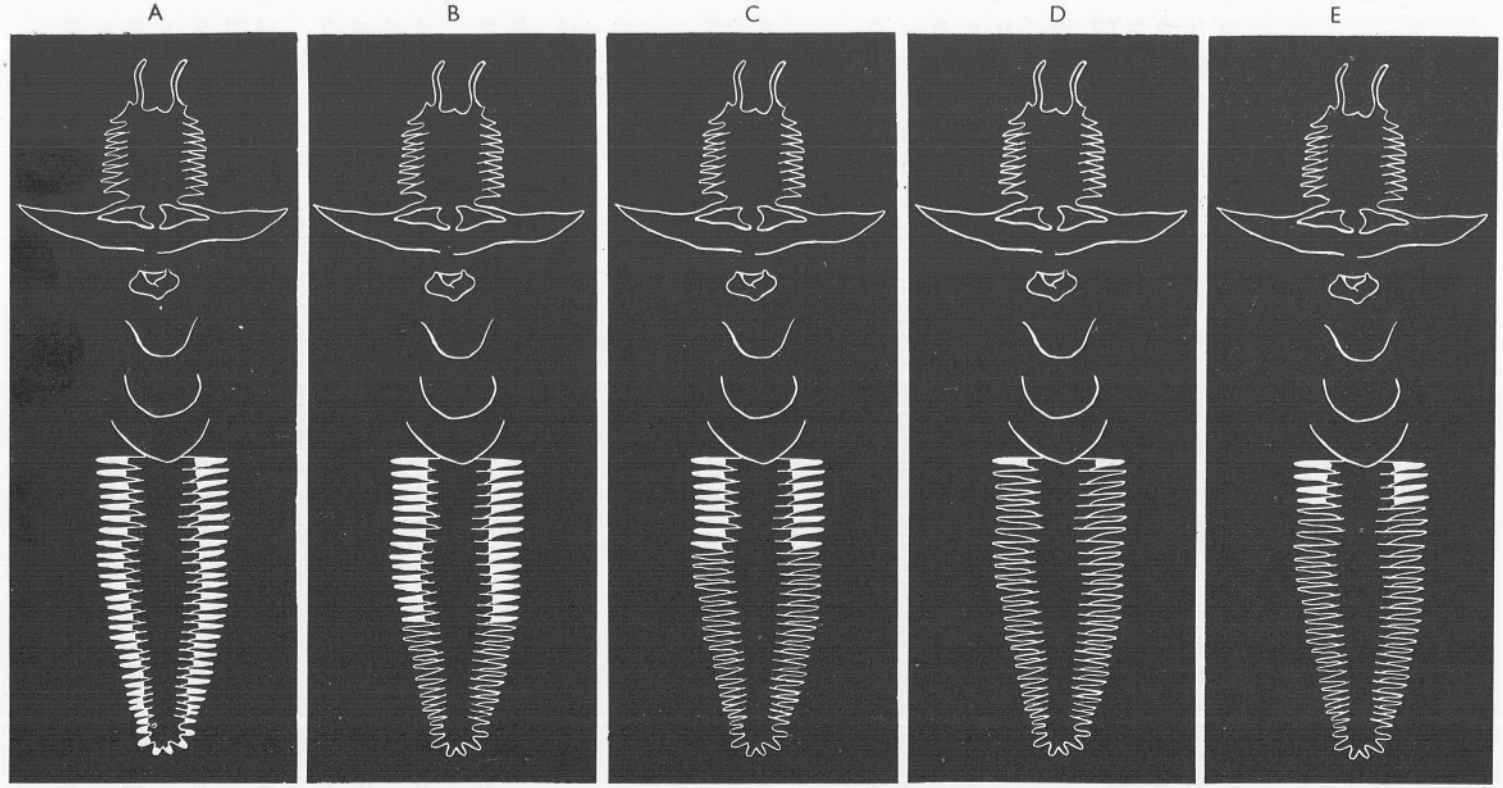


Fig. 23. The effect of altering the frequency and number of impulses on lighting of the posterior region of *Chaetopterus*. A, electrodes on segments I and II. Stimulation, 43 per sec. for 10 sec. Luminescence in all notopodia of the posterior region. B, electrodes on segments II and III. Stimulation, 10 per sec. for 10 sec. Luminescence in segments I–XV. C, electrodes on segments II and III. Stimulation, 10 per sec. for 2 sec. Luminescence in segments I–VIII. D, electrodes on segments I and II. Stimulation, 10 per sec. for 0·2 sec. Luminescence in segment I. E, electrodes on segments I and II of the posterior region. Stimulation, 1·2 per sec. for 40 sec. Luminescence in segments I–IV.

Specimen. 2. Electrodes on segments I–II of the posterior region.
Stimulation: 43 per sec.
Time: 0·2 sec. Segments I–II responded.
0·5 sec. Segments I–II responded.
1 sec. All posterior segments responded, but light weak.
10 sec. All notopodia of the posterior region were briefly luminous.

This spread of excitation is interrupted by cutting the nerve cord, and is therefore transmitted through the central nervous system. To explain these results it has been suggested that some process of internuncial facilitation occurs at higher frequencies (Nicol, 1952*b*). The present results show that the spread of excitation is also dependent upon the number of stimuli, and as the duration of the stimulatory period is increased, the luminous response spreads so as to involve more and more posterior segments.

Slightly different results are obtained by stimulating segments in the middle or hind end of the posterior region. When the middle of the posterior region is stimulated at low frequencies (1–5 per sec.) the appearance of light is usually confined to notopodia lying underneath and posterior to the electrodes. But on raising the frequency and number of impulses the light spreads to involve notopodia lying anterior to the electrodes. Here are two protocols.

Specimen 3. Electrodes on the terminal third of the posterior region.
Stimulation: 1 sec. burst at 5 per sec. Segments from the level of the electrodes to the hind end responded.
22 sec. burst at 5 per sec. All segments of the posterior region lighted up, anterior and posterior to the electrodes.

Specimen 4. Electrodes on the middle of the posterior region.
Stimulation: 3 per sec. for 20 sec. Only notopodia behind the electrodes responded.
5 per sec. for 20 sec. Only notopodia behind the electrodes responded.
10 per sec. for 20 sec. All segments of the posterior region responded.

In most of these animals it was observed that the light appearing anterior to the region stimulated was much weaker than that appearing posterior to that region.

The conclusion drawn from these experiments is that nervous transmission concerned in mediating the luminescent response takes place with much greater facility posteriorly than anteriorly through the nerve cord. Although transmission anteriorly does occur, there is a greater degree of resistance to it in postero-anterior pathways than in the reverse direction.

The build-up of a central excitatory state is presumably a synaptic phenomenon occurring at the junctions between segmentally disposed neurones arranged in linear sequence along the nerve cord. It may be assumed that at low frequencies, below 5 per sec., the excitatory state dissipates in the intervals between stimuli, but that at higher frequencies the rate of build-up of excitation exceeds the rate of decay. The degree of facilitation attained will depend on the frequency and number of impulses, both

factors determining the level of excitation reached. It appears that a single impulse, or a group of impulses at low frequencies, is insufficient to override the synaptic resistance between the segmentally arranged neurones, and that a burst of impulses is necessary to overcome these barriers. The number of segments responding can be interpreted as the external manifestation of facilitatory processes occurring at sequential synapses, each being a barrier in series to the next, and introducing a degree of decrement in stepwise series.

Correlated with this physiological interpretation it has been observed that the nerve cord possesses large tracts of longitudinally directed fibres, coursing from one segment to the next. Besides minute fibres, giant axons have also been noted, up to 7 μ in diameter. These probably only extend short distances, however, since Bullock (1948) could find no electrical evidence for giant fibres in this species.

Discussion

Peripheral augmentation of the luminescent response due to increasing the frequency or duration of stimulation manifests itself in heightened light intensity, often in augmentation of the total amount of light produced, and as an acceleration in the rate of increase of light intensity. The light produced is taken to be an adequate indication of the strength and temporal progress of events in the underlying response mechanisms. The response curve, of course, represents the progress of some chemical reaction, presumably the oxidation of photogenic material, and it only indirectly reveals the course of the physiological processes of secretion. To interpret this curve it is necessary to consider its relationship to the secretory process. Unfortunately, there are no critical biophysical data available for the oxidation of the luminescent secretion of *Chaetopterus*, and experiments with *Cypridina* (Ostracoda) extracts can only be cited for comparison. Harvey and his colleagues (1940) studied the luminescent reaction produced by mixing *Cypridina* extracts, and they found that the light emitted by this material reached its maximum in about 0·03 sec., and the decay time for a fall to half intensity occupied 0·5 sec. These experiments were carried out with refined extracts, and it is obvious that the reaction in *Cypridina* preparations proceeds at a much faster rate than in the luminescent material of *Chaetopterus*, as released by the normal animal (see p. 129). The secretion of *Chaetopterus* does not become luminescent until it is discharged from the cell and this in itself must introduce a time lag so that the luminescent response is not contemporaneous with the secretory process. Under optimal conditions it may be presumed that the chemical reaction will proceed rapidly to completion, but the luminescent secretion of the light glands in the aliform notopodia is actually released into a dense body of mucus that greatly retards gaseous diffusion, and, consequently, the oxidation of the photogenic material.

A study of the latent period is more informative. When the aliform

notopodial light glands are stimulated, the average latent period is 4 sec., as determined by measurements of the response curves. This latent interval of 4 sec. could be occasioned by the latent period of the effector, by the time consumed in the response of the effector, and by delay in the course of the chemi-luminescent reaction. In several experiments in which the effect of raising the frequency was investigated, stimulation consisted of short bursts of 0·5–4 sec. (p. 116). With these short bursts, lying within the latent period, a definite augmentation of the response was obtained on increasing the rate of stimulation. Other evidence indicates that augmentation of the response is due to summation. Since this process can take place well within the latent period of the response, it is clear that the luminescent response first becomes evident several seconds after the sequence of events in the underlying mechanism has taken place. On these premises it is now possible to predicate that most of the interval that elapses between the delivery of the first electrical shock, and the first appearance of light, is due to the slow progress of the chemiluminescent reaction in the material discharged upon the surface of the animal.

Since luminescence becomes evident considerably later than the sequence of events taking place in the underlying mechanism of secretory discharge, the response curve will not reveal detailed information about the temporal course of events in the secretory structures. On the other hand, its height and slope reflect the magnitude of the underlying secretory processes, and give a delayed, smoothed, and aggregate portrayal of the events preceding it.

The cytomorphological appearance of the photogenic glands is that of oval or elongated cells closely packed with eosinophilic granules (see Nicol, 1952*a*, for details and references to literature). These are arranged as a closely packed mass of paraplasm occupying most of the cell interior, and invested by a thin layer of cytoplasm. The mechanics of secretion in such a cell appear to be poorly understood, and present a problem common to many glandular cells of that type, for example, muciparous cells containing a mucous plug. It may be suggested that excitation causes the superficial protoplasm of the cell to contract, and this process leads to the expulsion of the cell contents; or that excitation initiates changes resulting in imbibition of water and a rise in internal pressure, again causing the photogenic mass to be squeezed forth. In either case nervous excitation of the glandular cell would be direct, and would result in a rise in internal pressure. Other possible mechanisms that can be suggested are the existence of myo-epithelial elements about the cell-body, as obtains in the oral glands of mammals, or of muscle elements between the epidermal gland cells as Eisig (1887) has described in the epidermis of capitellids. The mechanism in these instances would be indirect and neuro-muscular in nature.

In conjunction with the physiological investigations a considerable amount of histological work has been done on the photogenic glands and cells of

Chaetopterus. Specimens, fixed in a variety of ways, have been stained and impregnated with silver by silver-on-the-slide techniques. This work is summarized as follows.

The thin layer of protoplasm investing the photogenic cells stains rather feebly, probably owing to its tenuity. In preparations triple-stained with Heidenhain-azan this protoplasmic investment is coloured orange or reddish like the muscle fibres in the same sections. Treatment with silver (Holmes's method after Bouin fixation) sharply delimits this layer as a distinct deeply

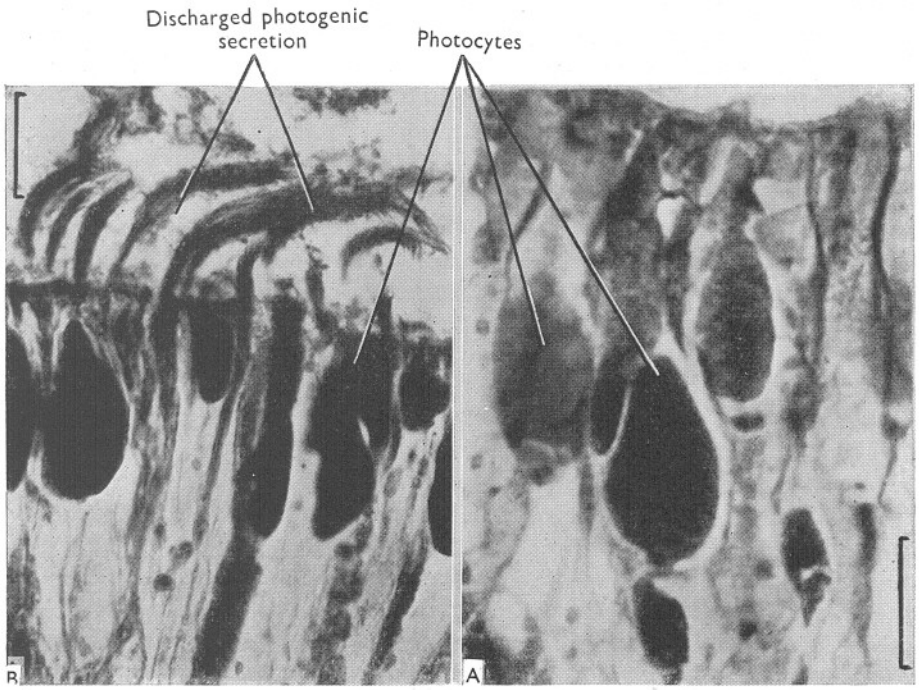


Fig. 24. A, section across an aliform notopodium to show some photogenic cells. Bouin fixation; impregnated with silver by Holmes's method. B, section across a notopodium of the posterior region to show photogenic cells caught in the act of secreting. Fixative, formol-sea water; stain, Heidenhain-azan.
The scale in both sections corresponds to 25 μ.

impregnated sheath about the photogenic material (Fig. 24A). The possibility that the protoplasmic investing layer contains fibrous oriented protein similar to that in muscle was considered, and sections were examined in polarized light. This material had been fixed in formalin or Bouin's fluid and was examined unstained. Under crossed Nicols and with a red gypsum plate the photogenic cells displayed no anisotropy although muscle fibres in the same sections were markedly birefringent. It was noted, however, that the protoplasmic layer about the photogenic cells in these unstained preparations was

strongly refractile. Moreover, careful examination of the epidermis has provided no evidence for the existence of myo-epithelial cells or epidermal muscle fibres.

This study, unfortunately, is inconclusive, but some preparations were obtained in which the photogenic cells were caught in the act of discharging their secretion (Fig. 24B). These sections give the impression that the photogenic material is being forcibly squeezed out of the cell. It is tentatively proposed that the protoplasmic investment of the photogenic cells is a contractile structure, capable of compressing and causing the evacuation of the photogenic cells when excited. The postulation of an organization of this kind permits the physiological results obtained in the present investigation to be interpreted as manifestations of the activity of contractile units, excited by nerve impulses, and such an interpretation permits comparison with results obtained in neuro-muscular physiology.

The augmented luminescent response obtained by increasing the number of stimuli or frequency of stimulation can be explained by a process of summation in the effector organ (light gland). Certain of the experiments were designed to reveal the possible existence of a facilitatory mechanism, with negative results. If facilitation were operating it would be expected that two stimuli would have an appreciably greater effect than one stimulus, even after allowing for partial exhaustion of the luminescent secretion. However, such an effect was not discovered.

The events that are considered to be taking place during stimulation of the photogenic glands on the aliform notopodia of *Chaetopterus* are depicted diagrammatically in Figs. 25 and 26, and are interpreted as follows. An electrical stimulus gives rise to a nerve impulse that activates the photogenic cells, causing them to contract, and results in the expulsion of enough secretion to produce visible luminescence. A single impulse causes a strong response that evacuates a large part of the cell contents. After this response has exhausted itself, a second impulse acting on the cell would initiate changes in the same response-mechanism, but since the paraplasmic contents of the cell are now only a fraction of their former level, the response will also appear as a fraction of the former intensity (Fig. 25). Subsequent stimuli, spaced at intervals so as to allow recovery of the effectors, should evoke responses of geometrically decreasing magnitude. This seems to be realized in actuality, and would explain the progress of fatigue under repetitive stimulation.

A series of impulses gives rise to a series of contractions which summate with one another to produce a heightened response (Fig. 26). A large part of the secretion would be expelled with the first contraction, and each subsequent contraction would have a relatively smaller effect. Two contractions then would produce only a little more secretion than a single contraction, and a whole series of contractions would be necessary to increase greatly the amount of luminescent material. Similarly, raising the frequency would

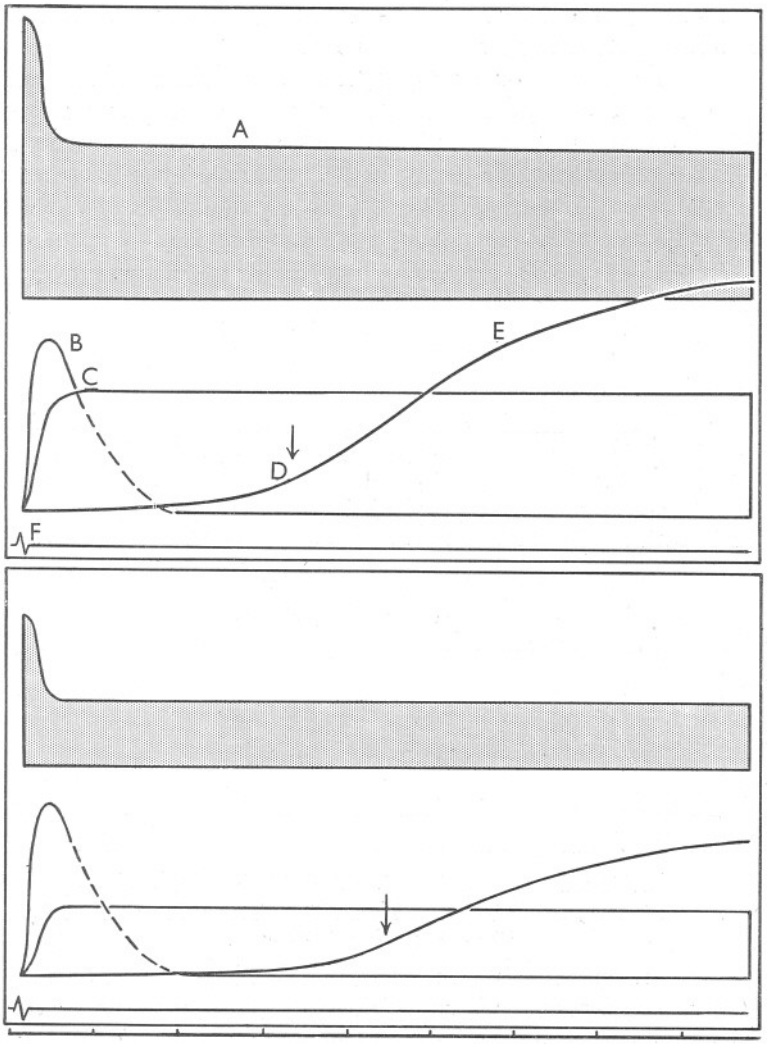


Fig. 25. Diagrams intending to illustrate the possible series of events taking place during the luminescent response of *Chaetopterus*. It is postulated that a stimulus (shown on the bottom line) excites the nerve fibres supplying the light gland and causes the gland cells to contract (illustrated as the first response curve on the left). This brings about the discharge of photogenic material and causes partial evacuation of the glandular cells. Both the secretion of photogenic material, and the residual amount remaining in the cells, are represented. Some time after the secretion of photogenic material, the photochemical reaction attains sufficient velocity to be revealed as a measurable luminescent response (threshold indicated by vertical arrow). A second impulse, after the effect of the first has died away, results in less secretion since the secretory content of the glandular cells is less. A, content of photogenic material in gland cells. B, contraction of gland cells. C, amount of photogenic material secreted. D, threshold sensitivity of recording apparatus. E, luminescent response. F, stimulus. Time scale, 1 per sec.

result in greater secretion since the individual contractions could summate before relaxation ensued or became advanced.

The rate of rise of the luminescent response curve remains to be dealt with. The slope of the curve becomes steeper as the frequency and number of impulses are raised, and it is related to the height of the response, i.e. an increase in the rate of rise of light intensity is followed by an increase in the maximal light intensity reached. This is probably due to the fact that as the contractile response becomes stronger, more secretion is poured out, and the

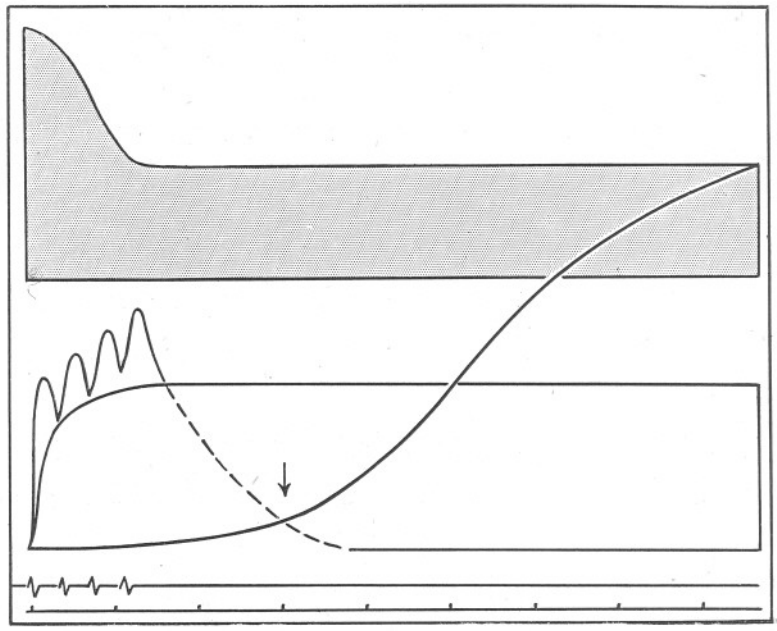


Fig. 26. Diagram, as in Fig. 25. A burst of impulses produces summation and augmented secretion.

velocity of the ensuing chemiluminescent reaction is governed by the initial concentration of the photogenic material. If this is so, it would be expected that the latent period would be reduced when the response becomes greater, and this is borne out by inspection of records of augmented responses. In Fig. 7A, for example, the latent period was reduced from 4·6 to 3·4 sec. when the duration of stimulation was prolonged from 0·2 to 5 sec.

The salient fact that emerges from this investigation is that the luminescent reaction is a triggered response. In the light glands of segment XII, a single impulse as well as a burst of impulses evokes luminescence. It may therefore be concluded that any effective natural stimulus, sufficient to excite sensori-neural pathways, will produce a response, without the necessity of reinforcement by repeated stimulation. In some specimens of *Chaetopterus* it has been possible to produce an enhanced effect with a second period of stimulation

at a higher frequency or duration, but in many of the animals investigated the second response was actually lower than the first, and fatiguing of the light response is an invariable accompaniment of repetitive stimulation. Moreover, recovery from fatigue is extremely slow, a matter of hours rather than minutes. The nature of the effector organ itself, therefore, precludes the possibility of the nervous system exercising any effective control over the levels of response on frequently repeatable occasions.

Although the magnitude of the initial response would depend on the conditions of stimulation, nevertheless, any excitatory stimulus results in the appearance of light. It has been suggested that luminescence in the aliform notopodia of *Chaetopterus* may be in the nature of a sacrifice lure associated with autotomy of the anterior region (cf. Joyeux-Laffuie, 1890), and, under this interpretation, the significance of luminescence as a triggered response becomes intelligible. It would be of value to the animal to produce a maximal amount of light initially, when stimulation reached or exceeded threshold, and actual variations in a few millilamberts at the low levels of brightness involved, would probably have little survival value as long as the response exceeded the visual threshold of the photoreceptor concerned.

In contrast to the events occurring peripherally, where a single impulse produces a pronounced luminescent response, the operation of facilitatory processes can be observed as the result of stimulating the nerve cord. These processes are manifested by an extension of the luminous area as the rate and duration of stimulation are raised. Since the response is observed as an extension of the field of luminescence involving more and more glandular units, it is not obscured by the intervention of glandular fatigue. The underlying mechanism seems to consist of a progressive increment of an excitatory state at synapses in the nerve cord. Such a mechanism could obviously provide a way of regulating the magnitude and extent of luminescence in posterior segments in response to natural stimulation, but the part that it might play in the normal life of the animal still awaits determination.

Summary and Conclusions

The nervous regulation of luminescence in *Chaetopterus* has been investigated by making use of controlled electrical stimulation and photoelectric recording. To achieve the latter a multiplier photocell has been used in conjunction with a galvanometer or oscilloscope, and camera.

A luminescent response is evoked in the aliform notopodia by a single stimulus or by a battery of stimuli. Due to the intervention of fatigue under repetitive stimulation it is difficult to obtain reproducible results, but in favourable preparations it has been possible to secure an enhanced response to increase in frequency and duration of stimulation above a rate of 1 per sec. Similar results were obtained by localized stimulation of the nerve cord, and

by peripheral stimulation of preparations from which the nerve cord had been removed. It is, therefore, concluded that the photogenic glands are being stimulated through their nerve fibres, and that the enhancement or augmentation of the response is not dependent upon the central nervous system, but takes place peripherally in the glandular tissue itself.

In experiments with two closely spaced stimuli no greater response was obtained than with a single stimulus. After making allowance for individual variation in the intensity of the response and partial exhaustion of the light glands following one initial stimulus, these results are interpreted as demonstrating that peripheral facilitation plays no part in the luminescent response. Any augmentation of the response due to prolonged or high frequency stimulation can be explained satisfactorily by summation in the effector organs.

The blocking effect of a number of anaesthetics on the luminescent response was determined. Chloretone, M.S. 222, stovaine, procaine, eucaine, cocaine and isotonic magnesium chloride all greatly diminish the luminescence which can be evoked by peripheral stimulation, and prevent excitation of the photogenic glands when the nerve cord is stimulated. Ether at 0·1 % had little effect. The small response under anaesthesia to peripheral stimulation could be progressively increased by raising the voltage. It is concluded from these results that the anaesthetics block nervous transmission while still allowing the glandular cells to respond directly to electrical stimulation. The assumption is made that the anaesthetics do not materially reduce the responsiveness of the glandular cells. It follows that peripheral stimulation achieves its effect in large part through nervous excitation, but a small amount of direct excitation of the gland cells is also involved.

Augmentation of the luminescent response also was obtained in narcotized specimens by increasing the number of stimuli. This is ascribed to summation in the photogenic cells as the result of direct stimulation.

The temporal characteristics of the response curves and the rate of rise of light intensity have been critically examined. It is found that under a higher frequency of stimulation or more prolonged stimulation there is an increase in the rate of rise of light intensity, as well as an increment of light intensity, and an increase in the total amount of light. The rate of rise is directly related to the intensity of the response. The latent period is also reduced by increasing the period of stimulation. The argument is developed that the response curve reveals the course of oxidation of extruded photogenic material, that light appears subsequent to the series of events involved in photogenic secretion, and that the luminescent curve is only an indirect indication of the magnitude of the events preceding it. The rate of rise of light intensity and the length of latent period are indices of the velocity of the chemiluminescent reaction, which in turn is governed by the initial concentration of the photogenic material.

Besides peripheral augmentation of the luminescent response, a process of central facilitation has been discovered in the nerve cord. This is most apparent in the posterior region of the body, and manifests itself as an increase in the number of segments responding as the duration or frequency of stimulation is raised. The threshold frequency lies at about 5 per sec. In addition, luminescence spreads posteriorly with greater facility than anteriorly, indicating greater resistance to postero-anterior transmission.

The effects of certain drugs on the luminescent response have been investigated. Atropine and *d*-tubocurarine fail to abolish the luminescence evoked by electrical stimulation. Acetylcholine does not appear to affect the magnitude of the response due to electrical stimulation, whereas eserine produces an enhanced response in a minority of specimens. In conjunction with the positive effect of acetylcholine in inducing luminescence in *Chaetopterus*, the augmentative effect of eserine provides suggestive evidence of a transmitter role for acetylcholine.

Histological examination of the photogenic glands has yielded inconclusive results. The epidermis is supplied with abundant nerves lying beneath the photogenic cells, and these fibres are well positioned to mediate secretion. The photogenic cells are surrounded by thin protoplasmic layers which stain like muscle, but lack birefringence which might be expected in fibrous protein. No myo-epithelial elements or muscle fibres were seen in the epidermis. The suggestion is made that the protoplasmic investment of the glandular light cells is contractile, and expresses the cellular contents. A mechanism of this kind explains the physiological results obtained in the present investigation.

The implications of some of these results on the physiology of bioluminescence and the nature of control in the normal life of the animal are discussed. In particular, it is pointed out that the luminous response of the aliform notopodia is a triggered response which can be produced by a single impulse. The spread of the response-area in the posterior region under increased stimulation raises the possibility of normal control of luminescence in that region of the body by alterations in the frequency and number of nerve impulses.

ADDENDUM

Since sending this paper to press I have had the opportunity of reading a paper by Hasama (1941) which describes experiments he has carried out on luminescence in *Chaetopterus variopedatus*. After faradic stimulation Hasama notes that the luminescent response lasts 10–40 sec. and that the duration of luminescence remains constant and independent of the duration of stimulation when the latter exceeds a certain time. No data are offered to support this rough generalization, which is merely a roundabout way of describing fatigue. With the use of non-polarizable electrodes this author has recorded potential

changes on the surface of the light-producing regions during luminescence. The resultant monophasic potentials appear to correspond with the luminescent response and are separable from muscle action potentials. Hasama believes that these potentials are electrical manifestations of the chemiluminescent reaction. Two kinds of cells are described in the epidermis of luminescent regions, viz. type 1 cells which contain granules staining well with toluidine blue, and poorly with eosin; and type 2 cells which stain with eosin and possess cilia. Hasama claims the first type are characteristic of luminescent regions, and are the photocytes, but for reasons which I have presented in earlier papers I believe that the photocytes are peculiar granular eosinophilic cells, lacking cilia. These cells are completely overlooked by Hasama. Harvey's recent book (1952) appeared too late to be consulted in the present work.

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INVESTIGATIONS ON THE MICROFAUNA INHABITING SEAWEEDS ON ROCKY COASTS

(UNTERSUCHUNGEN ÜBER DIE ALGEN-BEWOHNENDE MIKROFAUNA MARINER HARTBÖDEN)

IV. STUDIES ON THE VERTICAL DISTRIBUTION OF THE FAUNA INHABITING SEAWEEDS BELOW THE PLYMOUTH LABORATORY

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(Text-figs. 1–4)

CONTENTS

Introduction, Material and Methods

The present study forms part of a more extensive one on the ecological factors which govern the distribution of the microfauna inhabiting seaweeds on rocky sea-coasts. This part of marine ecology is lagging far behind other branches. I wish to stress two points in particular: first, that our understanding of the composition of the littoral (intertidal) fauna would increase very much if this fauna can be linked with the true infralittoral fauna, and, secondly, problems involving the synecological aspects of marine biology cannot be approached before more is known about the autecology of the animals composing the cryptofauna (i.e. those living in the shelter of algae) and the

causal factors determining their occurrence and distribution within a given area under stated conditions. On this point the work of T. A. Stephenson is especially relevant: as a result of his extensive investigations on the composition of the intertidal flora and macrofauna he has come to stress the importance of autecological rather than of biocenological work on the shore (Stephenson *et al.* 1942; Stephenson, T. A. & A., 1949). Moreover, he has clarified the intertidal terminology and introduced the most useful terms 'supra-' and 'infralittoral fringe'. Since his terminology appears to be the best suited to meet the needs of ecologists, I hope it will be generally applied in future. Most work on littoral ecology has been limited to macro-organisms, but valuable data on the ecology of microfaunas of seaweeds have been supplied by Colman (1940), Kitching, Macan & Gilson (1934), and Dahl (1948). Some ideas on the causes affecting the composition of faunas have been brought forward by Remane (1933, 1940), but with main reference to the bottom fauna. Such 'causal analysis', applied to the microfauna of algae attached to the bottom, is a primary aim of the present study. Dahl (1948) has called attention to the importance of the quantity of detritus, but otherwise almost nothing has previously been attempted in this line.

To establish a numerical basis for comparisons between intertidal and infralittoral samples, a uniform collecting technique is essential. On a rocky substratum the only useful method seems to be that of diving. The same conclusion was reached by Kitching *et al.* (1934), who used a diving-helmet to examine a sublittoral gully in Wembury Bay. Since it was my intention simply to study the population of single tufts of weed, it was possible to carry out the sampling under water (infralittoral and littoral during high water) using nothing but glasses (made by Draeger, Lübeck), flippers (made by Pirelli, Milano), and a belt with pieces of lead fixed to it. The sampling could be carried out only when the sea was fairly calm. A one-litre jar was carried and placed carefully over the clump of weed to be examined, which, when the whole was inside the jar, was torn off at the base as quickly as possible, the hand following and closing the opening of the jar immediately. Since all animals living in seaweeds have a tendency to fasten rather than to loosen their hold if their habitat is disturbed (reaction to wave-shock?), it is likely that my samples contained almost the whole population of the clump. The alga was carefully shaken in formalin, branch by branch, and the derived material examined under a dissecting microscope. All animals were picked out and counted. The nematodes, which were collected for special study, were transferred to glycerin-jelly and mounted on slides for examination.

The whole material was collected just below the Plymouth Marine Biological Laboratory, on the rocks adjoining the men's bathing place (Tinside). The limestone rocks here are broken and fissured, and supply habitats of varying degrees of shelter. For a description of the area see Evans (1947*b*, p. 176:

Tinside to West Hoe). It is only necessary to add that the deposition of sediment is rather heavy along this piece of shore, with important effects. Wave-action being fairly strong the sediment is distributed unevenly, being accumulated in dense tufts of weeds like *Gelidium corneum*, while tall and shrub-like weeds as *Fucus serratus* may be almost free of silt.

The Rivers Tamar and Plym discharge into Plymouth Sound and cause a variable reduction in the surface salinity of the sea water. This effect, however, is large only in winter. Fluctuations in the volume of the more erratic Plym particularly affect Tinside. The silt-laden surface skin of fresh water penetrating far into the Sound, after spells of heavy rain, is a familiar sight from the Laboratory.

No continuous measurements of salinity have been made at Tinside itself, but a fairly accurate picture can be obtained by extrapolating from Milne's data (1938). The salinity values for Tinside can be taken as identical with those for Drake's Island, or somewhat less during wet periods. It is evident that in winter quite large falls in salinity may occur, and appreciable differences daily between high and low water. However, the present work did not start until after 2 or 3 months of settled summer conditions and the winter can be ignored.

From April onwards it can be said that high-water salinities are normally between 32 and 34 ‰ (Typically 33 ‰), and low-water salinities between 31 and 32·5 ‰. The daily (or rather 12 hr.) fluctuation is normally less than 2 ‰. After 2 or 3 days of heavy rainfall inland a temporary fall below 30 ‰ may occur at low tide, but is not likely to have occurred in the summer of 1950 up to the time the work had been completed.

The conditions at Tinside, therefore, as applied to this study, can be regarded as truly marine with some polyhaline influences (see Dahl, 1948). This view is, moreover, supported by the character of the fauna, which is purely marine without any brackish component.

As regards the relation of shore organisms to tidal levels in the Plymouth area, reference is made to Colman (1933), Moore (1935), Evans (1947*b*), etc., who were dealing primarily with the macrofauna and flora. Certain heights and ranges are taken from these authors.

All tidal levels here are given in metres and are referred to *Chart Datum* for the Plymouth area (see The Admiralty Tide Tables). The position of some of the standard levels (see Hartley & Spooner, 1938) is as follows:

E.H.W.S.T.	+5·10	E.L.W.N.T.	+1·78
M.H.W.S.T.	4·78	M.L.W.N.T.	1·40
M.H.W.	4·26	M.L.W.	0·75
M.H.W.N.T.	3·73	M.L.W.S.T.	+0·06
E.H.W.N.T.	3·24	E.L.W.S.T.	−0·47
M.T.L.	2·49		

The seaweeds investigated in this work were collected in the following ranges (which should not be taken as the actual distribution limits):

Ceramium sp.	+2·75 to +0·80
Lomentaria articulata	+1·90 to +1·75
Fucus serratus	+2·00 to +0·20
Porphyra laciniata	+1·95 to +1·50
Gigartina stellata	+2·00 to +1·00
Cladophora rupestris	+3·25 to +2·50
Gelidium corneum	+2·75 to +1·10
Nitophyllum punctatum	−0·70 to −3·00

The material was obtained during a stay at the Plymouth Laboratory between 14 July and 17 August 1950. I wish to thank all members of the Laboratory's staff, above all Mr F. S. Russell, F.R.S., not only for their readiness to help and for all sorts of advice, but also for the spirit prevailing at Plymouth which makes work so easy and the Laboratory itself the most excellent of its kind in Europe. My visit was made possible by financial support from several private institutions and the 'Bundesministerium für Unterricht', at Vienna.

My special thanks are due to Dr A. G. Lowndes, Plymouth, and Prof. L. Pesta, Vienna, who determined the copepods dealt with here. I am also grateful to Dr H. Caspers, Hamburg, for the identification of the chironomid larvae, and, for taxonomic help, to Dr Mary Parke (algae), Dr Vera Fretter (gastropods), Mr G. M. Spooner (amphipods), all of Plymouth, and Prof. A. La Greca (polychaetes), Naples. My thanks are also due to Mrs A. Volsøe for her assistance in writing the paper in English, and to Mr G. M. Spooner for his critical rearrangement and correction of the manuscript.

General Remarks on the Vertical Distribution of the Fauna of Intertidal Seaweeds

The vertical distribution of littoral animals and plants has been studied best in macro-organisms. The zonation of seaweeds, barnacles, limpets, periwinkles, etc., and thus the degree of exposure to air and wave-action which they can endure, is more or less directly observable on the shore. This is due not only to the size of the organisms, but also to the fact that the rocks which serve as a substratum for them may not change appreciably in composition and texture down the length of the shore. The influence of the substratum on the distribution of these organisms may be almost negligible, and the vertical gradients of air-exposure and surf-action reveal themselves clearly in the well-defined zoning (or distribution limits) of the animals and plants in question.

The situation is quite different for micro-organisms inhabiting seaweeds.

The different species of algae serving as substratum for the microfauna show such different features in height, shape and consistency that exposure and wave-action, even within a very restricted area, by no means act uniformly on their inhabitants. It is obvious that the animals inhabiting a dense tuft of *Gelidium corneum* or *Lichina pygmaea* are much more sheltered against desiccation or wave-shock than those living on *Fucus serratus* or *Ascophyllum nodosum*. Therefore, if we suppose that both types of plants might extend over the same vertical range on a rocky coast, the upper level of some littoral animals might well be much higher in *Gelidium* and *Lichina* than in *Fucus* and *Ascophyllum*. Hence it follows that the vertical distribution of the microfauna could be studied best within one single kind of seaweed extending over a sufficiently large vertical range. Numbers of animals per weight or area-unit of the plant from different levels would then be directly comparable.

It is, however, not very often that the conditions are suitable for this kind of investigation in the littoral and upper infralittoral: even within a very limited area of the seashore there is normally a variety of algae some of them forming very narrow belts. To get a fairly true and complete picture of the vertical distribution of the microfauna in the whole area under consideration, the populations of different seaweeds must be studied together. A direct numerical comparison between samples from different weeds is hardly possible owing to their great differences in structure. The best that can be done is to express the vertical distribution of the microfauna in terms of *average 'dominance-values'* (see p. 158) from as many and as different samples as possible. This procedure, it should be remembered, cuts down the differences between single populations and gives an average picture only of the vertical distribution, the reliability of which can be improved by increasing the number of samples.

Different organisms are subjected in a different way to the factors of tidal exposure. The prototypes of littoral zonation: seaweeds, barnacles, periwinkles, etc., are either sessile, hemi-sessile or very slowly moving organisms. They cannot counteract rapid changes of environmental conditions by moving about. We have to presume, therefore, that the animals must be able to endure any condition to be expected in the inhabited area (as proved, for example, by Jacubowa & Malm, 1931, for several bottom-animals, in their ability to withstand anaerobic conditions). In the littoral area the habitat of these animals is either a well-defined 'zone' or has at least one well-defined upper or lower limit. The same conditions should apply to members of the microfauna which are truly sessile, e.g. Bryozoa and Hydrozoa, or hemi-sessile like the tubicolous polychaetes, amphipods (*Corophium*) and isopods (*Tanais cavolinii*); or, indeed, to all animals with slow powers of movement. The latter, it is true, might find shelter in the denser parts of the algae they inhabit, or in minute crevices of the substratum filled with sediments inaccessible to bigger animals, and so possess the ability of counterbalancing

the challenge of the environment if this becomes too unfavourable, but the movements are too small to effect the average vertical distribution on the shore. A great deal of the microfauna belongs to this class, e.g. nematodes, ostracods, small molluscs like *Rissoa* and *Lasaea*, halacarids, etc. There are other animals which would be able to swim or to crawl quickly and to change place during, say, the rise and fall of the tide, but which do not in fact do so, since they are hardy enough to stand any change in the environmental conditions. In this respect they behave like hemi-sessiles. Among them may be included several amphipods, especially *Hyale* spp., which are well-adapted inhabitants of the littoral seaweeds on all rocky coasts.

Finally, there is a group of animals not only able to move relatively far and fast, but also using this ability, i.e. compensating for changes in the environmental conditions by active motion over comparatively great distances. Many of the harpacticids and some amphipods like *Stenothoë monoculoides* seem to belong to this group. The study of their average vertical distribution does not give a true picture of their actual distributions, which might be quite different under different conditions, for example during high and low tide.

Results of the Survey at Tinside

The Population Counts from Different Algae

Gelidium corneum

For investigating the vertical distribution of the microfauna inhabiting a single species of alga in the Tinside area no better prototype could be chosen than *G. corneum*. Not only does it extend over a sufficiently large vertical range of about 1·65 m., but also, due to its dense, tuft-like shape, it contains an almost incredibly large fauna, whereby the numerical comparison of different samples is facilitated.

The results are given in Table I. The numbers of specimens are referred to 1 g. of living alga weighed in dry condition after pressing between pieces of cloth. The samples are arranged from left to right in descending sequence of tidal level. As was to be expected, the number of specimens in the different samples varies very much, from 343 in G-14 to 2818 in G-4. This variation is due in a very slight degree only to differences in the vertical position of the sample. In my opinion the most important factor is the silt content. An attempt was made to estimate the amount of silt by recognizing five arbitrary classes, ranging from 0 to 4. Plotting number of specimens against these classes brought out an evident correlation, at least for nematodes, sabellids and oligochaetes. This agrees with Dahl (1948). This interesting subject is, however, beyond the scope of the present paper. It is treated, at least as far as the nematodes are concerned, in my previous papers (Wieser, 1951, 1952).

In spite of fluctuating values, the distribution of several species throughout the range of the zone shows an obvious vertical gradient. For that purpose it

TABLE I. ANIMALS INHABITING SEVENTEEN SAMPLES OF *GELIDIUM CORNEUM* PER G. DRY WEED

(Note. It has not been possible to determine the oligochaetes. Possibly more than one related species is involved.)

No. of sample	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	G-12	G-13	G-14	G-15	G-16	G-17
Height (m.)	+2·75	2·75	2·50	2·50	2·00	2·00	2·00	1·90	1·80	1·75	1·60	1·55	1·50	1·50	1·50	1·40	1·10
Sediment-classes	3	0	2	2	3	3	1	3	1	3	3	1	1	1	3	2	4
Weight (g.)	1·3	2·2	1·2	1·0	0·8	1·4	1·3	1·2	1·4	1·3	1·6	2·0	0·6	0·6	1·6	1·2	0·8
Nematoda	1270	215	665	600	964	657	470	638	286	658	700	172	373	103	487	370	835
Copepoda	140	48	23	26	180	19	55	65	30	140	113	72	171	51	185	12	14
Ostracoda	130	25	17	32	280	21	41	30	88	119	87	23	40	45	103	7	19
Amphipoda:																	
Hyale nilssoni	5	25	6	18	10	2	10	.	1	.	1	1	.	.	.	2	26
Corophium spp.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	12	9
Stenothoë monoculoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	35	18
Polychaeta:																	
Fabricia sabella	38	.	29	26	614	311	217	483	4	548	420	142	375	40	270	150	44
Amphiglena mediterranea	.	.	.	.	.	.	.	.	1	.	.	.	.	1	.	5	.
Oridia armandi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Grubea pusilla	.	.	.	.	125	8	2	3	.	4	3	1	.	.	3	6	.
Syllis armillaris	.	.	.	.	.	.	3	.	.	.	.	1	.	.	.	.	.
Polydora hoplura	.	.	.	.	.	.	.	2	.	.	.	.	.	.	3	3	.
Exogone gemmifera	.	.	.	.	.	.	.	1	.	.	.	.	.	.	2	.	.
Cirratulus cirratus	.	.	.	.	.	.	.	1	.	.	.	.	.	.	6	.	.
Aonides oxycephala	.	.	.	.	.	.	.	.	.	.	.	.	1	.	2	.	.
Odontosyllis ctenosoma	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
Capitellides giardi	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Oligochaeta	225	130	.	12	230	258	77	64	70	292	172	32	139	2	50	89	90
Halacarida	14	7	13	28	14	6	5	20	6	21	43	4	21	12	3	62	227
Hyadesia sp.	.	1	.	3	3	.	10	2	.	.	1	.	.	.	.	.	.
Chironomidae (larvae)	58	51	70	114	129	24	48	60	23	100	54	40	75	50	45	10	9
Gastropoda:																	
Littorina obtusata	.	.	2	1	.	.	4	.	4	.	.	.	.	.	.	1	3
Skeneopsis planorbis	3	.	14	83	14	5	7	10	3	.	30	5	21	.	1	50	83
Cingulus cingillus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Rissoa parva	.	.	.	4	.	.	2	.	.	.	.	.	.	.	.	.	.
Indet.	2	.	1	3	.	.	.	.	.	36	.	.	6	25	.	.	.
Pelecypoda:																	
Lasaea rubra	685	265	348	1864	164	371	120	20	78	3	2	.	5	9	6	50	431
Mytilus edulis	16	3	10	4	5	10	2	5	3	1	2	3	5	2	3	8	12
Isopoda + Anisopoda:																	
Idothea neglecta ?+*granulosa*	.	.	.	.	.	.	.	.	.	.	.	.	11	.	.	5	10
Jaera marina	.	3	.	.	.	2	1	.	.	.	.	.	.	.	.	.	.
Naesa bidentata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Munna sp.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Tanais cavolinii	32	2	.	.	4	3	1	.	.	.	23	.	.	3	.	7	23
Pantopoda:																	
Phoxichilidium femoratum	.	.	.	.	1	.	1	1	.	.	.	.	.	.	.	.	.
Total	2618	775	1208	2818	2737	1697	1079	1409	597	1925	1651	497	1243	343	1173	885	1853

is convenient to combine several samples and to consider their average number of specimens.

The most interesting change is the decline in number of *Fabricia sabella* between +2·0 and +2·5 m. Up to +2·0 m. the average number of specimens is 263 per g. dried weed, while it drops to 25 in the four uppermost samples at +2·50 and +2·75 m. It appears, therefore, that a 'critical level' for the species may exist between +2·00 and +2·50 m. This level may be taken and tested with respect to other species or groups. In Table II are given the average values for stations G-1 to G-4 compared with those for G-5 to G-17. Three of the more numerous types are selected—*F. sabella*, Oligochaeta and *Lasaea rubra*. Each deserves comment.

(i) *Fabricia sabella*. I do not hesitate to call the level between +2·0 and +2·5 m. a true 'critical level' (in the sense of Colman and Evans) in the Tinside area for this species. The bulk of the population does not extend beyond this level (which does not preclude the penetration of some specimens into higher zones). It is difficult to compare my figures with those of Colman (1940) from Wembury Bay, since that area shows some important ecological

TABLE II. AVERAGE POPULATION DENSITY PER G. OF DRIED WEED

Height (m.)	... +2·75 to +2·50 m.	+2·00 to +1·10
Fabricia sabella	25	263
Oligochaeta	94	117
Lasaea rubra	790	97

differences from Tinside. Colman reports a few *F. sabella* (which also at Wembury penetrates farther upshore than any other polychaete) from *Fucus spiralis* about 1 m. higher up than the upper level of my *Gelidium corneum*, and a great number of specimens from a single sample of *Ascophyllum nodosum*+*Polysiphonia lanosa*, about +3·30 m. Then it is not until the *Laminaria* holdfasts that *Fabricia sabella* makes its appearance again in great numbers (this habitat being the lowest and most sheltered in Colman's samples).

(ii) *Oligochaeta*. The vertical gradient has apparently no significant influence on the distribution of this (or possibly two) species. These animals show a strong affinity to rich silt-content, and vertical differences could not be detected with certainty in the seventeen *Gelidium* samples. In Colman's tables, also, the oligochaetes (*Lumbricillus pumilus* and *L. scoticus*) show a somewhat irregular distribution with no apparent correlation with tidal exposure.

(iii) *Lasaea rubra*. The difference in numbers between the four higher and the thirteen lower samples is more striking than in any of the species investigated. It should, however, be noted that the lowest samples (G-17), whose content of sediments was extremely high, also contained an unexpectedly high number of *Lasaea*. My figures agree fairly well with Colman's statement to the

effect that his four samples of the lichen *Lichina pygmaea* (between +2·68 and +4·13 m.) contained almost incredible numbers of *Lasaea rubra*, while the species did not occur farther down. The relation between the shape of the weeds and their populations (Wieser, 1951) should also be kept in mind: *Gelidium corneum* and *Lichina pygmaea* are very alike in shape and structure, and both may harbour large populations of this lamellibranch for the same reason. (Colman has found an average of about 945 specimens per 1 g. damp *Lichina*, while I got about the same number in my four highest *Gelidium* samples but per 1 g. of dried weed: thus, Colman's figures are still higher; although, if we remember that in my *Gelidium* samples the numbers of nematodes, polychaetes, oligochaetes, copepods and ostracods were often higher and sometimes not very much lower than those of *Lasaea rubra*, while in Colman's samples *Lasaea* is absolutely dominant, the *Gelidium* of Tinside proves to be still richer in living organisms than the *Lichina* of Wembury Bay. As a matter of fact, the number of organisms in *Gelidium corneum* is comparable with those of rich soils which—according to Franz (1950) and not to the authors mentioned by Colman—often reach several million specimens per $m.^2$).

There are several other types which might illustrate the three possible relations to the factor of tidal exposure, viz. (i) the decline in number downshore, (ii) the decline in number upshore, and (iii) the more or less even distribution. Thus to the first belong *Hyale nilssoni* (although low down at G-17 a fairly large number was found), *Hyadesia* sp., probably *Jaera marina* and the chironomid larvae (*Clunio marinus* + *Trichocladius* cfr. *vitripennis*, not separated). For the latter (average of seventy-eight specimens above, fifty-one below +2·0 m.) there may be a critical level at about the lower limit of *Gelidium corneum*, which also accords with Colman, who did not find any insect larvae below +1·20 m. It should be mentioned that on all seashores which I have had the opportunity to investigate I have always found chironomid larvae among those animals which could be regarded as typical inhabitants of the higher intertidal zones.

To the second group belong *Corophium* spp., *Stenothoë monoculoides*, and all Polychaeta errantia (none of which passes above the +2 m. level). To the third group belong *Mytilus edulis*, most of the common nematodes like *Anticoma limalis*, *Thoracostoma figuratum*, *Enoplus communis* (the dominant species in nearly all samples), *Dolicholaimus marioni*, *Halichoanolaimus robustus*, *Monoposthia costata*, *Chromadora nudicapitata*, etc. (see Wieser, 1951); and probably *Tanais cavolinii* and the two Halacarida (most probably *Rhombognathus pascens* and *R. seahami*).

The Leaf-Like Algae Porphyra laciniata *and* Nitophyllum punctatum

It might be permissible to study numerically the microfauna not only within one species of seaweed but also within a single morphological type.

If it is agreed that it is the shape and consistency of the weed that is of prime importance to the composition of its fauna, two different species of algae of similar shape can be treated as a single species. At any rate in the very simple case of two seaweeds with flattened, uniform thalli like *Porphyra laciniata* and *Nitophyllum punctatum* there can hardly be any objection to this method. In the Tinside area *Porphyra* extends to about +2·0 m., while *Nitophyllum* is a typical infralittoral species which I have collected down to −3·0 m. The vertical distribution of the microfauna can thus be examined over a range of about 5 m. on a more or less comparable substratum. What is

TABLE III. DOMINANCE-VALUES OF NEMATODES IN THREE SAMPLES OF *PORPHYRA LACINIATA* AND FOUR SAMPLES OF *NITOPHYLLUM PUNCTATUM*

(*P.* = *Porphyra*; *N.* = *Nitophyllum*.)

No. of sample	L-1 to 3	L-4	L-5	L-6	L-7
Height (m.)	+1·50–1·95	−0·70	−1·20	−1·20	−3·00
Alga	*P.*	*N.*	*N.*	*N.*	*N.*
Anticoma limalis	.	.	.	.	5
Enoplus communis	16	9	4	18	15
Dolicholaimus marioni	5	.	.	.	.
Oncholaimellus diodon	.	.	.	.	8
Oncholaimus dujardini	5	.	.	.	.
Total Enoplidae	26	9	4	18	28
Cyatholaimus demani	5	5·5	6	4	4
Desmodora serpentulus	.	.	.	4	.
Monoposthia costata	5	.	2	.	.
Parasabatiera similis	.	.	2	.	.
Total Cyatholaimidae + Desmodoridae	10	5·5	10	8	4
Spilophorella paradoxa	.	2	.	.	.
Euchromadora tridentata	5	3·5	2	4	4
Hypodontolaimus inaequalis	.	.	2	.	.
Prochromadorella paramucrodonta	.	7	2	22	.
P. neapolitana	.	2	4	.	.
Neochromadora poecilosomoides	.	16	26	22	33
Chromadora nudicapitata	32	18	2	7	8
C. brevipapillata	.	29	47	15	19
C. macrolaima	10	.	.	.	.
Heterochromadora germanica	16	.	.	.	.
Prochromadora longitubus	.	2	.	.	.
Total Chromadoridae	63	79·5	84	70	64
Theristus setosus	.	5·5	.	4	4
Total Monhysteridae	.	5·5	.	4	4
No. of specimens examined	19	55	47	27	27

still more important to this study is that the change in the fauna which takes place between the intertidal and infralittoral zones can now be investigated. Altogether, I obtained three samples of *Porphyra laciniata* between +1·50 and +1·95 m. and four samples of *Nitophyllum punctatum* between −0·70 and −3·00 m. The results are given in Tables III and IV. For the nematodes in Table III, I have to confine myself to dominance-values, since I was unable to study the whole collection. Therefore only a rough picture of the composition of the fauna is given. The three samples of *Porphyra* are pooled.

TABLE IV. NUMBER OF SPECIMENS PER 50 G. OF DRIED WEED IN THREE SAMPLES OF *PORPHYRA LACINIATA* AND FOUR SAMPLES OF *NITOPHYLLUM PUNCTATUM*

(+ = common; + + = very common. *P.* = *Porphyra*; *N.* = *Nitophyllum*.)

	Littoral			Infralittoral			
No. of sample ...	L-1	L-2	L-3	L-4	L-5	L-6	L-7
Height (m.) ...	+1·95	1·50	1·50	−0·70	−1·20	−1·20	−3·00
Alga ...	*P.*	*P.*	*P.*	*N.*	*N.*	*N.*	*N.*
Weight (g.)	35	22	16	3·4	9	5	2·9
Nematoda (see Table III)	23	22	10	2130	462	380	544
Copepoda	4	90	95	1605	885	490	1071
Ostracoda	.	.	.	60	38	50	34
Amphipoda:							
Hyale nilssoni	2	2	.	.	.		.
Jassa falcata	.	2	6	15	15		136
Stenothoë monoculoides	.	.	3	.	.		.
Aora typica	.	.	.	60	112		.
Nannonyx goesi	.	.	.	30	.		.
Leucothoë spinicarpa	.	.	.	15	.		.
Corophium spp.	.	.	.	1020	455		102
Apherusa bispinosa	.	.	.	.	28		.
Polychaeta:							
Amphiglena mediterranea	.	4	.	.	.		.
Oridia armandi	.	.	.	45	.		17
Platynereis dumerilii	.	4	3	840	70		34
Lagisca extenuata	.	.	.	75	.		17
Odontosyllis ctenosoma	.	.	.	330	21		.
Exogone gemmifera	.	.	.	60	7		34
Grubea clavata	.	.	.	75	7		.
Autolytus aurantiacus	.	.	.	.	21		.
Pterosyllis formosa	.	.	.	.	7		.
Sphaerosyllis hystrix	.	.	.	.	7		.
Halacarida	.	2	3	.	126	30	34
Chironomida (larvae)	7	2	3	.	.	.	.
Gastropoda:							
Littorina obtusata	.	4	3	.	5	.	.
Rissoa parva	.	2	6	180	38	80	51
Tricolia pullus	.	.	.	.	11	30	.
Pelecypoda:							
Lasaea rubra	2	.	.	.	.	.	.
Mytilus edulis	.	.	.	60	5	.	.
Isopoda:							
Idothea neglecta ? + *granulosa*	18	12	.	15	.	.	.
Munna sp.	.	.	.	15	.	.	.
Bryozoa:							
Membranipora membranata	.	.	.	+ +	.	.	.
Tunicata:							
Botryllus schlosseri	.	.	.	+ +	+	+	+
Total no. of specimens	56	146	132	6630	3400		2074

In Table IV the number of specimens is given per 50 g. *of dried weed.* Sample L-4 was completely overgrown with *Membranipora membranata* and *Botryllus schlosseri*, and was extremely rich in specimens. The amphipods and polychaetes in samples L-5 and L-6 had to be treated together.

These two tables seem to show some significant contrasts between the littoral (intertidal) and infralittoral zones. Foremost, the increase in total population, in the descent across the low-water level, is most striking. The average number of specimens in the three *Porphyra* samples is 111 per 50 g. as against 3026 in the four *Nitophyllum* samples. Likewise I found twenty species above as against forty species below C.D. (excluding copepods, ostracods and halacarids). Some of the species found in the *Nitophyllum* samples do not reach a maximum until the infralittoral zone is reached (although some of them might well extend farther upshore in denser weeds where they are better sheltered from desiccation—see p. 149 above). Amongst the nematodes are *Neochromadora poecilosomoides*, *Prochromadorella paramucrodonta* and *Chromadora brevipapillata*. Most of the polychaetes, as for example *Lagisca extenuata*, *Exogone gemmifera* and *Grubea clavata*, as well as the tunicate *Botryllus schlosseri*, and the snail *Tricolia pullus* appear to belong to this group. Colman's data agree with respect to polychaetes and tunicates, since it is only in his lowest and most sheltered samples of the *Laminaria* holdfasts that the Polychaeta errantia suddenly occur, being exceedingly rich here both in specimens and species (amongst them *Lagisca extenuata* and *Exogone gemmifera*). The tunicates similarly are strictly confined to the *Laminaria* holdfasts. Comparably, with regard to the true littoral species, viz. *Hyale nilssoni*, the chironomid larvae, and *Lasaea rubra*, the conclusions drawn from the *Gelidium corneum* samples find further support from those of the leaf-like algae (or, if the data seem too scanty to be confirmative, at least they do not contradict them). *Hyale nilssoni*, as well as *Lasaea rubra* and the insect larvae in Colman's samples, are confined to the upper part of the littoral region.

It is no doubt primarily due to the shape of the leaf-like weeds that the difference in the littoral and infralittoral populations, and analogously the difference in the littoral and infralittoral ecological conditions, can be so clearly demonstrated. The flattened thalli of these weeds offer almost no protection to their inhabitants. It is thus natural that they are deprived of animals in zones where the degree of tidal exposure is great, while as soon as the environmental conditions become more favourable (as in the infralittoral zone and especially beneath the protective canopy of tall algae like *Laminaria*) life reappears in the abundance typically associated with surfaces below the level of the sea.

The Tufted Algae: Ceramium *sp.*, Cladophora rupestris *and* Lomentaria articulata

These three weeds occupy much of the tidal zone. Altogether twenty samples were collected ranging from +0·8 to +3·25 m. The results are given in Table V. The three algae differ somewhat in their structure, but as a whole I think their populations can be compared numerically without

TABLE V. TUFTED ALGAE: *CERAMIUM* SP. (*CE.*), *CLADOPHORA RUPESTRIS* (*CL.*) AND *LOMENTARIA ARTICULATA* (*LOM.*), ANIMALS PER G. OF DRIED WEED

(N.B. Samples T-12, 13 and 14 held a particularly large quantity of sediment.)

No. of sample	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12	T-13	T-14	T-15	T-16	T-17	T-18	T-19	T-20
Height (m.)	+3·25	3·25	2·75	2·60	2·50	2·50	2·50	2·50	2·50	2·50	2·25	2·00	2·00	1·90	1·75	1·60	1·60	1·40	1·40	0·80
Seaweed	*Cl.*	*Cl.*	*Ce.*	*Cl.*	*Ce.*	*Cl.*	*Ce., Cl.*	*Cl.*	*Cl.*	*Cl., Ce.*	*Ce.*	*Ce.*	*Ce.*	*Lom.*	*Lom.*	*Ce.*	*Ce.*	*Ce.*	*Ce.*	*Ce.*
Weight (g.)	0·9	0·9	0·5	1·8	1·0	2·0	0·8	0·15	1·2	0·4	2·0	0·5	0·5	2·0	3·6	2·0	1·7	3·5	2·6	1·7
Nematoda	91	84	20	17	120	53	172	28	4	55	93	1400	1096	31	249	450	40	71	116	70
Copepoda	27	10	34	34	16	9	29	7	21	42	3	86	48	4	4	27	4	105	3	20
Ostracoda	10	14	44	17	32	11	20	13	9	67	.	88	92	15	3	6	2	1	1	.
Amphipoda:																				
Hyale nilssoni	28	25	2	14	34	20	33	28	12	25	.	8	.	3	2	.	.	1	.	.
Stenothoë monoculoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Corophium spp.	.	.	.	.	.	.	.	.	.	.	.	.	6	.	.	.	.	1	.	3
Jassa falcata	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Polychaeta:																				
Fabricia sabella	1	2	.	.	.	1	.	.	.	2	.	172	168	13	.	11	.	1	.	2
Oridia armandi	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.
Grubea pusilla	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	3	.	.	.	.
Odontosyllis ctenosoma	.	.	.	.	.	.	.	.	.	.	.	4	.	.	1	.	.	.	.	.
Amphiglena mediterranea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	5	.	.	.	.
Oligochaeta	15	25	.	.	3	.	.	.	.	.	1	116	18	3	2	.	.	.	1	.
Halacarida	14	17	.	2	3	2	7	7	2	7	.	68	10	4	15	2	.	2	.	2
Hyadesia sp.	.	.	.	.	1	.	.	.	.	.	1	.	.	3	9	2	5	1	.	.
Chironomida (larvae)	25	27	10	35	34	31	85	14	2	152	4	28	22	11	4	27	12	6	3	4
Gastropoda:																				
Skeneopsis planorbis	.	.	.	.	3	1	.	7	.	.	.	182	.	25	1	.	2	1	.	.
Rissoa parva	.	.	.	.	.	.	.	.	.	.	1	10	.	.	.	.	1	3	.	1
Littorina obtusata	.	.	.	2	1	1	.	6	.	.	.	6	.	3	.	.	.	3	.	.
Cingulus cingillus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Indet.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	23	.	1	.	.
Pelecypoda:																				
Lasaea rubra	261	239	42	8	4	83	325	56	2	20	.	234	.	1	14	62	.	1	.	.
Mytilus edulis	.	.	.	.	.	.	.	.	.	.	.	12	6	1	3	3	.	.	.	.
Isopoda + Anisopoda:																				
Idothea neglecta ?+*granulosa*	.	.	.	14	1	3	4	13	3	5	3	.	16	2	2	2	3	6	1	8
Jaera marina	2	.	2	.	1	1	.	.	.	.	.	8	.	.	.	.	.	.	.	.
Naesa bidentata	.	.	.	.	.	.	.	.	.	.	1	.	.	.	2	.	.	.	.	4
Tanais cavolinii	.	.	.	2	.	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.
Total	474	443	154	145	253	217	676	179	55	377	107	2428	1482	119	312	623	68	205	125	116

important adjustments for the differences between them. The results agree satisfactorily with those obtained from the other algae, and only brief comment is necessary.

Decline in number downshore. The distribution of *Hyale nilssoni* agrees particularly well with what has been said above. The average lower level seems to lie at about +2·0 to +2·5 m. with some allowance, of course, for individual irregularities and special ecological conditions. In the tufted algae there is an average of 22 specimens per 1 g. dried weed above, against 1·4 below +2·5 m. Again, the distribution of *Lasaea rubra* agrees fairly well. The bulk of the numbers is at any rate to be found above +2·0 m. The samples T-1 and T-2 (+3·25 m.) with their average number of about 250 per g. almost approach the centre of the area which according to Colman is occupied by *L. rubra* in Wembury Bay—i.e. between +2·68 and +4·13 m. For the chironomid larvae *Clunio marinus* and *Trichocladius* cfr. *vitripennis* the results derived from *Gelidium corneum* (p. 153) also apply here: the lower level seems to be situated at about +1·10 m., which accounts for the rather slow decline in numbers in the present samples. There is, nevertheless, a marked difference between the samples above and those below 2·5 m. (41·5 against 12 specimens per g.).

Decline in number upshore. The 2 m. level proves again to be critical for all polychaetes. From Table IV it is suggested that *Rissoa parva* has the centre of its distribution decidedly lower than the lower level of the tufted algae, probably in the infralittoral zone (where it has been found, too, by Kitching *et al.* 1934), although single specimens can go up to about +2·50 m.

Even distribution. This appears to be shown by *Idothea neglecta* and the halacarids, the numbers of other species being too scarce for any conclusions to be drawn. Referring to data, as yet unpublished, I can add here most of the common nematodes, especially *Enoplus communis*, *Chromadorella parapoecilosoma*, *Heterochromadora germanica* and *Chromadora nudicapitata*.

'Average' Distribution

As already noted (p. 149), when data are presented of the fauna of seaweeds of very different structure, the numbers per unit weight tend to reflect the effect of the different substrata on the fauna rather than that of the vertical gradient. To overcome this difficulty it is usual to express the occurrence of the animals as a percentage of the total (giving so-called 'dominance values') and to compare as many samples as possible, aiming at an average picture of their vertical distribution. This procedure is followed in the present section, in which the vertical zoning is examined of amphipods, polychaetes and nematodes, in the littoral and upper infralittoral zones, regardless of the different algae from which they have been taken.[1]

[1] The data of this section are not only based on the samples dealt with in the previous chapter but of all those listed in the Appendix. It was, however, impossible to study every specimen; only a fraction of each sample has been examined taxonomically, the number of which is referred to in the tables of this section.

Amphipoda

The vertical distribution of the ten species found (excluding *Corophium* spp.) is given in Table VI. It is possible to compare these data with the distribution of the amphipods at Wembury combining Colman's and Kitching's results, from collections obtained between 1930 and 1932. Kitching obtained his samples by diving to about 10 ft. below C.D.

TABLE VI. DISTRIBUTION OF AMPHIPODS IN THE TINSIDE AREA. THE FIGURES GIVE PERCENTAGE OCCURRENCE AT EACH LEVEL ('DOMINANCE VALUES')

Height (m.)	+3·50 to +2·75	+2·75 to +2·00	+2·00 to +1·50	+1·50 to 0	0 to −3·00
Hyale nilssoni	100	87·3	46·7	7·0	.
Apherusa jurinei	.	11·1	10·3	16·2	15·7
Jassa falcata	.	0·3	31·7	18·4	33·2
Stenothoë monoculoides	.	.	10·0	51·0	9·3
Gammarus locusta	.	.	1·3	.	.
Pleonexes gammaroides	.	.	.	7·7	.
Apherusa bispinosa	.	.	.	.	4·0
Aora typica	.	.	.	.	29·0
Nannonyx goesi	.	.	.	.	6·2
Leucothoë spinicarpa	.	.	.	.	3·1
No. of samples containing amphipods	4	8	20	13	4
No. of specimens	108	199	166	276	49

TABLE VII. DISTRIBUTION OF AMPHIPODS IN WEMBURY BAY, COMPUTED FROM COLMAN (1940) AND KITCHING *et al.* (1934). FIGURES GIVE PERCENTAGES OF TOTAL AMPHIPODA (DOMINANCE VALUES)

	Colman			Kitching
Height (m.)	+4·63 to +3·50	+3·50 to +1·50	+1·50 to about 0	about 0 to −3·30
Hyale nilssoni	100	97·3	8·7	.
Melita sp.	.	0·2	.	.
(?) *Marinogammarus obtusatus*	.	0·2	.	.
Amphithoë rubricata	.	1·7	0·5	.
Microjassa cumbrensis	.	.	3·0	.
Pleonexes gammaroides	.	.	1·6	.
Hyale pontica	.	.	0·6	.
Stenothoë monoculoides	.	.	0·5	.
Tritaeta gibbosa	.	.	0·3	.
Leucothoë incisa	.	.	0·3	.
Biancolina cuniculus	.	.	0·17	.
Jassa falcata	.	.	50·3	22·6
Apherusa jurinei	.	0·5	9·4	6·4
Elasmopus rapax	.	.	7·1	4·5
Microdeutopus damnoniensis	.	.	5·0	1·8
M. chelifer	.	.	1·0	
Podocerus variegatus	.	.	5·3	0·17
Eurystheus maculatus	.	.	4·0	1·3
Lembos websteri	.	.	2·3	1·0
No. of samples	13	14	17	11
No. of specimens	466	618	621	593

To make this comparison I computed the dominance values of the amphipods from Colman's tables and from Kitching's *Distomus-Halichondria* association. The Caprellidae were omitted, and from Kitching's tables only those species were extracted which were also represented in Colman's samples. I am fully aware of the objections which might be made to this combining of samples taken at different times and by different methods but, nevertheless, the results, set out in Table VII, are not discordant.

Owing to differences in the ecological conditions and positions of samples (algae extending farther upshore, no samples taken between +2·00 and +1·50 m.) the zones of height applied to Colman's data differ from those used for Tinside. Furthermore, the number of specimens and of species found at Wembury is much higher than that found at Tinside, mainly due to the extremely rich *Laminaria* holdfasts and to the fact that the lower zone in Wembury has been more thoroughly investigated. Nevertheless, it appears as if the distribution of *Hyale nilssoni*, *Apherusa jurinei* and the few *Pleonexes gammaroides* agrees fairly well in the two areas, although *Apherusa jurinei* is distinctly more common at Tinside than at Wembury. Kitching's *Jassa* '*dentex*' should be merged with *J. falcata* (see Sexton & Reid, 1951), so *J. falcata* is about as abundant in the infralittoral and lowest littoral zone in Wembury as at Tinside, though it reaches higher upshore in the latter area. The increase in number of species downshore seen in both areas is only to be expected for a marine group of animals.

TABLE VIII. DISTRIBUTION OF POLYCHAETES AT TINSIDE, AS IN TABLE VI

Height (m.)	+3·25 to +2·75	+2·75 to +2·00	+2·00 to +1·50	+1·50 to about 0	About 0 to −3·00
Fabricia sabella	100	100	61·2	32·4	.
Grubea pusilla	.	.	22·7	6·5	.
Amphiglena mediterranea	.	.	8·5	7·8	.
Syllis armillaris	.	.	1·9	0·5	.
Polydora hoplura	.	.	0·8	4·0	.
Cirratulus cirratus	.	.	0·3	1·8	.
Syllis krohni	.	.	0·5	.	.
Capitellides giardi	.	.	0·16	.	.
Odontosyllis ctenosoma	.	.	3·0	24·5	20·4
Platynereis dumerilii	.	.	0·8	13·2	56·1
Exogone gemmifera	.	.	0·8	0·7	6·0
Oridia armandi	.	.	0·3	5·6	2·0
Grubea clavata	.	.	.	1·8	5·1
Autolytus aurantiacus	.	.	.	1·3	2·6
A. prolifer	.	.	.	0·2	.
Spionidae sp.	.	.	.	0·5	.
Aonides oxycephala	.	.	.	0·2	.
Lagisca extenuata	.	.	.	.	5·1
Pterosyllis formosa	.	.	.	.	1·0
Sphaerosyllis hystrix	.	.	.	.	1·0
Phyllodocae maculata	.	.	.	.	1·0
No. of samples (only those with polychaetes)	1	5	26	19	4
No. of specimens	2	23	628	447	119

Species of the genus *Hyale* contribute to the fauna of the highest algal zones on most rocky seashores. For example, in the Mediterranean we find *Hyale prevosti* and *H. nilssoni* f. *stebbingi* to be the dominant amphipods for this 'biotope' and on the Chilean coast *H. hirtipalma, grandicornis* and one or two other species. (For this information I am indebted to Dr E. Dahl, who kindly permitted me to quote it from his yet unpublished material.)

TABLE IX. POLYCHAETA FROM WEMBURY. (SEE TABLE VII)

(+ = less than 0·1 %.)

	Colman			Kitching
Height (m.)	4·63 to 3·50	3·50 to 1·50	1·50 to about 0	about 0 to about 3·30
Fabricia sabella	100	98·3	54·2	4·4
Spirorbis borealis	.	1·5	7·0	2·8
Nereidae	.	0·2	See species	
Amphiglena mediterranea	.	.	10·6	.
Sphärosyllis erinaceus	.	.	6·1	.
Polydora giardi	.	.	6·0	.
Micromaldane ornithochaeta	.	.	5·8	.
Oridia armandi	.	.	4·3	.
Capitellides giardi	.	.	1·4	.
Polydora ciliata	.	.	0·6	.
P. caeca	.	.	0·4	.
Pholoë minuta	.	.	0·17	.
Exogone gemmifera	.	.	0·17	.
Odontosyllis ctenosoma	.	.	0·14	.
Dodecaria concharum	.	.	0·14	.
Sphaerosyllis ovigera	.	.	0·1	.
Eulalia bilineata	.	.	+	.
Eteone picta	.	.	+	.
Grubea limbata	.	.	+	.
Eusyllis lamelligera	.	.	+	.
Exogone brevipes	.	.	+	.
E. verrugera	.	.	+	.
Perinereis cultrifera	.	.	+	.
Polydora hoplura	.	.	+	.
Heterocirrus alatus	.	.	+	.
Polycirrus calientrum	.	.	+	.
Potamilla torelli	.	.	+	.
Jasmineira elegans	.	.	+	.
Hydroides norvegicus	.	.	+	.
Pomatoceros triqueter	.	.	0·5	22·0
Syllis gracilis	.	.	1·0	0·9
Trypanosyllis zebra	.	.	0·4	2·8
Platynereis dumerilii	.	.	0·17	12·5
Syllis variegata	.	.	0·17	1·4
S. armillaris	.	.	0·17	2·0
S. prolifera	.	.	0·1	0·6
Sabellaria spinulosa	.	.	+	4·4
Dasychone bombyx	.	.	+	1·2
Potamilla reniformis	.	.	+	0·6
Lagisca extenuata	.	.	+	0·3
Syllis ferruginea	.	.	+	0·3
No. of samples	2	6	13	11
No. of specimens	6	871	7975	358

TABLE X. NEMATODA FROM TINSIDE, FROM ALL SAMPLES EXAMINED (DOMINANCE VALUES)

(+ = less than 0·1 %.)

Height (m.)	+4·50 to 3·25	+3·25 to 1·50	+1·50 to 0	0 to −3·00
Leptosomatidae:				
Anticoma limalis	.	6·5	5·5	2·0
A. pellucida	.	0·5	+	.
Leptosomatum bacillatum	.	.	+	.
Thoracostoma figuratum	.	2·4	1·0	.
Th. (Pseudocella) trichodes	.	0·2	+	.
Oxystomatidae:				
Trefusia longicauda	.	0·3	.	.
Thalassoalaimus tardus	.	+	.	.
Halalaimus gracilis	.	+	.	.
Phanodermatidae:				
Phanoderma albidum	.	+	0·3	.
Enoplidae:				
Enoplus communis	62·0	30·0	36·4	13·0
Dorylaimidae:				
Dolicholaimus marioni	.	3·8	0·4	.
Syringolaimus striaticaudatus	.	+	+	.
Oncholaimidae:				
Krampia acropora	.	0·1	0·3	.
Cavilaimus macramphis	.	.	0·1	.
Pontonema vulgaris	.	+	.	.
P. donsi	.	+	0·15	.
Metoncholaimus demani	.	+	0·1	.
Oncholaimus dujardini	.	+	0·75	.
Oncholaimellus diodon	.	+	.	2·0
Oncholaimide juv.	.	.	+	.
Enchelidiidae:				
Eurystomatina filiformis	.	.	+	.
Symplocostoma longicollis	.	+	.	.
Catalaimus maxweberi	.	0·5	0·8	.
Cyatholaimidae:				
Cyatholaimus demani	1·4	0·5	1·5	6·0
Paracanthonchus coecus	.	4·0	0·3	.
P. kreisi	.	+	.	.
Choanolaimidae:				
Halichoanolaimus robustus	.	1·7	0·4	.
Desmodoridae:				
Desmodora serpentulus	.	4·8	1·0	1·0
D. scaldensis	.	1·0	+	.
Xenodesmodora porifera	.	+	.	.
Monoposthia costata	.	1·0	0·5	0·5
Microlaimidae:				
Crassolaimus bipapillatus	.	+	.	.
Comesomidae:				
Parasabatiera similis	.	.	.	0·5
Chromadoridae:				
Spilophorella paradoxa	.	1·8	2·8	0·5
Spilophora gracilicauda	.	0·3	0·7	.
Chromadorina parva	.	1·0	0·3	.
Euchromadora vulgaris	3·0	1·0	0·1	.
E. tridentata	.	0·3	+	3·5
Hypodontolaimus inaequalis	.	0·4	0·4	0·5
Prochromadorella neapolitana	.	.	.	1·5

TABLE X (*continued*)

Height (m.)	+4·50 to 3·25	+3·25 to 1·50	+1·50 to 0	0 to −3·00
Chromadoridae (*cont.*):				
Prochromadorella mediterranea	.	+	.	.
P. paramucrodonta	.	+	0·8	0·8
P. macro-ocellata	.	0·8	.	.
P. obtusidens	.	1·3	.	.
Chromadorella parapoecilosoma	.	2·6	1·0	.
C. microlaima	.	0·3	0·6	.
Neochromadora poecilosomoides	.	+	0·5	17·0
Chromadora nudicapitata	32·0	13·5	34·0	10·0
C. brevipapillata	.	.	0·2	29·5
C. macrolaima	.	0·6	0·1	.
Heterochromadora germanica	.	10·1	4·0	.
H. granulo-pigmentatus	.	0·8	0·2	.
H. cervix	.	0·3	.	.
Prochromadora longitubus	.	+	0·2	0·5
Axonolaimidae:				
Odontophora setosa	.	0·1	.	.
Araeolaimidae:				
Araeolaimoides paucisetosa	.	+	.	.
Camacolaimidae:				
Camacolaimus tardus	.	2·1	1·1	.
C. conicaudatus	.	+	+	.
Halaphanolaimidae:				
Dermatolaimus membranatus	.	0·6	.	.
Linhomoeidae:				
Linhomoeus elongatus	.	0·2	0·3	.
Paralinhomoeus lepturus	.	+	.	.
Metalinhomoeus typicus	.	+	.	.
Monhysteridae:				
Theristus acer	.	2·0	1·0	.
T. normandicus	.	+	.	.
T. setosus	.	.	.	4·0
Theristus sp.	.	+	.	.
Monhystera parva	.	+	0·2	.
M. luisae	1·1	.	.	.
M. refringens var. *britannica*	0·5	+	0·2	.
M. disjuncta	.	+	0·8	.
No. of samples	3	33	11	4
No. of specimens	262	4160	1327	156

Polychaeta

In Table VIII is summarized the distribution of the polychaetes at Tinside (the serpulids were not counted and are, therefore, omitted). It must be stressed that the absence of species in the lowest zone is not at all conclusive since only relatively few specimens and only from one single biotope, viz. *Nitophyllum punctatum*, were studied.

By the same method as above the data from Colman's tables and Kitching's *Distomus-Halichondria* association have been extracted (Table IX). Still more striking than in Amphipoda is the abundance of species and individuals in the *Laminaria* holdfasts which makes this habitat almost incomparable with any other. Despite the differences between Tinside and Wembury Bay,

in number of specimens studied and in the habitats sampled, two facts seem to be fairly well established. First, *Fabricia sabella* is the polychaete with by far the highest power of resistance to exposure, and it dominates the higher zones. Secondly, the level at about +2 m. is critical for nearly all other species, indicating that most polychaetes are fairly susceptible to exposure.

Nematoda

The distribution of the nematode fauna in the Tinside area has been dealt with in a previous paper (Wieser, 1951). It has been shown that nematodes are more dependent on the shape of the algae on which they live and on the silt content than any of the groups examined. The study of the vertical distribution is somewhat hampered by these facts since we are even less sure about the 'causae efficientes' of the presence or absence of a given species than in other animals.

All the available data, however, are summarized in Table X, giving the vertical distribution of the seventy species found at Tinside amongst 5945 specimens picked out from fifty-one samples. The uppermost and the lowermost zones were not so well studied as the two middle zones, and their data are thus less reliable. These deficiencies remembered, attention may be called to a few points.

(i) The most evenly distributed species are *Enoplus communis*, *Cyatholaimus demani* and *Chromadora nudicapitata*. (ii) In my opinion there are several species which can be called true infralittoral forms, viz. *Neochromadora poecilosomoides*, *Chromadora brevipapillata*, possibly *Theristus setosus* and *Prochromadorella neapolitana*. (iii) In two genera very closely related species seem to replace each other in the upper and lower part of the shore respectively, viz. *Euchromadora vulgaris* (high) and *E. tridentata* (low) and *Chromadora nudicapitata* (high) and *C. brevipapillata* (low).

Critical Zones

Colman (1933) and Evans (1947*a*, *b*) introduced and applied the term 'critical level' which accounts for the observation that 'certain levels (of the intertidal region) have been shown to be more critical than others in connexion with the distribution of intertidal plants and animals' (Evans, 1947*b*). Colman and Evans studied critical levels only in macro-organisms. In micro-organisms inhabiting seaweeds the problem becomes more complicated since the effect of the substratum, i.e. the seaweeds, on the vertical distribution of the fauna has to be taken into account. Where certain algae reach their upper limit most of the animals living among them will also find there the limit for their penetration into the intertidal zone. It is, however, not established whether this is due to the same change in the degree of tidal exposure which causes

the disappearance of the algae or to the fact that they are dependent on the presence of the latter from a purely mechanical point of view. I therefore want to apply the term 'critical level', as far as the microfauna is concerned, only to those limits which do not correspond with the disappearance of algae. It can, however, be concluded from the data presented above that changes in the algal fauna may reflect the influence of the substratum to a greater degree than found in organisms attached to rocks. Differences in shape of the seaweeds cause the 'critical level' to oscillate somewhat in different habitats of the same area so that one could rather speak of a critical 'zone'. Or, in other words, the range of variation of the upper and (or) lower limits of certain animals seems to be greater in species inhabiting seaweeds than in those living as hemi-sessile or slowly moving animals on the surface of the rocks.

The five critical levels distinguished by Evans (1947*a*, p. 211 *et seq.*) are as follows: (1) between M.L.W.S. and E.L.W.S., where the majority of intertidal species achieve their lower limits; (2) between M.L.W.S. and M.L.W.N., which marks the lower limits of certain other intertidal species; (3) just above M.L.W.N., where several sublittoral species reach their upper limits of penetration into the intertidal zone; (4) just below M.H.W.N., marking the upper limit of one set of intertidal species; (5) between M.H.W.S. and E.H.W.S., where a further set of intertidal plants and animals achieve their upper limits. According to what has been said above I must leave out levels nos. 4 and 5, since they would only concern species which disappear together with their seaweeds. Furthermore, levels (1) and (2) which overlap even in Evans's figure (his p. 213) must be regarded as one as far as the microfauna is concerned. This I call 'Zone A', which is situated between M.L.W.S. and M.L.W.N. in the area under consideration. In this zone the true intertidal species which were found reach their lower limits.

I recognize an analogue of Evans's level no. 3, which I call 'zone B' and which is situated between E.L.W.N. and M.T.L. This is slightly higher upshore than Evans's level, for which the reason is believed to be the dense tufts of *Gelidium corneum* which allow several species to penetrate farther into the intertidal region than they do in any other weeds examined. In this 'zone B' a set of infralittoral animals reaches its upper limit.

A third 'zone C' deserves mention which either has no counterpart in Evans's survey or must be regarded as the lower part of his level 3 (though in my samples well distinct from 'zone B'). It marks the upper limit of another set of infralittoral species. It happens here to coincide with my 'zone A', but it should not be assumed that it necessarily does everywhere.

Summing up, the following 'critical zones' may be distinguished in the Tinside area:

(A) Between M.L.W.S. and M.L.W.N., where several intertidal species reach their lower limits.

(B) Between E.L.W.N. and mean tide level, marking the upper limit of a set of infralittoral species.

(C) Between M.L.W.S. and M.L.W.N., where another set of infralittoral species achieves its upper limit.

The results are given in Fig. 1. It is only meant to show the one (upper or lower) limit of the species concerned that falls within the critical zone. This limit is indicated by broken lines marking somewhat deliberately the oscillations of the zone. The line 'bulk of *Fabricia sabella*' indicates the sudden

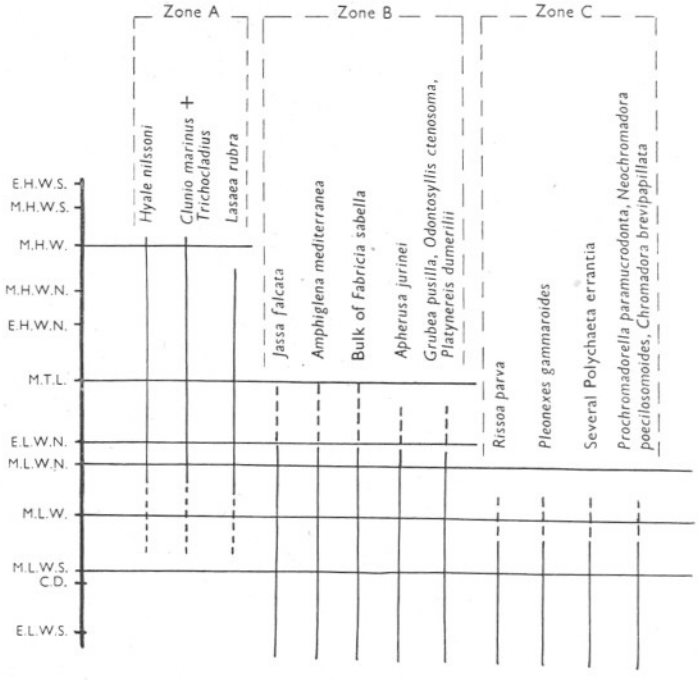

Fig. 1. Distribution of certain faunal types in relation to critical levels (see text for further explanation).

decline in number of individuals of this species at about the +2·0 m. level (Table II), though single specimens might reach farther upshore.

The following comparisons can be made with the data on the macrofauna published by Colman (1933), Evans (1947*b*) and Yonge (1949).

The species of zone A (*Hyale nilssoni*, *Clunio marinus*, *Trichocladius* cfr. *vitripennis* and *Lasaea rubra*) are the counterpart of *Littorina littorea*, *L. obtusata*, *Patella vulgata*, *Osilinus lineatus*, *Chthamalus stellatus*, *Ascophyllum nodosum*, and *Fucus vesiculosus*; the species of zone B (*Jassa falcata*, *Apherusa jurinei*, *Amphiglena mediterranea*, *Grubea pusilla*, *Odontosyllis*

ctenosoma, *Platynereis dumerilii*) approximately coincide in their distribution with *Gibbula cineraria*, *Rhodymenia palmata*, *Gigartina stellata* and *Chondrus crispus*.

The most interesting species, however, are those which achieve their upper limit in zone C (*Pleonexes gammaroides*, *Rissoa parva*, *Oridia armandi*, several Polychaeta errantia, and nematodes like *Prochromadorella paramucrodonta*, *Neochromadora poecilosomoides* and *Chromadora brevipapillata*), since they correspond with the organisms of the 'infralittoral fringe' (Stephenson, T.A. & A., 1949), i.e. species which are typically infralittoral but nevertheless occupy a small fringe in the lower part of the intertidal region. To them belong *Laminaria digitata*, *Himanthalia lorea*, *Pyura stolonifera* and most probably *Verruca stroemia* and *Calliostoma ziziphinum*, quoted by Colman (1933). Furthermore, Yonge's 'average low tide level' (1949) coincides with the upper level of the infralittoral fringe and therefore, also, with my zone C (as upper limit of a set of infralittoral species) and zone A (as lower limit of some intertidal species). Briefly, this level between M.L.W.N. and M.L.W.S. appears to be the most critical throughout the intertidal area: it is a true 'turning-point' of the highest ecological significance.

'Dynamics' of Vertical Distribution

In the previous sections the distribution of the microfauna was dealt with from a purely 'static' point of view, i.e. the upper or lower limits of certain species or their level of maximum abundance was given by using the average values of several samples disregarding the state of changes (rhythmical or sporadic) which might occur in the environment. The result is an *average distribution* of the species in question which fully corresponds with the *actual distribution-area* in sessile organisms, to a very great extent also in hemi-sessiles, slowly moving and highly euryoecous species. But with active and (relatively) stenoecous species care should be taken not to mix together samples which have been taken under quite different environmental conditions (as, for example, height of tide), since by this method it is impossible to detect movements which may counterbalance environmental changes if they become too unfavourable. In this case a more discriminating method should be applied and samples taken under different conditions should be kept separate. The term 'actual distribution-area' therefore is meant to comprise all changes in the distribution of a given species correlated with fluctuations in the environment. Naturally, certain restrictions have to be made, since over sufficiently long periods even sessile organisms extend or restrict their area of distribution for the special requirements of reproduction. Therefore only short-period fluctuations and their effect on some species will be discussed. Similar behaviour has been thoroughly investigated in movements of marine and limno-plankton in connexion with changes of light intensity, etc.

As is well known, tidal movements are of great importance in the littoral region. In areas with a big tidal range, in particular, extensive movements of the more mobile animals may occur with the rise and fall of the tide, but these are almost unknown in the microfauna. Watkin (1941) has shown the changes occurring in the arthropod fauna of a sandy intertidal area during high water. Two sorts of movements were found: the passive (and active?) upward transport by the rising tide of animals living in deeper water, and the (active) migration of true sand-dwelling species of the intertidal area into the waters above. With qualifications this can be compared with Remane's division into 'horizontal' and 'vertical migrants' (1940, p. 107).

It was possible to detect similar differences in the distribution of certain animals between low and high water on a rocky coast, such as at Tinside. This could be proved by collecting samples in several localities during low tide, and, by diving, during high tide. If the same differences of distribution occurred in all samples they could be regarded as significant. Naturally, not all the algae examined gave the same results. For example, in the small tufts of *Gelidium corneum* it was not possible to detect any differences in the composition of the fauna between high and low water. The most obvious differences were seen in the tall and shrub-like seaweeds *Gigartina stellata* and *Fucus serratus*. These weeds are most liable to desiccation when the tide is out and it is quite understandable that a set of mobile animals should leave them in this state and occupy them again on the succeeding flood. Those animals which cannot counteract the challenge of the environment in this active manner will mostly—as has been suggested in the general remarks at the beginning of this paper—find shelter in minute crevices, between the tiny roots and branches of epiphytes and epizoids.

I think it possible to distinguish two modes of migration within the fauna of seaweeds according to whether the animals are able to swim or merely able to crawl about. To illustrate the second condition I would refer to *Littorina obtusata*, the 'average' distribution of which is confined to the lower two-thirds of the intertidal area. The numbers of this species in twelve samples of *Gigartina stellata*, six of them taken during high tide, the other six during low tide, is shown in Fig. 2. Each column represents a different station within the area investigated. The whole column shows the number of specimens (per 20 g. dried weed) of the high-water sample, the black part that of the low-water sample at the same location. Thus the white part of each column represents the surplus of the flood samples over the ebb samples, and this appears to be quite significant.

These data strongly suggest that the snail carries out movements synchronous with the rise and fall of the tide. During high water it is more numerous on the fronds than during low water, when it seeks shelter within the denser and lower part of the seaweeds and in crevices of the rocky substratum nearby, escaping therefore detection if the seaweed is collected.

These movements are explicable if we keep in mind that *Littorina obtusata* feeds directly on algae and therefore the submerged and slowly floating fronds provide a much better opportunity than the dry and shrunken shrub to which tall algae like *Gigartina* are reduced during low water.

That it is the degree of humidity of the substratum and of the snail itself which causes these migrations is supported by the results of Haseman (1911), who found the same oscillatory movements corresponding to those of the tides in *Littorina littorea* at Woods Hole, Mass. This species feeds on small algae growing on rocks. It never crawls on dry surfaces and it has been shown by various experiments that 'the primary directive force for rhythmical

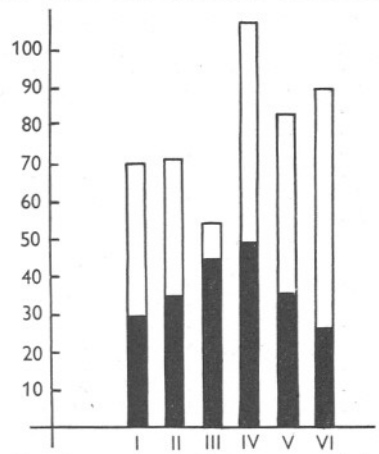

Fig. 2. Numbers of *Littorina obtusata* in six low-water and six high-water samples. Each column represents a different station. On the ordinate the number of specimens per 20 g. of dried weed. (For further explanation see text.)

movements is the surface film of water' (Haseman, 1911, p. 120). These vertical movements shown by *Littorina* make them comparable with Remane's 'vertical migrants', i.e. the species inhabiting an intertidal sandy area, which, when the tide recedes, crawl downwards into the interstitial spaces of the sand-grains, thus remaining in a zone of optimal humidity.

A similar mode of distribution, at least in appearance, is found in several copepods. But, since we know that copepods can swim ('bivagil' according to Remane, 1940, p. 191), in addition to probable movements within the seaweed itself, there are possibly more extensive migrations from one level to another, following the falling tide downshore and rising again with the flood. I am inclined to regard this type of migration as prevailing in the copepods in view of the abundance in plankton catches made by night of the species which are known to inhabit seaweeds (observation of Dr E. Dahl on the Swedish west coast, unpublished).

The present material was not adequate for studying several species separately, mainly because of the patchiness of their distribution (see also

Colman, 1940, p. 147). The distribution of the individual species is given in Table XI, while in Fig. 3 all the species are treated together. Again the high-water samples are represented by the whole columns and the low-water samples by the black part of them. (Owing to accidental loss of the tubes, a full examination of no. III and no. I from low water was not made.)

TABLE XI. OCCURRENCE OF COPEPODS IN TWELVE SAMPLES OF *GIGARTINA STELLATA*, DIVIDED INTO HIGH-WATER AND LOW-WATER SAMPLES

(+ = present; + + = very common.)

	High water						Low water					
No. of sample ...	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Dactylopodia vulgaris	.	+ +	.	.	+	.	.	.	.	+	+ +	+ +
Idya minor	+ +	.	.	.	.	.	.	.	.	.	.	.
I. graciloides	.	.	.	+	.	.	.	.	.	.	.	.
Zaus spinatus	.	.	.	+	+	+	.	.	.	.	.	.
Saccodiscus littoralis	.	.	.	.	.	.	.	.	.	.	+	.
Rhynchothalestris rufocincta	.	.	.	.	.	.	.	+	.	.	.	+
Parathalestris clausi	.	.	.	.	+	+ +	.	+	.	.	+ +	.
P. harpacticoides	.	.	.	+	+	.	.	.	.	.	.	.
Laophonte similis	.	.	.	.	.	.	.	.	.	+	.	.
L. inopinata	.	.	.	.	.	.	.	.	.	.	.	+
Heterolaophonte sp.	.	.	.	.	.	.	.	+	.	.	.	.
Amphiascus sp.	.	.	.	.	.	.	.	.	.	.	.	+
Pseudonychocamptus koreni	.	.	.	.	.	.	.	.	.	.	+	+
Parastenhelia spinosa	.	.	.	.	.	.	.	+	.	.	.	.
Ameira longipes	.	.	.	.	.	.	.	+ +	.	.	.	.
Harpacticidae juv.	.	.	.	.	+	.	.	.	.	.	+	.
Oithona helgolandica	.	.	.	+	.	.	.	.	.	.	+	.
Acartia clausi	.	.	.	+	.	.	.	.	.	.	.	.
Total no. of specimens per 20 g.	31	53	32	65	50	31	12	14	30	14	25	18

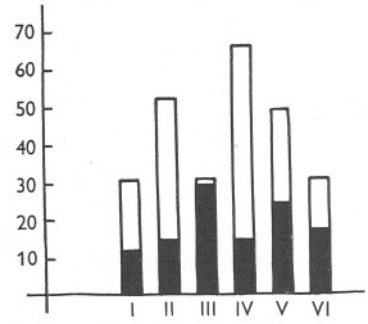

Fig. 3. Total Copepoda numbers at six stations, as in Fig. 2.

Since the copepods leave their seaweed cover with the receding tide and occupy it again on the flood we should reckon them (at least those species mentioned in Table XI) amongst Remane's 'horizontal migrants', though, with the exception of *Oithona helgolandica*, they do not come in from off-shore but only from lower levels of the algal zone. Nevertheless, a 'horizontal gradient' takes part in the movement and makes the analogy suggestive.

Finally, the vertical distribution of the amphipod *Stenothoë monoculoides* has been studied in twenty-four samples, ten of which were taken during high water, from *Fucus serratus* and *Gigartina stellata*. The results are given in Fig. 4 and Table XII. Since the collecting was carried out more thoroughly than in the former examples, I can give the exact height above C.D. of the samples taken. Within a limited vertical range 2 to 5 samples were collected, the average value of which is represented by the columns in Fig. 4.

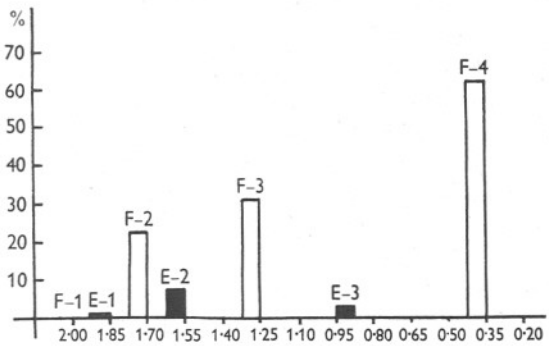

Fig. 4. Distribution of *Stenothoë monoculoides*. Every column represents the average 'dominance value' of two to five samples within a limited vertical range. The mean vertical position of the samples above C.D. is shown by the position of the columns on the abscissa (metres above C.D.). Black columns represent low-water, white columns high-water samples.

TABLE XII. DISTRIBUTION OF *STENOTHOË MONOCULOIDES*. SPECIFICATION OF ALL TWENTY-FOUR SAMPLES EXAMINED (FOR FURTHER EXPLANATION SEE TEXT)

	Low water			High water				
Station no. ...	E-1	E-2	E-3	F-1	F-2	F-3	F-4	Total
No. of *Gigartina* samples	3	3	2	1	2	2	0	13
No. of *Fucus* samples	2	2	2	1	1	1	2	11
Range of height above C.D. (m.)	2·0	1·9–1·4	1·3–0·7	2·0	1·8	1·6–1·0	0·7–0·2	2·0–0·2
No. of specimens in each sample	1, 0, 0, 0, 0	14, 2, 0, 0, 0	1, 1, 0, 0	0, 0	39, 2, 0	5, 72, 0	5, 20	162
Average dominance value of all samples	1	8	3	0	23	31	62	.

This time the dominance value of *Stenothoë monoculoides* in the whole amphipod population is given along the ordinate, since the algae were overgrown by epiphytes (*Elachistea fucicola*) and epizoids (*Membranipora membranacea*, *Dynamena pumila*) to very different degrees, making it impossible to compare the numbers per weight-unit. (The numbers as well as 'dominance values' are given in Table XII.) The data clearly show that the occurrence of the amphipod in the two algae considered, and between +2·0 and +0·2 m., is much more abundant during high than during low water. Furthermore, there might also be decrease in the density of the population upshore during high water, pointing to the same susceptibility to desiccation which causes

emigration from the intertidal area with the fall of the tide. For these reasons *Stenothoë monoculoides*, too, should be regarded as a 'horizontal migrant'.

Summary

The vertical distribution of the microfauna inhabiting seaweeds in the Tinside area of Plymouth Sound has been discussed from various points of view. Samples were collected by diving below water-level (infralittoral and intertidal during high water) to ensure uniformity in the method of collecting.

A very strong influence of the substratum on the distribution of the fauna was observed. The extent to which different powers of locomotion of the animals concerned might help to counteract environmental changes is discussed.

The fauna of *Gelidium corneum* was studied in seventeen samples between +2·75 and +1·10 m. (above C.D.). Examples of the three possible relations to the factor of tidal exposure shown by littoral animals—(i) decline in number downshore, (ii) decline in number upshore, and (iii) more or less even distribution—are given.

The fauna of the two leaf-like algae *Porphyra laciniata* and *Nitophyllum punctatum* was studied in seven samples extending from +1·95 to −3·00 m. A most striking increase in population density was observed passing below low-water mark. Some species are indicated which are confined to the infralittoral or the littoral samples respectively.

The three tufted algae: *Ceramium* sp., *Cladophora rupestris* and *Lomentaria articulata* were studied between +3·25 and +0·8 m. in a total of twenty samples. Further support for the results gained from the *Gelidium* samples was obtained. The 'average' distribution of amphipods, polychaetes and nematodes in all samples studied is given. A comparison with published data on the fauna of Wembury Bay (Colman, Kitching, etc.) shows interesting agreement in some points.

It is pointed out that 'critical zones' for the distribution of the microfauna might occur in the area studied. Three zones are suggested, viz. zone A, between M.L.W.S. and M.L.W.N., where several intertidal species reach their lower limits; zone B, between E.L.W.N. and M.T.L., marking the upper limit of a set of infralittoral species; zone C, between M.L.W.S. and M.L.W.N., where another set of infralittoral species achieves its upper limit.

For each zone examples are given and comparisons drawn with the results obtained by Colman, Evans and Yonge.

The term 'dynamics of vertical distribution' is introduced, taking into account that various species might have a very different distribution in the intertidal area according to the state of the tide. The differences in the distribution between low and high water are suggested for *Littorina obtusata*, several copepods and the amphipod *Stenothoë monoculoides*. The agreement with the results obtained by Haseman, Watkin and Remane is discussed.

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Appendix. List of all Samples Examined. Specification, Position, Numbers of Amphipods, Polychaetes, Nematodes and Total of Specimens.

Alga	Sample no.	Height relative to C.D. (m.)	Weight (g.)	Degree of silting (0–4)	Epi-growth (0–4)	Covered with water (+) or not (−)	Amphipods	Polychaetes	Nematodes	Total of specimens
Gelidium corneum	G-1	+2·75	1·3	3	0	−	6	49	1650	3400
	G-2	2·75	2·2	0	0	−	54	.	472	1697
	G-3	2·50	1·2	2	0	−	8	34	798	1427
	G-4	2·50	1·0	2	0	−	18	26	600	2816
	G-5	2·00	0·8	3	0	−	8	591	771	2186
	G-6	2·00	1·4	3	0	−	2	445	920	2364
	G-7	2·00	1·3	1	0	−	14	288	511	1307
	G-8	1·90	1·2	3	0	−	4	587	765	1685
	G-9	1·80	1·4	1	0	−	2	8	400	829
	G-10	1·75	1·3	3	0	−	.	723	856	2505
	G-11	1·60	1·6	3	0	−	1	675	1200	2714
	G-12	1·55	2·0	1	0	−	2	289	344	997
	G-13	1·50	0·6	1	0	−	.	227	224	749
	G-14	1·50	0·6	1	0	−	.	24	62	203
	G-15	1·50	1·6	3	0	−	1	458	779	1870
	G-16	1·40	1·2	2	0	−	60	196	450	1106
	G-17	1·10	0·8	4	0	−	42	35	668	1480
Cladophora rupestris	T-1	+3·25	0·9	0	0	+	25	1	82	427
	T-2	3·25	0·9	0	0	+	22	2	76	398
	T-4	2·60	1·8	0	0	−	25	.	32	240
	T-6	2·50	2·0	0	0	−	40	1	107	426
	T-8	2·50	0·15	0	0	+	4	.	4	24
	T-9	2·50	1·2	0	0	+	14	.	5	67
	T-10	2·50	0·4	0	0	+	10	1	22	152
Ceramium sp.	T-3	+2·75	0·5	0	0	−	1	.	10	77
	T-5	2·50	1·0	0	0	−	34	.	120	253
	T-7	2·50	0·8	0	0	+	27	.	138	542
	T-11	2·25	2·0	0	0	−	.	.	187	210
	T-12	2·00	0·5	2	0	−	4	93	700	1216
	T-13	2·00	0·5	2	0	−	3	84	548	741
	T-16	1·60	2·0	0	0	−	.	36	900	1246
	T-17	1·60	1·7	0	0	−	.	.	66	111
	T-18	1·40	3·5	0	0	−	3	2	247	690
	T-19	1·40	2·6	0	0	−	.	.	302	322
	T-20	0·80	1·7	0	0	−	8	2	118	190
Lomentaria articulata	T-14	+1·90	2·0	1	0	−	6	32	62	233
	T-15	1·75	3·6	1	0	−	6	7	708	930
Porphyra laciniata	L-1	+1·95	35·0	0	0	−	1	.	13	32
	L-2	1·50	22·0	0	0	−	2	4	10	66
	L-3	1·50	16·0	0	0	−	3	1	3	44
Nitophyllum punctatum	L-4	−0·70	3·4	0	4	+	76	95	142	444
	L-5	−1·20	9·0	0	1	+	90	205	84	376
	L-6	−1·20	5·0	0	1	+			38	131
	L-7	−3·00	2·9	0	1	+	14	6	32	121
Gigartina stellata	S-1	+2·00	18	0	0	+	2	10	25	212
	S-2	2·00	35	0	1	+	23	61	95	719
	S-3	2·00	45	0	1	−	19	95	52	493
	S-4	2·00	40	0	?	−	1	19	7	114
	S-5	1·80	15	0	1	+	5	37	48	245
	S-6	1·80	22	0	2	+	66	20	36	445
	S-7	1·80	40	0	2	−	57	143	196	992
	S-8	1·80	55	0	3	−	16	154	273	881
	S-9	1·50	17	0	?	+	4	9	18	211
	S-10	1·50	3	0	2	−	1	17	5	28
	S-11	1·40	32	0	2	+	67	39	51	528
	S-12	1·40	40	0	4	−	30	52	119	580
	S-13	1·30	20	0	3	+	128	12	24	420
	S-14	1·25	60	0	4	−	85	213	588	1629
	S-15	1·00	40	0	2	−	20	159	259	650
Fucus serratus	F-1	2·00	135	0	1	−	.	23	205	347
	F-2	2·00	24	0	1	−	2	3	18	67
	F-3	1·90	8	0	3	+	1	25	127	191
	F-4	1·90	25	0	0	+	1	2	52	100
	F-5	1·60	13	0	0	+	.	3	1	12
	F-6	1·50	20	0	0	−	.	1	16	23
	F-7	1·50	16	0	0	−	4	13	11	77
	F-8	1·40	65	0	1	−	1	19	56	128
	F-9	1·00	165	0	1	−	26	8	123	307
	F-10	1·00	21	0	0	+	.	.	1	10
	F-11	0·70	75	0	2	−	32	34	135	464
	F-12	0·70	28	0	3	+	27	16	200	423
	F-13	0·20	25	0	3	+	19	26	228	437

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE SMALLER MARINE ALGAE

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(Plates I and II)

Introduction and Methods of Isolation

Our lack of knowledge of nanoplankton organisms is due largely, no doubt, to the necessity for examining them alive and the comparative difficulty of observing them, and also to their being, apparently, of no more than indirect importance in the ecology of fishes, except when their occasional presence in great abundance has been accompanied by unfavourable hydrographical changes. An adequate survey of life in the sea is bound to take them into account. Since they are frequently abundant, at least in inshore waters, and propagate rapidly, they probably form an important constituent of the food of adult filter-feeding invertebrates. They are likely to be of still greater importance as the essential food of many planktonic larvae, particularly in the early stages. Oyster larvae (*Ostrea edulis* L.) can ingest nothing larger than about 10 μ and appear to rely for food on minute flagellates (Cole, 1936, 1939; Bruce, Knight & Parke, 1940). The food of other marine larvae has not been closely investigated.

Routine estimates of nanoplankton abundance have for some years formed part of investigations into the factors associated with good growth and survival of oyster larvae in the oyster-breeding tanks of the Ministry of Agriculture and Fisheries at Conway and in oyster-producing estuaries of Cornwall and Essex. The method used has been to concentrate a water sample quantitatively, by filtering through a Gradocol collodion membrane with the aid of an exhaust pump, and then to count the organisms in subsamples of the concentrate, using a haemacytometer (Cole & Knight Jones, 1949). The work has been hampered by lack of information concerning the systematics of nanoplankton organisms. The majority of those observed are of undescribed species and many belong to new genera. The affinities of some of the commonest minute flagellates are obscure. The present account is the initial result of efforts which are being made to fill this gap in our knowledge.

Most flagellates alter in shape and lose their flagella on treatment with many fixatives and become virtually unrecognizable, though 1% osmic acid may give satisfactory results; they must therefore be examined alive. Since they are rapidly motile for long periods and can be studied adequately only under

a 2 mm. objective, the work has so far been confined to forms from which thick cultures could be obtained.

The culture medium used has been sea-water Erdschreiber (see Gross, 1937). Serial dilution of sea-water samples into test-tubes of Erdschreiber proved to be the most useful method of isolating new cultures. This method, outlined below, is based on that used in the bacteriological examination of water supplies (Ministry of Health, 1940).

For a nanoplankton of from 1 to 30 organisms per mm.^{3} (the density most frequently encountered), a preliminary dilution of 1/100 was made; 1 ml. of the sea-water sample was added to 99 ml. of sterile sea water in a sterile stoppered bottle and shaken by inverting twenty-five times. Fifteen test-tubes, plugged with cotton-wool, sterilized and each containing 9 ml. of sterile Erdschreiber, had previously been prepared and arranged in three batches of five. With a sterile pipette 1 ml. of the preliminary dilution was added to each test-tube of the first batch. Then, with a fresh pipette, the contents were mixed by sucking up and down ten times and 1 ml. was transferred to a tube of the second batch. This process was repeated for the remaining four tubes in each of the first and second batches, using the same pipette. Then with a fresh pipette and following the same procedure, 1 ml. was transferred from each test-tube of the second batch to a tube of the third batch.

The three batches of test-tubes then contained, respectively, dilutions of 1/1000, 1/10,000 and 1/100,000, i.e. approximately 10, 1 and 0·1 mm.^{3} of the original sea water. They were placed in windows facing north. Exposure to direct sunlight, even for short periods when the sun was low, or for a few minutes only when the sun was high, often proved fatal to small flagellates. A series of test-tubes hung in a south window, but not exposed to direct sunshine, did not develop such a variety of organisms as a similar series in a north window. Apparently the wide temperature variations and the high light intensity in south windows are unfavourable to many forms. Cultures maintained at Nottingham grew well when kept in a cabinet at 12° C., under constant illumination from two 20-W. warm white tubes.

It was found that thick cultures usually developed in some of the test-tubes from the dilution series after 1–2 months in summer and 2–4 months in winter. Those of the first batch generally contained mixtures of organisms, while those of the third batch were often blank, but a few uni-algal cultures were usually obtained from the second and third batches.

Subcultures were made every 2 or 3 months, into either test-tubes or 150 ml. flasks. Although cultures in the larger vessels usually appeared more thriving and required less frequent attention, it was necessary, because of lack of space, to use test-tubes for the routine maintenance of cultures. It was found that many organisms could be maintained in mixed cultures, provided subculturing was carried out frequently. Some were subsequently isolated from these by making dilutions at favourable times. Some non-motile forms and larger

flagellates were isolated by picking out single cells or groups of cells with fine pipettes under a high-power dissecting microscope, followed by washing in successive drops of sterile medium.

Platymonas apiculata n.sp. was positively phototactic when first observed, and a few thousand specimens, introduced into a large volume of sterile sea water, and left undisturbed, assembled on the side nearest to the light in a visible green cloud. Successive washings, taking advantage of this habit, produced a culture which contained only one other flagellate.

Plates of solid medium (Erdschreiber with 2% agar) were prepared and inoculated with diluted sea water. No growth of motile flagellates was obtained, but *Nitzschia ovalis* Arnott, *N. longissima* Ralfs, *Synechocystis bacillaris* n.sp., and an encysted strain of *Platymonas apiculata* were isolated by this method.

The quantitative dilution method described above is similar to that used by bacteriologists for estimating the numbers of bacteria in water samples. A count of the number of test-tubes developing cultures in each of the three batches indicates the probable number of organisms in the original sample. This can be found from statistical tables in the Ministry of Health's publication (1940), which were recalculated and provided with their standard errors by Swaroop (1938). With nanoplankton organisms and present-day culture methods it is unwarranted to assume that all the organisms introduced into the test-tubes survive and multiply, and this method has given estimated nanoplankton densities less than those obtained from haemacytometer counts of concentrated samples.

In the taxonomic study of these organisms three things have greatly simplified their examination. The first was the discovery of a new method of staining flagella. A drop of a suspension of the organism was placed on a slide and to this was added a drop of 1% osmic acid followed by a drop of a new methylene blue dye which was kindly supplied by Messrs Imperial Chemical Industries Ltd. This dye is alkaline and soluble in sea water. It was found that the flagella almost always stained a bright blue and the contents were often little altered.

The second discovery is due to Reynolds (1950), who has devised a solution which induces, in many algae, the formation of zoospores. This solution has been most useful in the identification of *Ulothrix* and other filamentous forms.

The third discovery was that, if glass slips were inserted in the culture solutions in the test-tubes, many of the non-motile organisms readily grew on them and they could subsequently be withdrawn, examined and mounted, more or less permanently, either stained or unstained. Inoculations of a new culture could be made with a splinter of a glass slip chosen visually under a dissecting microscope and the glass slip with adhering algae could be inserted in the spore-inducing solution with minimum disturbance.

The author's very best thanks are due to Mr Knight Jones, who was largely responsible for the introduction and the section on methods of isolation; also to him and Dr H. A. Cole for supplying and maintaining many of the cultures of the organisms here described; to Prof. Pringsheim for suggestions in the early stage of this work; to Dr M. Parke for cultures and suggestions; and especially to his friend Mr N. Y. Sandwith for great help in preparing the Latin diagnoses.

CHLOROPHYCEAE

CHLAMYDOMONADACEAE

Platymonas West

P. apiculata n.sp. (Pl. I, figs. 1–5).

Cellula compressa, a fronte visa late elliptica antice apice profunde biloba, flagellis 4 e sulco cylindrico exorientibus; chromatophoro campaniformi juventute laevis sed senectute granulis cum pyrenoide basali et multis amyli granulis dispersis praedito; stigmate singulo parte anteriore inserto, aurantiaco; cysto 1–2 cellulis cum membrana externa crassa et stratifera praedito. Cellulis 7·5–10·5 $\mu \times$ 6·5–8·5 $\mu \times$ 4·5–5 μ.

Cells elliptical or narrowly oval, somewhat compressed, apex two-lobed with a deep furrow from which the flagella arise; pyrenoid large, conspicuous, situated at base of cell; chromatophore campanulate, single, bi-lobed, smooth in young individuals but coarsely granular in cells from old cultures. Eye-spot situated in upper portion of cell, orange; cells contain also numerous starch grains and two to six deeply staining granules near the furrow similar to those recorded by Carter (1937) for *P. tetrathele* West; flagella four, arising from base of furrow, 1–1½ times as long as cell; cysts common in culture, one- or two-celled, surrounded by a thick, sometimes stratified, cell wall with an apical papilla.

In the shell-fish tanks at Conway, North Wales, in an aquarium at Lowestoft, and in the estuaries of the Helford River and the Crouch, apparently common.

This organism bears considerable resemblance to *P. gracilis* Kylin (1935) in size and position of eye-spot, but the apex is two-lobed and the presence of a papilla, usual in *Chlamydomonas*, has been recorded in this genus only for *P. cordiformis* (Carter) Korch. (Skuja, 1948) and it may well be present in other species. Carter (1937) is doubtful whether the lobing of the apex used by Kylin to divide the genus is a sound taxonomic character, but in all the strains of this new species which have been examined there is no doubt about the two-lobed apex and it would therefore appear correct to distinguish it from *Platymonas gracilis*.

The four strains of this organism which have been isolated vary somewhat in their behaviour in culture solutions. Every strain sooner or later becomes

encysted but, even under what are apparently identical conditions, the time that elapses before all motile forms do so varies with every culture. In young vigorous cultures the chloroplast is almost smooth with very few starch grains but, on ageing, starch grains become more numerous and large green granules collect in considerable numbers in the cells.

The processes of division and encystment seem to be as follows. A cell becomes quiescent and sheds its flagella, while at the same time the compression becomes less obvious and the apical lobe shallow, so that, very shortly, the cell wall is entire and the cells scarcely compressed. Division takes place at an oblique angle and as the cells grow they move round, so that in mature cells the dividing wall is often horizontal. The daughter cells acquire flagella while they are still within the parent cell, and may at times be seen moving about before liberation. Liberation occurs by the splitting in half of the mother cell wall. When the cultures are old, single-celled cysts are the rule. These are not compressed, have a thick stratified cell wall and are filled with starch granules and other large spherical bodies which may possibly be oil.

CHLORELLACEAE

Chlorella Beijerinck

Unicellular green forms, of spherical or ovoid shape, are among the commonest of algae found developing in liquid cultures. Several such forms have been isolated from the River Crouch and elsewhere. All those now described have been under observation for a long time and have been grown in several strengths of solution, including Reynolds's spore-inducing solution, and the only form of reproduction which has been observed was by the production of non-motile bodies.

Subgenus *Euchlorella* Wille

C. salina n.sp. (Pl. I, figs. 11–14).

Cellula sphaeroidea, membrana externa tenui; chromatophoro laete viridi clare, phialiformi, parietali, cellulam fere implente; pyrenoide centrali, magna. Incrementum est per cellulae materis in 8 cellulas filias divisionem. Cellula 4–7 μ diametro.

Cells spherical; surrounded by an obvious but thin, smooth, cell wall; chromatophore saucer-shaped, almost filling cell, bright green, finely granular; pyrenoid central, large, surrounded by starch sheath.

Propagation by the successive division of the mother cell into eight daughter cells which free themselves by the bursting of the parent wall.

This organism developed in cultures from the Conway tanks. It bears a superficial likeness to *C. vulgaris* Beijerinck, but the cell wall is apparently thinner and the chromatophore rather different in shape. As it is a marine organism, it seems reasonable to distinguish it as a separate species.

C. stigmatophora n.sp. (Pl. I, figs. 30–34).

Cellula sphaeroidea vel aliquantum elongata, membrana externa tenui; chromatophoro laete viridi claro, phialiformi, cellulam fere implente sed depressione terminali praedito; pyrenoide manifesta, amyli capsula circumdata centrali nisi in cellulis elongatis, stigmate fusco, in cellulis parvis singulo, stigmatibus in cellulis majoribus 2 vel ultra plerumque terminalibus. Incrementum est per cellulae matris in 2 vel 4 cellulas filias divisionem. Cellula 4–6 μ diametro.

Cells spheroidal to somewhat elongate, 3–5 μ diameter, bright green, surrounded by smooth, thin cell wall; chromatophore saucer-shaped, filling most of cell but with depression at one end, rather granular; pyrenoid conspicuous, medium sized, central, though in the elongate cells rather to one end, surrounded by starch sheath; stigma single in young cells, two or more in the larger cells, usually at one end.

Propagation by division of mother cell into two, or more rarely four, daughter cells which are liberated by the bursting of the cell wall.

This was first isolated by Dr Parke (Bruce *et al.* 1940) from a sea-water sample collected at Port Erin, Isle of Man, and there referred to as *Chlorella* sp. Subsequently it has been found in the River Crouch, Essex.

In its size and method of division this plant is not unlike *C. spärckii* Alvik (1934), but it is clearly marked off from all other described species of *Chlorella* by the presence of at least one dark brown body which is interpreted here as a stigma.

Subgenus *Chloroideum* Nadson

C. ovalis n.sp. (Pl. I, figs. 15–22).

Cellula ovata vel ellipsoidea, membrana externa tenui; chromatophoro laete viridi, parietali, paulum lobato, cellulam $\frac{3}{4}$ implente. Pyrenoide nulla. Incrementem est per cellulae matris in 8 cellulas filias divisionem. Cellula 3–5 $\mu \times$ 5–10 μ.

Cells ovoid to ellipsoidal, surrounded by a smooth hyaline cell wall; chromatophore parietal, bright green, smooth, slightly lobed, occupying about $\frac{3}{4}$ of the cell and, especially in young individuals, with a hyaline space at one end. Pyrenoid absent.

Propagation by successive division of the mother cell in individuals of 10 μ diameter into eight daughter cells which free themselves by the bursting of the cell wall.

First isolated from the River Crouch, Essex, and apparently common in that district.

This differs from *C. spärckii* Alvik in the division of the mother cell into eight and not two daughter cells. Another closely related species is *C. saccharophila* Kruger (1894), but from the somewhat inadequate description his organism seems to be 15–20 μ diameter with a granular chloroplast.

C. marina n.sp. (Pl. I, figs. 6–10).

Cellula ovata, membrana externa tenui, glabra; chromatophoro laete viridi, parietali, lobato, granuloso fere totam cellulam implente. Pyrenoide nulla sed corpore ut videtur olei in cellulis maturis. Incrementum est per cellulae matris in 8 vel 16 cellulas filias divisionem. Cellula 4–6 $\mu \times$ 7–10 μ.

Cells ovoid, bright green, surrounded by smooth, hyaline cell wall; chromatophore an irregular parietal plate occupying almost all the cell, markedly and finely granular. Pyrenoid not observed, but in the mature cells definite hyaline bodies are present which appear to be oil.

Propagation by the successive division of individuals 10 μ in diameter into eight or sixteen daughter cells which are liberated by the bursting of the cell wall.

This organism appeared in the cultures of a flagellate originally isolated at Port Erin by Dr Parke.

This species is closely allied to the last from which it differs in its granular chromatophore and spherical oil-like bodies.

Nannochloris Naumann

N. maculatus n.sp. (Pl. I, figs. 23–25).

Cellula sphaerica vel elongata, membrana externa tenui laevi; chromatophoro pallide viridi phialiformi, granuloso fere totam cellulam implente. Pyrenoide nulla visa; sed granulis amyli et oleorum. Incrementum est per cellulae matris in 2 cellulas filias divisionem. Cellula 3 μ diametro.

Mature cells spherical or slightly elongate, surrounded by smooth cell wall; chromatophore pale green, cup-shaped, finely granular, occupying most of cell. No pyrenoid has been observed, but the cells contain both starch grains and other refractive bodies which are probably oil. Cells 3 μ diameter.

Propagation by division of the cell into two individuals of about 3 μ diameter.

First isolated from River Crouch, and common in saline ditches of that district.

The genus *Nannochloris* was established by Naumann (1931) to include two very minute *Chlorella*-like organisms in which the cells divided only into two, and there was no evidence of a surrounding mother-cell membrane. As far as can be ascertained in such small cells, the above species has this same characteristic, while in addition it has been possible to ascertain, in part, the internal structure. Whilst it is about the same size and shape as Naumann's *Nannochloris coccoides*, its occurrence in sea water appears sufficient evidence that it is a different species.

N. atomus n.sp. (Pl. I, figs. 27–29).

Cellula minuta spheroidea membrana externa tenui; chromatophoro pallide viridi phialiformi, cellulam fere implente; pyrenoide non visa. Incrementum est per cellulae matris in 2 cellulas filias divisionem. Cellula 3 μ diametro.

Cells spheroidal, 2–3 μ diameter, pale green surrounded by smooth thin cell wall. Chromatophore saucer-shaped, filling almost the whole cell, finely granular. Pyrenoid not observed.

Propagation by division of cells 3 μ diameter into two daughter cells.

First isolated in dilution cultures from River Crouch and apparently widely distributed in that district.

This is another very minute member of the Chlorellaceae which has the same general characters as the last except that it is even smaller and the numerous starch grains and similar bodies are apparently absent.

Stichococcus Naeg.

S. cylindricus n.sp. (Pl. I, fig. 26).

Cellula cylindrica membrana externa tenui, laevi; chromatophoro laete viridi, parietali, laevi, cellulam totam implente. Pyrenoide nulla; nucleo minuto. Incrementum est per cellulae matris in 2 cellulas filias divisionem; filamento brevissimo, facile fracto. Cellula 2 $\mu \times 3$–4 μ.

Cells cylindrical with rounded truncate ends, cell wall thin, hyaline; chromatophore parietal, bright green, smooth, completely filling cell; pyrenoid absent; nucleus small, difficult to detect.

Propagation by the transverse division of the mother cell into two equal halves; filament very short, easily fragmenting. Cells 2 $\mu \times 3$–4·5 μ.

Isolated from River Crouch, Essex.

This organism in many respects occupies a position intermediate between a species of *Nannochloris* such as *N. bacillaris* Naum. and *Stichococcus bacillaris* Naeg. which shows a similar slight tendency to form filaments. A very thin mother-cell wall can occasionally be distinguished between the cells, though it appears to be short-lived. Absence of such a mother-cell wall is one of the chief characters of the former genus. Species of *Stichococcus* are usually terrestrial and this form, being both aquatic and marine, is clearly a well-marked new species.

CHRYSOPHYCEAE

CHROMULINACEAE

Chromulina Cienkowsky

C. pusilla n.sp. (Pl. II, fig. 42).

Cellula minuta, sphaeroidea dorsiventraliter paulum depressa, chromatophoro singulo, parietali, phialiformi, fulvo; flagello singulo, 2–3 μ longo ut videtur secus marginem superiorem lateris applanati inserto; corpore fusco centrali. Cystis sphaeroideis membrana externa laevi. Cellula 1–1·5 μ diametro.

Cells rounded, with definite dorsi-ventral flattening, 1–1·5 μ diameter, periplast firm. Chromatophore single, parietal, saucer-shaped, filling most of

cell. Flagellum single, 2–3 μ long, apparently inserted along upper edge of flattened side; chromatophore brownish green, single, apparently a parietal plate, filling about two-thirds of cell. The only other cell structure which can be made out in this small organism is a central dark granule the nature of which it is difficult to decide. Cysts endogenous, rounded, wall smooth, unsculptured.

Propagation by vertical division of the cells.

This, or some organism closely related to it, is apparently widely distributed, as it has been obtained repeatedly in cultures from Conway, the Helford River, Cornwall, and the River Crouch, Essex. The greatest density recorded by the serial dilution method previously described was 3500 per ml. in October 1946 from Cornwall. In addition, minute flagellates probably of this species are regularly observed in plankton counts from the Conway tanks and the Essex estuaries.

The assignment of this organism to its true position is a matter of some difficulty as, in so small a species, the cell contents are very hard to define. The general brownish colour of the suspensions in mass and the greenish brown colour of the individuals, together with the simple organization of the cells, suggest a close relationship to *Chromulina*, an alliance also confirmed by the apparent formation of cysts. Against placing the organism in this genus is the unilateral compression of the cells and the apparently lateral insertion of the flagellum. Though these are important characteristics, the fact that *Chromulina* includes species of varied shape seems to justify the inclusion of this species within the genus.

C. pusilla differs from *C. pleiades* Parke (1949) in having one chromatophore only, as well as in the absence of vibrating bodies, and it differs from this and *C. parvula* Conrad (1930) by its flattened side, and from all other described species in its very small size.

OCHROMONADACEAE

Pavlova n.gen.

Cellula solitaria, natante, nuda periplasto differentia carente praedita forma mutabili; 2 flagellis, dissimilibus, plusminusve lateraliter insertis.

Cells solitary, motile, naked with undifferentiated periplast; strongly metabolic. Flagella 2, unequal, inserted more or less laterally.

P. gyrans n.sp. (Pl. II, figs. 35–38).

Cellula mutabili, elongata, aut ovata, aut amoeboidea, compressa, posteriore acuta; chromatophoris 2, magnis, fulvis, lateralibus, cum multis granulis dispersis; flagellis 2 dissimilibus, plusminusve lateraliter insertis; altero longiore apicem versus, altero breviore extrorsus provecto; parte apicali anteriore vacuolo contractili et stigmate singulo rubro praedita; parte basali posteriore granulis leucosini 1–2 praedita. Cellula 3–6 $\mu \times 4$–10 μ.

Cells strongly metabolic, either elongated, ovoid or amoeboid, usually compressed, base acute; chromatophores two, large, lateral, not extending to the base, dull yellow, granular; flagella two, unequal and inserted at some distance below the apex, the larger directed towards the apex and the shorter outwards; a conspicuous red stigma and a small contracting vacuole are present in the apical region; at the posterior end one to three leucosin bodies are present.

Propagation is by longitudinal division in the non-motile stage.

Isolated from the Helford River, Cornwall, in 1947.

This flagellate is most closely allied to the metabolic forms of *Ochromonas*, but differs in the sublateral insertion of the flagella, a character deemed of sufficient importance to separate it from this genus.

The metabolism of the individuals is so marked and the shape so varied that the form of the cells is anything from cylindrical compressed to almost spherical and amoeboid. Only the most frequently observed shapes are illustrated (Pl. II, Figs. 35–38).

Of the two flagella the one directed forward has a somewhat slow undulatory motion and can be seen without difficulty. It is about twice as long as the cell. The smaller flagellum is directed outwards and has a rapid up and down movement; in length it about equals the width of the cell and is very much more difficult to detect than the longer flagellum. The movements of the organism are very varied. Sometimes it twists and turns on its own axis, or goes forward with a vibratory motion, while at other times it moves rapidly in one direction. Or it may execute a combination of any of these movements.

A palmelloid state has been occasionally observed in cultures. The outline of the cells is then very irregular, but stigma and leucosin are very conspicuous.

The cells are very sensitive to all fixatives, in which they either become spherical or burst. In excess light on the microscope slide the cells behave as follows. Motion ceases and the elongate cells become oval or spherical, the long flagellum floats away and part of the hyaline contents of the cell is extruded through the periplast to form elongate or club-shaped 'pseudopodia'. Some individuals do not immediately lose their power of motion but continue to move with pseudopodia protruding. Apparently this form of the organism may best be considered a pathological condition.

CRYPTOPHYCEAE

CRYPTOMONADACEAE

Hillea Schiller

H. marina n.sp. (Pl. II, figs. 39–41)

Cellula spherica vel ovata, lateraliter depressa; depressione lata haud profunda a margine apicali lateris applanati deorsum per quartam cellulae partem

recte decurrente; 2 flagellis disparibus ex apice depressionis exorientibus; chromatophoro singulo, disciformi; parietali, fulvo, lobato granulis praedito; nucleo posteriore manifesto; pyrenoide centrali. Cellula $2\mu \times 2{\cdot}5\mu$.

Cells spherical to ovoid with distinct lateral flattening, rounded or tapering to the base; a broad shallow depression runs vertically from the apical margin of the flattened side and dies out one-quarter of the length of the cell; flagella two subequal, arising from apex of depression; chromatophore single, parietal, lobed, granular, dull yellow; nucleus distinct, situated towards the base; pyrenoid central. Fission not observed.

Isolated from sea water in the shell-fish tanks at Conway, North Wales.

This organism is very small and its complete structure is not easy to resolve. Its salient features are the subequal flagella, the pyrenoid and the shallow depression. The first two are characters of the Cryptomonads. The depression is very shallow and rudimentary and no trichocysts can be seen on its margin. It can hardly therefore be considered a furrow. Schiller (1925) describes a rather larger organism from the Adriatic, *Hillea fusiformis*, which apparently has a depression on one side and a conspicuous nucleus. The species now described appears to have characters sufficiently similar to justify its inclusion in the same genus. In shape and size, however, it is quite unlike *H. fusiformis* Schiller.

Cryptochrysis Pascher

C. fulva n.sp. (Pl. II, figs. 43–45).

Cellula nuda, natante, ovata dorsiventraliter depressa, asymmetrica; sulco bene notato a margine anteriore paulo infra apicem perduas tertias partes paginae lateralis decurrente; ordinibus trichocystorum duobus in margine sulci; deest gula; 2 flagellis disparibus in sulco insertis; chromatophoro singulo, magno, plicato, lobato, fulvo; pyrenoide plerumque singula vel in cellulis majoribus 2, centrali, amyli capsula circumdata; nucleo parvo centrali vel posterius inserto. Cellula $3–4\mu \times 5–7\mu$.

Cells naked, actively motile, ovoid in dorsal view, slightly flattened on one side though sometimes tapering to the base or with a somewhat truncate apex. A well-marked furrow runs obliquely from a short distance below the apex and dies out two-thirds down the lateral surface; two lines of trichocysts on edge of furrow; gullet not observed; flagella two, subequal, $10–12\mu$ long, arising from lower edge of furrow; chromatophore single, large, folded, lobed, greenish brown; pyrenoid usually single, or two in larger cells, central or one at either end, surrounded by a starch sheath; nucleus small, situated in middle or lower part; contractile vacuole apical, small, single. Multiplication by longitudinal division in motile stage and possibly also in the palmelloid state.

Isolated from the sea water in the shell-fish tanks at Conway, North Wales, 1946.

As noted by Pringsheim (1944) for other species, this and the two following

organisms form palmelloid states in culture, in which the cells are irregularly massed, and the contents are of a richer brown than in the motile cells, containing what appear to be oil granules as well as the pyrenoid. This condition appears to correspond very closely with the palmelloid state of the alga described by Reinisch (1911) as *Phaeocapsa marina* = *Phaeoplax marinus* Pascher (1911), and there seems to be little doubt that the two organisms are very closely related; in fact, a somewhat rounder apex and a sublateral insertion of the flagella seem to be the chief distinguishing points of my species. While the conditions which bring about this palmelloid state have not been ascertained the history of one culture may be relevant. Isolated in April 1948 it continued motile and gave satisfactory subcultures until 9 September when a brown deposit was seen in the original culture. This was found to be the palmelloid state together with a number of motile cells. A subculture made from this produced only the palmelloid state from 2 October onwards and was discarded in the following March. In the meantime the original culture remained in the palmelloid state with no vestige of a motile cell from October 1948 to May 1949, when motile cells were again seen, being released singly from the palmelloid cells. Meantime two other more recent cultures which had also been palmelloid had become full of motile cells though some 3 months younger. All these cultures, and any subcultures made from them, remained motile throughout the summer months.

It thus seems that the organism can multiply both in the motile and palmelloid stages, and the question arises whether the genus *Phaeoplax* should be retained. The ease with which other algae are known to form strange palmelloid states in culture, the inconstancy in nature of the formation of such structures, as for example the mucilage tubes in certain diatoms, and the frequency and apparent longevity of the motile state, are against the retention of the genus; the fact that multiplication is possible in the palmelloid state is the chief point in its favour. This and the two following similar organisms apparently do not possess a gullet and are thus included in the genus *Cryptochrysis*, and it seems logical that the species described by Reinisch should be called *C. marina*.

The chief distinguishing characters of this species are the single chromatophore, the general shape, and the sublateral insertion of the flagellum.

C. lateralis n.sp. (Pl. II, figs. 48–50).

Cellula nuda, natante, ovata vel obconica lateraliter depressa, asymmetrica, postice acuta; sulco bene notato de margine dorsali triente infra apicem fere usque ad basin paginae ventralis fere recte decurrente; ordinibus trichocystorum duobus in margine sulci; deest gula; 2 flagellis disparibus in sulco insertis; chromatophoris 2, magnis, lobatis, fulvis; pyrenoide singula, centrali, amyli capsula circumdata; cellulis senioribus granulis amyli dispersis praeditis; parte cellulae inferioris hyalina, granulis vel ultra refractivis proedita; nucleo postice inserto; cellula $3–4\mu \times 5–7\mu$.

Cells ovoid to obconical in dorsal view, slightly compressed with lateral flattening, tapering to the base; a well-marked furrow runs almost vertically from the ventral margin, one-third from the apex and dies out almost at the base on the lateral surface; two lines of trichocysts on edge of furrow; gullet not observed; flagella two, subequal, arising from the lower edge of furrow, 5–10 μ long; chromatophores two, large, lobed, filling most of cell, greenish brown; pyrenoid usually single, medium, obvious, surrounded by starch sheath, more or less centrally placed; older cells with scattered starch grains in addition; contractile vacuoles not observed. Lower portion of cell hyaline containing one or more oscillating refractive granules. Multiplication by longitudinal division, in motile state.

Isolated in August 1946 from the shell-fish tanks at Conway.

This species also forms palmelloid states in culture similar to the previous species. Though the insertion of the flagella is almost as low as in the Nephroselmidaceae, this organism is more closely related to the previous species than to the former group and can be easily distinguished by the shape, the two chromatophores, and the almost vertical furrow.

C. virescens n.sp. (Pl. II, figs. 46, 47).

Cellula nuda, natante, ovata, dorsiventraliter depressa; sulco lato bene notato margine dorsali circiter tertia parte infra apicem exoriente atque fere usque ad mediam paginam lateralem oblique decurrente; ordinibus 2 trichocystorum haud bene notatorum in sulco; deest gula; 2 flagellis subaequalibus margine sulci insertis, 6–10 μ longis; chromatophoro singulo totam cellulam occupante, viridi, glabro; pyrenoide singula, magna, basali; granulis in cellulis senioribus obviis; cellula 3–4 $\mu \times$ 5–7 μ.

Cells naked, roundly ovoid, somewhat flattened on ventral side, asymmetrical; a wide well-marked furrow runs obliquely from the lateral margin, about one-third from the apex and dies out about the centre of the lateral surface; two lines of faint trichocysts on the edge of furrow; gullet not observed; flagella two, subequal, arising from the edge of furrow, 6–12 μ long; chromatophore single filling the whole cell, green, smooth; pyrenoid single, large, basal; globules possibly oil are present in older individuals. Multiplication by longitudinal division, apparently in the motile state.

Isolated on one occasion from the River Crouch, Essex.

This species can be recognized at once by its colour which, in spite of the wide variation, is unusual in the Cryptophyceae. The furrow is very wide and shallow and this, in apical view, gives the cell a reniform appearance. The dorsiventral compression is also unusual. Nevertheless, its structure with furrow, subequal flagella, large pyrenoid and general appearance is clearly that of a member of the genus *Cryptochrysis*.

Cryptomonas Ehrenberg

C. acuta n.sp. (Pl. II, figs. 51–53).

Cellula nuda, natante, elliptica, dorsiventraliter depressa, basi acuminata vel acuta, apice acuta vel rotundata; sulco bene notato margine ventrali fere quarta parte infra apicem exoriente atque fere usque ad basin fere recte decurrente; ordinibus trichocystorum in sulco valde distinctis; gula sulci tret-quartras partes longitudinis occupante; 2 flagellis subaequalibus in stulco insertis 12–15 μ longis; chromatophoris 2 magnis leviter lobatis, totam cellulam basi excepta occupantibus, granulis paucis exceptis laevibus, fuscis; pyrenoide magna centrali vel parte anteriore inserta, amyli capsula circumdata; nucleo parvo, parte anteriore inserto, granulis basi cellulae oscillantibus. Cellula 12–15 $\mu \times$ 4–6 $\mu \times$ 5–7 μ.

Cells actively motile and slightly metabolic, elliptical with some dorsiventral flattening, base acuminate or acute, apex acute or rounded; a well-marked furrow runs almost vertically from the ventral side a quarter of the length from the apex and dies out almost at the base; trichocysts lining the furrow very distinct; gullet in furrow and three-quarters of its length; flagella two, subequal, arising from the upper edge of the furrow, 12–15 μ long; chromatophores two, large, slightly lobed, occupying all except basal portion of the cell, smooth except for a few granules, brown; pyrenoid large, central or in the upper portion, surrounded by starch sheath; nucleus small, in upper part of cell; basal part of cell hyaline and filled with oscillating granules. Multiplication by longitudinal division, apparently only in non-motile state.

Isolated both from the River Crouch and the Conway tanks, but apparently not common.

The gullet in this species is not very marked and it may prove to be intermediate in this respect between the genera *Cryptochrysis* and *Cryptomonas* and justify the suggestion of Pringsheim (1944) that it is unreasonable to keep them separate. Oscillating granules similar to the well-known structures in desmids have now been recorded in Chrysophyceae by Carter (1937) and Parke (1949), and this species is an interesting example in the Cryptophyceae. Similar structures, though not actively oscillating, were also apparently present in *Cryptochrysis lateralis* (see p. 187).

The distinguishing features of this form are the tapered ends, a shape which is rare in this genus and shown only by *C. gracilis* Skuja (1948), the oscillating bodies and the almost vertical furrow. The sublateral origin of the flagella is also unusual. The presence of a pyrenoid is the most obvious distinction between this and *C. gracilis* Skuja.

This plant is very sensitive to light and certain chemicals. It disintegrates within 5 min. under the microscope, the pathological symptoms being the extrusion of the protoplast in the form of pseudopodia. It does not change its shape on treatment with iodine but immediately assumes a spherical form on the addition of New Methylene Blue.

MYXOPHYCEAE

CHROOCOCCALES

Several very minute blue-green organisms with the cells apparently not enveloped in a mucilaginous mass appear from time to time in cultures. One such organism has been identified with *Synechococcus elongatus* Naeg., while others are suggestive of *Pediochloris* Geitler and *Tetrachloris* Pascher, members of the so-called Chlorobacteriaceae. They are often very minute and it is possible that, before their nature can be fully elucidated, methods akin to those used in the taxonomy of bacteria will have to be adopted. The following, however, appears to be a well-marked undescribed species.

Synechococcus Naegeli

S. bacillaris n.sp. (Pl. II, fig. 54)

Cellula parvula, singula vel e filamentis brevissimis constante, fere spherica, ovata vel cylindrata; deest vagina mucilaginosa; periplasto firmo, mucoso, granulis tenuibus coerules viridibus cellulam implentibus. Incrementum est per cellulam in 2 cellulas transverse divisam. Cellula $1{\cdot}5\,\mu \times 1{\cdot}7$–$4{\cdot}5\,\mu$.

Cells single or in very short filaments, not enveloped in a sheath of mucilage, almost spherical, ovoid or cylindrical, longest immediately before division; cell wall firm, apparently a thin mucous coat; contents finely granular, uniform, blue-green. Propagation by transverse division of the cells.

First noticed in June 1947 in a tube inoculated with a greenish brown colony from a culture from the Conway tanks growing on a solid medium.

With its nearly spherical cells this species occupies a position between the genera *Synechocystis* Sauvageau, in which all the cells are spherical, and *Synechococcus* Naegeli, in which the shape varies between ovoid and cylindrical. In size it is nearest to *S. elongatus* Naeg. (also isolated at Conway) but the cells are only shortly cylindrical and never elongated.

Summary

Sixteen species of marine algae have been isolated by dilution culture from various coastal waters and are described. They include seven flagellates.

Four are species of *Chlorella* (*C. stigmatophora* n.sp., *C. salina* n.sp., *C. ovalis* n.sp., *C. marina* n.sp.): two are species of the closely allied genus *Nannochloris* (*N. maculata* n.sp. and *N. atomus* n.sp.) and one is a species of *Stichococcus* (*S. cylindricus* n.sp.). A new species of the Chlamydomonadaceae (*Platymonas apiculata* n.sp.) is also described. Two others are members of the Chrysophyceae; a very minute form 1–$2\,\mu$ diameter, namely *Cromulina pusilla* n.sp., and an Ochromonad with lateral flagella (*Pavlova gyrans* n.gen. et sp.). The Cryptophyceae are represented by three species of *Cryptochrysis* (*C. fulvus*, n.sp., *C. lateralis* n.sp. and *C. virescens* n.sp.), a species of *Hillea* (*H. marina* n.sp.) and a species of *Cryptomonas* (*C. acuta* n.sp.). A minute blue-green algae, *Synechococcus bacillaris* n.sp., is also described.

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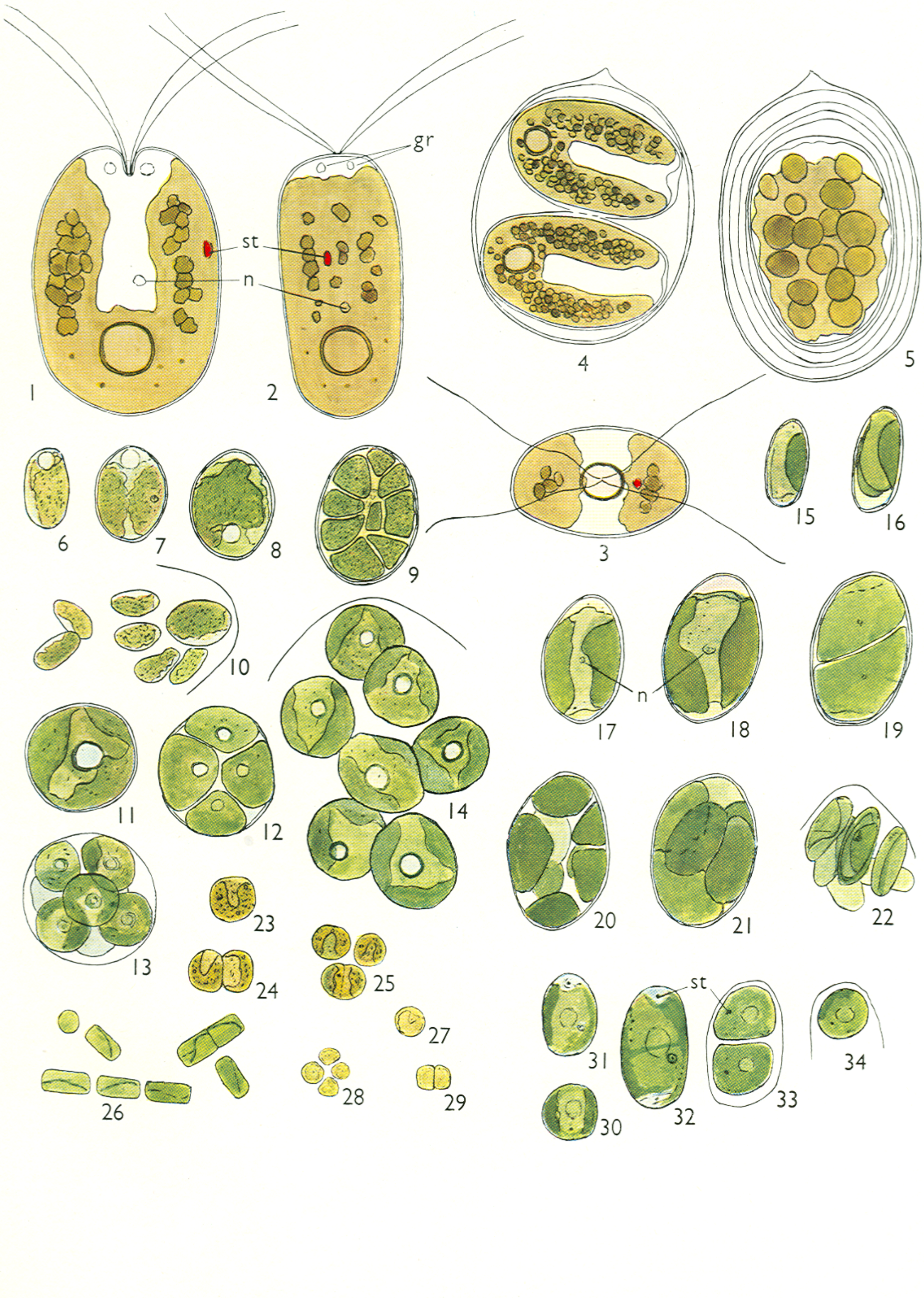
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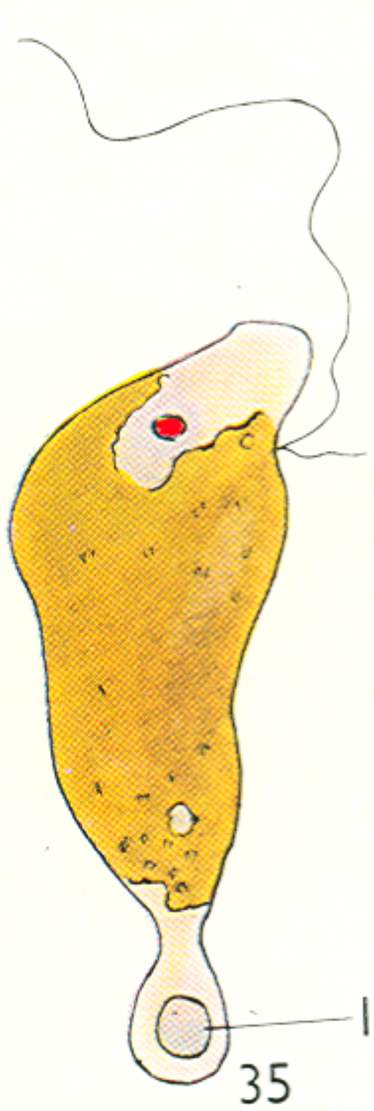

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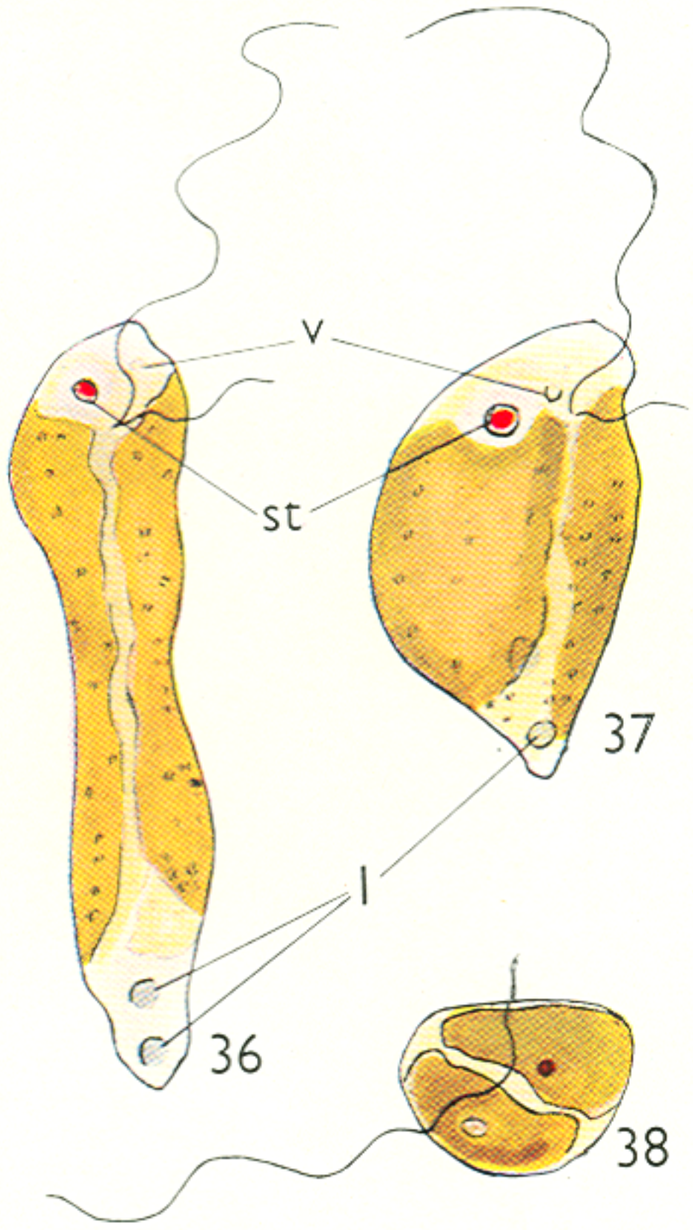

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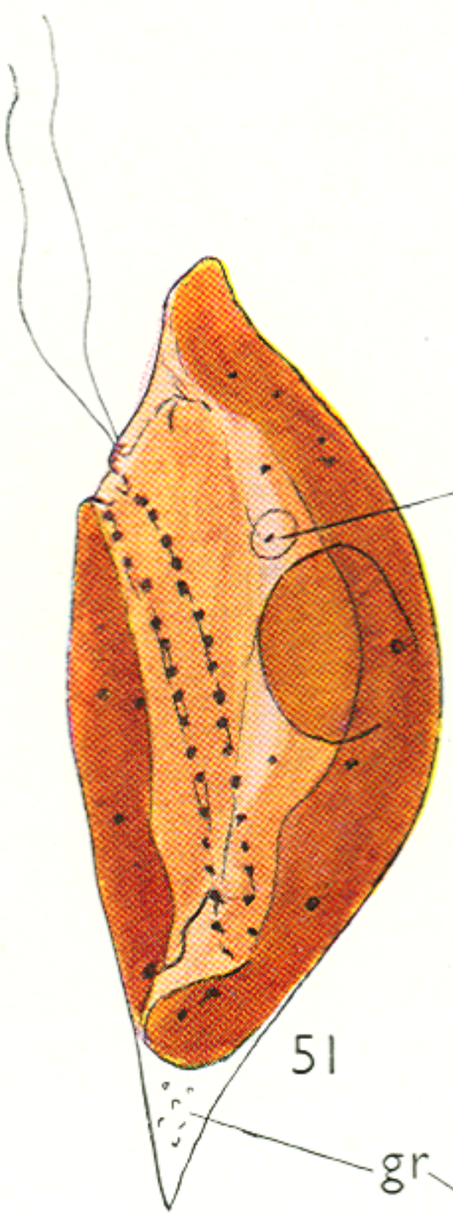

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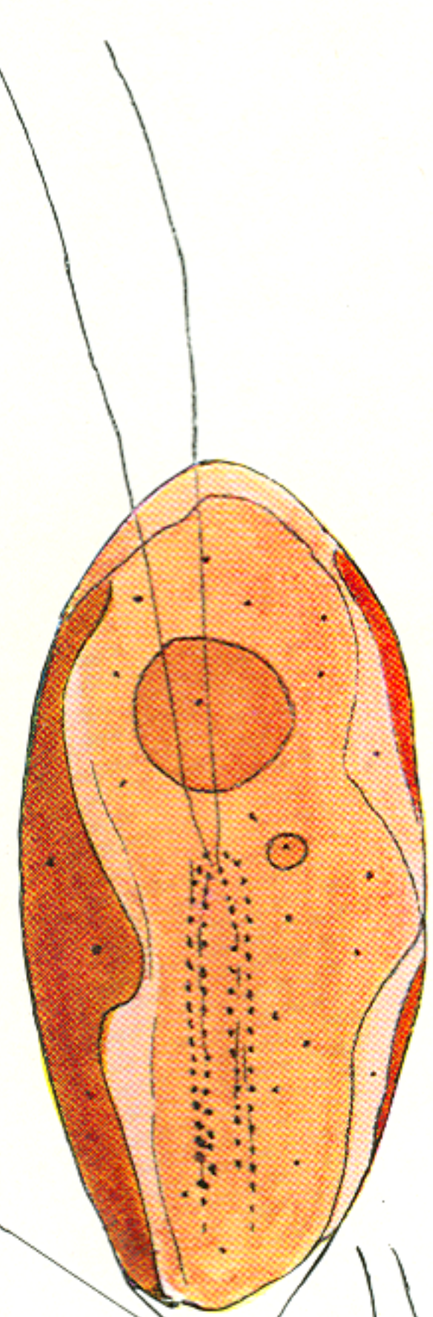

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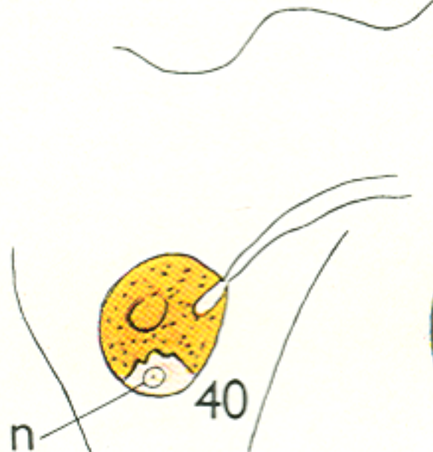

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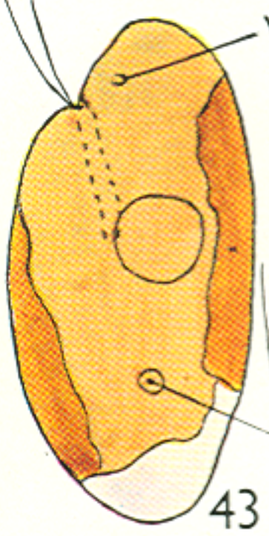

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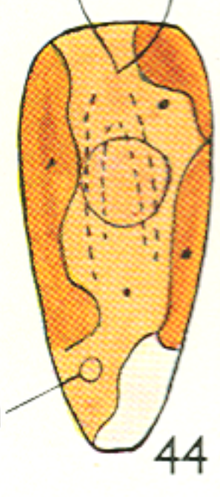

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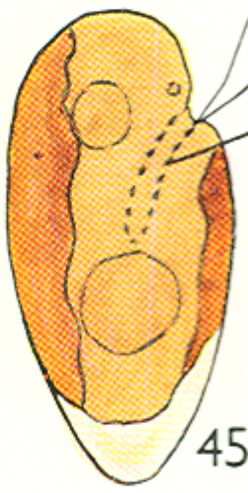

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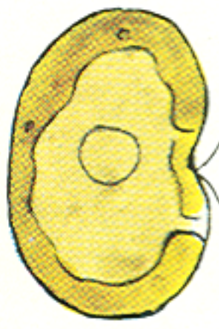

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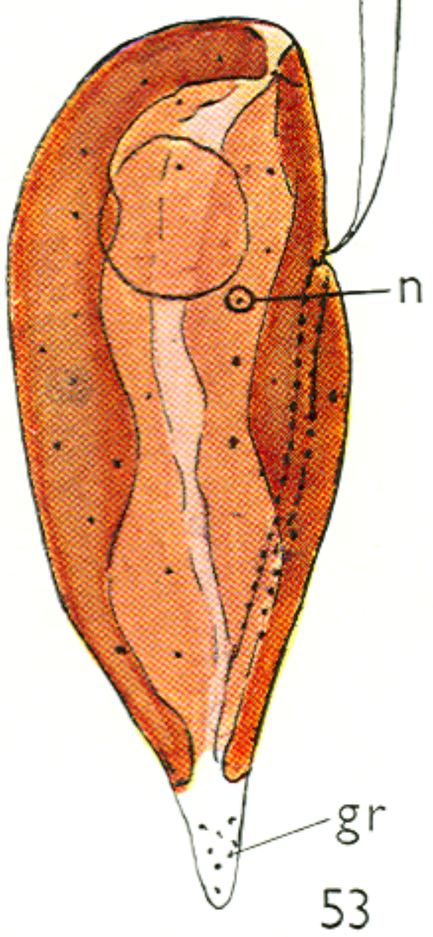

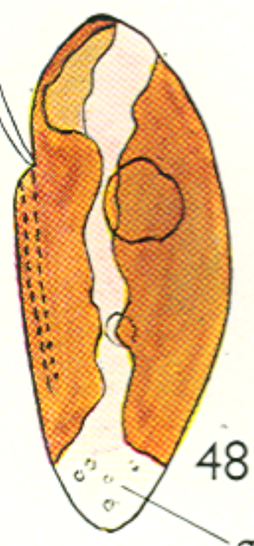

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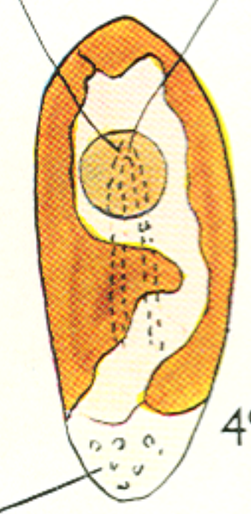

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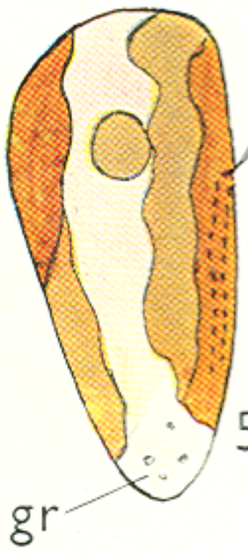

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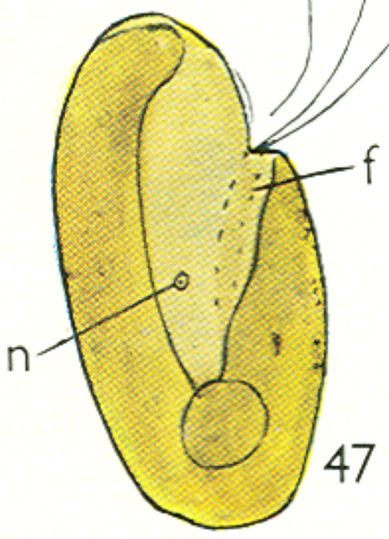

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EXPLANATION OF PLATES I AND II

PLATE I

(*gr.* = granules; *l.* = leucosin; *n.* = nucleus; *p.* = pyrenoid; *st.* = stigma; *cy.* = cyst.)

Figs. 1–5. *Platymonas apiculata* n.sp. Fig. 1, front view; fig. 2, side view; fig. 3, anterior view; fig. 4, cyst; fig. 5, older single-celled cyst with stratified wall. All ×4500.

Figs. 6–10. *Chlorella marina* n.sp. Figs. 6–8, cells of varying size; figs. 9, 10, stages in division. All ×2250.

Figs. 11–14. *Chlorella salina* n.sp. Figs. 12–14, stages in division. All ×2250.

Figs. 15–22. *Chlorella ovalis* n.sp. Figs. 15–18, cells of different size; figs. 19–22, stages in division. All ×2250

Figs. 23–25. *Nannochloris maculatus* n.sp. ×2250.

Fig. 26. *Stichococcus cylindricus* n.sp. ×2250.

Figs. 27–29. *Nannochloris atomus* n.sp. All ×2250.

Figs. 30–34. *Chlorella stigmatophora* n.sp. Figs. 32–34, stages in division. All ×2250.

PLATE II

Figs. 35–38. *Pavlova gyrans* n.gen. et sp. Figs. 35, 37, two individuals in lateral view; fig. 36, another individual, ventral view; fig. 38, anterior view. All ×4500.

Figs. 39–41. *Hillea marina* n.sp. Fig. 39 dorsal view; figs. 40, 41, lateral view. All ×4500.

Fig. 42. *Cromulina pusilla* n.sp. ×4500.

Figs. 43–45. *Cryptochrysis fulva* n.sp. Figs. 43, 45, lateral views; fig. 44, ventral view. All ×4500.

Figs. 46, 47. *Cryptochrysis virescens* n.sp. Fig. 46, lateral view; fig. 47, anterior view. Both ×4500.

Figs. 48–50. *Cryptochrysis lateralis* n.sp. Figs. 48, 50, lateral views; fig. 49, ventral view. All ×4500.

Figs. 51–53. *Cryptomonas acuta* n.sp. Fig. 51, lateral view; fig. 52, another individual, ventral view; fig. 53, the same, lateral view. All ×4500.

Fig. 54. *Synechococcus bacillaris* n.sp. ×4500.

THE AQUARIUM AND SEA-WATER CIRCULATION SYSTEM AT THE PLYMOUTH LABORATORY

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(Plate I and Text-figs. 1–4)

Introduction

The aquarium or tank room, to which visitors are admitted on payment of a small charge, was not designed primarily for public display, but was intended principally to facilitate scientific observations on the habits and life histories of marine animals. This original purpose it has never lost, but of recent years it has increasingly catered also for the steadily growing number of people interested in natural history, and for numerous classes of school-children brought by their teachers. Since the aquarium was re-opened in November 1946 attendances have shown a big increase over comparable pre-war figures.

The aquarium is over sixty years old. The fact that during the whole of that time the same tanks, the same reservoirs and much of the same piping have continued to serve without major trouble is a tribute to its designer and builders. The circulating system still in use was invented by W. Lloyd and was widely adopted for the public aquaria which were a popular feature of many towns towards the end of the last century. The system was also adopted at Naples. That is not to say, however, that the design and construction at Plymouth are ideal. Long experience, greater knowledge and changing function all emphasize the desirability of alterations for the benefit of the inhabitants of the tanks, for greater efficiency in working and for better viewing conditions for the observers, be they naturalists or general public.

Some improvements have been made since the war, during the repair and re-establishment necessitated by the damage suffered during air-attacks in 1941. These chiefly concern the erection of new rockwork in several of the large tanks, and the provision of boards along the top edges of the latter to shield the viewer's eyes from window-glare. Electric lighting for dull days has also been provided.

That the reputation of the Association's aquarium stands high is shown by the numerous inquiries concerning its size, construction and maintenance received during the post-war years. Promoters and architects for aquarium schemes at home and abroad have written, or called for information and

advice. Some have even brought plans for criticism. It is probable that not a few large public aquaria projected or building have been influenced in design by ideas and suggestions based on experiences at Plymouth. This world-wide interest in aquarium construction and management, and the rising numbers of visitors to our own aquarium, implies a need for a fairly detailed published account of the Plymouth aquarium as it exists to-day. Many details are equally applicable to marine aquaria anywhere, and should therefore be of interest to planners of all large installations.

The Aquarium or Tank Room

The last fairly detailed account published is that by Allen & Harvey (1928). The dimensions they gave were from an old plan and differ a little from those given here, based on new measurements. Widths and lengths of tanks are given to the nearest $\frac{1}{4}$ ft.; these measurements are all internal. The reader will find it helpful to refer frequently to the plan and section shown in Text-figs. 1 and 2.

The tank room or aquarium (Pl. I) is situated on the ground floor of the south building. It is a single large room measuring internally 70 ft. × 30 ft. (Allen & Harvey give the width as $34\frac{1}{2}$ ft., but this includes the thickness of the walls), with a minimum ceiling height of $11\frac{1}{4}$ ft. The ceiling consists of seven shallow vaults. The room is entered and left by a wide doorway at the west end (seen in the photograph). A private doorway at the east end is normally kept locked.

South-Side Tanks

The whole of the south side is occupied by nine tanks built close against the wall. All are a little over 4 ft. wide (front to back) and $4\frac{1}{4}$ ft. high with a water depth of about $3\frac{1}{4}$ ft. Their lengths, in order from the west end, are $10\frac{1}{2}$, 10, $15\frac{1}{4}$ ft. and six tanks averaging about 5 ft. each. The viewing panels or openings of the four cast-iron frames holding the glass fronts are all about 4 ft. $7\frac{1}{2}$ in. long × 3 ft. high. The first two tanks have two viewing panes each (one frame holding all four), the third three (one frame). The plate-glass panes are all $1\frac{1}{16}$ in. thick and the slate backs, sides and bottoms are $1\frac{1}{2}$ in. thick. The slate bottoms of these tanks are $2\frac{1}{4}$ ft. above the tank-room floor. The lower edges of the viewing panels are $29\frac{1}{2}$ in. above floor-level.

North-Side Tanks

On the north side of the aquarium there are three large tanks, all about $5\frac{1}{4}$ ft. high with a water depth of about $4\frac{1}{4}$ ft. Their lengths and widths, in order from the east end, are $15\frac{3}{4} \times 9$, 31×9 and $15\frac{3}{4} \times 5$ ft. The first two back against the north wall, but the last has a $4\frac{1}{4}$ ft. wide passage between it and the wall; this passage contains four shallow service tanks. A doorway between the

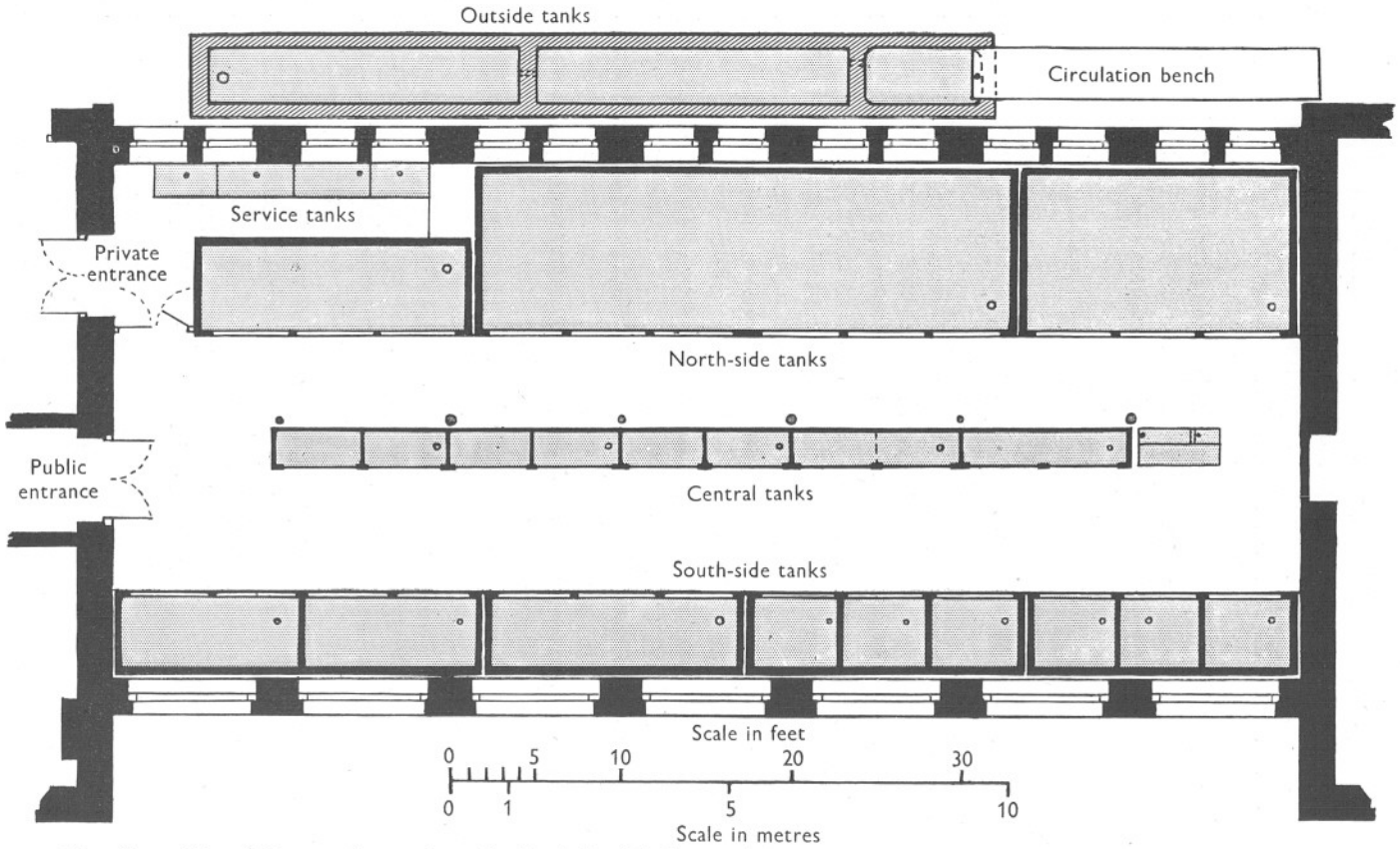
west end of this tank and the west wall of the room excludes the public from this passage and serves as a private entrance to the aquarium.

The viewing openings of the four cast-iron frames (each of three openings) holding the glass fronts of these north-side tanks are all about 4 ft. $7\frac{1}{2}$ in. long $\times$ 4 ft. high. The two shorter tanks have three glass panes each, the longest six. The plate-glass panes are all $1\frac{1}{16}$ in. thick and the slate backs, sides and bottoms are 2 in. thick. The internal bottom-level of these tanks is 2 ft. 2 in. above tank-room floor-level. The lower edges of the viewing panels are about 29 in. above floor-level.

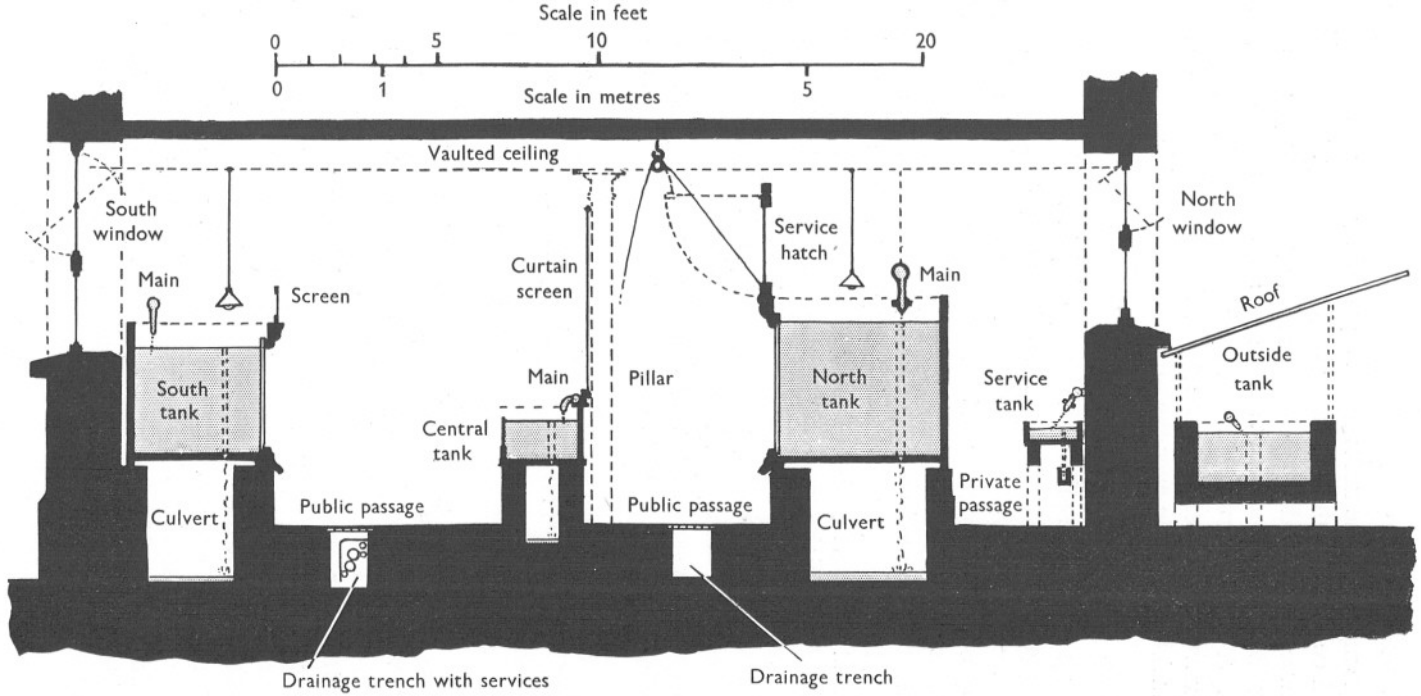
Glazing

The plate-glass panes of the north- and south-side tanks are not set in grooves. The cast-iron frames have machined facings which, when a pane is to be set, are covered with a suitable composition. The bottom edge of the pane is placed on two small composition-covered hardwood blocks, one at each end, and is eased into contact with the composition on the facing along the bottom edge of the frame. The whole pane is then raised into the vertical position and pressed home against the composition all round. It is then tightened against the composition with the aid of wooden shores, battens and folding wedges until the composition begins to ooze out all round in front. The tank is now flooded and the rising water pressure forces the pane in more firmly still. Eventually no more composition is squeezed out and battens and boards float to the surface. To ensure that no air pockets remain in the sealing composition a wooden wedge is used to ram in all round as much composition as can be forced between the glass and the facings of the iron frame. A wooden turn-buckle fixed to the top of the frame ensures the safety of the glass should it accidentally be pushed backwards when the water-level in the tank is lowered during cleaning or other operations. When the tank is full the pressure of water is amply sufficient to keep it in place.

For many years the sealing composition was a mixture of white lead ($7\frac{1}{2}$ lb.), powdered whiting ($1\frac{1}{4}$ lb.) and russian tallow ($\frac{1}{2}$ lb.). This mixture eventually sets hard and may crack away from the glass, giving rise to leaks. When a pane had been set for several months it was no longer advisable to lower the water level, or to empty the tank, without being prepared to reset the pane. More recently the commercial preparation 'Glasticon' (manufactured by Industrial Engineering Ltd., Mellier House, Albemarle Street, London, W. 1) has proved superior; it does not dry hard and therefore does not crack away from glass or frame. It has the additional advantage that should a leak develop (a rare occurrence) caulking can often be undertaken successfully from the *outside*. This preparation is now used for all the tanks and over a period of five years has given no trouble.



Text-fig. 1. Plan of the aquarium and outside circulation. Tanks containing sea water stippled. The small circles inside the tanks mark the positions of the overflows.



Text-fig. 2. Section through the aquarium near the west end. Sea water stippled.

Central Pillars and Screen

Down the middle of the tank room a row of six iron or steel pillars support the vaulted ceiling and the first floor of the laboratory. These are centrally placed between the outside walls of the building, but because the north side tanks are wider than those on the south side they appear to be sited asymmetrically. A screen or curtain of dark painted fabric is hung between these pillars to prevent the reflexion in the north-side glass panes of the south-side windows which are visible over the tops of the south-side tanks.

Central Table Tanks

Backing up against the pillars and the curtain is a series of five slate table tanks with glass fronts facing south. They are nearly 10 ft. long and $2\frac{1}{4}$ ft. wide and have a height of $1\frac{1}{2}$ ft. at the front, being some inches higher at the back and sides. The top edges of the ten $\frac{1}{2}$ in. plate-glass panes held in grooves in the slate are rounded and polished, and are only $3\frac{1}{2}$ ft. above floor-level. An adult is thus able to bend over the tank and inspect the contents from above, in addition to viewing them from the front if he stoops down. The water-level in these tanks varies from 8 to 15 in. according to the nature of the display. Most of the tanks are divided into two by a central partition. At the east end of the series a shallow wooden table tank has been added and above it are two small wooden tanks with glass fronts and backs so that they may be viewed from both sides. There is also a small glass-fronted slate tank above the central table tanks near the middle of the series. This is seen in the photograph in Pl. I, but has not been indicated in the plan (Text-fig. 1.)

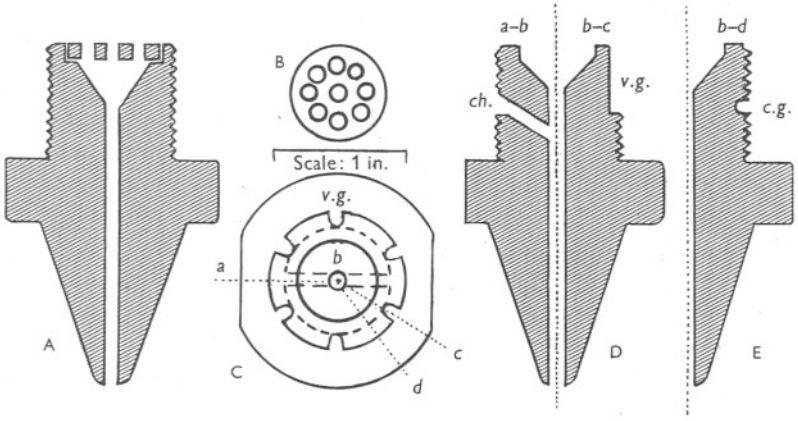
Designers of new aquaria should note that open table tanks are not entirely satisfactory for public display; they are a temptation to boys and some adults to interfere with their contents.

Culverts

The tanks are all built over culverts (originally termed 'circulating reservoirs') which receive the overflow water and return it to the reservoirs. The culverts on the north and south sides are about 3 ft. 9 in. high and almost as wide as the tanks above. Entry to the south-side culvert is through the floor of the westernmost tank; it is normally covered by a large slate sealed around the edges. This is an unsatisfactory arrangement, for the culvert can only be entered when this tank is drained. The north-side culvert can be entered by the west end of the largest tank at any time; the culvert of the central table tanks cannot be inspected at all. Under the two largest tanks on the north side a series of 9 in. brick pillars are staggered down the centre of the very wide culvert to support the weight of the tank floor near the middle. All culverts are lined with asphalt.

Inflow nozzles

Sea water pumped from the reservoirs enters the main tanks through a series of vulcanite nozzles situated generally towards the backs of the tanks and a few inches above water-level. Some nozzles are controlled by stopcocks. The smallest tanks are each supplied with two nozzles, the others with more according to size. Two nozzles are an absolute minimum for safety, owing to the risk of occasional blockage by foreign bodies. A blocked nozzle may not be noticed for hours, especially during the night, and there is a real danger of fishes in a tank dying before the absence of circulation to that tank is noticed.



Text-fig. 3. Diagrams of vulcanite nozzles modified to delay blockage by debris. A, vertical section of a nozzle provided with a perforated Perspex plate, shown in plan at B. Another method of modification, devised by Mr A. N. Bennett, is shown at C–E. C, plan from above; D, sections *a–b* and *b–c* as indicated on C. E, section *b–d* indicated on C. Six vertical grooves, or channels, *v.g.*, are cut through the screw thread to conduct water to a circular groove, *c.g.* From opposite sides of this circular groove are bored two downwardly sloping holes or channel-ways, *ch.*, to tap the central bore of the nozzle. Until the entrances to the central bore and all six vertical channels are blocked the nozzle continues to function.

Blockage of the nozzle is caused generally by pieces of sponges (*Halichondria bowerbanki* and *Sycon coronatum*) breaking off from growths in the pipes. This is a constant source of trouble. Most nozzles are now provided with a perforated disk fitted internally across the full bore of the pipe (Text-fig. 3 A, B), or have several channel-ways cut so that all channel-ways have to become blocked before the nozzle ceases to function (Text-fig. 3 C–E). This latter method, devised by Mr A. N. Bennett, the engineer in charge of pumps and pipes, has proved very satisfactory.

The water passing through a nozzle is forced down into the tank as a strong jet, often carrying with it an inverted fountain of bubbles. It is certain that only a fraction of this water reaches the bottom, much of it being quickly lost down the overflow, which draws off surface water. To lessen this loss of

newly injected circulation water there have in most tanks been placed one or more pipes of 3–4 in. bore, hung vertically from just above surface-level to within a few inches of the bottom. Water from one or more nearby inflow nozzles is conducted by rubber tubing into the top of each such pipe which ensures that the whole of it is led to the bottom. This arrangement has improved the health of the inhabitants of all tanks where it is in use. In the central table tanks glazed earthenware pipes, painted with black bitumastic paint, serve a similar purpose and keep the surface free from the ripples which, before they were used, obscured much of the view.

Overflow pipes

Each of the tanks has a single overflow pipe at one side and usually to the front. This is a vulcanite pipe of bore varying from 1 to 4 in. according to the tank. Its lower end is tapered and fits into a vulcanite seating, tapered to receive it, in the bottom of the tank. To empty a tank the overflow pipe is simply pulled up and the water rushes out through the hole thus opened in the tank floor. Overflowing water drains into the culvert under the tanks and is thereby returned to the reservoir in use, flowing into it at the surface against the north wall. The depth of water in the wide airy culverts (there are ventilators to the culverts through their walls from the outside of the building) increases from a fraction of an inch to 1 or 2 in. towards the reservoir end; good aeration therefore takes place before the water reaches the reservoir.

To obviate flooding in the event of an overflow pipe becoming blocked there are perforated vulcanite grids let into the slate sides of the tanks just above water-level. A stoppage of the overflow pipe results in a slight rise in the water-level of the tank which then overflows into its neighbours on either side.

Drainage Trenches

The floor of the aquarium in the public gallery contains two trenches 18 in. wide and 18 in. to 2 ft. deep, running the whole length of the tank room about 2 ft. in front of the north and south tanks. They are covered by iron gratings level with the rest of the floor, which is of concrete. In addition to containing various service pipes for the laboratory they function as drains running to waste and will carry away water overflowing on to the floor. They are useful when a tank is cleaned by siphoning, as described below (p. 206).

Lighting

Tanks are normally lit only by daylight through the windows. The tanks of the south side receive most light, but direct sunlight is now diffused by ground glass fitted into the south-side window frames. Formerly, when the windows were of clear glass, narrow sunbeams passed through the tanks and their dazzling brilliance made it difficult to see anything outside their range.

On the north side the lighting is always diffused and, except on bright days, is insufficient to light the tanks adequately. The smallest tank on this side is especially dark for, owing to the passage behind it, direct light from the windows hardly penetrates into it and what it does get is mainly reflected from the white ceiling above. Various devices have been tried to improve the lighting of this tank; at the present time independent electric lighting by three 60 W. daylight bulbs raises the intensity of the illumination to near that of the largest tank next to it, and is proving more satisfactory than reflectors.

The top front edge of the row of south-side tanks is only $6\frac{1}{4}$ ft. above floor-level and before the war the eyes of visitors were dazzled by direct light from the sky seen through the high arched windows behind the tanks. If the eyes were shielded with the hand, or the rim of a hat, visibility into the tanks was greatly increased, details not before visible becoming apparent. Therefore, during the reconstruction after the war, a 14 in. high plywood screen was erected along this top front edge so that the tops of the windows could no longer be seen from a normal viewing position. It was not possible to carry this screen close up to the ceiling (as was done on the north side) on account of the central table tanks. These are lit only by light passing over the screens to be reflected from the white ceiling above them. The screen is painted white on the window side to reflect light on to the backs of the tanks which formerly were barely visible. The screen is easily removable in sections to facilitate siphoning or other work.

The north-side screen almost reaches the ceiling, leaving a narrow gap for ventilation. At intervals there are hatchways for feeding, glass-cleaning and similar purposes. If ever the central table tanks are removed it would be advantageous to treat the south side in the same way, namely to extend the present low screen to near the ceiling and to provide appropriate hatchways. If this were done the central screen between the pillars would no longer be needed and the aquarium would become a fine hall lit only by light passing through the tanks.

In addition to the special electric lighting of the dark tank on the north side, electric lighting has recently been installed over all tanks (except the table tanks) for use on dull days, or after dark. Ordinary 60 W. pearl bulbs (not daylight type) in white plastic reflectors, approximately one to every glass pane, give very good illumination; they hang a few inches above water-level towards the fronts of the tanks. The general appearance of the tanks after darkness with these lights on is better than their appearance by day without them. This is probably due almost entirely to the direction of the light, coming from the top front of the tank instead of from the back. By day the side of the fish away from the observer is more brightly illuminated than the side which is on view. Only a few semi-transparent organisms are enhanced by back-lighting in the absence of frontal lighting.

Decorative Rockwork

To relieve the bare appearance of the slate tanks, and to improve living conditions for the inhabitants, most of the large tanks have rockwork built into them. Some of the original rockwork, dating from the time the tanks were first erected, can be seen in the largest tank on the north side. This original rockwork consists of lumps of calcite stuck together and to the slate walls with pitch. No attempt was made to achieve a natural effect. In 1946, when war damage to the aquarium was repaired, new rockwork was added to several of the tanks and much of the old rockwork reinforced with concrete. For the new rockwork, which was built by our own staff, waterworn limestone was obtained from the foreshore at Cattedown (district of Plymouth) and care was taken to arrange and cement the slabs to give an appearance of natural stratification and to provide suitably shaped holes for octopus, lobsters, conger eels, etc. In one tank stalagmitic rocks have been used with good effect. The floors of the tanks are covered with sand or shell gravel or with water-worn pebbles in accordance with the species kept in them. Wherever slate backs and sides are uncovered by rockwork they are painted over with 'Bituros', a black bituminous paint used for drinking-water tanks and manufactured by Wailes-Dove Bitumastic Ltd., Hebburn, Durham.

Labelling

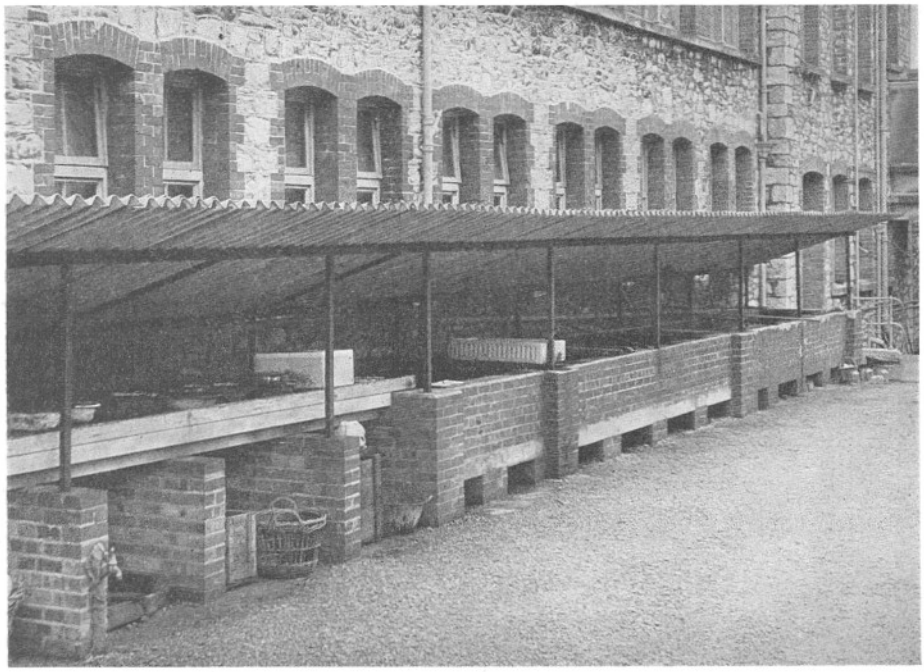
Lead frames containing labels are screwed to moulded wooden sills on the north- and south-side tanks. Name labels are constructed by pasting a printed paper sheet face down on to glass and backing with a sheet of white opal glass stuck on with paraffin wax. Picture labels are painted and lettered in water colour on good quality drawing paper and sandwiched between thin sheets of Perspex sealed around the edges. This is undertaken by a firm specializing in the process. Such Perspex labels should be permanently waterproof.

Outside Circulation

In the yard between north and south laboratory buildings there are three asphalt-lined brick and concrete storage and acclimatization tanks and a circulation bench for bowls (Text-fig. 4). They are built against the north wall of the south building and are screened from sky and rain by a pitched roof of corrugated asbestos but are otherwise open to the outside air. Two of these measure internally approximately 18 ft. 6 in. × 3 ft. 6 in., the water depth being 1 ft. 6 in.; they can be divided into smaller compartments by movable wooden partitions. The third tank is only 7 ft. long, but is similar in width and depth. Water is supplied by a series of jets from an iron pipe. Although very rusty, this pipe has already given adequate service for several years.

Laboratory Tanks

In the main laboratory on the first floor are tanks of various sizes for storage and research. There are eight rustless-steel framed tanks with 1 in.-thick slate bottoms and sides and $\frac{1}{2}$ in.-thick plate-glass fronts. The three largest measure 4 ft. × 2 ft. 4 in. × 1 ft. 6 in. deep externally. There are also sixteen porcelain sinks, 2 ft. × 1 ft. 6 in. external measurements, eight 10 in. deep externally, four 8 in. deep and four 6 in. deep. Two shallow table tanks, made



Text-fig. 4. View of the outside circulation showing portion of circulation bench and the three asphalt-lined brick and concrete tanks. The two sink-tanks are temporary additions.

from $1\frac{1}{2}$ in. thick teak measure externally 7 ft. × 5 ft. × 9 in. and 5 ft. × 3 ft. × 9 in. The water depth is only $6\frac{1}{2}$ in. With such a relatively great surface area one or two small nozzles suffice to keep a great variety of small animals alive. These tanks are indeed among the most successful for small organisms. Such tanks are unfortunately not suitable for public display owing to the ease of access to their contents.

Most of the tanks just described can be seen in photographs reproduced by Russell (1948, plate XIX). In these photographs it will be observed that provision is also made for giving circulation to bowls, etc., placed on slate circulation benches.

Overflow water from these laboratory tanks and from the circulation benches runs directly into exhibition tanks in the public aquarium on the ground floor below. This is a bad arrangement, because from time to time silty water produced by activities in the laboratory clouds the exhibition tanks into which it is discharged and adds considerably to the labour of siphoning needed to keep them reasonably clean.

In conjunction with the Specimen Supply Department in the north building there are four sink tanks and a small circulation bench. Formerly this had its own separate pump to supply water from one of the reservoirs, but of recent years an iron pipeline has been installed to supply it direct from the main pump.

The Reservoirs

Sea water is pumped into the tanks from one of two large reservoirs, each 37 ft. × 21½ ft., holding water 11 ft. deep. They are used alternately. The reservoirs were excavated in the solid limestone north of the aquarium at the time the latter was built; they were completed with concrete and lined with asphalt. They lie below general ground-level and are roofed over with a concrete flat to shield them from light and rain. Each holds about 55,000 gallons, or about two and a half times the total volume of water in the tanks, including those in the main laboratory on the first floor and the large specimen storage tanks in the yard outside.

Overflow water from the aquarium is discharged into the reservoir in use at the surface in a corner by the north wall. The outflow to the pumps is sited about 3 ft. from the bottom not far from a corner on the south wall. A complicated swirl is set up throughout the reservoir and there is no completely stagnant corner. In some parts the water moves extremely slowly; in other places and at different levels it moves much faster (determined by drift-bottles). It seems possible that some of the water returned from the aquarium is drawn off again to the pumps before water which has been in the reservoir for a longer time. The swirl must hinder the settlement of slowly sinking detritus brought from the tanks and much of this detritus is pumped back into the tanks again (see p. 209 for a suggested method of dealing with this problem).

The Engine Room

The engine room is in the basement and its floor is about level with that of the reservoirs. Circulation is maintained by one of two centrifugal pumps driven by electric motors. The older pump is a cast-iron 2 in. pump driven by a 3 h.p. motor; the newer one is a cast-iron 2½ in. pump driven by a 2 h.p. motor. Water drawn from one or other of the reservoirs at a point in the south wall about 3 ft. above the bottom is pumped at over 50 gallons a minute against a head of about 40 ft. through 2 in. cast-iron glass-lined piping to the main vulcanite pipes serving the tanks.

The pump impellers are of cast-iron mounted on stainless-steel spindles. A cast-iron 2 in. centrifugal pump driven from shafting by a 5 h.p. Crossley gas engine is a stand-by in the event of electrical failure. Except for two periods of about 1½ hr. in the morning and for ½ hr. in the evening, for cooling motors and for maintenance, pumping continues day and night.

The engine room also contains two air-compressors, but of recent years compressed air has had little use in the tank room though it is supplied to, and has its uses throughout, the laboratory.

Pump House

Reservoirs are periodically emptied, hosed down, and refilled from the sea. For this purpose there is housed in a small brick and concrete building on the rocks just above sea-level, which is about 97 ft. below laboratory ground-level, a cast-iron 4 in. centrifugal pump driven by a 14 h.p. motor. This is capable of delivering about 215 gallons a minute against a head of 150 ft. and can fill a reservoir in about 4 hr. A good spring tide in calm dry weather is chosen, and pumping commences 1–2 hr. before high water and continues for about the same time afterwards. Usually part of the volume required is pumped on one tide and the remainder the next day. The water is drawn through a suction rose situated near low-water mark at the base of the rock on which the pump house is built. The Shone's Ejector mentioned by Allen & Harvey (1928) was dismantled years ago, as was the last of the 'Otto' gas engines.

Maintenance

Treatment of the Stored Sea Water

During the summer one reservoir may be emptied, cleaned and refilled every month, but during the winter several months may go by without new water being obtained from the sea. Sometimes reservoirs are merely lowered a few feet and then filled up. In normal practice a reservoir is in use for 1 week, while the other rests. At the end of the week there is a change over. The reservoir passing out of use is then limed, that is to say, a bucket is one-third filled with quicklime and slaked under a freshwater tap. The slaked lime is spread as evenly as possible over the reservoir and sinking through the water restores the pH to that of natural sea water. It has been found advantageous to treat (with slaked lime) the reservoir actually in use about the fourth day, especially during hot weather. Water newly drawn from the sea often decreases in pH more quickly than old established water which has been frequently limed. This limed water is very satisfactory for ordinary aquarium purposes. For notes on the chemistry see Atkins (1931) and Cooper (1932).

Temperature

There is no means of artificially heating or cooling the aquarium water, its temperature varying with the seasons. The upper and lower limits are roughly 17–18° C. in summer and 7–8° C. in winter, occasionally being exceeded in both directions. For the fauna kept in our tanks temperatures above 16° C. and below 9° C. are undesirable. Quite a number of species suffer when the temperature changes rapidly (2–3° C. in a week), especially near the upper and lower limits.

The water flowing through the outside circulation bench and tanks, exposed to the outside air, is almost invariably cooled down and returned to the reservoir at a lower temperature than that from the rest of the system, the general effect being to cool the whole. Very rarely in summer the reverse may happen. In hard frosty weather it has sometimes been necessary to cut off this outside circulation to conserve the heat of the main system.

Salinity

Few data on the salinity of the aquarium water are available. The salinity of the water pumped at high tide is about 35 ‰, but increases somewhat with storage; for instance Cooper (1932) mentions a figure as high as 38·0 ‰, and Brown (1929) had previously given figures 37·0–37·9 ‰. It has never been necessary to add fresh water to make up for that lost by evaporation, and no effects on the animals attributable to increased salinity have been noted.

Servicing the Large Tanks

There are no service galleries behind the large north- and south-side tanks in the Tank Room and all work, such as feeding, glass cleaning and siphoning has to be carried out over the front of the tanks from a ladder propped up against them. On the south side the attendant can make his way along the backs of the tanks via the internal window sills, on the north side along an inconvenient cat-walk of planks placed across girders just above water-level (the two largest tanks only). There is insufficient head room to walk upright on this cat-walk.

Glasses are cleaned once or twice a week internally with a scrubbing brush attached to the end of a pole, or with a straight edge of Perspex on a long handle. This latter will remove quite hard growths without scratching the glass. Old newspaper serves for the exterior of the panes.

Silt which accumulates on the bottom of the tanks is removed by siphoning with a rubber hose, the siphoned water running to waste through the drainage trenches in the aquarium floor. Sand or fine gravel brought over from the tank is trapped in a galvanized iron bath into which the siphoned water first discharges. It is sometimes useful to fit a large funnel on to the end of the hose inside the tank. By choosing one of the right size flocculent silt may be

separated from fine gravel. A tap on the outer end of the hose, to regulate rate of water flow, can be of assistance in this.

Feeding

The main food used in the aquarium is the common squid, *Loligo forbesi*, obtainable at Plymouth in quantity most of the year round. The uncooked white mantle flesh of this animal, cut into suitable sized pieces, is relished by almost all the species kept, while the heads are eaten by the conger eels and nursehounds. The contents of ovaries dispersed in the water have proved excellent for feeding very small fishes such as young grey mullet. At times, when squid is not to be had, herring, mackerel, conger eel and some white fishes are used. The oily fishes tend to foul the water and the white fishes are not relished and are generally not eaten at all unless really fresh. Iced fish obtained from the fishmonger is often left uneaten by hungry fishes. Worms (*Nereis diversicolor*) are also an excellent food for smaller fishes.

A few animals need a specialized diet. Octopuses and cuttlefishes must be supplied with living crabs or prawns, although they can occasionally be induced to take dead fishes. John Dories generally require whole fishes but can be trained to take squid, especially if it be cut into an elongate shape. Pipefishes need living plankton. The overflow from a main laboratory tank leads into the pipefish tank and jars of living plankton, after satisfying the needs of workers in the main laboratory, are tipped directly down this overflow. Most fishes need to be fed at least twice a week, but daily is too often. Successful feeding demands care and attention to the varied ways of catching prey natural to the different species kept. A complete account would occupy several pages.

General Remarks and Suggestions

During the last few years a number of plans for proposed new aquaria have been submitted for criticism. A common fault has been that the sizes of proposed tanks have been too small, especially widths in relation to lengths. Except for quite small fishes a width of 3 ft. (commonly adopted) is insufficient, especially when it is proposed to fix rockwork inside on the back. Quite apart from matters concerning the health of the fish it is often forgotten that a tank full of water viewed through the glass appears to be considerably less wide than it actually is. While for viewing small organisms lying at the back of the tank this may even be an advantage, an aquarium consisting solely of apparently narrow tanks fails to give that illusion of an underwater world which is one of the charms of a properly designed aquarium.

There is no doubt, too, that large tanks are better for their inhabitants and give less trouble than do small tanks. In general the smaller the tank the more often does it require attention and the more likely are the results of a temporary stoppage of circulation to prove fatal. Some small animals,

vertebrate and invertebrate, which have never done well in our smaller tanks have flourished and lived a full natural life in our larger ones. Thus the little Two-spot Goby (*Gobius flavescens*), which never lived for long in small tanks, does extremely well in the largest tank of all. Presumably in a very large tank the goby readily finds scraps of food left over by the big fishes, as well as small worms, crustaceans, etc.; it is itself too small for the big fishes to eat. A great improvement in health and vigour has often been noted when fishes such as pollack, whiting, red mullet and sea-bream have been transferred from tanks on the south side to north-side tanks with several times the capacity.

A tank floor need not be level; it could be constructed to slope downwards from the front towards the back. This would give increased depth (at the back) without undue pressure on the glass. It may also slope upwards when it is desired to construct a series of rising terraces for anemones, sea-fans and other sedentary organisms.

Another point is the size of reservoirs. In a closed circulation the bigger the reservoir in relation to the inhabited tanks the better. During the war when most of our big tanks were broken and empty, the inhabitants of those which remained did noticeably better, and delicate organisms survived longer than they did before the war or do now, and sometimes even bred. As the capacity of one of our reservoirs is roughly two and a half times the capacity of the tanks it seems reasonable to conclude that a reservoir capacity double or treble this would give noticeably improved results. The relative proportions needed are influenced, of course, by the density of stocking, by liming, filtration and other treatments of the water, but there seems little reason to doubt that in designing a closed circulation aquarium it is advisable to construct the reservoirs on as generous a scale as possible. Such reservoirs must be darkened to inhibit the growth of water-clouding phytoplankton organisms; they should not be tanks exposed to the atmosphere and thereby able to gain or lose heat relatively rapidly. In the same way exhibition tanks should not be exposed to the sun or directly to the outside air; the whole system should be enclosed in a building. If the reservoirs be well insulated and if the building itself be centrally heated there should be little difficulty, in our climate, of maintaining a reasonable temperature during the winter months.

No public aquarium should be designed without service galleries behind the tanks. Such galleries not only greatly ease and expedite attention to inflows and overflows and all work of feeding, cleaning, addition and removal of specimens, but their lack can lead to difficulties on crowded days when it becomes scarcely practicable to give emergency attention from a ladder over the front of a tank. If during a Bank Holiday anything goes wrong it may not be possible for several hours to do anything to put it right.

Similarly, all aquaria should be provided behind the scenes with tanks for acclimatization and excess stock, living food and for sick fishes. It is bad

practice to put freshly caught fishes into the exhibition tanks. Even those that will survive often show bruises, torn fins and other injuries during the first 2 or 3 weeks and a damaged fish always attracts attention. Acclimatization tanks must be large and deep, otherwise they will fail in their purpose. Storage tanks and tanks for sick fishes can be appreciably smaller. In cases of infectious sickness the overflow water should run to waste; it may be wiser not to attempt to keep the fish alive at all.

A frequent inquiry concerns filtration; should the water be filtered? Many well-known aquaria do possess elaborate filter beds and the water in their tanks is crystal clear. This condition seems to suit most fishes, but some invertebrates, and especially filter-feeding invertebrates, do not seem to do so well. The filter-feeders could be provided with suitable cultures of micro-organisms, but on the whole for them the water is better not filtered. It should, however, be allowed to sediment while passing through the reservoir. This could be achieved by partitioning the reservoir so that it becomes in effect a long broad passage way, repeatedly bent back upon itself, through which the water would flow slowly from inflow to outflow. Overflow water from the aquarium would be conducted through a very broad pipe, or shaft, straight to the bottom at one end of the passage, and water to the pumps would be drawn off above the bottom at the far end. During the hours the water would take to traverse the passage sediment would be deposited on the bottom. Sedimentation would remove unwanted detritus without removing swimming micro-organisms.

A disadvantage of encouraging filter-feeders is the likelihood of sponges and other organisms growing in the pipes through which the sea water is pumped to the tanks. The blockage of nozzles from pieces of sponge has already been mentioned (p. 199). The trouble would not be so great if pipes were provided with some easy means of access at all bends and elbows; internal growths could than be scraped off from time to time.

In a permanent installation pipes conveying water under pressure need to be made of vulcanite or some other material not affected by sea water. This applies equally, of course, to all parts in contact with the circulating sea water. Copper, brass, zinc and galvanized iron are the metals most likely to be encountered in constructors' plans. In sea water they dissolve to a sufficient extent to poison marine organisms. Pure lead is permissible in small quantity but should be avoided if possible. In our experience vulcanite pipes and cocks have proved perfectly satisfactory; some of the original ones are still in use after sixty years of service. On the other hand, stainless, or rustless steel pipes installed to supply new tanks erected in the main laboratory during reconstruction in 1938–9 rusted through, especially at bends, in only a few years, though stainless-steel has been satisfactory for the outside framework of small slate and glass tanks and for pump spindles. Ordinary iron piping, in spite of extensive rusting (which is harmless) has given better service and

is very satisfactory for a temporary installation which is expected to last only for a few years. Internal rust must be scraped out from time to time. Rubber hose can also be used for temporary erections. Some of the newer plastic materials may prove excellent for sea-water aquarium pipes but we have had no experience with them. Pipes of transparent or translucent materials would need to be darkened to prevent algal growths inside.

For overflow water not under pressure ordinary earthenware drain piping, or asbestos pipes or troughs may be used.

Finally, there is the appearance of the tanks to be considered. The aim should be to present the animals in surroundings as natural to their species as is possible; this is not only good showmanship but is also beneficial to the animals. Carefully chosen natural rocks placed in position with due regard to their normal geological formation, sand or gravel or pebbles on the bottom (not too deep a layer), whatever is suitable, can greatly enhance the attractiveness of the display, but over-elaboration should be avoided. It is not desirable that the rockwork should attract the eye away from the fish. All inflows and overflows should be concealed. Inflows, of which even the smallest tanks should have at least two, can be placed in the forward corners of the tank, behind rockwork or anywhere else out of sight. Inflow nozzles should discharge into wide pipes passing from just above surface-level to near the bottom (see pp. 199–200) to ensure good circulation throughout the tank. Overflow water should pass through a hole in the back of the tank and should draw off surface scum. There is no need for unsightly pipes stuck into a seating on the tank floor.

Tanks can always be emptied by siphoning, but a plug could be provided in the tank floor in case of need. Aeration by compressed air forced through pieces of cane, or through diffusers manufactured for the purpose, should not generally be required, but when the temperature is high can be used with advantage. One use is to assist circulation in the tank by creating an upward current from the bottom. This is most effective if the air be bubbled up through a wide tube opening near the bottom and just below the surface. It should be noted that water supersaturated with air, or containing an excess of fine air bubbles, may not be good for the health of the fish. It may be a cause of the gas blisters which from time to time occur in the eyes, fins and skin of some fishes.

Excess daylight must be avoided if too vigorous a growth of algae is to be prevented. A slow growth of small red weeds can be permitted and will occur if the amount of daylight allowed is not reduced too much in intensity. Electric lighting for dull days and at night should be fitted, especial care being taken to ensure perfect insulation and water-proofing. Lights should be situated well forward (see p. 201), and close to the water surface, but it is a matter for choice whether fluorescent tubes or ordinary bulbs are used. The former give a shadowless light, the latter throw shadows and when the surface

of the water is rippled there is produced a play of light on rocks and sand which adds liveliness to the appearance of the tank.

In designing the layout of an aquarium care is necessary to ensure that no bright reflexions obscure the view. Windows, doorways, brightly lit tanks opposite, and even white labels reflected in the glass can distract from and obscure the tank contents. All paint-work should be very dark, for the same reason.

The slate backs and sides of the Plymouth tanks where not covered by rockwork are painted black (see p. 202). This is not always ideal. The system adopted at the Danish Aquarium at Charlottenlund has much to recommend it. Tanks are lined with various blue and green toned semi-opaque or enamelled glass, enhancing the brilliance of the tank, and showing off some fishes to better advantage than does black. A system of false backing, a sheet of ground or semi-transparent glass, perhaps lightly coloured, placed a few inches in front of the real back of the tank gives an impression of distance, especially if fish can penetrate behind the false back to be dimly seen. The edges of the false back must be concealed.

There are no guard-rails at Plymouth and on crowded days people crush up against the glass and hide the labels from view. A leaning rail in front of the tanks should always be provided, as it not only protects the glass and labels but is a definite comfort to the visitor.

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EXPLANATION OF PLATE I

The aquarium viewed from a height of 7 ft. close to the wall at the east end. Left to right: south-side tanks with low anti-dazzle screen along front top edge; cast-iron gratings covering drainage trench in floor; central table tanks with small glass-fronted slate and wooden tanks above; six central pillars with curtain screen hung on them (three sections of the screen near to the camera have been removed); north-side tanks with service hatch in screen open and service ladder in position. The public entrance, closed by double doors, is at the far end. The pipes crossing the ceiling in the middle distance are sea-water mains and overflows to and from tanks in the main laboratory above. Some details of the electric lighting to the tanks are also visible.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Onset of Shortening in Striated Muscle

By B. C Abbott and J. M. Ritchie

Journ. Physiol., Vol. 113, 1951, pp. 336–45

When a striated muscle is stimulated at one point, the excitation travels away from that point at a finite speed. The whole of the muscle is active when the wave of contraction has travelled the length of the muscle. Doubt has been cast as to whether the electrical wave associated with membrane excitation travels at the same velocity as the wave of contraction.

Isolated muscles from dogfish, frog and toad at 0° C. were stimulated (*a*) simultaneously at many points along their length, and (*b*) at one end only. The time course of unloaded isotonic shortening during maximal twitches was recorded photographically. When simultaneously stimulated all over the muscles began to shorten at their maximum speed at the end of a latent period. When stimulated at one end the speed built up gradually, but to the same maximum value, so that the shortening-time curves run parallel: the curves are separated in time by half the propagation time of the contraction wave along the muscle. It is shown experimentally that in the muscles of all three animals studied the propagation velocities of contraction and excitation waves are identical. When a muscle is stimulated in saline the propagation velocity is appreciably greater than when in air. B.C.A.

The Localization and Analysis of the Responses to Vibration from the Isolated Elasmobranch Labyrinth. A Contribution to the Problem of the Evolution of Hearing in Vertebrates

By O. Lowenstein and T. D. M. Roberts

Journ. Physiol., Vol. 114, 1951, pp. 471–89

Vibration responses in the form of impulse discharges can be recorded from nerve twigs leading from part of the macula sacculi, the macula neglecta, and the lacinia of the macula utriculi of the isolated elasmobranch labyrinth. The otolith-bearing part of the macula utriculi, the posterior portion of the macula sacculi and the adjoining macula lagenae do not respond to vibrational stimuli. They contain gravity receptors only. An appreciable number of the

sense endings show a resting activity in the absence of vibrational stimulation. There exists, however, convincing evidence that, at any given time, many sensory units are quiescent. These can be recruited to take part in the vibrational responses, and they show a considerable range of thresholds. Under the obtaining experimental conditions vibration responses were recorded to stimulus frequencies extending rarely higher than 120 cyc./sec. Vestibular microphonics were observed up to a signal frequency of 750 cyc./sec. but only responses in the form of nerve impulse discharges are accepted as evidence for vibration sensitivity. At low intensity stimulation the response consists of an increase in the discharge frequency of the 'spontaneously' firing units. Higher intensities lead to the recruitment of previously quiescent sense endings and to a marked synchronization of the response frequency with that of the stimulus. This synchronization closely resembles the responses described for the mammalian cochlea where it occurs at the lower end of the audible spectrum. Adaptation to sustained vibrational stimulation and a 'silent period' after cessation of prolonged stimuli have been observed and the latter has been quantitatively analysed. It is claimed that the theoretical implications of these results may be of considerable importance in relation to the problems of the evolution of hearing and pitch discrimination in vertebrates. O.L.

The Life-History of the Multiform Species *Jassa falcata* (Montagu) (Crustacea Amphipoda) with a Review of the Bibliography of the Species

By E. W. Sexton and D. M. Reid

Journ. Linn. Soc. London (Zool.), Vol. xlii, 1951, pp. 29–91

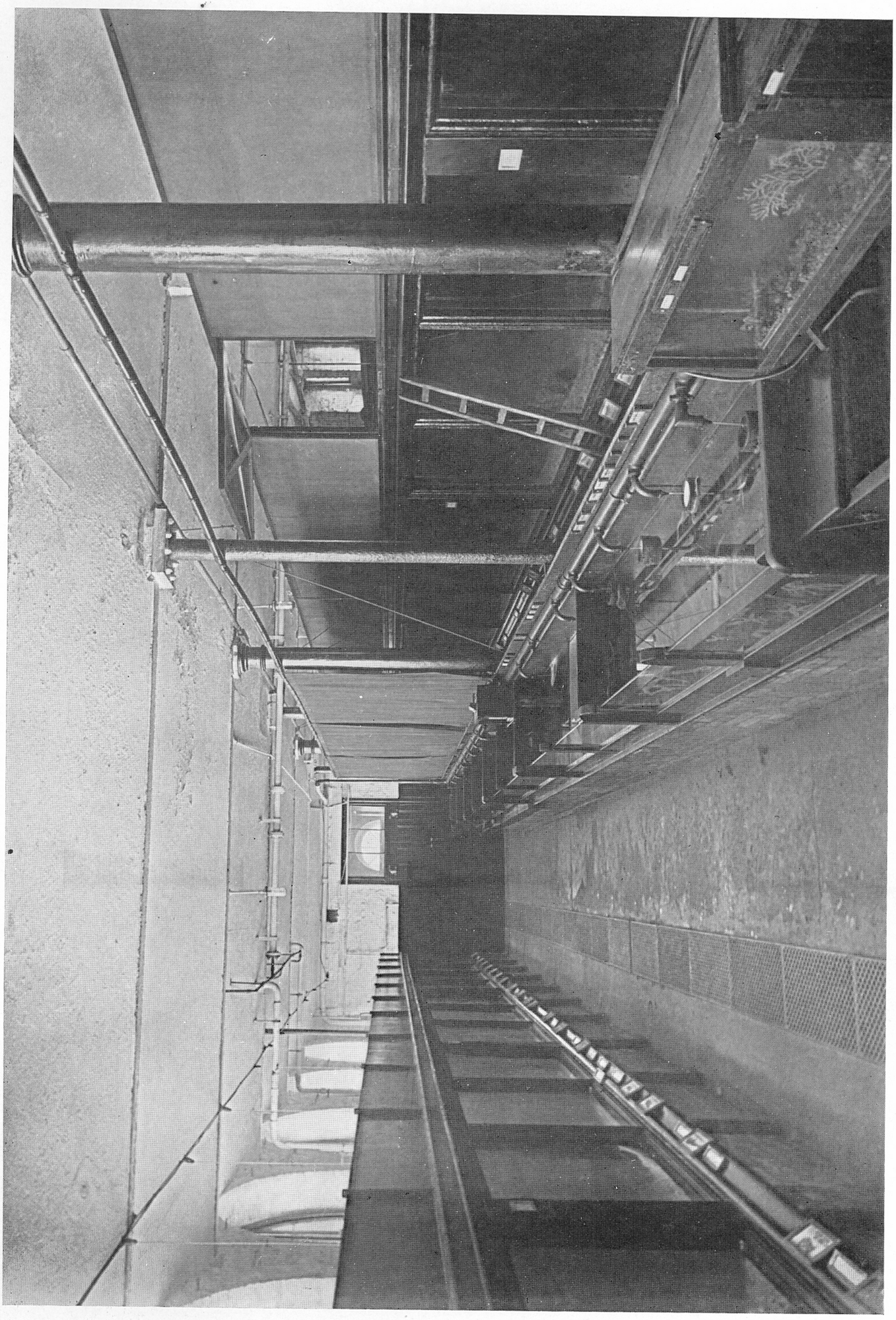
It has been shown by rearing and breeding experiments that the amphipod *Jassa falcata* (Montagu 1808) is a polymorphic species.

The species falls into two main classes or divisions, characterized particularly by the differing setation and shape of the second antennae, and the second gnathopods of the males. These divisions are called here, in accordance with their appearance, the Broad and the Narrow Forms. There is a third Form, in which the antennal characters of both the other Forms are combined with either the Broad gnathopod or the Narrow one.

In addition a number of Minor variants occur within the limits of the two main divisions.

The species also shows male intersexuality.

The synonyms of the species are summarized, and a full review of the bibliography given.



THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £23,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the Laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. An account of the Laboratory and the scope of the work undertaken there will be found in Vol. XV (p. 735) and Vol. XXVII (p. 761) of this *Journal*.

The Laboratory is open throughout the year and its work is carried out under the supervision of a Director and with a fully qualified research staff. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology and physiology. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the Laboratory.

TERMS OF MEMBERSHIP

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the Library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill Plymouth.

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