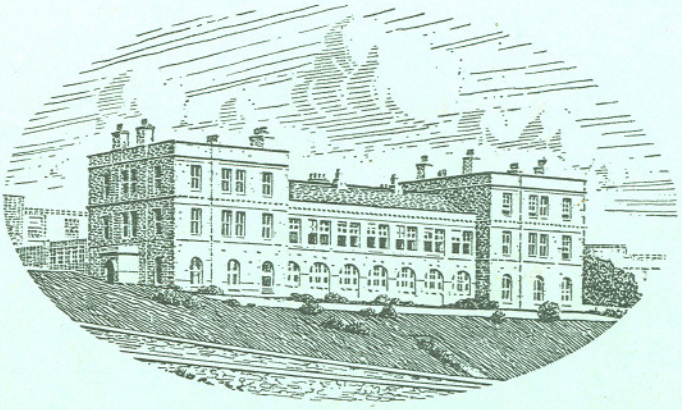
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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THE DEVELOPMENTAL STAGES OF *LERNAEOCERA BRANCHIALIS* (LINN.)

By Nora G. Sproston
From The Laboratory, Plymouth

(Text-figs. 1–6)

CONTENTS

INTRODUCTION

The remarkable life history of *Lernaeocera branchialis* was first described in detail by Claus (1868*b*), who also recognized the close relationship of the old family Lernaeidae, highly modified though it is, to the Caligidae (1868*a*); more recently this close similarity has been emphasized by Gurney (1934). Though the genus *Lernaeocera* is so common on gadoid fishes, the larval forms from pleuronectids are not frequently recorded; indeed, none has yet been found in the western hemisphere. Andrew Scott (1901) obtained abundant material from flounders from the Piel Hatcheries, but his descriptions are not in very great detail, and he leaves some doubt as to the number of stages passed through during metamorphosis.

Wilson's (1917) account is largely based on that of Scott, from whom he borrows his figures. He gives an original description, however, of a nauplius and a metanauplius of a species which he identifies with *L. branchialis*, which he obtained from a gadoid at Woods Hole. In the opinion of Schuurmans Stekhoven (1936*a*) there are so many points of discrepancy between his description and that of the European forms, that (in 1937) he renamed the

American form *L. wilsoni* (Wilson, 1917). Stekhoven gives adequate descriptions and figures of many species of the genus (1935, 1936*a*, 1936*b*, 1937), but he concerns himself only with adult (mature) females; though he refers (1935) to the finding of larval forms on *Pleuronectes flesus* off the Belgian coast and again at Helder (1936*b*) he does not describe them.

During the examinations of flounders from inshore waters near Plymouth, referred to in a previous paper (Sproston & Hartley, 1941*a*), about a hundred developmental forms of this parasite were collected, and the study of them has served to elucidate many points which were not clear from Claus's account and at the same time has raised new problems. It has been possible during the present studies to correct some details in the existing descriptions of *Lernaeocera* larvae, and an attempt has been made to follow the exact way in which the peculiar suspensory 'filament' of the chalimus stages arises. A comparison of the length data of the various stages has shown a somewhat unusual type of growth curve.

These investigations have been carried out during the tenure of the University of London Post-Graduate Research Studentship at the Marine Biological Laboratory, Plymouth. My thanks are due to the Director and staff of the Laboratory for their encouragement and practical help, and to Dr R. Gurney, who has read the manuscript and given his helpful advice.

The Nauplius

In a previous paper I have mentioned that it was possible to keep the mature female *Lernaeocera* alive for periods up to 9 days in circulating sea water in the laboratory, provided that no injury was done to the parasite during the dissection from the vascular tissues of the host. When the eggs were ripe and the nauplii began to emerge the parasite was transferred to a plunger jar, and some of the nauplii were taken for immediate examination and their moulting into the copepodid form was observed microscopically. Hatching was also observed, but only one form of nauplius was found and no nauplius was ever seen to moult into a second nauplius stage. The enormously long uniseriate egg-strings hatch very slowly, so that some days may elapse before all the nauplii have been liberated—unlike *Chondracanthus lophii*, whose almost equally long multiseriate egg-strings fragment and hatch within a few hours. Hatching eggs have been found throughout the year, and as concluded elsewhere, breeding is continuous in this as in some other parasitic copepods (Sproston & Hartley, 1941*a*). Unfortunately, owing to war-time fishing conditions, it was not possible to obtain flounders for infection experiments, but the identity of the copepods reared in the laboratory with those found from time to time on the gill-tips of flounders left no doubt that the forms described from the latter habitat were *Lernaeocera branchialis* (Linn., 1767) Blainville, 1822.

It may be mentioned that whereas the nauplii soon after hatching swam

freely in the circulating sea water and even tended to prefer the surface levels, when about to moult they sank to the bottom. The resulting copepodids, though actively swimming, also tended to keep near the bottom of the container: they would often cease swimming and crawl, largely by a 'hand-over-hand' movement of their chelate second antennae, which during the more active swimming were kept folded back along the under side of the cephalothorax, and so were invisible from above. This observation may be correlated with the behaviour of the intermediate host, the flounder, and it is interesting in that it supports an analogous observation by Gurney (1930). He found that the nauplii of *Nicothoë astaci* and *Chondracanthus lophii*, after a brief period of activity at all levels in the aquarium, sank to the bottom where they remained until moulting. It may not be insignificant that all three species are about to seek hosts which live on the bottom.

Claus did not describe or figure the nauplius stage of *Lernaeocera*, and though Van Beneden (1861, figs. 7, 8) gave two figures, neither these nor his descriptions are sufficiently detailed to be of much value. Wilson (1917) described two nauplius stages: a more or less rhomboidal nauplius 0·45 mm. long and with a maximum width of 0·40 mm. (pl. 12, fig. 107), and a metanauplius 0·55 mm. long, with a maximum width of 0·25 mm. which occurs near the anterior end (pl. 10, fig. 88). The metanauplius differs in shape from the nauplius, and has two pairs of limb rudiments in the form of bud-like protrusions on either side of a small median process at the hind end of the tapering body. No such form was found in the present material.

Andrew Scott (1901, pl. 4, fig. 2) shows 'a newly hatched nauplius' which is broadly club-shaped and in many ways resembles Wilson's metanauplius, especially in the slight indication of two segments near the narrow truncated hind end; the length is given as 0·45 mm., which agrees with Wilson's rhomboidal nauplius in size.

Fig. 1 represents a typical nauplius from the present material from Plymouth, and though there was rather a wide range of size, 0·345–0·405 mm. (see Fig. 6*A*) —the mean of ten random samples being 0·371 mm. long with a maximum width of 0·2–0·25 mm.—it was markedly smaller than either Scott's or Wilson's material. The general colour is slightly yellowish due to the contained fat globules. Irregular chromatophores occur laterally at about the middle two-thirds of the animal; they are of a dark puce colour, and a still darker pigmentation surrounds the eye. The nerve mass, chiefly below this, is bilobed and stains deeply with haematoxylin. Neither my speci-

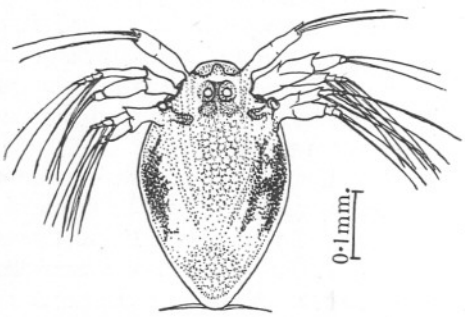

Fig. 1. Nauplius (stage I) of *Lernaeocera branchialis*—dorsal aspect.

mens nor Scott's show limb rudiments near the hind end and I saw no free nauplius showing segmentation, though I have fixed specimens in the process of moulting into copepodids in which such faint segmentation is seen under the skin at the hind end. It seems that in my material the metanauplius stage has been suppressed and is passed through very rapidly just before the emergence of the copepodid. This takes place some 24 hr. after hatching from the egg at laboratory temperatures.

The usual three pairs of appendages are found in the nauplius, though the labrum could not be made out, and a pair of stiff setae, 'balancers', occur at the hind end, usually held at right angles to the body axis, though they have some degree of freedom posteriorly. Pedaschenko (1898, pl. 5, fig. 169) figures them with small articular bosses, but I am unable to confirm this (he also shows small basal joints on the first antennae).

The *first antennae* arise from the sides of a somewhat flattened frontal region by a narrow isthmus, and there is a faint indication of segmentation into two equal joints: they are not so distinct as Scott shows them, and in Wilson's figure of the nauplius the antennae are unjointed and bear two small spines on the posterior subterminal region which I have not seen. In all my specimens there is a short sharp spine on the anterior angle of the tip which also has two long setae. Between these appendages there is a small median papilla under the skin, slightly ventral, which stains darkly with haematoxylin; in nauplii about to moult it is distinctly but minutely papillose. I suspect this of being the precursor of the 'rostral gland' which secretes the peculiar attachment apparatus of the later (chalimus) stages.

The *second antennae* arise somewhat ventrally in a shallow depression on the anterior lateral margins; the basal joints narrow abruptly at their insertion, and there is a short anterior spine on the broad distal end of this joint which gives rise to a four-jointed exopodite and a three-jointed endopodite. The exopodite is more slender and the first joint is about one and a half times longer than the other three together, each of which bears a long seta—four in all. The endopodite is much stouter, and the first two joints are about equal; the first bears a short anterior spine, and the end joint is short and stout with two long setae.

The rami of the second and third appendages have a considerable degree of freedom: Fig. 1 is a camera lucida drawing showing the position of the rami of the second appendage on the right side interchanged; this appendage has a marked tendency to be displaced ventralwards, and the endopodite on the left side is seen in perspective.

The third pair of appendages is similar to the second and the rami bear four and two setae respectively. The proportions of the segments differ slightly, the first joint of the exopodite being shorter and about equal to the remaining three, and the endopodite has a long basal joint and two short ones following it. At the base of the third appendage is what appears to be an excretory gland: a pear-shaped granular mass which stains deeply with haematoxylin and has a non-staining lumen which seems to open on to the basal segment of

this limb on its anterior side. In some specimens some indication of a similar aperture could be made out on the base of the second antenna, but no underlying gland was visible.

In his account of the developmental stages of *Thersitina gasterostei*, Gurney (1913, p. 422) states that the maxillary gland was present in the third nauplius (the antennary gland was present in the second nauplius), but even in the later nauplius stages of this copepod he was unable to make out the second maxilla. Owing to the telescoping of the early stages of *Lernaeocera*—a single copepodid following a single nauplius stage—it may be that the antennary gland, which is the normal excretory gland of the copepod nauplius, has only a transitory existence and that it is soon replaced by the maxillary gland of the later stages, this being present even in the nauplius in which there is no second maxilla as yet developed on which it can open. This would mean that at this stage external development is lagging behind internal development. The gland is apparently functional, since it has a clearly defined lumen, and it discharges through a specially formed aperture on the base of the third appendage (the mandible), which in this sense has the functional significance of a second maxilla. Unfortunately, it was not possible to make out the further development of this gland[1] in the copepodid stage, and its aperture has not yet been seen in any of the later stages of this species. When more material becomes available it will be possible to settle this question by serial sections and dissection.

The Free-swimming Copepodid

Large numbers of free-swimming copepodids (Fig. 2*a*) were obtained from nauplii moulting in plunger jars in the laboratory, and others exactly similar were taken from time to time throughout the year on the gill-tips of the flounders caught near the mouths of the estuaries at Plymouth. This stage shows a remarkable variation in size, the length varying from 0·385 to 0·633 mm. with an average of 0·484 mm.; but only three individuals were longer than 0·55 mm., and these will be referred to later when the rate of growth is considered. In spite of the difference in size there was no difference in form of these copepodids, so that whether there is a moult or not in the copepodid stage, it is not thought that more than one stage form can be represented.

The cephalothorax is about five-eighths of the body length and has a strong equatorial line of segmentation behind the second maxillae, and other intermediate folds are present which hint at segmental boundaries. The cephalothorax in all the developmental forms shows a clear ventral infolding along the lateral margins, particularly in the anterior half, as shown in the figures. The mouth-parts in the early stages are not easy to see and they have been omitted from many of the figures. The first antennae are indistinctly five-articled and

[1] Colonel R. B. S. Sewell, with whom I discussed this matter, made the interesting suggestion that it is more likely to represent the primitive segmental organ of the mandibular somite which has become the functional excretory organ in this type.

are abundantly beset with fine setae, the terminal joint bearing four stronger setae; the second joint is the longest, though this is not obvious, especially in the later stages when the first antenna has a tendency to be bent ventrally and laterally along the fold of the cephalothorax, so that in many of the figures it is seen in perspective. These appendages arise immediately in front of the strong chitinized bars which run forward from the anterior lateral angles of the cephalothorax, bifurcate in front, and support the wide basal joints of the chelate second antennae. The second antennae therefore arise only slightly ventrally to the anterior margin of the cephalothorax; they are the main prehensile organs and have a remarkable degree of freedom—rather more than 180°—for at this stage the rectangular rostrum has not yet developed to restrict some slight movement dorsally. Their independent movement in the vertical plane has already been referred to when they crawl over a solid substratum. On finding a gill-tip of a flounder the chelae take a firm hold and this is seldom relinquished, so that it becomes the anchorage of the animal until the adult free-swimming stage (VII) is reached. Owing to the violent currents in the gill chamber the copepod is twisted and twirled on its support; sometimes it is washed off, but though it swims for a time it will soon take a fresh hold. Such currents were imitated for the purpose of observation by removing the gills of flounders to watch-glasses of sea water under the microscope and a one-way current provided by a pipette. The specimen, of which Fig. 2*a* is a camera lucida drawing, was fixed in situ and shows the left chela turned through 180° on its own axis, a compensatory movement just before the animal lost its hold. The gill filaments were grasped by the now well-formed second maxillae, so that the mouth-parts were kept in close contact with the tissues on which they browse, in spite of currents.

Two pairs of well-formed biramous swimming legs are present, the first on the last thoracic segment fused in the cephalothorax and the second on the first free thoracic segment. The second free segment is of characteristic shape and bears two long stout spines on its posterior lateral corners which represent the third pair of legs; the third free segment is also characteristic in shape and represents the pregenital and genital segments; the fourth is the abdomen and bears two relatively large caudal laminae each with five long setae. The exopodite and endopodite of the first pair of legs are two-jointed and the first joint is produced into a fine spine; the distal joint of the exopodite in both limbs bears an outer spine and five long setae, but the endopodite in both bears five long setae only. In the second pair of legs the rami are as yet only one-jointed, but the short spine between the joints of the adult limb is already formed.

The First Chalimus Stage and the Suspensory Mechanism

At the time of moulting from the copepodid a chitinous secretion appears to come from the mid-frontal region and to be extruded as a laterally flattened thread between the now closely clinging second antennae into the surrounding

gill tissue. It penetrates the latter in two diverging filaments—apparently through the perforations made by the claws. The rest of the secretion falls dorsally and laterally forming the hood which is at first attached to the newly formed cephalothorax, enclosing in it the new second antennae and the distal joint of the old pair which remains attached to the gill, inside the hood, and embedded in the chitinous mass. Ventrally the secretion falls as a pear-shaped mass to the level of the chelae of the new antennae.

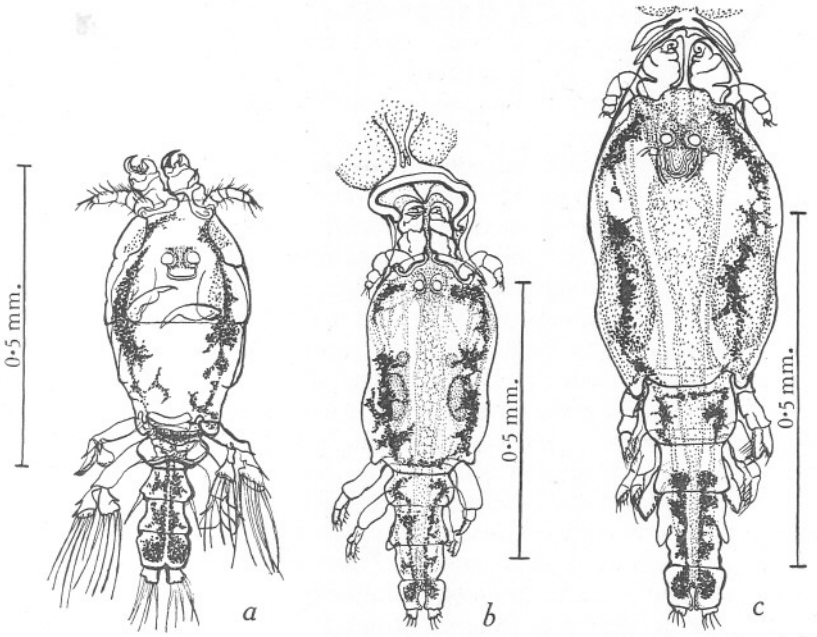

Fig. 2. *a*, Free-swimming copepodid (stage II)—ventral aspect of female. *b*, first chalimus (stage III)—dorsal aspect of male. *c*, second chalimus (stage IV)—dorsal aspect of male.

Soon after this moult has been completed, the movement of the chalimus causes the hood to crack along the transverse line joining it to the dorsal region of the head, and occasionally the dorsal triangular hood section breaks off, but usually the hoods remain attached by their apices to the common filament and the particular stage can be determined by counting these, as Claus has already shown (1868*a*). For example, the fourth chalimus (Fig. 3*b* below) has four hoods, that of the last moult being still attached to the carapace.

Fig. 2*b* shows the first chalimus stage with hood (which was formerly attached to the dorsal region of the copepodid during the moulting period) broken away, but the old chelae are hidden under the chitinous plate: the new hood has broken away except for its lateral edges. The two intrusions of chitin into the gill tissue are seen and in between them is a fine filament—the axial thread of the suspensory apparatus. The ventral pear-shaped masses are best seen in the lateral view of the fourth chalimus stage (Fig. 3*b*).

It seems, therefore, that it is justifiable to refer to the fixed stages of *Lernaeocera* and its allies as 'chalimus stages', since in all essentials they are so similar to the chalimus stages of the Caligidae. Gurney (1934) has given an admirable account of the developmental stages of *Caligus labracis* and *C. centrodonti* and the mode of attachment to their host, but in these as in other Caligidae the arrangement is much simpler, for the sole attachment is by a long filament, secreted once and for all by the first chalimus at the time of moulting from the copepodid. It suspends the animal well away from the host tissues and none of the appendages are involved as they are in *Lernaeocera*; the only addition at each succeeding moult is a small bulb cementing the new frontal region on to the last bulb, and the number of bulbs is an indication of the stage.

A curious intermediate condition is found in *Pennella varians* as illustrated by Wierzejski (1877, pls. 32, 33). These larvae were found on cephalopods and they are very similar, except in the shape of the forepart of the cephalothorax and the wider spread of the free thoracic region and limbs, to the parallel forms of *Lernaeocera*. The strong chelate second antennae begin by suspending the copepodid; but on its moulting they appear to lose hold of the tissues and the secretion alone suspends the first chalimus, leaving a small knob at the top of the conical chitinous funnel, which is much narrower than in *Lernaeocera* and does not include the second antennae but is well anterior and dorsal to them. A bulb of secretion is formed at each moult, but no succession of hoods is left as in *Lernaeocera*. Dedifferentiation of the chelae and other appendages and their loss of segmentation is seen in these chalimus stages as in the present material, but whereas this is at its maximum in the second and third chalimus stages in *Lernaeocera*, it begins earlier in *Pennella*—the first and second stages showing maximum dedifferentiation. Similarly, in *Sarcotretes scopeli*, which is probably a near relative of *Pennella*, Jungersen (1913) has shown four chalimus stages which all appear more retrograde than any stages of the other two genera; the second antennae are more ventrally placed in the copepodid and remain ventral and rather feeble until the free pelagic stage which follows the fourth chalimus. In this species there seems to be no question of the involvement of the anterior appendages in the suspension filament, and the arrangement is somewhere between that of *Pennella* and *Caligus*, differing from the latter in that the filament appears to arise from a broader base as in *Pennella* and so appears as a conical thread; but unlike either no clearly formed bulbs are seen, and certainly no succession of hoods as in *Lernaeocera*. Jungersen admits to using caustic potash (KOH) for the removal of these forms from the fins of *Scopelus glacialis*. This drastic treatment may have dissolved some of the more delicate secretion if this is not pure chitin, for he says: 'In all the present pupal stages I find the structure [of the filament] to be identical.'

To return to the structure of the first chalimus of *Lernaeocera* the most striking change is in the loss of the long-swimming setae on the two biramous legs, the change in shape of the free thoracic segments, and the appearance of

a stump in the place of the long spine representing the third leg. The anal laminae are relatively smaller and their setae are also short and poorly developed: only four of them could be made out in this and the following stages. The rami of the limbs have lost their fine structure, though those of the second leg are now two-jointed. No new segment has been added to the thorax.

The Second Chalimus: Stage IV

There has been little change in general shape (Fig. 2*c*) since the last moult, but dedifferentiation has increased. The second antennae are swollen masses with their segmentation shown only by folds, and in the legs it is becoming more indistinct, that of the third leg being barely discernible, though this shows a notched inner margin with vestigial setae. There is an indication of the third free segment dividing into the pregenital and genital segments, and the former shows the rudimentary stump of the fourth pair of legs. In the specimen depicted the dorsal part of the hoods has been broken off, but the axial filament is well shown arising below the bases of the antennae and passing up between them. The mouth tube has not joined up and the mandibles, which are relatively long at this stage, are seen at the sides; in the next stage they are seen within the mouth tube which is in the process of being formed. The second maxillae and maxillipedes have been omitted from these figures to avoid confusion. All the chalimus stages are illustrated by males, though there is only a very slight difference in the shape of the hind body in the female and she is always larger (see Fig. 6 A).

The Third Chalimus: Stage V

The general shape of this stage (Fig. 3*a*) is similar to the last, but some of the appendages are beginning to redifferentiate, though the second antennae appear to be more regressive than in the preceding stage and their segmentation is only indicated by notches; the distal ends of the tips of this appendage belonging to previous moults are seen below each hood—the suspensory apparatus is shown in optical section, the dorsal parts being omitted for clarity. The free segments of the thorax are less clearly marked; but there is an indication of the presence of five, and this is confirmed in the next stage (Fig. 3*b*) in which the genital segment of the adult is clearly shown to be composed of two segments. (This has already been formulated for the Caligidae by Gurney (1934).) Fig. 3*a* shows the emergence of the fourth pair of legs, which are clearly two-jointed at this stage though the small distal blade-like joint has no setae.

The Fourth Chalimus: Stage VI

There is again little change in shape (Fig. 3*b*), but the segmentation of all parts is better defined than in the previous chalimus stage; the limbs, though not functional, are beginning to resume their characteristic form and the setae

are slightly stronger, as are also those on the caudal laminae and first antennae. The second antennae have again recognizably chelate ends. The mouth tube is nearly closed and the first maxillae can be seen at its sides, though its bipartite structure is not apparent at this stage. The second maxillae are both shown,

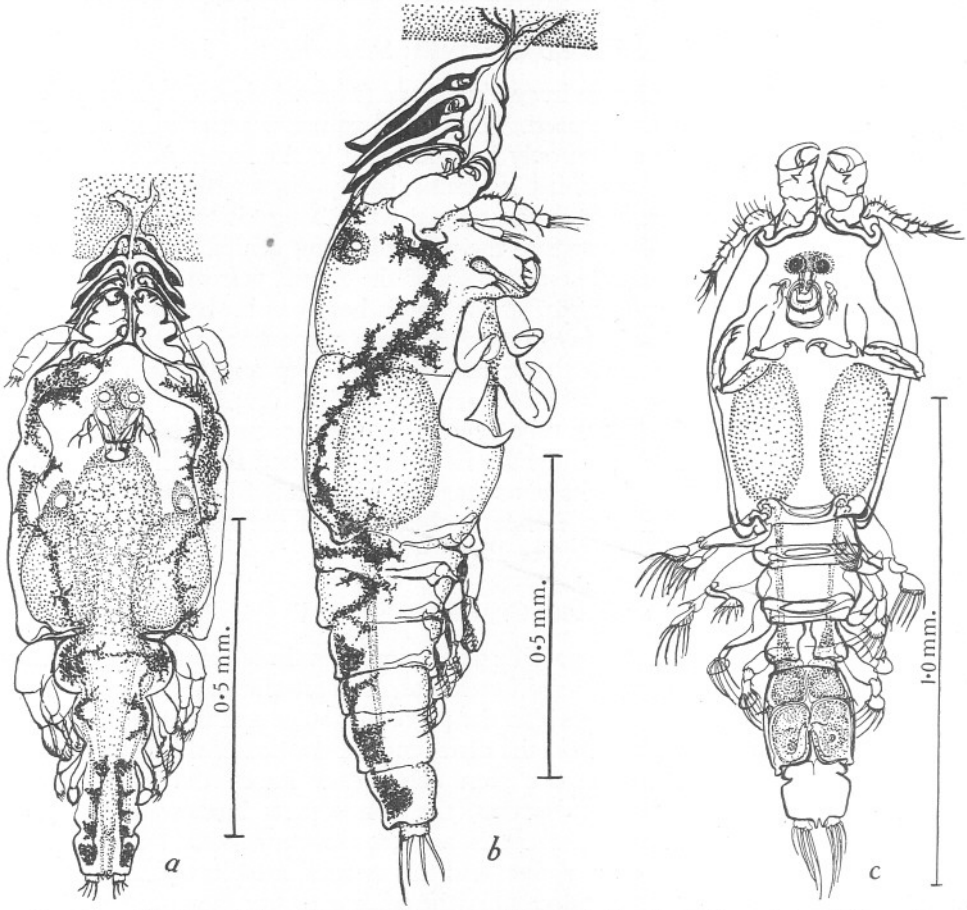

Fig. 3. *a*, Chalimus (stage V)—ventral aspect of male. *b*, fourth chalimus (stage VI)—male from the right side. *c*, adult free-swimming male (stage VII)—ventral aspect, the pigmentation has been omitted.

the distal joints reflexed on the middle ones; they are still rather swollen and lack the characteristic shape of the adult (and copepodid stage). The maxillipedes are relatively even more swollen and are quite unlike the long slender limbs they become at the next moult; indeed, they resemble the adult second maxillae in general outline. They bear no spines or claws.

The Adult Male: Stage VII

There has been a marked change in shape (Fig. 3*c*) at this moult, and the body form of the copepodid has returned: the setae on the swimming legs are not quite so long, but in fine structure they resemble those of the early stage very closely. The two additional pairs of legs are uniramous and clearly three-jointed; they bear four long setae and a shorter one. Each ramus of the first two pairs of legs bears five long setae and a shorter spine on the outer side, and as foreshadowed in the copepodid each ramus is two-jointed.

The *genital segment* is particularly interesting in the male, for, as mentioned above, its two component segments were indicated quite clearly in the last chalimus stage: they have now coalesced into a barrel-shaped segment containing the spermatophore sacs which open near the median line in two spout-like tubes whose function is not quite clear. It is difficult to make out whether these are the internal openings (? communications with the vas deferens), for they are probably not the external openings for the discharge of the large spermatophores, which Scott holds are beneath the posterior corners of the genital segment. The heavy pigmentation in this region makes it difficult to see the relationships of these structures clearly. The characteristic disposition of the chromatophores has been shown in all the preceding figures, but since the intense dark pigmentation is so heavy in the male, it has been omitted from this figure. That the genital segment does represent two segments is shown in this species almost as well as in the male of *Caligus diaphanus* as figured by Gurney (1934, fig. 17); it is a double segment bearing the vestiges of legs 5 and 6. In the male of *Lernaeocera* the vestige of leg 5 is not easy to see in all specimens, but in some a small spine can be seen arising from a minute boss beneath the cuticle, though sometimes the spine does not reach the surface. Leg 6 is represented by the small indented knob on the ventral side of the posterior corners of the genital segment, but I have not been able to see a seta on this in any of my specimens. Immediately below the genital segment is a short segment preceding the terminal trapezoidal abdominal segment to which it evidently belongs. The anus is terminal and there are two small triangular processes immediately ventral to it; the setae on the anal laminae are four in number, the two innermost are the longest and the two outer, and slightly dorsal, are much finer and shorter.

The first antennae are five-partite as in the copepodid stage and the second joint is longer than the rest, all being richly supplied with short setae; the terminal joint bears four stronger setae and a small curved one. The second antennae are very similar to those in the copepodid, though their relative size is slightly greater and they are very strongly chitinized; they are three-jointed and the terminal joint bears a strong short outwardly directed spine at about the middle of its width on the ventral side.

The mouth-parts. The mouth tube and its immediate appendages do not differ in the two sexes, and they are shown in Fig. 4*a*. It is difficult to state

with certainty the exact morphological entities composing the mouth tube, for though it is usually stated that it is formed by the fusion of the upper and lower lips, such an explanation seems too facile. Gurney (1930, fig. 2) figures the mouth tube of the copepodid larva of *Nicothoë astaci* in side view: the lateral suture can be seen, through which the 'masticatory process' of the mandible has already entered the closed tube, while the basal joint of the protopodite remains outside, slightly lateral and anterior to the base of the first maxilla. This lateral suture can be seen in the fourth chalimus stage of *Lernaeocera* (Fig. 3*b*). In Gurney's fig. 4, of the mouth-parts of the adult female *Nicothoë*, a U-shaped chitinous thickening can be seen projecting posteriorly from the dorsal edge of the tube; a similar heavily chitinized rectangular bar is present in *Lernaeocera* (Fig. 4*a*), which seems to be identical with what was identified as the upper lip in the earlier stages (Figs. 2*a*, 2*c* and 3*a*). In the adult it can be traced forwards and inwards, where it bifurcates, the anterior branch passing directly outwards, apparently joining the base of the first maxilla, and the other passing backwards dorsal to the mouth tube and within it forming the lateral borders of the mouth itself, continuing as a flattened concave rim round the lower edge of the mouth aperture to join its counterpart on the other side. The upper border is not similarly fortified, but seems to be flexibly opposable on to the former by virtue of the oblique sheets of muscle fibres in the walls of the mouth, which are attached distally round the orifice. This inner chitinous structure I take to be part, at least, of the lower lip (labium), though it is relatively smaller than this structure in the earlier stages (shown in Fig. 2*c*).

That the labrum and labium are separate entities from the mouth-tube proper, is indicated by Wierzejski (1877) in his figure of these structures side by side in the third chalimus stage of *Pennella varians*. He shows (pl. 33, fig. 16) the tube itself as complete and, apparently separate from it, the labrum and bipartite labium within it. The mandibles are also enclosed in the tube at this stage as they are (for the first time) in *Lernaeocera*. In the mouth tube of the adult *Pennella varians* (pl. 33, fig. 17) the lips are not indicated, nor are they included in any of Brian's figures of the developmental stages of *P. sagitta* (1929). That the mouth tube is more likely to be composed of more than one modified sternite and therefore involves more than the 'paragnaths'—the labrum and labium—is suggested by its enormous development in the mature adult *Lernaeocera* (the figures of which, reproduced in Stekhoven's paper (1936*a*), so nearly agree with my own that they will not be repeated here). Stekhoven's figs. 3*c* and 4 on pl. 2 show the first maxillae carried more than half-way up the oral cone in *L. branchialis*, as do also those of Brian (1929, pl. 5, fig. 9) for *Pennella sagitta*.

If the mouth tube were formed largely from anterior and posterior moieties, its plane of symmetry would be transverse, whereas it is clearly vertical throughout in the adult (Fig. 4*a*). Three pairs of strongly chitinized half-hoops support the structure, which is composed of a thinner membrane with apparent lacunae (or even thinner areas of chitin) along the posterior axis; at

least this is so in the adult stages on the flounder (stage VII), for in the mature female, after the final moult on the gadoid host, the mouth tube appears more evenly and far more heavily chitinized. The distal rim of the tube is a narrow membrane edged with minute prickle-like processes. In Wierzejski's figures, referred to above, the three pairs of chitinous bands are seen in an earlier stage of their development in *P. varians* as small projecting lobes within the tube, but not yet applied to its walls. Their late development in this species and their arrangement strengthens my suspicion that part of the mouth tube, at least,

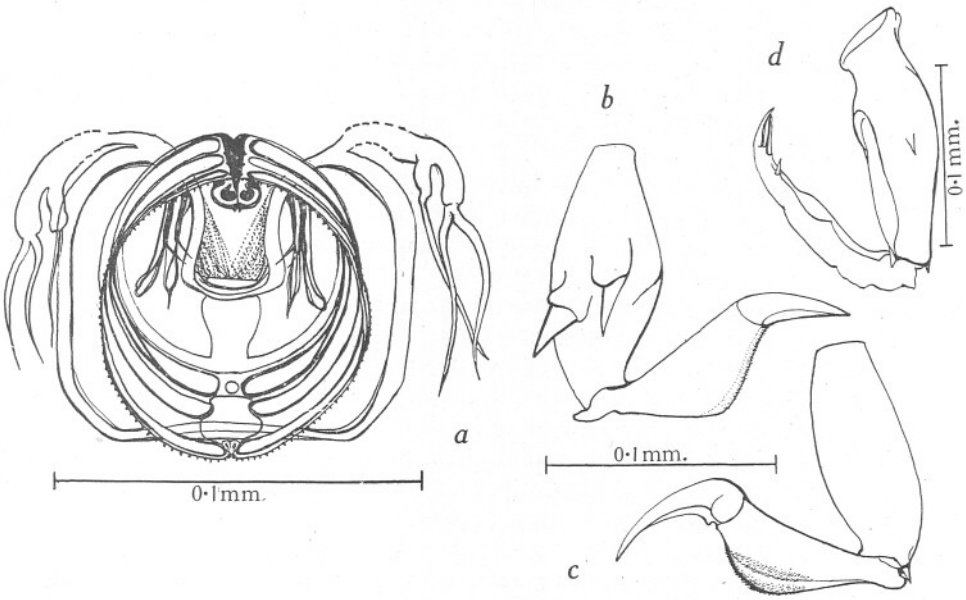

Fig. 4. *a*, Mouth tube in situ, showing first maxillae outside and the bipartite mandibles inside: a composite drawing from stage VII specimens. *b*, right second maxilla of female. *c*, left second maxilla of male. *d*, left maxilliped of male; all from the ventral aspect (stage VII).

represents the highly modified body of the mandibles. 'The masticatory process' of the mandible, which alone is said to be developed in parasitic copepods, is in *Lernaeocera* fairly well developed, but it is very different from that of the Caligidae (and Lernaeopodidae), in which it is a single lancet with an inner dentigerous margin. In the third chalimus stage of *Lernaeocera* (Fig. 3*a*) the mandibles arise from the same origin as the incipient mouth tube, and comprise a stout basal joint with two fine lancet-like setae at its tip. In the adult the basal joint is only seen with difficulty at the bottom of the anterior part of the mouth tube, but the bipartite distal portions are more easily seen as they reach about half-way up the tube. Both rami are slightly concave and about half-way up give off a long sharp spine directed inwards, above which they are sheath-like; the inner ramus ends in a sharp point formed by the rolled-in

edges of the sheath. The outer ramus is an open sheath, and its thickened edges sometimes appear to project as two terminal spines owing to the tip being bent back. I am inclined to think that it was this appearance which led Leigh-Sharpe (1930, p. 336, fig. 5 *Mn*) to depict the mandible of *Lernaea barbicola* (mature female) as a bluntly rounded limb with two short spines: for at certain foci I have obtained almost exactly this view of the outer ramus in *Lernaeocera*. There has been much conflicting description of the mandibles of this group of copepods: Wilson (1917, p. 81), in stating the subfamily characters of Lernaeocerinae (female), says 'the mandibles are unknown', and regarding those of the male, 'antennae and mouth-parts as in the female'. Whereas in dealing with the external morphology of the entire family (p. 24): 'The mandibles are simple curved claws or spines, one-jointed and entirely devoid of teeth'; and in describing *L. branchialis* ('copepodid male') he states 'mandibles in the form of a long and slender spine'. This must have been copied from some previous author, for he does not include males in his material. Even Claus (1868*b*) figures the mandibles, rather indistinctly, as a pair of double broad spines attached to the sides of the mouth tube and within it (which is in no serious disagreement with my own figure).

A. Scott (1901, p. 37) seems to have mistaken the first maxillae for mandibles, for he says they are not enclosed in the mouth tube, though he goes on to say that they are situated at the base of the lateral surfaces of the conical tube of the mouth and consist of two parts, the basal joint being cylindrical and the second joint flattened and terminating in a broad blade, which is serrated on the inner margin. I have seen nothing like this in any of my specimens, nor do Scott's figures support this description. From his figures it appears as one of the broad setae of the first maxilla. Stekhoven neither describes nor figures them; he admits that they are exceedingly difficult to see.

The *first maxilla* (Fig. 4*a*) is in two parts, an inner short joint gradually tapering into a long seta, and an outer joint also tapering off, this time into two long setae. In the chalimus stages the outer joint is relatively larger and stouter (Fig. 3*a*).

The *second maxilla* is clearly three-jointed, and it is particularly interesting in that it is different in the two sexes: that of the female (Fig. 4*b*) bears two very stout spines on the ventral side of the first joint, this also showing a faint transverse fold so that it may in reality be composed of two or three joints. No spines or indication of subdivision into more joints are found in the male appendage (Fig. 4*c*), though a small spine is present on the outer corner of its distal end. The penultimate joint in both sexes is somewhat triangular, and on the outer edge there is a shallow groove, the distal half of which is thickly beset with minute spines—these are rather more extensive in the male than in the female. The end joint is clawed, and in the male it is rather more curved. In the adult (stage VII) of both sexes these last two joints appear to be immovably articulated, and sometimes the suture is difficult to see; this is also true for the copepodid (Fig. 2*a*), but in the intervening chalimus stages

the joints lose their characteristic shape and they are flexed on one another (Fig. 3*b*).

The second maxillae, like the first, seem to be remarkably uniform in related genera: the above description agrees almost exactly with the figures of Wierzejski (1877, pls. 32, 33) of *Pennella varians*, where the two spines are shown on the proximal joint of the second maxilla of the female alone. The appendages of Brian's specimens of *P. filosa* (1912, pp. 16–18, pls. 3, 6) and of *P. sagitta* (1929, loc. cit.) are also in agreement in all but minute details.[1]

The *maxillipedes* are only present in the males, and I have not been able to make them out before the fourth chalimus stage; those of the adult male are shown in Fig. 4*d*. They are comparatively long and thin and superficially two-jointed. The proximal joint is the more robust, and like that of the second maxillae may be, in reality, composed of two or more joints, for there is a spine, directed slightly outwards, about the middle of its ventral face; there is also a short spine on its outer angle distally. On the inner face of this joint there is a long concavity and a corresponding one on the inner face of the distal joint which is about the same length. It is thought that this groove has a function in connexion with insemination, and the use of this pair of limbs during copulation is referred to below. There are a number of slight folds along the margin of the distal joint which may indicate its component segments, but they are indistinct. About a quarter of its length from the tip the concavity of the limb ceases, and at this point there is a small spine directed inwards; three longer spines occur at the end—two straight ones subterminally and a curved one terminally, all three being closely opposed.

The Adult Female: Stage VII

Though her own gonads are not yet mature, the female at this stage (Fig. 5*a*) has attained, superficially, the same degree of structural development as the sexually mature male; it is the stage in which copulation takes place, and it is therefore justifiable to call her 'adult'. The ensuing metamorphosis involves superficial dedifferentiation, for the second time, and the final assumption of an entirely different form several times larger than the present one. The cephalothorax is slightly larger than that of the male and is oval, the maximum width being equatorial rather than anterior to the equator as in the male. The appendages are all similar to those of the male with the exceptions already mentioned, and in addition the swimming legs have slightly longer setae and their

[1] It therefore appears that Leigh-Sharpe's figure (1935, p. 108, fig. 1) labelled as the antennule of *Saucissona sauciatonis* is the second maxilla. It shows a three-jointed appendage ending in a blunt claw and having one blunt spine on the basal segment. Apart from the likeness of this figure to a second maxilla of *Lernaeocera* and its allies, its resemblance to the first antenna of any copepod known to me is remote. Leigh-Sharpe (1935) created a new genus for this species (represented by a single immature female) which he considers to be congeneric with *Lernaeocera lumpi* (T. Scott, 1901). Scott's species was described from a single mature female from the gills of *Cyclopterus lumpus*. The separation of the new genus rests on the absence of the familiar flexures in the body of the mature females.

basal joints are longer and more slender. The distribution of setae differs slightly from that in the male and copepodid stage and is as follows: leg 1, 7+spine, 6+spine; leg 2, 6+spine, 6; leg 3, 6+spine; leg 4, 5 (in legs 1 and 2 the exopod is cited first).

The thoracic segments have lost the characteristic shape seen in the cope-

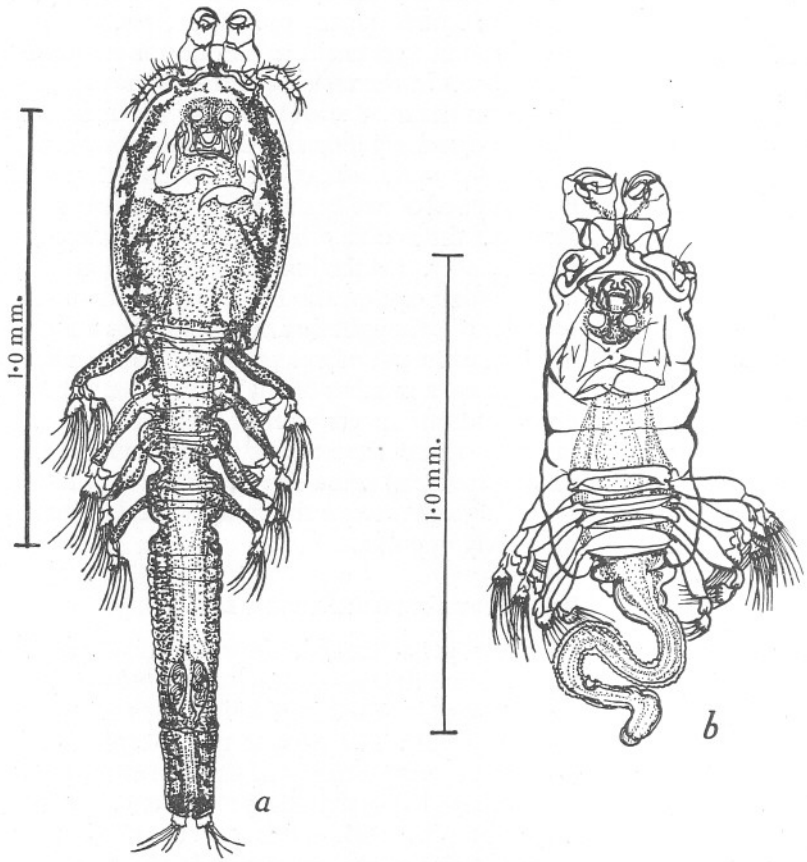

Fig. 5. *a*, Adult female (stage VII)—dorsal aspect, showing spermatophores within the hind body which has not yet expanded to its full length for this stage. *b*, adult metamorphosing female from the gill region of the gadoid host (the first antennae have broken off)—ventral aspect.

podid and adult male, and they are rounded in outline except the pregenital which is slightly quadrangular. The genital segment is without the vestigial appendages of somites VI and VII which were found in the male: it is enormously elongated with a crenulated outline which indicates the region of imminent expansion. Copulation takes place immediately the male comes along, even though the female has barely finished moulting, and she be still

attached to the gill in her original position with the chitinous attachment apparatus adhering to the mid-subrostral region. As soon as copulation is over the genital segment elongates very rapidly until it is slightly longer than the rest of the body. By this time the female has let go her hold on the tissues of the flounder and has begun to swim actively; but she does not make much progress, for she is not a good swimmer and relies mainly on currents to waft her out of the gill cavity of the flounder and up to the water inhabited by her next host—a suitable gadoid. From time to time she will use her anteriorly projecting second antennae to rest on the gills of the flounder before quitting them for a pelagic life. The rostrum is rather large and rectangular in the adult and prevents any dorsal movement of the second antennae, which are held rigidly forwards.

During copulation the male first grasps the genital segment of the female, about a third of its length from the proximal end, by means of his chelate second antennae: so firm is his grip on the still soft cuticle of this region that a papilla is raised and some injury done, for when the male retires the papilla persists for a time but finally subsides when the cuticle is stretched in elongation. Specimens fixed at this time and stained show an area of penetration of the dye around the papilla, elsewhere the cuticle is impervious to all but the fiercest stains. Insemination is effected by the male applying himself parallel to the body of the female on her ventral side, which he does with the further aid of the maxillipedes. His body is then flexed vigorously back and forth several times, the spermatophores suddenly appear and are shed opposite to the vulva, where partly by pressure of his body and by the manipulation with the maxillipedes which have now released their hold (the flexed body tearing unmercifully on the cuticle of the female), the spermatophores are introduced into the vulva where they remain intact. I have not been able to see them discharge or migrate higher up the oviducts. The external opening of the vulva is a transverse elliptical fold, but just inside it are two small apertures leading to the oviducts.

The segmentation of the abdomen is not clear; it has several transverse striations and is, like the limbs and thorax, very heavily pigmented. The anlage of the ovaries can be seen indistinctly in the hinder region of the cephalothorax, and the descending oviducts can also be made out. Cement glands appear to be situated in the lateral fields of the genital segment, but details are obscured by pigmentation.

The Adult Female: Stage VIII

I have been unable to find A. Scott's 'pennella stage'—the fertilized female with the long hypertrophied, though straight, genital segment—on the gills of gadoids (Fig. 5*b*). Though Scott found only a single specimen (on the gills of *Gadus merlangus*), Stekhoven has been fortunate in finding what he considers to be a complete series on the gills of the cod (Stekhoven & Punt, 1937). These authors do not figure the specimens showing the formation of the antlers, but

they agree with A. Scott (1901) that the latter are produced, and the body elongates (to about eight times the length of the cephalothorax) before flexure begins.

There is, however, some variation in this stage. Stekhoven found one specimen in 1936 of length 13 mm. and a cephalothorax to hind-body ratio of 1 : 8·0; the genital segment had a right-angled flexure and antlers were present. Whereas another found by him on 13 May 1937 had a length of 14 mm. and a ratio of 1 : 8·85, yet the body was straight and there were no antlers. I also found a specimen of total length 14 mm., on 14 November 1940 on *G. pollachius*, in which there was a right-angled flexure in the genital segment, and the second flexure had progressed some 30°; also, not only were antlers present but they had each dichotomized. All specimens found by me on *G. pollachius* and *G. merlangus*, with one exception, have had antlers; and in all the flexure of the body had already begun. There seems to be a moult immediately the impregnated female settles herself on the gadoid, and there may be another later, but the specimen in Fig. 5*b* is, I feel sure, the stage immediately following the pelagic female. I have found only one, on 29 April 1940, situated in the usual position in the anterior angle of the fourth gill-arch of *G. pollachius*. It was hanging on to the tissues surrounding the blood vessels at the base of the gill filaments by means of the second antennae. It was dissected away without damage (except for the loss of the distal segments of the second antennae), and I think it was probably dead, though there were no signs of decomposition. It was fixed immediately, without pressure, in Bouin Duboscq, and no apparent shrinkage occurred. I am inclined to think that moulting had just occurred, as the chitin was very soft and the gut appeared empty; it thus seems that the parasite had probably just arrived on the gadoid after its pelagic (starving) phase and had not yet had its first meal of gadoid blood. It is possible, of course, that this is the final moult and that growth by swelling, on imbibition of gadoid blood, was just about to take place; at all events, the most striking feature of this specimen is the typically flexed genital segment of Stekhoven's later stages, yet the antlers have not yet formed.

Though the cephalothorax is no longer than that of the larger females of stage VII, it is of a different shape, narrower and with parallel sides, and all the leg-bearing segments have become incorporated in it. There are transverse folds across the soft fore-body, and the four radiating folds beneath the nerve mass appear to be the beginning of the dorsal antler, though nothing is visible of the lateral antlers. The first antennae have broken off and their basal joints alone remain. The mouth tube was rather larger than previously and became bent forwards during the mounting of the specimen, and the upper lip is seen above it. The genital segment was very soft and its cuticle more finely crenulated than in stage VII; the opening of the oviduct through which the egg-strings will emerge later is clearly seen at its distal end. It is remarkable to find, at this early stage, that the four right-angled flexures of the mature female are completed. The existence of this specimen is a demonstration of the extreme

variability in the order of development of the maturing stages of the female of *Lernaeocera branchialis*. It may be mentioned that the anus appears to be closed in this specimen: the abdomen ends in two lateral flaps, but the lumen of the rectum does not persist to the extremity in mature specimens. The significance of this has been examined at length in a previous paper (Sproston & Hartley, 1941*b*).

The Rate of Growth

The measurements of over a hundred specimens are represented in graphical form in Fig. 6 A, B. Wherever possible the sexes have been distinguished, but doubtful individuals are shown unsexed. The arithmetic means of the total lengths are indicated for each sex, and the mean of all the specimens at each stage is also shown by a short horizontal line. It is curious that my measurements in all instances should be less than those of Claus (1868 *a*, *b*); but one reason for this is that in expressing the total length of the body I have omitted the projecting second antennae, and where present, the attachment apparatus and rostrum. The anterior limit of the body proper I have taken to be the transverse line across the front of the cephalothorax marked by the bifid ends of the chitinous bars supporting the second antennae. The posterior limit of the cephalothorax is taken at the distal edge of the hindermost coupler of the first pair of swimming legs (except in the specimen in Fig. 5*b*). The hind end of the body is measured to the tips of the anal laminae, excluding their setae.

In order to test the applicability of Przibram's (1931) law to the growth of the larval stages of *Lernaeocera*, the length of the cephalothorax was chosen as the most convenient part of the body for measurement in all the stages II–VIII; but when it was compared with the total length of the body in all the specimens, the ratios were found to be highly variable. The high degree of scatter of the values for the cephalothorax can be seen in the graph (Fig. 6 B), and the standard deviations of these measurements are given in Table I for comparison

Table I. Measurements of the Developmental Stages of *Lernaeocera branchialis*

Stage	Mean length of body mm.		Standard deviation from the mean		Mean length of cephalothorax mm.		Standard deviation from the mean	
I	0·371		0·0145		—		—	
II	0·484		0·0449		0·297		0·0465	
III	0·609		0·0431		0·353		0·0386	
IV	0·732		0·0529		0·426		0·0375	
	♂	♀	♂	♀	♂	♀	♂	♀
V	0·797	0·920	0·0529	0·0183	0·461	0·498	0·1072	0·0302
VI	0·937	1·047	0·0559	0·0277	0·506	0·537	0·0410	0·0683
VII	1·255	1·700 (1·246–1·91)	0·1188	—	0·517	0·619	0·1176	0·0492

with those of the total body length. Owing to the unreliability of the measurements of the cephalothorax, and the fact that it does not show isogonic growth,

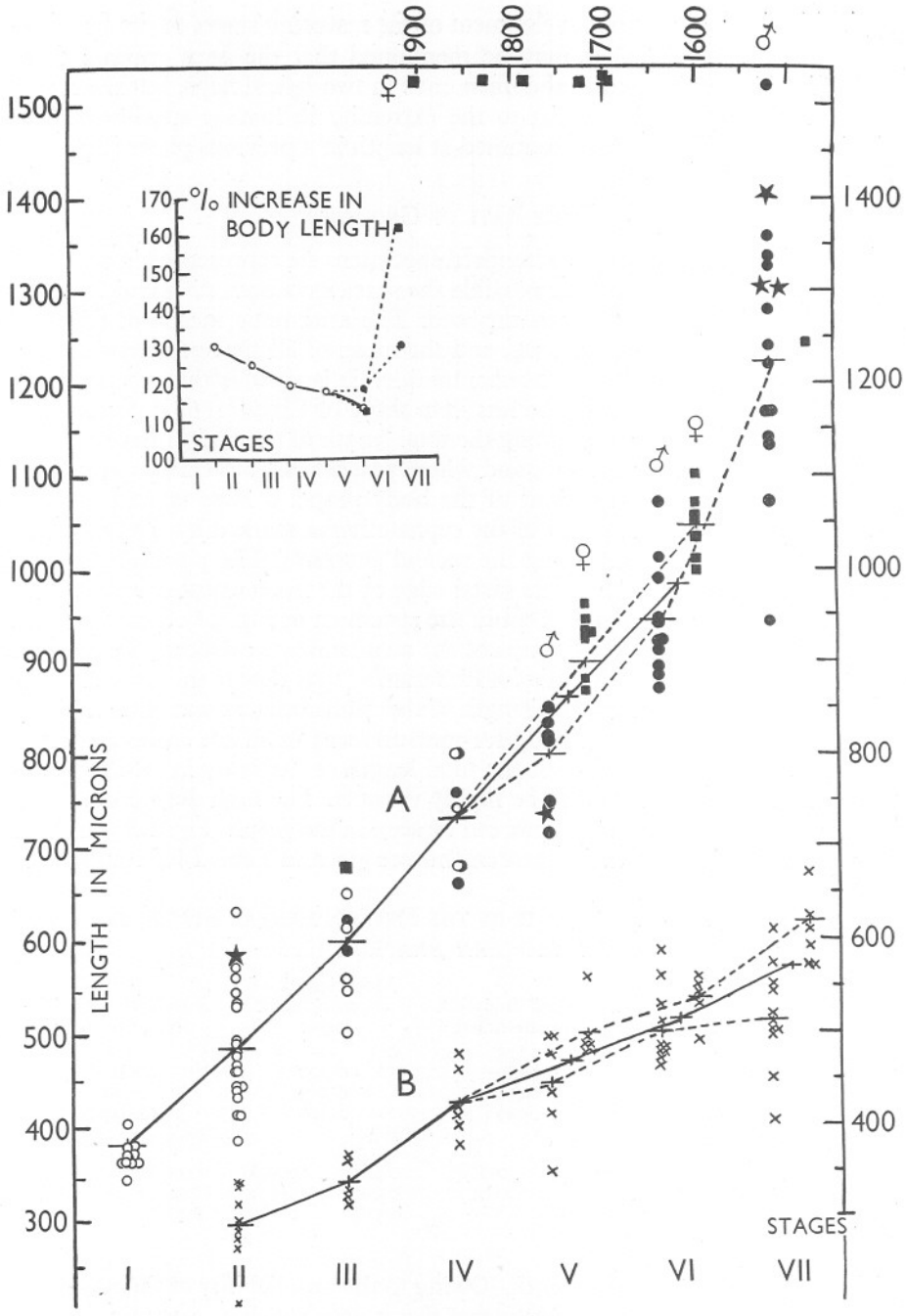
1900
1800
1700
1600
♂
♀
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
LENGTH IN MICRONS
1400
1200
1000
800
600
400
STAGES
I
II
III
IV
V
VI
VII
A
B
% INCREASE IN BODY LENGTH
170
160
150
140
130
120
110
100
STAGES
I II III IV V VI VII

Fig. 6.

the values could not be used in the usual allometric expressions to examine relative growth. Neither could the length of those appendages common to all stages be used, since their measurement in the retrogressive chalimus stages is obviously unreliable.

When the means of the simple lengths of the body, stage by stage, are plotted the result is rather surprising, for instead of obtaining a logarithmic curve—as others have done with the higher Crustacea (Harrison (1940), for example for the caprellid *Pseudoprotella phasma*)—the result is a straight line. The growth approximates to a straight line even when the nauplius is included (stage I) and holds good up to the adult stage VII. There is some irregularity when the sexes are considered separately: the males grow more slowly up to the third chalimus stage, then the curves are parallel until the adult form is reached, and the growth is more rapid. The females grow more quickly than the unsexed earlier stages, and in the final stage on the flounder there is a sudden increase in length of the genital segment, which of course causes the growth curve to bend steeply upwards. In the smallest female shown (1·223 mm. long) the genital segment had only just begun to elongate, and the animal was still attached to the gill tissue by the chitinous apparatus, and though it had only recently moulted fertilization had taken place. However, it is very interesting to note that this specimen is very nearly the same length as the average adult male (see Table I, in which minimum and maximum length are given for stage VII females).

When rate of increase in length is expressed graphically (Fig. 6, inset), it is seen to decrease uniformly up to stages V–VI and then to show a marked increase in both sexes. It is interesting and rather remarkable that this decrease in rate of growth should be so uniform, especially as it is generally assumed that one or more stages are omitted in the early phases of the life history. For instance, there is a marked jump in development, from the structural point of view, between the nauplius and the copepodid with its two pairs of biramous legs and well-developed hind body.

Fresh problems are raised in comparing the rates of growth of presumably related and unrelated copepods. For simplicity, a growth factor has been

Fig. 6. Graphical representation of the growth of the developmental stages of *Lernaeocera*.—A. Shows the total body lengths of the measured series: ordinates represent the lengths in microns and the abscissae the growth stages in seven columns, the sexes being separated into subcolumns V–VII. Unsexed individuals as open circles, males as black circles and females as black squares. The ordinates for stage VII have been continued at right angles along the top of the diagram to include those females in which the hind body had become extended. The arithmetic mean lengths for the total individuals for each stage are shown by a horizontal line and for the separate sexes by a longer line; these values are connected by continuous and dotted lines respectively to show the mean growth-rate. The stars represent the lengths of individuals from hosts other than *Pleuronectes flesus* and are not included in the mean values. B. A similar diagram for the lengths of the cephalothorax: these show a greater variation and a slower growth rate as compared with the body as a whole. Inset: diagram illustrating the rate of increase in body length from stage to stage —see Table II column 1—symbols for sexes as above.

obtained by dividing the length of one stage by the length of the preceding stage and multiplying by 100. In Table II this is shown for *Lernaeocera* (also in Fig. 6, inset), for *Caligus centrodonti* (measurements taken from Gurney, 1934), also for two free-swimming copepods, the fresh-water *Diaptomus castor* (from Gurney, 1940, p. 283, table III) and the marine *Calanus finmarchicus* (from Marshall, 1933, p. 126).

TABLE II. PERCENTAGE INCREASE IN LENGTH OF *LERNAEOCERA BRANCHIALIS* DURING DEVELOPMENT AS COMPARED WITH THAT OF OTHER COPEPODS

Stage	*Lernaeocera branchialis* (Sproston)		*Caligus centrodonti* (Gurney, 1934)		*Diaptomus castor* (Gurney, 1940)		*Calanus finmarchicus* (Marshall, 1933)	
I								
II	131		114		—		—	
III	126		115		134		122	
IV	120		154		130		122	
	118		157		130		119	
V	♂	♀	♂	♀	♂	♀		
	119	114	178	154	120	131	117	
VI							♂	♀
VII	130	162	—	—	124	119	—	112

As with *Lernaeocera*, the rate of increase in length decreases in the free-living forms *Diaptomus* and *Calanus*, and though the growth factors for the sexed forms vary rather markedly, the gradient of the curve is about the same for *Diaptomus* and *Lernaeocera*, and for *Calanus* it is more uniform though flatter. Gurney (1928) found that the growth factors for *Eurytemora velox* decreased rather rapidly from stage to stage as compared with those of *Diaptomus*. On the other hand, the rate of growth in *Caligus centrodonti*—a parasitic form more nearly related to *Lernaeocera*—increases throughout the life cycle. This increase shows two high jumps: the first is between the first and second chalimus stages when there is no discontinuity in morphological development and only a slight alteration in shape. Between the third and fourth chalimus stages in the female there is a very slight drop in the curve, but for the male there is a further increase in growth rate (but only about half the previous increment) and yet there is again no morphological gap between the two stages; and the change of body form is almost negligible. Such a contrast in the growth behaviour in two nearly related forms is difficult to explain.

It is clear from the foregoing that a discontinuity in the growth-factor curve has no necessary correlation with development: it can neither be used as an indication of abnormal moults (Marshall, 1933), nor of the suppression of certain developmental stages in the life history (inverse induction from the *Lernaeocera* results). Gurney (1928) points out that apart from the growth factor changing from moult to moult, there is a marked individual variation in the free-living copepods which he studied; this has since been confirmed by Marshall (1933) in her work on *Calanus finmarchicus*. Gurney (1940, p. 283,

table IV) shows that the growth factor varies irregularly for different parts of the body of individuals of the same sex at the same stage of development, and that these variations are not correlated with the growth factor for the body length, nor this latter with the average of the growth factors for selected parts of the same body.

When the body lengths of *Lernaeocera branchialis* and *Caligus centrodonti* are plotted logarithmically as functions of the growth stages, their difference in behaviour is at once apparent: the curve for *Lernaeocera* is by no means a straight line, but is a curve with a very gentle convexity. That for *Caligus*, on the other hand, is markedly concave—the steep gradient is, however, only developed after the first chalimus stage, and this, incidentally, is the juncture at which accelerated growth is seen in the cephalothorax of *Lernaeocera* (Fig. 6 B).

Size Variation

From Fig. 6 A the high degree of scatter of the lengths of the various stages is apparent, even though the means of these measurements give a uniform growth curve. It is not possible to group the individuals into two or more size classes in the various stages. At the same time the degree of scatter does not increase uniformly along the stage scale (see column 2, table I) if exception be made for the stage VII females—for which the scatter is due to the operation of a continuous variate within the group—the progressive elongation of the genital segment. The differences in size of the individuals belonging to one stage cannot be explained on the basis of seasonal variation. As an example of this apparently capricious variation in the size of the adult males: an exceptionally small specimen (0·944 mm.) was taken from a flounder on 27 December 1939, while from the same fish another male was taken measuring 1·335 mm. The largest male (1·52 mm.) was found during April, but in the same month other small males were found, including one only 1·075 mm. long.

The appearance of abnormally large copepodids from time to time throughout the year on the gills of the flounder may possibly find their explanation in delayed metamorphosis. Nauplii measured at random from different hatchings in the laboratory showed very little variation in size, and the copepodids obtained from them under experimental conditions had a uniformly small size. It is conceivable that the largest copepodid (0·633 mm. long, found on 13 February 1940) was one that had moulted without metamorphosing, owing to a prolonged pelagic existence in search of a suitable host. Some little support is given to this suggestion by Gurney & Lebour (1941), who found giant larvae of littoral decapods in oceanic plankton, and hint that metamorphosis is delayed owing to the unsuitable habitat: the larvae have got lost, as it were, and when they moult they do so only to emerge as larger forms of the preceding stage.

Whether such giant copepodids give rise to abnormally large chalimids is unknown, though this may be the explanation of the large forms among the

later stages in my material (see Fig. 6 A). Attractive as this hypothesis is, it makes the explanation of the abnormally small forms all the more difficult.

Larvae resembling *Lernaeocera* from other Hosts

During a visit to Roscoff in September 1938 I found a copepodid and a third chalimus stage which in every way resembled *Lernaeocera branchialis*, except that their size was rather wide of the average for these stages (the specimens are marked by stars on Fig. 6 A). I hesitate to identify them with this species because they were found on the gills of *Solea solea* (L). Since then (Sproston & Hartley, 1941 *a*), numbers of pleuronectids (other than *Pleuronectes flesus* and including *Solea solea*) have been examined carefully for larvae in those localities where *Lernacocera branchialis* is common, but none was found except on *Pleuronectes flesus*.

During April 1940 occasional specimens of *Cyclopterus lumpus* were brought into the laboratory, but the only copepods found on the gills of these fish were three adult males (on one fish) which resembled those of *Lernaeocera branchialis*. The only differences were very slight—in the shape of the cephalothorax —but the specimens were larger than the average for the males of *L. branchialis*. These specimens are also marked by stars on Fig. 6. No character could be found which would separate these larvae into distinct genera or even species, though the specimen referred to above (footnote, p. 455) as found by T. Scott, and since called *Saucissona lumpi* by Leigh-Sharpe, may possibly be the corresponding mature female to these males. On the other hand, it is unusual to find males on the final host, and in the present instance no females were present. The possibility that these males are conspecific with Scott's female cannot be ruled out, but if this is true the almost exact correspondence of the males with *Lernaeocera* shows that Scott's specimen can scarcely be relegated to a separate genus.

At least two examples exist in the Lernaeoceridae of the developmental forms and males occurring on the same host as the mature females: Brian (1929, p. 14) describes all the forms of *Pennella sagitta* on a single host, *Antennarius histrio*, from the Sargasso Sea. A still more surprising record is that of Stekhoven (1936 *a*, pp. 17–18, fig. 16), who found *Lernaeocera lusci* as a mature female in the gill chamber of *Solea solea*, the same host on which I found larval forms of *Lernaeocera* sp. at Roscoff.

Stekhoven suggests that the characters of the second maxillae may prove to be specific criteria in the genus *Lernaeocera*: an excellent suggestion, because these appendages occur well developed in the copepodid, in the free-swimming male and female, and also sometimes in the mature female (very often, however, they are broken off in this stage). I have, unfortunately, been unable to use these characters, for in all my material the appendages appeared identical —except for sexual differences—and I have not found forms exactly corresponding to Stekhoven's figures.

Summary

The morphology of a complete series of developmental stages of *Lernaeocera branchialis* is described. Seven stages are passed through before reaching the gadoid (final) host.

The structure of the mouth tube and appendages is discussed in detail and compared with those of related genera in which the resemblances are close.

The free single nauplius and copepodid stages are immediately followed by four chalimus stages on *Pleuronectes flesus*; these show some dedifferentiation though segmentation is not lost. An explanation is given for the peculiar suspensory mechanism of the chalimus.

A second dedifferentiation follows the second well-developed pelagic phase in which the adult form is attained and copulation takes place. Some irregularity is noted in the details of the retrogressive metamorphosis of the maturing female on the gadoid host.

The rate of increase in body length in *Lernaeocera* decreases regularly up to the assumption of the adult form (stage VII). In this respect it is similar to the free-living copepods *Diaptomus*, *Eurytemora* and *Calanus*, but is in strong contrast to *Caligus centrodonti* which has an increasing growth rate up to the fourth chalimus stage. In the latter at this stage there is a marked acceleration in the growth rate unaccompanied by any change in shape or any other discontinuity, whereas in *Lernaeocera* there are early discontinuities in development, yet the growth rate is continuous when expressed graphically: it is a straight line rather than a logarithmic curve as would be expected from our knowledge of the higher crustacea.

The size of the female is greater than that of the male: that of the female overlapping the male of the succeeding stage. Variations in size of the larvae in a stage group are not seasonal, and an explanation is offered for giant larvae.

Similar lernaeocerid larvae and males to those of *Lernaeocera branchialis* on *Pleuronectes flesus* were found on *Solea solea* and *Cyclopterus lumpus*. No characters of generic or specific significance could be found which would serve to separate them from *Lernaeocera branchialis* so that their identity is not determined.

REFERENCES

BRIAN, A., 1912. Copépodes parasites des Poissons et des Échinides provenant des Campagnes scientifiques de S.A.S. le Prince de Monaco 1886–1910. *Res. Camp. Sci. Monaco*, Fasc. 38.

—— 1929. Copépodes parasites de Poissons et d'Annélides. *Res. Camp. Sci. Monaco*, Fasc. 76.

CLAUS, C., 1868*a*. Ueber die Metamorphose und systematische Stellung der Lernaeen. *Sitzungsb. Ges. Nat. Marburg*, No. 2, pp. 5–13.

—— 1868*b*. Beobachtungen ueber *Lernaeocera*, *Peniculus* und *Lernaea*. Ein Beitrag zur Naturgeschichte der Lernaeen. *Schrift. Ges. Nat. Marburg*, Bd. 9, Suppl. Heft. 2, 32 pp.

GURNEY, R., 1913. Some notes on the parasitic copepod *Thersitina gasterostei* Pagenstecher. *Ann. Mag. Nat. Hist.*, (8), Vol. XII, pp. 415–24.

GURNEY, R., 1928. Dimorphism and rate of growth in Copepoda. *Internat. Rev. d. ges. Hydrobiol. u. Hydrogr.*, Bd. 21, pp. 189–207.

—— 1930. The larva of *Nicothoë astaci* and its systematic position. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 453–9.

—— 1933. *British Freshwater Copepoda.* Ray Society, Vol. III (Family Lernaeoceridae, pp. 334–6).

—— 1934. The development of certain parasitic copepoda of the families Caligidae and Clavellidae. *Proc. Zool. Soc. London*, pp. 177–217.

—— 1940. Some notes on the biology of the Copepod *Diaptomus castor*. *Ann. Mag. Nat. Hist.* (11), Vol. VI, pp. 277–83.

GURNEY, R. & LEBOUR, M. V., 1941. On the larvae of certain Crustacea Macrura, mainly from Bermuda. *Journ. Linn. Soc.*, London, Vol. XVI, pp. 89–181.

HARRISON, R. J., 1940. On the biology of the Caprellidae. Growth and moulting of *Pseudoprotella phasma* Montagu. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 483–93.

JUNGERSEN, H. F. E., 1911. On a new Gymnoblastic Hydroid (*Ichthyocodium sarcotretis*) epizoic on a new parasitic copepod (*Sarcotretes scopeli*) infesting *Scopelus glacialis* Rhdt. *Vid. Medd. Natur. Foren. Copenhagen*, Bd. LXIV, pp. 1–33.

LEIGH-SHARPE, W. H., 1930. *Lernaea* [*Lernaeocera*] *barbicola* n.sp., A parasitic copepod of *Barbus* sp. from the Transvaal. *Parasitology*, Vol. XXII, pp. 334–7.

—— 1935. Some new and rare Lernaeidae (Copepoda) from Plymouth. *Parasitology*, Vol. XXVII, pp. 107–10.

MARSHALL, S. M., 1933. On the biology of *Calanus finmarchicus*. II. Seasonal variations in the size of *Calanus finmarchicus* in the Clyde Sea-Area. *Journ. Mar. Biol. Assoc.*, Vol. XIX, pp. 111–38.

PEDASCHENKO, D. D., 1898. Die Embryonalentwickelung und Metamorphose von *Lernaea branchialis*. *Trav. Soc. Impér. Nat. St. Pétersbourg*, Vol. XXVI (4), No. 7. (German text: pp. 247–307.)

PRZIBRAM, H., 1931. *Connecting Laws in Animal Morphology*. University of London Press.

SCOTT, ANDREW, 1901. *Lepeophtheirus* and *Lernaea*. *L.M.B.C. Memoirs*, No. 6.

SCOTT, THOMAS, 1901. Notes on some parasites of fishes. *19th. Ann. Rep. Fish. Bd. Scotland*, Pt. III, Sci. Invest., pp. 120–53.

SPROSTON, N. G. & HARTLEY, P. H. T., 1941*a*. The ecology of some parasitic copepods of gadoids and other fishes. *Journ. Mar. Biol. Assoc.*, Vol. XXV, pp. 361–92.

—— —— 1941*b*. Observations on the bionomics and physiology of *Trebius caudatus* and *Lernaeocera branchialis*. *Journ. Mar. Biol. Assoc.*, Vol. XXV, pp. 393–417.

STEKHOVEN, JR., J. H. SCHUURMANS, 1935. Copepoda Parasitica from the Belgian Coast. *Bull. Mus. Roy. Hist. Nat. Belg.*, Vol. XI, No. 7, pp. 1–13.

—— —— 1936*a*. Copepoda Parasitica from the Belgian coast (including some habitats in the North Sea). *Mem. Mus. Roy. Hist. Nat. Belg.*, No. 74, pp. 1–20.

—— —— 1936*b*. Beobachtungen zur Morphologie und Physiologie der *Lernaeocera branchialis* L. und der *Lernaeocera lusci* Bassett-Smith (Crustacea parasitica). *Zeits. Parasitenk.*, Bd. VIII, Heft 6, pp. 659–96.

STEKHOVEN, JR., J. H. SCHUURMANS & PUNT, A., 1937. Weitere Beiträge zur Morphologie und Physiologie der *Lernaeocera branchialis* L. *Zeits. Parasitenk.*, Bd. IX, Heft 5, pp. 648–68.

VAN BENEDEN, P. J., 1861. Recherches sur la Faune littorale de Belgique: Crustacés. *Mém. Acad. Roy. Belg.*, T. XXXIII, 174 pp.

WIERZEJSKI, A., 1877. Ueber Schmarotzerkrebse von Cephalopoden. *Zeitschr. f. wiss. Zool.*, Bd. XXIX, pp. 563–82.

WILSON, C. B., 1917. North American Parasitic Copepods belonging to the Lernaeidae with a revision of the entire family. *Proc. U.S. Nat. Mus.*, Vol. LIII (No. 2154), pp. 1–150.

ON THE FECUNDITY OF SOME GAMMARIDS

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(Text-figs. 1, 2)

CONTENTS

INTRODUCTION

There is available comparatively little information regarding the fecundity of gammarids. Sexton (1913, 1928) makes some remarks upon the reproductive capacity of *Gammarus chevreuxi*, *G. pulex* and *G. locusta*. Recently she and Spooner (1940) have made an exhaustive taxonomic study of the marine littoral forms, and include brief records of the size of the broods in each species. The present investigation was undertaken with the object of making a statistical study of the fecundity of several species with special reference to the relation between the body weight and fecundity.

The great profusion of marine fauna, particularly of littoral species, on the north coast of Wales is well known (Jackson, 1940). The number of species of *Gammarus* and *Marinogammarus* previously recorded as occurring here is seven, namely, *G. pulex*, *G. locusta*, *G. zaddachi*, *G. duebeni*, *G. chevreuxi*, *M. marinus* and *M. obtusatus*.

In this investigation, the specimens were collected mainly from the upper part of the intertidal zone at the following two places along the Bangor coast of the Menai Straits: (1) the shore on either side of the pier at Bangor, (2) the stream (Gorad-y-Gyt) flowing towards the Straits. Altogether a total of fourteen samples was obtained, nine from the former and five from the latter. The dates of collection are as follows:

Shore collection	Stream collection
Sample I: 27 Nov. 1940	Sample X: 16 Nov. 1940
,, II: 11 Dec. 1940	,, XI: 20 Nov. 1940
,, III: 28 Jan. 1941	,, XII: 22 Nov. 1940
,, IV: 10 Feb. 1941	,, XIII: 9 Dec. 1940
,, V: 6 Mar. 1941	,, XIV: 7 Mar. 1941
,, VI: 19 Mar. 1941	
,, VII: 19 May 1941	
,, VIII: 20 May 1941	
,, IX: 29 May 1941	

An examination of the material reveals the occurrence of six species[1] as shown in Table I.

TABLE I

Species	Habitat	Abundance
M. marinus (Leach)	marine	very abundant
M. obtusatus (Dahl)	,,	abundant
M. finmarchicus (Dahl)	,,	rare
M. stoerensis (Reid)	,,	abundant
G. duebeni Lilljeborg	brackish water	very abundant
G. chevreuxi Sexton	,,	very rare

It will be seen that *M. marinus* and *G. duebeni* dominate the shore and the stream respectively. Not infrequently the former was also encountered in the stream collection. The conclusion that these two species can be found in association has been noted by Beadle & Cragg (1940). It is noteworthy that the distribution of *M. obtusatus* appears to be very restricted in this region, occurring only in a particular area where there is sewage discharge. *M. stoerensis* is usually found in company with *M. obtusatus*, but on one occasion a female was obtained from the stream area. It shows that *M. stoerensis*, though a marine species, prefers to inhabit a place with freshwater influx as already noticed by Sexton & Spooner (1940). All these species are found to be widely distributed in this country, especially *M. marinus* and *G. duebeni* (see Crawford, 1937; Sexton & Spooner, 1940, etc.).

I wish to express my thanks to Prof. Brambell for advice and criticism. My thanks are also due to Mr Cragg for having placed at my disposal a list of species recorded by him and for his kind help in various ways.

BODY WEIGHT AND FECUNDITY DATA

Owing to the extremely small numbers of females which were obtained, *M. finmarchicus* and *G. chevreuxi* are excluded from this investigation. The mean values of the body weight[2] and the fecundity of other species, together with the standard deviations, are shown in Table II.

It will be seen that the mean and the standard deviation of the body weight vary with the species, being highest in *M. marinus* and lowest in *M. stoerensis*. The former is nearly nine times as heavy as the latter.

As regards mean fecundity, the species fall into the following order: *M. stoerensis* > *M. marinus* > *G. duebeni* > *M. obtusatus*, the difference between the first and the last being fifteen eggs per brood.

By comparing these two sets of data, it may be seen that the size of a species bears no relation whatever to the reproductive capacity, as shown plainly in *M. stoerensis*. Although it is the smallest species, its mean fecundity is

[1] Their identification has been confirmed by Mrs Sexton to whom my thanks are due.

[2] Before weighing, the eggs were taken out of the brood pouch and counted. The animals were then drained thoroughly by means of blotting paper for a certain time depending on the size of the animal.

greater than that of the others. Sexton (1928) obtained the same result from her investigation into the reproduction of various species of *Gammarus*, stating: 'The number of young in a brood varies with the species, but the size of the species is no gauge of either the size or the number of young. *G. chevreuxi* is

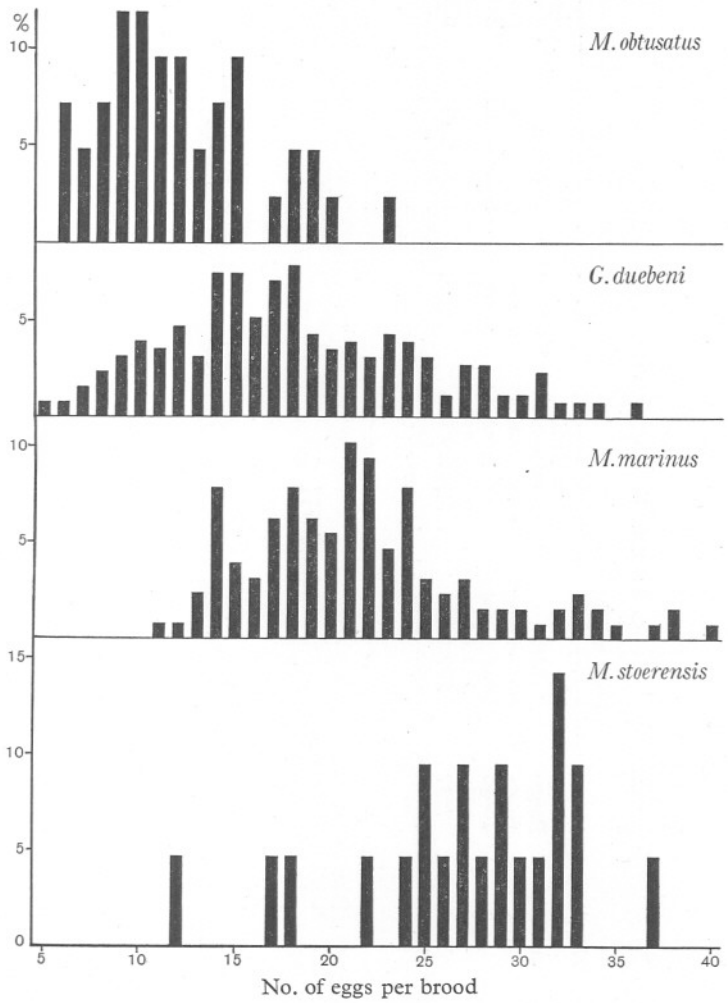

Fig. 1. Percentage frequency distribution of fecundity of various species.

a small species compared with *G. pulex*, yet it produces up to 63 young in a brood, whilst the highest record for *G. pulex* is only 28.'

A comparatively small difference exists in the maximum fecundity of the four species examined; the maximum is highest in *M. marinus* with forty eggs per brood and lowest in *M. obtusatus* with only twenty-three eggs per brood.

TABLE II

Species	Number of animals	Body weight (mg.)			Fecundity (no. of eggs per brood)		
		Range	Mean	Standard deviation	Range	Mean	Standard deviation
M. marinus	128	19–63	37·71 ± 0·91*	10·3 ± 0·64*	11–40	21·68 ± 0·53	6·02 ± 0·38
M. obtusatus	42	10–30	18·17 ± 0·64	4·18 ± 0·46	6–23	12·02 ± 0·63	4·09 ± 0·45
M. stoerensis	21	3–6	4·52 ± 0·16	0·75 ± 0·11	12–37	27·1 ± 1·32	6·04 ± 0·93
G. duebeni	254	7–27	15·29 ± 0·21	3·34 ± 0·15	5–36	18·13 ± 0·42	6·63 ± 0·29

* Standard errors.

TABLE III

Species ...	*G. duebeni*			*M. obtusatus*		*M. marinus*		
Sample no.	X–XII	XIII	XIV	I–II	VII–VIII	III–IV	V–VI	VII–IX
Date	16–22 Nov.	9 Dec.	7 Mar.	27 Nov. and 11 Dec.	19–20 May	28 Jan. and 10 Feb.	6–19 Mar.	19–29 May
No. of animals	30	102	122	22	20	15	73	40
Mean fecundity	13·7 ± 1·01	15 ± 0·45	21·8 ± 0·57	10 ± 0·64	14·2 ± 0·9	19·9 ± 1·2	22·3 ± 0·71	20·9 ± 0·97
Standard deviation	5·53 ± 0·71	4·58 ± 0·32	6·36 ± 0·41	2·99 ± 0·45	2·99 ± 0·45	4·64 ± 0·85	6·03 ± 0·5	6·12 ± 0·68

The largest broods recorded by Sexton & Spooner (1940) for *M. marinus* and *M. obtusatus* number fifty and twelve respectively.

The percentage frequency distribution of fecundity in the various species is represented by histograms in Fig. 1. It will be seen that the modal class is by no means the same in the four species, being 9–10 in *M. obtusatus*, 18 in *G. duebeni*, 21 in *M. marinus* and 32 in *M. stoerensis*. This diagram provides a clear picture of the reproductive potentiality of each species.

As already mentioned in the introduction, the collecting period extended from 27 November to 29 May for the 'shore' samples and from 16 November to 7 March for the 'stream' samples. In order to show whether there exists any seasonal variation in the fecundity of various species, another figure (Fig. 2) has been drawn in which each month is represented by a separate histogram. It is evident that the fecundity does vary with season, being generally higher in the spring than in the winter months, as is also shown clearly in Table III. The phenomenon is best displayed in *G. duebeni* in which the modal class for each month is apparently different, being 9 for November, 18 for December, and 23 for March.

That the mean fecundity is on the whole higher in the spring than in the winter is not unexpected, because in the former period external conditions, such as temperature and food, are obviously more congenial. It is found in *Daphnia* that the size of a brood produced by a female in the laboratory can be increased by both higher temperature (up to a certain limit) (Berg, 1931) and rich food (Ingle, 1933). The increase in fecundity in the spring has no connexion with the age of the animal, because an analysis of the material reveals that a female of the same weight generally produces less eggs per brood in the winter. It is unfortunate that the collecting period does not extend beyond May, so that no comparison can be made between the mean fecundity of various months of the year. Such an investigation has been carried out in other animals. Berg (1931), studying the productivity of *Daphnia* in nature throughout the whole year, finds that the maximum fecundity occurs in May.

Practically nothing is known regarding the breeding of these species in nature except in *G. chevreuxi*, which has been found to breed all the year round (Mrs Sexton, private communication). In the present investigation pregnant females of *M. marinus*, *M. obtusatus* and *G. duebeni* were present in all the samples collected, showing that these species are capable of breeding in the winter as well as in the spring.

Relation between body weight and fecundity

The species used in this investigation are *M. marinus*, *M. obtusatus* and *G. duebeni*, because the number of females obtained is relatively larger in these species. Their body weight and fecundity data are shown in Table IV. It will be seen that although there appears to be a great variation in the fecundity of certain body-weight classes, particularly so in *G. duebeni*, the general tendency

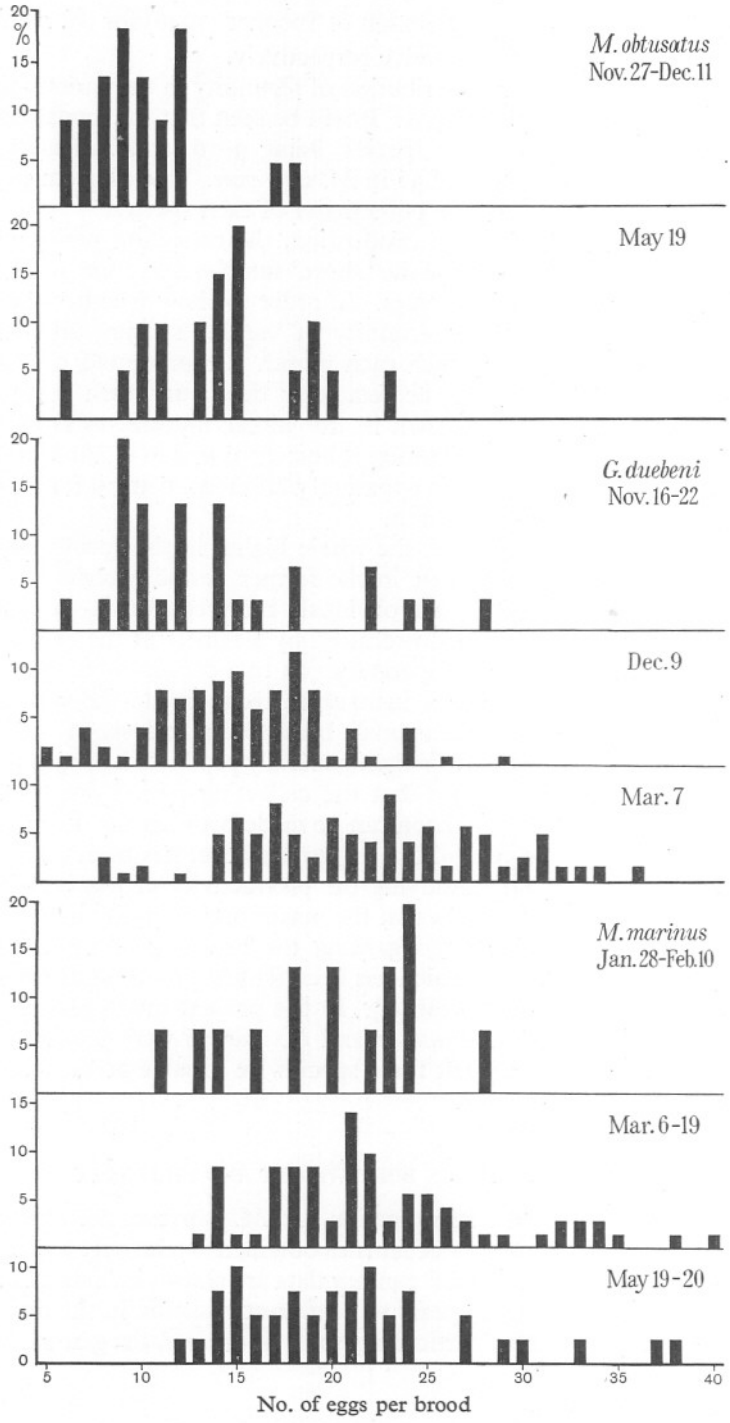

Fig. 2. Monthly percentage frequency distribution of fecundity of various species.

TABLE IV

Fecundity (no. of eggs per brood)	*M. obtusatus*				*M. marinus*										*G. duebeni*				
	10–15	16–20	21–25	26–30	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	6–10	11–15	16–20	21–25	26–30
36–40	.	.	.	.	.	.	.	.	.	1	.	1	2	.	.	.	2	.	.
31–35	.	.	.	.	.	.	.	.	.	1	3	3	1	1	.	2	10	.	.
26–30	.	.	.	.	1	.	1	.	3	3	1	1	2	1	.	5	14	4	.
21–25	.	.	.	1	1	1	4	7	10	10	8	1	2	.	2	22	16	6	1
16–20	.	1	4	1	2	4	13	7	5	5	2	.	.	.	3	41	23	3	1
11–15	3	10	4	.	.	2	9	6	2	1	.	.	.	.	2	42	20	3	.
6–10	9	9	.	.	.	.	.	.	.	.	.	.	.	.	4	18	8	.	.
1–5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.

Body weight (mg.)

is towards an increase in fecundity with an increase in body weight. Sexton (1928) obtained the same result from her experimental investigation into the reproduction of *G. chevreuxi*, stating 'the number of young in a brood increases as the female grows'. A comparison of these three tables reveals that the trend is most marked in *M. obtusatus* and least in *G. duebeni*. In the latter the positive correlation between the body weight and the fecundity does not appear to hold for the heavier groups exceeding 20 mg. in body weight. These larger females are found to be present in all the samples collected. No such tendency to reduction in fecundity of older individuals is discernible in the other two species, probably due to lack of sufficient material, especially in *M. obtusatus*.

The relation between fecundity and body weight in the three species is measured statistically by estimating the value of the correlation coefficient. The result is shown in Table V.

TABLE V

Species	No. of animals	Correlation coefficient (r)	Test of significance of r	
			t	p
M. marinus	128	+0·668	7·524	<0·01
M. obtusatus	42	+0·713	4·566	<0·01
G. duebeni	254	+0·383	5·494	<0·01

It is noteworthy that the correlation coefficient of *M. obtusatus* is nearly twice as high as that of *G. duebeni*. It is expected because, in the latter, the positive correlation between the body weight and the fecundity appears to break down after the 19 mg. body-weight class. The observed correlations are definitely significant because p is less than 0·01.

The relation between body weight and fecundity has been studied in other animals by several investigators (Wynne-Edwards, 1929; Gregory, 1932; Stone, 1938). Their result shows that fecundity bears an almost linear relationship to the body weight of the animal studied. The same holds true of the relation between body length and fecundity (Allen, 1895; Stone, 1938; Hickling,

1940). All these investigators agree that the productivity increases as the animal grows. The present investigation reveals, however, that the linear relation between the body weight and the fecundity does not hold for the higher body-weight groups and that fecundity reaches a maximum at a certain body weight or age, as seen in the case of *G. duebeni*. This finding is substantiated by the outcome of the breeding experiments. In *G. chevreuxi*, it is found that after reaching its maximum, the size of broods produced by a female becomes greatly reduced towards the end of the reproductive period (Sexton, 1928). In *Daphnia*, the reproductive capacity drops to a low level after attaining its peak at about the 12th–14th instar (Ingle, Wood & Banta, 1937; Banta, 1939). It thus leads to the conclusion that the correlation between body weight and fecundity is positive at first and then becomes negative after the latter has reached its maximum at a certain age.

Summary

1. The number of species found in this investigation is six, of which four are marine and two brackish water.

2. The mean value of the body weight and the fecundity varies with the species. The size of a species bears no relation whatever to the reproductive capacity.

3. The fecundity is subject to seasonal variation, being generally higher in the spring than in the winter months.

4. In general, a positive correlation exists between body weight and fecundity of the individuals of each species. This rule does not, however, apply to *Gammarus duebeni*, in which the fecundity appears to fall after reaching its maximum at the 19 mg. body-weight class.

REFERENCES

Allen, E. J., 1895. The reproduction of the lobster. *Journ. Mar. Biol. Assoc.*, Vol. iv, pp. 60–70.

Banta, A. M., 1939. Studies on the physiology, genetics and evolution of some Cladocera. *Washington, Carneg. Instn., Dept. of Genetics*, Paper No. 39.

Beadle, L. C. & Cragg, J. B., 1940. The intertidal zone of two streams and the occurrence of *Gammarus* spp. on South Rona and Raasay (Inner Hebrides). *Journ. Anim. Ecol.*, Vol. ix, pp. 289–95.

Berg, K., 1931. Studies on the Genus *Daphnia* O. F. Müller with especial reference to the mode of reproduction. *Vidensk. Medd. Naturh. Foun. Kdh.*, Vol. 92.

Crawford, G. I., 1937. The fauna of certain estuaries in West England and South Wales, with special reference to the Tanaidacea, Isopoda and Amphipoda. *Journ. Mar. Biol. Assoc.*, Vol. xxi, pp. 95–106.

Fisher, R. A., 1930. *Statistical Methods for Research Workers*. 3rd edition. Edinburgh.

Gregory, P. W., 1932. The potential and actual fecundity of some breeds of rabbits. *Journ. Exp. Zool.*, Vol. lxii, pp. 271–85.

HICKLING, C. F., 1940. The fecundity of the herring of the southern North Sea. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 619–32.

INGLE, L., 1933. Effects of environmental conditions on longevity. *Science*, Vol. LXXVIII, p. 511.

INGLE, L., WOOD, T. R. & BANTA, A. M., 1937. A study of longevity, growth, reproduction and heart rate in *Daphnia longispina* as influenced by limitations in quantity of food. *Journ. Exp. Zool.*, Vol. LXXVI, pp. 325–52.

JACKSON, L. H., 1940. An account of the littoral fauna of the Anglesey coast of the Menai Straits from the Tubular Bridge to Menai Lighthouse. *Anglesey Antiquarian Soc. Trans.*, pp. 100–10.

SEXTON, E. W., 1928. On the rearing and breeding of *Gammarus* in laboratory conditions. *Journ. Mar. Biol. Assoc.*, Vol. XV, pp. 33–55.

SEXTON, E. W. & MATTHEWS, A., 1913. Notes on the life history of *Gammarus chevreuxi*. *Journ. Mar. Biol. Assoc.*, Vol. IX, p. 546.

SEXTON, E. W. & SPOONER, G. M., 1940. An account of *Marinogammarus* (Schellenberg) Gen. nov. (Amphipoda), with a description of a new species, *M. pirloti*. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 633–82.

STONE, U. B., 1938. Growth, habits and fecundity of the *Ciscoes* of Irondequoit Bay, New York. *Trans. Amer. Fish. Soc.*, Vol. LXVII, pp. 234–45.

WYNNE-EDWARDS, V. C., 1929. The reproductive organs of the herring in relation to growth. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 49–65.

THE AMERICAN WHELK TINGLE, *UROSALPINX CINEREA* (SAY), ON BRITISH OYSTER BEDS

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(Text-figs. 1–10)

CONTENTS

INTRODUCTION

Although it is probable that the American slipper limpet (*Crepidula fornicata* L.) was introduced with consignments of American oysters about 70 years ago (see Robson, 1929; McMillan, 1939), it was not until 1928 that it was known that the American oyster pest *Urosalpinx cinerea* (Say) had also become established on British oyster beds. The first record of the occurrence of *Urosalpinx* is that of Orton (1927) who figured American tingles in an article relating to the native rough tingle *Ocenebra erinacea* (L.), the two forms being confused. Later, Orton & Winckworth (1928) corrected this mistake and definitely recorded *Urosalpinx* for the first time. Subsequently, Orton (1930) has recorded the finding of specimens of *Urosalpinx* among material collected in 1920. It is almost certain that this pest had been established in one or two places for several years before it was recognized. The confusion between *Urosalpinx* and *Ocenebra* still persists among many oystermen, who lump them together as tingles, drills, or borers, different terms being employed in different districts.

In 1939 the writer was informed that several tingles had been found a week previously among American oysters received by an east coast oyster merchant. It is not known whether they were alive, but the possibility of fresh introductions will exist as long as American oysters are imported.

In presenting this paper I have to thank Mr R. E. Savage for criticism and advice and Mr H. H. Goodchild for the photograph on p. 483. I am also indebted to a large number of east coast oystermen who have supplied specimens or information, and in particular to Mr F. E. Wombwell and the

foremen and dredgermen of the Tollesbury and Mersea Native Oyster Fishery Company, who assisted me in every way possible.

Distribution

In America *Urosalpinx* occurs from Cape Cod to Florida (Galtsoff, Prytherch & Engle, 1937), while it has also been introduced into San Francisco Bay and to Bermuda.[1] Along the English coast its distribution has not been fully worked out. The two main centres of distribution appear to have been Brightlingsea and West Mersea, Essex (Fig. 1), where for many years American oysters have been laid down. It is very abundant in the River Blackwater and in all the creeks running into the Blackwater to the south of Mersea Island. These creeks are leased to a large number of separate planters, most of whom have at some time or other dealt in American oysters. In Brightlingsea Creeks and in the River Colne *Urosalpinx* is also abundant. Farther south it occurs sparingly in the River Crouch and more abundantly in the River Roach and in the creeks around Paglesham. It seems likely that *Urosalpinx* was recently introduced into the Roach-Crouch river system, probably not long before 1934, for local dredgermen then noted the occurrence of a new type of tingle. These men suggested that they were brought with winkles (*Littorina*) or oysters from West Mersea, the winkles, unobtainable in quantity in the Crouch, being used to keep the oyster pits free of weed. *Urosalpinx* evidently multiplied rapidly, for in one year (1936–7) the company working the grounds in the River Roach paid £9. 5*s*. 0*d*. for tingles collected, at the rate of 1*s*. per 1000, equivalent to approximately 185,000 tingles, many of which, possibly even the majority, would of course be *Ocenebra* and *Nucella*.

On the Kentish coast *Urosalpinx* occurs on the Whitstable beds at the mouth of the River Swale, but it does not thrive on these beds in the same way as in the Essex rivers. The beds at Whitstable are practically in the open sea, and the bottom is hard and quite different from that in the Essex creeks. It clearly does not represent a very favourable habitat for *Urosalpinx*, for it has never become abundant despite the fact that numerous oysters from Essex are laid down annually at Whitstable and small tingles must be carried with them. There is no evidence that *Urosalpinx* occurs in the River Medway, for although I have not dredged there, numerous inquiries among oystermen who work in the Medway, and the showing of specimens, elicited only negative answers, although American oysters have been laid down in the area on several occasions. The beds at Faversham in the River Swale are said to carry no tingles.

Although *Crepidula* now occurs from the Humber to Dorset and the Isle of Wight, and has recently been carried in small numbers to the River Yealm, south Devon, although it may not have established itself there, there are no authenticated records of *Urosalpinx* outside of Essex and Kent. It must be admitted, however, that no intensive search has been conducted in other areas,

[1] Needler has recently recorded (*Bull.* LX, *Fish. Res. Bd. Canada*) the occurrence of *Urosalpinx* in the Northumberland Strait area of the Atlantic coast of Canada.

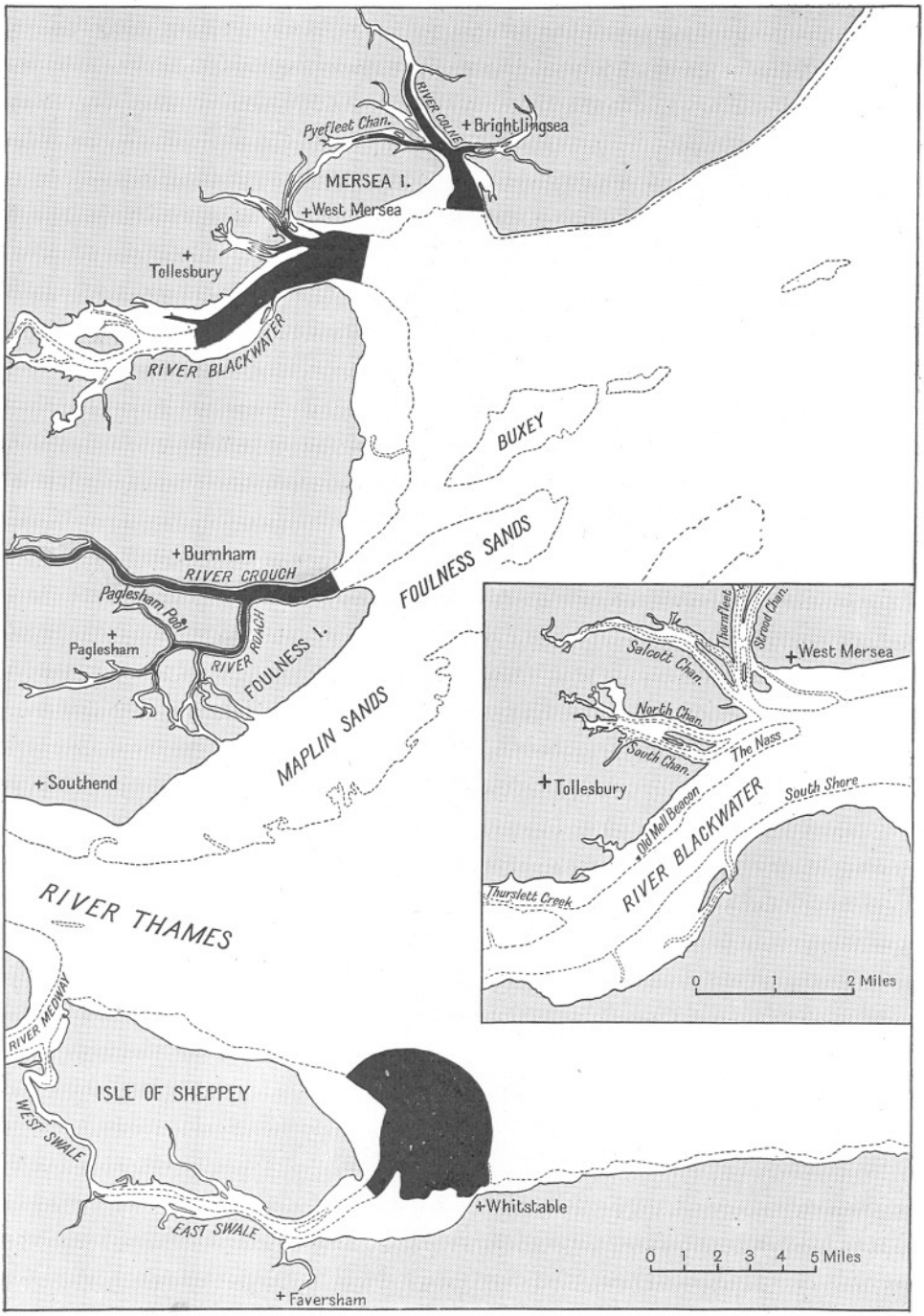

Fig. 1. Chart of the known distribution of *Urosalpinx cinerea* on British oyster beds (from Admiralty Chart). Inset—the Blackwater estuary.

and American oysters have in the past been laid down at several points on the east, south, and north-west coasts, e.g. Cleethorpes and the Orford River on the east, Poole Harbour, Emsworth and Bosham creeks, and the River Yealm on the south, and the Menai Straits in the north-west, while many years ago the beds at Hayling Island were used for wintering oysters from the east coast. If, therefore, as Orton & Winckworth (1928) think, *Urosalpinx* was introduced many years ago with *Crepidula*, an opinion with which the evidence collected by the present writer does not entirely agree, there is a good chance of it occurring wherever American oysters have been laid down. Since *Urosalpinx* lays its eggs in capsules from which the fully formed young tingles emerge, the larval stages being passed in the capsule, it is clear that to infect new localities tingles or their spawn must be carried there along with oysters or cultch.

Until the end of 1939 *Ocenebra erinacea* occurred in fair numbers along with *Urosalpinx* on the east coast oyster beds. Samples of tingles obtained from the River Blackwater in 1938 and 1939 included from 6 to 12 % of *Ocenebra*. Following the extremely cold winter of 1939–40 and the only slightly less cold winter of 1940–1, *Ocenebra* has disappeared from the Blackwater beds; during a fortnight's dredging in May 1941 not a single live specimen was encountered, although empty shells were common. Empty shells of *Urosalpinx* are, on the other hand, only occasionally found, and it seems that the 'great frost' of 1939–40 did not kill off any significant number of *Urosalpinx*; this is hardly surprising, for in its natural home on the Atlantic coast of the U.S.A. it is subjected to much greater extremes of cold and heat than are ever recorded here.

The progressive replacement of *Ocenebra* by *Urosalpinx* in the River Blackwater is well illustrated by some figures given by Orton & Lewis (1931). After the severe winter of 1929 *Ocenebra* was practically wiped out, while *Nucella* was much reduced in numbers—*Urosalpinx*, on the other hand, appeared actually to increase (Table I).

TABLE I. SHOWING THE PERCENTAGE OF *OCENEBRA*, *UROSALPINX* AND *NUCELLA* IN DREDGED CATCHES FROM THE RIVER BLACKWATER (FROM ORTON & LEWIS, 1931)

	Urosalpinx %	*Ocenebra* %	*Nucella* %	Total
1928	7·8	41·2	51	1739
1929	71·2	0·2	28·6	584
1930	77·9	0·11	22	5467

Dredged catches taken in 1941 contained only 1–5 % of *Nucella*, so that this species has also decreased markedly in recent years. During the period 1930–8 *Ocenebra* increased until in 1938 it formed 6–12 % of the catch; it may therefore once again re-establish itself in the Blackwater by migration or transport from outside.

In any river system *Urosalpinx* appears to favour the shallow creeks rather than the main river, and muddy bottoms rather than those of hard gravel or sand. The hard flats off Whitstable seem to be definitely less favourable than, for example, the muddy creeks at West Mersea. Federighi (1931) has shown that the range of salinity tolerated by *Urosalpinx* is much greater than that under which the cultivation of the European native oyster (*Ostrea edulis*) can be carried on.

Migration and movement generally in *Urosalpinx* has been studied in America by Federighi (1931) and Galtsoff *et al.* (1937) with somewhat conflicting results. Federighi, employing tagged drills planted on oyster bottoms at low water, found that even after one month the tagged drills had not moved more than 10–15 ft. from their original situation. It is stated that this was not due to the presence of unlimited food, for drills placed on a hard bottom 20 ft. from an oyster bed did not move towards it. Federighi notes also that contiguous oyster beds in Hampton Roads, Va., which had been left undisturbed for two years, were infested by drills in different quantities; he therefore concluded that significant migration does not take place. Experiments made by Galtsoff *et al.* (1937) did not confirm Federighi's conclusion. Marked drills were released on different types of bottom devoid of oysters and shells, and wire bags, some containing seed oysters and others containing only shells, were planted on the bed at different distances from the drills. In all cases more drills migrated to the bags containing seed oysters than to those containing no live oysters. These experiments were conducted over a period of several months and were thought to show definitely that the migration of drills was influenced by the position of food and that they tend to move towards it at the rate of at least 150 ft. in 48 hr. Galtsoff *et al.* therefore conclude that migration may play an important part in the distribution of drills. It is as well to remember, however, as these authors point out, that under natural conditions the behaviour of *Urosalpinx* is determined by the combined and sometimes antagonistic effects of several factors and cannot be attributed to a single cause.

There is a general belief among Essex oystermen that tingles are able to detect the presence of freshly laid oysters and to move on to them, and several instances were related of tingles which appeared suddenly on oysters laid down at low-water mark on ground previously free of this pest. This move is, however, observed during the breeding season, and it might be argued that the tingles moved on to the oysters from the surrounding clean bottom for the purpose of depositing their spawn. During 1941 a wooden trough 215 cm. long, coated inside with pitch and filled with sea water, was used at Conway to study the movements of marked drills. Food—spat or brood oysters, or barnacled stones—was placed at varying distances from the centre, towards one end of the trough, and the drills lined up at the centre and their movements noted at intervals of 30 min. or more. Ten drills were employed in most experiments and water temperatures were recorded. When the illumination over the whole surface of the trough was the same it was found that the drills

moved at random and showed no ability to detect the presence of food or tendency to move towards it. This was true even when the distance between the drills and the food was reduced from the maximum of about 105 cm. to as little as 35 cm. These experiments were repeated a great number of times with identical results. Until steps were taken to even out the intensity of light by appropriate shading, the drills tended to move out of the sun in bright weather and take up a position on the shady side of the trough. Individual drills varied considerably in the degree and rate of their movements, but the maximum rate of creeping for a distance of not less than 90 cm. was 3 cm./min., at a temperature of 14·4° C. This compares favourably with the figure given by Federighi, 2·6–2·8 cm./min. at 26·5° C. This high rate of creeping was only observed once, and in the majority of experiments the most active drill covered the distance of 105 cm. to the end of the trough in 1–1½ hr., a rate of creeping of 1·17–1·75 cm./min. This rate was observed on occasions at all temperatures between 13 and 23° C. At temperatures from 23 to 25° C. only very slight movement occurred, above 25° C. none. Such high temperatures are probably never experienced on British oyster beds, but were frequently exceeded in the laboratory experiments of Federighi without apparently causing any inconvenience to the drills.

Drills moving consistently in one direction at the rate of 1·5 cm./min. would cover a distance of 43·2 m. in 48 hr. The rate of travel observed under natural conditions by Galtsoff *et al.* was 150 ft. (45·72 m.) in 48 hr. This was the minimum rate of travelling for the drills that reached the bags containing seed oysters, and presumed that they travelled in a straight line. It seems certain, therefore, that *Urosalpinx* is capable of moving at this rate; but it has not yet been satisfactorily established that it is able to detect the presence of food and move towards it, and it seems unlikely, from the limited evidence available, that migration is of more than local significance in the distribution of the species.

Growth

The shell dimension most useful in the study of growth rates in gastropods is the height from the tip of the siphonal canal to the top of the spire; this dimension has been employed in this study. In most localities in America the adult *Urosalpinx* is stated (Galtsoff *et al.* 1937) to average slightly over 2·5 cm. in height, but at Seaside, Va., specimens as large as 6 cm. in height are said to be very common. Federighi (1931) found that the largest females from Chesapeake Bay measured 3·3 cm. and the largest males 2·9 cm., but the most usual size was from 2·1 to 2·5 cm. The giants from Seaside, Va., appear to parallel the giant forms of *Nucella lapillus* described by Moore (1936) from the British Museum collection, having been obtained originally from Minehead and Swanage. The present writer has subsequently collected a number of such giants from the River Crouch. The peculiar environmental conditions leading

to the production of these giant forms are not known, but in some gastropods parasitism is a factor of importance (Rothschild, 1936).

Urosalpinx on British oyster beds considerably exceeds the size generally reached in America. The largest individuals obtained were 4·3 cm. in height, and the only one sexed of this size was a female. The largest male measured 3·9 cm. Males over 3·5 cm. are always rare, but very large females are not uncommon on grounds which have not been worked intensively. This increase in size of an American form in British waters is also well shown by the slipper limpet, *Crepidula fornicata*.

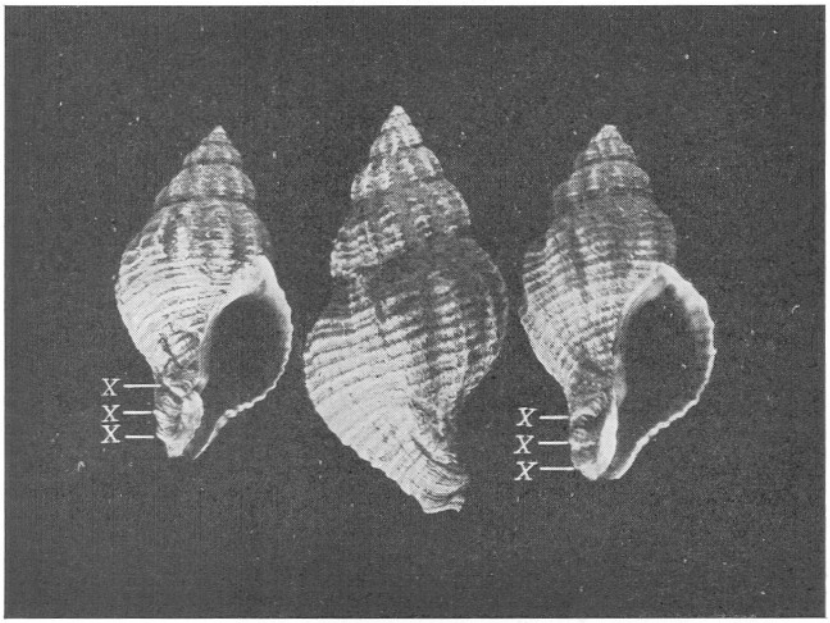

Fig. 2. *Urosalpinx cinerea* Say, showing growth marks (*X*) on the shell bounding the siphonal canal: × 1½.

Apart from the general observation that females grow larger than males the American work contains no information on growth rate or duration of life. This observation is equally true of *Urosalpinx* on British beds, and in the following analysis of growth males and females have been treated separately. Size-distribution curves of catches of females usually show a number of closely approximated peaks, and it appears that the annual increment in height is small after the first few years. This impression is confirmed by a study of the growth marks which may frequently be clearly seen on the tip of the shell bounding the siphonal canal (Fig. 2). Such growth marks in large shells are usually separated from the extreme tip and from each other by 0·1–0·3 cm. Considerable overlapping of successive year groups must therefore occur, and

this seriously impedes or prevents altogether the fixing of the position of the peaks in the frequency curves without recourse to further analysis. For this reason wherever possible the analysis of size-distribution curves has been carried out by the freehand drawing method advocated by Buchanan-Wollaston & Hodgson (1929).

TABLE II. SIZE DISTRIBUTION OF *UROSALPINX* SAMPLES COLLECTED FROM THORNFLEET, RIVER BLACKWATER DURING 1941

Shell height	24 May 1941		10 June 1941		8 July 1941		26 August 1941	
cm.	Females	Males	Females	Males	Females	Males	Females	Males
1·6	1	—	1	—	—	—	—	—
1·7	—	—	1	—	—	1	—	—
1·8	1	1	—	—	1	—	—	—
1·9	—	1	—	—	—	1	—	—
2·0	7	1	2	1	3	1	—	—
2·1	—	2	—	2	2	1	—	1
2·2	4	1	1	—	1	2	—	—
2·3	2	—	3	3	5	1	—	—
2·4	1	1	5	2	4	3	2	—
2·5	4	3	15	14	12	5	1	3
2·6	6	9	27	22	19	8	2	2
2·7	12	5	46	27	53	13	3	4
2·8	12	13	55	24	36	23	6	6
2·9	14	9	45	32	46	17	4	4
3·0	6	17	43	24	31	21	3	6
3·1	8	7	34	16	16	9	5	4
3·2	3	3	12	5	7	7	2	3
3·3	5	2	17	4	7	3	—	—
3·4	2	1	4	—	2	3	1	1
3·5	1	—	1	—	—	—	1	—
3·6	3	1	1	—	—	—	—	—
3·7	1	—	3	—	—	—	—	—
3·8	—	—	—	—	—	—	—	—
3·9	1	—	—	—	—	—	—	—

During 1941 a series of four samples of tingles was collected from the beach in Thornfleet (a creek running into the River Blackwater, Essex, near its mouth) at approximately monthly intervals from May to August (Table II). The first lot was collected on 24 May, when tingles were first appearing on the shore at the beginning of the spawning season, and the last on 26 August, when many had already migrated to deeper water. On each occasion all visible tingles were hand collected from stones, old drain pipes, mooring chains, etc., in the region of low-water mark of spring tides. On the same day that the first shore sample was collected a further lot was obtained by dredging in the channel adjacent to the beach. In Fig. 3 the size distribution of the females contained in the four monthly samples from Thornfleet are shown, those of May, June, and July being resolved into their components. The August sample was too small to permit of such treatment, while the May sample contained also those females dredged in Thornfleet on the same day. It will be seen that the modes of successive year groups are separated by an interval of about 0·2 cm., or slightly more in the younger age groups, and rather less in the older ones.

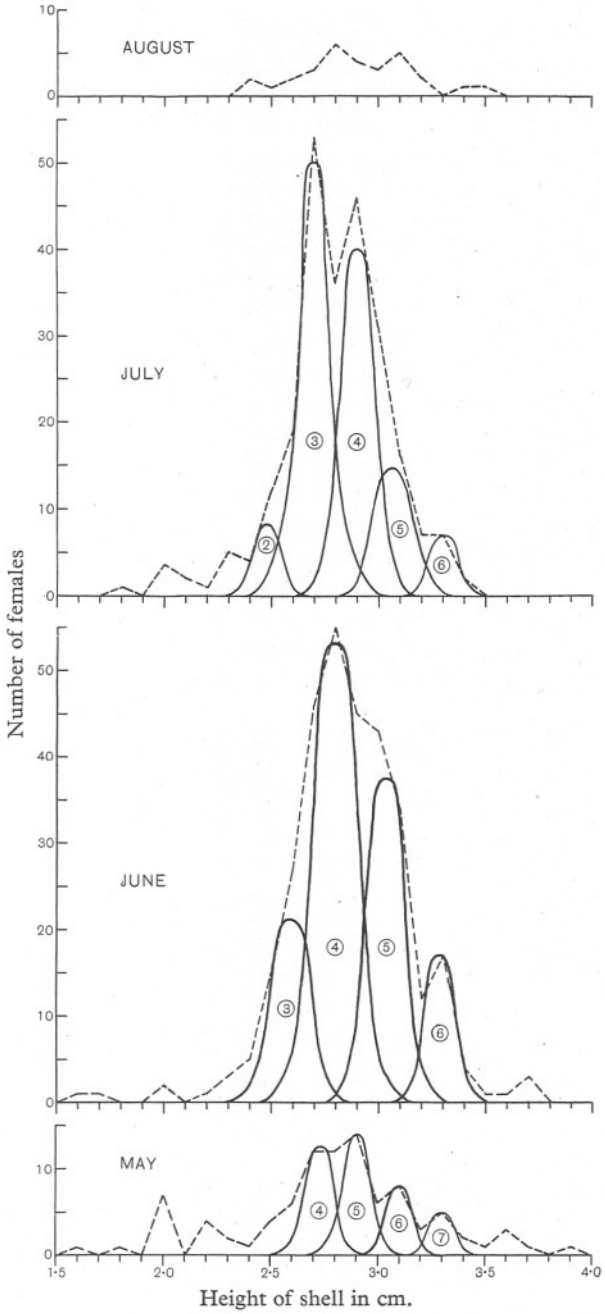

Fig. 3. Size-distribution curves of females from samples of *Urosalpinx* hand collected from the beach of Thornfleet, River Blackwater during 1941. The May sample also includes those dredged in the adjacent channel on the same day. In this and subsequent figures the numbers in the circles represent the probable age of each year group.

The comparison of the May and July groups shows the growth during 1941, up to 8 July, to have been rather less than 0·2 cm., as would be expected.

Comparison of the May and July samples shows the presence in July of two modes on the left-hand side not represented in May. In July these modes lie at 2·47 and 2·7 cm. respectively; but the sample also contains tingles as small as 1·8 cm. which cannot belong to the age group with the mode at 2·47 cm. The latter mode therefore must belong to the 2-year-old group, while the smaller tingles would belong to the 1-year-old class with a mode somewhere in the region of 2·0 cm. This 1-year-old group, which must numerically be the strongest, is very poorly represented on the shore during the summer.

Tingles hatched from egg capsules in July, and reared on small oyster spat in a plunger jar at Conway during 1941, reached a maximum height of 1·2 cm. by the end of the feeding period, the mode of the group being at approximately 1·0 cm. These tingles would start the following season at this size and might be expected to reach about 2·0 cm. by July, i.e. by the end of their first year. Dredged samples of tingles collected during the summer which contain fair numbers of very small tingles usually have a suggestion of a peak around this figure; unfortunately, as yet no method has been devised for collecting adequate samples of these small tingles, the majority of which pass through the rings of an ordinary oyster dredge.

That a growth rate similar to that observed in 1941 prevailed among female tingles in other years is shown by Figs. 4 and 5, which show the results of

TABLE III. SIZE DISTRIBUTION OF *UROSALPINX*

Shell height cm.	River Blackwater 15 July 1939		Thurslett Creek R. Blackwater 15 May 1940	River Roach 13 May 1939
	Females	Males	Females	Females
1·6	—	—	—	1
1·7	—	—	—	3
1·8	—	—	—	2
1·9	—	—	—	7
2·0	—	—	—	8
2·1	2	2	1	4
2·2	1	2	1	7
2·3	4	6	1	5
2·4	7	4	1	1
2·5	11	7	4	13
2·6	14	7	11	12
2·7	23	14	16	15
2·8	17	9	27	19
2·9	16	9	23	13
3·0	12	5	30	9
3·1	9	2	21	14
3·2	2	1	38	7
3·3	7	—	23	14
3·4	1	—	13	8
3·5	2	—	19	3
3·6	1	—	5	2
3·7	—	—	8	—
3·8	—	—	1	3
3·9	—	—	1	2
4·0	—	—	3	1

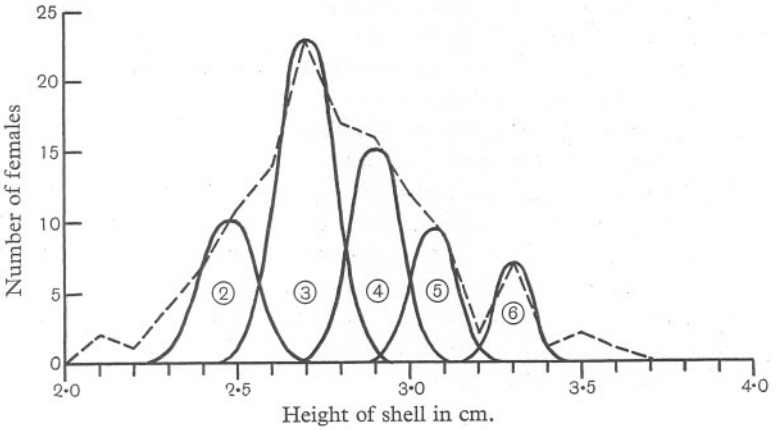

Fig. 4. Size-distribution curves of females from a sample of *Urosalpinx* dredged from the River Blackwater on 15 July 1939.

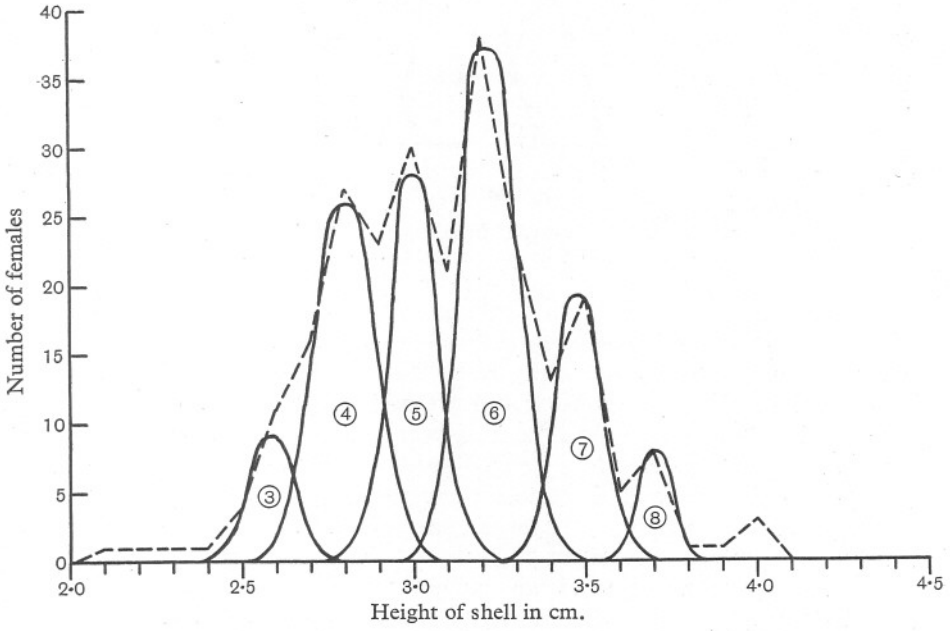

Fig. 5. Size-distribution curves of females from a sample of *Urosalpinx* dredged from Thurslett Creek, River Blackwater, on 15 May 1940.

analysing samples of tingles (Table III) dredged from the River Blackwater in 1939 and 1940. Both samples show a series of successive modes at intervals of about 0·2 cm., the position of the modes agreeing reasonably well with the corresponding sample of the year 1941, when due allowance is made for the lateness of the season in that year.

Although a number of samples of tingles have been collected from other rivers in Essex, only one (included in Table III) contains sufficient females to permit of the resolution of the frequency curve into its components. This sample was obtained in 1939 from the River Roach, a tributary of the Crouch, and is shown in Fig. 6. Successive modes are rather more distant than in the Blackwater collections, being approximately 0·25 cm. apart, indicating a more rapid growth rate in the River Roach. At least seven age groups are represented in this sample, the first mode representing most probably the 3-year-old group (cf. Fig. 2).

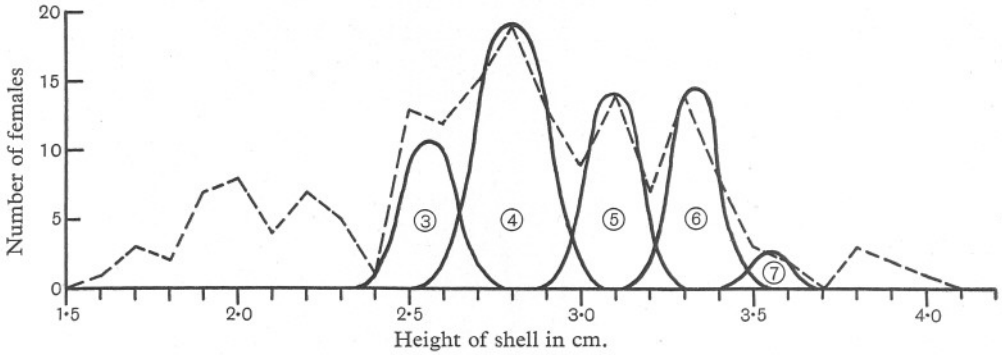

Fig. 6. Size-distribution curves of females from a sample of *Urosalpinx* dredged from the River Roach on 13 May 1939.

Summarizing the information obtained concerning growth rate in female tingles, from both size-frequency curves and the study of growth marks, it seems probable that in July, when a new batch of young emerges from the capsules, the 1-year-old group would average about 2·0 cm. in height, the 2-year-old 2·5 cm., 3-year-old 2·7 cm., 4-year-old 2·9 cm., 5-year-old 3·1 cm., 6-year-old 3·3 cm., 7-year-old 3·5 cm., 8-year-old 3·65 cm., 9-year-old 3·8 cm., 10-year-old 3·9 cm., and thereafter successive age groups would occur at intervals of 0·1 up to 4·3 cm., the largest size recorded. It seems probable that this 4·3 cm. specimen was about 14 years old.

Frequency curves derived from catches of male tingles obtained from Thornfleet in 1941 (see Table II) when analysed show successive modes at intervals of 0·2 cm. or rather less (Fig. 7). The July sample contains representatives of six age groups without taking into account tingles below 2·3 cm. in height. As in the females from these catches, the July sample shows two modes not present in May, so that the first mode in May must comprise indi-

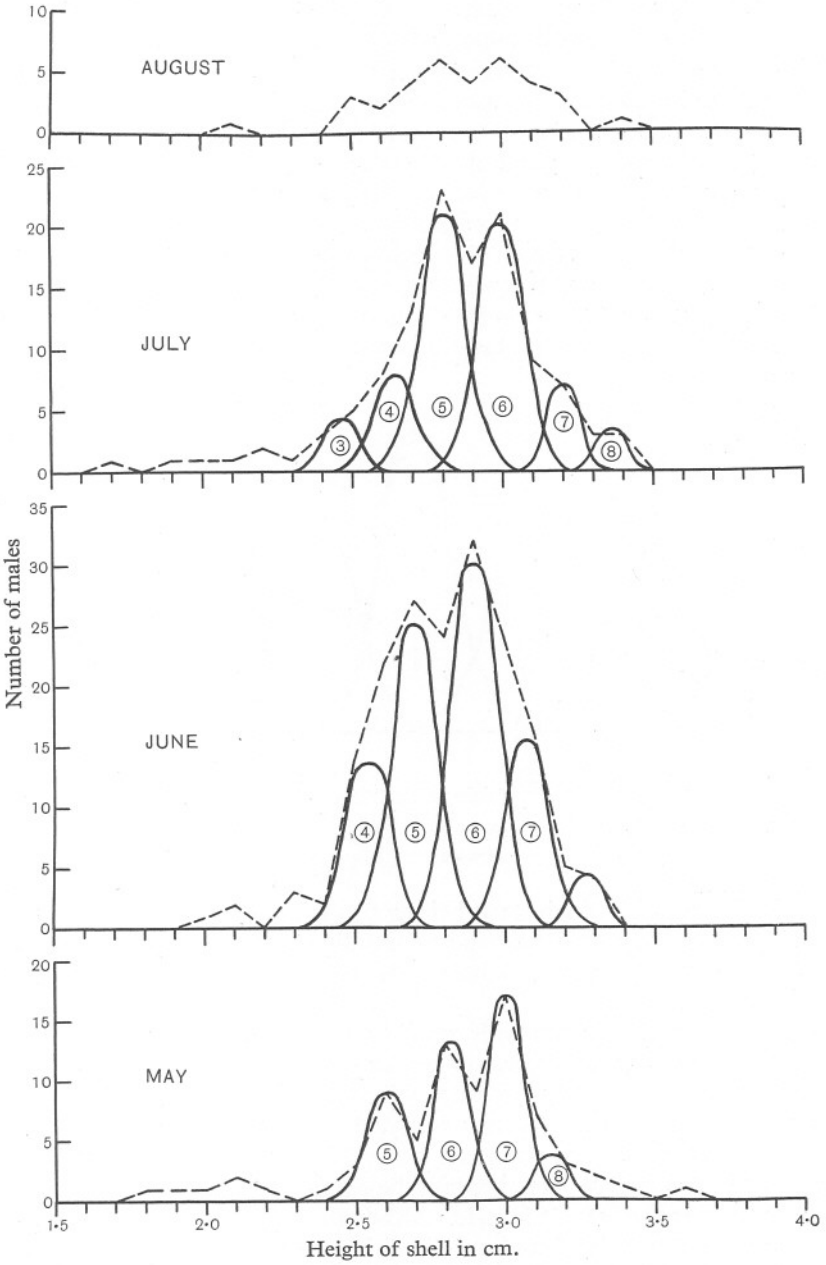

Fig. 7. Size-distribution curves of males from samples of *Urosalpinx* hand collected from the beach of Thornfleet, River Blackwater, during 1941. The May sample also includes those dredged in the adjacent channel on the same day.

viduals at least 3 years old; evidence presented below shows that they are in fact older. The July sample contains tingles down to 1·7 cm. in height which cannot belong to a group with a mode of 2·46 cm., consequently the latter must consist of individuals at least 2 years old. Comparison of Figs. 7 and 8, the latter being the analysis of a sample taken in July 1939 from the River Blackwater (see Table III), reveals an extra mode at 2·3 cm. in the 1939 sample in addition to one at 2·51 cm. corresponding to the first mode (2·46 cm.) in the July 1941 sample. When it is remembered that the 1941 season was very late the slight discrepancy between the position of the modes in the two years is explained. The July 1941 frequency curve (Fig. 7) would therefore, if complete, show a further mode slightly below 2·3 cm., but this age group could not reasonably contain individuals as small as 1·7 cm., the smallest size in-

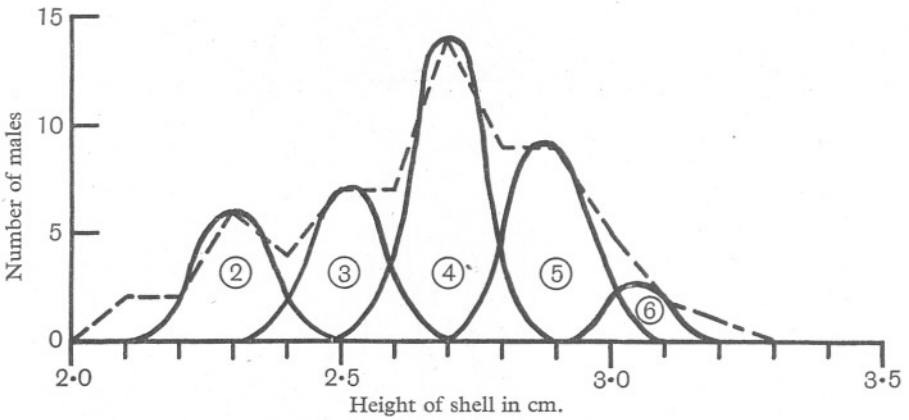

Fig. 8. Size-distribution curves of males from a sample of *Urosalpinx* dredged from the River Blackwater, 15 July 1939.

cluded in the sample, which must belong to a still earlier group. We therefore conclude that the first mode shown by the July 1941 sample (Fig. 7) represents the 3-year-old group and consequently the first mode in May 1941 represents the 5-year-old tingles.

In males growth thus proceeds roughly as follows: 1st summer, 1·0 cm.; 1st year, 1·0–1·8 cm.; 2nd year, 1·8–2·3 cm.; 3rd year, 2·3–2·5 cm.; 4th year, 2·5–2·7 cm.; 5th year, 2·7–2·9 cm.; 6th year, 2·9–3·1 cm.; 7th year, 3·1–3·25 cm.; 8th year, 3·25–3·4 cm.; 9th year, 3·4–3·55 cm.; 10th year, 3·55–3·65 cm.; and thereafter at about 0·1 cm. per annum. The largest male so far collected, viz. 3·9 cm., would therefore be about 13 years old, a similar age to that deduced for the largest female. Although both males and females up to this extreme age may be found on occasion, no substantial number of males over 3·6 cm. or females over 3·9 cm. have been collected, so that the approximate duration of life in *Urosalpinx* is 10 years.

Parasites

During the examination of dredged samples of *Urosalpinx* for sex seven specimens have been found containing what appears to be a very specialized arthropod parasite, probably an isopod, in the liver region. All the tingles so affected came from the oyster beds in the mouth of the River Blackwater. In addition one individual from the same river has been found with the gonad completely riddled with rediae containing cercariae agreeing exactly with *Cercaria sensifera*, which Stunkard & Shaw (1931) have described in *Urosalpinx* from Woods Hole, U.S.A. These authors point out that it is possible that *Cercaria sensifera* is identical with *C. purpurae* (Lebour, 1912), found in *Nucella lapillus* in British waters, although the survival and establishment of an alien trematode is by no means impossible.

Breeding

As in several other carnivorous gastropods the eggs of *Urosalpinx* are deposited in tough horny capsules each of which contains several eggs. The capsules are attached to the substratum in clusters and from them fully formed young tingles emerge. The egg capsules have been described and compared with those of *Nucella* and *Ocenebra* by Orton & Amirthalingam (1929), while the actual method of formation of the egg capsule and the structure of the organs involved has been studied by Fretter (1941) in a variety of British stenoglossan gastropods, including *Ocenebra* and *Nucella*. The deposition of egg capsules usually begins during late April or early May and continues during June and July. Spawning reaches its maximum during June and thereafter declines. During 1940 the first few capsules were found in the River Blackwater in the second week in April, but although the winter had been exceptionally severe the spring was milder than usual. In 1941, when the spring months were exceptionally cold, no spawn was found until the third week of May. During the last fortnight in May, when the water temperature rose steadily from 11·6 to 13·4° C., the number of tingles dredged in the act of spawning and the total amount of spawn caught per day increased steadily. It seems probable therefore that spawning begins each year in British waters when the sea temperature in its seasonal rise reaches 12–13° C. This estimate agrees well with the figure given by Galtsoff *et al.* (1937) for Delaware Bay, U.S.A., where capsule deposition is stated to begin when the water temperature has reached 13·9° C. The bulk of the spawn is deposited during May and June; but small numbers of freshly laid capsules may be found in August, e.g. a sample of spawn collected from the River Blackwater on 27 August 1940 contained about a dozen freshly deposited capsules—a small number contained embryos in early stages of development, but the great majority of the capsules had already hatched or were in process of hatching. The earliest date at which capsules have been seen from which the young tingles had emerged

is 2 July (1940); as already mentioned, the first few capsules laid in that year were seen during the second week in April. Several batches of spawn obtained in June 1940 showed no embryos advanced beyond the early shelled stages. In this year, although a few capsules were seen in April no substantial quantity was deposited until May. We may therefore conclude that incubation of capsules under natural conditions occupies about two months.

Further observations made in 1941 confirm the above estimate. Deposition of capsules began in the Blackwater during the third week in May, spawning being in full swing by the first week in June. No hatching had occurred in samples obtained in June, no capsules containing embryonic stages later than morulae; but samples of spawn procured on 23 July showed a large number of capsules with young tingles emerging. The period of incubation was therefore 7–8 weeks.

In the laboratory, incubation of capsules deposited by tingles in experimental tanks at Conway has been carried out in plunger jars, with and without frequent changes of water. The periods of incubation observed were 27–32 days at an average temperature of 22·6° C., and 44–50 days at an average temperature of 18·3° C. According to the records given by Orton & Lewis (1931) of sea temperature in the River Blackwater during the four years 1926–8 and 1930, the mean during the period mid-May to the end of August, when the spawn of *Urosalpinx* is being incubated, varies between 13·5 and 19° C., so that the period of incubation under natural conditions in this river might be expected to exceed slightly the period of 44–50 days recorded above. This expectation accords very well with the period of about two months deduced from observations of the time of commencement of spawning and hatching. In years when the water temperature rises rapidly during spring, spawning may begin in April, but it is not usually general until May or, in late seasons such as 1941, until June. Emergence of young tingles therefore generally occurs about the middle of July.

Federighi (1931) found that the average period of incubation under laboratory conditions of *Urosalpinx* capsules from Hampton Roads, Va., U.S.A., varied between 36 and 44 days with a mean of about 40 days. Water temperature in these experiments varied from 22 to 28° C. Galtsoff *et al.* (1937) give the period of incubation in Delaware Bay as varying from 21 to 53 days. In American waters spawning of *Urosalpinx* apparently occurs throughout the summer and autumn in many localities: e.g. in Delaware Bay it is stated that spawning begins early in April when the water temperature reaches about 14° C. and continues until late November. In Cape Cod waters two separate periods of spawning were observed, the second occurring during the second half of September. There was no evidence until 1941 that a second period of spawning ever occurred in this country, but in that year tingles kept in a tank at Conway spawned intermittently during late August and September, the last capsules being deposited on 30 September. During the latter part of this period water temperature fluctuated between 13 and 17° C. During

September groups of 11, 10, 12 and 14 capsules were laid, all of which, as will be seen later, are less than the usual number deposited in one laying early in the season.

Not a great many opportunities have occurred of noting the number of capsules deposited by a single tingle at one laying, but about a dozen such groups which have been noted during the normal spawning season have ranged from 20 to 35, with an average of 25 per female. This is in fair agreement with the work of Federighi (1931) in America, who found that an average of 28 capsules was deposited by isolated females. Galtsoff *et al.* (1937) state that in Delaware Bay females may deposit as many as 50 capsules, which are grouped in clusters. It is possible therefore that the groups noted above, averaging 25 capsules, may form but a part of the total production of the drills concerned. In practice two or more drills are frequently found spawning together on the same shell, while a large stone or pile of old chain at or about low-water mark may harbour a dozen or more spawning drills. This does not indicate, I think, a social habit among drills but merely the close occupation of the available satisfactory spawning sites. Capsules are deposited at the rate of three or four per day. Orton (1930) states that there is an inshore migration of *Urosalpinx* in spring and early summer, and that the species spawns heavily in shallow water. This is very noticeable in the river Blackwater, but very large numbers spawn also at all depths, and the selection of a spawning site seems to be governed mainly by the presence of abundant 'clocks', stones, or other firm objects, rising well clear of the bottom and offering situations free from silt. *Urosalpinx* kept in a tank at Conway deposited capsules most frequently on the vertical sides of the tank or in situations which were overhung.

On American oyster beds the average number of embryos per capsule is stated to be 9 in Hampton Roads, Va., the numbers varying from 3 to 20, and 8 in Delaware Bay, varying from 0 to 20. On British beds *Urosalpinx* is more prolific, for 1423 capsules from different localities averaged 11·74 embryos per capsule; of this total 823 capsules contained early stages and 600 contained shelled veligers practically ready to hatch. The early stages averaged 12·47 embryos per capsule and the shelled stages 10·74 per capsule. There is therefore a mortality during incubation of 1·73 embryos per capsule, or 13·9%. This figure is much lower than that of Federighi (1931), who found only an average of 5·1 larvae emerging per capsule (the average number of eggs being 8·8)—a loss of 42%. The number of embryos per capsule may vary from 1 to 29 in this country, but in capsules from one parent there is little variation.

If the average number of capsules laid per female in this country is assumed to be 25, from each of which on the average 10·74 drills emerge, then each female drill gives rise to 268·5 young. It is probable that the actual figure is somewhat higher, as Galtsoff *et al.* (1937) have shown that after depositing one group of capsules females may move away and later deposit further batches. It seems likely therefore that from the capsules laid by one female on British beds not less than 300 young drills emerge.

In order to determine at what age and height of shell deposition of egg capsules begins, 21 tingles varying from 1·8 to 2·4 cm. in height were placed in a floating wooden wire-covered cage in one of the tanks at Conway towards the end of June 1941 and a daily examination made for egg capsules. Barnacles were provided as food. Thirteen of the 21 small tingles survived the summer and only three were noted to deposit egg capsules. The largest group of capsules deposited numbered only 13, the others being considerably smaller. Of the 13 tingles remaining at the end of the summer only three were females; these were the largest of the group and were almost certainly 2 years old. The result of this experiment was inconclusive, but suggests that 2-year-old females below 2·5 cm. in height at the beginning of the season deposit a small number of egg capsules. In other lots of tingles two individuals measuring 2·4 cm. and one measuring 2·5 cm. have been noticed in the act of spawning; each of these laid only a small number of capsules in late summer. On the other hand, during May 1941, when breeding was just beginning, it was noticed that the majority of the females 2·2 cm. or less in height had undeveloped gonads and accessory reproductive organs and appeared to be definitely immature. It seems therefore that some of the largest of the tingles below 2·5 cm. in height in July and August, which are probably 2 years old, or possibly unusually small 3-year-olds (see Fig. 2), do spawn to a slight extent, but it is highly probable that the amount of spawn deposited is insignificant in relation to the production of older tingles. The question is of some practical importance, since such small tingles are only retained in very small numbers by the dredge and at present there is no other practicable method of removing them from the beds below low-water mark.

Among the numerous very large females examined only two or three have been found with the gonad in an exhausted condition. None of these was below 4·1 cm. and one was 4·3 cm. in height, the largest size yet recorded; on the other hand, individuals up to 4·1 cm. have been observed in the act of depositing capsules. The latter could not be less than 10–12 years old, so that the reproductive life of each female may extend over 7 years (ignoring the first 2 years when some capsules may be deposited), in each of which she may give rise to 300 young.

It is very noticeable from Fig. 3 how the dominant age group changes during the season among the female tingles collected from the beach in Thornfleet, River Blackwater. In Fig. 9 the composite May sample shown in Fig. 3, which is made up of two separate catches, the one dredged and the other hand collected on the same day, is split up into its components (see Table IV), males and females being graphed separately. The females in the shore sample are seen to belong principally to one year group, which by comparison with Fig. 3 is seen to be the 4-year-old class, with smaller numbers of 3- and 5-year-old tingles. The sample dredged on the same day in the channel adjacent to the beach shows that 5-, 6-, and 7-year-old tingles were also present in numbers in the creek as well as of course large numbers of 1-, 2-, and 3-year-old females

which would not be picked up in quantity by the dredge; but the first tingles to come to shore for spawning were mainly 4 years old. The males accompanying these females belong mainly to the 5- and 6-year-old groups (see Fig. 7), with a few younger individuals, but the dredged sample shows that numerous older drills remain in the channel.

In June the 4-year-old females (mode at 2·8 cm.) are dominant on the shore with comparatively small numbers of 3-year-olds and a few smaller tingles, but a month later, in July, the 3-year-olds have enormously increased and form the dominant group, although older tingles are also fully represented, while

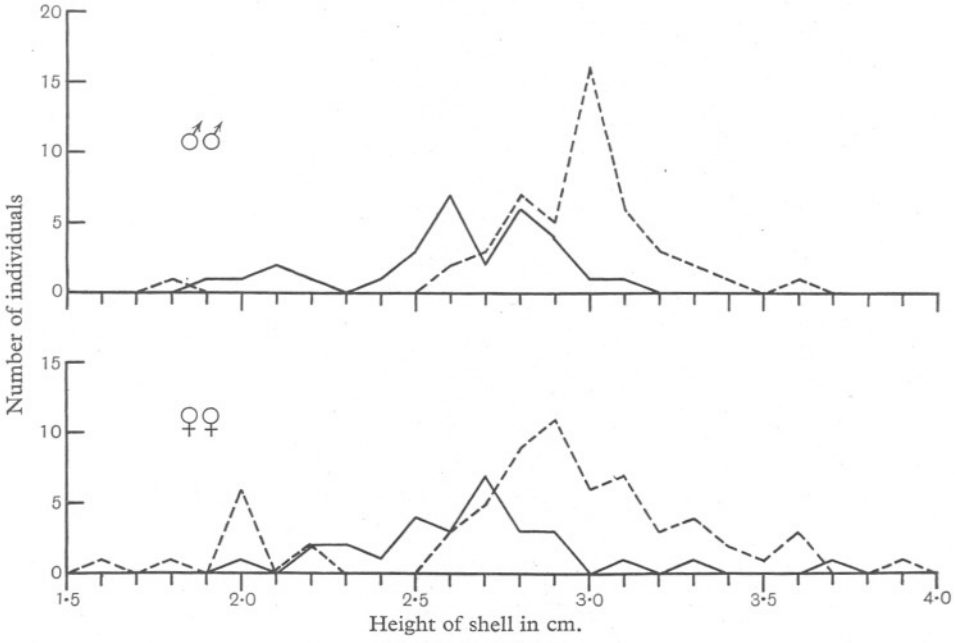

Fig. 9. Comparison of hand-collected (continuous line) and dredged (pecked line) samples of tingles collected on the same day (24 May 1941) from Thornfleet, River Blackwater.

there are a few 2-year-old and even younger tingles. Among males the tendency is similar, for as the season advances the younger age groups appear on the shore in increasing numbers, but the proportion of 1- to 4-year-old males is never commensurate with their numerical strength on the beds.

It should be remembered that these samples from Thornfleet are hand collected and therefore representative of the population on the beach at the time. The females have moved inshore primarily for the purpose of spawning, and it appears therefore that the 3-year-old females spawn about a month after the older groups, while only a few of the 2-year-old and smaller tingles migrate inshore. It has been noted that when such small tingles were isolated some deposited egg capsules, but each drill produced only a fraction of the usual

number laid by mature individuals. These facts, taken in conjunction with the absence of significant numbers of 1- and 2-year-old females from the spawning population on the beaches, tend to prove that full spawning does not occur until the tingles are 3 years old.

TABLE IV. SIZE DISTRIBUTION OF DREDGED AND HAND-COLLECTED SAMPLES OF *UROSALPINX* FROM THORNFLEET, 24 MAY 1941

Shell height cm.	Dredged		Hand-collected	
	Females	Males	Females	Males
1·6	1	—	—	—
1·7	—	—	—	—
1·8	1	1	—	—
1·9	—	—	—	1
2·0	6	—	1	1
2·1	—	—	—	2
2·2	2	—	2	1
2·3	—	—	2	—
2·4	—	—	1	1
2·5	—	—	4	3
2·6	3	2	3	7
2·7	5	3	7	2
2·8	9	7	3	6
2·9	11	5	3	4
3·0	6	16	—	1
3·1	7	6	1	1
3·2	3	3	—	—
3·3	4	2	1	—
3·4	2	1	—	—
3·5	1	—	—	—
3·6	3	1	—	—
3·7	—	—	1	—
3·8	—	—	—	—
3·9	1	—	—	—

SEX RATIO

American work gives no indication of any abnormality in the sex ratio of catches of *Urosalpinx*. It has already been recorded, however (Cole, 1941), that the sex ratio in dredged catches from British oyster beds shows some striking peculiarities. Table V gives the proportions of the two sexes in all dredged catches of *Urosalpinx* obtained from the River Blackwater during the years 1938–41. Allowance should be made when studying this table for the fact that the 1941 season was characterized by an abnormally cold spring, the season being reckoned as roughly a month later than usual on the Blackwater. Bearing this in mind it is clear that, generally speaking, males and females are equally abundant in dredged catches of tingles at the outset of the season, but thereafter the proportion of females rapidly increases reaching a peak of over 90% during June and declining again in July and August to approximately 50%. It will be noted also that the seasonal curve of percentage of females follows very closely the intensity of spawning. This suggests that as the females move about and take up their spawning positions they become more

TABLE V. DREDGED CATCHES OF *UROSALPINX* FROM THE RIVER BLACKWATER

Date	Locality	Females	Males	Total	% females
7. iv. 38	R. Blackwater	56	55	111	50·5
10. iv. 40	Thornfleet, R. Blackwater	13	13	26	50·0
2. v. 40	Thornfleet, R. Blackwater	82	58	140	58·1
8. v. 40	Thurslett Creek, R. Blackwater	124	19	143	86·7
15. v. 39	Thurslett Creek, R. Blackwater	55	13	68	80·9
15. v. 40	Thurslett Creek, R. Blackwater	246	33	279	88·2
15. v. 39	South Shore, R. Blackwater	69	28	97	71·1
19. v. 41–27. v. 41	Thurslett—Old Mell Beacon, R. Blackwater	213	75	288	74·0
20. v. 41–29. v. 41	S. Shore, R. Blackwater	58	24	82	70·7
21. v. 41	Nass End, R. Blackwater	65	61	126	51·6
24. v. 41	Thornfleet, R. Blackwater	65	47	112	58·0
10. vi. 41	Thurslett Creek, R. Blackwater	84	13	97	86·6
13. vi. 40	R. Blackwater	57	3	60	95·0
17. vi. 41	Thurslett—Old Mell Beacon, R. Blackwater	112	7	119	93·1
24. vi. 41	Thurslett—Old Mell Beacon, R. Blackwater	149	22	171	87·2
2. vii. 40	R. Blackwater	81	30	111	73·0
15. vii. 39	R. Blackwater	129	68	197	65·5
26. viii. 40	R. Blackwater	92	68	160	57·5

susceptible to capture by an oyster dredge, while the males remain in positions where the dredge rarely captures them. It is known (see Federighi, 1931) that females tend to crawl up on to objects raised off the sea bottom to deposit their spawn, and in these situations they may be particularly liable to capture; but it is highly probable that on most grounds the scraping bar of the dredge digs slightly into the bottom, for the contents of a dredge bag usually includes some mud and a good deal of fine gravel and shell fragments. Nevertheless, female tingles upon objects slightly raised off the bottom may be reasonably considered as particularly liable to be included in a dredge bag; it is therefore likely that the proportion included will increase as the intensity of spawning increases and more females take up spawning positions. It is curious, however, that the males are not with the females or at least crawling among the cultch in search of food. Such evidence as we possess concerning the behaviour of the male tingles does in fact suggest that they are to be found with the spawning females. A sample hand collected on 24 May 1941 from stones, old drain pipes, mooring chains, etc., on the beach at low-water mark in Thornfleet, River Blackwater, includes practically equal numbers of males and females (Table VI), showing that at the beginning of the spawning season males are also on the move and may be found on raised objects alongside the females. The dredged catch from the channel adjacent to the beach, collected on the same day (see Table V) contained only 42 % of males. Slightly later in the year at the height of the spawning season similar collections off the same raised objects in Thornfleet gave a preponderance of females (see Table VI), but males were also very numerous, and the percentage of females did not approach the high figures shown by dredged catches at this season. At the end of

TABLE VI. HAND-COLLECTED SAMPLES OF *UROSALPINX* FROM THORNFLEET, RIVER BLACKWATER

Date	Females	Males	Total	% females
24. v. 41	29	30	59	49·2
10. vi. 41	316	176	492	64·2
20. vi. 40	33	23	56	58·9
8. vii. 41	245	119	364	67·3
26. viii. 41	30	34	64	47·0

August another collection from the same raised objects gave approximately equal numbers of males and females. These hand-collected samples show that males are to be found along with spawning females on raised objects throughout the breeding season, at least on inshore grounds; it is all the more remarkable therefore that dredged catches from offshore contain so few males at this season. The difference in size between males and females of the same age will lead to some selection of females by the dredge, for it is clear from the size-distribution curves of dredged catches (e.g. that shown in Fig. 4) that drills below about 2·7 cm. are particularly liable to be lost through the meshes of the dredge bag. Thus the first three year classes of males with modes at *ca.* 1·8, 2·3, and 2·5 cm. at midsummer are likely to be missed, whereas only the first two year classes of females with modes at *ca.* 2·0 and 2·5 cm. are equally liable to pass through the meshes.

The continued collection of large numbers of females and small numbers of males during normal dredging would, in a population with a normal sex ratio, bring about a considerable alteration in the proportion of the two sexes in that population within a few years. For this reason on well-worked grounds such as those in Thurslett Creek, River Blackwater, one might expect to find, at least occasionally, a preponderance of males, yet in the nineteen dredged samples listed in Table V the highest proportion of males is 50%. In a previous note on this question (Cole, 1941) I have mentioned the possibility that sex change in a proportion of the population might be responsible for the observed abnormalities in the sex ratio. There is, however, no evidence whatever to support this hypothesis. During 1941, 20 drills caught in the act of spawning were isolated during the summer and examined in October; 19 were female and one had died. Careful search has been made among thousands of drills for individuals with characters intermediate between those of males and females, but none has been found, yet the sexes are easily recognizable and the penis is quite a large organ, so that if variations occurred in this organ they would be easily detected. The inference to be drawn is that sex change does not occur.

FEEDING

It is extremely difficult to evaluate the destructiveness of tingles, since it is impossible to determine the number of very small oysters killed on the beds. By keeping tingles enclosed with oysters of various sizes some idea of the

potential damage may, however, be obtained. This has been done at Conway during the summers of 1940 and 1941. Known numbers of tingles have been enclosed in wooden wire-covered floating cages with ample supplies of either 1- or 2-year-old oysters, and daily counts made of the quantity destroyed. Maximum and minimum water temperatures were also recorded. Drilling may begin as soon as the water temperature exceeds 11–12° C.; this usually occurs during April or early May, but very little feeding occurs at this time as the drills are actively spawning. In June, when the peak of the spawning season has been passed, intensive feeding begins and continues until the water temperature again drops below 11–12° C., usually towards the end of October.

TABLE VII. RATE OF DESTRUCTION OF SPAT AND BROOD OYSTERS BY *UROSALPINX*

Type of oyster	Year	No. of tingles	Oysters destroyed	Period of feeding days	Rate per day per tingle
1-year-old spat	1940	22	701	96	0·332
,, ,,	1940	36	173	10	0·481
,, ,,	1941	39	2168	127	0·438
2-year-old brood	1940	12	45	109	0·034
,, ,,	1941	30	92	98	0·032

In Table VII are given the rates of destruction of 1-year-old spat and 2-year-old brood oysters recorded during 1940 and 1941. For these experiments mixed lots of tingles varying in age from 3 to about 8 years were employed, all collected originally from the River Blackwater. In Fig. 10 the daily rate of destruction of 1-year-old spat in 1941 is correlated with the average daily water temperature. It will be noted from Table VIII that the rate of destruction of spat during July and August is considerably above the final average figure for what was practically the whole feeding period. It is very noticeable how the rate of feeding fell away immediately the water temperature dropped below 14° C. This figure may be regarded as the minimum at which full feeding activity begins. There was no close correlation between water temperature and rate of feeding until mid-July when the breeding season was practically over. During the short period between 22 July and 6 August spat were destroyed at the rate of 0·90 per tingle per day. There is a fairly good general correlation between water temperature and rate of feeding from mid-July onwards, but it is apparent that there is also a seasonal rhythm to some extent overriding the effect of temperature; tingles feed voraciously immediately after the completion of spawning, but thereafter a general decline in the rate of feeding sets in, although it rises and falls also in response to changes in water temperature.

The average duration of the feeding period on the east coast oyster beds may be deduced from records of sea temperature in the River Blackwater covering the five years 1926–30 given by Orton and Lewis (1931). It is apparent that on the average the water temperature exceeds 14° C. about mid-May and again

TABLE VIII. OYSTER SPAT DESTROYED PER DAY DURING 1941 BY 39 *UROSALPINX*, TOGETHER WITH THE MEANS OF THE DAILY READINGS OF MAXIMUM AND MINIMUM WATER TEMPERATURE

Date	Spat destroyed	Average water temperature ° C.	Date	Spat destroyed	Average water temperature ° C.	Date	Spat destroyed	Average water temperature ° C.
June			July			Sept.		
17	1	17	30	36	15·5	10	12	16
18	0	18·5	31	30	15·75	11	26	15·5
19	0	18·5				12	16	14·5
20	0	18·5	August			13	15	14·25
21	0	16	1	36	16·5	14	18	No record
22	0	No record	2	31	18	15	13	14·5
23	2	18	3	40	18	16	11	14
24	4	19	4	31	17·5	17	10	14·5
25	8	19·5	5	48	15·5	18	20	15·25
26	6	19	6	27	13·25	19	8	14·5
27	10	19·25	7	24	13	20	22	14
28	13	18·5	8	16	14	21	8	No record
29	18	No record	9	19	15	22	23	14
30	9	19	10	22	15·75	23	12	14
			11	23	16·5	24	10	14·25
			12	30	16·75	25	10	14·25
July			13	29	15·5	26	13	15·25
1	16	21	14	24	15	27	9	15·5
2	17	21·25	15	26	14·5	28	12	16·25
3	32	22	16	21	13·75	29	9	16
4	27	18·75	17	19	14·5	30	8	15
5	10	19	18	21	15·75			
6	32	19·5	19	24	16·75			
7	15	20	20	22	16·75	Oct.		
8	17	19	21	16	16·25	1	2	13·5
9	27	19	22	11	15·75	2	0	13
10	21	20	23	17	16	3	3	13
11	28	20·5	24	16	15·25	4	3	12·5
12	12	21	25	17	15·25	5	1	No record
13	26	20·5	26	27	16·75	6	1	12·5
14	44	21·5	27	13	16·5	7	4	13·5
15	37	21·25	28	15	15·25	8	1	14
16	19	18·75	29	12	16	9	1	14·75
17	23	16·75	30	11	14·75	10	4	14·75
18	31	17	31	15	15	11	6	14·75
19	23	17				12	3	No record
20	16	No record	Sept.			13	2	13
21	31	16·5	1	28	16	14	2	11·5
22	28	15·75	2	22	16	15	2	11·5
23	29	15·25	3	21	16·25	16	1	10
24	37	17·75	4	24	17	17	1	11
25	45	19·25	5	22	17·25	18	1	10
26	32	18·75	6	31	18	19	2	No record
27	37	No record	7	22	17·5	20	3	11·5
28	43	18·5	8	19	17	21	3	10·75
29	22	18	9	29	17	22	2	10

falls below this level early in October. The duration of the feeding period is therefore approximately 5 months. Assuming an average rate of destruction of 0·385 spat per day, one tingle feeding exclusively on such 1-year-old spat would destroy 59 spat. Valuing these spat at four to the penny, a conservative estimate, the potential saving for each tingle removed is 1*s.* 3*d.* per season.

According to Federighi (1931), *Urosalpinx* from Hampton Roads, Va., U.S.A., begins to feed when the temperature rises above 15° C. and ceases when the temperature falls below 10° C. Similar studies in Delaware Bay (Galtsoff *et al.* 1937) indicate that drilling ceases altogether at 9·5° C. Drilling had not ceased completely at Conway when the water temperature fell below 11° C. (Fig. 10), but activity was practically negligible at temperatures below 13° C. Galtsoff *et al.* (1937) obtained a good correlation between the number of oysters destroyed in baskets let down on to the oyster beds in New Jersey and the prevailing water temperature.

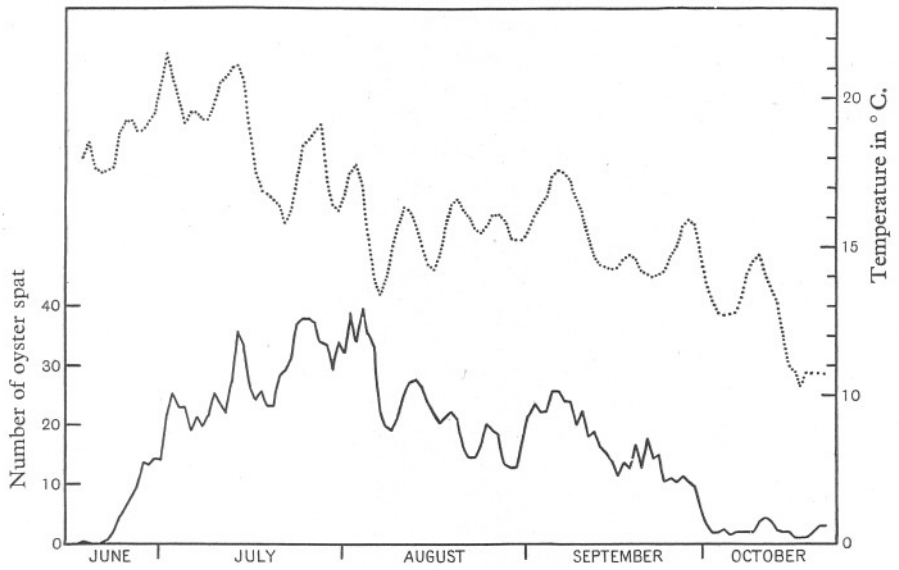

Fig. 10. Daily rate of destruction of oyster spat by 39 *Urosalpinx* during 1941 correlated with water temperature. Figures of both temperature and spat destroyed have been smoothed in threes.

The rate of destruction of spat and larger oysters as determined by American workers is considerably higher than that observed in the experimental cages at Conway. In the U.S.A. the ravages of *Urosalpinx* have been found to vary in different localities. In North Carolina waters a single *Urosalpinx* is stated (Galtsoff *et al.* 1937) to destroy 30–200 oysters in a season, depending on their size; the basis for this statement is not given. Certain other general statements are given, such as that of Nelson (1931), who states that in Delaware Bay *Urosalpinx* kills $1,000,000 worth of oysters annually, and that of Galtsoff *et al.* (1937) who say that there are many localities in Long Island Sound and in Chesapeake Bay where 60–70% of the seed oysters are destroyed annually. These round figures are much on a par with the assertion by Essex cultivators that at least half the spat in the Blackwater is killed annually by tingles, a

figure repeated by Orton (1937); there is a great lack of precise information on the subject. The only reasonably precise American determination of the rate of drilling is that of Galtsoff *et al.* (1937), who state that during a 9 weeks season in New Jersey waters each drill destroyed on the average 0·34 adult oysters per week. These workers also state that over 300 spat may be destroyed in a single season by each drill; the basis of this estimate is not given, but one suspects that the rate of destruction of spat has been determined for a short period in midsummer and multiplied by an appropriate factor depending on the relative length of the period of observation and the season of activity. If this is so, the estimate of total damage is clearly much too high, as it has been shown here that the maximum rate of drilling is only maintained for a short period in midsummer after the completion of spawning.

In order to determine the rate of destruction of newly settled spat by young tingles, 20 tingles which had been hatched under laboratory conditions were isolated with a plentiful supply of oyster spat which had settled the same season. The tingles averaged approximately 0·35 cm. in height, while the oyster spat varied in diameter from 3 to 7 mm. The experiment was conducted in a plunger jar with sea water of normal salinity, and the average water temperature during the period of the experiment was 20° C. A total of 162 spat was drilled and destroyed during a period of 140 hr. by the 20 young tingles, giving an average rate of 1·39 spat per day (24 hr.) per tingle. This experiment proves that freshly emerged tingles may cause extensive havoc amongst newly settled spat. At this stage the tingles are for practical purposes immune from capture. Young tingles feeding entirely on such small spat might destroy as many as 100 each during the period July to September inclusive.

Orton (1927), in the course of some preliminary experiments, states that the average time taken for *Urosalpinx* to drill through an oyster was 5·7 days; the size of these oysters is not given, nor the water temperature, but it is implied that the oysters were larger than brood size. It is not suggested, however, that *Urosalpinx* consumed oysters continually at this rate, but that the average rate of drilling in 15 cases was as stated above. His figure for brood oysters 1–2 in. long was 4·1 days (10 oysters), but eight brood oysters nearly bored through had been drilled for an average of 4·5 days before being abandoned. Orton gives a general estimate of the time taken to drill a brood oyster as 5–6 days. This is very different from the average rate of destruction of such oysters as determined at Conway, viz. 33 days; this does not mean that each drill takes 33 days to destroy an oyster, for drills do not continuously attack one oyster after another; considerable periods of inaction occur, so that the rate of drilling, as distinct from the rate of destruction, may be much the same as Orton observed.

The high rate of destruction of adult oysters observed in America (see above) is all the more remarkable when we recall that on the average no less than 33 days was needed to destroy one 2-year-old oyster under experimental conditions at Conway. We are forced to conclude either that *Urosalpinx* has changed

its habits under the conditions on British beds or that the shell of *Ostrea edulis* presents a much more effective barrier to *Urosalpinx* than that of *Gryphaea virginica*. In this country the general belief among oystermen is that the American tingle is principally an enemy of the spat and early brood stages and that damage to marketable oysters is negligible. Careful observations on the beds and food-preference experiments at Conway confirm this view.

Food preference has been investigated by enclosing samples of tingles in floating cages with two or more sorts of food and noting daily the number of drills attacking each kind of food. Materials offered as food have been oysters of all ages from early settled spat to 3 years old, mussels, and barnacles. The drills used were obtained from the River Blackwater and were all over 2·5 cm. in height. Certain general conclusions emerge quite clearly from these experiments. Drills offered barnacles or spat oysters (1-year-old) divided their attention about equally between the two kinds of food. Drills offered spat oysters and mussels destroyed practically all the spat before attacking the mussels. When the spat were all finished the mussels were quickly destroyed, drilling occurring chiefly near the thin edge of the shell. Similarly barnacles were more attractive than mussels. Drills offered both spat and brood oysters (2 years old) destroyed practically all the spat before attacking the larger oysters. The latter were then attacked and slowly consumed. It was observed also that tingles showed a distinct preference for spat of thumb-nail size, not attacking very small spat until all the large spat had been destroyed.

We may therefore conclude that oyster spat and barnacles form the principal foods of *Urosalpinx* on the beds in the River Blackwater, but that larger oysters may also be attacked. Similar experiments made in America by Galtsoff *et al.* (1937) placed mussels ahead of seed oysters in the list of foods attractive to drills, barnacles being the most attractive of all. The lack of attractiveness of mussels to drills from the River Blackwater is probably due to the comparative scarcity of mussels in this river system. Orton (1929) has shown that *Ocenebra erinacea*, the British rough tingle, will attack most readily those food species which occur commonly in its normal habitat and that *Ocenebra* from areas where oysters do not occur will not readily attack oysters while other foods are available. It is possible therefore that *Urosalpinx* from, say, Whitstable, where mussels are abundant on certain parts of the ground, would exhibit a strong preference for this food. It is clear that in the River Blackwater *Urosalpinx* is primarily an enemy of the spat stage of the oyster.

Galtsoff *et al.* (1937) conclude from observations of feeding habits that drills are able to detect the presence of food and move towards it. As already noted, careful experiments made at Conway did not confirm this conclusion.

Economic Considerations

Since *Urosalpinx* has no free-swimming stage in its life history, and it is probable that migration is of no more than local significance, it is dependent for distribution upon transportation by human or other agencies to fresh areas.

There is therefore, theoretically, a good chance of localizing this pest and of controlling it in areas where it has already secured a foothold. The latter include, as already noted, the estuaries of the Blackwater, Colne and Crouch and the Kentish Flats off Whitstable. At present two methods of control are practised, in the first place all tingles seen are picked out of dredge hauls and taken ashore and, secondly, all spawn noted is collected and dried. Owing to the general depression in the oyster trade, brought about by the heavy mortality experienced as a result of the severe winters of 1939–40 and 1940–1, the amount of dredging done by the few boats that are now employed is insufficient to keep the pest in check, let alone eradicate it. The present slightly enhanced prices due to the general scarcity of oysters would normally result in rather more intensive working of the grounds; but this is hardly possible now on account of the shortage of suitable men and the operation of certain defence restrictions, e.g. the closing of parts of the oyster beds on the east coast, thus forming reservoirs in which tingles can multiply undisturbed. In normal dredging very few tingles less than three years old are captured, although these are most numerous on the grounds, simply because they are too small to be retained by the dredge bag, the rings of which are an inch across, and further, the close sifting of the dredge contents which is necessary to collect the smallest tingles is not in practice carried out. With the limited amount of labour employed and the scarcity of oysters on the grounds it is necessary to dredge and cull out as expeditiously as possible in order to secure enough oysters to pay for the day's working and consequently thorough sorting of the contents of the dredge bag is hardly possible. Further, many grounds on the east coast, partly as a result of infestation by *Crepidula* and partly as a result of the dying out of the *Zostera* which formerly clothed and consolidated the banks, have in recent years become rather muddy and it is necessary therefore to wash or 'dock' the dredge bag several times, by lifting it almost clear of the water and then dropping it back, in order to wash out mud and facilitate culling of the contents. Trials made by the writer when working on muddy ground at the mouth of the Blackwater showed that washing of the dredge to the extent necessary to free it from mud resulted in the loss of between 75 and 100% of the tingles. It has been recommended therefore that when dredging to clean fouled ground the usual docking of the dredge before shooting on deck should be omitted and any washing necessary carried out on deck with the aid of buckets. This is a slightly more tedious process but the catch of tingles is likely to be at least trebled.

Since *Urosalpinx* spawn hatches after an incubation period of 6–8 weeks, and the bulk of the spawn is deposited during May and June, hatching of spawn usually begins in early July. Consequently the period from the beginning of spawning, i.e. when the tingles first become active after the winter period of quiescence, until early July is the time during which maximum effort should be made to collect both tingles and spawn. After the beginning of July much of the spawn will have hatched. From other points of view the period

from mid-April to early July is a favourable one for working the beds. It serves admirably for the collection and concentration on fresh ground of oysters destined for sale, both when the latter are to be sold as food in the autumn and when they are destined for replanting in other localities. This period is also the most favourable for cultivating the ground, picking out slipper limpets, and working the cultch over so as to render it suitable for picking up a spatfall in the period July to September.

The inefficiency of the dredge in collecting tingles below 3 years old is not so serious a matter as at first appears, for it is probable that the amount of spawn deposited by the small tingles is insignificant in relation to the production of larger tingles. Any reduction in the size of the rings forming the belly of the dredge will inevitably reduce the number of oysters caught per man on grounds carrying a lot of shell, due to the rapid clogging which will occur, and the increased numbers of tingles caught must be balanced against the reduction in the yield per dredge of oysters. It is, however, the practice in the Blackwater to use slightly smaller rings on certain grounds.

Although it is now generally recognized among oystermen on the east coast that tingles are a serious pest, much of the damage done passes unnoticed, for it is only when the clock of a brood or half-ware oyster is found with one of the valves drilled that visible evidence of the damage done is obtained. This will not occur very often, for serious damage to brood and larger oysters only occurs when spat is absent. In this country tingles are mainly an enemy of the spat, but a drilled spat is very rarely seen, for the drilled valve, usually by the nature of the situation of the spat the flat valve, generally becomes detached and broken up almost immediately after the spat gapes. Practically the only evidence of the activity of tingles is therefore the failure of the spatfall to show up the following season. Similarly it is practically impossible to detect the damage done by freshly emerged tingles among the tiny spat of the season; yet it has been shown that these small tingles may destroy these spat at an astonishing rate. Since no method is known or is likely to be devised for trapping these very small tingles the best way of controlling their activities is to prevent them emerging by collecting the spawn before the beginning of July. It will be noted that the time of emergence of tingles coincides with the beginning of the normal spat settlement period.

Until a few years ago a bonus was paid to dredgermen on the basis of the number of tingles and clumps of spawn brought in. This has now been discontinued, partly because of the difficulty of checking the actual numbers and partly because the extra payment for tingles was found to result in what was considered to be undue attention being paid to this aspect of the work on the beds to the detriment of the numbers of oysters taken per man per day. It would probably pay to re-introduce this system where the main task involved is the cleaning and cultivation of ground rather than the collection of oysters. The payment involved was small, e.g. 1*s*. per 1000 on the River Roach. The amount paid out during the year 1936–7 was £9. 5*s*. 0*d*., representing a total

of 185,000 tingles and spawn. The main operation on which the company was engaged at the time was the clearing of the ground prior to laying down a large consignment of oysters from Brittany, and the ground was being intensively worked by a large number of boats. The following season payments fell to £3. 11*s.* 6*d.*, representing only 71,500 tingles, showing that tingles may be greatly reduced by dredging alone when intensive working of the ground is possible.

As mentioned earlier a conservative estimate of the value of the damage due to a single tingle feeding entirely on oyster spat is 1*s.* 3*d.* per season, while should the tingle prove to be a female and be removed before spawning the potential saving is much greater. It is possible therefore to justify on financial grounds any reasonable sum spent on tingle control, for a man engaged solely upon this work and being paid £3 per week needs to catch only eight per day to pay his way on the basis of the above estimate. When it is realized that on some beaches in the Blackwater river system a man can collect 500 tingles in a few hours from around low-water mark, then there can be no doubt that the detachment of men from normal dredging operations for the work of hand-picking on such beaches is well justified.

In the U.S.A. special tingle traps and dredges have been the subject of large-scale experiment with very encouraging results (see Galtsoff *et al.* 1937). Some of the simpler methods of control advocated in America are already incorporated in normal practice over here. These methods all aim at the removal of drills from oysters after dredging and include screening, forking, hand-picking, etc. This is of course already carried out on British beds; no oyster-man in this country knowingly throws a tingle back once it has come on board.

Another method of control strongly advocated by Galtsoff *et al.* (1937) is the use of special dredges fitted with a perforated pan to retain tingles but allow oysters to escape. Although these special dredges have never been tried in England the consensus of opinion is that they would clog up immediately on the clay grounds of the Essex rivers. They might work well on the Kentish Flats, but tingles do not yet present a severe problem there and present methods of control are fairly adequate.

Several different kinds of drill traps have been tried in America, with apparently considerable success. One type of trap takes advantage of the habit of the drills of congregating on objects raised above the bottom for the purpose of depositing egg capsules. Small concrete pillars, wire bags filled with shells, bunches of large shells or tin cans wired together, all are stated to attract spawning drills. They are laid out attached to lines and buoys and fished every few days, being taken up and moved to fresh areas as the catch diminishes. The use of such traps is to be strongly recommended in this country for the habits of the spawning tingles are the same; further, half-sections of large drain-pipes laid down on the beach at low water of spring tides in Thornfleet have attracted large numbers of drills and show that trapping can be carried out with success.

SUMMARY

The American whelk tingle, *Urosalpinx cinerea* (Say), a gastropod enemy of oysters, has been introduced to Britain with American oysters and is now established in the rivers Blackwater, Colne, Crouch and Roach in Essex and on the Kentish Flats off Whitstable. Evidence available concerning the migratory powers of *Urosalpinx* is conflicting, but it is probable that migration is of local significance only in the spread of this pest. To infest new areas tingles or their spawn must be transported by human or other agency, for *Urosalpinx* has no free-swimming larval stage, the eggs being deposited in capsules from which fully formed young tingles emerge.

Females of *Urosalpinx* grow more quickly than males and reach a larger size. In males the annual increase in shell height never exceeds 0·2 cm. after the first 3 years, and is generally rather less. In females an annual increment of 0·2 cm., or slightly more, is general up to the age of about 7 years, after which it gradually falls. Males and females of the same age differ in height by about 0·25 cm. Growth marks are frequently to be seen on the shell and are of value in assessing growth rates. In both sexes a maximum age of 13–14 years may be reached. *Urosalpinx* reaches a much greater average size in Britain than in its natural habitat on the Atlantic coast of the U.S.A.

Females may deposit some spawn during the first 2 years of life, but the amount is insignificant in relation to the production of older tingles. Spawning begins when the water temperature in its seasonal rise reaches 12° C. Adult females may deposit an average of 25 egg capsules at a single laying, but it is possible that further capsules are deposited later in the season. The bulk of the spawn is deposited in May and June on British beds, but a few freshly laid capsules may sometimes be found in August and September. The average period of incubation is about eight weeks. Young tingles usually begin to emerge early in July. The average number of young emerging from each capsule is 11·74. *Urosalpinx* appears to be more prolific on British oyster beds than in America.

Urosalpinx from the River Blackwater exhibits a preference for oyster spat and barnacles over other foods. Oysters larger than spat are not seriously damaged when spat are available. Oyster spat and barnacles are about equally attractive. Tingles confined with oyster spat destroyed them at an average rate of 0·385 per tingle per day over the whole feeding period, but, for a period during July, spat were destroyed at the rate of 0·9 per tingle per day. Two-year-old oysters were destroyed at the rate of 0·033 per tingle per day. The rate of destruction is correlated with water temperature. Drilling may begin when the water temperature exceeds 11–12° C., but little feeding occurs while the tingles are breeding in May and June. Feeding usually ceases early in October and tingles remain quiescent throughout the winter until the water temperature again reaches 12° C., when spawning begins. Freshly emerged

tingles may cause much damage among oyster spat which has recently settled.

Control of this pest is discussed in the light of the information available concerning its biology and distribution.

REFERENCES

BUCHANAN-WOLLASTON, H. J. & HODGSON, W. C., 1929. A new method of treating frequency curves in fishery statistics, with some results. *Journ. Cons. Int. Explor. Mer.*, Vol. IV, pp. 207–25.

COLE, H. A., 1941. Sex-ratio in *Urosalpinx cinerea*, the American oyster drill. *Nature*, Vol. CXLVII, p. 116.

FEDERIGHI, H., 1930. Control of the common oyster drill. *U.S. Bur. Fish., Econ. Circ.* 70, pp. 1–7.

—— 1931. Studies on the oyster drill (*Urosalpinx cinerea*, Say). *Bull. U.S. Bur. Fish.*, Vol. XLVII, pp. 85–115.

FRETTER, V., 1941. The genital ducts of some British stenoglossan prosobranchs. *Journ. Mar. Biol. Assoc.*, Vol. XXV, pp. 173–211.

GALTSOFF, P., PRYTHERCH, H. F. & ENGLE, J. B., 1937. Natural history and methods of controlling the common oyster drills (*Urosalpinx cinerea* Say and *Eupleura caudata* Say). *U.S. Bur. Fish., Fish. Circ.* 25, pp. 1–24.

LEBOUR, M. V., 1912. A review of British marine cercariae. *Parasitology*, Vol. IV, pp. 416–56.

MCMILLAN, N. F., 1939. Early records of *Crepidula* in English waters. *Proc. Malacol. Soc. London*, Vol. XXIII, p. 236.

MOORE, H. B., 1936. The biology of *Purpura lapillus*. I. Shell variations in relation to environment. *Journ. Mar. Biol. Assoc.*, Vol. XXI, pp. 61–89.

NELSON, J. R., 1931. Trapping the oyster drill. *N. J. Agric. Exp. Stat. Bull.* 523, pp. 1–12.

ORTON, J. H., 1927. The habits and economic importance of the rough whelk-tingle (*Murex erinaceus*). *Nature*, Vol. CXX, pp. 653–4.

—— 1929. Habitats and feeding habits of *Ocinebra erinacea*. *Nature*, Vol. CXXIV, pp. 370–1.

—— 1930. On the oyster drills in the Essex estuaries. *Essex Naturalist*, Vol. XXII, pp. 298–306.

—— 1937. *Oyster Biology and Oyster Culture*. London.

ORTON, J. H. & AMIRTHALINGAM, C., 1929. The oyster drills on English oyster beds. *Nature*, Vol. CXXIV, pp. 298–9.

ORTON, J. H. & LEWIS, H. M., 1931. On the effect of the severe winter of 1928–29 on the oyster drills of the Blackwater estuary. *Journ. Mar. Biol. Assoc.*, Vol. XVII, pp. 301–13.

ORTON, J. H. & WINCKWORTH, R., 1928. The occurrence of the American oyster pest *Urosalpinx cinerea* (Say) on English oyster beds. *Nature*, Vol. CXXII, p. 241.

ROBSON, G. C., 1929. On the dispersal of the American slipper limpet in English waters. *Proc. Malacol. Soc. London*, Vol. XVIII, p. 273.

ROTHSCHILD, M., 1936. Gigantism and variation in *Peringia ulvae* Pennant 1777, caused by infection with larval trematodes. *Journ. Mar. Biol. Assoc.*, Vol. XX, pp. 537–46.

STUNKARD, H. W. & SHAW, C. R., 1931. The effect of dilution on the activity and longevity of certain marine cercariae, with descriptions of two new species. *Biol. Bull.*, Vol. LXI, pp. 242–71.

THE DISTRIBUTION OF *ARACHNACTIS ALBIDA* M. SARS IN THE CELTIC SEA

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(Text-fig. 1)

The present paper is an account of the distribution of *Arachnactis albida* (Fam. Arachnanthidae, Carlgren, 1924) in the area south of Ireland and in the western approach of the English Channel during 1937–9. This larval Cerianthid, the adult of which is at present unknown, is a North Atlantic planktonic species. It has been collected by numerous scientific expeditions, the records of which indicate a centre of distribution between the Faroes and the Hebrides with a range extending along the Atlantic seaboard of north-western Europe from the Barents Sea to south-west of Ireland, and as far west as long. 20° W. (Carlgren, 1906, 1924). It has been taken in the northern North Sea frequently, but in the southern North Sea the only record is of a solitary specimen found off the Norfolk coast (lat. 50° 02′ N., long. 1° 40′ E.) by Leloup (1929, 1931), who attributes its occurrence there to a south-going coastal current. There is no valid record of the species in the English Channel or the Irish Sea, but off the west coast of Ireland specimens have been taken from many points, especially towards the south-west in Valentia Harbour (Browne, 1900) and off Tearaght Light (Bourne, 1920). This is the southernmost point (lat. 51° 27′ N., long. 11° 51′ W.) at which it has been recorded by previous workers. According to Carlgren's investigations (1906), several of the records of *A. albida* in the International Bulletins refer to other species (Kramp, 1913), and the records in the *Bulletin Planktonique* are not reliable as *A. albida* has been confused with *Synarachnactis bournei*[1] (Ostenfeld, 1931, p. 632). The records show that the species occurs mainly in open oceanic seas and not in the relatively more enclosed waters of the English Channel, Irish Sea and the southern North Sea (excepting Leloup's single record). Bourne, who investigated the specimens collected off south-west Ireland, suggested a subcentre of distribution in that area (near lat. 51° N.), but he emphasized that 'the possibility must not be overlooked that the larvae and adult or quasi-adult forms taken off the south-west coast of Ireland belong to a species or sub-species distinct from *A. albida*' (Bourne, 1920, p. 45). His statement is

[1] This is the species of *Arachnactis* which occurs at Plymouth in spring and summer (vide *Plymouth Marine Fauna*, 1931, p. 87). *A. albida* has not been found at Plymouth. Bourne's record (1890, p. 321), cited by many authors, actually refers to *Synarachnactis bournei* which is the larva of *Cerianthus lloydi* (vide Bourne, 1920, p. 29). The statement in *Journ. Mar. Biol. Assoc.*, Vol. VII, p. 203, 1904, concerning the occurrence of *Arachnactis albida* in spring at Plymouth is a mistake, repeated by Pax (1934), who has overlooked the correction in the 1931 edition of the Plymouth Marine Fauna.

based on evidence of 'differences in size, colour(?), distribution and minute anatomy' between the specimens from the south-west coast of Ireland and examples from the Faroes. This problem is discussed in the present paper.

The specimens of *Arachnactis* taken during 1937–9 in the Celtic Sea[1] can be referred with certainty to the species *A. albida*. A careful analysis of distribution in the area was made not only in view of the new locality but also because there is no accurate information concerning its distribution towards the south. The present data extend the range of the species as far south as lat. 48° N. off the mouth of the English Channel and indicate the existence of a localized subcentre of distribution to the south of Ireland in the neighbourhood of lat. 50° N., long. 10° W. Its occurrence to the south and east of this new centre can probably be correlated with water movements.

The material for this study was obtained in 1937–9 during a series of spring and summer cruises undertaken in the course of the Plymouth mackerel investigations and covering the area of the south-western continental shelf. The plankton samples were collected by means of half-hour oblique hauls with the 2 m. stramin ring-trawl. A thorough examination was made of the material obtained in June 1939, for which year hydrographical data were also available. The larvae were picked out from each haul and their total numbers counted except at those stations where they occurred in swarms; in the latter the total was estimated by subsampling. The plankton collections of the other cruises were then examined in order to determine whether the centre of distri-

TABLE I. *Records of* Arachnactis albida *from the Celtic Sea, arranged according to months*

Cruise	Station no.	No. of *Arachnactis*	Cruise	Station no.	No. of *Arachnactis*
March 1939	5	0	June 1939[2]	2	1
	18	0		3	1
				6	44
April 1938	19	0		7	3
	20	0		8	4
	21	0		9	1
				10	1050
April 1939	23	0		11	600
	24	1		12	1
				14	2
May–June 1937	5	0		15	47
	8	0		18	2
	10	0		19	2
	15	0		27	5
June 1938	6	0	July 1937	5	0
	13	1		8	102
	18	3000		10	0
	28	0			
	30	0			

[1] 'Le terme de "mer Celtique" fut employé pour la première fois par E. W. L. Holt, pour désigner l'étendue marine qui recouvre le plateau continental au sud de l'Irlande et à l'Ouest de l'entrée de la Manche' (Le Danois, 1938). Though we have not been able to find it in any of Holt's published papers, this term is used here owing to its great convenience.

[2] See Fig. 1 for the fourteen stations at which no larvae were obtained in this cruise.

bution was similarly located in each year and also to ascertain the time of year at which the larvae begin to appear in the plankton. Owing to the large number of samples this examination was not made station by station: it was limited to certain hauls from the areas in which *A. albida* was abundant in June 1939 and to certain other hauls from areas barren of larvae in June 1939. This was considered fully sufficient, since all the samples had previously been examined minutely in the course of the mackerel work and the presence of

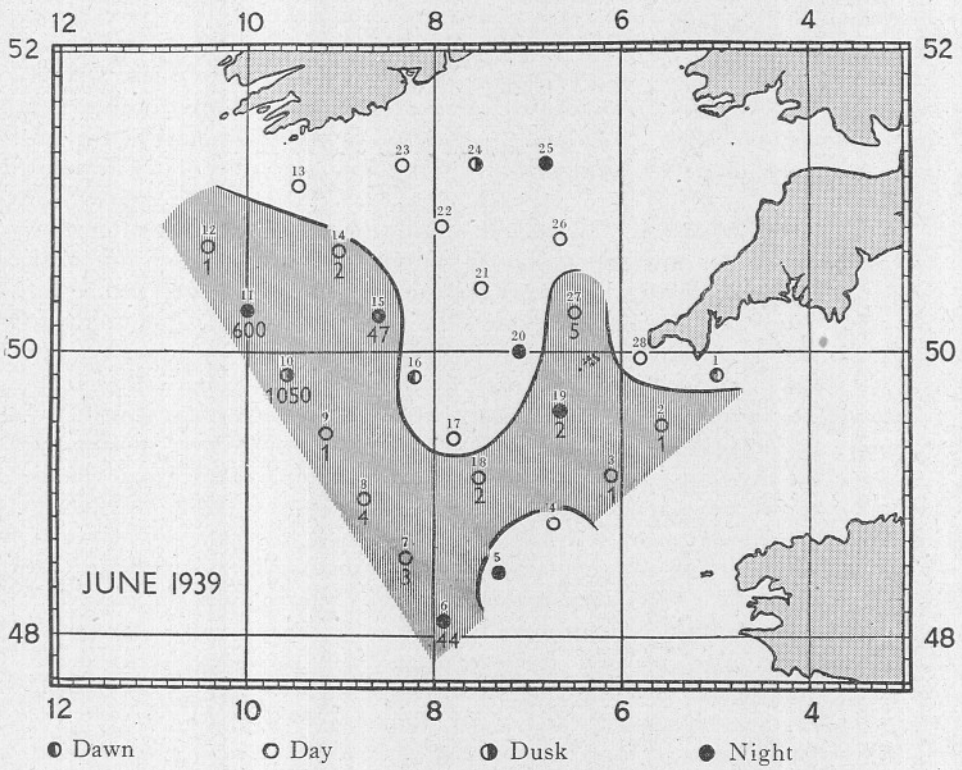

Fig. 1. Distribution of *Arachnactis albida* in the Celtic Sea in June 1939. Shaded area shows regions where larvae were obtained. Circles indicate station positions (differently shaded according to time of haul) with the station numbers above. The number of larvae obtained (if any) is shown below each circle.

Arachnactis larvae had been noted at the time. It is therefore unlikely that their occurrence in any numbers escaped notice. These results are summarized in Table I.

The numbers of *A. albida* obtained in June 1939 are shown in this table and in Fig. 1. The species was widely distributed over the western, southern and eastern margins of the area investigated—almost completely surrounding a region barren of larvae which extended southwards from the St George's and Bristol Channels (Sts. 13, 16, 17 and 20–26). The largest catches were taken

at Sts. 10 and 11, where 1050 and 600 specimens respectively were recorded. Elsewhere only small numbers occurred, but 44 larvae were taken at St. 6, which is noteworthy since it is the southernmost record of the species. The large hauls at Sts. 10 and 11 suggest the existence of a distribution centre in this region. Since, however, both day and night hauls were taken during the cruise, this indication cannot be established without first determining whether or not the observed horizontal distribution was affected by diurnal vertical migration of the larvae. This is especially important since Sts. 10 and 11 were worked at dusk and just before dawn. Sts. 6 and 15, where the next largest catches were made (44 and 47 specimens), were worked just before dawn and at midnight respectively. Unfortunately there is little information relating to the diurnal behaviour of *A. albida*, and the question can therefore only be further investigated by a critical examination of the data available in the present records.

In June 1939 *A. albida* was taken at fourteen stations. A total of twenty larvae were taken at nine day stations in contrast to over 1700 from one dusk and four night stations (Sts. 6, 10, 11, 15 and 19). The four largest catches of *A. albida* were all from night stations. This suggests that the large numbers taken in night hauls were probably due to migration of the larvae into the upper layers of water during darkness. Apart, however, from any temporary night-time increase in numbers of larvae near the surface, the existence of a distribution centre in June 1939 is indicated by the marked difference between the large total of 1650 larvae from Sts. 10 and 11 and the much smaller total of only 91 from the two next largest night catches at Sts. 6 and 15. Vanhoffen (1895) has noted the occurrence of *A. albida* in swarms at the surface in daytime. A daylight haul in July 1937 contained 102 specimens, and it is significant that about 3000 *A. albida* were taken in a daylight haul in June 1938 at a point less than 5 miles distant from St. 10 of June 1939. This recurrence in two consecutive years (1938 and 1939) of dense *Arachnactis* patches in the same locality and at the same time of year affords strong evidence of the existence of a breeding centre in the neighbourhood of lat. 50° N., long 10° W.

All the larvae taken in June 1939 were at fairly early stages of development. The youngest example had only five marginal and two oral tentacles, a stage very similar to that illustrated by Bourne (1920), which is the youngest larva recorded. The oldest individuals had eleven marginal and six oral tentacles. Of the 1050 specimens from St. 10, a critical examination of two subsamples showed that about 10% were early stages in which the directive tentacle had not appeared, about 2% possessed more than nine marginal tentacles, and the bulk of the material belonged to intervening stages in which the directive was recently formed and distinctly smaller than the other marginal tentacles. Larvae from Sts. 11 and 15 showed about the same percentages. Larvae from Sts. 6 and 27 were mostly earlier stages with less than eight marginal tentacles, and the single specimens obtained at Sts. 2, 3 and 9 were damaged, though certainly identifiable as *A. albida*. No *A. albida* were taken on

the June 1937 cruise. This may in some measure have been due to the fact that the area investigated did not extend farther west than long. 8° 30′ W. But in July 1937, 102 specimens were taken at a point about 50 miles south-east of the June 1939 centre. Most of these larvae were older stages than those found in June 1939; they had more than six oral tentacles, and the marginals had begun to drop off prior to the settlement of the larvae.

On the whole the records of 1937–9 show considerable agreement in regard to the locality of the dense concentrations of *A. albida*—a fact which points to the existence of a localized centre of distribution in the area investigated. It is also evident that in this area early June is the period of abundance, whereas in the Faroe-Hebrides region, July and August are the months during which the species has been most commonly taken. The single larva taken in April 1939 (about 20 miles west of St. 10 of June 1939) is the earliest record from the Celtic Sea. It would appear that in the south adults of *A. albida* begin to breed earlier than in northern latitudes, an observation which is in accord with the breeding behaviour of many other species.

It is probable that the centre of distribution off the south-west of Ireland is not as localized as is indicated by either Bourne's or the present records. Further work in the area may reveal that it covers a very much wider region embracing both these sets of records; or that the position of the centre of distribution shifts with the changes in flow of the Atlantic Stream (Harvey, 1930) or with changes of other controlling factors not yet known.

The *Arachnactis* obtained in the Celtic Sea during 1937–9 all belong to the species *A. albida*. The external appearance of the larvae, the arrangement of tentacles, colouring and disposition of the mesenteries as revealed by dissection of older individuals agree completely with the descriptions of *A. albida* given by Carlgren, Vanhoffen and others. The earlier stages show the characteristic brown coloration at the tips of the tentacles and on the throat. Examination of *A. albida* from the Faroe Channel[1] and comparison of them with the Celtic Sea specimens showed complete agreement in regard to external features.

It has also been possible to examine and compare a large series of larvae collected from the west coast of Ireland[2] with the Celtic Sea material. Some of these were taken to the north in the neighbourhood of Cleggan Head, Co. Galway, others to the south near Tearaght Light, the original locality of Bourne's specimens. From this comparison there can be no doubt that the larvae from the west coast of Ireland are identical with those from the Celtic Sea. It is therefore necessary to re-examine Bourne's suggestion of a distinct Irish species or subspecies which, it will be recalled, was based on differences

[1] These specimens were supplied through the courtesy of Capt. A. K. Totton of the British Museum.

[2] These specimens were collected during May–September 1890–1905 by the Irish Fisheries Department, and were kindly handed over for examination by Mr W. J. Rees. They were formerly part of the late Mr E. T. Browne's collection.

in distribution, size, colour and certain details of micro-anatomy. The Celtic Sea records, together with those from the west coast of Ireland and the numerous records from the Faroe-Hebrides area clearly indicate that *A. albida* has a continuous distribution along the western seaboard of the British Isles. The specimens from the neighbourhood of Cleggan Head do not appear to have come to Bourne's notice. This may possibly account for Bourne's statement that the 'northern form' has a distribution entirely separate from that of the 'Irish form'. With regard to colour, Bourne stressed the absence from his specimens of the brown coloration of the throat region which is typical of *A. albida*. He examined the specimens several years after their collection and noted that the throat coloration might have been lost since capture. In this connexion, it may be mentioned that the larvae obtained in June 1939 clearly showed the characteristic brown coloration at the tips of the marginal tentacles and in the throat, whereas this had already begun to fade, especially from the throat, in the larvae taken during the earlier cruises of 1937 and 1938. All the Celtic Sea larvae were examined in 1941. This possibility of fading during storage considerably reduces the importance which Bourne attached to the colour differences between his specimens and typical *A. albida*. The third difference noted by Bourne is that of size. His specimens from the south-west coast of Ireland were smaller than larvae from the Faroe Channel. On the whole, the Celtic Sea larvae were also smaller when compared with the measurements given by Vanhoffen and others. Considerable size variation among larvae of the same developmental stages was noticeable in the Celtic Sea material and also in the specimens from the Faroe Channel. This slight size difference alone cannot be considered sufficiently important a criterion upon which to base a specific or subspecific difference. Finally, the minor anatomical differences observed by Bourne are not of value from a systematic standpoint, and the morphology of the typical *A. albida* as described by Carlgren, Vanhoffen and others, is essentially the same as that of the Irish specimens studied by Bourne. It would thus seem that there is no justification for the separation of the *Arachnactis* from the south-west of Ireland into a species or subspecies distinct from the *A. albida* of the Celtic Sea and the Faroe-Hebrides region.

The distribution of *A. albida* in the Celtic Sea in June 1939 seems to show some correlation with the salinities of the surface layers (for chart of salinities at 5 m., vide Mare, 1940, p. 477). The larvae were present where salinities were greater than 35·2 $^{o}/_{oo}$ but were entirely absent from the central tongue of lower salinity water ($<35{\cdot}2$ $^{o}/_{oo}$), extending southwards from the St George's and Bristol Channels. The centre of distribution lay in high salinity water ($>35{\cdot}4$ $^{o}/_{oo}$) which was present at all depths along the edge of the continental shelf. Salinities at depths less than 25 m. probably varied little, but at about 25 m. there were indications from both salinity and temperature values of a sharp discontinuity. In the deeper water of 50 m. there was less correlation between salinity and the occurrence of larvae.

Matthews (1914), Harvey (1929, 1930) and others have given a general description of the cyclonic circulation of water in the Celtic Sea and its bearing on the distribution of many species is reviewed by Russell (1939). The salinities observed in June 1939 support this picture of circulation, and the correlation between surface salinities and the occurrence of larvae indicates that water movements in the area may have a marked influence on the dispersal and distribution of *Arachnactis*. The small numbers of larvae obtained outside their centre of distribution (Sts. 2, 3 and 19 off the Channel mouth and St. 27 north of the Scillies) were probably carried there by the cyclonic current.

It is of interest to note that Mare (1940), working on the phytoplankton of the Celtic Sea, found some evidence of mixing of Atlantic and Channel water close to the Scillies in the occurrence in April 1939 of both oceanic and neritic species of diatoms. In June 1939 a surface patch of high salinity water ($>35{\cdot}5$ ‰) at St. 27 again indicated the presence of water of mixed origin in the Land's End-Scillies channel, but Mare states that no phytoplankton species was especially associated with it. Some few *A. albida* were, however, taken at this station; they were all early epiplanktonic stages which may well have been carried there by water movements.

It is probable that the adult of *A. albida* is a benthic species (Carlgren, 1931). In the Celtic Sea adult examples, liberating larvae in May and June, may be expected to occur near the edge of the continental shelf in the area round lat. 50° N., long. 10° W.

It remains to be seen from future work if the correlation between water movements and the occurrence of *A. albida*, here indicated, is reliable, for the species may then prove to be a useful summer indicator of oceanic water in the Celtic Sea.

The authors wish to acknowledge their indebtedness to the Director and Staff of the Marine Biological Laboratory, Plymouth, where the work was carried out. P. G. Corbin also wishes to make acknowledgements to the Trustees of the Leverhulme Research Fellowships for a research grant which enabled him to carry out his share of the work.

Summary

The distribution of *Arachnactis albida* in the Celtic Sea in 1937–9 is described. The species appears to have a definite centre of distribution in the neighbourhood of lat. 50° N., long. 10° W. The larvae have been taken as far south as lat. 48°, and eastwards up to the mouth of the English Channel, but they are absent from the tongue of low salinity water continuous with the St George's and Bristol Channels. Their distribution in the remaining area can be correlated with the cyclonic circulation.

The larvae obtained from the Celtic Sea are specifically identical with those found off the west coast of Ireland and with the typical *A. albida* of the Faroe-Hebrides region.

REFERENCES

BOURNE, G. C., 1920. Observations on *Arachnactis albida* M. Sars. *Quart. Journ. Micr. Sci.*, Vol. LXIV, pp. 27–65, pls. 3–5.

BROWNE, E. T., 1900. The fauna and flora of Valencia Harbour on the west coast of Ireland. I. The pelagic fauna (1895–98). *Proc. Roy. Irish Acad.*, Ser. III, Vol. V, pp. 667–93, pl. 19.

CARLGREN, O., 1906. Die Aktinien Larven. *Nordisches Plankton*, Bd. VI, Lf. XI, pp. 65–89.

—— 1924. Die Larven der Ceriantharien, Zoantharien und Aktiniarien. *Wiss. Ergebn. Deutschen Tiefsee Expedition, 'Valdivia'*, Bd. XIX, Lf. VIII, pp. 341–476.

—— 1931. On some Ceriantharia. *Ark. Zool.*, Bd. XXIII*a*, No. 2, pp. 1–10.

DANOIS, E. LE, 1938. *L'Atlantique: Histoire et vie d'un Océan*, pp. 1–291. Paris.

HARVEY, H. W., 1929. Hydrodynamics of waters south-east of Ireland. *Journ. Cons. Int. Explor. Mer*, Vol. IV, pp. 80–92.

—— 1930. Hydrography at the mouth of the English Channel 1925–28. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 791–820.

KRAMP, P. L., 1913. Résumé des observations sur le plankton 1902–8. Pt. III. Coelenterata. *Bull. Planktonique Cons. Int. Explor. Mer*, pp. 537–8.

LELOUP, E., 1929. Sur la présence de l'*Arachnactis albida* M. Sars dans le sud de la Mer du Nord. *Ann. Soc. Roy. Zool. Belgique*, Vol. LX, pp. 22–3.

—— 1931. Contribution à la repartition des Cerianthaires dans le sud de la Mer du Nord. *Bull. Mus. Hist. Nat. Belgique*, Vol. VII, No. 2, pp. 1–10.

MARE, M. F., 1940. Plankton production off Plymouth and the mouth of the English Channel in 1939. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 461–82.

MARINE BIOLOGICAL ASSOCIATION, 1931. *Plymouth Marine Fauna*. 2nd edition.

MATTHEWS, D. J., 1914. The salinity and temperature of the Irish Channel and the waters south of Ireland. *Fish. Ireland Sci. Investigations*, 1913, Pt. IV, pp. 1–26.

OSTENFELD, C. H., 1931. Résumé des observations sur le plankton des mers explorées par le conseil pendant les années 1902–1908. *Cons. Int. Explor. Mer, Bull. Trimestriel*, Pt. IV, pp. 601–72.

PAX, F., 1934. *Die Tierwelt der Nord- und Ostsee*, Lf. XXIV, Teil IIIe_1: Anthozoa, pp. 1–80.

RUSSELL, F. S., 1939. Hydrographical and biological conditions in the North Sea as indicated by Plankton organisms. *Journ. Cons. Int. Explor. Mer*, Vol. XIV, pp. 171–92.

VANHOFFEN, E., 1895. Untersuchung über Anatomie und Entwicklungsgeschichte von *Arachnactis albida* M. Sars. *Bibl. Zool.*, Hft. XX, Lf. 1, pp. 1–14.

A STUDY OF A MARINE BENTHIC COMMUNITY WITH SPECIAL REFERENCE TO THE MICRO-ORGANISMS

By Molly F. Mare

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(Text-figs. 1–3)

CONTENTS

Introduction

The object of these investigations is to make some contribution to the understanding of that part of the marine food cycle which takes place on the sea bottom. A marine mud sediment, off Rame Head near Plymouth, under relatively open sea conditions was chosen for this study. A preliminary account[1] is first given of the quantities and types of micro-organisms found in the surface layers of the deposit. In such a place organic matter is continually settling, and under aerobic conditions a rich and varied population of micro-organisms is present. An attempt has been made to treat these

[1] Owing to the war further samples could not be obtained.

micro-organisms as a community. Special attention was paid to the surface layer because it is an important feeding zone for detritus-eating members of the macrofauna, and a subsidiary problem was the question of the relative importance of detritus and micro-organisms as the actual food supply of the macrofauna. Under the term *detritus* is included all material of living origin at any stage of disintegration and decomposition, from recently dead plant or animal or part thereof to the finest particle. The final stages of some of the products of decomposition, being colloidal or true solutions, are not included under the term; they are likely to be of food value to bacteria and minute saprophytes.

A brief census was also made of most of the larger fauna. A census of organisms over the whole range of individual size is a necessary preliminary to the understanding of quantitative food relationships throughout the community of micro-organisms and larger animals. It is also necessary for assessing the relative importance of the various groups in the utilization of deposited phytoplankton and detritus and the extent to which loss of organic matter from circulation is prevented. Fig. 1 shows the main phases of the complex marine food cycle. The parts with which this paper is specially concerned are indicated by heavy type. The part which takes place in the overlying water masses has been described for the Plymouth area by Harvey, Cooper, Lebour & Russell (1935), and data on the phytoplankton crop and its consumption during the period when these bottom investigations were in progress is given in Mare (1940). The turn-over in the water (shown at the top of Fig. 1) is probably rapid and considerable (Gardiner, 1937, Harvey, 1940, 1942), but in shallow water events on the bottom merit some consideration although their relative importance in the general cycle is as yet unknown.

The productivity of the sea bottom was the subject of important contributions by Petersen and Boysen Jensen (1911, 1914, 1918, 1919) and Blegvad (1914). These workers were concerned with the macrofauna, its sources of food supply and its utilization as fish food. There have since been numerous papers dealing with the macrofauna and some with other aspects of the subject. An investigation of the food of the macrofauna made in the Plymouth area by Hunt (1925) showed clearly the need for quantitative work on the smaller organisms of the benthic fauna and flora. Several workers have studied copepods, nematodes, foraminifera, etc., in submarine deposits and also the bacteria; but except for qualitative work on the protozoa by Remane (1933) and Lackey (1936) no ecological study has previously been made of the protozoa and algae of the marine benthos.

On the bottom is a fauna of detritus eaters and predators which may be separated for convenience according to size or weight into three groups. Associated with these differences is another factor, the generation time, which, though by no means easy to determine, varies enormously between the groups and is important in considering the role of the various organisms in the community. A new terminology is needed, and these groups are here designated

the *macrobenthos*, *meiobenthos*[1] and *microbenthos*. The macrobenthos is equivalent to the macrofauna of the bottom, and would also include large attached

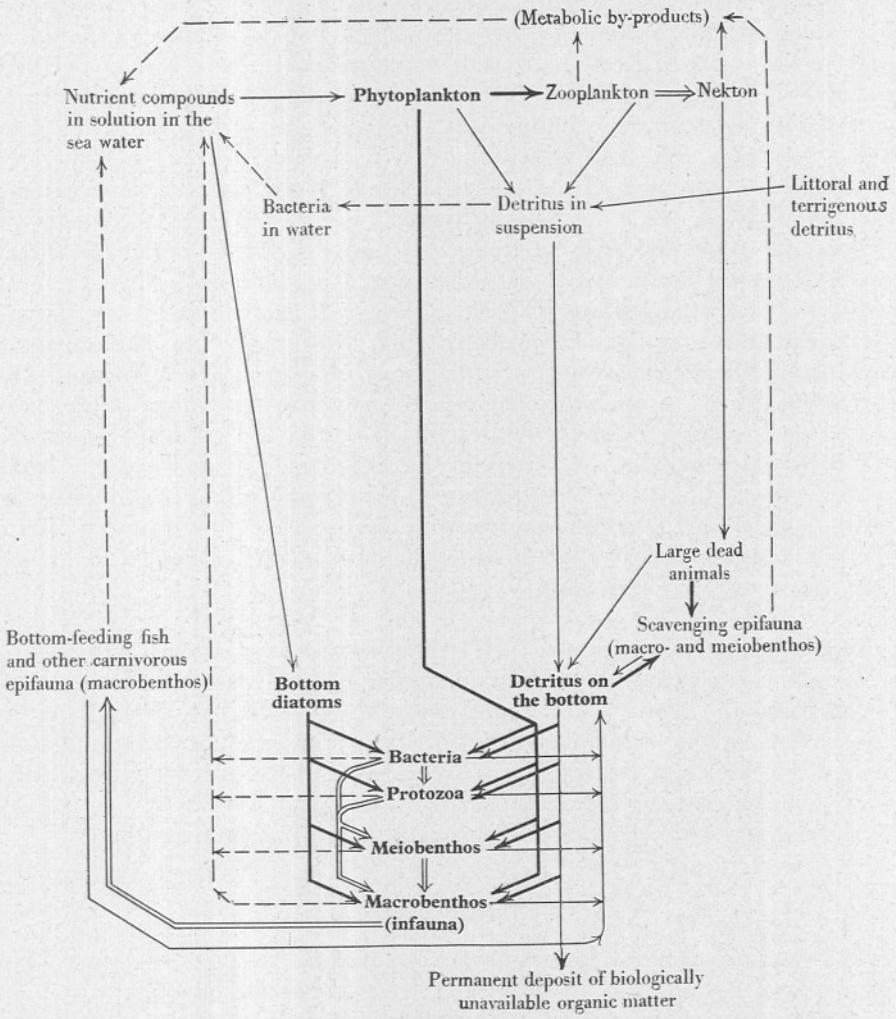

Fig. 1. Diagram of the main phases of the marine food cycle. Above are stages which take place in the water and below are those which take place on the bottom surface and in the deposit. Heavy type indicates the material with which this paper is concerned. Plant and detritus consumption, ⟶; predatory relationships ⟹; metabolic changes, — — →; other changes, ⟶.

algae in habitats where they occur. The meiobenthos here comprises the fauna of intermediate size, such as small crustacea (copepods, cumaceans, etc.) small polychaetes and lamellibranchs, nematodes and foraminifera. The

[1] Greek *meion*, smaller.

microbenthos comprises all the small organisms—protozoa (excluding foraminifera, i.e. ciliates, amoebae and flagellates), bottom diatoms and bacteria. The word micro-organisms here denotes all the members of the microbenthos. The fauna of intermediate size has been called the microfauna by Krogh & Spärck (1936) and by Rees (1940), and Remane used the term to include small metazoa and some protozoa. In a freshwater deposit Rawson (1930) employed a similar usage, including small metazoa and such protozoa as were found by direct searching under the term microfauna; some small metazoa appeared also in the lists of the macrofauna and he did not consider algae and bacteria. This use of the term seems rather unsatisfactory and inadequate for the present study. Bacteria are frequently called the micro-organisms of the sea bottom, but the omission of protozoa and diatoms has been largely on account of lack of knowledge of these groups. The exact limits of the macro-, meio- and microbenthos will probably vary according to the habitat under consideration and the methods which have to be employed for collection. The two larger groups can be separated according to size, depending on the mesh of the sieves employed for their extraction from the deposit. The groups can also be separated on the basis of weight, the weights of the microbenthos being deduced from volumes. The difference in generation time provides a further reason for separating the foraminifera with the small metazoa from the microbenthos. For a more exact definition of the limits of the groups in the habitat here described see Table VIII, p. 545.

This work was carried out at the Plymouth Laboratory while I was holding a Newnham College Research Studentship and a grant from the Department of Scientific and Industrial Research. I wish to thank the Director of the Laboratory for the numerous facilities given me, and to thank the staff, particularly Mr G. A. Steven, and visiting research workers for many helpful discussions during the course of the work, and also Prof. J. Gray, Dr M. Rosenberg and Dr J. E. Smith for helpful criticism of the typescript.

The Habitat

The station at which regular samples were taken is about one mile west-south-west of Rame Head, to the west of Plymouth Sound, at a depth of about 45 m. The conditions are those of a relatively shallow open coast, where the bottom is sometimes considerably disturbed by storms. The habitat thus differs from any previously described in detail by workers on the meio- and microbenthos, such as bottom stations in the Clyde worked by Moore (1931) and Lloyd (1931) and from the sheltered Danish waters.

Samples were collected by means of a weighted metal tube into which fitted a glass lining tube, with a plate valve at the upper end. This core sampling apparatus was a modification of that designed by Moore & Neill (1930). It brought up cores 5–15 cm. long with the surface layers practically undisturbed.

The deposit at the Rame station was a sandy mud, fawn to grey in colour. The amount of coarse material (5–0·5 mm. in diameter) was very variable in different samples, but the fraction of greatest weight was always made up of particles 0·25–0·1 mm. in diameter, and very fine silt (from 40μ to less than 1μ in diameter) contributed about 25 % of the dry weight and was, of course, important in determining the texture. There was no clear demarcation into zones within the depth sampled.

All water was rapidly expelled by heating at 110° C. The surface layers were more fluid than the rest, which retained its form when removed from the tube. In the surface $\frac{1}{2}$ cm. layer the water content was about 57 % of the wet weight and 30–33 % at a depth of 5 cm. It was noticed that in a set of samples collected after rough weather the loose texture of the surface mud extended appreciably farther down the cores. The volume of interstitial space was found to be approximately the same as that occupied by the particles. The fine silt lying between the larger particles could easily be brushed aside by motile members of the microbenthos, for which the microscopic topography is an important factor. Active holotrichous ciliates about 28 × 10μ in size were common, and hypotrichous forms, up to 50μ long, have been seen. Nematodes and polychaetes would burrow between the larger particles and ingest some of the finer ones. Harpactacid copepods seemed to be restricted almost entirely to the less compact surface layer. This restriction was probably also correlated with oxygen supply.

The total organic carbon content was about 1 % of the dry weight of the deposit, as determined by a wet dichromate oxidation method, slightly modified from Anderson (1939). Coal was frequently detected in samples of the deposit from this and other stations near the port of Plymouth, but fortunately it was scarcely oxidized by the method here used. The presence of coal as a contaminant in bottom samples has received little mention previously (see Trask, 1932). By no means all the organic detritus on the sea bottom is of equal use to the fauna or to the bacteria. Waksman & Hotchkiss (1938) and Anderson (1939) have found that the biologically utilizable fraction, as indicated by the oxygen consumption of micro-organisms oxidizing it and feeding on it in culture, represents only a small part (about one-sixth to one-tenth) of the organic matter found by the dichromate method.

Only on prolonged standing in the laboratory did the cores develop irregular black patches due to the presence of sulphide; it was therefore thought probable that the habitat normally contained some free oxygen in the surface layers.

Microscopic investigation of the deposit gave a further picture of the environment of the micro-organisms. Mud was frequently compacted into faecal pellets by the activities of the small polychaetes and lamellibranchs. No deposition of recognizable green faecal pellets of planktonic copepods, such as was found by Moore (1931) in the Clyde, was detected, although such pellets were common in the water. Large particles in the deposit consisted of siliceous

and calcareous skeletal fragments often with pores or cavities. While these still contained organic remains they would probably cause aggregations of bacteria and protozoa therein (thus similar difficulties in dispersing the micro-organisms prior to counting arose with this material as with land soil). The following list was typical of the objects encountered, and it gives an indication of the sources of supply to the region.

	Origin
Moss shoots and leaves (rare fragments)	Terrigenous
Wood with medullary rays (rare fragments)	
Oak leaf (rare fragments)	
Laminaria stipe (rare fragments)	Littoral benthic
Red and brown filamentous algae (fragments)	
Hydroid spp. (fragments)	Benthic (including intertidal)
Polyzoan spp. (fragments)	
Sponge spicules	
Echinoderm spicules	
Polychaete chaetae	Planktonic or benthic (some indigenous)
Crustacean setae	
Crustacean limbs, empty	
Diatom frustules	
Ostracod valves	Benthic (some indigenous)
Foraminifera, mostly dead	
Living ciliates and flagellates *very* rarely seen	Indigenous fauna
Living copepods, nematodes, etc.	
Living diatoms:	
Gyrosigma sp., *Navicula* sp.	Indigenous benthic or tychopelagic
Paralia sulcata	Tychopelagic
Coscinodiscus sp., *Biddulphia regia*	Planktonic
Thalassiothrix Nitzschioides, *Bacillaria paradoxa*	
Faecal pellets, ovoid, consisting of mud	Products of the smaller macrobenthos

Note. The *littoral zone* is here used to include the intertidal region and down to the limits of attached vegetation. The Rame station is regarded as being in the *sublittoral zone*, in which it now seems very probable that pennate diatoms are present and active (see pp. 528 and 535). The term *tychopelagic* is applied to organisms which are associated with the bottom and may be washed up into the plankton. Diatom nomenclature is that used by Lebour (1930).

The nature and quantity of the organic fragments occurring on the bottom is dependent on the depth, the distance from land and on the local conditions of settlement. A few samples taken with the Hunt vacuum grab (Hunt, 1926) from coarse deposits farther seawards and taken from intertidal stations at Wembury and Laira illustrated the influence of these factors and also showed an interesting distribution of planktonic and bottom diatoms (see p. 533). The occurrence of the latter was associated with differences in light intensity. Hunt (1925) deduced that phytoplankton was in all probability the chief ultimate source of supply of organic matter to the bottom in the Plymouth region.

It should be noted from the above list that protozoa, bacteria and small diatoms were hardly ever encountered in direct searching, even though alive and active and found by other means to be very numerous. Petersen and Boysen Jensen (1911) stated as a result of direct microscopic examination that "Of living organisms apart from bacteria and higher animals there are but few; one or two living bottom diatoms were met with." Krogh and Spärck

(1936) gave values for the numbers of "infusoria" found by direct searching, which from the results obtained by another method at Rame would appear to be far too small, but perhaps they refer only to very large ciliates. Investigators of the soil protozoan population have found direct searching to be valueless, as protozoa have a definite affinity for the soil particles, adhering closely to them so that they are hardly ever seen. The relatively enormous number of silt particles compared with the living organisms would also tend to make such a method very unsatisfactory for assessing the numbers present. The number would change appreciably before many samples could be counted.

The Microbenthos

A study has been started of the types, numbers and distribution of the micro-organisms and the quantity of living matter present in the surface layers of the mud deposit at the Rame station. A little information about the part played by these micro-organisms in the food cycle is here collected: it has been shown that indigenous photosynthetic organisms are present on the sea bottom; the importance of bacteria as converters of organic debris is known chiefly from the work of Waksman *et al.* and ZoBell *et al.*; data are here given on the feeding habits of some of the protozoa of the Rame microbenthos. The ways in which bacterial and protozoan populations interact, and particularly the influence of protozoa on bacterial activities associated with the regeneration of nutrient salts, are important subjects which have yet to be explored in marine habitats.

There has as yet been little ecological work on populations of micro-organisms treating them as communities rather than as collections of individuals. The most extensive work of the type indicated above has been carried out by Cutler, Crump & Sandon and others at Rothamsted on soil populations. Special attention was there paid to the determination of the numbers of organisms present, to fluctuations in abundance, and the influence of protozoa on bacterial activities affecting soil fertility. Picken (1937) described the structure of some freshwater protozoan communities, laying stress on the inter-relationships of the organisms with each other on the basis of food chains, and their dependence on the structure of the habitat. In a distributional study of the microscopic benthic fauna of a Michigan lake, Moore (1939) noted the effects of depth, type of deposit and of seasonal changes in the oxygen concentration on the numbers and species of such protozoa as were observed by direct search.

In marine habitats, the nannoplankton was investigated extensively by Lohmann (1908, 1911), who determined the numbers and volumes of all the minute organisms of sizes comparable with the microbenthos, and placed them in relationship with the rest of the plankton. The predatory activities of protozoa in the water are held by Waksman & Hotchkiss (1937) and others to be important in limiting the numbers of bacteria normally found in sus-

pension in the sea. Lackey (1936) made qualitative studies of the protozoa from the water and from the bottom and discussed their possible roles, pointing out that protozoa have an important function in the turn-over of organic matter. For example, a dead copepod is soon invaded by bacteria, followed by ciliates, which, after escape from the carapace, may serve as food particles of convenient size for metazoan filter feeders. 'The holozoic and saprophytic forms attack organic debris in suspension and constantly convert it to protoplasm in part and accomplish its mineralization in part. If they do not compare with the bacteria in numbers, their size is a compensation for this.' He also suggested that the smaller and presumably saprozoic flagellates may act as 'food condensers' for the larger forms.

The only members of the microbenthos which have so far been investigated in detail are the bacteria. Lloyd (1931), Waksman *et al.* (1933 *et seq.*) and ZoBell *et al.* (1936 *et seq.*) have investigated bacterial numbers, distribution and physiological types in sea water and in bottom deposits. They have been concerned with the bacteria and with the net effects in nutrient salt regeneration and have not investigated the influence of protozoa in the benthic community.

The possibility that marine fungi might be present on the bottom was investigated by Sparrow (1937), who found no typically marine forms there, and stated that it was doubtful if fungi are present in a trophic state or take any part in the decomposition of organic detritus.

An attempt is here made to fill in one of the gaps in our knowledge of the organisms of the sea bottom, and investigations have been made in detail on the protozoa and algae present on the bottom. Some work on bacteria has been included.

Census Methods for Micro-organisms

Routine Procedure

The routine procedure for obtaining a sample and for its subdivision was as follows. The core sampling apparatus used has been described on p. 520. A sterile glass tube was fitted for taking each sample and at least ten good cores were obtained. Since the surface area of each core was 2·54 cm.^{2}, census data of the micro- and meiobenthos (expressed as numbers per gram of dry deposit or numbers per unit area) are from mud derived from an area of 25·4 cm.^{2}, unless otherwise stated. Full precautions were taken against contamination by extraneous organisms in the laboratory and on board ship. A flow of water through the tube during its descent was, however, inevitable; but despite this, calculations showed that there could have been no significant contamination by organisms from the water. No contamination was possible during ascent, with the glass tube plugged at the bottom by the core of mud (the bottom of which was discarded) and closed at the top by the valve. Immediately on being brought on deck, sterile corks were put in both ends of the tube. The cores

were transported vertically in an ice box and used immediately on arrival in the laboratory (2–3 hr. after being taken). The supernatant water was siphoned off each in turn to just above the surface of the mud, and the level of $\frac{1}{2}$ cm. down the core was marked with a rubber band. Sterile water was then added and the top $\frac{1}{2}$ cm. of mud with this water siphoned over into a sterile burette, where the volume was measured before discharge into a flask. The open ends of the sample tube and the flask were protected by sheet rubber hoods. In this way none of the rather fluid surface layer was lost and contamination in the whole process was found experimentally to be quite negligible. Any organisms migrating up or thrown into suspension just above the surface of the mud were included with those living in the surface layer. The suspension was used first for setting up the bacterial count and then for the protozoan and algal census. An aliquot sample of 50 c.c. was also taken to ascertain the dry weight of the mud. The total volume of mud and water from ten cores was usually about 350 c.c., containing about $\frac{1}{25}$ g. of mud per cubic centimetre of suspension.

Census Method for Bacteria

Work on bacteria was restricted to assessing the number of aerobes present (without further distinction of types), and to evaluating the bacterial contribution to the total bulk of living matter. Bacteria were plated from dilutions of approximately 1/2500 and 1/25,000. After a comparison of several media, including those used by Waksman *et al.* (1933) and ZoBell & Anderson (1936), it was found that the medium giving the highest counts was that used by Waksman with the interesting addition of a trace of iron:

Sea water	1000 c.c.	K_2HPO_4	0·05 g.
Bactopeptone	1 g.	$FeCl_3$ (anhyd.)	0·0125 g.
Glucose	1 g.	Agar	15 g.

The optimum *p*H was found to be 7·5 (as shown by cresol red corrected for salt error). The suspension was shaken for 3 min. immediately before plating to disperse aggregations of bacteria. Incubation was at room temperature and the plates were observed after 14 days, by which time significant differences were easily detected. There are so many factors slightly affecting the numbers of bacterial colonies counted that it was considered safe only to compare plates set up on the same day.

The plate count represents only a small and not necessarily a constant fraction of the total number of bacteria present in the habitat, owing to the impossibility of finding a medium or conditions in which all types of bacteria will develop. Direct count methods were tried but found inapplicable to a marine sediment. Data obtained by workers on other habitats (soil, fresh water and sea water) show that their plate counts represent something of the order of one-hundredth of the numbers they obtained by direct counts. The latter, however, include dead as well as living bacteria.

Census Method for Protozoa and Algae

A dilution culture method, modified from that used by soil microbiologists (see Cutler, 1920; Cutler *et al.* 1922), was used to count the protozoa and algae. The suspension of mud was diluted by stages and 1 c.c. samples plated out into Petri dishes containing a suitable medium. The dilution stages were in orders of two or four with, respectively, one or two plates at each dilution. A range of dilutions from approximately 1/25 to 1/102,400 was necessary to include all the protozoa and to 1/6,553,600 to cover all the diatoms. The medium which was found to encourage the greatest variety of species was made up as follows:

Sea water	1000 c.c.	Cystine or methyonine	10 mg.
$NaNO_3$	0·1 g.	Gluconic acid	2 mg.
Na_2HPO_4	0·02 g.	$Fe^{\cdots}$	0·1 mg.
Sea mud extract	100 c.c.	$Mn^{\cdot\cdot}$	0·02 mg.

The nitrate and phosphate are necessary for plant growth. The sea-mud extract was made by autoclaving mud from the Rame habitat, in a similar way to the soil extract used by Gross (1937). The last four ingredients had recently been found advantageous to the growth of diatoms by Harvey (1939). This medium was found to give a much greater variety of species than media richer in organic matter, in which bacterial growth was liable to be excessive. By keeping the cultures in a dull light at 13° C., a satisfactory gradual succession of species was obtained. The plates were examined at weekly intervals, samples being removed with a fine sterile pipette for recognition of species.

From the presence or absence of any particular species down the dilution series the number of positive plates for that species is recorded, and from this the original concentration of the species can be calculated (see Cutler *et al.* 1922, table iii, p. 341). It is assumed (and there is definite evidence in some cases) that any single organism is capable of giving rise to a population which on examination of the dishes will be detected. However, with a wide variety of species in mixed culture (see p. 528) each of the individuals of a species in a dish sometimes inevitably fails to multiply. Some allowance can be made for such gaps from a table[1] of the probabilities of occurrence of the various types of end-point in the dilution series. For estimates of the total population, the filling in of obvious culture gaps is essential. These estimates are in any case *minimal* values for the population, and with standardized conditions and technique, valid *comparisons* can be made between parallel series from different places and between successive samples from the same place. The significance or otherwise of the differences obtained is tested on the number of positive plates, reliance being placed on those species which show few or no culture gaps. A difference of at least three plates is significant for any single species, and therefore where such a difference occurred, the numbers of that species per gram of deposit were also significantly different. Since groups of species compared showed the same tendency for high or low figures these smaller grouped differences were usually significant. The method was tested as

[1] A table kindly prepared for my use by Mr G. M. Spooner.

follows: it was used to count a ciliate population of known density, and in each of two tests with three parallel dilution series the number of positive plates was within one plate of the expected number. In making two parallel estimations of the same natural mixed population, technical variations were found to be insignificant.

In the tables on pp. 530–533, the numbers are given per gram of dry mud. The volume of an organism has been calculated from its dimensions, using individuals of average size found in culture. The volumes of all species have been summed to give the volume of protoplasm (in cubic millimetres) contributed by a group such as ciliates or amoebae to the total bulk of living matter forming the microbenthos.

Limitations of Methods

A clear distinction must be drawn between the value of these census methods for comparative purposes and for giving absolute numbers. Although they cannot be made very accurate, with standardized technique they are adequate for comparative work when large differences are involved. The absolute number of living bacteria lies between the number deduced from the plate count and approximately 100 times that number. The census method used for the other micro-organisms includes both the trophic forms and any resting stages there may happen to be on the bottom, but it is a minimal value for the total number of organisms because an unknown number of species may have failed to develop under the conditions provided. At present there is no more adequate method available.

A comparison of direct and culture counts, made on a sand deposit, illustrated the difficulties and limitations of both census methods. For protozoa and small diatoms the culture method gave much higher figures; but only a few of the larger planktonic diatoms seen in direct search had responded to culture conditions because some of the vegetative cells were too moribund to reproduce; others may have found the conditions or the media unsuitable. There was, however, some development of resting spores. By culturing a sample of detritus freshly deposited from sea water into a glass dish, in which only a few species were found by direct observation, Lackey (1936) obtained several other species of protozoa and algae by excystment. One must therefore be very cautious in discussing results obtained only by culture methods, but for members of the microbenthos, direct examination has led to erroneous results (see p. 522).

Types of Organisms found and their Habits

Bacteria

Rods and cocci were common, including motile forms. Measurements indicated that the average volume was about 1 cu. μ. This figure was used by ZoBell & Feltham (1938) and Baier (1935) for calculations of an available food supply. The physiological types have been investigated by

several workers, recently by Waksman *et al.*, ZoBell *et al.* and Lloyd. ZoBell (1939) reviewed the subject of bacteria in deposits.

Protozoa and Algae

The species of protozoa and naviculoid diatoms have in most cases been known by numbers. Taxonomic studies have been omitted, as these would have very much enlarged the scope of the work. Some ten species of flagellates, three species of amoebae, two of ciliates and about twenty species of diatoms occurred regularly in the cultures and showed fairly consistant behaviour. It was usual for about 40–50 species in all to be present in the mixed cultures of the dilution series. To this must be added the bacteria developing in these cultures. It is therefore not surprising that irregular gaps in the dilution series, due to culture failure, were apt to occur, especially among forms developing late.

The general sequence of events in one culture dish was as follows:

(1) Bacteria developed rapidly during the first week.

(2) The small colourless flagellates were present at the end of the first week—spp. II, IV, V (see p. 529 and Fig. 2).

(3) These were soon followed by another group of colourless flagellates—spp. VI, VII, XI, XII.

(4) Meanwhile planktonic diatoms were rapidly increasing and reached a maximum about the second or third week according to the light intensity. They then decayed.

(5) This gave rise to abundant organic matter, causing a further increase in the bacterial population. Most of the first group of small colourless flagellates had by this time died down, but not infrequently spp. II, IV and V persisted and were very abundant.

(6) Another group of flagellates, some containing coloured food vacuoles appeared about the third week. These (spp. I, XX, X and others) were rather larger than the first group.

(7) Amoebae were occasionally found early in the month, but they did not become abundant until there was plenty of plant debris. Some appeared to feed directly on the plant debris while others contained bacteria in their food vacuoles.

(8) During the decay of the planktonic diatoms, naviculoid diatoms and species of *Nitzschia* and *Gyrosigma* developed and usually remained active until well after the fourth week. In the winter months with low light intensity these bottom diatoms were conspicuous earlier in the sequence. Probably their compensation point (the light intensity at which the rate of photosynthesis and respiration are equal) is much lower than that of the planktonic forms.

(9) Ciliates only occurred at the more concentrated end of the series. They usually appeared early and were persistent.

(10) If coloured flagellates occurred, it was towards the end of the month. These were probably members of the nannoplankton.

(11) After prolonged standing occasional red, green or brown algae developed, presumably from spores of littoral species.

Under the term 'bottom diatoms' are included pennate species, mainly naviculoids, which are not normally part of the net plankton. Varieties of *Nitzschia closterium* have been present so constantly that they have been included, though possibly they should be regarded as tychopelagic. Other

tychopelagic forms, such as *Paralia sulcata* have been included with the planktonic diatoms. The distinction drawn has been partly one of convenience, separating those species which develop early in the cultures and were generally found in suspension from those which developed more slowly and usually crawled on the bottom. Members of this latter group were the only diatoms to develop under conditions of low light intensity. On account of its good growth in poor illumination, it is reasonable to include *N. closterium* with the bottom forms. The following species of planktonic diatoms occurred frequently in the cultures: *Thalassiosira gravida*, *Th. nana*, *Sceletonema costatum*, *Chaetoceros* spp., *Asterionella japonica*, etc.; the *Rhizosolenia* spp. were for the most part conspicuous by their absence. These species of planktonic diatoms did not reflect the changes in species seen in net plankton hauls made regularly over the Rame station. This discrepancy may be due to culture conditions, to the development of some species from resting spores independently of the season, to the hardiness of the vegetative cells of a few species, or possibly to some selective consumption by the zooplankton.

Some of the organisms mentioned above closely resembled species previously described, but identification is tentative. Others, while they cannot be named, were readily recognized by their characteristic behaviour and simple morphology:

FLAGELLATES.

II resembles *Bodo angustus* 8–12 μ long, cf. Lemmermann (1914).
IV is a *Monas* sp. 4–6 μ long.
V is a *Bodo* sp. 4–5 μ long.
VI is a Choanflagellate 4 μ long.
VII resembles *Rhynchomonas nasuta* 5 μ long, cf. Griessmann (1914).
XI resembles *Rhynchomonas mutabilis* 10 μ long, cf. Griessmann (1914).
XII is a *Bodo* sp. 2–3 μ long.
I is a *Bodo* sp. 16 μ long, flattened.
X resembles *Telonema subtilis* 6–8 μ long, cf. Griessmann (1914).

AMOEBAE. Normal types, 5–10 and 15–30 μ diameter (excluding pseudopodia) and *limax* type *ca.* 16 μ long.

HELIOZOA. An occasional species, *ca.* 5 μ diameter.

CILIATES. *Uronema marinum*, 20–25 μ long, cf. Kahl (1935).
Lembus pusillus, 20–30 μ long, cf. Kahl (1935).
Trochilia sp., 16–20 μ long, cf. Kahl (1935).
Mesodinium pulex, 15–20 μ long, cf. Kahl (1935).
Hypotrichous species up to 50 μ long.

Several of these species are by no means restricted to the bottom habitat, they have been cultured from water samples and some have been found on a sandy shore. From Lackey's list of protozoa, classified according to habitat—floating, attached or in debris and on the bottom—it seems possible that some of the protozoan species may be only casual inhabitants of the bottom. In culture many of the flagellates and ciliates swim freely in the water, and it should not be supposed that individuals are necessarily confined to the deposit under natural conditions.

Results of Culture Census of the Microbenthos

The following tables summarize the results[1] of sampling between January and June 1939. Samples taken previously, while the technique was being standardized, are (so far as can be judged) in reasonable agreement with these later results. The comparisons given in Tables I–IV are based on minimal numbers, and the figures always indicate the order of magnitude of the numbers and quantities involved rather than their exact values. Of these organisms the bacteria, protozoa and some of the bottom diatoms may be regarded as constituting the true microbenthos. The planktonic diatoms are best regarded as a part, and probably only a small part, of the deposition on the bottom, which with detritus forms the main food supply of the benthos. Attention is again drawn to the limitations of the methods here employed (see p. 527). The results are still tentative, as there has not been sufficient opportunity to confirm any of the conclusions, which for the present are made on single or relatively few estimations.

Horizontal Distribution

Table I records the results of an attempt to investigate the local horizontal variations in numbers. The samples were taken on 24 January. Numbers per gram of dry mud from the top $\frac{1}{2}$ cm. layer from the surface of five tubes are compared with those from another five tubes. The cores were grouped at random from a set of samples taken at the Rame station. Some part of the

TABLE I. HORIZONTAL VARIATION

	Cores 1–5			Cores 6–10		
	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud
Bacteria (plate count)	?	345,000	0·0003	?	2,050,000	0·002
Ciliates	2	15	0·0001	2	75	0·0003
Amoebae	4	2,335	0·0013	4	6,160	0·005
Flagellates	17	14,900	0·0028	13	79,080	0·015
Bottom diatoms	11	16,440	0·0046	10	46,270	0·0049
Planktonic diatoms and algae	17	21,140	0·0083	18	49,000	0·007
Total vol. of living matter			0·017 c.mm			0·035 c.mm.

difference in the total number of bacteria and of protozoa per gram may be due to the fact that rather less mud was taken off the surface of the cores 6–10 than off cores 1–5, there being probably some vertical gradation in abundance. Even if bacteria and protozoa were entirely concentrated in the extreme surface, irregularities of depth of sampling could not make the numbers per gram in cores 6–10 as much as twice those in 1–5. Therefore there must be very considerable local variations, particularly among flagellates and bacteria. Diatoms are much more evenly distributed; this fairly even distribution has been confirmed in another count of diatoms alone. In subsequent comparisons

[1] Full tables, illustrating the method of calculating the results (see p. 526), are available in a thesis placed in the Cambridge University Library and in the Library of the Marine Biological Association.

ten cores were mixed, and it is hoped that local irregularities of distribution were thereby eliminated. Owing to difficulties of sampling it was not practicable regularly to obtain more cores.

Vertical Distribution

Table II summarizes the results of a comparison of the micro-organisms found in the surface $\frac{1}{2}$ cm. layer of mud at the Rame station with those found at a depth of 2·5–3·0 cm. in the same cores. The sample was taken on 27 April.

TABLE II. VERTICAL VARIATION

	Surface, 0–0·5 cm.			Deeper layer, 2·5–3·0 cm.		
	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud
Bacteria (plate count)	? 12	710,000	0·0007	? 12	145,000	0·00015
Ciliates	2	12	0·00003	1	7	0·00007
Amoebae	3	6,850	0·0005	2	230	0·0003
Flagellates	15	49,800	0·0034	10	2,840	0·0018*
Total protozoa		(56,660)			(3,080)	
Bottom diatoms	13	118,090	0·012	8	34,160	0·0025
Planktonic diatoms and algae	13	346,420	0·33	12	23,940	0·0079
Total vol. of living matter			0·35 c.mm.			0·013 c.mm.

* The full tables show that the volume of flagellate protoplasm at the lower depth is almost entirely due to the presence of relatively few large dinoflagellates belonging to a single species (4% of the total number of flagellates giving rise to 90% of the volume). It is most unlikely that this species was in an active state when buried. If it were omitted from the list or a volume smaller than that of the active vegetative cell used in the calculations, the volume of flagellate protoplasm in the deeper layer would be much smaller than that in the surface layer. This is in better agreement with the numerical differences.

The surface layer is seen to be much richer in all groups than is the layer deeper in the core. It is obvious that planktonic diatoms contribute by far the greatest bulk to the total volume of living matter, especially on the surface. It is this rich layer which is of particular importance to many detritus eaters.

Seasonal Variations

Seasonal variations in the numbers of diatoms present in the surface layer were followed from winter to summer conditions. Variations in the numbers of protozoa could not be considered as seasonal variations since there were obviously large local variations, the greatest difference between samples being that shown in Table I. It is possible that there may be short-period fluctuations in numbers of protozoa and bacteria similar to those found in the soil by Cutler *et al.* (1922), but no attempt was made to investigate this point.

Separate stages in the changes in abundance from winter to spring and summer conditions are not significant, but the general drift is significant. There is a fourfold increase in numbers of bottom diatoms, and they are of considerable importance in the winter. Owing to the very great rise in the number and the greater bulk of planktonic species, these become overwhelmingly important in the spring and summer.

Hunt stated that bottom diatoms 'have been found in sufficient quantity in the stomachs of various animals to suggest that they contribute a very important ultimate food factor for the animals of such a region'. The lack of

TABLE III. SEASONAL VARIATIONS IN THE NUMBERS OF DIATOMS

All samples from the surface layer at the Rame station.

Date 1939		Planktonic diatoms (Pl.) No. of spp.	+ve pl.	No. per g.	Vol. per g.	Bottom diatoms (B.) No. of spp.	+ve pl.	No. per g.	Vol. per g.	Total vol. of proto-plasm per g.	Ratio of vol. Pl. : B.
24 Jan.	*a*	13	52	26,000	0·0083	11	54	26,000	0·0046	0·013	1·7 : 1
	b	15	51	16,000		8	34	6,400		0·013	
	c	15	55	48,000	0·0076	10	42	46,000	0·0049		
6 Mar.	*d*	15	74	71,000	0·026	12	46	14,000	0·0042	0·042	8 : 1
	e	16	67	178,000	0·048	10	49	11,000	0·0049		
16 Mar.	*f*	14	60	196,000	0·030	12	70	99,000	0·015	0·045	2 : 1
3 Apr.	*g*	17	69	112,000	0·19	12	66	84,000	0·018	0·21	10 : 1
27 Apr.	*h*	13	80	364,000	0·33	14	65	118,000	0·012	0·34	33 : 1
31 May	*i*	15	75	604,000	1·28	11	54	156,000	0·019	1·30	67 : 1

a and *b*, each from five cores (see Table I).
c, from an independent culture series using mud from *a* and *b*, from ten cores.
d and *e*, each from five cores. *f*–*i*, each from ten cores.

knowledge as to their abundance and seasonal variation in numbers has now to some extent been remedied. Owing to the lack of agreement between the planktonic species (see p. 529) occurring on the bottom and in the water, a detailed comparison cannot be made between the numbers deposited and the phytoplankton crop.

Regional Distribution

Samples were collected from different types of deposit and from various depths. A comparison was made between a Rame mud sample taken on 16 March and an Eddystone shell-gravel sample taken on 14 March, so that the cultures were running simultaneously in the same light intensity. The coarse deposit was collected with the Hunt vacuum grab, which takes an unstratified sample from an area of about 42 cm.^{2} and to a depth of about 2 cm. on sands and gravels. Figures are given per gram of total deposit, as in the probable absence of a distinct surface layer this is the form most comparable with the Rame figures. No bacterial estimation was made, because the Hunt grab in its present design is unsuitable for bacterial sampling. The number of bacteria per gram of total deposit is likely to be low in shell-gravel.

All groups are significantly less abundant in the shell-gravel than in the mud. A deposit-eating member of the shell-gravel fauna would, however, probably ingest only the fine particles; the proportion of living matter in this small silt fraction is quite as high as at Rame. Smith (1932) recorded the presence of animals feeding both selectively and non-selectively on bottom material and also, of course, of carnivores. Suspension feeders are, however, the members

of the macrofauna most common on the Eddystone shell-gravels. The figures in Table IV indicate that there is an appreciable deposition of planktonic diatoms, and so presumably of other organic matter; water movement over the bottom will keep some of this material in suspension. It is possible that some of the organisms here recorded per gram of deposit were actually in suspension

TABLE IV. REGIONAL VARIATION IN DIFFERENT TYPES OF DEPOSIT

	Rame mud (regular station), sample taken 16 March			Eddystone shell-gravel, sample taken 14 March		
	No. of spp.	No. per g. of dry mud	Vol. of protoplasm in c.mm. per g. dry mud	No. of spp.	No. per g. total deposit	Vol. of protoplasm in c.mm. per g. dry deposit
Ciliates	2	30	0·0002	2	8	0·00003
Amoebae	3	5,680	0·0033	3	680	0·00036
Flagellates	12	11,614	0·0013	6	600	0·000022
Bottom diatoms	12	99,470	0·015	11	7,680	0·007
Planktonic diatoms and algae	16	196,000	0·030	6	38,590	0·006
Total vol. of living matter, excluding bacteria, per g. total deposit			0·050 c.mm.			0·013 c.mm.

immediately over the bottom and brought up with the supernatant water which is unavoidably collected by the vacuum grab.

A few other samples, though not directly comparable with the preceding results, gave a preliminary indication of the distribution of diatoms on the bottom. Planktonic diatoms were much less frequent on coarser deposits seawards from the Rame station, and they were not found in sand on the shore at Wembury nor in the estuarine mud at Laira. Naviculoid diatoms had a surprisingly wide distribution in the sublittoral zone, at least during the summer. For example, *Cocconeis* sp. occurred quite abundantly with several other species as well, on the sandy bottom at Station E 1, 20 miles from the land and at a depth of 72 m., but under clearer water than at Rame. In a sample[1] taken at the mouth of the English Channel (at 49° 9′ N., 6° 7′ W.) from a depth of 113 m. no planktonic diatoms were found, only one large naviculoid diatom species developed in culture and a few other moribund cells of tychopelagic and bottom species were seen. Owing to the unavoidable delay of 6 days after sampling before examination and the setting up of cultures it is difficult to be certain of the reason for the dearth of plants, but it seems probable that the limit of active plant life on the bottom had nearly, if not quite, been reached at this station. The exact significance of the commonly accepted idea of a lighted benthic zone down to a depth of about 100 fm. (the sublittoral zone) needs more thorough investigation.

The Quantity of Microbenthos relative to the total Organic Matter in the Deposit

In order to assess the contribution that the microbenthos makes to the total food supply available to the detritus-eating macrofauna an estimate would have to be made of the absolute population density. Since this is at present

[1] Kindly taken for me by Mr G. A. Steven.

impossible, an arbitrary method has been employed of obtaining from the culture census figures something more nearly approaching a maximal value for the population. From this extremely rough approximation it has been estimated that, if the organic carbon at Rame be taken as 1 g. in 100 g. dry mud, then in the surface $\frac{1}{2}$ cm. layer only about 0·3–0·03 % of this carbon is derived from bacteria, protozoa and bottom diatoms; at least a further 2·5–0·015 % comes from recently deposited and still living planktonic diatoms, according to the season. At a depth of 2·5–3·0 cm. below the mud surface, a single sample indicated that only about one-fifth of the volume of microbenthos was present compared with that at the surface, and probably there would be even smaller quantities at greater depths.

Comparison with other Habitats.

Some of these results are supported by comparison with those of other workers. Data from the western Atlantic coast given by Reuszer (1933) show the richness of the bacterial population in the extreme surface layer of a mud deposit compared with the numbers present at a depth of 2·5 cm. in the mud and with those found in the top 2·5 cm. of a sand deposit. The organic content of the mud was considerably greater than that at Rame, but despite this the numbers of bacteria were of the same order in the two habitats. Reuszer found up to a hundredfold variation between the bacterial content of different cores and concluded that dense aggregations of bacteria might be associated with recently deposited organic matter. Lackey (1936) gave figures which showed the richness of the protozoan fauna associated with freshly deposited detritus, and in stressing the importance of the surface layer of mud for the activities of the protozoa, stated that the surface of mud cores from shallow water contained 'an abundance of flagellates and ciliates', whereas samples from a sandy bottom yielded few protozoa. Remane (1933), on the other hand, in comparing the number of species of ciliates in sand and mud deposits in the shallow waters of Kiel Bay (maximum depth 20 m.) found very few ciliates in the mud. It is, however, probable that the black mud there was of a stickier consistency than that at Rame. The sand habitat in Kiel Bay contained many species of ciliates, but the population density is unknown. On this sand there was a rich flora of bottom diatoms.

In freshwater deposits Moore (1939) found by direct examination that protozoa were most numerous in the top centimetre of the cores and that the numbers decreased rapidly to extinction at about 5 cm. below the surface on 'muck' bottoms. Pennak (1940) made brief mention of the micro-organisms found in a sandy beach of a large freshwater lake: in general composition and in the importance of ciliates, coloured flagellates and also rotifers, there was considerable similarity to the microbenthic community found in sand on the sea shore at Wembury. Micro-organisms present in arable soil at Rothamsted (see Cutler *et al.* 1922) were more numerous than in the Rame deposit, a

difference possibly associated with the smaller amount of available organic matter in the sea bottom. Groups occurred in the same order of abundance (bacteria, flagellates, amoebae and ciliates) in both habitats, but when account is taken of size the amoebae were possibly relatively more important than flagellates in the soil, whereas there was more nearly an equality of volumes of these two groups in the Rame deposit.

Food Relationships

Diatoms have been shown to constitute a large part of the microbenthic community. It is important to consider whether or not they are able to photosynthesize when on the bottom. Jenkin (1937) found that the compensation point for *Coscinodiscus excentricus* (a planktonic diatom requiring low light intensity) was at 45 m. in July near the Eddystone, over cleaner, sandier ground than the Rame station. In Jenkin's experiments the light intensity at 45 m. was about 0·5 % of that at the surface. Poole & Atkins (1929) found the light intensity very close to the sandy bottom, at 70 m. at station E 1, in March, April and May to be respectively 0·016, 0·098 and 0·121 % of that at the surface; on this deposit (sampled in May) a small naviculoid, *Cocconeis* sp., was found to be abundant. In the immediate neighbourhood of the bottom over mud there must be a rapid decrease in light intensity. It is therefore probable that there is little or no production by planktonic diatoms in the vicinity of the bottom at the Rame station. Since bottom pennate diatoms have a lower compensation point than planktonic species it seems probable that sufficient photosynthesis for net production can be carried on by bottom diatoms when lying on the extreme surface and to a greater extent when they are washed into suspension. These diatoms may be buried by falling debris or by disturbances of the bottom due to the epifauna or burrowers, but since most bottom diatoms are motile some will regain the surface. Small coloured flagellates have also been found on the bottom, but it is thought that they are really members of the nannoplankton, and nothing is known of their light requirements.

Some deductions concerning the food habits of protozoa, when under culture conditions, can be made from observations on the dishes of the dilution series and from other cultures. It was observed for instance that both *Uronema marinum* and *Lembus pusillus* throve in pure cultures on a diet of bacteria, but that in the mixed cultures of the dilution series, while *Uronema marinum* continued to feed almost exclusively on bacteria, *Lembus pusillus* nearly always had coloured food vacuoles, containing the remains of diatoms or coloured flagellates. Thus it seems that these two ciliates differ in their food preferences. To what extent such preferences would be apparent in the natural environment would be dependent on the relative abundance of bacterial and plant food. Both ciliates probably ingest detritus. A large *Bodo* species was observed on one occasion to have been ingesting numerous small chlamydomonads, but generally the plant-eating flagellates and also ciliates and amoebae

were dependent on diatoms already partially disintegrated by autolysis or bacterial action and flagellates have been observed ingesting chloroplasts inside frustules of decaying diatoms. Under natural conditions it is probable that these plant-eating forms are capable of subsisting on a variety of diets; possibly they require more abundant organic particles than do the smaller flagellates. The small flagellates mentioned in stages (2) and (3) of the culture series (p. 528) were observed to contain particulate food; they tended to remain on or near the bottom surface of the dish unless there was dead organic material in suspension. Some clearly showed aggregation round organic particles. It is thought that they are chiefly bacterial feeders and probably also saprophytic. Sandon (1932), while not doubting the possibility of saprophytic nutrition among the Protomastigina, does not regard it as definitely proved. Some of the colourless euglenids seen occasionally in these cultures may well be saprophytic. As far as is known amoebae always depend on the ingestion of solid food; the way in which they grazed the film of bacteria on the bottom of a Petri dish made patches of amoebae easy to detect with a dissecting microscope. Soil amoebae are known to show distinct preferences for various types of bacteria. Singh (1941) has demonstrated such preferences in both pure mixed culture and in sterilized soil, but has found that the amoebae are not so destructive of edible species in the soil cultures. This further illustrates that predator-prey relationships may well be quite different when 'refuges' are present, as was shown by Gause (1934).

The interaction of protozoa and bacteria may be of importance in several ways in the turn-over of organic matter in a deposit. The predatory activities of amoebae and ciliates may exert a marked influence on the bacterial numbers, depending on the relative volumes and rates of reproduction of the predators and prey. These predatory activities, if excessive, would decimate the bacterial population. They may, however, only be sufficient to keep the bacterial numbers at or near such a level that each bacterial cell exhibits its maximum chemical efficiency; such an effect has been found by Cutler & Crump (1929) and by Meiklejohn (1930, 1932) with pure cultures of bacteria and soil amoebae or ciliates. Protozoa and bacteria may compete for available organic matter, either dissolved or particulate (see Fig. 2, legend).

Great care must be exercised in deducing from results of culture experiments any relationships in the natural habitat. In cultures here set up no attempt was made to reproduce exactly the natural conditions. A culture dish of the dilution series was an isolated habitat with only very sparse mud particles. It usually had a large initial supply of organic food followed by the naturally produced abundant plant food, which resulted from a light intensity far in excess of that in the natural habitat. When small doses of organic food were added at weekly intervals the protozoan culture succession was delayed, though the additions were not accurately adjusted to maintain a balanced population. When cultures were grown under much reduced light intensities, thus approaching more nearly the natural conditions of the habitat, only a few

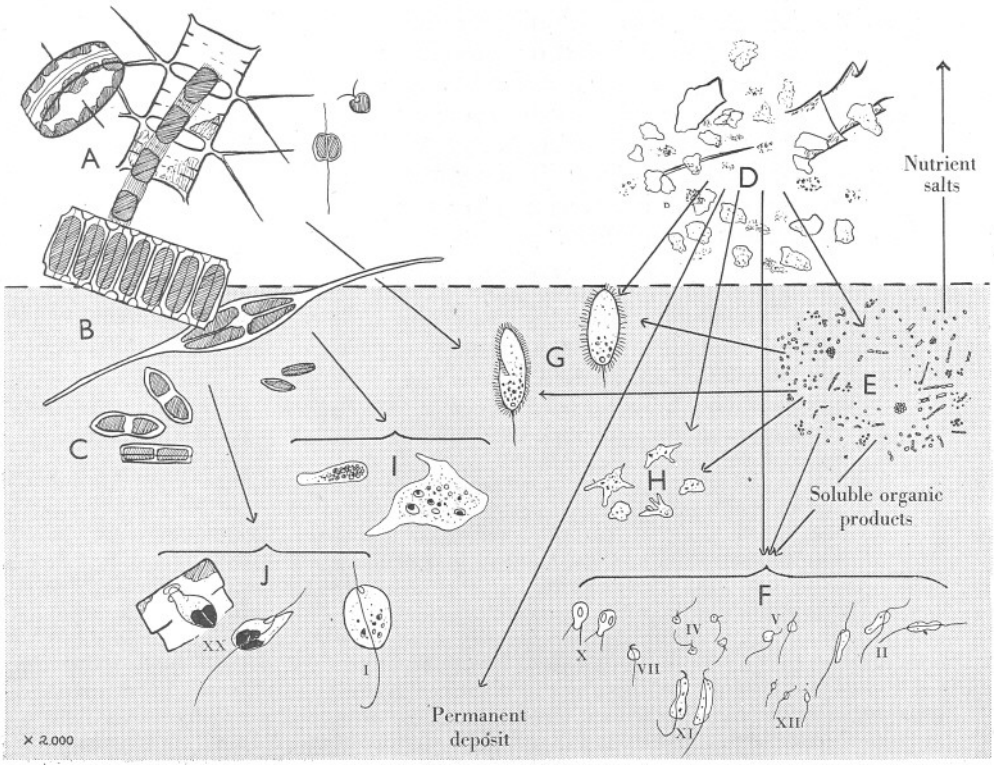

Fig. 2. Food chains in the microbenthos. This diagram represents probable food relationships between supplies dropping from the water and organisms in the surface layer of the mud. The organisms represented are mentioned on pp. 528 and 529 and are drawn to scale (1 mm. $= 2\mu$). Relative abundance cannot be adequately represented. A. Phytoplankton—diatoms and coloured nannoplanktonic flagellates. B. Tychopelagic diatoms. C. Bottom naviculoid diatoms. D. Detritus—animal and plant remains and faecal material already disintegrated and partly decomposed in the water. E. Bacteria—by means of extracellular enzymes they utilize detritus, converting part into their own protoplasm, liberating nutrient salts to the water, and freeing some soluble organic matter which may be used by other bacteria or by flagellates. F. Small colourless flagellates—they ingest bacteria and possibly detritus and may also live saprophytically. G. Ciliates—*Uronema marinum* is known in culture to digest bacteria and probably also takes detritus. *Lembus pusillus* ingests bacteria and if plant remains are abundant, has many coloured food vacuoles. H. Small amoebae—bacterial feeders, probably also ingesting detritus. I. Amoebae—a large normal and a limax type are often seen with coloured food vacuoles, they probably also take other types of food. J. Large colourless flagellates—with coloured food vacuoles, one is shown inside a dead diatom. Plant-eating protozoa cannot ingest whole diatoms, but depend on fragments produced by autolysis and bacterial action. The resistant residues of detritus are permanently buried in the mud.

naviculoid diatoms developed and the growth of planktonic species was prevented.

It is clear that the groups of the microbenthos are by no means independent of each other. Only the broadest outlines of qualitative relationships in the habitat are suggested in Fig. 2. A determination of the relative abundance (see pp. 530–533) and of the rates of reproduction of the various species and characteristics of their behaviour under natural conditions are essential before further deductions can be made. It is suggested, however, that the possible influence of protozoa in the nitrogen cycle or in regeneration of phosphates in the sea should not be overlooked.

Summary of the Activities of the Microbenthos

It is helpful here to summarize the qualitative knowledge of the activities of the microbenthos in the turn-over of organic matter in a sub-littoral habitat. These activities may be subdivided as follows:

(1) *Production* of organic matter from inorganic compounds.

(*a*) Chemosynthesis by bacteria is known to play a part on the bottom (see ZoBell, 1939).

(*b*) Photosynthesis. The activity of the bottom diatoms will obviously depend on the light intensity, varying with the season and with the depth and clearness of the water. It is thought probable that there is some production, at least in the spring and summer, on the bottom at the Rame station.

(2) *Conversion* of organic matter from one form to another is chiefly carried out by bacteria. They change indigestible forms such as chitin (see ZoBell & Rittenberg, 1938) or celluloses into more digestible bacterial protoplasm. ZoBell & Feltham (1938) and MacGinitie (1932) have proved that some bacteria may serve as food for metazoa, and it is known that they are an important food for protozoa. The bacteria, however, also utilize the more available fractions of the organic detritus in their own metabolism, leaving indigestible residues known as marine humus (Waksman, 1933).

(3) *Accumulation* of soluble organic matter as protoplasm by bacteria and saprozoic protozoa is certainly an advantageous process for the metazoa. On the other hand, the concentration of small food particles by protozoa, though of possible advantage to metazoa obtaining food by filtration of large volumes of water, is an extravagant process in a mud habitat, because the protozoa themselves consume some of the organic matter which they accumulate and a deposit feeder ingests all small particles in whatever size or form they may be.

(4) *Consumption* of organic matter. All the microbenthos respire, and except for some of the bacteria involved in the nitrogen and sulphur cycles, they derive their energy from the oxidation of organic matter, whether it be external to them or their own protoplasm.

The Total Fauna and Flora per unit area of the Rame Habitat

A brief census was attempted of organisms of all sizes in a unit area of the Rame deposit, in order to place the quantity of the microbenthos in perspective with that of the larger fauna. This has only been partially achieved. The sampling has not been extensive enough to include satisfactorily the larger, scarcer members of each size group, nor has it been possible to take more than a few samples to determine the approximate abundance of the macro- and meiobenthos. Of the macrofauna only the infauna were collected.

The numbers and weights of the organisms present give a picture of the population at one time. Investigations of the type of food consumed link together the members of the community. To assess their relative importance in the turn-over of organic matter, it would be necessary to know their respiratory rates throughout the year, i.e. their rate of oxidation or consumption of organic matter. In considering the quantity of food which any one of the smaller groups may be providing for consumption by the larger fauna, the relative rates of growth and reproduction must be taken into account. This line of approach has been followed by previous ecologists, who stressed these important considerations (see Petersen, 1918; Boysen Jensen, 1919; Bornebusch, 1930; Thamdrup, 1935; and Krogh & Spärck, 1936). With the exception of Lohmann (1908, 1911) they have usually confined their attention to one or two size groups.

The Meiobenthos

Only a few core samples were examined to gain an approximate idea of the abundance of nematodes, harpactacid copepods, foraminifera, etc. Small crustaceans are known sometimes to leave the bottom and were occasionally seen to migrate up the sample tubes; the census includes all those which were taken in the sample tubes. In Table V are given the results of examination of sieved mud from two sets of samples taken (*a*) on 16 March 1939, and (*b*) on 31 May 1939.

TABLE V. NUMBERS AND DEPTH DISTRIBUTION OF MEIOBENTHOS

		Groups							
Sample	Depths in cm. below surface of core	Nematodes	Harpactacids	Ostracods	Nauplii	Cumaceans	Polychaetes	Lamellibranchs	Turbellarians
(*a*)	0·0–0·5	130	80	14	3	+	35	10	21
	0·5–1·5	100	—	—	20	—	10	—	—
	1·5–2·5	65	—	—	15	—	10	—	—
(*b*)	0·0–0·5	48	74	4	7	—	4	—	4
	0·5–1·5	36	—	—	—	—	—	—	—
	1·5–2·5	44	—	—	—	—	—	—	—

Table V shows the greatest abundance of animals to be in the surface $\frac{1}{2}$ cm. layer and the restriction of adult harpactacids to that layer; nematodes and very small polychaetes were the dominant organisms deeper in the cores. Living foraminifera were entirely restricted to the extreme surface layer and were not very abundant relative to the rest of the meiobenthos (Dr E. H. Myers, personal communication).

These observations of the numbers and distribution of the small fauna are in reasonable agreement with the findings of Moore (1931) in the Clyde and Krogh & Spärck (1936) in Copenhagen Sound.

Satisfactory determination of the true live weight of these small animals is not easy. The following procedure was adopted: 50–70 copepods were placed on a cover-slip and the animals were counted while as much as possible of the extraneous water was removed with a fine pipette. They were weighed quickly; the animals were then picked off with a needle, leaving salt crystals and any debris on the cover-slip, which was then weighed again. The chief errors were due to the presence of extraneous water and to loss by evaporation from the animals themselves; these errors may be large but they oppose each other. The method was tested by weighing some larger copepods—*Tigriopus* sp.—the true density of which had previously been determined by Mr A. G. Lowndes (to whom I am much indebted for permission to use his data and material). Determinations of the density on two samples had given the average weight of an individual as 0·042 and 0·039 mg. and direct weighings gave 0·043 and 0·041 mg. The results from the two methods agreed better than was expected. The density method is only applicable when small animals can be obtained in very large numbers free from debris. A supply of copepods and nematodes was derived from Laira, where they were of similar size to those occurring at Rame. The average weight of these copepods was 0·005 mg. each and the nematodes 0·003 mg. each. From the Rame habitat, less satisfactory determinations (made on fewer animals and when evaporation from the animals was strongly suspected) gave values of 0·0024 mg. each for copepods and 0·0017 mg. each for nematodes. Turbellarians disintegrated during preparation for weighing; it was judged by inspection that they would be about the same average weight as the nematodes and that polychaetes would be appreciably heavier. The total weight of the Rame meiobenthos given in Table IX has been estimated on the basis of the individual weights of animals obtained from Laira; it is therefore an extremely rough and temporary approximation. Krogh & Spärck (1936) gave figures for the 'microfauna' from which can be calculated the average weight of copepods, 0·1–0·135 mg. each and of nematodes, 0·1 mg. each. The relative weights are similar to the Laira and Rame values, but if these organisms are the same size in the three habitats it is very tentatively suggested that perhaps there is some error in their computation, presentation of data or in the determination of the wet weight. The values for all the groups are very high, and the weight given for 'Infusoria' (0·45–1·0 mg. each) seems impossible.

For its food supply the meiobenthos is dependent on detritus, on the microbenthos and on other members of its own group. The little that is known of the food and feeding habits is tabulated below, with the sources from which the information was obtained.

NEMATODES.	Guts often empty, occasionally containing a little fine silt and possibly organic particles	Own observation
	Ingest the whole of the substrate in freshwater muds	Baier (1935)
	Carnivores, detritus eaters and diatom eaters	Remane (1933)
OSTRACODS.	Probably mainly mud eaters	Remane (1933)

TURBELLARIANS.	May prey on nematodes	Remane (1933)
HARPACTACID COPEPODS.	No recognizable particles in amorphous mass in hind-gut	Own observation and from Mr R. Elmhirst
	Probably scavengers and carnivores, being quite capable of chewing selected pieces of food	Mr A. G. Lowndes, personal communication
FORAMINIFERA.	Solid particles ingested by Miliolidae	Own observation
	Some ingest solid particles, while others will grow on a diatom food supply but appear to feed by external digestion	Dr E. H. Myers and Dr M. W. Jepps, personal communications

The Macrobenthos

A brief investigation of the macrofauna of the Rame station has been made, and it may be amplified by the earlier survey of the region by Ford. The station lies nearest to his station no. 93 (see Chart to face p. 167, Ford, 1923). The community is very similar to that illustrated on p. 184 of his paper. Observations on feeding habits of macrofauna in this region were made by Hunt (1925).

Hauls were made with a Petersen grab on 12 July 1939, and the material washed through a piece of stramin netting which proved to be an effective method for the retention of the very numerous small polychaetes. Table VI

TABLE VI. THE MACROFAUNA OF THE RAME DEPOSIT

	No. per m.²	Av. wt. of each in g.	Wt. per m.² in g.	Feeding methods
Cucumaria elongata	7	5·5	38·5	S or nsD
Amphiura filiformis	15	0·07	1·05	sD
Synaptidae	4	0·3	1·2	nsD
Psammosolen chamasolen	2	5·0	10·0	S
Nephthys sp. / *Glycera rouxi*	13	0·3	3·9	C
Nemertea	3	0·5	1·5	C
Sagartia sp.	2	2·0	4·0	C
Milne-Edwardsia sp.	6	2·1	12·6	C
Gonoplax rhomboides	1	2·0	2·0	C
Total of larger macrobenthos			75·0	
Lamellibranchs:				
Abra alba	222	0·003	1·2	sD
Thyasira flexuosa	104			S
others	20			S or sD
Gasteropods	42			C or sD
Polychaetes:				
Scalibregma inflata	920	0·014	31·5	nsD
Aonides oxycephala	500	0·003		sD
Magelona sp.	120	0·027		sD and C ?
Trichobranchus sp.	250	0·023		sD
other tubicolous spp.	200	0·025		sD
errant spp.	60	0·01		C
fragments	—	—		—
Amphipods	50	0·002	0·1	sD
Cumaceans	20			sD
Total of smaller macrobenthos			33·0	

Types of feeding according to Hunt's classification:
C = carnivore. sD = selective deposit feeder.
S = suspension feeder. nsD = non-selective deposit feeder.
Carnivores, 27 g. The rest, 81 g.

gives the numbers and weights per square metre. For the larger animals ten hauls, each from 0·1 m.², were examined, and for the smaller animals five of these hauls were searched. Living weights obtained by direct weighing are given. Since the average depth of the grab sample is about 10 cm. some of the rapidly burrowing crustacea will have been missed; it is known, for example, from dredge hauls that *Callianassa subterranea* is present at that station. *Echinocardium cordatum* was also found only in dredge hauls. Nomenclature is that used in the *Plymouth Marine Fauna* (1931).

The great importance of small polychaetes in this area is clearly seen from Table VI. When generation time and potential reproductive capacity are borne

TABLE VII. POLYCHAETES FROM THE RAME DEPOSIT

(Sample collected 12 July 1939)

	No. per m.²		No. per m.²
Harmothoë longisetis (Grube)	r	Spionidae: indet.	r
Harmothoë lunulata (Delle Chiaje)	r	*Aonides oxycephala* (Sars)	500
Sthenelais limicola (Ehlers)	r	†*Magelona* sp.	120
Aphroditidae: indet.	r	Cirratulidae: indet.	40
Phyllodoce sp.	r	*Diplocirrus glaucus* Haase	r
Eulalia sp. possibly *E. sanguinea* (Oersted)	r	*Scalibregma inflatum* Rathke	920
Phyllodocidae: indet.	r	*Ammotrypane aulogaster* Rathke	20
Oxydromus sp.	r	*Notomastus latericus* Sars	9
Ophiodromus flexuosus Delle Chiaje	r	*Owenia fusiformis* Delle Chiaje	r
Nereis fucata (Savigny)	r	*Pectinaria* sp.	r
**Nephthys* sp. possibly *N. hystericis* McIntosh	8	*Ampharete grubei* Malmgren	r
		Amphicteis gunneri Sars	r
Nephthys sp. probably young *N. hombergi* Audouin & M. Edwards	8	Ampharetidae: indet.	r
		Melinna palmata Grube	20
Glycera rouxi Audouin & M. Edwards	10	*Terebellides stroemi* Sars	r
Goniada maculata Oersted	25	**Trichobranchus roseus* Malmgren	250
Lumbriconereis sp.	r	**Streblosoma bairdi* (Malmgren)	70
**Drilonereis filum* (Claparède)	r	Terebellidae: indet. tube only	r

r = about 1–5 per m.², i.e. 1–3 specimens taken.
* = new record for the Plymouth area.
† *Magelona* sp. has been provisionally identified as *M. cincta* (Ehlers) by Mr D. P. Wilson, who is engaged on a description of it and its larva.

I am much indebted to Mr D. P. Wilson for correcting and amplifying this list, as far as possible with limited material. Several interesting species need further investigation.

in mind, the polychaetes appear even more important than is immediately obvious from the weights. Details of this part of the fauna are listed in Table VII. Juvenile forms were common, but many were mature specimens of small species. The list includes several new records for the Plymouth area.

An examination of the gut contents of the bottom fauna and of the morphology of their feeding apparatus throws some light on their feeding methods and the parts of the habitat from which food was derived. Hunt's observations have largely been confirmed. A summary of the more interesting and important data is given below and the types of feeding are also indicated in Table VI.

Cucumaria elongata, whose weight in these samples comprised about a third of the total, may feed from material in suspension above the bottom but may also, according to Orton (1914), remain completely buried for long periods and presumably behave as a non-selective deposit feeder.

Scalibregma inflata is an important non-selective deposit feeder, which by means of its eversible pharynx ingests the surface or subsurface layers of the deposit. It may be of some importance in the habitat in transporting buried material to the surface. It occurred on the average at a density of one worm per 11 cm.^{2} of mud surface.

Melinna palmata, *Trichobranchus roseus* and *Aonides oxycephala*: the gut contents showed some selection of the finer grades of silt and occasional diatoms were found; this presumably indicates that they collect particles from the surface of the deposit by means of their ciliated tentacles.

Magelona sp. has a pair of tentacles armed with many small suckers. Besides silt, its gut contained fragments of small crustacea, indicating that the worm is in part either a carnivore or a scavenger. Larvae of *Magelona* are known to select lamellibranch larvae in the plankton (Lebour, 1922).

Nephthys spp. and *Glycera rouxi* rarely had any gut contents; when present they consisted of the remains of other polychaetes.

Gonoplax rhomboides: one specimen had been feeding on polychaetes; the gastric mill of another contained fragments of small lamellibranchs and numerous Foraminifera—*Quinquiloculina akneriana* (= *Miliolina seminulum*) and *Rotalia beccarii* had been selected; these foraminiferan species were among the commonest living on the deposit.

LAMELLIBRANCHS: as shown by Hunt, some species feed from the surface of the deposit and others from material in suspension just above the bottom, the latter habit causing a conspicuous selection of diatoms.

From Table VI it will be seen that about 25 % of the fauna is carnivorous, while the rest depends on deposited organic matter and possibly on the microorganisms associated with it. As pointed out by Hunt, the absence of recognizable remains of minute naked organisms is no proof that they are not ingested. Non-selective deposit feeders will ingest detritus and microbenthos and to some extent meiobenthos in the proportions in which they have been found in the general mass of mud. Selective deposit feeders, particularly those collecting material from the extreme surface, have access to rather more living and dead organic matter, while suspension feeders have a distinctly more nutritious food supply.

Diagrammatic Summary of the Quantities of Organisms in the Rame Deposit

The total population per unit area of the deposit is best summarized in a diagram. The diagram (Fig. 3) is intended to convey only the order of magnitude of the quantities of living matter present at one time and to illustrate a method of considering a population. It is built up from the few samples taken at various times from the Rame habitat and described in the preceding sections. It must be emphasized that the diagram is not by any means complete, first because the larger, and less frequent forms of each of the size groups have not been satisfactorily sampled, and secondly because the depth to which the samples have been taken is not sufficient to include all the organisms of each

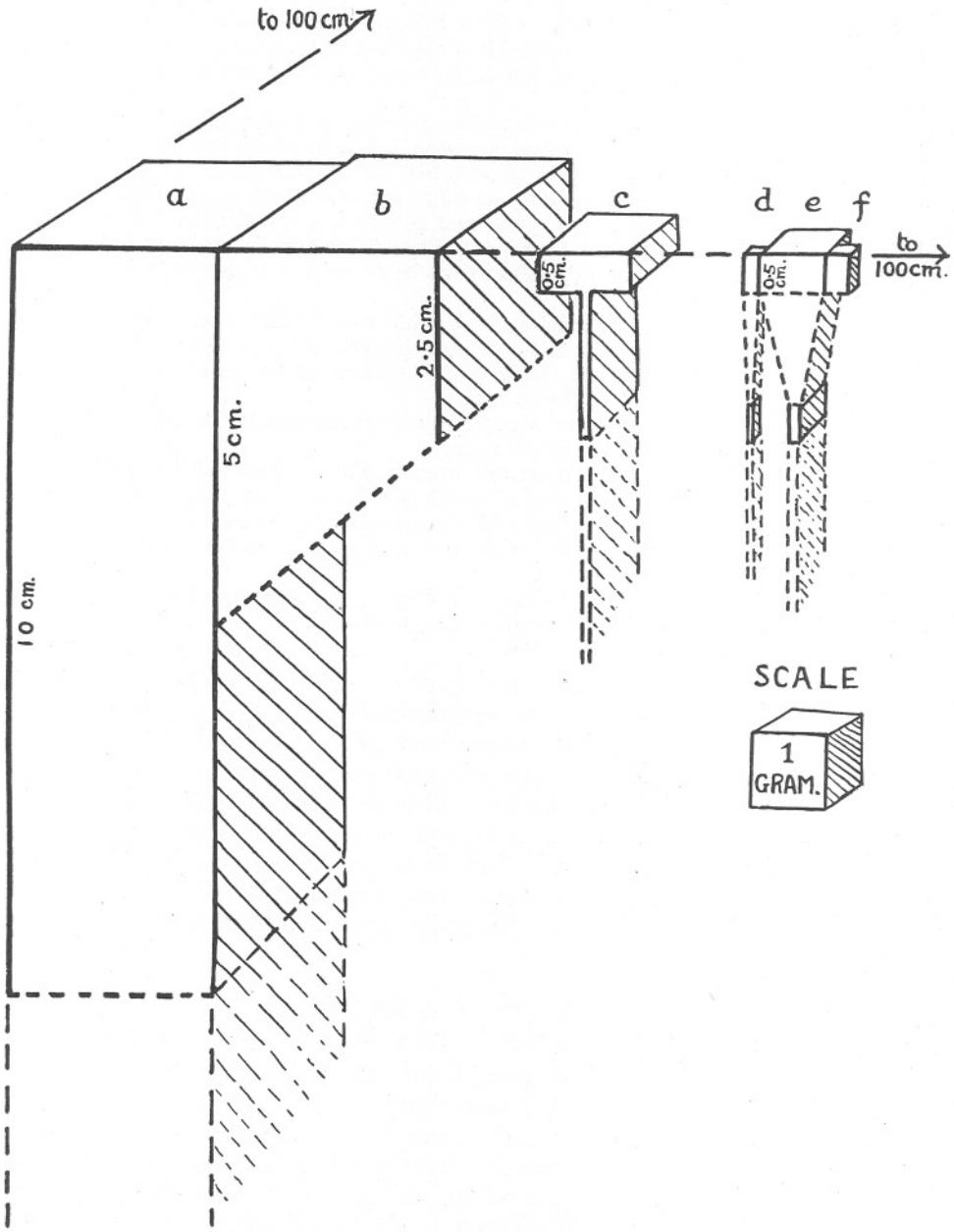

Fig. 3. The total amount of living matter in 1 m.² of Rame mud. *a*, large macrobenthos; *b*, small macrobenthos; *c*, meiobenthos; *d–f*, microbenthos: *d*, protozoa; *e*, bacteria; *f*, bottom diatoms. For full explanation see text and Table VIII and for numerical data see Table IX.

TABLE VIII

Group	Method of collection and analysis	Range of individual weight	Date of collection
Macrobenthos (*a*)	Peterson grab, average depth of sample 10 cm.	Over 100 mg. (up to 6 g.)	12. vii. 39
Macrobenthos (*b*)	Same sample, sieve 1 × 1 mm.	100–1 mg.	12. vii. 39
Meiobenthos (*c*)	Core sampler, sieve 0·1 × 0·1 mm.	0·05–0·0001 mg. usually to 0·001 mg.	16. iii. 39 to 31. v. 39
Microbenthos (*d–f*)	Core sampler, culture	1×10^{-4} to 1×10^{-9} mg.	27. iv. 39

TABLE IX

	Weight per m.² in g.	No. of individuals per m.²
LARGE MACROBENTHOS (*a*):	75	56
Cucumaria elongata, dominant by wt.		
Some burrowing crustacea missed below 10 cm.	+	+
SMALL MACROBENTHOS (*b*):	33	2,300
Small polychaetes dominant, probably restricted to top layer, surface to 2·5–5 cm.		
Some small polychaetes missed	+	+
MEIOBENTHOS (*c*):		
Numbers minimal; weights of some groups uncertain; total weight a very rough approximation.		
Variety of species—in surface ½ cm.	0·8	86,800
Nematodes and a few polychaetes, 0·5–2·5 cm.	0·35	60,000
Nematodes probably extending deeper	+	+
Foraminifera, a few living ones confined to surface ½ cm.	+	+
MICROBENTHOS:		
Large ciliates: some probably occurred, too scarce to be counted in culture	+	+
PROTOZOA (*d*):		
Minimal numbers, trophic and resting forms:		
In surface ½ cm.	0·02	283,000,000
At 2·5–3·0 cm.	0·01	15,000,000
BACTERIA (*e*):		
Plate count × 100; a very rough approximation to total live count: In surface ½ cm.	0·36	355,000,000,000
At 2·5–3·0 cm.	0·07	72,000,000,000
BOTTOM DIATOMS (*f*):		
Minimal numbers; only those on surface and probably capable of photosynthesizing are shown in diagram:		
In surface ½ cm.	0·05	590,000,000
At 2·5–3·0 cm.	0·01	140,000,000

PLANKTONIC DIATOMS, obtained by the culture method, represent an unknown fraction of the total diatoms deposited, either as resting spores or in a more or less moribund condition. The culture method showed 1·64 g. per m.² to be present in the surface layer on 27 April and 0·04 g. in the layer at 2·5–3·0 cm.; the highest figures obtained were about 25 g. of living planktonic diatoms per square metre down to a depth of ½ cm. The planktonic diatoms, with the less recognizable detritus are best regarded as the organic matter deposited on to the habitat from an external source, and not being indigenous members of the benthos they have not been included in the diagram.

group. These omissions are indicated in the diagram. Ideal sampling would give the numbers and weights of animals per unit area, e.g. per square metre, of deposit to the maximum depth of occurrence of the fauna, all samples being collected on the same date. The vertical distribution is indicated on the vertical faces of the rectangular blocks, the presentation being somewhat influenced by the methods of sampling. The square horizontal areas are adjusted to give volumes representing the total live weights of organisms in the different groups. The density is conventionally taken as 1, i.e. 1 c.c., is equivalent to 1 g. The blocks are represented in perspective, the depth and width of the front face being absolute measurements; the blocks are placed in one corner of the whole square metre. Epifauna is excluded. Data concerning the method of collection and subdivision into size groups are given in Table VIII and the actual figures from which Fig. 3 is built up are given in Table IX.

The Proportion of Organic Matter which consists of living Organisms

The total organic matter is about 1 %, i.e. 10 mg. per g. dry weight of the deposit at Rame. It is obvious that the largest animals would not be included when making chemical determinations, and the chance of encountering them is very small. The micro- and meiobenthos and possibly some of the small macrobenthos would, however, be included in such determinations. The present state of our knowledge of the components of the organic carbon in a deposit is summarized below. 'Maximal' values (see p. 534) for the microbenthos and diatoms are used. Table X gives an indication of the composition of the organic matter ingested by a non-selective deposit feeder:

TABLE X

		Proportion of the total organic matter
In the surface ½ cm.	True microbenthos	0·3–0·03 %
	Planktonic diatoms	At least 2·5–0·015 %
Deeper in the deposit	True microbenthos	1/5th to 1/10th of the above
	Planktonic diatoms	A smaller proportion
In the top 2·5 cm.	Meiobenthos	At least 0·02 %
Fraction biologically available to bacteria (see Waksman & Hotchkiss, 1938; and Anderson, 1939)		10–17 %
Resistant fraction, mostly finally buried		The rest

There is of course far more dead organic matter in the deposit than total organisms. The total indigenous fauna and flora amount to about 5–10 g. of organic carbon per square metre down to a depth of 10 cm., while the detritus amounts to about 1 kg. of organic carbon per square metre down to a similar depth. Of this, as seen in Table X, only a very small proportion consists of recently deposited or readily available food.

DISCUSSION

In assessing the relative importance of the different groups in the turn-over of organic matter, the determination of the numbers and weights of organisms of all sizes is only the first preliminary. The relative rates of respiration must

be taken into account. Krogh & Spärck (1936) suggested that the small animals, such as copepods and nematodes, use three or four times as much oxygen per gram of living tissue per hour as do members of the macrofauna, and it is well known that bacteria and protozoa have a much higher rate of respiration than metazoa (see Krogh, 1916; Heilbrunn, 1937, pp. 197–201; and Cook & Haldane, 1931). The smaller organisms therefore utilize relatively much more of the food supplies in the habitat than would appear from a direct comparison of quantities such as is made in Fig. 3.

It is impossible at present to do more than point out this important aspect of the subject because of the scarcity of data on the respiratory rates of the organisms concerned, and the complete lack of determinations under conditions of oxygen concentration, population density, food supply and temperature comparable to those found in the mud habitat. Some of the macrofauna may have an important mechanical effect on oxidation of organic matter in the deposit. For example, non-selective deposit feeders such as *Scalibregma inflata*, and forms such as tubicolous polychaetes and *Calianassa subterranea* which maintain currents of water in their tubes or burrows, may facilitate direct chemical oxidation or prolong the activities of aerobic bacteria.

The problem of the role of living micro-organisms in the food of detritus eaters is not yet capable of solution, because neither the food requirements nor the rate of feeding of any member of the Rame benthos have yet been worked out. It is to be expected that the quantitative food requirements and utilization will vary greatly between different species and with different modes of feeding. In so far as it throws any light on the subject, work (in progress) on *Arenicola marina*, a non-selective deposit feeder from the littoral zone, may be mentioned. From a study of this animal it seems that only a very small amount of food is required for maintenance (estimated from its respiratory rate), but that the quantity of micro-organisms ingested in a sand deposit is insufficient to meet this requirement. Account must be taken of the quantity of living food in a mouthful of ingested deposit, of the rate of feeding, of the population density of detritus eaters and hence of the interval which elapses before the same mud is again ingested. On the Rame ground it seems probable that, at the time of heavy deposition of planktonic diatoms, the quantity of diatoms present in the surface layers would be adequate for an animal feeding non-selectively and having a rate of feeding and food requirements of the same order as those of *Arenicola*. The quantity of true microbenthos and of planktonic diatoms found at other times of the year is such as to suggest that a non-selective deposit feeder would be largely dependent on dead detritic matter to form the bulk of its food supply. Selective deposit feeders and suspension feeders have access to a richer food supply in the extreme surface layer.

Although quantitatively inadequate to be of appreciable calorific value the microbenthos or the freshly deposited phytoplankton may be essential components in the food of the macrofauna in that they contain vitamins in all probability absent from detritus. This suggestion was first made by Hunt

(1925), but in the light of more recent work on vitamins (see also Fox, 1937) it requires modification in detail.

The food value of a population depends on the quantity present at any one time and the rate at which depletions are made good, i.e. the rate at which mud, in the form of faecal pellets, becomes part of the general habitat again and is reinvaded by such types of micro-organisms as are destroyed by passage through the gut of a metazoan. The rate of reproduction of the micro-organisms must also be sufficient for the invasion of newly deposited silt and detritus, if the community is to remain balanced. For bacteria and protozoa the food concentration, both local and general, plays so important a part in determining the rate of reproduction that it would be very difficult to find the normal rate in the habitat. From laboratory observations and literature (see Cutler & Crump, 1924; Baier, 1935; and Mare, unpublished thesis) it can be tentatively suggested that the generation time for bacteria is about 1–3 hr., for small flagellates about 10 hr. or less, for small ciliates 10–20 hr., and for bottom diatoms possibly about 2–3 days, under the conditions of light, temperature and food concentration in the Rame habitat. These rates, coupled with the quantities listed in Tables I–IV, make it probable that the reproductive rate would not be a limiting factor in the population density of the microbenthos providing that there were ample organic matter available. ZoBell & Feltham (1938) estimated that 'several milligrams of bacterial substance are synthesized per day in the top 5 cm. of 1 sq. metre of marine muds and although the majority is consumed by bottom animals the diet is perhaps supplemented by other types of food'.[1] It was shown in Table VI that 75 % of the Rame macrofauna was dependent on detritus and micro-organisms in and immediately above the habitat. The meiobenthos consists of both potential food supply and consumers. In comparing the annual potential food supply with the quantity of consumers, allowance must be made for the number of generations per annum of consumers, their food requirements for energy production and the extent to which the young stages draw on the food supply but fail to reach maturity. These factors are at present completely unknown.

The food relationships within the benthic community cannot be fully elucidated without reference to the relationship between the benthos and the water above the habitat, see Fig. 1. This subject requires an immense amount of knowledge accumulated from the work of many investigators, and the work already done could only be dealt with adequately in a separate review. Studies on these lines are much less advanced in the sea than in freshwater lakes, where the greater accumulation of data and the circumscribed water mass make it possible to estimate the total productivity of the water and the part

[1] The same authors (1942) investigated a very abundant bacterial flora, important in various ways, in a Californian tidal mud flat. Taking 10 million as the average number of living bacteria per c.c. of mud, they estimated that about 11 g. dry wt. of bacterial substance would be synthesized per day per cubic foot of mud, thus forming an important source of food in the habitat. It should be noted that bacteria are far more numerous in that intertidal habitat than in the sublittoral Rame ground and that probably the fauna is also more abundant.

utilized by the bottom fauna (see Rawson, 1930; Juday, 1940; and Deevey, 1941).

An outline of the balance-sheet of events on the sea bottom is given here, so that the small additions made in the present work may be seen in their correct places in the whole cycle. For an understanding of the annual cycle on the sea bottom, quantitative knowledge on the following subjects is required:

The annual deposition from the water, of plant, animal and faecal material.

The amount of photosynthetic production (if any) in the habitat itself (see p. 535).

The conversion of part of the detritus into protoplasm of micro-organisms and the mineralization of part. The numbers and quantities (see pp. 530–533) and the reproductive and respiratory rates when fully known would enable the annual production of living matter and the amount of turn-over of organic matter for which these organisms are responsible to be assessed.

The consumption of some of the living organisms and of detritus in oxidative metabolism by detritus eaters of all sizes. The numbers and weights (see pp 539–545) and the annual food requirements for energy production need to be known.

The accumulation of organic matter in the growth of the larger fauna, including the rate and efficiency of growth.

The utilization of detritus eaters by the carnivorous members of the community, as material for oxidation and growth.

The liberation of reproductive products to the water and the return of larvae to the habitat.

The removal of food from the habitat and of members of the community by scavengers and by predators from outside the habitat, e.g. by bottom-feeding fish. If the community remains approximately constant in total weight, the quantity removed is equal to the sum of the annual growth increments of such members of the fauna as are of a nature and size to serve as suitable food.

The return of nutrient salts and other important substances into the water as the result of bacterial activity and as excretory products of the fauna.

The loss from the food cycle of matter finally buried. This will consist of that resistant fraction which is biologically unavailable to bacteria of the upper layer and indigestible to the fauna. There may be some anaerobic decomposition of this material by bacteria deep in the deposit and perhaps slow autolysis; some marine humus may thus eventually be converted into petroleum.

Summary

An attempt has been made to deal briefly but quantitatively with all size groups of the fauna and flora in a marine mud deposit.

It has been necessary to propose the following new terminology: the *Macrobenthos*, which is here equivalent to the macrofauna, the *Meiobenthos*, under which term are included copepods, nematodes, foraminifera, etc., and the *Microbenthos*, comprising the rest of the protozoa, bacteria, bottom diatoms and other algae; planktonic diatoms and coloured flagellates also occur on the bottom in the region investigated but are not regarded as true microbenthos.

A quantitative ecological study of the microbenthos has been started and the habitat is first described, stress being laid on points of importance to the micro-organisms.

The census method for bacteria was an agar-plate method and that for the protozoa and diatoms was a dilution culture method modified from that used by soil microbiologists.

These methods give minimal values for the total population and may safely be used for comparative purposes. The results are still tentative.

Typical figures for the minimal numbers and volumes of living protoplasm per gram of dry mud in the top $\frac{1}{2}$ cm. layer are given in Tables I–IV.

The surface layer is much richer in all types of organisms than is the mud deeper in the cores. There is considerable local horizontal variation in numbers of bacteria and protozoa, suggesting dense aggregations.

Diatoms contribute by far the greatest bulk to the total volume of living micro-organisms; planktonic diatoms, particularly during the summer, completely outweigh the true microbenthos.

A seasonal variation has been shown in the numbers of bottom and planktonic diatoms. There was a fourfold increase in the quantity of bottom diatoms from winter to summer and while in the winter they were nearly as important as with the planktonic species (the ratio by volume being 1 : 1·7), the planktonic species became overwhelmingly important in the summer (the ratio becoming 1 : 67).

The distribution of bottom diatoms and other photosynthetic organisms in the littoral and sublittoral zones has been briefly investigated.

At the Rame mud station there are many more organisms per gram of total deposit than there are in the Eddystone shell gravel. The proportion of living matter in the fine fraction of the gravel is, however, much higher and of the same order as at Rame.

Food relationships in the microbenthos are suggested and it is pointed out that the protozoa are sufficiently numerous for their interaction with the bacterial population to be highly important.

The total volume of the true microbenthos present at any one time is extremely small. Even in the surface layers at the Rame station its carbon content was only about 0·3–0·03 % of the total organic carbon in the deposit. planktonic diatoms formed at least 2·5 % of the total organic carbon in the spring and early summer.

The meio- and macrobenthos were briefly investigated, the numbers and weights per m.^{2} being determined.

An interesting fauna of small polychaetes includes several new records for the Plymouth area.

The majority of the fauna ingest detritus either from the surface layer of the deposit or from the water immediately above the bottom.

The numbers and weights of most of the fauna and flora belonging to all size groups in the one habitat, as far as they were determined, are shown in Table IX and diagrammatically in Fig. 3. The relative amount of the living to the total organic matter present is given in Table X. These facts, when more adequately known and when combined with further qualitative observations on the food and feeding methods, will form a necessary basis for the discussion

of food relationships throughout the community. Taking into account the amount of microbenthos represented in Fig. 3 and its respiratory and reproductive rates, the micro-organisms are probably capable of playing a not inconsiderable part in the turn-over of organic matter in the habitat when compared with the larger fauna.

The information so far accumulated is of a preliminary nature, and the numerical data presented must always be taken as indicating the magnitude of the quantities involved and not their exact values. Differences are so great, however, that it is possible to draw tentative conclusions. As it is no longer possible to obtain samples from the original habitat, these conclusions are put forward now, subject perhaps to modification in the future.

REFERENCES

ANDERSON, D. Q., 1939. Distribution of organic matter in marine sediments and its availability to further decomposition. *Journ. Mar. Research*, Vol. II, p. 225–35.

BAIER, C. R., 1935. Studien zur Hydrobakteriologie stehender Binnengewässer. *Archiv Hydrobiol.*, Bd. XXIX, pp. 183–264.

BLEGVAD, H., 1914. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. *Rep. Dan. Biol. Stat.*, Vol. XXII, pp. 41–78.

BORNEBUSCH, C. H., 1930. The fauna of forest soils. *Forstliche Versuchswesen in Dänemark*, Bd. XI.

BOYSEN JENSEN, P., 1914. Studies concerning the organic matter of the sea bottom. *Rep. Dan. Biol. Stat.*, Vol. XXII, pp. 1–39.

—— 1919. Valuation of the Limfjord I. *Rep. Dan. Biol. Stat.*, Vol. XXVI, pp. 1–44.

COOK, R. P. & HALDANE, J. B. S., 1931. The respiration of *B. coli communis*. *Biochem. Journ.*, Vol. XXV, pp. 880–7.

CUTLER, D. W., 1920. A method for estimating the numbers of active protozoa in the soil. *Journ. Agric. Sci.*, Vol. X, pp. 135–43.

CUTLER, D. W. & CRUMP, L. M., 1924. The rate of reproduction in artificial culture of *Colpidium colpoda*. Part III. *Biochem. Journ.*, Vol. XVIII, pp. 905–12.

—— —— 1929. Carbon dioxide production in sands and soils in the presence and absence of amoebae. *Ann. Appl. Biol.*, Vol. XVI, pp. 472–82.

CUTLER, D. W., CRUMP, L. M. & SANDON, H., 1922. A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. *Phil. Trans. Roy. Soc.*, B, Vol. 211, pp. 317–50.

DEEVEY, E. S. Jr., 1941. Limnological studies in Connecticut. VI. The quantity and composition of the bottom fauna of thirty-six Connecticut and New York Lakes. *Ecol. Monogr.*, Vol. 11, pp. 413–55.

FORD, E., 1923. Animal communities of the level sea bottom in the waters adjacent to Plymouth. *Journ. Mar. Biol. Assoc.*, Vol. XIII, pp. 164–224.

FOX, D. L., 1937. Carotenoids and other lipoid soluble pigments in the sea and deep marine muds. *Proc. Nation. Acad. Sci. U.S.A.*, Vol. 23, pp. 295–301.

GARDINER, A. C., 1937. Phosphate production by planktonic animals. *Journ. Cons. Int. Explor. Mer.*, Vol. XII, pp. 144–6.

GAUSE, G. F., 1934. *The Struggle for Existence.*

GRIESSMANN, K., 1914. Über marine Flagellaten. *Archiv f. Protistenk.*, Bd. 33, pp. 1–78.

GROSS, F., 1937. Notes on the culture of some marine plankton organisms. *Journ. Mar. Biol. Assoc.*, Vol. XXI, pp. 753–68.

HARVEY, H. W., 1939. Substances controlling the growth of a diatom. *Journ. Mar. Biol. Assoc.*, Vol. XXIII, pp. 499–520.

—— 1940. Nitrogen and phosphorus required for the growth of phytoplankton. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 115–24.

—— 1942. Production of life in the sea. *Biol. Rev.*, Vol. 17, pp. 221–46.

HARVEY, H. W., COOPER, L. H. N., LEBOUR, M. V. and RUSSELL, F. S., 1935. Plankton production and its control. *Journ. Mar. Biol. Assoc.*, Vol. XX, pp. 407–41.

HEILBRUNN, L. V., 1937. *An Outline of General Physiology.*

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *Journ. Mar. Biol. Assoc.*, Vol. XIII, pp. 560–99.

—— 1926. A new method for quantitative sampling of the sea bottom. *Journ. Mar. Biol. Assoc.*, Vol. XIV, pp. 529–34.

JENKIN, P. M., 1937. Oxygen production by the diatom *Coscinodiscus excentricus* Ehr. in relation to submarine illumination in the English Channel. *Journ. Mar. Biol. Assoc.*, Vol. XXII, pp. 301–43.

JUDAY, C., 1940. The annual energy budget of an inland lake. *Ecology*, Vol. 21, pp. 438–50.

KAHL, A., 1935. *Die Tierwelt Deutschlands. Protozoa. I. Wimpertiere oder Ciliata (Infusoria)*, pp. 1–886.

KROGH, A., 1916. *The Respiratory Exchange of Animals and Man.* Monographs in Biochemistry.

KROGH, A. & SPÄRCK, R., 1936. On a new bottom sampler for investigation of the microfauna of the sea bottom—with remarks on the quantity and significance of the benthonic microfauna. *K. danske vidensk. Selsk. Biol. Medd.*, Vol. XIII, 4, pp. 3–12.

LACKEY, J. B., 1936. Occurrence and distribution of the marine protozoan species in the Woods Hole area. *Biol. Bull.*, Vol. LXX, pp. 264–78.

LEBOUR, M. V., 1922. The food of plankton organisms. *Journ. Mar. Biol. Assoc.*, Vol. XII, pp. 644–77.

—— 1930. *The Planktonic Diatoms of Northern Seas.* Ray Society, London.

LEMMERMANN, E., 1914. *Die Süsswasser-flora Deutschlands, Österreichs und der Schweiz.* Heft 1. *Flagellatae I. Pantostomatinae, Protomastiginae, Distomatinae.*

LLOYD, B., 1931. Muds of the Clyde Sea area. II. Bacterial Content. *Journ. Mar. Biol. Assoc.*, Vol. XVII, pp. 751–66.

LOHMANN, H., 1908. Untersuchungen zur Feststellung des vollständigen Gehalts des Meeres an Plankton. *Wiss. Meeresuntersuch. Abt. Kiel*, N.F., Bd. X, pp. 131–370.

—— 1911. Über das Nannoplankton und die Zentrifugierung kleinster Wasserproben zur Gewinnung desselben im lebendem Zustande. *Int. Rev. Hydrobiol.*, Bd. IV, pp. 1–38.

MACGINITIE, G. E., 1932. The rôle of bacteria as food for bottom animals. *Science*, Vol. 76, p. 490.

MARE, M. F., 1940. Plankton production off Plymouth and the mouth of the English Channel in 1939. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 461–82.

—— *The Significance of Micro-organisms and Organic Detritus in the Food Chains of a Marine Benthic Community.* Unpublished thesis.

MARINE BIOLOGICAL ASSOCIATION, 1931. *Plymouth Marine Fauna* (2nd edition).

MEIKLEJOHN, J., 1930. The relation between the numbers of a soil bacterium and the ammonia produced by it in peptone solutions; with some reference to the effect on this process of the presence of amoebae. *Ann. App. Biol.*, Vol. XVII, pp. 614–37.

—— 1932. The effect of *Colpidium* on ammonia production by soil bacteria. *Ann. App. Biol.*, Vol. XIX, pp. 584–608.

MOORE, G. M., 1939. A limnological investigation of the microscopic benthic fauna of Douglas Lake, Michigan. *Ecol. Monogr.*, Vol. 9, pp. 537–82.

MOORE, H. B., 1931. Muds of the Clyde Sea area. III. Chemical and physical conditions; Rate and nature of sedimentation; and Fauna. *Journ. Mar. Biol. Assoc.*, Vol. XVII, pp. 325–58.

MOORE, H. B. & NEILL, R. G., 1930. An instrument for sampling marine muds. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 589–94.

ORTON, J. H., 1914. On some Plymouth holothurians. *Journ. Mar. Biol. Assoc.*, Vol. X, pp. 211–35.

PENNAK, R. W., 1940. Ecology of the microscopic metazoa inhabiting the sandy beaches of some Wisconsin Lakes. *Ecol. Monogr.*, Vol. 10, pp. 537–615.

PETERSEN, C. G. J., 1915. A preliminary result of the investigations on the valuation of the sea. *Rep. Dan. Biol. Stat.*, Vol. XXIII, pp. 29–32.

—— 1918. The sea bottom and its production of fish food. *Rep. Dan. Biol. Stat.*, Vol. XXV, pp. 1–62.

PETERSEN, C. G. J. & BOYSEN JENSEN, P., 1911. Valuation of the sea. I. Animal life on the sea-bottom, its food and quantity. *Rep. Dan. Biol. Stat.*, Vol. XX, pp. 3–79.

PICKEN, L. E. R., 1937. The structure of some protozoan communities. *Journ. Ecol.*, Vol. XXV, pp. 368–84.

POOLE, H. H. & ATKINS, W. R. G., 1929. Photo-electric measurements of submarine illumination throughout the year. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 297–324.

RAWSON, D. S., 1930. The bottom fauna of Lake Simcoe and its role in the ecology of the lake. *Univ. Toronto Stud. Biol.* Ser. XXXIV. *Pub. Ontario Fish Res. Lab.*, Vol. 40, pp. 1–183.

REES, C. B., 1940. A preliminary study of the ecology of a mud-flat. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 185–99.

REMANE, A., 1933. Verteilung und Organisation der benthonischen Mikrofauna der Kieler Bucht. *Wiss. Meeresuntersuch., Abt. Kiel*, N.F., Bd. XXI, pp. 161–222.

REUSZER, H. W., 1933. Marine bacteria and their rôle in the cycle of life in the sea. III. Distribution of bacteria in the ocean waters and muds about Cape Cod. *Biol. Bull.*, Vol. 65, pp. 480–97.

SANDON, H., 1932. *The Food of Protozoa.* Pub. Faculty of Science, Egyptian Univ., Cairo.

SINGH, B. N., 1941. Selectivity in bacterial food by soil amoebae in pure mixed culture and in sterilized soil. *Ann. App. Biol.*, Vol. 28, pp. 52–64.

SMITH, J. E., 1932. The shell gravel deposits and infauna of the Eddystone grounds. *Journ. Mar. Biol. Assoc.*, Vol. XVIII, pp. 243–78.

SPARROW, F. K., Jr., 1937. The occurrence of saprophytic fungi in marine muds. *Biol. Bull.*, Vol. 73, pp. 242–8.

THAMDRUP, H. M., 1935. Beiträge zur Ökologie der Wattenfauna auf experimenteller Grundlage. *Medd. Komm. Havundersøg. Kbh. Serie Fisk.*, Bd. X, pp. 1–125.

TRASK, P. D., 1932. *The Origin and Environment of Source Sediments of Petroleum.* Pub. Amer. Assoc. Petrol. Geol.

WAKSMAN, S. A., 1933. On the distribution of organic matter in the sea bottom and the chemical nature and origin of marine humus. *Soil Science*, Vol. 36, pp. 125–47.

WAKSMAN, S. A. & CAREY, C. L., 1935. Decomposition of organic matter in sea water. I, Bacterial multiplication in stored sea water. *Journ. Bact.*, Vol. 29, pp. 531–43.

WAKSMAN, S. A. & HOTCHKISS, M., 1937. Viability of bacteria in sea water. *Journ. Bact.*, Vol. 33, pp. 389–400.

—— —— 1938. On the oxidation of organic matter in marine sediments by bacteria. *Journ. Mar. Research*, Vol. 1, pp. 101–18.

WAKSMAN, S. A., REUSZER, H. W., CAREY, C. L., HOTCHKISS, M. & RENN, C. E., 1933. Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms. *Biol. Bull.*, Vol. 64, pp. 183–205.

ZOBELL, C. E., 1939. Occurrence and activity of bacteria in marine sediments. In *Recent Marine Sediments*. Pub. Amer. Assoc. Petrol. Geol.

ZOBELL, C. E. & ANDERSON, D. Q., 1936. Vertical distribution of bacteria in marine sediments. *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 20, pp. 258–69.

ZOBELL, C. E. & FELTHAM, C. B., 1938. Bacteria as food for certain marine invertebrates. *Journ. Mar. Research*, Vol. 1, pp. 312–27.

—— —— 1942. The bacterial flora of a marine mud flat as an ecological factor. *Ecology*, Vol. 23, pp. 69–78.

ZOBELL, C. E. & RITTENBERG, S. C., 1938. The occurrence and characteristics of chitinoclastic bacteria in the sea. *Journ. Bact.*, Vol. XXXV, pp. 275–87.

THE DISPLACEMENT METHOD OF WEIGHING LIVING AQUATIC ORGANISMS

By A. G. Lowndes

(Text-fig. 1)

CONTENTS

Introduction

The work to be described constitutes the first successful attempt to weigh a living aquatic organism such as a fish, a prawn or an embryo without removing it from its aquatic environment. My primary object has been to find the *density of living aquatic organisms*, and this has been done throughout; but since it is essential to *weigh* before any form of quantitative analysis can be made, it would appear that this is the more basic problem. The determination of the *density* has as its final goal the problem of the distribution and the amount of energy required for that distribution, while the technique of weighing a living aquatic organism is fundamental in biochemistry and embryology and many other branches of biology.

The work was first started in 1936, but it was not till 1938 that I was able to give my whole time to it at the Plymouth Laboratory. For this work I received a generous Government grant through the Royal Society, together with

a similar grant from the British Association, and I am indebted to the Marine Biological Association for laboratory accommodation and all possible facilities. To all these bodies I wish to express my gratitude.

The war has brought many and serious interruptions to the work, and though in normal circumstances publication at this stage might well be thought premature, it has been thought best in existing conditions to give an account of the results so far obtained.

Methods

To find the density of an organism it is necessary to weigh it and to find its volume, and since many people have suggested that this can be done directly it is thought advisable to discuss the method briefly. A large prawn was known to weigh 5·253 g. with an error of less than ±0·007 g. It was weighed directly by placing it in a dry stoppered weighing tube and weighing the whole. Then the prawn was removed and the tube again weighed. The weight was found to be 5·663 g., indicating an error of 8%. The volume of the same prawn was known to be 4·80 ml. with an error of rather less than ±0·010 ml. Its volume was obtained directly by taking two 100 ml. graduated cylinders and rinsing them both with sea water. One was filled with sea water, the prawn inserted and the water levelled to the 100 ml. mark. The water was then poured into the other cylinder leaving the prawn behind, and it was drained for 15 sec. The volume of the prawn thus obtained was 6·0 ml., thus indicating an error of 24%. In nearly all the results obtained by the displacement method (to be described later) the weight was also found by the direct method and the percentage error given. Direct weighing forms a useful check, but for accurate work it is useless.

It has also been suggested that a fish can be weighed by weighing a tank of water and dropping the fish into it, but apart from the fact that it is not possible to dry the fish, or even to drain it properly without bringing about serious physiological changes, there is the difficulty of weighing a tank of water. The accuracy with which anything can be weighed depends primarily on the earth's gravitational constant, and it can be shown on theoretical grounds that the extreme limit is 1 part per 1,000,000. Thus it is not possible to weigh a kilogram to the fourth decimal place of a gram. In practice the seventh figure in any single weighing is meaningless. If a tank holding 10 l. of water is taken, and this amount is certainly not sufficient to cover a large dogfish, a lobster or a crab, a weight of nearly 20 kg. has to be dealt with, and since the displacement value of the majority of organisms in sea water is quite small, the difference in weight cannot be measured by direct weighing with reasonable success. Similarly, since a measuring cylinder always carries 100 divisions unless it is specially made, a litre cylinder having a diameter of about 3 in. will only read to 10 ml. A measuring cylinder which would take a large crab some 10 in. in length of carapace could only be read to about 100 ml. The above difficulties should be sufficient to indicate that a special technique is essential.

The Displacement Method

The general principle of the method I have adopted lies in the use of the specific gravity or density bottle, which is probably the most accurate of all volumetric apparatus. The bottle is weighed full, the animal inserted and the bottle again weighed. There is almost without exception an increase in weight, showing that all marine organisms have a density greater than that of sea water. The method can, however, only be rendered strictly quantitative if it is possible to obtain the volume of the organism and the density of the sea water together with the volume of the density bottle. The last two are easily obtained by standard methods and present no great difficulty. The important point is the accurate determination of the volume of the organism.

Volume Determination. If a density bottle is filled with sea water and the contents poured into excess of silver nitrate, a definite weight of silver chloride or rather silver halide is obtained. This precipitate can be weighed, and one is actually using what is probably the most accurate of all gravimetric determinations in standard analytical chemistry. If now the same bottle is again filled with sea water, a shrimp inserted, and the water again poured into excess of silver nitrate, a smaller weight of silver halide will be obtained. From these two weights it is possible to obtain the volume of the shrimp with extreme accuracy; but there is, however, the rinsing of the shrimp to be taken into account. In the first instance the bottle was rinsed with distilled water; but this cannot be used for rinsing the shrimp because of the difference in osmotic pressure between it and sea water. The shrimp must therefore be rinsed with a solution devoid of halide but having the same osmotic pressure as sea water. The obvious washing or rinsing solution is one of sodium nitrate to which a little calcium nitrate is added. In practice the shrimp is retained on a filter and rinsed quickly with the isotonic solution. The volume of the shrimp having been determined, its weight and density can be obtained and the shrimp can go back to the aquarium for further experimental work. An animal tissue would naturally be weighed in a suitable Ringer solution, otherwise the procedure is identical.

In actual practice the technique is rather laborious and less simple than it sounds at first. It consists, however, in the preparation of the isotonic solution of sodium nitrate and the making and standardization of suitable density bottles. Dealing with the solution first: 4 lb. of pure sodium nitrate are dissolved in about 8 l. of water. 100 ml. of pure nitric acid are neutralized with excess of calcium carbonate and the solution boiled and filtered into the sodium nitrate solution. The *p*H is brought up to that of sea water by adding a dilute solution of sodium carbonate. In practice this neutralization is quite easy, for on adding the sodium carbonate a precipitate is formed which dissolves less and less readily as one approaches the end-point. The freezing-point of the solution is determined accurately with the Beckman apparatus at different concentrations and a convenient table constructed. It is found that if 350 ml.

of the above solution are made up to a litre, a solution very slightly hypertonic to the Plymouth circulation sea water is obtained.

In dealing with organisms living in brackish water the freezing-point of the environment must first be ascertained and then the corresponding solution of nitrate made up. The solution should be as near isotonic with that of the environment as possible, but it should be hypertonic rather than hypotonic, for if the nitrate solution is hypotonic the organism will take in water and give out chlorides, while if it is hypertonic it will give out water, which will make no difference to the measurement of the volume at this stage, and the rate at which the organism will absorb chlorides will be so slow as to be negligible, apart from the fact that there is very little chloride available for it to absorb. It should also be remembered that the nitrate solution need only be in contact with the organism for a very short time, usually a few seconds or half a minute at the most. The bivalent calcium ion has the effect of slowing down the rate of exchange of ions considerably.

That this nitrate solution has no detrimental effect on the organisms is shown by the fact that they will survive being placed in it many times in succession. Thus the same specimen of the first larva of a lobster has been rinsed thoroughly in the solution six times during the one day. The same is true for *Anemonia sulcata* and for the sponge *Ficulina ficus*. If a young nursehound is removed from sea water, it soon forms within the pharynx quantities of mucus, but nothing of the kind appears when it is immersed in the nitrate solution.

Density Bottles. The greatest difficulties in the displacement method are those afforded by the density bottles. While it is easy to clean and dry a crucible and handle it with forceps, and finally leave it on the balance for some time before weighing, such things cannot be done with a density bottle containing a liquid, and the difficulty becomes far greater if the bottle contains a living organism which is to survive. The accuracy of the method is determined by the consistency of consecutive readings with a bottle which is filled, dried on the outside and placed on the balance. If the difference does not lie within a milligram it is clearly useless to weigh to the fourth decimal place. The difficulty has been long recognized and partly overcome by such devices as the Bousfield and Sprengel pycnometers; but these are useless for weighing anything but the very smallest organisms by the displacement method. If a 100 ml. density bottle when full of sea water can be weighed accurately to within a milligram the percentage error is small; but we are concerned with the displacement weight, or the difference between the bottle full and the bottle with the organism inserted, and since this seldom amounts to more than a few grams the percentage error may be considerable.

The density bottles used range from those holding 0·5 ml. to those holding nearly 8 l. The smaller bottles, which are always of the type shown in the diagram (Fig. 1*a*), are always placed for weighing in a close-fitting outer covering (Fig. 1*b*). The water evaporating from the bottle itself quickly saturates the relatively small space enclosed, while the rate of diffusion be-

tween the air in the outer case and the desiccated atmosphere of the balance itself is extremely slow. A density bottle of this type, placed in its outer case, showed no increase or decrease in weight at the end of half an hour on the most sensitive balance used. The outer case need never be removed from the balance case. Usually a reasonably accurate weighing can be made with one of these smaller bottles in 30 sec. at the most. The balances used with bottles up to 100 ml. are the air-damped Sartorious type or the new Oertling aperiodic type, or in the smallest bottles a Sartorious air-damped microbalance, these balances carrying a maximum load of 200, 100 and 20 g. respectively with corresponding sensitivities.

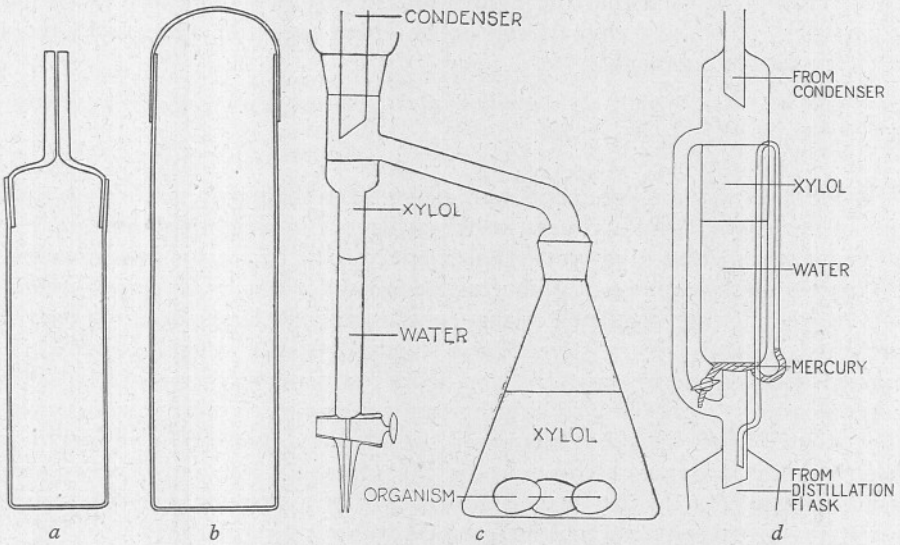

Fig. 1. *a*, type of density bottle used. *b*, outer case for taking the density bottle while being weighed. *c*, an ordinary Dean & Stark's apparatus used for the estimation of water. *d*, an ordinary Soxhlet Extractor used as a Dean & Stark's tube. The siphon tube is blocked with a little mercury. Water collects in the extractor and xylol automatically returns to the distillation flask.

For the weighing of prawns a special density bottle was made. Some long thin weighing bottles were found in the laboratory. They were just over 4 in. long and fitted with a good ground-glass stopper. A small V notch was made in the stopper with a file and the bottle carefully standardized by use of N.P.L. tables. It was easy to make up a closely fitting outer case. The volume of the bottle was about 35 ml., and since 1 ml. of sea water gives about 0·08 g. of silver halide precipitate, the whole contents of the bottle gave about 2·8 g. of the silver salt. This amount of silver salt is easily handled with modern sinter glass crucibles and can be weighed accurately. The limit of accuracy here is clearly the accuracy with which the bottle can be dried and weighed consistently. The only real test is the error obtained by taking the same prawn,

weighing it several times and comparing the result; but unfortunately this is not so simple as it sounds. No living aquatic animal is unaffected by handling, and in prawns scales or spines may be lost or the animal may excrete or feed during the process. Moreover, the aerated sea water frequently gives off bubbles of air and these may seriously affect the result. It is true that the air could be expelled from the sea water by first boiling it, but the animals are not living in freshly boiled sea water.

The following table shows the extremes obtained by weighing the same prawn four times by the displacement method and thus obtaining the extremes of weight, volume, density and sinking factor (the last-named is described later). It will be seen that the figures for the last two agree well to within 1 part per 10,000, and this appears to be about the limit of accuracy for a very favourable organism.

Volume ml.	Weight g.	Density 15·2° C.	Sinking factor	% error in volume
4·810	5·2597	1·0935	1066·2	0·026
4·795	5·2438	1·0936	1066·3	0·026

While the above measurements were being taken the prawn in question shed one of its uropods; this accounts for the difference in weight and volume, but as might be expected it has not seriously affected the density or sinking factor.

Apart from the expense, it is not feasible to work with a much greater weight of silver halide than that used in this experiment; if a larger density bottle is used, its contents are transferred to a standard flask, a suitable volume extracted with a pipette and the silver halide estimated gravimetrically, or in certain cases by titration. The limiting factor in this method is the accuracy of the pipette, which increases the error considerably, and if one resorts to a titration the percentage error may be considerable. The same prawn was again weighed by the displacement method, but a titration was substituted for a gravimetric estimation. This gave the following result:

Volume ml.	Weight g.	Density 15·2° C.	Sinking factor	% error in volume
4·717	5·1664	1·095	1068·0	0·77

From the above figures it will be seen that a titration is scarcely satisfactory, but much depends on what proportion of the volume of the density bottle is occupied by the organism. An example will make this clear. A full-sized prawn in berry will just go into an ordinary $\frac{3}{4}$ in. test-tube and occupy about three-quarters of the length. The volume of the test-tube level with the tip of the rostrum of the prawn is about 35 ml., but the volume of the prawn itself is only 5 ml. If the sea water from the test-tube is poured into a 250 ml. flask, in the one case 35 ml. of sea water will be added and in the other 30 ml., and the difference in these two solutions is too small to be estimated with any great degree of accuracy. The actual figures approximate to the following: If a 35 ml. density bottle full of sea water is taken and rinsed to fill a 250 ml. flask, then 25 ml. of this solution will be titrated by 19·6 ml. of $N/10$ silver nitrate. If the prawn, of an assumed volume of 5 ml., is inserted into the density bottle,

which is again washed out into the same 250 ml. flask, 25 ml. of this solution will be titrated by 16·8 ml. of the silver nitrate. Assuming the accuracy of the titration to be within ±0·02 ml. the percentage error is $\frac{0{\cdot}02 \times 100}{19{\cdot}6 - 16{\cdot}8} = 0{\cdot}71$, and to this must be added a further error of 0·02 due to dilution. If, on the other hand, the solutions are estimated gravimetrically, the figures are as follows: 35 ml. of sea water will give 2·8 g. of silver halide while 30 ml. will give 2·4 g. These weights can be measured accurately and in practice they will give the fourth place of decimals. The percentage error is therefore $\frac{0{\cdot}0001 \times 100}{2{\cdot}8 - 2{\cdot}4} = 0{\cdot}025$, and since there is no dilution this will be the total percentage error. Thus gravimetrically the percentage error is 0·025 compared with an error of 0·73 by titration. In practice I have almost entirely discarded titrations in this work.

It might be expected that an even greater degree of accuracy could be obtained as the result of this gravimetric work, but in general this cannot be done. Greater accuracy can only be obtained with such material as the early larvae of crabs or lobsters which can be packed into a density bottle very closely and made to occupy a large proportion of its volume. It must be remembered that the density bottle is used as a piece of volumetric apparatus, and this must put its own limit on the work. A density bottle cannot be graduated to the fifth decimal place of 1 ml., and it is being used with organisms and not dry chemicals. It should also be borne in mind that sea water itself is not a pure solution of sodium chloride.

For bottles holding over 100 ml. a special technique is required and practice has shown that excellent density bottles are easily made by taking good wide-mouthed bottles with suitable stoppers and cutting a V notch in the stopper. Excellent bottles for this purpose are made in the following sizes: 8, 12, 16, 32, and 64 oz. The 8 oz. bottle has an approximate volume of 315 ml. and the 64 oz. holds nearly 2 l. The stopper must be marked so that it can be replaced in exactly the same position each time, and the bottles must be accurately standardized by means of N.P.L. tables. Evaporation from these wide-mouthed bottles is a difficulty, but for reasons which need not be discussed it is far less in these bottles with a V notch than it is in those bottles with the ordinary perforated stoppers. For weighing with these larger bottles I have used two Oertling balances, the one taking a maximum load of 2 kg. and the other 5 kg. Weighing is here a much slower process, but since the animals are larger and more robust they can remain in one of these bottles for a longer time without suffering any apparent injury. With care the same animal can nearly always be weighed twice. The first time an approximate weighing is obtained; the animal is then returned to circulation for a short time, and on the second weighing an accurate figure is quickly obtained. If necessary, evaporation can be overcome completely by placing the large bottles in an outer covering as recommended for the smaller bottles; well-made tins can

be used. The disadvantage with the larger bottles is that the contents must be poured into a standard flask and an aliquot part extracted with a pipette, and this naturally limits the accuracy. In practice 50 ml. are extracted with the pipette and the silver halide estimated gravimetrically. These larger bottles are admirable for weighing nearly full-sized dogfish.

The work has now been extended so that the largest crabs and lobsters can be weighed, but this has involved a rather special technique. The density bottle presented considerable difficulty, but I was fortunate enough to obtain a large vessel with a really well-fitting stopper, made for the storage of sterilized instruments and dressings. By cutting two V notches in the stopper it was possible to convert it into a good density bottle. It held over $7\frac{1}{2}$ l. and had a mouth about 8 in. across. Two flasks of suitable size had to be obtained, and this presented some difficulty for they had to hold at least 10 l. The neck of one of them was carefully marked. It was unnecessary to standardize this flask provided there was some means of filling it up to the mark consistently. Finally, a suitable balance had to be obtained, and this was eventually supplied by Messrs Avery and Co., Ltd. It is capable of taking a load of well over 20 kg. and can be read to the nearest gram. The balance has the great advantage of being damped by a special kind of oil-bath and weighings are thus instantaneous.

With practice the jar could be filled and the weight read consistently to within a gram, and since the whole weighed just over 10 kg. the error was not more than 1 in 10,000. This apparatus was only used for the largest animals, and provided the displacement value was over 100 g. the percentage error in weight would be less than 1. Since any error in weight affects the weight and volume of the animal in question in the same direction and approximately to the same extent (1·025 : 1·0), the density and the sinking factor can be relied upon at least to the fourth digit. Titration would introduce far too great an error to be of any service.

The Limits of the Method. As an upper limit it may be stated that the largest lobsters to be obtained from the ordinary market and almost the largest crabs can be dealt with by the method outlined above. Nursehounds up to a metre long and even still longer congers can also be weighed, but the handling of these animals is difficult. The chief danger is that the large crabs or lobsters may break the jar with their claws. It is, however, the lower limit that is the most important, and in this respect the method is disappointing. The smallest density bottle used holds 0·5 ml., and so far I have found it impossible to place it consistently on the balance when full, with an error of less than 0·1 mg. This is about ten times the displacement value given by ten specimens of *Calanus*, and it is therefore impossible to weigh a single specimen of the copepod. It is possible, however, to obtain a reasonably accurate figure by placing 100 specimens in the bottle. Similarly, it has not been found possible to weigh satisfactorily a single specimen of the first larva of the lobster. On the other hand, it is quite easy to hatch out the first larvae of a single prawn in

berry and get a very accurate estimate of the density by weighing several hundreds of them at once.

Density. The Units Used

By density is meant the mass of unit volume, and the unit of volume is the millilitre and not the cubic centimetre. In all cases the densities have been calculated according to the formula

$$d_{4^\circ}^{t^\circ} = \frac{W'D}{W} - \frac{0{\cdot}0012\,(W'-W)}{W}.$$

$d_{4^\circ}^{t^\circ}$ means the density of the liquid at t° C. compared with that of distilled water at 4° C. (its maximum density). W is the weight of distilled water at t° C. and W' is the weight of sea water at the same temperature. The second part of the formula is the correction for the buoyancy of air. The formula is that given in *Practical Physical Chemistry* by Alexander Findlay (Longmans, London). The density of water at different temperatures is that given on p. 64 of the same book. Owing to the discovery of 'heavy water' the whole concept of pure distilled water has been changed. The distilled water used for standardization of apparatus in this work was obtained by distilling the Plymouth tap water first in an 'Atmos Still' and then redistilling it in a special still of Monax glass.

The Sinking Factor

Little information is conveyed by a determination of the density of the organism unless the density of the environment is determined at the same instant and under the same conditions of temperature. It is usually assumed that the density of sea water is 1·03, but this is scarcely correct for Plymouth sea water, and naturally the density varies with the temperature. What is required is a simple expression which will at once show the relation of the organism to its environment at the time of the determination. As an example: it was found that the density of a certain dogfish at a temperature of 17·2° C. was 1·0761, while the density of the environment at the same temperature was 1·02552. The obvious way of comparing these two densities is by means of the ratio $\frac{\text{Density of dogfish}}{\text{Density of the sea water}}$ or 1·0761/1·02552, which gives 1·0493. This, however, is not an easy number to bear in mind, and it is therefore multiplied by 1000 and the last figure discarded. The sinking factor of the dogfish in question would thus be 1049. A few examples of other results may be given. Only two determinations were made with the conger, and in both it was impossible to get a displacement value at all; in other words, the density of the fish was that of its environment, and the sinking factor (or S.F.) is therefore 1000. In the common stickleback the factor was found to be 1002, in the dogfish 1049, while in lobsters a sinking factor of 1140 has been obtained. This evidently means that a conger need make no effort to keep itself off the

bottom; a stickleback requires a little more effort, while a dogfish must exert a much greater effort. Lobsters require a great expenditure of energy to raise themselves off the bottom.

The Percentage of Water

Previously this was obtained with an organism such as a sponge by taking the sponge and rinsing it quickly in distilled water and drying it either in air, when it would largely decompose, or heating it in an oven at 90° C., in which case it would take days to acquire a constant weight and give off a good deal besides water and incidentally retain a good deal of its water. Alternatively, it might be dried for weeks in a vacuum desiccator.

The method I now use is a modification of that of Dean & Stark for finding the percentage of water in oils. The apparatus is quite simple and is of a standard type which can be obtained from any dealer in chemical apparatus. For the smaller animals the apparatus is that figured in the diagram (Fig 1 *c*). Assume for simplicity that one wants to find the percentage of water in some such animal as a mouse or a cockroach, both of which can be weighed on the balance without any appreciable error. The mouse is weighed, for preference in the distilling flask, and then covered with several times its volume of xylol. The whole is then heated over an electric hot-plate, and both xylol and steam are driven off and condensed and fall together into the graduated receiver. The two liquids are not miscible and the xylol floats on top of the water. Xylol distils at a temperature of just over 130° C., and so a good deal is driven off besides water, but any fats or oils remain in the xylol and do not affect the result. The point at which all the water has been driven off is clearly marked, for so long as water is coming off the xylol floating above the water is cloudy; distillation is therefore continued till a clear layer of xylol forms above the water. In actual practice it seldom takes more than an hour's distillation to drive off all the water.

Paraffin cannot be substituted for xylol as it does not have a constant boiling-point. This has been tested carefully, and at the end of an hour the boiling-point has usually risen to 160° C. and many of the tissues have begun to char. Glucose and starch are not affected by being distilled under xylol for a couple of hours, but cane sugar is and begins to char. In most cases it is sufficiently accurate to read off the volume of the water and take this as so many grams, but for more accurate determinations it is better to read off the scale, then empty the receiver completely and clean it. Then run in a little xylol and finally run in distilled water from a standard burette up to the previously observed scale mark. Then run the same volume of water from the same burette into a dry and weighed flask or weighing bottle and weigh again.

For larger animals the apparatus shown in the diagram (Fig. 1 *d*) is used. It consists of an ordinary Soxhlet extractor. This is first boiled out with xylol and the syphon tube is then sealed by putting a small amount of mercury in the extractor. The distillation is carried out as before, but the water which is

condensed collects at the bottom of the extractor. When the distillation is complete and the apparatus has cooled down, both mercury and water and some of the xylol are run out by means of the tap at the bottom of the extractor into a suitable graduated cylinder or possibly a standard flask. For ordinary work the heights of the top of the mercury and the top of the water are read, but for accurate work only the height of the water under the xylol is read, and then the mercury, water and xylol are run out. The mercury is dried with acetone and warm air and returned to the clean dry graduated cylinder. Some clean xylol is added and then distilled water is run in from a standard burette as before.

The process has many advantages apart from the great saving of time. Volatile substances such as oils which would come off below the temperature of boiling xylol are absorbed by the xylol. If a gas such as HCl comes off as the result of dissociation of the calcium and magnesium chlorides, it does not cause a loss in weight, for it dissolves in the water and the water is determined in the first instance by volume and not by loss in weight.

I recently determined the percentage of water in the ordinary hen's egg by this method. The distillation took less than an hour and the result obtained was 65·4 %, which agrees closely with the figure given by Lebbin, 65·16 % in 1900 as quoted in *Chemical Embryology* by Needham (Cambridge, 1931). Doubtless the percentage of water in a hen's egg will vary with age, etc.

With a sponge the technique is slightly different, but it is quite simple. First of all a rather extensive set of experiments had to be carried out to determine what weight of distilled water is to be obtained when 100 g. of the Plymouth Laboratory sea water is distilled under xylol by the method described above. The sea water in the tanks at Plymouth varies, but any serious alteration in its alkalinity is accompanied by a change in density, and this is automatically checked in finding the density or sinking factor of living aquatic organisms. Actually it was found that 100 g. of the laboratory sea water, having a density of 1·02518 at a temperature of 19·0° C., gave 96·8 (actually 96·796) g. of distilled water. This figure may or may not agree with the theoretical yield, but it includes the percentage experimental error which is far more important. The sponge is then weighed by the displacement method and returned to circulation for an hour. It is then transferred to the distillation flask, which has been weighed. If the sponge was known to weigh 50 g. and the contents of the flask weighed 100 g., there would be 50 g. of extraneous sea water present. If on distillation 80 g. of distilled water was obtained, that from the sponge would be $80 - 48{\cdot}4$ g. Thus the percentage of water in a living sponge is found. In the modified Dean & Stark method which requires the Soxhlet extractor some flasks had to be made with specially wide mouths and ground-glass adaptors, but otherwise the apparatus is again of a standard type.

In passing it may be well to note that the special method of distilling sea water is of some interest apart from anything else, for it is not possible by any known means to take sea water and evaporate it to dryness and obtain a

constant weight of residue. Sea water always contains both calcium and magnesium chlorides, and as the water is driven off these will dissociate and continue to give off HCl till both the calcium and magnesium are present as CaO and MgO. On distilling sea water below xylol a certain amount of dissociation always takes place and HCl is given off, but this makes no difference in the determination of the percentage of water, for as explained above the water is first measured by volume and not by weight. Contamination with xylol would in any case rule out the actual weighing of the water driven off.

The Percentage of silica and nitrogen

On distilling the sponge *Ficulina* under xylol it does not disintegrate but retains its shape in a rather remarkable manner. The sponge can then be removed, the xylol burnt off and the whole heated to redness in a silica basin. The residue is then digested with strong nitric acid and the silica finally filtered off on a Gooch crucible which is heated to redness for several hours and then weighed. Thus the percentage of silica is obtained. Similarly a weighed amount of living sponge can be digested with sulphuric acid and potassium sulphate and the nitrogen estimated by Kjeldahl's method. Calcite can be estimated by several methods, but so far the percentage of carbon has not been determined successfully.

Results obtained with the Different Organisms

Sponges and the Density of Protoplasm

	Density	Temp. ° C.	Calcite %	Density of protoplasm	Water %
Sycon coronatum	1·0925	14·6	7·88 Silica	1·0370	—
Halichondria panicea	1·068	15·0	5·08	1·0384	—
Ficulina ficus	1·0809	15·6	3·85	1·057	—
	1·077	15·6	3·98	1·056	—
	1·0805	16·5	5·506	1·049	81·6
	1·0815	17·0	5·35	1·0509	80·94

The above figures were those obtained for a number of sponges, but unfortunately it was not possible at the time to substitute gravimetric estimations for titrations. It is obvious that there is a great deal of work to be done in this direction, but it is felt that it must be postponed till more material can be obtained and longer periods devoted to it.

G. P. Bidder realized many years ago that if the density of a living sponge could be obtained and the percentage of either calcite or silica present as spicules estimated, it should be possible to obtain a close approximation to the density of the protoplasm itself. This assumes that a sponge is composed of little else besides spicules and protoplasm. We know that a sponge consists of other things, but since it is unlikely that their density differs greatly from that of protoplasm the above assumption is reasonably valid. On this assumption the density of protoplasm has been computed, the method being the following:

Sycon coronatum (Ellis & Solander)

Temperature throughout: 14·6° C.
Distilled water (filling density bottle) 50·9652 g.
Sea water (filling density bottle) 52·3331 g.
Sea water and sponge (filling density bottle) 53·0210 g.

Volume determination

Titrations 14·31 and 11·40 ml. with $N/10$ silver nitrate

$$\text{Vol. of sponge} = 50{\cdot}87 - \left(\frac{11{\cdot}40}{14{\cdot}31} \times 50{\cdot}87\right) = 10{\cdot}345 \text{ ml.}$$

$$\text{Density of sea water} = \frac{52{\cdot}3331 \times 0{\cdot}999190}{50{\cdot}9652} - \frac{0{\cdot}0012\,(52{\cdot}3331 - 50{\cdot}9652)}{50{\cdot}9652} = 1{\cdot}026055.$$

Weight of water displaced by sponge $= 10{\cdot}345 \times 1{\cdot}0260 = 10{\cdot}6145$ g.
Weight of water remaining in density bottle $= 52{\cdot}3331 - 10{\cdot}6145 = 41{\cdot}7186$ g.
Weight of living sponge $= 53{\cdot}0210 - 41{\cdot}7186 = 11{\cdot}3024$ g.

$$\text{Density of sponge} = \frac{11{\cdot}3024}{10{\cdot}345} = 1{\cdot}0925$$

Weight of calcite 0·891 g.
Percentage of calcite 7·88
Weight of silica 0·0351 g.

$$\text{Density of protoplasm} = \frac{W - (M' + M'')}{V - \left(\frac{M'}{2{\cdot}72} + \frac{M''}{2{\cdot}65}\right)}.$$

W, weight of living sponge; M'', weight of silica;
M', weight of calcite; V, volume of living sponge.

Density of calcite = 2·72.
Density of quartz = 2·65.

$$\text{Density of protoplasm} = \frac{11{\cdot}3024 - (0{\cdot}891 + 0{\cdot}035)}{10{\cdot}345 - \left(\frac{0{\cdot}891}{2{\cdot}72} + \frac{0{\cdot}035}{2{\cdot}65}\right)} = 1{\cdot}0371 = 1{\cdot}0371 \text{ at } 14{\cdot}6° \text{ C.}$$

I am greatly indebted to Dr Bidder for much advice and helpful criticism in connexion with these sponges.

CRUSTACEA

	Temp. ° C.	Sinking factor
Branchiopoda		
Chirocephalus diaphanus Prévost	11·3	1011
Artemia salina (Linn.)	10·0	988
Daphnia pulex (de Geer)	7·0	1017
Ostracoda		
Candona candida O. F. Müller	7·6	1025
Herpetocypris reptans Baird	8·8	1170
Copepoda		
Calanus finmarchicus (Gunnerus)	15·4	1029
" " "	15·4	1033
Anomalocera patersoni Templeton	16·2	1014
Diaptomus gracilis Sars	8·0	1023
Tigriopus fulvus (Fischer)	15·6	1060
Leptostraca		
Nebalia bipes (Fabr.)	7·4	1045
Amphipoda		
Gammarus pulex de Geer	20·0	1066
" " "	20·0	1088

	Temp. ° C.	Sinking factor
Mysidacea		
Hemimysis lamornae (Couch)	6·4	1075

Decapoda

	Water %	Error by direct weighing %	Temp. ° C.	Sinking factor
Leander serratus (Pennant)				
Female (not in berry)	65·08	73·7	15·2	1098·4
" " "	—	—	13·8	1086
Female (not in berry) (highly coloured)	73·14	—	15·2	1066
Female (in berry)	—	—	15·2	1068·8
First larva	—	—	15·2	1074
Palaemonetes varians (Leach)	—	—	10·8	1071
" " "	—	—	10·8	1049
Crangon vulgaris Linn.				
Female (in berry)	—	—	17·2	1083
First larva	—	—	15·5	1052
"	—	—	15·5	1039

Homarus vulgaris Milne-Edwards.

	Length in in.	Water %	Error by direct weighing %	Temp. ° C.	Sinking factor
Female late intermoult	7·5	66·8	—	16·6	1128
24 hr. after moulting	7·5	82·24	4·5	16·6	1050
Female, large encrusted	14·55	—	1·4	15·2	1137·1
Female, similar	14·55	—	2·6	15·2	1139·8
Female in berry	10·2	—	3·7	15·2	1129·3
Female in berry	10·5	—	4·2	15·2	1120
Male, shell clean	11·8	—	3·6	15·2	1140
Male, shell clean	8·4	—	2·4	15·2	1099
Embryos:					

	Water %	Temp. ° C.	Sinking factor
Fairly young: 2100 × 1862 μ 1980 × 1560 μ	51·2	15·2	1066·1
Slightly younger	43·24	15·2	1073
Much later, about to hatch: 2340 × 1980 μ 2250 × 1920 μ	—	15·2	1047

Portunus depurator (Linn.) Palmer

	Temp. ° C.	Sinking factor
Male, 4 specimens	16·6	1143
Female, not in berry	16·2	1133
Same specimen, in berry	16·2	1123
Embryos:		
White: 16 blastomere stage	15·2	1098
8–64 blastomere stage: Size 320 × 330 μ 340 × 340 μ	15·2	1090·2
Red	15·2	1070
Black, just before hatching	15·2	1047
1st larva (pre-zoaea)	15·2	1053
Megalopa (29 specimens)[1]	15·2	1065
Portunus puber (Linn.) Palmer: Males, 2 large	16·2	1136·7
Carcinus maenas (Pennant): Male	16·6	1177

[1] Weight of a single specimen 0·0024 g.

Cancer pagurus Linn.

	Width of carapace in cm.	Error by direct weighing %	Temp. ° C.	Sinking factor
Female	11·21	2·5	5·6	1091
Female	20·15	1·16	15·2	1176
Female, 2 hr. after moulting	10·68	—	16·0	1023·6
Female, same crab as above but 1 week later, abdomen still soft	12·7	—	15·2	1054
Male	6·8	5·6	15·2	1218
Male	10·13	3·0	5·6	1112
Male	10·5	2·5	5·6	1187
Male	15·5	0·98	5·6	1230
Male	23·75	1·2	15·2	1226

Fish

	Temp. ° C.	Sinking factor
Conger vulgaris Cuvier (27 cm. long)	10·0	1000
Gasterosteus aculeatus Linn.	10·0	1003
" " "	20·0	1002
Crenilabrus melops (Linn.)	13·8	1004·5
Lepadogaster bimaculatus (Donovan)	15·0	1047
Pleuronectes platessa Linn., 5 small specimens	6·8	1036·3
Cottus bubalis Euphrasén: Female	6·4	1046
Male (water 72·56 %)	6·4	1054

Scyllium catulus Cuvier (nursehound)

Two young male specimens were obtained from the trawl. One (A) was weighed and then distilled under xylol and the percentage of water estimated, while the other (B) was kept alive in the aquarium and weighed at intervals. Another specimen (C) was a young female. There is apparently no means of telling the age of the Selachii. One large male was brought in to the laboratory having a weight (by direct weighing) of 5763 g. and a length of 112 cm., while another weighed 4798 g. and had a length of 105·4 cm. By observing the growth rate of the small specimen (B) it was hoped to estimate the age of these larger fish, but unfortunately the death of the specimen after being kept alive for nearly 18 months put an end to this work for the time being[1]. The following results were obtained:

	Age weeks	Length cm.	Weight g.	Volume ml.	Density	Water %	Temp. ° C.	Sinking factor
A	—	24·1	41·34	38·51	1·0734	69·71	16·0	1046
B	—	21·6	31·36	29·32	1·0695	—	16·0	1042
B	17	24·4	49·07	45·53	1·0773	—	5·6	1047
B	45	27·5	72·74	67·68	1·0747	—	15·2	1048
B	52	28·0	92·13	85·67	1·0754	—	15·2	1048
C	—	28·5	68·85	63·89	1·077	—	16·2	1049·7

Scyllium canicula (Linn.) (dogfish)

Female	55·0	548·92	510·1	1·0761	—	17·2	1049

[1] The figures under B show that after one year there had been the following changes:

Increase in length 29·5 %
Increase in weight 193·8 %
Increase in volume 192·2 %
Increase in density 0·55
Increase in sinking factor 0·62 %

MISCELLANEOUS

	Water %	Temp. ° C.	Sinking factor
Amphioxus lanceolatus (Pallas)	—	13·2	1040
Nereis diversicolor O. F. Müller	—	7·4	1045
Nereis virens (Sars)	—	9·6	1033
Anemonia sulcata (Pennant)	88·19	9·5	1017·6
Frog, *Rana temporaria* Linn:			
Tadpoles, 14 days old	—	9·6	1014
			Density
Ovary, just before ova enter the oviduct	—	7·4	1·0962
" " " "	—	7·8	1·0987
Testis, at breeding season	—	8·0	1·0596
" " "	—	9·2	1·0596
" " "	—	9·8	1·056
Hen, *Gallus*, yolk from egg	—	15·0	1·0252

DISCUSSION OF RESULTS

Direct Weighing

The displacement method of weighing an aquatic organism is a lengthy one, but there seems to be no alternative way of obtaining accurate results. I very much hoped that experiment would show that with the larger animals the error due to direct weighing, or weighing the animal wet on the balance, would be small and more or less constant; but this is not so. It would be possible to remove a great deal of the adhering water by the application of a cloth or filter paper, but this does not solve all the difficulties. By draining the animal for a definite time it is possible to keep the error on the one side, and certainly if the error were approximately constant much time could be saved; but the figures show that this is not possible. Thus, two large lobsters of the same length and neither of which was in berry gave widely different percentage errors with direct weighing. With smaller crustacea in berry the percentage error may be enormous. Reference to the figures will show that in a prawn in berry the error was nearly 74 %.

Swimming

An animal can only swim freely so long as it can support itself, and in all the organisms examined so far, with the exceptions of *Artemia* and the conger, a definite effort must be made by the animal to keep itself up. I have been able to make one determination only of the sinking factor of *Artemia*; it appears that when it is swimming in strong brine, which is its normal habitat, it has to exert a muscular effort to keep itself *below* the surface. This state of affairs is not confined to *Artemia*, for in addition to many of the aquatic insects which take a supply of air below the surface of the water with them, the common stickleback, *Gasterosteus aculeatus* may be mentioned. This fish in fresh water has a sinking factor of 1002, which means that it must make an effort to keep itself off the bottom. Frequently the fish can be seen resting on the bottom, and it often feeds on crustacea, many of which have a high sinking factor. I have observed this fish after a big feed of ostracods unable to lift itself up at

all. The stickleback can also be transferred suddenly from fresh water to sea water without any apparent injury, and this means that for a period the fish is less dense than its environment or in other words its sinking factor is less than 1000. I have observed that when treated in this manner the fish exert a great deal of energy in keeping themselves down, and it takes about 6 hr. before they are again capable of resting on the bottom.

The amount of work which must be done by a fish to keep itself off the bottom must depend on its sinking factor and, as stated above, if this is 1000 no effort is required. It is possible to show on theoretical grounds that it is not possible for an aquatic organism to remain in a state of hydrostatic equilibrium with its environment; but it appears that in the conger the difference between its density and that of its environment is less than 1 part per 1000.

The limitation of cilia as organs of locomotion was pointed out by Gray in 1928 (*Ciliary Movement*, Camb. Univ. Press), and it should be clear that cilia can only sustain and propel an organism provided its sinking factor is below a certain figure. What that figure is remains to be determined. The first larvae of echinoderms certainly have a low sinking factor and this is true of trochophores, but I look forward with great interest to finding the highest sinking factor attained by free-swimming veligers or the later larvae of echinoderms or any organism supported by cilia alone. What has been said about cilia is also true of limbs or other appendages moved by muscles. So far as fish are concerned it appears that the limit has been attained by the dogfish or nurse-hound, and the figure appears to be very close to 1050; but it must be remembered that the method of obtaining the correct sinking factor has only just been perfected. Few figures are available for dogfish, and at present living dogfish cannot be obtained at Plymouth. No determinations for the skates or rays have yet been made, but it is interesting to note that the sinking factor of such teleosts as the young plaice, *Lepadogaster* and *Cottus*, which only swim with difficulty, is below that of the free-swimming dogfish.

With crustacea the sinking factor attains a far higher figure. 1098 has been recorded for the prawn, *Leander*, which normally swims by means of its pleopods, while 1140 has been obtained for the lobster, an animal which is certainly capable of raising itself off the bottom even if it does not actually swim. *Portunus* reaches an even higher figure, 1143, but neither of the two animals last mentioned can be described as pelagic, and it is doubtful if this term can be applied to prawns or shrimps which spend a great deal of their time resting on the bottom. Truly pelagic malacostraca are difficult to secure, especially at the present time, but they will probably be found in due course among the euphausids or mysids. It is interesting to note that *Hemimysis lamornae* has a sinking factor of 1075, while that of the first larva of the prawn, which is certainly pelagic, is 1074.

A point of considerable interest arises with regard to crustacea in berry, and the work may throw considerable light on the habit of carrying the eggs or embryos which is so prevalent among the crustacea. At first sight it would

appear that the female is considerably hampered by having to carry this extra load, but this does not really represent the actual fact. In all the cases so far investigated the embryos have a sinking factor which is less than that of the adult and, thus, while a prawn in berry does actually weigh more than one not in berry, yet her sinking factor in berry is less than when she is out of berry. The load of eggs or embryos may impede her rate of swimming, but it has the compensating effect of causing her to make less effort to keep herself afloat. Thus it may be an advantage rather than otherwise for the female to carry her young. This, however, cannot be the cause of her doing so, for the far heavier crabs, which are quite incapable of swimming, also carry their eggs.

Reference to the figures on p. 568 will show that the sinking factor of the crustacean eggs and embryos is high. Cilia do not occur in the crustacea, but if they were to be found we should expect them first to occur in the early embryonic stages. The segmentation in the early stages of *Portunus depurator* is equal and holoblastic and the blastulae appear to be as symmetrical in their formation as those of *Echinus esculentus*. They are, however, much larger and never become free swimming by means of cilia; their sinking factor is in fact so high that they could not in any event be supported by cilia. It is true that the early embryos of *Portunus depurator* are considerably larger than those of *Echinus esculentus*, but the embryos of such organisms as *Cyclops* and *Diaptomus* are much smaller than many of the ciliophora or the flagellated organisms like *Volvox globator*. It appears then that there is something in the crustacean embryos which makes them abnormally heavy, but what this substance is apparently remains to be investigated.

Statements are made in many standard text-books that the density of the ovum in general is determined by the amount of yolk it contains, a large amount of yolk causing a high density. This, however, is incorrect, for the density of the yolk of the ordinary hen's egg has been obtained and found to be 1·0252 at 15° C., which agrees fairly well with the figure given by Baudrimont & de St Ange (1846), as quoted by Needham in *Chemical Embryology*, namely, 1·0288–1·0299. This means that if the yolk of the crustacean egg is in any way comparable to that of the hen a large amount of yolk should decrease the density or lessen the sinking factor, since the density of the yolk of the hen's egg is less than that of Plymouth sea water and less than that of protoplasm itself.

The density of the cytoplasm of the eggs of *Arbacia* according to Heilbrunn as quoted by Gray (*Experimental Cytology*, Cambridge, 1931, p. 63) is 1·03583. This is lower than the lowest figure which I obtained for the density of protoplasm in sponges, but even so it is higher than the density of yolk. Reference to Heilbrunn's paper reveals to my mind a rather serious objection, for to quote Gray: 'The specific gravity of the granules was determined by shaking the eggs to pieces; the resultant suspension was then centrifuged into sugar solutions of varying specific gravity to find a solution in which the granules just pass to the bottom of the solution.' But solutions of sugar which vary in

density must also vary in osmotic pressure. A hypertonic solution of sugar would withdraw the lightest substance in the granules, namely, water. This point seems to have been overlooked.

The eggs[1] of *Portunus depurator* and *Homarus vulgaris* appear to be very dense to begin with, and the sinking factor gradually becomes less and less, probably due to the absorption of water, till they assume a sinking factor of about 1050. At this stage the larvae become free, with a sinking factor of practically the same figure, and muscular limb movement readily supports them.

In passing it is interesting to note that the sinking factor of *Calanus* is so low that it is probably lower than that of its embryos. It is also an organism which undergoes extensive vertical migrations, and it therefore appears to be significant that the eggs are not carried by the female, at any rate in the later stages.

Nearly all the work on crustacea is, however, hampered by their undergoing ecdysis. A crab just after moulting had a sinking factor of 1023, while a week later its sinking factor was 1054. Months later it would attain a figure of 1230 according to the data already recorded, and until there is some means of ascertaining the state of the intermoult the use of the figures for the sinking factor is somewhat restricted. It seems that a rather laborious piece of work is indicated, namely, that of isolating a number of crustacea and weighing each individual periodically. An attempt was made to ascertain how far the sinking factor of *Palaemonetes varians* was effected by its migrating from sea water into brackish water; but the experiment consisted merely of finding the sinking factor of some individuals kept in brackish water and comparing it with data from those kept in sea water. The figures showed nothing and clearly any change would be masked by variation due to the state of the intermoult. An adult female freshwater crayfish, *Potamobius pallipes*, was investigated and compared with a young female lobster, neither being in berry. The result is given below, but it is doubtful whether it means very much at present.

	Crayfish, adult female	Lobster, length 7·5 in.
Sinking factor	1130	1128
Water	71·05 %	66·68 %

The highest figure reached by any freely moving animal is 1230 for the crab, *Cancer pagurus*, with a width of carapace of 15·5 cm. It is interesting to note that a much larger specimen with a width of carapace of 23·75 cm. gave 1226, while a much smaller specimen with a width of carapace of only 6·8 cm. gave 1218. The crabs appear to have higher sinking factors than the lobsters, but in spite of this they seem to have attained far greater activity on land.

No attempt will be made to discuss the function of the so-called swim-bladder of the teleost fishes; it is a fact that those fishes with a swim-bladder have a low sinking factor while those without it have a high one. Reference to the figures will show that the sinking factor of adult dogfishes and nursehounds appears to be remarkably constant, namely, 1050 ± 1.

[1] The word *egg*, though it is the one in general use, should really be replaced by *embryo*.

The Effect of Spines in Crustacea

A very noticeable characteristic of many of the pelagic crustacea, both fresh water and marine, is the outgrowth of the integument in the form of spines or setae, and the function of these has been the subject of a good deal of discussion. By some they are supposed to have a buoyancy effect, while it has also been suggested that their effect is to maintain or restore the centre of gravity of the organism to a certain position. Clearly the term *buoyancy* cannot be used of an organ or tissue which has a greater density than that of the environment, and the term should be restricted to such structures as bladders containing gas or possibly to oil-drops. I have discussed the effect of these spines on the centre of gravity in a previous communication and given my reasons for considering this theory untenable (Lowndes, A. G., *Proc. Linn. Soc. London*, Ses. 150, 1937–38, 25 March 1938, pp. 62–73). The chief effect of these spines is to slow down the rate of sinking. Spines and setae can have another effect however, for it is known that pelagic crustacea such as *Calanus* and *Diaptomus* as well as the nauplius and many decapod larvae have a slow gliding method of propulsion to which is added a sudden leaping or escape movement. *Calanus* itself when quiescent remains with its antennules extended and its body practically vertical. The sudden leap due to the action of the thoracic limbs which are otherwise motionless causes the animal to dart away suddenly in a plane at right angles to the vertical. If it darted off in the vertical direction when it was near the surface it would clearly leap out of the water (this actually happens in *Anemalocera patersoni*). *Calanus* is able to hang vertically by means of the long and characteristic plumose setae on the antennules. The effect of these setae thus is to cause the animal to sink slowly and in the most advantageous position so far as its escape movement is concerned.

Summary

The method by which the weight, volume, density and sinking factor of living aquatic organisms can be determined has been extended. By sinking factor is meant the ratio of the density of the organism to that of its environment at the time of the actual determination. The density of the environment is always raised to 1000, and the density of the organisms computed on this basis.

A quick and accurate method for determining the percentage of water in a living organism has been described and discussed.

On the assumption that a sponge consists of protoplasm and spicules only, the density of the protoplasm has been computed.

The sinking factor of several fish and of the embryos of some crustacea at different stages has been determined. One effect of the swim-bladder in teleost fishes is to reduce the sinking factor and thus facilitate swimming.

The effect of the sinking factor on swimming in general and the effects of spinous outgrowths in pelagic crustacea are discussed.

THE RELATION OF *GAMMARUS ZADDACHI* SEXTON TO SOME OTHER SPECIES OF *GAMMARUS* OCCURRING IN FRESH, ESTUARINE AND MARINE WATERS

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From the Plymouth Laboratory

(Plates I–III, Text-fig. 1)

CONTENTS

INTRODUCTION

Gammarus zaddachi, the species under discussion in this paper, is one of the most widely spread of the amphipods which inhabit the estuarine waters of northern Europe. It has been recorded in the river mouths and adjacent seas from the White Sea, along the Baltic, the coasts of Scandinavia, Prussia, the Netherlands, Great Britain and France and from the Crimean region also. Most of the early records are under the name of *G. locusta*, but many of these I have been able to revise, having had access to several large collections, housed in the British Museum, the Königsberg and Hamburg Museums and others.

This species has always been confused with *G. locusta*—why, it is difficult to say, as the characters are quite distinct. Their habitats also are different, *locusta* being a marine species, and *zaddachi* typically brackish water, though there may be a little overlapping at the seaward limit of its range. The discovery referred to below, of the difference in the development of the sensory armature of the cuticle in relation to the environment, may perhaps account for some of the confusion which has grown up round the two species, though not for all. Some may be due to incorrect identifications and the consequent incorrect data on distribution. In view of the immense range of *G. zaddachi* and its recognition and acceptance as a typical salinity indicator in ecological work, it is important that the whole subject should be cleared up once for all and the present paper is an endeavour to this end.

G. zaddachi was first established as a distinct species in 1912, when I found

and described ten specimens of it in a collection of Crustacea taken by Herr W. Klie in the harbour of Bremerhaven. I named it after Zaddach (1844), who was the first to draw attention to its difference from *G. locusta*, although he recorded it, with a query, under that species. I was enabled, however, before publishing my description, to examine Zaddach's original specimens preserved in Königsberg Museum, and thus confirm the distinctions between the two species.

The material from several collections available to me at that time consisted of about 2000 specimens of *G. zaddachi* taken in many of the northern European estuaries and ranging from the fresh water at the upper end, through variations of salinity to the mouth of the river where it entered the sea.

During the course of the investigations on the species a remarkable fact came to light, viz. that the difference in habitat was correlated with a difference in the external appearance, though the structure in all the forms was identical. The animals taken at the seaward limit of their range, the typical 'saline' estuarine form, were delicate and pellucid, the chitin so thin as to be semi-transparent, with comparatively very few hairs[1]; in consequence they presented a more spinose appearance when compared with the hairy 'fresh-water' form. The latter, living in fresh water in the upper reaches of rivers and in lakes, were more solidly built, opaque, with thicker chitin in the cuticle, and with dense clusters of long fine hairs on the appendages in addition to the spines. (For description and figures see pp. 593–602, Pls. I–II.)

In fact, on taking typical samples of the two forms from the extremes of the species range, the difference in their appearance was at first glance so great as to suggest two distinct species. Further observation, however, proved it to be entirely due to the greater development of the epidermal outgrowths (the 'sensory armature') in freshwater conditions. It must be emphasized that structurally the two forms are identical, and that it is not possible to find any point of distinction other than the increase of hairs. A further proof of their being the same species is provided by the specimens which I have called 'intermediates'. These occurred in different localities in varying salinities some with more hairs developed, some with less.

It may be said as a general rule that as the salt content of the water increases so the development of the hairs decreases. A very interesting instance of this is shown in a series of samples from the Elbe, ranging from the typical 'fresh-water' hairy form taken in the Hamburg Water supply, with numbers of intermediates in the early stages collected at different stations down the river, to the typical 'saline' form at the farthest seaward station in the estuary at its mouth. This distribution has since been amply confirmed in several English rivers, where good series of dredgings have been made throughout the whole length of the estuaries.

[1] In the general descriptions throughout the paper, the word 'hairs' is used as a comprehensive term to include all the varieties of sensory equipment such as the finest delicate hairs, and setae of all grades to strong bristles, as distinct from the spinose armature.

The more exact methods employed by recent workers, such as Serventy, Crawford, Spooner, Reid, Goodhart, and others, give clear-cut results as to the limitations caused by tidal influence on the distribution of the common north European *Gammarus*, *G. locusta*, *pulex*, *duebeni*, and *zaddachi*. The importance of these species in modern ecological work lies in their use as salinity 'indicators', and naturally any confusion as to their identity would nullify the value of the results.

As has just been said there has been great confusion in the past, which unfortunately persists to the present day. One of the principal causes seems to be the perpetuation of the errors of the old records in the identification of species, through acceptance of their statements without verification; and this has naturally given rise also to errors in the records of the distribution of the species. Inaccuracies of this kind have been handed down in the synonymy of a species by one Crustacea worker after another.

It is not surprising that in the early days mistakes were made. The number of species known to science was very small, and the descriptions and figures, while definite enough to distinguish one of these from the few others, proved quite inadequate for the purpose when applied to the numbers of closely allied forms found later. These forms could easily be fitted into the generalizations of the original diagnosis of the genus, but the finer specific distinctions were not noted, specimens of different species being only too often all labelled *G. locusta* if found in the sea, or *G. pulex* if taken in fresh water.

G. locusta, the oldest established species of *Gammarus*, was given by Linnaeus as the type of his genus; later authors, following him, redescribed the species, e.g. J. C. Fabricius (1775), O. Fabricius (1780), G. Montagu (1808), H. Milne Edwards (1830), Rathke (1836), Zaddach (1844), Brandt (1851) and many others, but as they were not all dealing with the true *locusta*, their accounts have added to the confusion instead of clearing it. In fact, it is impossible to-day to know with certainty which species is referred to by these early writers without having access to the original collections and examining the actual specimens described by them.

Another cause of confusion may perhaps be found in the theories formulated by certain carcinologists in their efforts to solve the species problem—theories built on very insecure foundations. An example may be given from Schellenberg (1937). The theory he puts forward seems to be that a new variety of a species may be formed directly by new modifications of its environment, and that conditions which work in the same way will produce similar changes, so that forms resembling each other are to be found living discontinuously in places far apart.[1] In the case he is arguing he considers that by a lowering of the salinity *G. zaddachi* is produced as a brackish-water

[1] Schellenberg (1937, p. 514) summarizes it thus: 'Mehrere Befunde bestätigen den Einfluss der äusseren Bedingungen auf den *Gammarus*-Bau. Gleichartig wirkende Bedingungen haben konvergente Formen hervorgebracht, so dass übereinstimmende Formen, diskontinuierlich an weit entfernten Stellen auftreten.'

variety of the marine *G. locusta*. He ignores the facts that the specific characters distinguishing *zaddachi* are as clear-cut and definite as those of *locusta*, and that the resemblances are simply generic. Even where the areas of their distribution overlap a little in the marine zone, *zaddachi* is as distinct from *locusta* there as it is from *G. pulex* in the fresh water at the other end of its range (see also p. 592).

Perhaps a further source of error may lie in the fact, pointed out to me by Prof. J. M. Pirlot (in litt., 6 February 1939), that even now another species, quite distinct but hitherto undescribed, frequenting a lower salinity than *locusta*, is still being mistaken for it and recorded under its name. He had described and figured it ready for publication just before the outbreak of war. Knowing how salinity conditions affect and limit the range of a species, I think it will be found later that many of the records which now puzzle ecologists, of the occurrence of *locusta* in unusually low salinities, can often be explained by the presence of this second species.

In the present paper I have limited my acceptance of the records of *G. zaddachi* to those that I could verify myself, though no doubt many others are correct in identification. There still seems to be such difficulty in recognizing the two forms of the species that I have described and figured them both in detail. The illustrations are taken from the original specimens, the 'saline' from Zaddach's collection at Königsberg, the 'freshwater' from the Hamburg water-supply pipes. They have been compared with hundreds of specimens from British rivers and estuaries and have agreed to the smallest detail. I have also noted the most striking points of difference from other species, *locusta*, *pulex*, *duebeni* and *wilkitzkii* (pp. 600–1).

I am greatly indebted to my daughter and to Dr E. J. Allen for the help I have received from them in the preparation of this paper.

Historical Review

The principal papers dealing with *G. zaddachi* are described below in chronological order. In tracing back the history of the species its records were found to be so involved with those of *G. locusta* from the earliest beginnings that it was impossible to extricate them without examining the specimens described. This I have been unable to do farther back than 1836 (Rathke).

The first reference to *G. locusta* is in an account by Linnaeus (1745, p. 260) of an amphipod taken on the seashore at Gothland, and named by him *Cancer macrourus coeruleus*. In the 10th edition of his *Systema Naturae* (1758), as also in later editions of this work, he has recorded it as *C. locusta*. For many years Linnaeus's work was the standard authority on the Crustacea, and the tendency of workers following him seems to have been to identify any marine *Gammarus* with his *locusta*, any freshwater one with his *pulex*. In an endeavour to define the specific characters with more exactitude, some of them amplified his description, e.g. J. C. Fabricius (1775, p. 418, under the name of *Gammarus*

locusta, syn. *Cancer locusta* Linné); O. Fabricius (1780, No. 231, *Oniscus pulex* syn. *Cancer locusta* of Linné); Montagu (1808, p. 92, *Cancer* (*Gammarus*) *locusta*); H. Milne Edwards (1830, pp. 367–9 as *Gammarus ornatus*, and 1840, p. 44).

From certain discrepancies in their accounts it can be seen, however, that they were dealing not with one and the same species but with several different ones. Four names, Linnaeus, Fabricius, Montagu or Milne Edwards, are quoted indiscriminately by later writers as the authority for the species *locusta*.

1836 **Rathke** described as *Gammarus locusta* an amphipod taken by him in the Black Sea, on the eastern half of the Crimea, living on the sand and under stones where the sand was still moist. He stated that, as he had no detailed characterization of the *G. locusta* which occurred in England and France, he might be mistaken in his identification, and he would therefore give a close description. The different appendages are described with notes on their proportions, size and colour, and figures given of the 2nd gnathopod, peraeopod 5, urosome, and telson. The figures are not distinctive, but fortunately Rathke's specimens were preserved at Königsberg Museum. Two tubes of them were sent to me for examination, the one, labelled '*G. locusta*, Krim, Rathke', contained three males of the true *G. locusta* (L.); the other marked '*Gammarus*, Krim, Rathke' had three *G. pulex* in it, and one ovigerous female *G. zaddachi*.

1843 **Rathke** (pp. 67–8) in this work records *G. locusta* Montagu from several places on the west coast of Norway, from Christiania, and Droeback at the head of the Fjord, and from near Danzig in the Baltic. He compared them, and found no difference between them, beyond the fact that the eyes in the Danzig specimens were rather broader in relation to the length of the animals. Neither could he see any further difference when comparing them with his specimens brought back from the Black Sea, except for a greater length of the hairs on the 2nd antennae of the Crimean animals, and a slight variation in the proportionate breadth of the rami of the 3rd uropod.

Fortunately, some of his specimens were still in Königsberg Museum, labelled '*Gammarus locusta*, Montagu, Norwegen; Rathke'. I examined the fifteen preserved. They were all fine examples of *G. zaddachi*, the 'saline' form, 9–22 mm. in length, males and ovigerous females.

1844 **Zaddach** (pp. 4–6) recorded a species of *Gammarus* from the Prussian coast, which he attributed, though with a query, to *G. locusta* Fabr. as described by Milne Edwards (1840, p. 44). He pointed out that his specimens differed from this account, notably in two characters, viz. the presence of clusters of long hairs on both the antennae, and the proportions of the rami of the 3rd uropods. He stated that if Milne Edwards's characterization were accurate, then this description of his Prussian animals would constitute a new species of the genus *Gammarus*. It is interesting to find Zaddach had noted the two most striking distinctions between the true *locusta* and these specimens from the Prussian coast. He was evidently reluctant to institute a new species, perhaps because, having investigated the collection of 'the celebrated Rathke', from the Norwegian coasts, he had compared the '*G. locusta*' represented there with his own animals and had found them identical in structure. That he was right in making this statement has just been shown above—the Norwegian specimens labelled *G. locusta* by Rathke were in fact all *G. zaddachi*.

I was able to examine Zaddach's collections (see Sexton, 1912) and establish beyond doubt the distinction of the species. I therefore named it in honour of Prof. Zaddach, as he was the first to draw attention to the characters peculiar to it.

1851 **Brandt** (pp. 132–5), in reporting on von Middendorff's Siberian collections, found a single specimen of *Gammarus* from the Doschkander River which flows into the Sea

of Okhotsk. He placed it under the Section 1. A. *a.* of Milne Edwards (*Hist. nat. d. Crust.*, 1840) defined as having the inner ramus of the 3rd uropod as large, or at least more than half as long as, the outer: he named it *G. locusta* with a query. He would, he said, have unhesitatingly attributed it to that species (the *G. locusta* of O. Fabricius) but for some differences in the proportions and greater hairiness of the antennae and the shape of the uropods, and also because of lack of knowledge of the structure of the 3rd pair, which was missing.

Brandt made a great effort to clear up the confusion in the synonymy of *locusta*, as he had found that several obviously different species had been included under this name. He traced the history of the species from the original diagnosis of Linnaeus, and discussed the various authors who had afterwards dealt with it, Roesel, Klein, Sulzer, Herbst, Pallas, Frisch, Otto Fabricius, J. C. Fabricius, Montagu, Kröyer, Leach, Desmarest and Rathke. The three last, he said, had accepted Montagu's determination (and this is borne out by Rathke's specimens being labelled '*G. locusta* Montagu'). He then referred to Milne Edwards's description of the species and the discrepancies between his account and those of O. Fabricius, and of Montagu. In discussing Zaddach's (1844) *Gammarus* from the Baltic he agreed with him that it could only doubtfully be assigned to *locusta* Fabr., since it showed several variations from Milne Edwards's description.

His conclusions throw little light on the subject, for they are based on the very inadequate descriptions and figures of the earlier authors, and not apparently on any of the actual specimens; but at least his work shows that by this time the need for more exact specific distinctions or definitions was clearly recognized.

1862 **Spence Bate** (p. 206, pl. 36, fig. 6) in the British Museum Catalogue, included under the name of '*Gammarus Locusta*' all the previous records, Linnaeus, Montagu, Milne Edwards, Rathke and Zaddach amongst others. He described the rami of the 3rd uropod as 'subequal'.

1863 **Bate** and **Westwood.** On p. 378 *G. locusta* is figured, practically the same drawing as in the British Museum Catalogue. The description is more detailed, and an effort is apparently made to combine the conflicting statements of the previous observers, without any realization that they related to different species. For instance, it is said 'the antennae have the peduncles strongly hirsute' (i.e. like *zaddachi*) but the figure shows them almost glabrous (i.e. like the true *locusta*); and the 3rd uropods 'have the branches subfoliaceous and nearly of one length, the inner one sometimes one-fourth or one-third smaller than the outer one' (i.e. including characters of both *locusta* and *zaddachi*).

1873 **Möbius** (pp. 118–19) recorded the occurrence of *G. locusta* L. from thirty-four stations in the Baltic, with the depths and nature of the ground on which the amphipods were found. As references he gave Linnaeus's *Syst. Nat.* and Bate & Westwood's description mentioned above. Some of these records no doubt apply to the true *locusta*, but that others are certainly of *zaddachi* I have been able to prove by examining the collection preserved at Königsberg Museum. The tubes sent to me were all labelled *G. locusta* and included several from the localities given by Möbius. One of these, marked '*Gammarus locusta* L. Kiel, Möbius, 1872, No. 6526' contained three specimens, a male and two female *zaddachi*, 13–15 mm. in length; two tubes from Zoppot, one also dated 1872, with forty-five *zaddachi* (saline form); three from the station near Memel with seventeen *zaddachi* (saline form); and mixed dredgings from Danziger Bucht, Zoppot, Hela, etc., various depths 1–25 m., 168 specimens, of which nineteen were *locusta* and 149 *zaddachi* (saline form).

1878 **Zaddach** (pp. 27–32) in this paper gives a full description of the species which he had previously identified as *G. locusta* Fabr. with a query, but which he now considers to be unquestionably that species. In his list of references he cites Fabricius, Spence

Bate, and Möbius. He gives a detailed account, with numerous figures, to illustrate the structure, the reproductive organs and the differences between male and female, and between the adults and the young and the changes at the various stages of growth. Of the two distinctive specific characters which he had emphasized in 1844 he mentions the long setae on the antennae, though without drawing particular attention to them; but, in discussing the second character, viz. the proportionate length of the two rami of the 3rd uropod, he affirms that his specimens differ definitely in this from the species character given by Spence Bate and Milne Edwards. Instead of 'the 2 rami of an almost equal length' they have the inner ramus the shorter of the two, about three-quarters the length of the outer ramus in adults and even shorter in the young.

Zaddach's specimens were preserved in the Königsberg Museum collection. I have examined thirty-six of his tubes of *Gammarus*, thirty-two of which were labelled *G. locusta*. They contained 648 specimens from a large area extending from the open sea near Memel, along the Baltic coasts westward to the Gulf of Danzig and Putziger Wick, and from near the shore to a depth of 25 m. Many rivers drain into this area, e.g. the Niemen, Pregel, Vistula, and the salinity is therefore low. It is interesting to note in confirmation of the statement previously made with regard to the range of the two species being strictly limited by the salinity that the seventy-one *locusta* of Zaddach's collecting were found in the higher salinity of the open sea, and the 577 *zaddachi* saline form, in the estuarine waters.

1886 **Kraepelin** (pp. 13–25) gives an account of the Hamburg water-supply system, which at that date drew its water from the Elbe above Hamburg, and of the living things that were able to enter it direct from the river owing to the lack of a central filter-plant. He took many samples of the contents of the underground pipes with the idea of perhaps finding blind forms living in the darkness, or modifications of the river animals caused by their life underground. Though unsuccessful in this, he obtained no less than fifty different genera in the fauna. Amongst the Edriophthalma he mentions *Asellus aquaticus* in all the samples, as occurring in thousands, and second to it only in numbers '*Gammarus pulex*'.

He points out that though the animals enter from the river, there is a dissimilarity between the faunas of the river and the supply pipes; that all air breathers and vegetable feeders perish in the latter, and only those survive which are provided with gills and feed on detritus. For these the conditions of life in the pipes are much more favourable than conditions in the river, the pipe fauna enjoying a much greater abundance of food, protection from its enemies, and only slight variations of temperature.

Some of this material collected by Kraepelin, and by the Director of the waterworks, was sent to me for examination. The specimens, all labelled '*Gammarus pulex*', numbered seventy-nine, and were all the typical freshwater form of *G. zaddachi*, the largest measuring 20–22 mm. in length. The illustrations of the freshwater form (Pls. I, II) were taken from one of these specimens.

1889 **Hoek**,[1] in preparing his list of the crustacean fauna of the Netherlands, was faced with the difficulty of the conflicting records of the characters and distribution of *G. locusta*. The earlier writers had accounted for the confusion by stating that the species was an extraordinarily variable one. Hoek was able, however, to show that this was caused, not by any variability of character or habitat, but by the fact that several distinct species had been included under the one name, *locusta*. He made a careful study of numbers of specimens from different localities, and as a result divided them into four well-defined forms, as follows:

[1] My thanks are due to Mr Van de Kasteele, the Dutch Consul in Plymouth, for his kindness in translating Hoek's paper.

(1) The 'true *G. locusta*', living in the sea near the coast, farther out than *marinus*, the typical coast form;

(2) 'Variety A', inhabiting brackish water, found by Hoek himself in the Haringvliet and other places at the mouth of the Rhine, and the Waal;

(3) 'Variety B' (= *G. pulex* Fabr.), from perfectly fresh water in brooks and ditches; and finally

(4) 'Variety C', another brackish form, distinct from variety A, collected by Ritzema Bos in 1871 in a slightly brackish ditch near Warffum. This was later identified by Sars as '*G. duebeni* Lilljeborg'.

All four forms are well characterized with figures of the head, pleon armature, 3rd uropods and telson of each. I have compared Hoek's description of the characters of variety 'A', and its distinctions from *locusta* with *G. zaddachi* and found the two forms in such complete agreement (see p. 597) that there seems little doubt but that they are identical. The habitat also, with the salinity, is where one would expect the saline form of *G. zaddachi* to flourish.

Hoek found 'variety A' very common in the Haringvliet, at Hollandsch Diep above and below the bridge at Moerdyk in 4–7 fm., and at Nieuwe Merwede, where, he says, the salt content was somewhat different in these three differently named parts of the same river. The highest salinity he recorded was in the Haringvliet, 8·9 °/₀₀ at 6° C., though usually it is here much lower, from 3 to 3·2 °/₀₀. In the other places named it varied from about 1–0 °/₀₀. He was unable to extend his investigation farther up the river, though he considered it probable that such animals might exist there, and that it would be very important to ascertain to which variety they belonged.

1890 to 1894 **Sars** (p. 499, pl. 1 and pl. 176, fig. 1) describes the true *G. locusta* of Linnaeus, figuring it as the type of an amphipod. He was the first to give an accurate representation of the whole of its structure. He identifies Hoek's 'variety B' with *G. pulex*, and 'variety C' with *G. duebeni*.

1893 **Dahl** (p. 168) records *G. locusta* as one of the commonest animals in the Elbe from Hamburg down to the North Sea, and of special importance as food for fish. He adds that the species is very plentiful in the Lower Elbe in spring, practically absent in autumn, giving as an explanation of this fluctuation in numbers the statement that *G. locusta* during the summer lives on the shore under stones and among mussels (*Mytilus*) attached to woodwork and goes in winter into deep water. The animals at Hamburg, he says, live in quite fresh water with another species, *G. pulex* L., recorded by Kraepelin. He considered *locusta* and *pulex* to be very closely related, and thought it probable the 'freshwater *locusta*' might prove to be a form transitional between the two. He therefore examined specimens from the sea, and some from Altona, taking as distinguishing characters the eyes and the two rami of the 3rd uropods but no such transitional characters could be recognized. Kraepelin showed him some of his material from the Hamburg water system which he had identified as *G. pulex* L. In Dahl's opinion the specimens were definitely *G. locusta*, and he adds that Kraepelin naturally did not expect these sea forms in fresh water, and therefore had paid no attention to the small differences which distinguish them. It has already been shown (p. 581) that Kraepelin's specimens from Hamburg were *G. zaddachi*.

1892 **Chevreux et Guerne.** The two papers given in the bibliography were published at the same time, May 1892, and contain practically the same matter, viz. a description of a new species of *Gammarus* from Lake Annecy and a review of the freshwater *Gammarus* of France. The account in the *Bull. Soc. Zool.* is the more detailed, and contains figures for a comparison of the new species with *G. pulex*.

The authors mention '*G. locusta* Linn.' as a marine form, able to adapt itself to life in quite fresh water. They state (p. 141) that it is 'extrêmement commun dans la Loire, en amont de Nantes à plus de 80 kilomètres de la mer et se trouve aussi dans les

rivières de la Corse et de la Provence, au voisinage de l'embouchure'. They consider that *G. locusta* has frequently been mistaken for *G. pulex* in similar conditions, and cite, in corroboration of this, Dahl's statement that the *Gammarus* of the Hamburg water supply, identified by Kraepelin as *G. pulex*, was in reality *G. locusta* 'ayant remonté l'Elbe'.

1899 **Chevreux** revised the list of the Amphipoda found on the oceanic coasts of France. It is interesting to note that he now separates the *Gammarus* of the Loire from *locusta* under the name of '*G. Duebeni* Lillj.', while giving the same locality 'au bord de la Loire, depuis Saint-Nazaire jusqu'en amont de Nantes'. The second locality mentioned, 'Prairie de Mauves', is on the river near Nantes.

But Chevreux was evidently not satisfied with his identification of the species, for after the publication of my paper in which I said that these animals were probably *G. zaddachi* (1912, p. 661), he sent me specimens from his collecting in the district, labelled '*G. Duebeni* Lillj.' and asked me to examine them and confirm the identification, if correct. Some were from 'Nantes, au bord de la Loire', some from 'Belle Île-en-Loire (eau douce)', but all, without exception, proved to be *G. zaddachi*.

1907 **Volk** gives an account of an investigation in the lower reaches of the Elbe on the effect of the sewage water from the towns of Hamburg, Altona and Wandsbek on the fauna and flora of the river. The area surveyed extended from the freshwater region of Gauert, 15 km. from the harbour of Hamburg and far above any contamination from the city drainage, down to the Third Lightship and Neuwerk Island in the estuary of the Elbe where, with high tide and the wind inshore, nearly the full salinity of the North Sea water is encountered. A chart of the district surveyed shows the tributary streams, canals and docks at Hamburg and Altona. Detailed descriptions are given of the different harbour basins, the nature of the ground, and the plant life found in them. Temperature, chemical analyses of the water, the biological collections and their quantitative distribution are also reported on.

Volk records the occurrence of '*Gammarus pulex*' in Altona Harbour, where the river receives the sewage of that town, and where it contains the typical fauna and flora found in sewage-polluted water having a low oxygen concentration. He states that, though an animal usually sensitive to contamination, it occurs here in surprising quantities. In view of the fact that the River Elbe receives the sewage of Hamburg also, the outfall being about 2 km. above Altona, he regards this occurrence of '*Gammarus pulex*' as supporting one of the general conclusions of his survey of the river which he expresses thus:[1] 'on the whole the biological relations are such that there can be no question whatever of a pollution of the Lower Elbe harmful to the fisheries, in consequence of the inflow of sewage from Hamburg, Altona, and Wandsbek.'

The '*Gammarus pulex*' collected at twenty-one stations in this Survey were sent to me for examination; all the specimens, 564 in number, proved without exception to be *G. zaddachi*, both forms, the freshwater and the saline, being represented.

1911 **Vanhöffen** (pp. 399–405) describes a collecting trip made by him in September 1911 to Pillau on the Frisches Haff for the purpose of obtaining specimens of the brackish water coelenterate *Cordylophora lacustris* Allm., the so-called 'prickly moss' of the fishermen. It forms dense masses in the eastern part of the Haff, where the water still has 1‰ salinity. Vanhöffen found it growing plentifully in the 'Graben' (or Moat) which runs in from the Haff at Pillau, separating the old port from the new part of the town, and ending inland.

[1] 'Alles in allem liegen die biologischen Verhältnisse so, dass von einer die Fischerei schädigenden Verunreinigung der Unterelbe durch die Sielergüsse von Hamburg, Altona und Wandsbek überhaupt keine Rede sein kann' (p. 54).

On preserving the material from the Graben, he found a rich animal life in the sediment, comprising no less than forty species, showing an interesting mixture of marine, brackish and freshwater forms, of which thirty were new records for the Frisches Haff. Amongst these thirty, he gives '*Gammarus locusta* L.', but says the specimens found were all young, judging by the small number of joints, four in the accessory flagella of the 1st antennae.

Vanhöffen sent me twenty-four of these specimens labelled '*Gammarus locusta* L.' On examination they proved to be all *G. zaddachi*, the saline form, measuring from 2 to 10 mm., the largest, strongly built and opaque, approaching the freshwater type.

1912 **Sexton** (pp. 656–65, 2 pl.). Some estuarine amphipods, taken by Klie (1913) in the old harbour at Bremerhaven, form the subject of this report. Amongst them were ten small specimens of a *Gammarus* new to science which I described as *G. zaddachi* n.sp. The largest specimen, a female 9 mm. long, was figured. I compared the animals with an estuarine form collected in the River Tamar at Plymouth in 1911, and with some specimens, labelled *G. duebeni*, from the Norman Collection in the British Museum from Suffolk, East Anglia, and found they were one and all the same species. The report was prepared, but just as it went to press, a great quantity of material became available, including, amongst others, Vanhöffen's collection from the Frisches Haff, and the estuary of the Oder; Zaddach's from Königsberg Museum (dealt with in a separate paper (1913)); and, in particular, the interesting series of samples referred to above under Kraepelin and Volk. In the reconstruction of the paper, the English samples were, through some oversight, omitted.

While this varied material was being investigated, the remarkable difference in the *appearance* of the animals was noted, and correlated with the conditions in which they lived. Those from fresh water had thick chitin in the cuticle, and long fine hairs conspicuously developed; those from brackish water, the 'saline form', were spinose with few hairs, and a thin semi-transparent cuticle. The figures illustrating the new species were taken from the latter form, the female being illustrated in this 1912 paper and the male in the next (1913). But, as so far only the 'saline' has been represented, and as so many observers have failed to recognize its identity of structure with the 'freshwater' form, I have figured both forms in detail in the present paper.

1913 **Sexton** (pp. 90–4, 1 pl.). An account is given here of the collection of *Gammarus* in the Königsberg Museum, with figures of the males of *G. locusta* (L.) and *pulex* (L.) for comparison with the male of *zaddachi*. Specimens of the true *locusta* and *pulex* from British collections and of *duebeni* sent by Prof. Sars were compared with the new species and the differences noted.

Some errors in the printing of the text and plate will be seen (e.g. two joints instead of three in the peduncle of the first antenna of *G. locusta*) which could have been avoided had the usual practice been followed of sending the proofs for correction.

1913 **Klie** here describes his study of the seasonal distribution of the crustacean fauna of the Old Harbour of Bremerhaven. He took a series of samples at regular intervals during the year March 1911 to March 1912, noting also the tides, salinity and temperatures. The water surface of the Old Harbour he says is only 7·2 hectares, with a depth at mean high water of 7·06 m. Its situation at the mouth of the Geeste, where the stream of the Weser meets it almost at right angles at the entrance, together with the narrow sluice entrance, 11 m., accounts for the relatively low average salinity of the water, 5 °/₀₀. This is subject to constant variation, since the flushing water of the sluice at each tide causes a variation of the water level in the harbour and basin of 1 m. or more, a change which becomes very obvious through the predominance of fresh water at the ebb, and through the entrance of sea water at the flood. An increase of salinity in the height of summer each year has been recorded, as also a decrease in autumn and winter, the explanation given of this phenomenon being the lowered freshwater inflow, and

the evaporation during summer. Klie quotes figures to show the variation: e.g. on 22 October 1910 the salinity was 3·35 °/₀₀, on the same date in 1911 it was 8·13 °/₀₀; on 18 December 1910 it was 1·80 °/₀₀, the corresponding value for the same date in 1911 being 6·32 °/₀₀. In the table of salinities he shows the range of the variation for one year, the lowest record being for March, 0·79 and 1·02 °/₀₀, temp. 7·2° and 7·1° at low tide; and the highest in September, 12·64 °/₀₀, temperature 17·6°, also taken at low tide. It is in conditions such as these that *G. zaddachi* flourishes, as has been observed many times, and Klie records its appearance in twelve samples, and states that it is very common and to be found in the same places throughout the year.

1915 **Tesch** (pp. 336–9), in his account of the Amphipoda collected by the 'Wodan' in the southern area of the North Sea, discusses at length two specimens, male and female, of a species of *Gammarus* from the open sea, which he could not identify with any of the known species. He refers to Hoek's work on *G. locusta* (see p. 581), in which he showed that the idea of the so-called 'variability' of the species was due to the inclusion of several different species under the one name, the 'true *locusta*' was not variable.

Tesch gives a very careful comparison of his two specimens (male 17 mm., female 15 mm.), with both the 'true' *locusta* and Hoek's '*locusta*, variety A', and shows that they agree with 'variety A' much more than with the typical form, in the following characters, viz. the proportions and the number of joints of the antennae in both sexes; the armature of the pleon segments 4–6, which differ from the typical form in not being produced dorsally in definite humps; the telson; the 3rd pair of uropods; and the proportionate length of the two rami, with their spines and hairs. Indeed, he says, that were it not for the inexplicable fact that the label of his tube of specimens gives the position of the place of capture as the open sea (St. H. 4 b., about 10 miles from the coast, and over a depth of 80 m.) and also for the knowledge that 'variety A' is recognized as a purely brackish-water form, he would unhesitatingly identify them with that form. He considers them quite distinct from *locusta* and therefore meriting a separate name.

1916 **Stephensen** (pp. 236–7, and 293) records *G. zaddachi* Sexton as new for Greenland, one specimen being taken in 1912 in a freshwater stream at Narssak Skovfjord, south Greenland. He says the specimen was small, 7 mm. long, and a little defective, but that the 3rd uropod and 4th sideplate were in excellent accordance with Sexton's figure.

1917 **Stephensen** (pp. 37–50, figs. 1–5) and

1918 **Stephensen & Ussing** (pp. 335–8, figs. 9 *a*, *b*). The *Gammarus* discussed in these papers were captured in Randers Fjord, Denmark, during an investigation undertaken with the particular object of seeing to what extent the variation in salinity influenced the fauna. The salinity range was from fresh water 0 °/₀₀ at the head of the fjord, to 24 °/₀₀ (bottom) and 18 °/₀₀ (surface) at the mouth. Stephensen records five species of *Gammarus*, viz. *G. pulex* L., *G. locusta* L., *G. zaddachi* Sexton, an intermediate form between *locusta* and *zaddachi*, and *G. duebeni* Lillj.; but, he states, the specific characters are so vague that it is most probable that all the species he mentions are in reality only one species (1917, p. 37).

G. locusta L. was taken only at the mouth of the fjord in 16–24 °/₀₀ salinity, but, in discussing its distinctive characters in order to illustrate how greatly the species can vary with the environment Stephensen described specimens of '*locusta*' from different parts of the world, from Spitzbergen, the Kara Sea, Greenland, Arctic Seas, Great Britain and east America. (The figure given, however, of a male '*locusta*' from the Kara Sea (1917, p. 40, fig. 3) is evidently the Arctic species *G. wilkitzkii* Birula (see Gurjanova, 1930, p. 588).)

Most of the specimens identified as *zaddachi* and an 'intermediate form' were taken in water of lower salinity. Stephensen, also, refers to Hoek's work on *locusta* and its varieties (1889), and unites *zaddachi* with Hoek's 'variety A', saying, however, that he

considers it is not a true species but only the brackish-water form of *locusta*, and that the forms merge into each other.

Later on he re-examined this collection (see 1927, p. 120) and attributed all the specimens to *G. locusta*, acknowledging *G. zaddachi* as a distinct species.

1922 **Schäferna** (p. 96, text-fig. 6), in describing new freshwater species of *Gammarus* from rivers, brooks and pumps in the Balkan peninsula, names one, *G. spinicaudatus*, as near to *zaddachi*. He apparently accepts Stephensen's view (1917) that *zaddachi* is a variety of *locusta*, and says that his new species also is connected with *locusta* 'but more progressed than the *G. zaddachi* form'. There are not, however, sufficient distinctive characters given in the summary or in the figures to enable one to judge of the correctness of his view.

1922 **Schlienz** (p. 215) says that in studying the *Gammarus* of the Lower Elbe he found a difficulty in obtaining dependable characters on which to separate the species. He quotes Stephensen (1917) as having had the same difficulty, especially in distinguishing between *locusta* and *zaddachi*, and as having eventually reached the conclusion that the brackish-water *zaddachi* described by Sexton was only a variety of *locusta*. Schlienz goes on to state that, in his opinion, Sars's description and figures of *G. campylops* Leach, Sexton's species *zaddachi*, and his own material from the Elbe, all deal with one and the same animal, and that the one established by Leach in 1813–14.

It may be well to note here that Sars's identification of his species with Leach's *campylops* was an error. This, indeed, can be seen by comparing his figures, especially that of the 3rd uropod (1890, pl. 176, fig. 2), with Spence Bate's drawing of the same appendage from Leach's type specimen in the British Museum (1862, pl. 37, fig. 3), which was confirmed by Walker (1911) and Calman. They pointed out that Leach's types had the unmistakable short inner ramus characteristic of *G. marinus* and that Sars's animals belonged to a different species and were probably some young specimens of *locusta*. It is curious that Schlienz did not see this himself. He had the reference to Spence Bate in Sars's description, and also all the specimens of *G. zaddachi* which I had identified, in Hamburg Museum, where he could have studied them. Later, when Walker's paper came into his hands, he argued that Sars must have had a brackish-water form of *locusta* before him which was evidently *zaddachi*; that Leach's '*campylops*' became a *nomen nudum*, but the name could be retained for Sars's species; that *zaddachi* as a name must drop out, and the species should have only varietal status, and be known in future as '*Gammarus locusta* Linn. var. *G. campylops* Sars'. This matter has been dealt with here rather fully, and also in Sexton & Spooner (1940, pp. 673–5), because it is one of those mistakes in systematic work which get carried on indefinitely in the history of a species and cause so much waste of time in refuting them.

1923 **Schlienz** (pp. 429–52) in this paper gives an account of the conditions in the Elbe Estuary, such as salinity, nature of the substratum, sewage water, etc., as they affect the higher Crustacea.

Collections were made at twenty-six stations from Lauenberg, 570 km. from the river source to Cuxhaven on the Estuary, 725 km. Three species of *Gammarus* are recorded, *G. locusta* (L.), *G. duebeni* Lillj., and *G. zaddachi* Sexton, referred to throughout as *G. locusta* Linn. var. *campylops* G. O. Sars. The latter species was taken at all the stations in the area investigated, even up to the main sewer. Far from avoiding polluted waters, it occurs in almost incredible numbers below the actual harbour region of Hamburg, and is especially numerous near Mühlenberg (Blankanese), and at the Estemündung, where suitable places of refuge are afforded by the fresh and brackish-water hydroid *Cordylophora lacustris* and the two pond weeds *Potamogeton perfoliatus* and *P. pectinatus*. A table of salinities shows the occurrence of *Gammarus zaddachi* in brackish water, 0·5–18·0 °/₀₀, with the lowest mean salinity as 0·37 °/₀₀; of *duebeni* from

4·40 to 18‰; and of *locusta*, mean salinity 18–32‰, lowest 13·15‰. Schlienz concludes that, while this species cannot be regarded as an indicator (Leitorganismus) for the impurity of the region in question, it still follows that, as it exists in such great numbers, it is an essential member of the living community of the mesosaprophytic zone and a considerable factor in the biological self-purification of the Elbe.

1926 **Szidat** (p. 9) enumerates the many rivers draining into the Kurisches Haff, to show the great inflow of fresh water. That the salinity of the mesohaline region is very low, he says, is demonstrated by the presence of *G. zaddachi* and *Cordylophora lacustris* on the Mole at Schwarzort, together with numerous freshwater forms. In his list of Amphipoda (p. 15) he gives '*Gammarus zaddachi* (Sexton) = *G. locusta* var. *campylops* (G. O. Sars)', as found in both the mesohaline and polyhaline regions, and as occurring abundantly at Memel.

1926 **Riech** (p. 36) gives practically the same range of salinity for *zaddachi* in the Frisches Haff. He states that it is extraordinarily numerous in the algal growth, with *Cordylophora lacustris* 'die Leitform des Brackwassers'; and that it is found in all the littoral of the mesohaline region, as well as at Pillau and other places in the polyhaline division.

1927 **Stephensen** (p. 120), in his revision of the Danish Amphipoda, acknowledges *Gammarus zaddachi* Sexton as a distinct species. He says it has never yet been taken in Denmark, and that the specimens from Randers Fjord, which he ascribed to it in 1917 (p. 41), proved on re-examination to belong without a doubt to *locusta*.

1928 **Sexton** describes a series of experiments, the aim of which was to ascertain whether or not cross-breeding could take place between closely allied species of the same genus, and, in particular, between species living in the same environment. For several years previously the material for these experiments had been in course of preparation.

Beginning in 1912 with the brackish *G. chevreuxi* it was discovered that this species was able to adapt itself to great changes of salinity and could be brought to live in any conditions from perfectly fresh water to supersaturated sea water, provided only that the change over was made gradually and sufficient time allowed for the animals to accommodate themselves to the successive increases or decreases of salinity. It was found that more time had to be allowed for the last stage—the decrease to fresh water.

Eventually six species were used, viz. *G. locusta*, marine; *G. duebeni* and *chevreuxi*, brackish; *G. pulex* fresh water; *Marinogammarus marinus*, marine littoral; and *M. stoerensis* brackish. Three stocks of each were kept going, in fresh water, in brackish and in full sea water, and throughout the work, carried on for several years through many generations, special attention was given to note what effect, if any, the changes might produce on the specific characters. The most striking point to emerge from these observations was the *inherent constancy of the specific types*. The species characters, far from being variable, were not affected in any degree by changes of temperature, salinity, food, or other conditions; growth stages could be accelerated or retarded without affecting the structure in any way; young hatched after a 10–day incubation compared with those which had taken 120 days showed no differences; and specimens brought in from the wild were identical in appearance and mated at once with those of the same species inbred in the laboratory for several generations. The characters remained stable, and were transmitted to their offspring without modification.

It was important first of all to establish the fact of this constancy of type, for in systematic work where there has always been difficulty in distinguishing between the young and immature of allied species of a genus, the suggestion has frequently been put forward that these forms might be varieties due to chance interbreeding. It was necessary therefore to investigate this question, and as some hundreds of animals were available in all the stocks, fresh, brackish and marine, a large number of reciprocal crosses were set out.

To sum up briefly: no results were obtained from any combination of *locusta*, *duebeni* and *marinus* with the other species, no matings took place, and in most of the

crosses the females were eaten. Out of fifty-seven animals male *pulex* × female *chevreuxi*, five matings were recorded, no eggs laid; in the reciprocal cross, out of 148 animals, fifteen females paired and five batches of eggs were deposited, all infertile. Out of 391 animals, male *stoerensis* × female *chevreuxi*, there were only two pairings, no eggs, and most of the females eaten; in the reciprocal cross, 468 animals, twenty-nine pairings took place, eight batches of eggs were laid, all infertile. With twenty-one animals, male *stoerensis* × female *pulex*, there were five pairings, no eggs, all the females eaten; in the reciprocal cross the results were the same.

These results show that even in the most favourable and carefully prepared conditions, with each pair accustomed to the same salinity, guarded from outside attack, and provided with a plentiful supply of food, not a single cross-bred young was produced. None of the eggs laid developed, but were discarded by the females before the end of the incubatory period.

1928 **Stephensen** (pp. 275–8, fig. 58), in a key to the genus *Gammarus*, reinstates *G. zaddachi* as a species. For the principal character distinguishing it from *G. locusta* he takes the 4th sideplate; in *locusta* much deeper than broad, lower portion almost rectangular, corners rounded; in *zaddachi* not deeper than broad, inferior margin almost semicircular. Other distinguishing characters given by him are: peduncle of antenna 1 longer in proportion than in any other known species of *Gammarus*, and thickly beset with clusters of setae graduated in length; dorsal humps not very distinct; and inner ramus of uropod 3 only about three-quarters as long as outer.

He states that the species has not yet been found in Denmark and gives its distribution as recorded in Sexton (1912, 1913).

1929 **Stephensen** (pp. 138–9; text-fig. 34—figs. 241–2). This is the same account of *Gammarus* with the key and the characters distinguishing *zaddachi* from *locusta*, as that already given in 1928. He adds a reference to the occurrence of *zaddachi* in the Frisches Haff (where it is known as the 'Krabbenplage'), in such masses as to injure the fishing nets.

1930 **Gurjanova** (pp. 241–4, fig. 9) gives a redefinition and figures of *G. wilkitzkii* (Birula), one specimen of which had been taken in Ob-Jenissei Bay and described by Birula in 1897 as a variety of *G. duebeni* Lilj. Stephensen (1917, fig. 3) figured it as *G. locusta* L., and later it was suggested as the Arctic form of *G. zaddachi*. Gurjanova here distinguishes it as an independent species, and points out the characters in which it differs from other northern *Gammarus*, e.g. the shape of the head with very deep sinus; the eyes broadly reniform, relatively shorter and broader than in the other species; the numerous clusters of long hairs on the lower margins of both antennae; the shape of the gnathopod hands; and the rami of the 3rd uropods almost equal, and beset on both sides with long feathered bristles. She gives the known distribution of the species, Ob-Jenissei Bay, Nordenskjöld Sea, and Stephensen's specimen from the Kara Sea. It inhabits seas of very low salinities, and reaches a great size, up to 48 mm.

Gurjanova collected *G. zaddachi* in large numbers in the White Sea, at the mouth of the Sewernaja Dvina, in quite fresh water. The salinity in the estuary during flood-tide does not exceed 7–10 $^{\circ}/_{\circ\circ}$ and falls during the ebb to 0 $^{\circ}/_{\circ\circ}$. She considers that *G. zaddachi* and other organisms (such as *Anurea cruciformis*, *Zostera*, etc.), which are common in both the Baltic and the White Sea in the river mouths, are relict forms indicating that formerly an interchange of the faunas of these seas must have taken place.

Dr Stephensen kindly sent me three male specimens of *Gammarus wilkitzkii* from east Greenland, 65½° N., 33° W., measuring respectively 30, 44 and 45 mm. It has been suggested that this species is the Arctic form of *zaddachi*, and certainly the dense clusters of setae on the antennal peduncles give a superficial resemblance; but the points of difference are well marked, and confirm Gurjanova's view that it is a quite independent species. It can be easily distinguished from *zaddachi*, by its strongly

built body, small head and eye, and by its appendages which are all longer and more slender in proportion, with the hair clusters denser and more numerous on the anterior portion of the body, less so on the posterior.

1933 **Gurjanova** (pp. 75–90), in a list of the Crustacea found in the mouths of several of the great northern rivers, in the Baltic, and in the Caspian Sea, again states that the fauna of the Northern Dwina (Sewernaja Dvina) mouth is nearer to that of the Baltic than to the fauna of the Siberian estuaries. The fact that *Mesidotea entomon entomon* and *Gammarus zaddachi* occur only in these two localities points to a former connexion between the Baltic and the White seas.

1932 **Poulsen** (pp. 1–12, figs. 1–5), when examining the invertebrates collected by the Danish Commission for Research of Sea and Fisheries, found a number of specimens of *Gammarus* in the material, captured in the waters between the Islands of Sealand, Möen, and Falster. To complete this material, collections were made in quite shallow water along the coasts.

Between the islands, the depth of the water does not usually exceed 3–4 m. and the salinity is low, generally varying between 7 and 10 °/₀₀, only exceptionally reaching 12–13 °/₀₀.

Three species of the genus were taken: *G. locusta* L. in great numbers all over the area; *G. duebeni* Liljeborg, well represented in the material; and *G. zaddachi* Sexton, found in only two localities, Stege Nor, Möen, and at Gaabense, Falster. Poulsen gives detailed descriptions of all three species, together with measurements, and figures. For the most characteristic features by which the three species can be quickly distinguished from each other, he gives: for *locusta*, the 1st joint of the 1st antenna carrying on its underside only one hair besides the distal cluster of hairs, and secondly, the distinct nodular projections on the three urosome segments: for *duebeni*, the numerous hairs on the dorsal part of the urosome; and for *zaddachi* the length of the peduncle of the 1st antenna, and the clusters of hairs on its underside; and the 'non-hairy dorsal parts of the 1st–3rd urosome segments'.

The characters hold good for comparison with the true *locusta*, but not for *duebeni*, since we now know that the freshwater form of *zaddachi* has as many if not more hairs dorsally; and similarly, not for *zaddachi* since both forms carry hairs as well as spines on the urosome and the description 'non-hairy dorsal parts' refers only to the most extreme development of the saline form. Poulsen evidently knew nothing of the difference in the development of the specific sensory armature in relation to the difference in environment (see also Crawford, 1937, p. 660).

1933 **Oldevig** (p. 199) includes *G. zaddachi* in his survey of Sweden's Amphipoda, for although, he says, it has not yet been taken in Swedish waters, it may probably be found there judging by the records of its occurrence near the southern coast of Norway. He gives its distinguishing characters and distribution, as noted by Stephensen (1928).

1933 **Palmer** (pp. 64–7) found *G. zaddachi* in large numbers, together with *G. duebeni*, in a shallow brackish-water dyke behind the sea wall at Tollesbury, Essex. They were all, as would be expected, of the saline form. The colour of the living animal is given as transparent yellowish green, varying a little in intensity, with bands of brown on the posterior margins of the body segments, antennae, peraeopods, and uropods; the oil globules on the sides of the pleon are dark purplish red. The eyes are normally black with white interommatidial pigment, but five instances out of the normal were seen among the five to six hundred specimens examined; in one, a female, the pigment was red not black, and four others had 'mosaic' eyes, i.e. some of the ommatidia red, some black, the first record of such an abnormality in the wild.

1933 **Bassindale** (pp. 297–8, 2 figs.) discovered *G. zaddachi* in the Tay Estuary during the investigations of the Water Pollution Research Board. Collections were made from

the Bridge of Earn, 32 miles from the sea, near the limit of tidal waters down to Newburgh below the confluence of the Rivers Earn and Tay. The water is for the most part fresh, the salinity at Newburgh, the lowest station at which *zaddachi* was taken, at high tide reached only 3–4 °/₀₀. 199 specimens were taken, and were sent to me for identification; 198 were *G. zaddachi*, the typical freshwater form; four of the specimens had abnormal eyes, described in detail in the paper. It is curious that two reports on abnormalities in the wild should appear in the same year, this one on irregularities of eye-structure, and Palmer's, referred to above, on irregularities of the pigment.

1934 Schellenberg (pp. 7–13; 2 text-figs.), in his report of the Amphipoda of the Baltic, gives for the genus *Gammarus* two well-defined species, the euryhaline *G. locusta* (L.) and the brackish-water *G. duebeni* Lilj., with *G. zaddachi* Sexton named as a brackish-water variety of *locusta*.

He discusses first the distinction between *locusta* and *duebeni*. Stephensen (1929) had characterized the latter by the numerous setae on the urosome, telson, and peraeopods. This, Schellenberg points out, is not a sufficient guide, and he adds the salient distinguishing character found in the hinder peraeopods, viz. the posterior angle of the basal joint—free in all three in *duebeni*, but only in the 3rd peraeopod in *locusta*; that, together with differences in the male gnathopod hands, marks them as separate species. He comes to the conclusion that *duebeni* is sharply separated from the other two species although it has been frequently confused with *zaddachi*. All the certain records of its occurrence are concerned with brackish water of several per mille salinity, and he therefore suspects that wherever '*duebeni*' is recorded in fresh water, it is really *zaddachi* that is in question.

His next argument that *zaddachi* has no standing as an independent species, but is only the brackish-water form of *locusta*, is difficult to follow. He does not appear always to recognize the differences between the two forms of *zaddachi*, freshwater and saline, nor the distinction between them and *locusta*, and while it seems evident that he has seen these various forms, I am inclined to doubt if all his material of *locusta* is of the typical true species, or in fact if some of it may not belong to Pirlot's 'low-salinity' 'voisine de locusta' type (p. 578). This view would appear to be supported by his statements (on pp. 9, 10), where, in comparing the two species, he fails to note the differences in the shape of the 4th sideplate, and other distinctive characters such as the gnathopod hands, the development and position of the setae clusters and spines, the proportions of the rami of the 3rd uropods, etc. The most obvious distinguishing specific character to which I drew attention (1913, p. 91) he disregards, although it is an infallible guide in all the growth stages as well as in the adult animal. It is as follows: 'in *G. zaddachi*, the peduncles and flagella of both antennae carry dense clusters of long, graduated, outstanding setae: while in *G. locusta*, the upper antenna is, as in *G. pulex*, almost glabrous.' I have given figures (Pl. I fig. 1; Pl. III, fig. 19) of this character, and of the gnathopod hands of the males of the two species which should suffice to show their distinction.

Another definitive character given by me (1912, p. 662), viz. the greater length of the antennal peduncle in *zaddachi* as compared with *locusta*, Schellenberg considers as of no use for the purpose, and quotes in support of his argument the incorrect figure, of a two-jointed antennal peduncle, from my 1913 paper (pl. iv, fig. 3) but, as stated above (p. 584), I was not responsible for this absurd error in the reproduction of my drawing.

In his discussion of the distribution and salinity range of the two species he says there is overlapping, but *locusta* he rightly regards as marine and *zaddachi* as typically estuarine. He notes that *zaddachi* flourishes in the violent fluctuations of salinity such as occur in river mouths, and that it also lives well in waters of almost constant but quite low salinity, e.g. up the Elbe around Hamburg.

Finally, Schellenberg mentions various authors and their identifications, and concludes that the following, amongst others, all refer to *zaddachi*: Hoek (1889) '*G. locusta*, variety A'; Hellen (1919) '*G. duebeni*'; Schlienz (1922), '*G. locusta* Linn. var. *G. campylops* (Sars)'; Szidat (1926), '*zaddachi* Sexton = *G. locusta* var. *campylops* (G. O. Sars)': and Neuhaus (1933), '*G. duebeni*'.

1934 **Serventy** (p. 203), in his account of the marine invertebrate fauna of Scolt Head Island, Norfolk, records three *Gammarus*; *locusta* from the lower tidal levels, a member of the *Zostera* community: *marinus* from the upper levels, amongst *Fucus*: and *zaddachi*, always under estuarine conditions, widely distributed on the east coast.

1935 **Serventy** (pp. 286–94), in his observations on *Gammarus zaddachi*, gives notes on its occurrence in the River Deben in Suffolk, and its relation to the other species of the district. A diagram shows the salinity of the 12-mile long estuary, and the zones of distribution of the three principal species: *G. locusta*, inhabiting the lower part and extending to the sea outside, is the dominant form only as far as the salinity remains above 25–30 °/₀₀. *G. zaddachi* occupies the zone of decreasing salinity to the limits of the tidal influence, a distance of 5 miles. The river is very small compared with the size of the estuary, and this zone is characterized not only by a very rapid fall in the salinity gradient but also by a great fluctuation in salinity between high and low water, in one place reaching 15 °/₀₀. So that here twice daily the salinity oscillates over a range of 15 °/₀₀; an area, therefore, as Serventy points out, of peculiar rigour for marine animals, and one 'that demands a special physiological constitution, including the development to a high degree of osmo-regulatory mechanisms in its denizens'. The third species, *G. pulex*, takes the place of *zaddachi* just above the limit of saline influence in the estuary.

Serventy examined a collection made by Gurney in East Anglia, in which *zaddachi* was found plentifully represented, the specimens identified by Gurney being correctly named, those by Norman wrongly called '*duebeni*' (see also Sexton, 1912, p. 584).

Another interesting investigation was carried out by him in the Tamar, where Percival (1929, p. 93) had recorded '*G. locusta*' as occurring along the whole length of the river, from the sea to practically fresh water (0·1 °/₀₀ at high tide). As none of Percival's animals had been preserved, these statements could not be checked with the actual specimens themselves. Serventy therefore decided to collect in the brackish water section of the river where Percival had recorded *locusta*, but where, from his figures of the salinity, that species certainly could not have been expected to occur, though *zaddachi* might have been. From these localities he obtained *zaddachi* only, and there was no sign of *locusta*. Through his kindness in showing me his collection I was able to confirm the identification.

1936 **Crawford** (p. 102) refers to *G. zaddachi* as the commonest brackish-water *Gammarus* in the River Tamar and its confluents, the Tavy and Lynher, and as occurring in every stream running into these rivers. His list of localities completes Serventy's (1935) account of the distribution of the species in the Tamar.

1937 **Crawford** (pp. 647–62), in a more extended report on the estuarine fauna of the west of England and south Wales, names six species of *Gammarus* as inhabiting these regions, viz. *marinus* Leach, and *locusta* (L.) marine, the former found at a higher tidal level among *Fucus*; *chevreuxi* Sexton, *duebeni* Liljeborg, and *zaddachi* Sexton, in the brackish-water zone, though penetrating on occasions into the fresh water; and *pulex* (L.) fresh water.

Crawford describes the conditions and plant life of the various rivers investigated and gives the most important published lists of the British brackish-water Crustacea, together with certain suggested corrections of their nomenclature.

1937 **Schellenberg** (p. 482), in the course of his remarks on the systematics of the freshwater *Gammarus*, raises the problem of the cause of the numbers of different forms which yet are genetically close to each other. He asks, are we dealing with differences due to environment of such a kind that particular environments transform in a definite direction the *Gammarus* stocks which occur there? He considers the question could be answered either by experiment or through investigations as to whether with the transition from one environment to the other, one form of *Gammarus* is also transformed into the other, or whether particular forms are specially adapted for particular environments. He states, though without advancing proof, that such a step-by-step transition is seen in the case of the marine *G. locusta* going over into the estuarine *zaddachi*, and that it is most likely the gradual freshening of the waters of the river mouths which brings about the increased bristling (see Sexton, p. 587).

Schellenberg definitely concludes that environmental conditions are able to influence markedly the structure of animals, and that conditions working in the same way have developed forms similar to each other living discontinuously at places far apart.

1937 **Höfken** (pp. 116–48, 6 text-figs. and 20 tables), noting the conclusions reached by Schellenberg (1934), has attacked the problem of the Baltic species of *Gammarus* (*G. locusta*, *duebeni* and *zaddachi*) first by a statistical-variation research on a series of natural populations, and, secondly, by rearing the animals under changed environmental conditions, particularly changed salt concentrations, so as to follow up how far the development of the bristles would be influenced thereby.

He took his 'populations' from eleven places of varying salinities: from the North Sea at West Helgoland, with a salinity of about 32 °/₀₀; Kiel Harbour, of about 17 °/₀₀; Wittower Fähre on Rügen, 7·2 °/₀₀; to Neukuhren on the Samland coast with a mean salinity of 6·5 °/₀₀. He collected also at several places in the Schlei Estuary on a fluctuating range from 18 to 3·6 °/₀₀, and finally from Barther Bodden at Zingst. The Zingst station differed from the others in having a fairly constant salinity, of 6 °/₀₀, and being connected with other ponds (Bodden) and the open sea only by small channels, it is cut off from the Baltic, and the interchange of water, in consequence, is slight.

In order to carry out the statistical research on the setae numbers, three characters were taken from the anterior, middle and posterior parts of the body, viz. the number of seta clusters on the 2nd joint of the peduncle of antenna 1; the number of setae in the anterior distal cluster on joint 4 of peraeopod 5 ('Pp. 7', in Höfken's numbering of the appendages); the number of spines and the number of setae on the telson; and the size of the gill on peraeopod 5 (='Pp. 7'). The whole material was divided into size groups, each 1 mm. apart; one size-group, e.g. 10 mm., included animals measuring 10–10·9 mm., the next 11–11·9 mm., and so on. The measurements were taken by stretching the animal on a millimetre scale, and counting from the tip of the head to the insertion of the telson.[1]

[1] To my mind, an arbitrary grading of the size by such a method must lead to inaccuracy in the results. If the growth stages had been used as a standard of measurement, instead of 1 mm. increases in length, absolutely correct figures could have been obtained for each stage of the male and the female, and of the mature and the immature. It has been shown before, in *Gammarus* (Sexton, 1924), that all the individuals of a species go through a number (different for each species) of growth stages, marked off from each other by a moult, before they reach the full adult development; that each stage is characterized by an increase in size, in the number of joints of the antennae and pleopods, in the number of seta or spine clusters on the appendages, and, in the female, in the number of eggs in the broods laid in the successive breeding periods. Both sexes go through the same stages till sexual maturity is attained, after which the development is different, but, it must be noted, at any given stage, mature or immature, all the animals at that particular point are exactly alike, even to the number of setae and spines. The specific characters are *constant*, variation as such being unknown.

I have not seen any of Höfken's material, but it is evident that he clearly recognizes the distinction between the species *locusta* and *zaddachi*, though it seems doubtful whether, in the low-salinity populations, he is dealing with the true *locusta*, or what is perhaps more likely, with Pirlot's species, the 'voisine de locusta' (p. 578).

The different points of the problem which he investigated are briefly:

(1) The question whether there is a continuous transition from *locusta* to *zaddachi*, and if so, whether it is connected with the geographical distribution. The conclusion from the statistical work on nine populations is that there is no continuous transition from one to the other, and that the whole material belongs to two different types, the one weakly and the other strongly bristled, 'the *Locusta* and the *Zaddachi Types*'. There were no intermediate forms found, i.e. animals in which all the characters take up an intermediate position between the types.

(2) The next point examined was the relation between the salinity of the habitats and the distribution of the types, and here, after quoting Schellenberg's summary of the observations of previous workers and his own, and Serventy's (1935) results on the Deben, Höfken analyses his 'populations' and sums up by largely confirming Schellenberg's conclusions. These are that 'above a certain salinity, about 7 °/₀₀, *G. zaddachi* occurs only when strong salinity variations take place; from 7 °/₀₀ downwards, even if these are lacking, *G. locusta* can still flourish in exceptional cases at 4 °/₀₀.'

The second method, breeding animals under changed salinity conditions very different from their original habitats, *locusta* in water of 6 °/₀₀, *zaddachi* in 18 °/₀₀ showed a far-reaching constancy of type unaffected by the changes in environment.

Höfken summarizes his results as follows: (1) A *locusta* type and a *zaddachi* type are to be distinguished; structurally they overlap each other, but each of them is in itself independent. (2) Each of these two types occupies its own area of distribution, which, however, in places overlaps that of the other type. (3) The distribution of the two types is chiefly dependent on the mean salinity and the salinity variations. (4) The *locusta* and the *zaddachi* types are by heredity fixed types, and invariable when exposed to changes in the salinity concentration.

Description of *Gammarus zaddachi*[1]

Figures have already been given of the saline form from Bremerhaven (male in 1912, female in 1913). The freshwater form is now figured for the first time, and compared with adult saline animals of the same size.

The freshwater male represented was from the Hamburg water-supply collection (p. 581), the saline male from Rauschen on the Baltic coast, Zaddach's collection. The figures of the true *G. locusta* (L.) (Pl. III), the species most often confused with *zaddachi*, are given to demonstrate their distinction.

The *body* is slender and laterally compressed, in the male larger, stronger, and more slenderly built and with stronger appendages than in the female. The chitinous cuticle varies in the two forms, being thick, strong and opaque in the freshwater, thin and semi-transparent in the brackish-water specimens. The former, also, carries a much denser supply of the characteristic long fine hairs on the antennae, peraeopods (particularly the hinder ones), pleon, 3rd uropods and telson.

[1] The terminology which I have used in this paper is that of Stebbing ('Amphipoda Gammaridea', Vol. 21 of *Das Tierreich*), and is as follows: body, consisting of head, peraeon, and pleon: head with 1st and 2nd antennae; peraeon, with seven pairs of legs, two gnathopods, and five peraeopods; pleon segments 1–3 with three pairs of pleopods, segments 4–6 (or 'urosome') carrying three pairs of uropods and telson.

The *body colour* varies a little, much darker in some specimens than others, but is usually a pale yellowish green with bands of faint brown on the posterior margins of the body segments, the antennae, and other appendages. The females are generally darker than the males, more of a greyish green. Rathke gives the colour of his Crimean specimens as like weakly coloured joiners' glue in the male, dark grey in the female. The lateral patches of 'red oil-globules' on the first three pleon segments are very noticeable, bright red to purplish in colour. These red spots, noted by all the observers, were considered a distinguishing character of *G. locusta* at one time, whereas it is now known they are more or less developed in all the species of *Gammarus*.

Size and Sex. Compared with others of the genus, *zaddachi* is a fairly large species. There are many records of the male in favourable conditions, attaining a length of 20–22 mm.; the females are smaller, the largest about 15–17 mm. Rathke gives 10 lines for his Crimean specimens, 10½ lines for the Norwegian animals. The measurements are taken along the dorsum with the specimen straightened out on a micrometer scale, from the tip of the rostrum to the insertion of the telson.

Sexual maturity is reached at about half-growth. Zaddach found many females ovigerous during the months of May and June 1865 in Putziger Wiek, and in August 1868 he collected four small females with eggs in Danziger Bucht, the largest about 9 mm. There are many records from Britain of the species breeding from February to May.

No differentiation between the sexes can be seen in the newly hatched young, nor can it be traced till the sexual characters begin to appear several growth stages before maturity. Each growth stage ends with a moult, and by examining these moults it is possible to ascertain the state of the animal's development. This is more easily traced in the female in the brood-plates, which are attached to the ventral surface of the 2nd to the 5th segments. When fully mature they show as large thin transparent lamellae, inset on the margins with long flexible fringing hairs which interlace to form the brood-pouch.

The number of stages before sexual maturity is reached in *Gammarus* varies with the species; in *locusta*, for example, eggs are laid after the 12th moult, in *pulex* after the 10th, in *chevreuxi* after the 7th, and so on; but the development of the brood-plates appears to follow the same line in all the species observed. To take an example (Sexton, 1924, p. 345): in *G. pulex* where, owing to the thickness of the chitin, the moulted cuticle retains its firm outlines, it was possible to trace four stages of the developing brood-plates before full maturity. They first appeared in moult 7 as minute, rounded, leaf-like plates with margins entire; in moult 8 they had lengthened and increased to three times the size; in moult 9 rudimentary hairs were present on the margins; in moult 10 there was a great increase in size, and in the number of the rudimentary hairs, and the chitin was hard; in the next stage, sexual maturity was reached, the brood-plates were fully developed, with the long fringing hairs. In *G. locusta* the chitin is very thin in the young animals and tears so easily

that it is difficult to find the first stages of the brood-plates; three were found, the stage of moult 10 in *locusta* corresponding with moult 7 of *pulex*; that of moult 11 with moult 9 *pulex*; and moult 12 with moult 10 *pulex* (i.e. the stage before full maturity).

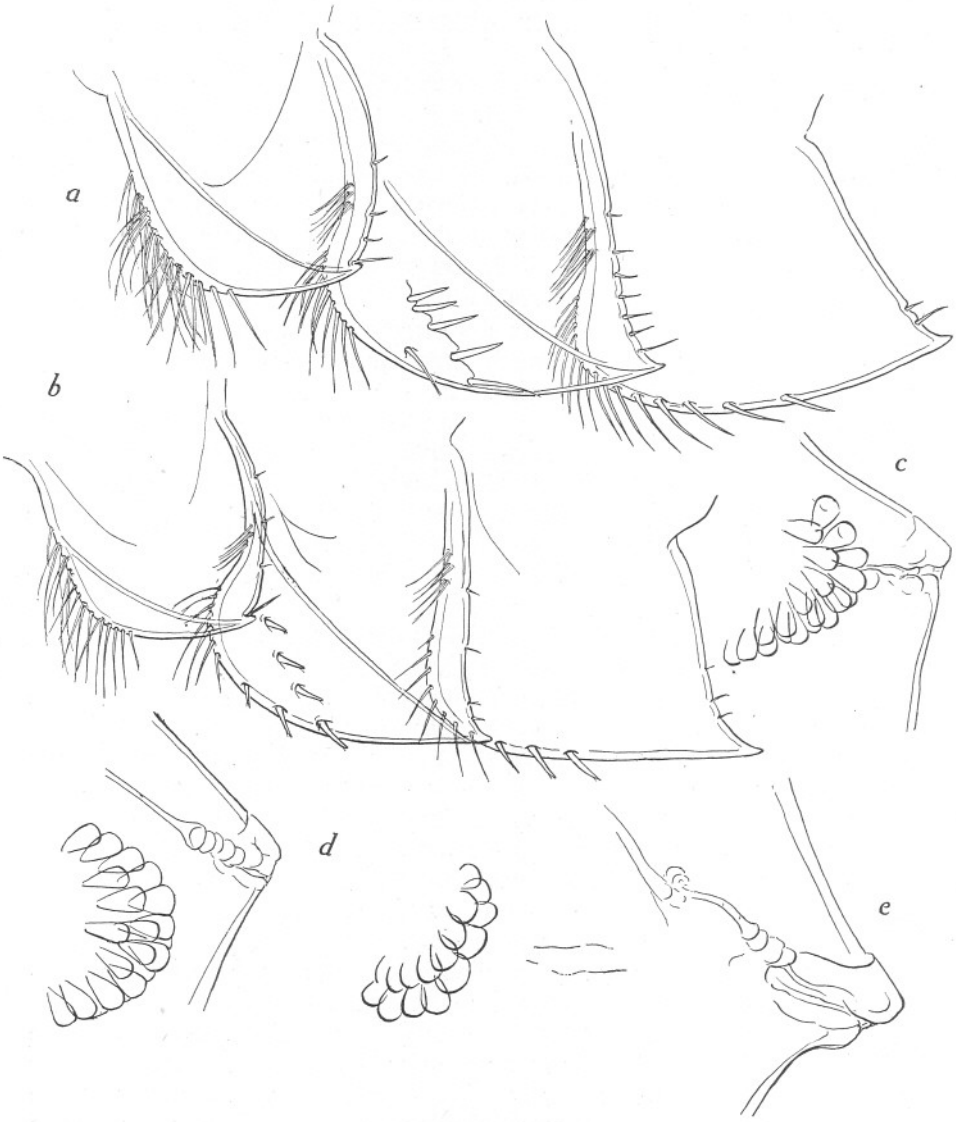
This point is emphasized here, because of the fact that certain female intersexes of *G. chevreuxi* showed an arrested development of the brood-plates, marked by a great reduction of size, and by the rudimentary condition of the fringing hairs, resembling the immature stage (e.g. in moult 9 of *pulex*). So close is the resemblance that some collectors have recorded the young immature females with partially developed lamellae as 'intersexes', but the mistake could have been avoided if the age of the specimens had been noted. In the young animal, the sensory armature is sparse, the gills and brood-plates are small, while the female intersexes are larger and much more setose than the adult normals, with the gills much bigger, and the brood-plates very small in proportion, when compared with them.

The *head* (Pl. I, fig. 1) measured along the dorsal line is about equal in length to the first two peraeon segments; in *locusta* it is definitely shorter. In both the freshwater and saline forms, the lateral lobes are obliquely truncate, with the upper angle produced, and subacute in the adults, rounded in the young and immature, as appears to be the case in the young of all the other species examined of the genus. Just below the angle and defining it is the indentation (Text-fig. 1*c*) described by Pirlot as 'l'invagination de l'organe frontal...creusée à la face interne du lobe lateral'. In *locusta* (Text-fig. 1*d*) the upper angle is sharply produced with the indentation small. A figure is given of this part of the lobe in *wilkitzkii* (Text-fig. 1*e*) showing the extreme development of both the angle and the channelled indentation.

Eyes (see Pl. I, fig. 1, and Pl. III, fig. 19) large, reniform, retinal pigment black with the interommatidial accessory pigment forming a thick white reticulation (not shown in the figures, as it tends to obscure the actual size of the lenses). The ommatidia are smaller and more numerous than in *locusta*, numbering about 170–180 in the largest males. In the newly hatched young the eyes are quite round, as Zaddach (1878) was the first to point out and figure, and consist of about eight ommatidia.

Side-plates smaller than in *locusta*, about as deep as the corresponding segments; 1–4 with two or three setules inset at the rounded anterior angle. The 4th (Pl. I, figs. 7, 9), which forms one of the distinguishing characters for the species, is as broad as deep, with the posterior expansion short and rounding into the almost semicircular margin, with three or four setules inset on the hind curve. In *locusta* the posterior expansion of the 4th is very deep, with the hind margin straight, and with about thirteen setules inset on the curve. In both forms of *zaddachi* the posterior margins of the 5th, 6th, and particularly the 7th side-plates are beset with long setiform spines, most numerous in the fresh-water animals.

Pleon, segments 1–3 (Text-fig. 1*a*, *b*): the 1st epimeral plate is deeply notched



Text-fig. 1. *G. zaddachi*, epimeral plates of pleon segments 1–3, from the males figured in Pls. I and II: *a*, the freshwater form; *b*, the saline form. ×23. *c*, *d* and *e*, upper angle of the lateral lobe of the head, right side, of *G. zaddachi*, *locusta* and *wilkitzkii* respectively, showing the opening connected with the frontal organ described by Pirlot, and the position of the eye in relation to the lobe in each species. ×75.

behind for the insertion of a seta, the 2nd and 3rd are acutely produced backwards, though not to the same degree as in *locusta*. The difference between the two forms is seen plainly in the armature, the freshwater form having many more hairs anteriorly, and fewer and more slender spines inferiorly.

Segments 4–6 (Pl. II, figs. 13, 18) slightly raised and rounded dorsally, each with the three spine groups. The number of the spines in these groups increases with age; the newly hatched young have only one spine in each group. In the adults the difference in the appearance of the two forms is more strongly marked here and in the hinder peraeopods than in any other part of the body, owing to the profusion of hairs developed in the freshwater animals. The spine formula for the saline form is usually 3 : 2 : 3; 3 : 2; 3; 2 : 2 : 2, with only a few short inconspicuous hairs between the spines, not exceeding them in length. Hoek (1889) gives the same formula for his '*locusta* var. A', and Tesch (1915) for his male specimen, and 2 : 2 : 2 on each of the three segments for the female. In the largest specimens from Rauschen the spines numbered 4 : 2 : 4; 4 : 2 : 4; and 3 : 2 : 3; and Vanhöffen's (1911) records are the same for his largest, with occasionally three instead of two in the first dorsal group. Rathke's largest Norwegian animals, 22 mm., had five in the lateral clusters of 4th and 5th segments, three in 6th.

In the freshwater form the spines are usually fewer, 2 : 2 : 2, on each of the segments, but the hairs are very numerous and very long, more than twice the length of the spines.

The *antennae* form one of the most striking distinguishing features of the species and the one most easily seen at first glance. In all the animals, saline as well as freshwater, both peduncles and the flagella carry on the under margin dense tufts or clusters of long stiff outstanding setae, some of each cluster extending far beyond the rest. The clusters in each joint are graduated in length to the distal angle, where the longest setae reach to three or four times the length of those in the proximal groups.

Antenna 1 (Pl. I, fig. 1) is not quite half the length of the body; this measurement, though formerly much used in diagnosis, cannot be considered satisfactory or reliable. The antennae seem particularly subject to injury, judging by the number of broken or regenerating flagella one finds on going through a collection. Antenna 1 is a little longer than antenna 2 in both sexes. The peduncle is unusually long for the genus, reaching in the adult to about half the length of the 5th peduncle-joint of antenna 2; in the young the peduncles of the two antennae are equal in length. The 1st joint, long and stout, is slightly longer than the 2nd in some specimens, subequal in others; the 3rd joint is rather more than half the length of the 1st; all three joints bear clusters of graduated setae on the under margin. As has just been said, it is a little difficult to give an exact number for the joints of the flagella. Zaddach has 25–35 for the primary and nine for the accessory; Hoek, for the male, 27–34, and 5–7; and for the female, 20–29 in the primary and 4–6 in the accessory. The largest males in the collections examined had 36–44 for the one and 7–9 for the other.

In addition to the long setae each joint in the primary flagellum from about the 5th carries a small stalked sensory filament on the under-surface.

There should be no mistaking this species for *G. locusta*, the characterization is so distinct. In antenna 1, for instance, the peduncle in *locusta* is noticeably shorter in proportion, extending only to the distal end of the 4th joint of the peduncle of antenna 2; both peduncle and flagellum are almost glabrous, very sparsely provided with small setae (Pl. III, fig. 19) and the accessory flagellum is much longer, 13–14-jointed in the male, with about forty-seven joints in the primary. Poulsen (1932) also pointed out that the 1st peduncle joint in *zaddachi* bore long graduated clusters of setae, while in *locusta* there is only one hair on the under-margin in addition to the distal cluster.

Antenna 2 (Pl. I, fig. 1) is stouter as well as shorter than antenna 1. The 4th joint of the peduncle is slightly shorter than the 5th, both having the characteristic graduated clusters of long setae. The flagellum is about as long as these two joints taken together, 15–19-jointed in the largest males, furnished with clusters of setae, similar to those on the peduncle, less numerous in the female than in the male. Calceolae (Pl. I, fig. 2) occur in both sexes on the proximal joints, generally on succeeding joints from about the 1st (or 2nd) to the 6th or 7th, then missing one joint, on the 8th or 9th; e.g. a male 15 mm. long with a 14-jointed flagellum, carried calceolae on the 1st to the 6th and 8th; in others of 17–19 mm. with 15-jointed flagellum the 1st–7th and 9th joints were thus provided. The largest freshwater males 20–22 mm. with 17–19 flagellum joints, have 11–12 calceolae.

Gnathopods. On comparing *zaddachi* with *locusta* it will be noted that the hands in the former are much broader in proportion to their length, with the palm less oblique and the palmar spines of the adult male different in structure and in position. In the older specimens of *zaddachi* the spines are stout, truncate and microscopically ridged on the top; whilst those which characterize *locusta*, and appear to be peculiar to it (Pl. III, figs. 21, 24), are flask-shaped, swollen at the base and constricted near the flat ridged top, with a strong central core. In *duebeni* the spines are strong, conical and pointed.

Gnathopod 1 (Pl. I, fig. 3). The hand of the male is oblong-oval, smaller than in gnathopod 2, and narrower in proportion to its length: palm and hind-margin are subequal; the palm crenulate, oblique, curving so as to end on the under-surface where the claw shuts down between the angle spine groups. One large truncate spine is situated midway on the palmar margin. The upper angle group contains six stout spines inset in pairs along the curve, the first pair consisting of one long sensory spine and one short and flat-topped, the other two pairs of short curved spines (Pl. I, fig. 4). The lower angle group also has six spines; one long sensory with two to three short curved ones inset facing the similar one on the upper side and a pair of the small bent spines below. The hind-margin carries about six rows of long stiff setae and serrated bristles.

In *locusta*, the hand of gnathopod 1 is smaller than in gnathopod 2, longer

and narrower in proportion than in *zaddachi*, about twice as long as wide; the palm very oblique ending on the under-surface. One of the large peculiar flask-shaped spines is inset in the mid-margin; in the upper angle row are three graduated spines with swollen bases and a slight constriction above; in the under angle row there are one long spine and four small stout curved ones (Pl. III, figs. 20–22). The hind-margin has six rows of setae and serrated bristles.

Gnathopod 2 (Pl. I, fig. 5). The hand of the male is larger and broader than that of gnathopod 1; it is roughly triangular in shape, the hind margin about two-thirds the length of the anterior margin and subequal with the palm. There is one large truncate spine in the mid-margin separated by a gap from the four graduated stout sensory spines at the palmar angle. A slight torsion brings the palm to end on the under-surface of the hand, where the claw closes down, with a pair of small stout spines near its tip. The hind margin carries nine to ten rows of long stiff setae and curved serrated bristles.

The female has one spine midway on the palm, and a more slender and pointed claw than the male.

In *locusta* (Pl. III, figs. 23, 24) also, the hand is decidedly larger than in gnathopod 1, but is narrower in proportion to its length than in *zaddachi*. It carries three of the peculiar flask-shaped spines on the palmar margin, one midway, and the other two spaced at intervals between it and the palmar angle. The upper angle row consists of six sensory spines inset in pairs. The claw impinges on the under-surface where, with a slight torsion as in *zaddachi*, the palm ends. The hind margin has about 11–12 rows of the long setae and bristles.

The *peraeopods* show a striking difference between the freshwater and the saline forms. The figures are taken from animals typical of the extremes of the species range to show the alteration in appearance caused by the development of the hairs particularly on the 4th to the 6th joints.

In the freshwater form (Pl. I, figs. 6, 7; Pl. II, figs. 10–12) these joints carry dense clusters of very fine long hairs mixed with long slender spines; whilst at the other extreme, the 'spinose' effect of the saline form (Pl. I, figs. 8, 9; Pl. II, figs. 15–17) is produced by the great reduction in the number and size of the hairs causing the spine clusters to show up conspicuously. The spines, too, are usually shorter and stronger and often more numerous than in the freshwater form. While these two types seem constant in their own particular environment, every grade of development of the sensory armature can be found in the 'intermediate' range, in the varying salinities, but whether these grades are correlated with the particular salinity of the locality in which they are captured, or whether they move up and down with the tidal movements in the estuaries, is not known.

The peraeopods in this species differ from *locusta* in various measurements, the 4th joint, for example, being much shorter in proportion and broader; it is longer than the 6th in peraeopod 1, about equal in peraeopod 2, and always shorter in the hinder peraeopods.

Comparison of the Adult Males of *Gammarus zaddachi* and the

	G. zaddachi Sexton, 1912	**G. locusta** (L.)
Head	As long as peraeon segs. 1 and 2 taken together. Sinus slight	Distinctly shorter than peraeon segs. 1 and 2. Sinus slight
Lateral lobes	Obliquely truncate, upper angle subacute; channelled indentation below connected with frontal organ	Angular; upper angle sharply produced
Eyes	Large, elongate, reniform, broader than in *locusta*; ommatidia smaller, 170–180. Black	Large, elongate reniform; ommatidia large, about 100. Black
Antenna 1	Peduncle long, reaching to half length of 5th ped. jt. of ant. 2; 1st and 2nd joints subequal; 3rd more than half length of 2nd. Thick clusters of graduated hairs on all 3 joints (8 on jt. 1: 8 on jt. 2; and 5 on jt. 3). Primary flagellum 38–44; accessory flagellum 7–9	Peduncle shorter, reaching distal end of 4th ped. jt., ant. 2; 1st jt. about as long as 2nd and 3rd combined; peduncle and flagella almost glabrous, only 1 seta on ped. jt. 1 under-surface. Primary flagellum 40–47, accessory flagellum 8–13 or 14
Antenna 2	Shorter than ant. 1. 4th ped. jt. slightly shorter than 5th; both carrying clusters of graduated hairs (9 on jt. 4; 10 on jt. 5). Flagellum 15 jts.; with similar hairs and calceolae	Shorter than ant. 1. 5th jt. about $\frac{1}{6}$ longer than 4th; both with tufts of long fine hairs (9 on 4th; 9 on 5th). Flagellum 24 jts.; also with similar tufts and calceolae
Side-plates	As deep as corresponding segs. 4th as broad as deep; expansion short, inferior margin almost semicircular	Nearly twice as deep as corresponding segs. 4th much deeper than broad; lower portion deep and almost rectangular
Pleon	Epimera of segs. 2 and 3, with hind-corners subacute. Dorsal humps only slightly raised	Segs. 2 and 3, hind-corners acutely produced. Dorsal humps elevated and prominent
Spine formula	3 : 2 : 3 3 : 2 : 3 2 : 2 : 2	4 : 3–5 : 4 3 : 4–5 : 3 3 : 3 : 3
	Numerous long fine hairs in all the groups	Spines and hairs shorter
Gnathopod 1	Hand smaller than in gnath. 2; oblong oval, broad in proportion to length; 1 truncate spine, mid-margin of palm; angle group of 6 spines inset in pairs. Claw closes on under-surface	Hand longer and narrower than in *zaddachi*; piriform; smaller than in gnath. 2; 1 flask-shaped spine on palmar margin; angle row of 3 spines. Claw much bent under
Gnathopod 2	Hand roughly triangular. 1 truncate spine on palmar mid-margin, separated by a gap from the angle row of 4 spines	Hand much broader and stronger than gnath. 1, narrower proportionately than in *zaddachi*. 3 flask-shaped spines on palm; angle group of 6 spines
Peraeopods	4th jt. shorter and broader in proportion than in others; hinder pps., basal jts. not much expanded; pp. 3 with hind-corner free; pps. 4 and 5 with spines inset at distal angle	Slender and elongate; hinder pps., only 3rd pp. with hind-corner free, produced to an acute angle
Uropods	Slender. Ur. 1 longest; inner ramus of ur. 3 always shorter than outer, about $\frac{3}{4}$ the length, with numerous long, feathered setae	Ur. 3 elongate, the 2 rami subequal in length, with numerous feathered setae and spines
Telson	Longer than broad; each lobe with apical group of 3 spines and hairs; subapical, 1 spine and 1 hair; subbasal, 2 spines and 1 hair	Elongate; longer than broad; apical group of 3 spines, 1 hair; subapical, 1 spine, 1 hair; subbasal, 3 spines, 2 hairs
Size	♂ 18·5–22 mm., ♀ 13–15 mm.	♂ 20–33 mm., ♀ 18–20 mm.

FOUR OTHER SPECIES WITH WHICH IT HAS MOST OFTEN BEEN CONFUSED

G. duebeni Liljeborg, 1851	**G. pulex** (L.), 1758	**G. wilkitzkii** Birula, 1897 (Arctic species)
Short; deep sinus	Very short in proportion; sinus rather deep	Small in proportion; $\frac{2}{3}$ the length of peraeon. segs. 1 and 2; very deep sinus
Vertically truncate; upper angle rounded	Vertically truncate, but less broad than *duebeni*; upper angle rounded	Upper angle acutely produced; very deep sinus
Rather smaller, reniform. Black	Small, rounded oval. Black	Small, broadly reniform, only twice as long as broad. Black
1st ped. jt. not as long as 2nd and 3rd combined, sparsely furnished with hairs. Primary flagellum 25–34; accessory flagellum short, 5–6 jtd.	1st ped. jt. not as long as 2nd and 3rd combined, almost glabrous. Primary flagellum 24–28; accessory flagellum short, 3–4 jtd.	Peduncle, 1st jt. longer and stouter than jt. 2; 3rd about $\frac{2}{3}$ as long as 2nd. Peduncle reaches to about $\frac{1}{3}$ of 5th ped. jt. ant. 2, all 3 jts. thickly beset with clusters of long hairs (8–9 on 1st jt.; 12–13 on 2nd; 7 on 3rd). Primary flagellum 54–56; accessory flagellum 8–10
Much shorter than ant. 1. 4th and 5th ped. jts. subequal. Flagellum stout, 11–15-jtd. with calceolae	Considerably shorter than ant. 1. 4th and 5th ped. jts. subequal; with numerous short straight setae like bottle-brush. Flagellum 12–13 jtd. with calceolae	A little shorter than ant. 1. 5th jt. of ped. longer and more slender than 4th; both ped. and flagellum with dense clusters of long hairs (12–13 on jt. 4; 13–14 on jt. 5). Flagellum 24–28 (broken). Calceolae seen on 21 jts.
Deeper than corresponding segs.; 4th as broad as deep; expansion deep, rounding into inferior margin	Deeper than corresponding segs., 4th as broad as deep; posterior expansion deep and subrectangular	A little deeper than corresponding segs.; 4th about as wide as deep; expansion very shallow, merging into semicircular inferior margin; angle hardly produced
Segs. 2 and 3, hind-corners little produced, acutely quadrate. Dorsal humps not much raised	Segs. 2 and 3 very slightly produced; hind-corners quadrate. Dorsal humps slightly raised	Segs. 2 and 3 not much produced. Dorsal humps not much produced, lateral clusters at a different level (farther back on segments)
3 : 2 : 3 3 : 2 : 3 3 : 2 : 3	1–2 : 2 : 1–2 1–2 : 2 : 1–2 1–2 : 2 : 1–2	3 : 2 : 3 3 : 2 : 3 2 : 2 : 2
Numerous long hairs	A few short hairs	Very numerous long straight hairs
Hand subequal in size to gnath. 2; slender, conical, pointed spine in mid-margin; palm long and very oblique; angle row of 3 spines, and a pair inset on the hind-margin	Hand about same size as in gnath. 2; piriform; palm very oblique, crenulated; stout rounded spine in palmar mid-margin, angle row of 6 spines, inset in pairs. Claw closes underneath	Hand smaller than in gnath. 2; triangularly ovate; the whole limb thickly clothed with long hairs, many dentate spines on joints; palm very oblique; 1 stout conical spine mid-margin; and angle row of 5 graduated similar spines. Claw closes on under-surface
Hand ovate, 1 slender pointed spine in mid-margin, another near angle row, almost continuous with it; angle row of 4 graduated pointed spines	Hand oblong, widening slightly at palm. Palm transverse and concave; 1 stout rounded spine in mid-margin; angle row of 3 spines	Hand very large and broad; palm almost transverse, only slightly oblique; 1 stout rounded median spine; and 5 graduated similar ones in angle row
Stouter; all 3 hinder pps. with hind corners free, produced in lobes; densely setiferous	Slender. 3rd pp. has hind-corner free and rounded; pps. 4 and 5 with expansion wide and convex; hind-corners free, though small	Slender and elongate, with fewer spines and hairs than in *zaddachi*, 5th the longest and very slender; hinder pps., basal jts. successively longer and narrower: pp. 3 hind-corner produced, subrectangular; 4th and 5th narrow to distal angle, where 2 long spines are inset
Ur. 3 inner ramus much more slender than outer, and not much more than $\frac{1}{2}$ its length; both rami densely setiferous, setae feathered	Ur. 3 long; inner ramus about $\frac{3}{4}$ the length of the outer; numerous long feathered setae	Ur. 3 inner ramus about as long as outer; both margins with long feathered setae
Shorter, broader and more spinose than in *zaddachi*; apical group of 4 spines and 7–9 hairs; subapical, 1 spine, 3 hairs; subbasal, 3 spines and 6 hairs	Rather small, and sparsely armed; apical, 2 spines, 2 hairs; subbasal, 1 spine; and 2 groups of 2 hairs each inset between	Longer and more slender in proportion; spines few, 2–3 in apical group; 1 subapical, and 1 subbasal
♂ 13–16 mm.	♂ 12–20 mm.	♂ 46–48 mm.

The basal joint in peraeopod 3 is expanded with the hind corner free and subacute; in peraeopods 4 and 5 it is narrowed distally, the hind corners not free but with one or two strong spines inset at the angle. In the older males the basal joints are long and very narrow, but in the females and immature they are always shorter and more expanded proportionately than in the adult male. The posterior margins of these joints in the saline form are inset with a few short hairs; in the freshwater forms the hairs are about three times the number and length.

The *uropods* (Pl. II, figs. 13, 14, 18). Uropod 1 is the longest; both uropod 1 and uropod 2 extend beyond the peduncle of uropod 3, and both are furnished with more spines in the saline form. In *locusta* the rami of uropod 3 are subequal in length; in *zaddachi* the inner ramus is always shorter, about three-quarters as long as the outer ramus in the adult, but the proportions vary with age and growth. In the newly hatched young, 1·5 mm., the inner ramus is not one-third the length of the outer. This character was first pointed out by Zaddach, as one of the principal features distinguishing his specimens from *locusta*. The figures show the development of the hairs and spines in the two forms.

The *telson* is about the length of the peduncle of the 3rd uropods and is longer than broad. Each lobe is armed, in the saline form, with an apical group of three spines and two or three short hairs; a subapical group of one spine and one short hair; and a subbasal group of two spines and one hair: in the freshwater form, the apical group has three spines and 9–10 long hairs; the subapical, two hairs; and the subbasal one spine and two hairs; the hairs more than twice the length of the spines (Pl. II, figs. 13, 18).

Summary

Gammarus zaddachi is perhaps the most prolific and widespread of all the estuarine amphipods known to occur in northern Europe, and inhabiting, as it does, the low-salinity estuarine zone and adjacent coasts, it has come to be recognized in recent ecological work as a 'salinity indicator'.

Unfortunately, there has been constant confusion with the other common species of *Gammarus*, *G. locusta*, *pulex*, and *duebeni*, which has been greatly complicated by the difference in the appearance of *zaddachi* according as it lives in a freshwater or a saline habitat. It is shown that this difference is entirely due to the sensory equipment, the greater production of hairs in freshwater conditions, and that the structure of the two 'forms' is identical.

The history of the species has been carried back as far as I have been able to trace it (1836) with the actual specimens, described in the different papers, and the more important of these papers are discussed. It will be seen that the material examined was derived from every country of northern Europe; from Russia, the White Sea, Crimea, and the Baltic, the coasts of Scandinavia, Germany, including the Hamburg water-supply, Denmark, the Netherlands, Great Britain and Ireland, and France as far up the Loire as Nantes.

Detailed descriptions and figures of both forms of *G. zaddachi* are given; and finally, a comparison is made between the species most commonly confused with it, the Arctic species *G. wilkitzkii* being included because of a suggestion recently made that it might be, not a distinct species, but merely the Arctic form of *zaddachi*.

REFERENCES

BASSINDALE, R., 1933. Abnormal eyes in wild *Gammarus zaddachi* Sexton. *Journ. Mar. Biol. Assoc.*, Vol. XIX, pp. 297–8.

—— 1941. The Invertebrate Fauna of the Southern Shores of the Bristol Channel and Severn Estuary. *Proc. Bristol Nat. Soc.*, Ser. iv, Vol. IX, Amphipoda, pp. 167–75.

BATE, C. SPENCE, 1862. *Catalogue of the Specimens of Amphipodous Crustacea in the Collection of the British Museum*, p. 206. London.

BATE, C. SPENCE & WESTWOOD, J. O., 1863. *History of the British Sessile-eyed Crustacea*, Vol. I, pp. 378–83. London.

BRANDT, F., 1851. *Reise in den äussersten Norden und Osten Siberiens während der Jahre* 1843 *und* 1844. Bd. II, Krebse., pp. 132–5. St Petersburg.

CHEVREUX, E., 1899. Révision des amphipodes de la côte océanique de France. *C.R. Assoc. franç. Avancem. Sci.*, 27me Sess., pp. 474–84.

CHEVREUX, E. & J. DE GUERNE, 1892*a*. Sur une espèce nouvelle de *Gammarus*, du lac d'Annecy et sur les amphipodes d'eau douce de la France. *C.R. Acad. Sci., Paris.*

—— —— 1892*b*. Description de *Gammarus Delebecquei* nov.sp. du lac d'Annecy, suivie de quelques remarques sur les Amphipodes d'eau douce de la France. *Bull. Soc. Zool. France*, T. XVII, pp. 136–42.

CRAWFORD, G. I., 1936. Additions to the Plymouth Marine Fauna (1931) in the Crustacean Orders Tanaidacea, Isopoda and Amphipoda. *Journ. Mar. Biol. Assoc.*, Vol. XXI, pp. 95–106.

—— 1937. The Fauna of Certain Estuaries in West England, and S. Wales with Special Reference to the Tanaidacea, Isopoda and Amphipoda. *Journ. Mar. Biol. Assoc.*, Vol. XXI, pp. 647–62.

DAHL, FRIEDR., 1893. Untersuchungen über die Thierwelt der Unterelbe. *Sechster Bericht d. Komm. z. Wissenschaftl. Untersuchung. d. deutschen Meeres in Kiel* 1891, Bd. III, pp. 151–85.

FABRICIUS, J. C., 1775. *Systema Entomologiae*. Flensburgi et Lipsiae.

FABRICIUS, OTTO, 1780. *Fauna Groenlandica*. Hafniae et Lipsiae.

GURJANOVA, EUPRAXIE, 1930. Beiträge zur Fauna der Crustacea-Malacostraca des arktischen Gebietes. *Zool. Anz.*, Bd. 86, pp. 231–48.

—— 1933. Contribution to the Fauna of Crustacea-Malacostraca of the Obj-Enisej Bay. *Explor. de la Mer U.S.S.R.*, T. 18, pp. 75–90 (English summary).

HELLEN, 1919. Zur Kenntnis der Amphipoden-Fauna Finnlands. *Medd. Soc. Fauna Flora Fennica*, Bd. 45.

HÖFKEN, ULRICH, 1937. Statistische und experimentelle Untersuchungen über die Variabilität von *Gammarus locusta* (L.). *Kieler Meeresforsch.*, Bd. II, pp. 116–48.

HOEK, P. P. C., 1889. Crustacea Neerlandica. *Tijdsch. Neder. Dierk. Ver.*, Ser. 2, D. II, pp. 170–234.

KLIE, W., 1913. Die Crustaceen-Fauna des Alten Hafens zu Bremerhaven. *Internat. Rev., Biol. Supp.*, Bd. VI (12 pages).

KRAEPELIN, KARL, 1886. Die Fauna der Hamburger Wasserleitung. *Abh. a. d. Geb. Naturwiss. Verein in Hamburg*, Bd. IX, pp. 13–25.

LINNAEUS, C., 1745. *Ölandska och Gothlandska resa.* Stockholm och Upsala.
—— 1758. *Systema Naturae*, 10th and later editions. Stockholm.
MILNE EDWARDS, H., 1830. Recherches pour servir à l'histoire naturelle des Crustacées Amphipodes. *Ann. Sci. Nat.*, T. XX, pp. 353–99.
—— 1840. *Histoire naturelle des crustacées comprenant l'anatomie, la physiologie, et la classification de ces animaux*, T. III. Paris.
MÖBIUS, KARL, 1873. Die wirbellosen Thiere der Ostsee. *Jahresber. Comm. Wissenschaftl. Untersuch. d. deutschen Meeres in Kiel* 1871. Translation, DALLAS, 1873. On the invertebrate animals of the Baltic. *Ann. Mag. Nat. Hist.*, Ser. 4, Vol. XII, pp. 81–9.
MONTAGU, GEORGE, 1808. Description of several Marine Animals found on the South Coast of Devonshire. *Trans. Linn. Soc. London*, Vol. IX, p. 92.
OLDEVIG, HUGO, 1933. Sveriges Amphipoder. *Göteborgs Kung. Vetenskaps- och Vitterhets-Samhälles Handlingar. Femte Följden*, Ser. B, Bd. 3, No. 4, pp. 197–9.
PALMER, RICHARD, 1933. Notes on *Gammarus zaddachi* Sexton from Essex with observations on Eye-variations in this species. *Ann. Mag. Nat. Hist.*, Ser. (10), Vol. XI, pp. 64–7.
PERCIVAL, E., 1929. A Report on the Fauna of the Estuaries of the River Tamar and the River Lynher. *Journ. Mar. Biol. Assoc.*, Vol. XVI, pp. 81–108.
POULSEN, E. M., 1932–3. On a Collection of *Gammarus* from the waters South of Sealand (30. 5. 1932). *Vidensk. Medd. Naturh. Foren. Kjøb.*, Bd. 94, pp. 1–12.
RATHKE, HEINRICH, 1836. Zur Fauna der Krym. *Mem. K. Akad. Wiss.*, Bd. III, pp. 82–4 (of original issue). St Petersburg.
—— 1843. Beiträge zur Fauna Norwegens. *Verhandl. d. K. Leopold-Carol. Akad. d. Naturf.*, Bd. XX, pp. 67–8.
RIECH, FRITZ, 1926. Beiträge zur Kenntnis d. litoralen Lebensgemeinschaften in der poly- u. mesohaline Region d. Frischen Haffs. *Schr. der Phys.-ökon. Ges. z. Königsberg i. Pr.* Bd. LXV, pp. 32–47.
SARS, G. O., 1890. *Crustacea of Norway.* I. *Amphipoda.* p. 499.
SCHÄFERNA, KAREL, 1922. *Amphipoda Balcanica. Societas scientiarum bohemica*, T. II (with English summary, p. 96).
SCHELLENBERG, A., 1934. Zur Amphipoden-Fauna der Kieler Bucht. *Schr. d. Naturwiss. Ver. für Schleswig-Holstein*, Bd. XX, pp. 1–16.
—— 1937. Kritische Bemerkungen zur Systematik der Süsswassergammariden. *Zool. Jahrb., Abt. Syst.*, Bd. 69, pp. 469–516.
SCHLIENZ, WALTER, 1922. Systematische Bemerkungen zu den *Gammarus*-Arten aus Nord-deutschen Flussgeschwellen. *Zool. Anz.*, Bd. LIV, pp. 215–17.
—— 1923. Verbreitung und Verbreitungsbedingungen der höheren Krebse in Mündungsgebiet der Elbe. *Arch. Hydrobiol.*, Bd. 14, pp. 429–52.
SERVENTY, D. L., 1934. The Marine Invertebrate Fauna, in *Scolt Head Island.* Cambridge, pp. 196–213.
—— 1935. Observations on *Gammarus zaddachi* Sexton, an Estuarine Amphipod and Associated Forms. *Internat. Rev.*, Bd. 32, pp. 285–94.
SEXTON, E. W., 1912. Some brackish-water Amphipoda from the mouths of the Weser and the Elbe, and from the Baltic. *Proc. Zool. Soc. London*, pp. 656–65.
—— 1913. On a Collection of *Gammarus* from the Königsberg Museum. *Schr. der Phys.-ökon. Ges. z. Königsberg i. Pr.*, Bd. LIV, pp. 90–4.
—— 1924. The moulting and growth stages of *Gammarus* with descriptions of the normals and intersexes of *G. chevreuxi*. *Journ. Mar. Biol. Assoc.*, Vol. XIII, pp. 340–401.
—— 1928. On the Rearing and Breeding of *Gammarus* in Laboratory Conditions. *Journ. Mar. Biol. Assoc.*, Vol. XV, pp. 33–55.

SEXTON, E. W. & SPOONER, G. M., 1940. An account of *Marinogammarus* (Schellenberg) gen.nov. [Amphipoda] with a description of a new species *M. Pirloti*. *Journ. Mar. Biol. Assoc.*, Vol. XXIV, pp. 633–82.

STEPHENSEN, K., 1916. Zoogeographical Investigation of certain fjords in Southern Greenland with special reference to Crustacea, Pycnogonida, and Echinodermata. *Medd. om Grønland*, Bd. LIII, pp. 236–7.

—— 1917. On a Collection of *Gammarus* from Randers Fjord Denmark. *Vidensk. Medd. fra Dansk Naturh. Foren.*, Bd. 68, pp. 37–50.

—— 1927. Revideret Fortegnelse over Danmarks arter af Amphipoda (3. Del Gammaridea. Fam. Gammaridae tel Fam. Podoceridae (Dulichiidae aut) Caprellidea). *Vidensk. Medd. fra Dansk Naturh. Foren.*, Bd. 84, pp. 115–20.

—— 1928. *Danmarks Fauna*. Storkrebs. II. Ringkrebs. I. Tanglopper (Amphipoder). pp. 275–8. København.

—— 1929. *Die Tierwelt der Nord- und Ostsee*. Lief. XIV, Teil Xf. (Amphipoda). pp. 138–9.

STEPHENSEN, K. & USSING, H. J., 1918. Krebsdyrene i Randers Fjord. In *Randers Fjord Naturhistorie*, kap. V.E., pp. 335–8. København.

SZIDAT, LOTHAR, 1926. Beiträge zur Faunistik und Biologie des Kurischen Haffs. *Schr. der Phys.-ökon. Ges. z. Königsberg i. Pr.*, Bd. 65, pp. 5–31.

TESCH, J. J., 1915. Die Amphipoden der zuidelijke Noordzee verzameld met de 'Wodan'. *Rapp. Rijksinst. Vissch. underzoek*, D. I, pp. 336–9.

—— 1922. Amphipoden: in *Flora en Fauna der Zuiderzee*, pp. 326–36. Helder.

VANHÖFFEN, E., 1911. Beiträge zur Kenntnis der Brackwasserfauna im Frischen Haff. *Sitz. Ber. d. Ges. Naturf. Freunde*, pp. 399–405.

—— 1917. Die niedere Tierwelt des Frischen Haffs. *Sitz. Ber. Ges. Naturf. Freunde*, pp. 113–47.

VOLK, RICHARD, 1907. Mitteilungen über die biologische Elbe-Untersuchung des Naturhistorischen Museums in Hamburg. *Verhandl. d. Naturw. Ver. in Hamburg*, Bd. 3, XV, pp. 1–54.

WALKER, A. O., 1911. On *Gammarus campylops* Leach. *Ann. Mag. Nat. Hist.*, Ser. (8), Vol. 7, pp. 397–9.

ZADDACH, E. G., 1844. *Synopseos Crustaceorum Prussicorum Prodromus*. pp. 4–6. Regiomonti.

—— 1878. Die Meeres-Fauna an der preussischen Küste. *Schr. der Phys.-ökon. Ges. z. Königsberg i. Pr*, 19. Jahrg., pp. 26–32.

I have not been able to consult Tesch, 1922, *and Vanhöffen*, 1917.

EXPLANATION OF PLATES I–III

PLATE I

Adult male, *Gammarus zaddachi* Sexton

Figs. 1–7 show the freshwater form, from Hamburg Water-Supply; Figs. 8 and 9 the saline form from the Baltic.

Fig. 1. Head and antennae. ×23. (White reticulation not shown in eye.)

Fig. 2. One of the calceolae from the flagellum of antenna 2. ×140.

Fig. 3. Gnathopod 1.

Fig. 4. Distal portion of hand of gnathopod 1. Under-surface, showing the torsion of the palm, the upper and under angle groups of spines with the claw closed down between them. (The long setae are omitted for the sake of greater clearness.) ×50.

Fig. 5. Gnathopod 2, gill outlined. ×23.

Fig. 6. First peraeopod, freshwater. ×23.

Fig. 7. Second peraeopod, freshwater. ×23.

Fig. 8. First peraeopod, saline, for comparison. ×23.

Fig. 9. Second peraeopod, saline, for comparison. ×23.

PLATE II

Adult male, *Gammarus zaddachi* Sexton

Figs. 10–14 freshwater form; Figs. 15–18 saline form; from the same males as in Pl. I.

Fig. 10. Peraeopod 3. ×23.

Fig. 11. Peraeopod 4. ×23.

Fig. 12. Peraeopod 5. ×23.

Fig. 13. Pleon segments 4–6 with uropods 1 and 2, peduncle of 3rd, and telson. ×23.

Fig. 14. Uropod 3. ×23.

Fig. 15. Peraeopod 3, saline, for comparison. ×23.

Fig. 16. Peraeopod 4, saline, for comparison. ×23.

Fig. 17. Peraeopod 5, saline, for comparison. ×23.

Fig. 18. Pleon segments 4–6, showing dorsal spines, uropods and telson. ×23.

PLATE III

Adult male, *Gammarus locusta* (L.)

Magnification less than in Pls. I and II.

Fig. 19. Head and antennae; white reticulation not shown in eye. ×12.

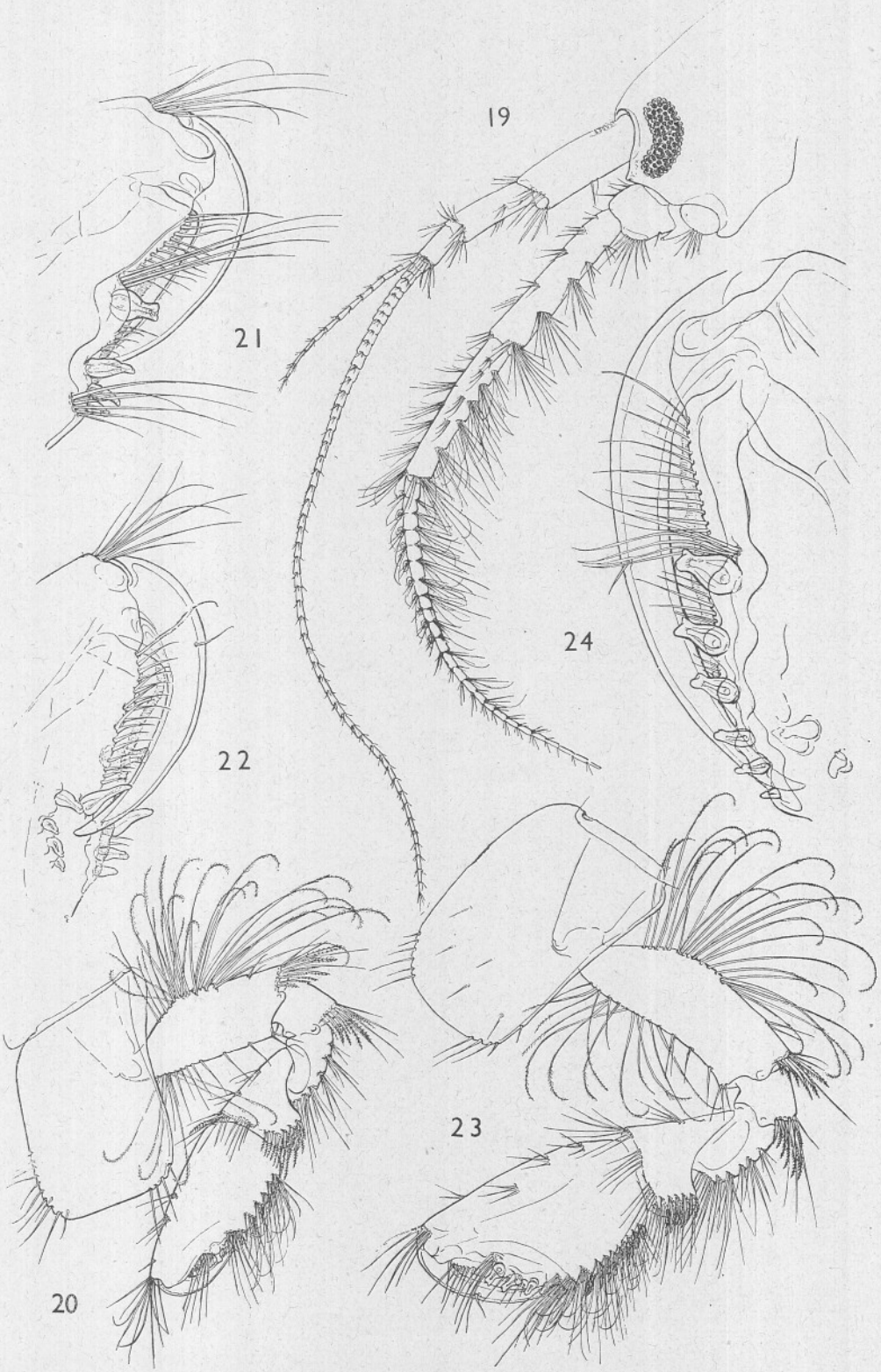
Fig. 20. Gnathopod 1. ×12.

Fig. 21. Distal portion of hand of gnathopod 1, showing palmar spines—upper surface. ×28.

Fig. 22. Distal portion of hand of gnathopod 1, under-surface. ×28.

Fig. 23. Gnathopod 2. ×12.

Fig. 24. Distal portion of hand of gnathopod 2 with palmar spines; turned over slightly to show palmar angle more clearly; long hairs omitted. ×28.



E. W. SEXTON del.

STUDIES ON *POLYSTOMELLA* LAMARCK (FORAMINIFERA)

By Margaret W. Jepps

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late Leverhulme Research Fellow

(Plates IV and V and Text-figs. 1–9)

CONTENTS

Part I. Introduction and Methods, with an Account of Feeding

Introduction

There must be a vast number of biologists who, like myself, were taught as students a life history of *Polystomella*[1] based on the careful observations of J. J. Lister (1895, 1906) with the additions which he accepted from the work of Schaudinn (1895 *a*, p. 59; 1903, p. 500 in the *Arbeiten*[2]). To others beside myself it must have come as somewhat of a shock to realize later on the incompleteness of these observations; and most particularly the slender basis on

[1] I am not convinced that the last word has been said concerning the most acceptable name for this organism, and therefore continue to use the name so familiar in Great Britain, in spite of the fact that American and some other writers are now calling it *Elphidium* Montfort as advocated by Meek & Hayden (1864).

[2] All the page references in this paper to the works of Schaudinn refer to the collection known as the *Arbeiten*, and not to the isolated papers as they originally appeared in various periodicals.

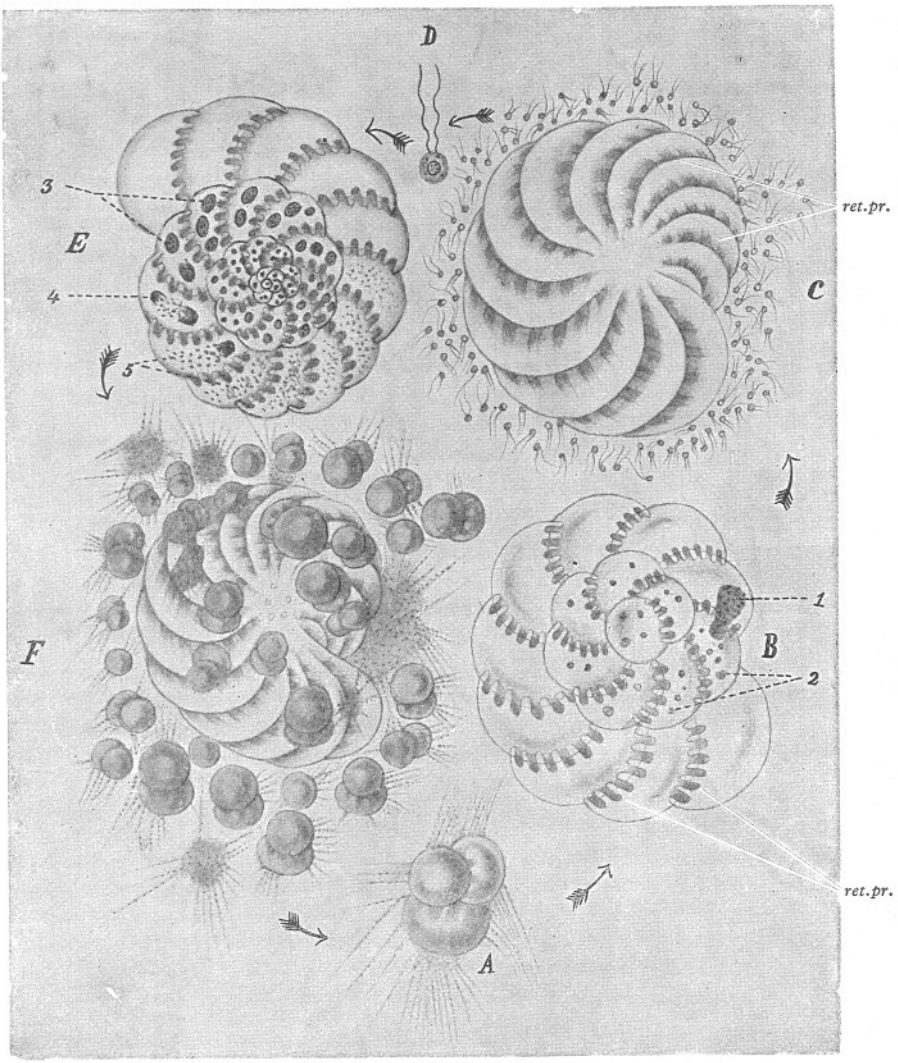
which rests the special contribution of Schaudinn, including what might be called the crisis of the life history, the process described by him as a form of sexual reproduction.

The story of the life cycle of *Polystomella* is to be found in numerous text-books of zoology, notably in the excellent short account of the Foraminifera contributed by J. J. Lister himself in 1903 to the *Treatise of Zoology* edited by E. Ray Lankester, and still more briefly in Graham Kerr (1921). In the latter, of course, the whole story is given, including Schaudinn's addition of 1903. In Text-fig. 1 is reproduced from Arnold Lang's *Lehrbuch* (1901, p. 208) an illustration which according to its legend was sketched for this text-book by Schaudinn. This figure shows a series of stages in the life history of *Polystomella*. At A is a young megalospheric form, with its large spherical initial chamber, the megalosphere, and two others, which grows into the adult at B, still having only a single nucleus. At C the protoplasm has produced a large number of flagellate swarm spores, each provided with a nucleus and two flagella. These leave the parental shell and give rise to the microspheric form at E, with a number of nuclei and a small initial chamber, the microsphere. At F its protoplasm has crept out of the shell and divided up to form a comparatively small number of megalospheric offspring, as at A, thus completing a life history which includes a sexual (megalospheric) followed by an asexual (microspheric) generation. According to Schaudinn (1895*a*, p. 62) the alternation of the two generations is not always quite regular, for the offspring of a microspheric form may occasionally be multinucleate, and produce another asexual generation. Schaudinn claims to have observed this in three out of 4300 specimens of *Polystomella*, and points out that similar irregularity occurs more frequently in other Foraminifera, quoting *Orbitolites* and *Peneroplis*. This is believed[1] to be the case in some few Foraminifera (e.g. *Orbitolites*, *Cornuspira* and 'several other genera', Lister, 1903, p. 74: in *Rotalia* and *Cristellaria*, Lister, 1895, p. 442: also reported in other Miliolidae by Hofker, see 1930*b*, p. 28) and it may happen in *Polystomella*. But Schaudinn did not say how he made this observation—it would be a matter of special difficulty in this genus, as pointed out by Lister (1903, footnote to p. 74), and nobody has repeated it since. It might be added that once the protoplasm has left the shell it is very rarely possible to distinguish between microspheric and megalospheric individuals. I have seen in a very old emptied shell at Plymouth the initial chamber (a megalosphere) indicated by the rich growth of diatoms inside the membrane which persists after decalcification, but it is usually invisible.

Text-fig. 1 is especially interesting because it includes (at D) the only figure of a swarm spore of *Polystomella* ever published by Schaudinn. At E also is shown a dividing nucleus of which we shall have more to say later.

Two years afterwards in 1903 (p. 500) Schaudinn added his description of

[1] But it is by no means certain in the absence of adequate measurements, etc., in the light of Føyn's analysis of *Discorbina villardeboana* (1936*b*, 1937). In this species the ranges of size of the initial chambers of megalospheric and microspheric forms overlap, so that it might, for all we know, be possible for a microspheric (asexual) parent to produce megalospheric (sexual) young having initial chambers of an equal or even of a smaller size.



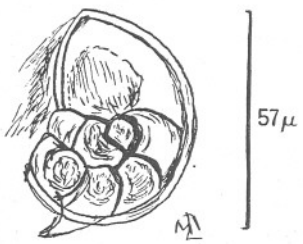
Text-fig. 1. Dimorphism and alternation of generations in *Polystomella crispa*. The arrows indicate the direction of the life cycle. A, young megalospheric individual; B, full-grown megalospheric individual, decalcified; C, megalospheric individual in the act of spore-formation, the protoplasm leaving the shell as flagellispores; D, flagellispore more highly magnified; E, microspheric individual decalcified; F, microspheric individual in the act of producing amoeboid young. 1, nucleus; 2, chromidia; 3, nuclei; 4, nucleus 'in multiple fission to produce chromidia'; 5, chromidia; *ret.pr.*, retral processes. (From Lang, after Schaudinn). After Parker and Haswell, *Textbook of Zoology*, 2nd ed., Vol. 1, p. 57. By courtesy of Messrs Macmillan and Co., London.

Three of the four guide-lines marked *ret.pr.* do not indicate the retral processes correctly. The processes are unshaded in all the figures and are projections from the concave side of each chamber.

the actual union of two swarm spores (gametes), each from a different (megalospheric) parent, to initiate the microspheric generation, which continued to develop in his culture as far as the five-chambered stage, by which time the nucleus had usually divided more than once, and then it died.

Schaudinn made extensive studies in marine aquaria which, it seems, grew up in a rather haphazard way and were, when they succeeded, probably more natural than if more elaborate and controlled methods had been employed. It is possible that by some lucky set of chances he had the good fortune to witness what so many others have striven in vain to see in more than one form of protozoon. There is, however, a disconcerting lack of corroborative evidence in the way of detail and good figures; and we find, for example, on the preceding page of the *Arbeiten* categorical statements which now appear to us almost preposterous about the 'Kopulation' of well-known intestinal flagellates. The question then arises whether he might not also have been mistaken in his interpretation of the observations under review. Further, a young microspheric *Polystomella* at the five-chambered stage might not show easily recognizable features by which it could readily be distinguished from the young of other Foraminifera. It has already been noticed, for example, that the retral processes (see Text-fig. 1 B, C) which are so characteristic of this genus are not produced by the earliest chambers in the microspheric phase (Schaudinn, 1895*a*, p. 61; Lister, 1895, p. 418). In my whole mounts of decalcified older specimens I find that one or two retral processes begin to appear on the protoplasm about the 9th–12th chamber (Schaudinn gives the 20th–25th chamber as the first of the series to show the processes; yet he refers to young *microspheric Polystomella* of 8–15 chambers without the least description of their appearance (1895*a*, p. 59)).

In April 1940, in the course of some experiments at Plymouth with the flagellate swarm spores of *Polystomella*, I found in one culture the little shell shown in Text-fig. 2. It was watched for 2 days, but unfortunately showed no sign of life. The circumstances in which it was found were such that it might quite well have been a microspheric *Polystomella* of some eight or nine chambers—a much earlier stage than any so far figured (Lister (1895) shows a young specimen of about twenty chambers in his pl. vi, fig. 7). A study of stained decalcified older specimens shows that it is quite comparable as regards its size and shape with the first eight chambers of an adult microspheric *Polystomella* (but also of some other species of Foraminifera). It was during the season of the supposed sexual reproduction, and early on the previous day three *Polystomella* had emitted their swarms of flagellate spores in a Petri dish under conditions which were as normal as possible, excepting that no food was supplied, so that one might have expected



Text-fig. 2. From a sketch by Dr M. V. Lebour of a small (? *Polystomella*) shell seen in culture. See p. 660.

to obtain some early stages of the next generation. There might have been, as can be said after making the experiments already referred to, an even greater likelihood of a very young microspheric *Polystomella* being brought in from the sea on the shell of an adult. Although these were always carefully washed, and the resulting cultures indicated that a surprisingly high degree of cleanliness was obtained, it was found that a small organism (usually a ciliate, flagellate, or small amoeba) might occasionally remain on the shell and multiply in the culture. (No other Foraminifera, as a matter of fact, had ever appeared in a long series of cultures of this kind). On the whole it does not seem justifiable, however, to identify the little shell confidently as a young *Polystomella* in our present state of ignorance of the appearance of the early stages of the microspheric phase. It is quite conceivable that it was the young of some other species with spirally arranged chambers.

On 15 March 1895, Lister wrote, 'there is no direct evidence of the conjugation of zoospores or the mode of origin of the microspheric form in a dimorphic species'; and this may perhaps still be said of *Polystomella*, although the story usually told seems very likely to be true. The only other Foraminiferan in which observed fusion of flagellate gametes has been described, leading to the development of the asexual generation, is also recorded by Schaudinn (in *Trichosphaerium*, 1899, p. 163). Again he describes the act of union of the flagellate gametes of *Gromia dujardinii* (1894*a*, p. 49; 1899, fig. on p. 163), although he did not succeed in rearing the offspring. Winter (1907, p. 19) claims to have seen the union of flagellate 'gametes' in *Peneroplis*, and le Calvez gives an account of the fusion of the 'flagellispores' in *Iridia lucida* (1938, p. 207); but neither followed the development of the zygote although the latter actually obtained a new generation in his culture. These few records constitute the only 'direct evidence of the conjugation of zoospores' in the group in spite of a great deal of work by competent workers on a number of forms of which the published life histories seem otherwise complete. This point will be further discussed.

There is, it appears, an unexpected diversity in the stages of the life history in the Foraminifera, since Myers has brilliantly demonstrated the fusion of *amoeboid* gametes in *Patellina corrugata* (1935*a*) and in *Spirillina vivipara* (1936), to give rise to the asexual generations of these two species. From his studies (1935*b*, 1936) it seems that the terms *megalospheric* and *microspheric* are not universally applicable to the sexual and asexual phases respectively even of the more typical dimorphic Foraminifera. In these two, for instance, the initial chamber (or proloculum) is not clearly divided off from the rest of the shell; and the diameter at the beginning of the spiral is actually somewhat less in the sexual than in the asexual form. For general purposes, then, it is preferable to call them by some other names such as *sporont* and *schizont*. In the present work the terms *megalospheric* and *microspheric* are retained, as they are eminently suitable in the case of *Polystomella*, though even here they have lost their widest implication.

The present studies, which it is hoped to continue when circumstances again become favourable, were undertaken with the idea of contributing something towards the demonstration of the life history of *P. crispa*, the species studied by so many generations of students in the laboratories of Great Britain. The possibility of more intensive work was provided when the Court of the University of Glasgow granted me leave of absence for the academic year 1938–9, thus enabling me to avail myself of the award of a Leverhulme Fellowship for the year. I am very glad to have this opportunity of recording my sincere gratitude to the University Court; and to the Leverhulme Trustees who have, through their Secretary Dr L. Haden Guest, M.P., so graciously expedited the work. The year was spent in the laboratory of the Marine Biological Association of the United Kingdom at Plymouth, beside the Sound where lie the famous beds of *Polystomella* whence J. J. Lister obtained much of his material, and which supply the teaching establishments of the British Isles. I am indebted to a number of benefactors who kindly bestowed on me the use of their tables in the course of the year; to a Committee of the British Association for a total period of six months; to the following Worshipful Companies of the City of London—the Clothworkers (2 months), the Fishmongers, Goldsmiths, Mercers, and Grocers (1 month each); and to the Fishmongers for another month in the Spring Vacation of 1940 when I was again at Plymouth. I offer my best thanks to Dr Stanley Kemp, F.R.S., Director of the laboratory at Plymouth, and to all those on his staff who helped me in so many ways; their friendly welcome added a great deal to the achievement and to the pleasure of the time I spent with them. Almost all my material has been collected by Mr William Searle, and I owe much to his skill and long experience for supplies in all weathers. Mr Searle continues to collect under the shadow of war in the English Channel and over Plymouth Sound. I am very grateful also to those other friends and colleagues who made it possible for me to be freed for the year from my usual routine duties, and even encouraged me to make a serious attack on a piece of work so many would have put aside as a forlorn hope. Amongst them I would especially mention the late Head of this Department, Sir John Graham Kerr, F.R.S., M.P.; Dr E. J. Allen, F.R.S., lately Director of the Plymouth Laboratory; and Mr Edward Heron-Allen, F.R.S., who gave me much material and moral support from the British Museum of Natural History. To Prof. Edward Hindle I am also grateful for some apparatus and special facilities for the continuation of the work at Glasgow, where I have in addition the ready assistance of the Director of the Marine Station at Millport (Firth of Clyde) in collecting the local material.

Material

Of the several good collecting grounds for *Polystomella* in the vicinity of Plymouth I found the material from the Drake's Island ground the cleanest and richest (Heron-Allen and Earland's Station I; see the Marine Biological Association's *Plymouth Marine Fauna*, 2nd edition, p. 34). All the catches

brought in for me during the year I was at Plymouth came from there. After the middle of March 1939 the '*Gammarus*' went out specially and brought the material straight back early in the morning. Since this ground ceased to be available in September 1939 my *Polystomella* have been collected at the station known as 'White Patch', nearer the east end of the Breakwater.

A widespread and much the most plentiful species in this neighbourhood is *P. crispa* L., and, without claiming any special qualifications as a systematist on the shells of the Foraminifera, I believe that all my work has been done with it. This is probably only of importance in the experiments with the flagellate spores, and in order to be reassured particularly at this point I have called in the skilled assistance of Mr Arthur Earland, F.R.M.S. I shall refer to his opinions in the course of these studies; and gladly take this opportunity of thanking him for his ready help.

Fixation of Material

Experience had shown that the usual methods of fixation give very poor results with *Polystomella*. This is presumably due, at least in part, to the heavy calcareous shell of complicated form, inside which most of the massive protoplasm is only freely accessible through very small openings until the calcium carbonate is dissolved away; this takes quite an appreciable time—several hours if the reaction is not to go on with such violence as to break the structure up. So that apart from the old antithesis between good fixation of nucleus and of cytoplasm respectively there are further difficulties to be overcome, and these increase with the size of the specimen to be fixed. One such difficulty is only too obvious after fixation: a more or less violent protoplasmic movement is apt to occur, the cytoplasm surging from one chamber to the next, the nuclei often becoming drawn through the foramina between the chambers and fixed there in a great variety of bizarre shapes—even though their inner structure may be quite well preserved. That these distorted nuclei were not all caught in the act of passing through from chamber to chamber, as has been suggested, is clear because of their constant relative incidence according to the method employed. It may be that the difficulty in obtaining an undisturbed fixation is also partly due to an extreme sensitivity and very quick movement of the protoplasm. A living *Polystomella* may move very swiftly, the long fine pseudopodia being very active outside the shell, while the cytoplasm in the chambers may be seen at times to be in rapid circulation.

A great many trials were made with most of the usual fixatives and variants of them, including corrosive acetic mixtures, with and without alcohol, Zenker, Susa, trichloracetic acid, Bouin, with and without urea and chromic acid, Gilson, Carnoy, Flemming and other osmic acid methods. It is not proposed to describe them all in detail. None of them gave perfectly reliable results with large *Polystomella*, and each has its fairly constant defects; but a very great improvement has been found possible, at any rate for some stages of the life history.

Cold fixatives, i.e. up to room temperatures, are as a rule hopeless for the nuclei. After such the nuclei often show no internal structure at all, being fixed only on the surface at one side, thus producing a figure more or less like that of an open empty tow-net. The fixation is much improved by heat, especially if the degree is carefully selected to reduce protoplasmic streaming to a minimum. Hot fixatives, on the other hand, have the disadvantage that their application dislodges more of the foraminal plugs ('bouchons' of le Calvez, 1938, p. 236, see p. 625 and Text-figs. 6, 7), sometimes causing them to be found lying loose in the cytoplasm of the terminal chambers, and sometimes to be thrown right out of the creature.

Corrosive acetic mixtures have been the classical fixatives for Foraminifera ever since both Schaudinn and Lister chose them for this purpose, using them 'warm' (Lister, 1895, p. 414). Lister's figures show the limitations of the method, as do his remarks in the text about protoplasmic disturbances (p. 416), the radial arrangement of the cytoplasm round about the nuclei (p. 418), and the absence of a nuclear membrane (p. 419). After trying many modifications I prefer a 6% solution of corrosive sublimate in sea water, to which I add 5% of glacial acetic acid, used at a temperature of 60–65° C. The specimens are put with a pipette into the fixative previously heated in a closed dish on a water bath, and after 5–10 min. the dish is removed to the bench and left to cool for an hour or so. Decalcification proceeds, and the *Polystomella* may be lifted towards the surface by the bubbles coming off the shells. Inspection shows, however, that they do not actually rise to the surface because as soon as the bubble touches the surface film it is stopped, even if it is not discharged; there is no risk of the specimen drying up as it hangs below in the liquid, and it is perhaps better to leave these apparently floating specimens alone. If decalcification is not completed in the fixative it is usually finished off in 70% alcohol with 3% of nitric acid, after thorough washing in 30, 50 and 70% alcohols in succession, a few drops of iodine solution being added to the last. Fixation by this method is not free from the imperfections already mentioned, though they are reduced as far as possible. The result is fairly good and of course excellent for staining. I do not find the addition of alcohol any advantage, nor the increase of the acetic acid to 25%. Higher and lower temperatures give worse results. I have not been so successful with the method which gave Myers (1935*a*) such good preparations of *Patellina*[1]—although I

[1] I had the great pleasure of seeing some of Dr Myers's fine preparations at Plymouth, as he and Mrs Myers arrived from California quite unexpectedly in the summer of 1938. They decided to work at *Polystomella* while they were at Plymouth, and we agreed to continue our separate researches, which lay along such different lines, quite independently, although for a time under the same roof. It is a pity that our records cover the same year—but our coincidence was purely fortuitous. I greatly appreciated their offer to show me their special paraffin embedding bath before its publication—part of the technique they had evolved in their long experience with the Foraminifera, and which enables them to deal with the astronomical numbers of *Polystomella* on which their results are based. I found the apparatus very useful in sectioning my much smaller sets of specimens, although for special individuals, of course, solitary embedding is to be preferred.

found that his Schaudinn's solution (corrosive acetic with alcohol, containing 25% acetic acid instead of the usual 4–5%) gives slightly better results at higher temperatures (80–90° C.) than at 60–65° C.; my less drastic mixture has the advantage also at the higher temperature.

Better effects were obtained with a modified Zenker's solution, viz. sea water, 100 c.c.; corrosive sublimate, 5 g.; potassium bichromate, 2·5 g.; with 5% glacial acetic acid added immediately before use (and omitting the usual sodium sulphate).[1]

This solution was best used at 40–50° C. for about 10 min. and then left for about 2 hr. on the bench to cool. Care must be taken afterwards to wash very thoroughly in water to avoid a precipitate in alcohol. The excess sublimate is removed with iodine in the usual way. On the whole the cytoplasm and nuclei look less disturbed after this fixation. Most of the nuclei, especially in the megalospheric form, are more often nicely rounded, showing a distinct membrane. Radiations in the surrounding cytoplasm are usually absent at this temperature, but in a few cases there is a small shrinkage space about the nucleus.

The only other useful results with large *Polystomella* were obtained with osmic acid methods. 'Flemming' was used by Lister with some success, though he barely mentions it in his published work; and from his results it was probably used warm, though he does not say so. Various modifications of these solutions were therefore given a thorough trial, the acetic acid always being added at the time of using.[2] They all produced much the same result.

In the cold, i.e. at room temperature (about 14° C.) or using ice (about 2–3° C.), after several hours' application, the cytoplasm was excellently fixed, the fatty constituents being preserved and giving it the crowded appearance it has in life;[3] but the nuclei are very bad, often showing the townet shape and no internal structure at all. There was unfortunately a tendency to violent surging of the protoplasm from one chamber to the next, no doubt rendered very conspicuous by the subsequent good fixation. When the solutions were used hot (35–40° C.) the nuclei were as usual greatly improved, but although a few good ones were seen, on the whole they are wrinkled and not so well fixed as with Zenker or corrosive acetic. Once or twice I noticed a slight exudation of protoplasm from the shell similar to that which occurs when a living *Polystomella* is put into fresh water (see p. 620, footnote 4). It was seen after the use of strong Flemming, made up in fresh water and used at room temperatures or higher.

[1] After an interesting talk with Mr J. Z. Young on the speed of ions and fixation (Young, 1935) I doubt whether the sea water is any improvement with corrosive acetic or Zenker—but with the latter at least its use saves trouble.

[2] 'Strong', 'weak', and an intermediate solution of Meves (see Gatenby & Cowdry, p. 377) were tried; also other variations with more or less chromic or osmic acids, and Føyn's mixture made up in sea water (1936*a*, p. 275). Various times (1½–21 hr.) seemed alike in their effect.

[3] It is hoped to analyse the cytoplasmic inclusions in a later part of these studies.

When desirable before staining it was found quite satisfactory to bleach in hydrogen peroxide solutions either whole *Polystomella* or sections blackened by fixation in osmic acid.

It was noticed that with the osmic solutions the nuclei were not pulled through the foramina, and some experiments were made with the object of attempting to combine the advantages of these solutions with those of the corrosive acetic mixtures with which the derangement of the nuclei is a most serious drawback. The *Polystomella* were allowed to crawl on well-cleaned microscope slides; and when their pseudopodia were well extended the slide was cautiously inverted over osmic vapour. After the desired exposure the slides could be transferred face downwards to other reagents, or the *Polystomella* washed off with sea water and immediately subjected to further treatment. (Incidentally this method was used to make whole preparations of *Polystomella* with extended pseudopodia, with or without subsequent treatment to dissolve the shell or to improve the fixation of its contents.) In the cold the best results were obtained with an exposure to the vapour for 10 min., followed by immersion in 6 % corrosive sublimate with 5 % acetic acid, in sea water. But as usual there was a great improvement when the *Polystomella*, after exposure to osmic vapour, was washed into the corrosive acetic at 60–65° C. With the osmic vapour the canal system which runs through the shell (see Lister, 1903, p. 65) is particularly well shown up, being well fixed and slightly darkened; also the cytoplasm, particularly in the outer chambers. Sometimes the whole effect, including that on the nuclei, was very good, notably in the case of one or two large microspheric *Polystomella*, which are usually very difficult subjects. The protoplasmic disturbances seem to be reduced, more foraminal plugs remain in situ, but on the whole the fixation of the nuclear structure is not improved by osmication before hot corrosive acetic and indeed may be rendered worse. It seems likely that the exposure is rarely just right, and it may be unequal in different parts of a single large specimen.

The rather complicated mixture known as Heidenhain's 'Susa',[1] used at 40° C., produced some good nuclei, but tended to give a washed out general appearance to the preparation. Small *Polystomella*, below twenty chambers or so in a megalospheric specimen, or thirty in a microspheric one, are more easily fixed. With these the best results have on the whole been obtained with 'Susa' at 40° C.

If for any reason it is not possible to use the fixative hot, a preliminary treatment with osmic vapour may be advised before corrosive acetic. Next to corrosive acetic with or without osmication, perhaps Zenker gives the best results in the cold.

Most of my material was fixed in Zenker or corrosive acetic, both used hot,

[1] See Brontë Gatenby (1937, p. 74). Water, 80 c.c.; corrosive sublimate, 4–5 g.; sodium chloride, 0·5 g.; trichloracetic acid, 2 g.; glacial acetic acid, 4 c.c.; formol, 20 c.c.; or Romeis's simplification of the same (Romeis, 1928, p. 80), saturated aqueous corrosive sublimate, 25 c.c.; 5 % trichloracetic acid, 20 c.c.; formalin, 5 c.c. The instruction is to proceed direct from these fixatives to several changes of 80–90 % alcohol.

excepting small specimens for which I often used 'Susa', also hot. The fixation was quite satisfactory as regards staining properties, which were still good after a year or more in 70% alcohol when it was unfortunately necessary to keep the specimens unstained for so long. An interval not longer than a month was aimed at in the case of Feulgen's stain, however, and when this was exceeded the fact will be noted. For staining whole mounts borax carmine, well differentiated, gives good results; and picrocarmine even better, especially for osmic acid preparations. Ehrlich's haematoxylin may be used for very small specimens only. Sections are generally stained with Ehrlich's haematoxylin and eosin. Mann's stain gives very beautiful results with thin sections (8μ), the foraminal plugs coming out yellow, while the chromatophores, to which the colour of the living animal is due, are pink.

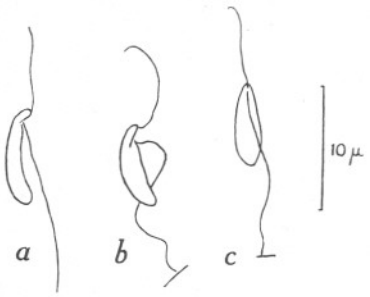
Cultures

It was obviously desirable to have *Polystomella* growing in cultures, and this has been achieved with a fair measure of success. Further experiments are contemplated with a view to improving the results already obtained. *Polystomella* lives, grows, apparently remains in good condition, and proceeds with its life history as long as it is kept in a pasture of flourishing diatoms. To this end I used the medium known as 'Føyn's Erdschreiber'—sterile sea water + salts to which an earth extract is added—with excellent results.[1] I did not attempt to free my cultures from bacteria, nor from small flagellates, both of which might have been useful in the feeding of the Foraminifera. The diatoms were grown in Petri dishes, and small numbers of *Polystomella* (up to four or five adults or about twenty young) lived here as long as the diatoms were plentiful and of a good healthy brown colour—usually 3 or 4 weeks. Larger numbers or growing up families were kept in cultures grown in larger receptacles, glass pneumatic troughs measuring 9–10 in. in diameter and 4–5 in. high being found very suitable for cultures 2–3 in. deep; and flat-sided glass museum jars with at least one polished side for purposes of observation with a horizontal microscope. The diatoms were usually grown at room temperatures, excepting in the hottest summer weather at Plymouth, but the *Polystomella* cultures were kept at various temperatures as will be noted in the course of this paper. A moderate light suits most diatoms; on dark winter days extra light was sometimes supplied from ordinary electric lamps overhead, and in the summer at Plymouth the light close to the window, even though this faced north, seemed too bright and the cultures were moved farther away. The rate of growth of the diatoms naturally varies with conditions of light, temperature, the density of the sowing and the state of the diatoms themselves,

[1] I am very glad to take this opportunity of expressing my best thanks to Dr Fabius Gross, Lecturer in Zoology in the University of Edinburgh, for so kindly showing me his technique for the culture of planktonic diatoms, both at Plymouth in 1936 and in Edinburgh in 1938. See Gross (1937), where he gives the recipe for the culture medium which I also used for bottom diatoms, viz. sterile sea water, 1000 c.c.; soil extract, 50 c.c.; $NaNO_3$, 0·1 g.; $Na_2HPO_4.12H_2O$, 0·02 g.

and it is a matter of experiment to determine the time necessary to produce an adequate feeding ground. Sometimes the life of a culture was prolonged by renewing the culture medium in the dish.

Several different diatoms were cultivated, including one or two planktonic forms, but the most suitable type was found to be the motile bottom-living Pennatae which would spread evenly over the surfaces covered by the culture medium, preferably rising up the sides of the vessel as well as covering the floor. I had the good fortune to catch an ideal form in November 1938 by crushing in a watch-glass of Erdschreiber one of the empty *Polystomella* shells from the sea, which at Plymouth sometimes contain crowded and almost pure cultures of a single diatom of this kind, often along with nematodes and various protozoa. From this source was obtained without further purification a culture containing a single species of naviculoid diatom, which was identified in a subculture of some months later as *Navicula mutica* Kutz. var. *Cohnii* (Hilse) van Huerck.[1] There was also one easily recognized flagellate which persisted unchanged, though its numbers might fluctuate, throughout the year in which the cultures were kept going. It was 8–10μ long, with a very rapid dancing motion, and no tendency to amoeboid action, keeping its shape unchanged except for slight movements of the mobile anterior end. The results of a somewhat cursory examination are shown in Text-fig. 3 and Pl. V, fig. 1, from which it is clear that our flagellate may be included in the genus *Bodo*. It does not agree very closely with the description of any of the well-known species—from freshwater habitats. Its shape and activity place it nearest to *B. celer* Klebs (1892, p. 313). No contractile vacuole was observed, nor any attempt at encystation, but this is not surprising in a marine flagellate. Neither was it seen to feed by sucking out other protozoa, but there was small opportunity of this in my cultures, unless it were extensively cannibalistic. I shall refer to it simply as *Bodo* sp.



Text-fig. 3. *Bodo* sp. living, from culture of *Navicula mutica*. *a*, swimming freely, *b* and *c*, slightly shortened and thickened as when anchored by long trailing flagellum, which also shows a tendency to wind spirally round the body.

The *Navicula*, on the other hand, unfortunately underwent a progressive deterioration in the series of subcultures. At first (December 1938) it was a plump oily diatom of the usual naviculoid shape, about 20μ in length and about 6μ wide in the middle, of a rich brown colour. It had considerable motility which enabled it to spread evenly over surfaces. The motility de-

[1] I am indebted to Mr R. Ross of the British Museum of Natural History for this identification. He says, however: 'I am not really satisfied about the *Navicula*, and it would be better to query the identification for the present.' The uncertainty is probably to be explained by the gradually increasing abnormality in the subcultures described in the text.

creased with age, and the diatom became enclosed in a thick (? mucous) coat to which bacteria adhered. When the colour of the growth changed to a yellower shade it was due for replacement as a feeding ground for *Polystomella*.[1] The most suitable temperature seemed to be about 55–60° F. (14–16° C.); at higher temperatures the growth came faster, but it was paler; lower temperatures gave a slower growth but of a richer brown. In the winter with a moderately thick sowing a Petri-dish culture at Plymouth would be ready for use after about a month, while the old one would need replacing—in the spring and early summer the plates developed in half this time or less. Almost from the beginning it was noticed that there was an admixture of shorter cells in the cultures—down to 10μ or so in length—which tended to grow in short chains or little heaps; and at times these would predominate in a patch of diatoms, the general growth habit at the same time becoming rough and not attached to the substratum. I believe, however, it was a pure culture, and that I obtained at various times a smooth growth of 18–20μ diatoms from a rough growth of short ones and vice versa. This went on throughout the summer, but I believe the original size of the longest diatoms was not regained after a while. In August 1939 it is noted at 16μ; by the following February it did not seem to exceed 10–12μ. In the spring of 1940 the cultures got much worse after a very dark winter in Glasgow, finally producing only very slowly a few cuboidal cells 5–6μ across, in little heaps, and the strain was abandoned. The shape of the cells had degenerated until they could at times not be recognized as naviculoid diatoms at all.

It was very interesting to see in February and March 1940 the *Navicula mutica* cultures which Mr D. P. Wilson had very kindly maintained for me at a lower temperature after I left Plymouth in September 1939. They appeared also to have lost their original size, but had not then declined so far as my diatoms which had been cultivated for use and much more vigorously; by the autumn, however, they seemed almost as bad as mine were in the spring.

Unfortunately, time did not allow of a closer investigation of the diatom cultures I was growing as food. I thought that the dying out might be due to an increasing unsatisfactoriness of the periodic auxospore phases owing to some imperfection in the conditions of cultivation.

The only other diatom I cultivated at all extensively for use at Plymouth was *Synedra tabulata* (Ag.) Kütz.[2] This was a beautiful golden brown form 40–50μ long, not a naviculoid and not motile. Therefore it did not spread well in the cultures but grew in heaps lying loosely here and there on the floor of the dish, excepting when it was spread about by browsing *Polystomella*. It was culti-

[1] As M. Schultze remarks (1854, pp. 20, 24), the colour of *Polystomella* depends on its state of nourishment, and agreed as far as his chemical investigation went with that of the diatoms on which it largely fed (the diatomin of Nägeli).

[2] Also identified by Mr R. Ross. This diatom appeared in a culture of *Enteromorpha* sp. (kindly named for me by Miss E. Stanbury, of the Technical College, Plymouth) which came from an aquarium taken by Dr Gross from Plymouth to Edinburgh some months earlier.

vated in Erdschreiber from November 1938 to July 1939, when it was given up since it had been showing irregularities for some time.

Latterly one or two other bottom-growing diatoms, some from the *Corallina* shore pools at Millport[1] and some at least naviculoid, have been tried at Glasgow, and have seemed more or less satisfactory as food for *Polystomella*; but I have not come across any which gives such a beautiful even growth as did *Navicula mutica* at its best. These cultures of 1939, which could be grown to any required density on any clean glass surface, gave me the opportunity for many hours of fascinating observation of living *Polystomella* going about their business, the results of which I hope to record in these studies. As Max Schultze wrote of his work in 1854, p. vii: 'Der Hauptzweck dieser Arbeit ist, durch Mittheilung treuer Beobachtungen über den Bau und die Lebenserscheinungen der beschalten Rhizopoden der Meere unsere bisher so lückenhafte Kenntniss derselben zu ergänzen.'

The *Polystomella* were taken from a D-netting which was given a preliminary washing at sea. As soon as the catch arrived in the laboratory it was put through a sieve to remove large particles, and well washed with 'outside sea water'[2] in a large flat white enamel dish until as much as possible of the remaining mud, etc., was removed. After a few hours the *Polystomella* had crept up on to the surface of the catch and some up the sides of the dish, whence they could be easily picked off with a pipette. The shells chosen for culture were again cleaned; first with a small brush[3] under a binocular, and then washed with a pipette in several changes of filtered outside sea water.[4] After this treatment it was found as a rule that no other protozoa were introduced into the cultures, the protoplasm of the *Polystomella* being withdrawn inside the shell so that nothing appeared to stick to it so long as it was kept agitated. If, however, it is left still for a few minutes the protoplasm emerges and pseudopodia are put out almost at once.

It was sometimes desirable to be able to recognize individual *Polystomella* without having each one isolated in a culture by itself; and also to know when growth had taken place, this being most clearly indicated by the formation of additional chambers to the shell. Since the shell is not normally external to the cytoplasm it seemed better not to risk an attempt to mark the shell itself in any way. Very often individuals could be put together which showed some marked difference—a set of small chambers due to irregular growth, or a protuberance,

[1] These pools support a small population of living Foraminifera of several species, including some small *Polystomella*. Mr Earland has examined a number of these and assures me they are all *P. crispa* of a small size (under 1 mm.) and often showing spines at the shell periphery, or with a tendency to make very thin shells with rather bloated chambers and no keel—probably ill-developed forms surviving under hard conditions.

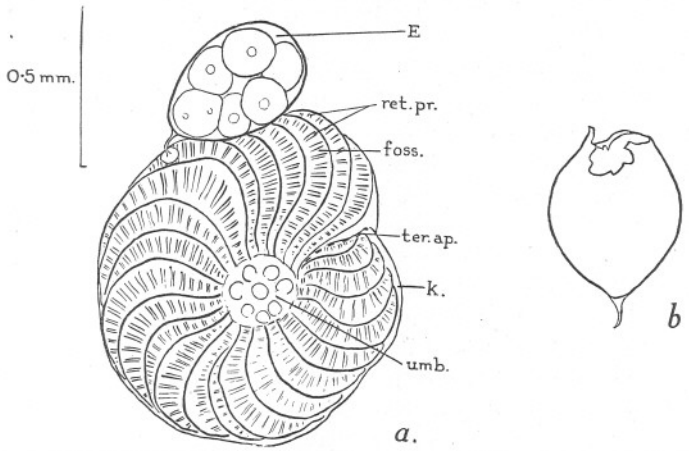
[2] Brought in from outside the Breakwater in carboys (cf. Lister, 1903, pp. 69, 70).

[3] A red sable brush no. 00, for miniatures, is very suitable. It has a fine point, can be sterilized in boiling water, and stands hard treatment well.

[4] Fresh water may not be used as it causes the protoplasm to swell and ooze out at the terminal apertures and from the fossettes (see Pl. IV). This swelling in fresh water also occurs in other Foraminifera.

for example, would serve as an identification mark, or as a landmark in a growing shell. But best of all were clear signs in the form of stalked egg cases or cocoons which occur not infrequently attached to the *Polystomella* shells at Plymouth.[1] These are almost always empty as shown in Text-fig. 4 *b*, but usually remain firmly fixed by the basal plate of the stalk.

These cocoons were found still occupied in April and May 1939, from the Drake's Island ground; in October 1940 and in June 1941 from 'White Patch'. They will continue with their development and hatch in sea water or diatom cultures in Petri dishes in the laboratory. The earliest cocoon contained 6–8 rounded objects (see Text-fig. 4 *a*) in which no movement could be detected, and no change in 10 days. Then, fearing that the contents might be



Text-fig. 4. *a*, *Polystomella* (the shell only being represented) bearing a turbellarian egg-case; *b*, emptied egg-case of turbellarian. *E*, egg case with developing embryos; *foss.*, fossette (d'Orbigny); *k.*, keel of shell; *ret.pr.*, ridges between the fossettes and over retral processes of the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

dead and disintegrating, I opened the egg case, and in it found eight little embryos with ciliated cells which gave them a slow rotation. In more developed specimens about half a dozen planarian embryos could be seen crawling over one another inside the cocoon; they were white in colour, each with two dark eyes. Hatching has occurred a few days after collection by way of an irregular hole at the distal end of the egg case, as many as eight little worms about half a millimetre long emerging. These were still quite white, and swam rapidly by means of their cilia or crept actively about the dish, while keeping constantly in the spot of brightest light. They were offered diatoms, etc., in the cultures, *Polystomella*, small copepods, alive and dead, pieces of a freshly

[1] Also on a miliolinid (*Quinqueloculina*) from 'White Patch' in June 1941. It is not suggested that they necessarily have any special relation to the Foraminifera, although they are often so firmly fastened to the organic basis of the shell that they survive decalcification by acid. When they are found adrift from their substratum they proceed with their development and hatch as usual.

killed small gasteropod, and segmenting eggs of a mollusc: but were not observed to feed on anything of a size visible with a magnification of about 40. They disappeared about a week after hatching.

There is no operculum on the egg case, and it is interesting to note in one emptied case which was mounted on a slide that the chitinous capsule seemed somewhat macerated in the vicinity of the hole, i.e. its usually obscure tesselated pattern had become very obvious, as if a process of solution had been involved in the liberation of the young turbellaria.

It is probable that the 'gestielte, ansehnlich grosse häutige Beutel mit gerissenen Öffnungen am Rucken ihrer Schale festgeheftet' found by Ehrenberg (1839, pp. 109, 133, and pl. ii, fig. 1*g*) on *Geoponus stella-borealis* (= *Polystomella striato-punctata*) from Christiania in Norway, and on *Nonionina germanica* from Cuxhaven were also turbellarian cocoons—although I never saw one with the opening near the stalk; nor one with two or three openings such as he describes (1839, p. 168). M. Schultze (1854, p. 28), on the other hand, suggests that these were tests of the ciliate *Cothurnia*, such as he saw on the same species of *Geoponus* from Cuxhaven in the spring of 1851.

These cocoons cannot at present be ascribed to any known species of turbellarian. Of those already known they look most like minute representatives of the triclad bdellourid cocoons which so far have only been found infesting the gill-books of *Limulus*, the king crab, in both its eastern and its western areas of distribution (see, for example, Wilhelmi, 1909, p. 120). The egg cases on *Polystomella* are not flattened as are the bdellourid cocoons to fit into their special habitat between the leaves of the *Limulus* gill-books.

This is the only animal in any sense parasitic on a living *Polystomella* in my material. I have seen small algae growing on a shell during life, but the *Polystomella* already seemed inactive and soon signs of life ceased altogether.

Feeding

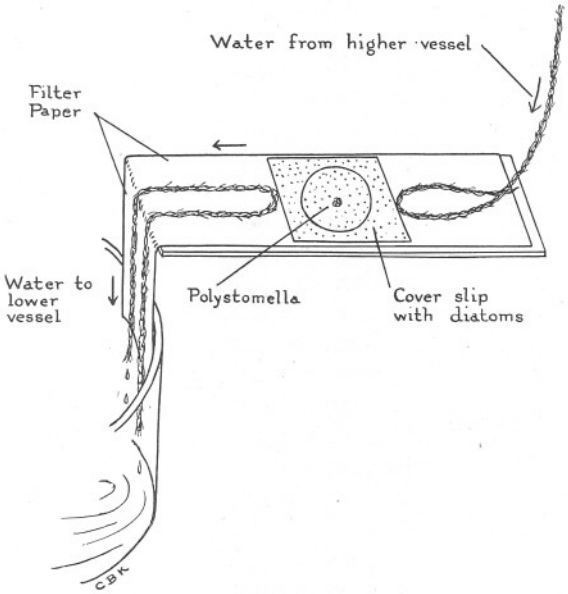
Observations were made in Petri-dish cultures with the aid of a Greenough binocular, and on the sides of taller vessels with a hand lens or a horizontal microscope. Finer details were seen by using a modification of an old method for continuously irrigating a culture over a microscope slide.[1] *Navicula mutica* was grown on cover-slips lying in a Petri dish, and when sufficiently covered, but not too thickly overgrown, the cover-slips were ready for use. A hole was punched[2] in a strip of filter paper (or two or three slips superimposed), and this was laid on a slide and moistened with sterile sea water. A clean *Polystomella*, preferably young on account of its greater transparency and activity, was placed on the slide in the middle of the hole and covered with sea water, then with a cover-slip with the diatom culture growing on its lower surface—the upper side having been previously dried off. The irrigation is very simply effected by strands of white (or 'natural') wool. I found it could easily

[1] See Schaudinn (1895*b*, p. 10). Also described by Lister in Vol. II of his laboratory notebooks (1892–1905).

[2] A sharp corkborer was used for this.

be kept going for several days at least on the microscope stage when a single strand brought the sea water from a higher level on to the slide, and two strands led it away from the opposite side of the cover-slip into a lower vessel (see Text-fig. 5).

All that is necessary is to keep the upper vessel filled, and to wash the wool about twice a day in fresh water, rinsing it in sea water before it is replaced in position. In such an arrangement I have had a young *Polystomella* feeding for a week, in the course of which two new chambers were added, while protoplasmic activity inside and outside the shell could be observed under high magnifications. This activity was seen to be speeded up when the circulation was set going.



Text-fig. 5. Irrigation of living *Polystomella* on a microscope slide.

In spite of its massive shell *Polystomella* may be fairly mobile—quite often moving along as much as 1 mm. in 10–15 min.[1] by means of its very vivacious pseudopodia, which at the time are engaged in a great deal of other activity. This restlessness is not on the whole an advantage in working with cultures. I thought at times that the *Polystomella* tended to move towards a moderate light; but such a tendency, if it really exists, is not strong enough to be useful in controlling their movements under ordinary conditions. Dujardin (1835) also found the direction of incident light to be without influence on the positions taken up by his Foraminifera; but Verworn (1889, p. 40) claims that *P. crispa* moves slowly towards the source of diffuse daylight. I could not dis-

[1] This figure agrees well with the speed of 4–8 mm. per hour recorded by Dujardin for his '*Vorticialis*' = *Polystomella* sp. (1841, p. 258).

cover that their paths bore any relation to a current, for example, during irrigation. They do not appear to seek out good feeding grounds, except by random exploration; nor to stay on them when found, excepting temporarily while actually feeding. They creep up the vertical sides of culture vessels and may spend days crawling under the surface film where their food may be scarce, and whence they are not too easily dislodged once they have established a good set of pseudopodia there.

The pseudopodia of various Foraminifera, including those of *Polystomella*, have been described several times already, and from different points of view (see e.g. Dujardin, 1841; Schultze, 1854, p. 16; Verworn, 1889; Schaudinn, 1893, 1895 *b*; Bütschli, 1894; Lister, 1903, p. 48; Winter, 1907, p. 49; Rhumbler, 1909, p. 251; de Saedeleer, 1932; Sandon, 1934; W. J. Schmidt, 1937; le Calvez, 1938).

Polystomella has pseudopodia of the *reticulose* type, like most of the group; that is to say they are narrow, almost thread-like, branched and continually anastomosing, usually by way of side streams, although occasionally two pseudopodia have been seen to flow together. Even in the finest end-twigs there is evidence of an active circulation, to and fro and across, in the lively movements of the many small granules which normally course up and down the pseudopodia of a well-fed healthy specimen. Sometimes a single granule will suddenly reverse its direction and jostle a way back a certain distance amongst the others which are in the meantime keeping on its former path.[1] This takes place even close behind the steadily advancing hyaline tip of an extending pseudopodium, or in one which is slowly contracting in the act of drawing food towards the shell; and the coloured excretory granules (to be described later), being caught up in the same movement, go to and fro on their way to rejection by the pseudopodia. The pseudopodia of *Polystomella* arise for the most part from the thin covering of cytoplasm which passes through the minute pores found all over the shell between the tubercles (Text-fig. 9*b*) and comes to lie outside it—but also directly from the protoplasm inside the shell via the terminal apertures, sometimes stretching right across the last chamber which is often not filled by the animal; and from the external openings of the canal system in the fossettes and on the umbo (Pl. IV and Text-fig. 4*a*). They may be shot out a short distance into the water like little rockets, and with the granules chasing up and down,[2] wave about like minute feelers, bending, undulating, quivering, and putting out side branches which meet and fuse and so establish the reticulum. This spreads, often in the form of a cone based on the shell, until in half an hour or so bundles of pseudopodia may lie

[1] The animation of the pseudopodia could best be shown in a film, and it was hoped to make one in collaboration with Mr A. G. Lowndes, formerly of Marlborough College, Wilts, whose ultra-rapid cinema-photomicrography (1935) has given such good results with small flagellates. A beginning was made, but the project has had to be abandoned for the present. See Pl. V 9, 10, 11.

[2] Rhumbler (1909, p. 255) ascribes a spontaneous movement to the individual granules in addition to that due to the cytoplasmic streaming.

more or less all round the shell reaching out for rather more than its diameter on every side, or for two or three times as far in one direction as the case may be (see Pls. IV, V).

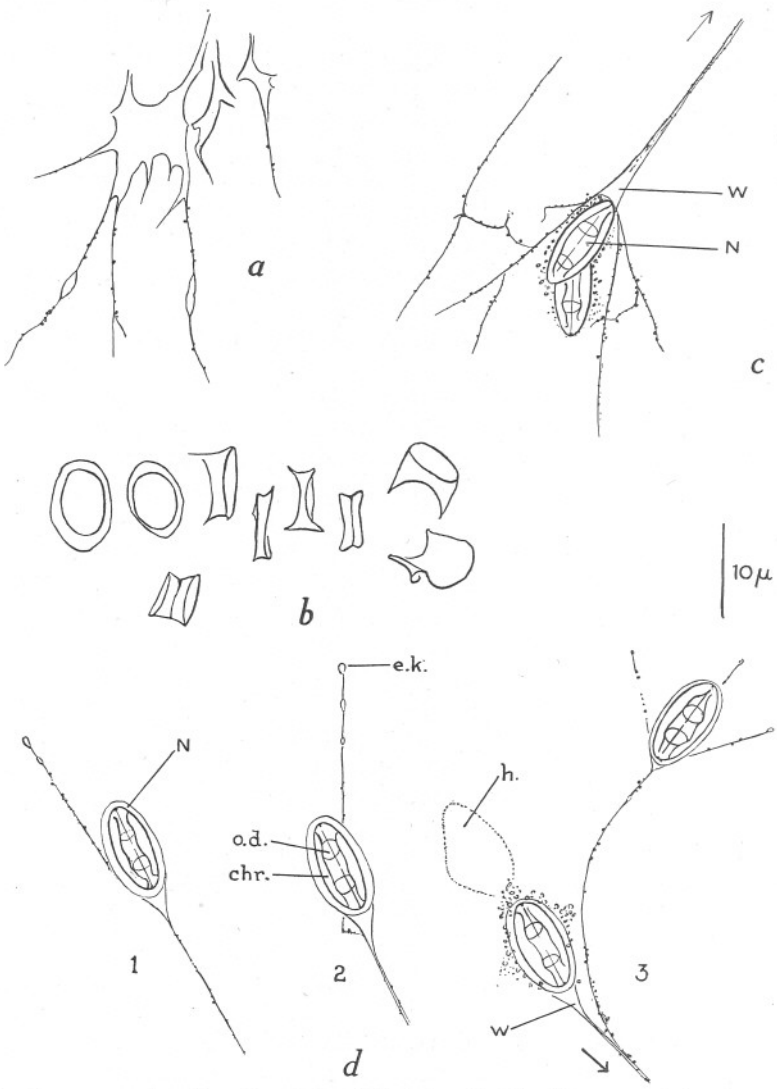
The pseudopodia seem to be bathed in mucus, which is left behind as a trail after their withdrawal. Here and there in the pseudopodia are spindle-shaped varicosities, hyaline, not very refractile, varying in size up to several μ in length. They are deformable, divisible, etc., and moved along in the general streaming of the protoplasm. Sometimes a small one may remain close to the tip of a pseudopodium forming a kind of 'end-knob' (cf. Winter, 1907, p. 50) which may be sticky (Text-fig. 6*d*). Sometimes they spread out to make small webs where the pseudopodia branch, or flattened knots where several lie near together (Text-fig. 6*a*). They become very abundant in the pseudopodia at certain times—at a particular stage of the life cycle or after injury—and may be of a mucous nature.

The circulation goes on in the pseudopodia as long as they are extended, and in a well-developed reticulum is a very wonderful sight. Normally the pseudopodia are withdrawn by a reversal of the process of extension, but when necessary at lightning speed,[1] leaving behind droplets of various sizes (? of mucus) in their tracks.

I could not make out a definite axial thread in the pseudopodia of *Polystomella*, such as is present in those of the Heliozoa for example, although it seems probable that a more fluid surface layer, in which the granules principally circulate, gradually passes over into a core of more solid protoplasm. The pseudopodia show a fairly high degree of stiffness; they extend in a straight line as a rule, and may stretch unsupported through the water for a distance at least two or three times as great as the shell diameter.

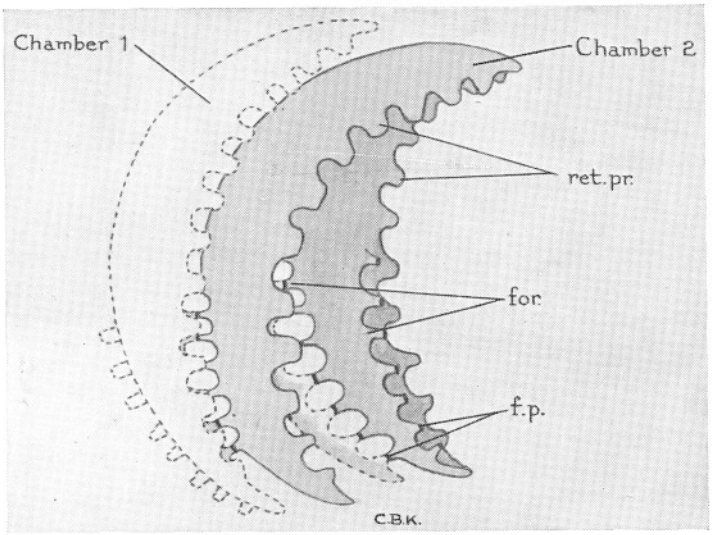
Polystomella depends for its nourishment more than usually on its pseudopodia, for not only does it come in contact with food and catch it by means of them, but the process of digestion also takes place here. No solid food is as a matter of fact ever found inside the shell. Williamson (1849, p. 166) refers to 'very minute siliceous organisms—placed in positions indicating the possibility of their occupying the interior of the segments' in his dried specimens; these were probably the same as his '*Cocconeis*' of 1852 (p. 126), 'frustules of minute Diatomaceae' which he had 'recently found' in the interior protoplasm of a *Polystomella*—none too large to have passed through the terminal apertures of the shell—and which he evidently regarded as food. They are almost certainly loose foraminal plugs (see Text-figs. 6, 7). Lister, in his laboratory notebooks (1892–1905), several times mentions 'algae', 'parasitic algae', especially in the terminal chambers, 'with thick walls' or 'collapsed', and his sketch in Vol. I (opposite p. 111) puts it beyond doubt that these were in reality foraminal plugs, cast off probably at fixation (see p. 627). In 1895 (pl. vi, fig. 10) he figures the same structures in situ, without comment. Le Calvez (1938, p. 236)

[1] These movements should be regarded as a resultant of cytoplasmic streaming and true stretching and contraction, as suggested by Schmidt (1937, p. 590).



Text-fig. 6. *a*, pseudopodia of an injured *Polystomella* showing a great development of ? mucous varicosities and webs; *b*, foraminal plugs sketched after ejection; *c*, pseudopodia showing a bundle of diatoms being drawn towards the shell in the direction of the arrow; *D*, 1, 2, 3, sketches at intervals showing the extraction of a small portion of the diatom culture on which the *Polystomella* was feeding, as described in the text, p. 628. *chr.*, chromatophore of diatom; *e.k.*, sticky end-knob; *h.*, hole in pasture; *N.*, *Navicula mutica*; *o.d.*, oil drop; *W.*, web attached to food mass.

first attempted to describe them fully, in *Planorbulina mediterranensis*. According to le Calvez the foramen is gradually closed up by a deposit which appears as a ring at the narrowest point and spreads across the opening to form a 'bouchon'. Periodically the plugs come loose and are carried out of the animal by the cytoplasmic currents. The cycle is synchronous throughout all the chambers. My observations on *Polystomella* indicate that the same thing



Text-fig. 7. Showing the protoplasmic structures filling two chambers of the shell. *Chamber* 1 (outline dotted), *chamber* 2 (shaded), protoplasm in chambers; *for.*, protoplasm in foramina before and behind chamber, forming bridges by way of which each chamber communicates with its neighbours except in so far as the bridges are closed by the foraminal plugs *f.p.*, in situ; *ret.pr.*, protoplasmic retral processes underlying transverse ridges on exterior of shell. The canal system lies in the shell substance between these structures; a spiral canal, leaving the initial chamber and following the ends of the series of chambers, runs in the umbo on either side, giving off (*a*) in the septa, meridional canals which pass out between the retral processes and the foramina and have branches opening into the fossettes, and (*b*) several vertical canals which open in depressions on the umbo. After Williamson (1849), and see Carpenter (1860).

happens here also, the plugs being composed of some 'chitinous' material, staining as le Calvez says with chromatin stains, including Feulgen, and showing in favourable specimens at all the foramina from the initial chamber outwards in both microspheric and megalospheric phases. Le Calvez does not give details of his observations on the shedding of the 'bouchons'. As already noted they are loosened by fixatives, especially when used hot, but they are certainly also cast at times in the absence of such unnatural treatment, for they appear along with the excretory granules in the dejecta of the living animal (see Text-fig. 6*b*). Their function seems at present quite obscure. (William-

son's reference in 1849 (p. 165) to a constriction seen at times in the foramina of *Polystomella* must indicate a stage in the laying down of these plugs.)

When the pseudopodia of a *Polystomella* in the course of its wanderings come across a movable solid body they may get hold of it, and by subsequent appropriate shortening gradually draw it up towards the shell, perhaps pulling it in a millimetre or so in 10–20 min. Sometimes the object may go backwards and forwards on the way as the pseudopodium stretches and contracts again, and sometimes it is abandoned altogether. A food body may be seized in either of two ways. I have seen one come against the side of a pseudopodium which suddenly bends over it—thus enclosing it—or the tip of the pseudopodium may attach itself to the food body—perhaps by the aid of a sticky 'end-knob'—spreading out against it in a fanwise manner and apparently flowing more or less around it. Then it seems as if the food is actually pulled along towards the shell, while new pseudopodia may arise beyond it and go further afield (see Text-fig. 6*c*). It was most interesting to watch pseudopodia pulling on *Navicula* growing in a carpetlike mass of bacteria, etc., until a little patch would suddenly give way and be dragged out of the carpet, leaving a hole where it had been, as shown in Text-fig. 6 *d*, 1–3, while the patch was drawn away towards the shell. When the pseudopodia are working fast the collection of food (? embedded in mucus) may be enough to form a good 'feeding cyst' completely covering the shell in an hour or two. Inside this, the pseudopodia being withdrawn so far, digestion of the meal goes on. After an interval pseudopodia again appear outside the feeding cyst and the *Polystomella* begins to emerge. Sometimes a new chamber is first formed inside the cyst; and the following laboratory notes give an account of an emergence which I watched, from a cyst which happened to be thin, mainly composed of small granules with only a few diatoms in it. As is usually the case it was somewhat thicker over the terminal face of the last chamber, and this part is referred to as the 'face-mask'. '8/8/39, 9.45 p.m. R. had moved a very little way back from 'face-mask', but still in slough. Large numbers of rather straight parallel pseudopodia over face and parts of cyst nearby—i.e. above and below 'face-mask'. Lengthened as if pushing, while great numbers of pseudopodia on opposite side [of shell] pulled, and *Polystomella* slid backwards out of thin granular slough—pseudopodia passed easily through this.'

There had probably been but little feeding in this particular cyst, but this does not affect the mode of emergence, which may, however, be above or below the 'face-mask' instead of directly opposite it. Emergence from a complete covering of food is usually at the periphery—probably a line of weakness in the feeding cyst, and the point at which the strong keel of the shell could be most useful in forcing a way through. There is another record in July 1939 of a *Polystomella* which had also made a new chamber inside its cyst, this time mainly composed of *Navicula mutica*, and then had left it backwards as usual. The cast slough was examined. The majority of the *Navicula* in the cyst looked normally healthy, but amongst them were large numbers of empty

frustules, the two valves of many of them being separated. There were also coloured excretory granules, about 2μ in diameter, especially in the 'face-mask'. This is the usual constitution of a discarded feeding cyst of *Polystomella*; inside this one the creature had evidently taken a meal, excreted, and made a new chamber. In Pl. V, figs. 4–6, are reproduced sketches made during life of another *Polystomella* which was watched as it fed on the planktonic diatom *Nitzschia*, while wandering on the side of an upright museum jar. First there was an abandoned feeding cyst (Fig. 4) composed, as could be judged from its colour, largely of diatoms; then another slough of a brighter golden hue was left behind (Fig. 5), largely made up of excretory granules. The *Polystomella* itself is seen moving off (Fig. 6). It seems that feeding may take place at any time of day or night. The interval between one meal and the next may be very short—a *Polystomella* is on record as making and discarding two feeding cysts in 7 hr.—or it may be a matter of days, according to circumstances. The actual digestion of the contents of a *Navicula* for example would seem not to take a long time, as so few are ever found in a condition intermediate between normality and the empty open frustules. These were observed mostly over the flat surfaces of the shell in one case—the undigested diatoms tending to lie near the periphery of the cast cyst. All my attempts to witness the process of digestion in detail have so far been in vain; and from what has been said it will be plain that success in this must depend on a very lucky chance, as it evidently takes place close to the outside of the shell, either in pseudopodia or in the external protoplasmic layer which is at times thick enough to enclose diatoms for example. It is interesting to note that the cast cysts of *Polystomella* feeding in *Synedra* cultures also show emptied frustules, although this genus has no open raphe. The valves may however be pierced by pores.

The 'coloured excretory granules' already mentioned are of special interest because they are such a conspicuous feature of the Foraminifera as a group. They are known as 'xanthosomes' on account of their amber colour (see Jepps, 1926; le Calvez, 1938, p. 268). They may be seen in trails (tracks of pseudopodia) and clumps in diatom cultures, etc., in which *Polystomella* have been feeding, as well as in their discarded feeding cysts. They also appear, at times in very great numbers, scattered through the cytoplasm of the living animal, or collected in certain parts of it. In *Polystomella* they vary in size up to 5μ or so across, and are often slightly angular in contour. Reference has already been made to their presence in the pseudopodia (p. 624), where they go about in the circulation for a time and are ultimately left behind by the protoplasm retreating from the mucous trail. We have seen also that they are sometimes thrown off in a more or less complete cyst-like covering. Like the foraminal plugs which are sometimes thrown out with them, they are too large to pass through the fine pores of the shell. They may be seen massed in the 'face-mask' about the terminal apertures (foramina) of the last chamber, in the fossettes between the retral processes, or in the depressions on the umbo

of the shell. Both xanthosomes and foraminal plugs are present in the protoplasm of the canal system in a number of my mounted specimens. The former can be shown in the terminal apertures as well as in the position of the fossettes; while the latter tend to collect in the latest formed chambers and perhaps usually pass out by the foramina, although in at least one preparation appearances suggest that they also may be able to leave the shell by openings into the fossettes. Excretory granules have actually been seen on their way out in strong pseudopodia emerging from the fossettes of the last two chambers and from the terminal foramina of a *Polystomella* under irrigation. Thin discarded feeding cysts often show a pattern representing the fossettes of the lower side of the shell in little heaps of excretory granules. The conclusion is that these solid bodies pass out of the shell by one or other of these two routes —and therefore that the canal system of *P. crispa* certainly has openings into the fossettes as described for the larger *P. craticulata* (up to about 5 mm. in diameter, fossilized), from the coast of Australia by W. B. Carpenter (1860), although he specifically denied it for *P. crispa*. It is easy to understand how Carpenter came to pass them over. I could not be quite certain in my preparations of more or less normal whole *Polystomella* that the meridional canals (see Text-fig. 7 legend) bore lateral branches which might open into the fossettes until I had seen them most clearly displayed in one of a set which had been living in a culture of *Synedra* for some months. Owing possibly to more than one cause, the protoplasm was badly diminished and disorganized—the nucleus had disappeared—but the canal system seemed swollen out and full of staining granular material so that it was very conspicuous indeed, even to these ultimate branches, one to each fossette, especially where it had broken away from the remains of the chambers. The canals are of course to be seen fragmentarily in sections of *Polystomella*. My observations on the canal system confirm the findings of Lister (see 1903, pp. 65, 66, with fig. 9).

It is often stated that the pseudopodia of Foraminifera have the power of killing their living prey suddenly as if by the action of some specific poison (Schultze, 1854, p. 23, for *Polystomella* and *Gromia* feeding on 'kleine Infusorien'; Winter, 1907, p. 10, for *Peneroplis* feeding on small crustacea; and others). I have seen no evidence of this in *Polystomella*; but I have not yet offered small crustacea as food, and Winter says the poison of *Peneroplis* is not so effective against 'Infusorien' and 'Flagellaten'. As already indicated my *Polystomella* fed mainly on diatoms, though there were usually plenty of small zoomastigine flagellates ('*Bodo* sp.') about. Occasionally, however, I have seen these being drawn towards the shell. They continue their movements for some time, even after they begin to look rather abnormal, and seem to die gradually in the course of their transport. This agrees with the observations of Rhumbler (1909, p. 253), although I cannot agree that the movement of the prey is necessary to its capture as he goes on to suggest (quoting Verworn, 1889, pp. 148–9). We have seen that non-motile diatoms, for example, are caught, and apparently used as food. Besides these, *Polystomella* frequently takes

inanimate objects up to its shell, such as small sand grains, filter-paper fibres in the irrigation experiments, etc., and manufactures a more or less complete covering out of them. Although I have a general impression that *Polystomella* did to a certain extent choose diatoms, and particularly *Navicula mutica* as food in the cultures, it is difficult to say anything more definite about its power of selection in the face of the probability that the function of the cyst may be partly protective (see p. 642). Further, although Cryptomonads and Chlamydomonads when available were also incorporated in the cysts, in spite of a search no evidence was obtained that they had been used as food; but even if they had been, neither would leave as conspicuous an indigestible residue behind as the frustules of a diatom. Some other Foraminifera, including the pelagic forms, are reputed to feed to a large extent on animal food, especially small copepods (see for example Rhumbler, 1900, p. 2; Winter, 1907, p. 10), and it is hoped, when opportunity offers, to try the effect of a more mixed diet on *Polystomella*.

Foraminifera have long been famous for their tenacity of life, at least in certain circumstances (Schultze, 1854, pp. 21, 31). Although at times their appetites seem large, some of them, including *Polystomella*, can survive long periods of more or less complete starvation. When short of food *Polystomella* slowly loses its brown colour, at any stage of its life history, often becoming patchy and then always paler until in 3 or 4 weeks it may be almost white. If it has not degenerated too far, the colour will return when food is again taken, a week or 10 days of good living making a noticeable difference. As pointed out by Rhumbler (1909, p. 255 footnote) the granules gradually disappear from the pseudopodia of a starved *Polystomella*; all the cytoplasm in fact seems to become thinner in its consistency, and inside the shell it is seen to decrease in extent. Lister (1895, p. 422) gives figures to show that starvation also induces a shrinkage of the nucleus relative to the cytoplasm as indicated by the number of chambers filled at the time of fixation. It is a pity that his two groups of *Polystomella* were collected at different seasons, viz. 118 fixed fresh from the sea in May and June, and forty-eight collected in October and fixed in January after 3 months in the laboratory on short rations; because, owing to the annual cycle of which Lister was unaware, there is also an age factor involved, though it is not known that this actually affects the result. The curves come out remarkably clearly for so comparatively few specimens, and in spite of the fact that the number of chambers is only a very rough indication of the *amount* of protoplasm present since they vary a good deal in size.

Part II. The Biennial Cycle, including an Account of the Life of a Megalospheric Brood in Culture and a description of the Formation of a New Chamber

The Microspheric Phase

It is well known that microspheric *Polystomella* often undergo asexual reproduction in a laboratory culture if provided with a modicum of food.[1] Schaudinn 'mentioned' the event (1894*b*, p. 42) and referred to it again with a few particulars (1895*a*, p. 59; 1903, p. 499). In 1895 Lister gave in his Postscript no. 2 (p. 445) an incomparably succinct account of the process after observing 'some hundreds of cases'. This was amplified in 1903 and illustrated with the now familiar figures (pp. 67–9). Later references (Lister, 1906, p. 7; 1907, p. 492) only added that some 200 young are commonly produced in a brood. Heron-Allen published extracts from Lister's Notebooks (1892–1905) giving further details of the actual observations, the most important being that 'in specimens whose protoplasm has begun to emerge, faintly stained round nuclei 10μ in diameter are found in the clear protoplasm of the terminal chamber' (1930, p. 7). This supports Lister's statements that there are nuclei in the young megalospheric individuals from the beginning, although, curiously, he had originally come to the same conclusion as Schaudinn that the nuclei of the microspheric parent had completely disintegrated into a chromidial form before reproduction set in. I believe that this conclusion may be due, partly at any rate, to imperfect fixation, because of a very severe reaction to disturbance, perhaps accompanied by actual injury to the protoplasmic mass in the course of securing it for fixation at a particular moment. Emergent protoplasm which was detached from the walls of a culture dish either showed no nuclei at all or nuclei somewhat altered in appearance, so as to make them even more easily concealed amongst the deeply staining chromidial bodies which are very conspicuous at this time. On the grounds of analogy and general theory one can accept complete nuclear dissolution only with the greatest reserve until it is demonstrated to be true beyond all question. There is no need for further discussion of the inaccuracies of Schaudinn's account of the process since they are fully dealt with by Lister (1903, pp. 69, 70).

Perhaps the sensitivity referred to above may account at least in part for the fact that we have as yet no description of any nuclear division in *Polystomella*. It is of course notoriously rare to come on most species of protozoa with nuclei in a state of division; and apart from the sporulation phase there are anyway only a few nuclear divisions in the long life history of a *Polystomella*. The structure of the nucleus was described by F. E. Schulze (1877, p. 17, pl. ii), and its position about midway in the series of chambers of the megalospheric phase of *P. striatopunctata* indicated. The nuclei of the microspheric phase are similar in appearance and are spread through the chambers forming approximately the middle third of the series, those in the outer chambers (as pointed

[1] And occasionally if no food is provided.

out by Lister) being larger as a rule than the others. It may be said on the whole that the nucleus of a large megalospheric *Polystomella* is more likely to be packed full of nucleoli of a smaller size, which, however, may vary a good deal inside one nucleus, while, as noticed by Lister (1895, p. 419), those of the microspheric form tend to fewer nucleoli of a larger size, especially the larger ones in the outer nucleated chambers. No positive reaction to Feulgen's stain was obtained in the nucleus of either form. Both Lister and Schaudinn believed them to increase in number by direct (amitotic) division as the chambers increased. As 'good evidence' for this Lister indicates their disposition in the chambers in pairs of similar size and appearance, sometimes united by a narrow bridge as if in the act of dividing with absolutely no visible rearrangement of their components (1895, p. 419). These observations are easily confirmed, but I believe they may be found susceptible of a different explanation. It does not, however, seem profitable to discuss the matter further at present. After examining whole mounts of some 125 and sections of over 200 microspheric *Polystomella*, many of them in April and May when reproduction is at its height, I am still unable to give the smallest indication of any division figure. Some of these specimens were fixed soon after collection from the sea, i.e. in a few hours' time during which they were kept as far as possible at sea temperatures. Others were cleaned and put to feed in diatom cultures for periods up to a fortnight, and afterwards fixed at various times of day and night. Some were starved and then fed; a further trial of this procedure is greatly to be desired.

Nuclear division in microspheric *Polystomella* seems to go on at any time of year if we may depend on size differences, etc., of the nuclei as indications of recent multiplication. Such indications are most often seen towards the middle of the series in each *Polystomella*, where the nuclei tend to be more numerous and smaller; and are evident in specimens collected in October and November as well as in April when asexual multiplication is generally imminent. If averages obtained from my comparatively small numbers can be trusted, there is not much increase in the number of chambers through the winter months, but the nuclei have multiplied from say thirty-three in the autumn to forty-four (varying $\pm$ 10) in some fifty-four chambers by the middle of April (thirty-seven specimens, 11 April 1940). About two further divisions for each nucleus are then all that are necessary to produce the number required by the brood of megalospheric young. The highest number of nuclei shown in my preparations is about 116 in a *Polystomella* of fifty-five chambers collected in March, the protoplasmic body after decalcification measuring a little over 1·5 mm. in diameter,[1] and having as many as a dozen or more nuclei in some of the

[1] My measurements refer to what is usually the greatest diameter, along a line passing lengthwise through a chamber a little before the last of the series. I regret that many were made before I realized that the official plane of measurement runs across the shell parallel to the last chamber. In counting the chambers I have reckoned the 'globular swelling' referred to by Lister (1895, p. 418), which lies next to the initial chamber as the second markedly smaller chamber of the series, which it undoubtedly is. The third chamber is roughly as big as the initial chamber, and thereafter there is generally speaking a steady increase in size in the

chambers towards the middle of the nucleated series. Another *Polystomella* of the same size (in August) had seventy-six chambers, the biggest number I ever found; and the largest specimen (in the April collection) with sixty-eight chambers measured 1·8 mm. after decalcification. Microspheric *Polystomella* tend to reach a large size on the average, although some megalospheric forms also attain the same dimensions. I have no record of asexual reproduction in a *Polystomella* that was not over 1 mm. across in the shell and probably comprised at least fifty chambers.

It seems that Lister was working with a population of a smaller size or an earlier maturity—for he quotes (1895, p. 419) as one of his largest microspheric specimens a *Polystomella* of forty-seven chambers which measured 800μ across (? after decalcification); while a few figures in his Notebooks (1892–1905) give an average of thirty-six chambers for ten reproducing microspheric individuals.

The *Polystomella*, which usually show signs of starvation by the end of winter,[1] are able to feed again as soon as the diatoms begin to increase in the spring. Their colour improves and growth again takes place. By early in March 1939 it was noted that they had begun to look a little browner in the collections, and by the end of the month some of them had taken on a rich brown colour. From now on it was possible to make a partial separation of megalospheric and microspheric forms. Apart from the fact that one may often effect a slight concentration of the microspheric *Polystomella* by selecting those of the largest size, in April they could be collected by taking those of the richest colour and those which showed the greatest recent growth. This was apparent in the shape of the creatures, because their newly formed chambers were conspicuously large, raising the contour of the shell into a high bulge as they spread over the whorl below. This feature is actually shown in Lister's figures (1903, pp. 63, 67) without comment. Though it is good enough to be useful in choosing specimens for cultures, the test is by no means infallible even at this season. Young or small megalospheric *Polystomella* which feed and grow at a greater rate, naturally tend to exhibit what we may call the microspheric figure, and there are always some larger ones of both kinds which are also exceptions to the rule. The degree of success in diagnosis of living *Polystomella* which was attained by these criteria is indicated by one or two examples. On each of two

series. It might be pointed out here that the initial chamber of the microspheric form differs from all other chambers of both forms when seen in decalcified specimens in that it is enclosed in a thickish capsule, like a cyst wall with a single pore which is the foramen. It would obviously be more significant to give measurements of this capsule where possible rather than of the more variable cytoplasm which may not fill it, although the capsule itself appears shrivelled in many preparations. As Lister (1895, p. 418) observed, the first three or four chambers are also different in that they are arranged in a spiral, which may be either right-handed or left-handed. Does this represent an ancestral form of the creature? (See Lister, 1903, pp. 135–7; Tan Sin Hok, 1935; Ovey, 1938).

[1] It will be very interesting to compare my account of the annual cycle, based on general observation and cultures, with that of Earl H. Myers derived from his statistical investigation of very large numbers of specimens, during the same year at Plymouth.

occasions in May about thirty 'microspheric' *Polystomella* were picked out, mostly by the shape, and there were some which could not be assigned to either form. Only about 10 % were correctly selected. Better results were obtained in April by choosing the darkest brown specimens, especially the largest ones, when a 60 or 70 % success was reached. It is plain that the method could not be applied to individual specimens. It is usually stated that there are only about 3 % of microspheric forms in the population, and this is true of the greater part of the year. In the month of March 1939 the percentage rose from this value to about 25 %, and in April it continued to rise to about 30 %. This was due to the fact that the megalospheric forms had been sporulating[1] from January on, and so gradually disappearing from the living population. At first only a few *Polystomella* were reproducing, the peak of sporulation occurring late in March or early in April. It was perhaps at their point of highest frequency that the shape of the microspheric forms was most distinctive; later in May the choice became confused by the presence of some megalospheric young, and perhaps by the darkening of the colour of some older ones after more prolonged feeding.

A few comparable figures are given in Table I for small collections of *Polystomella* from the 'White Patch' ground in 1940.[2]

TABLE I

Date	% microspheric form	Total collection
3. i. 40	10	250
29. ii. 40	17–18	90
5. iv. 40	about 50	94
18. vii. 40	about 7·0	about 300
21. x. 40	4·5	424

Some time in April the microspheric *Polystomella* in turn reached the peak of their season for reproduction by a form of schizogony, and in turn began to show a notable reduction of their numbers.[3] This asexual reproduction began early in February in my cultures fed on *Navicula mutica* for a little over a month, and in the sea by the middle of April there were already a large number of young megalospheric forms, 0·5 mm. or so in diameter, offspring of this process. On 26 April I selected twelve *Polystomella*, measuring 1·2–1·6 mm. in diameter, as likely to be microspheric from their colour and shape, and nine of them produced young asexually in cultures of *Navicula mutica* between 5 and 22 May (at least two of the other three were megalospheric). During this time there was unfortunately an unavoidable interval in my observations, and when I returned to them on 22 May the high season for schizogony seemed to be past, although it occurred in my cultures later on, and probably continued

[1] This process will be discussed in a later section of this series.

[2] No great value must be placed on the exact numbers given. The samples were not especially taken for this purpose, nor all in the same way.

[3] Heron-Allen (1914, p. 9, footnote) refers to the finding of 'a very large number' of two- and three-chambered megalospheric young on the Drake's Island ground in April 1929.

in the sea through the summer months.[1] From June onwards large *Polystomella* of any kind were scarce in the catches, and of these a fairly high proportion were microspheric. Of eighty specimens in August 6% were microspheric, and young of 10–20 chambers were still common in October. By the end of June some young had reached the size of the smaller specimens of the year before, i.e. a millimetre or so in diameter, but they could still often be distinguished by their browner colour. Towards the end of July a feeding experiment indicated that less food was being taken and that the rate of growth had very much slowed down, and in August I could no longer pick out 'young' and 'old' specimens amongst those of the larger sizes.

Of the *Polystomella* from 'White Patch' to which reference is made in Table I about 100 were put to feed in a culture of diatoms on 20 July 1940, and eight out of twelve microspheric individuals reproduced before 27 August, while a similar experiment in October gave no reproduction at all.

Development of the Megalospheric Phase

Some of the young megalospheric *Polystomella* produced in my cultures were reared for varying periods; and some account will now be given of the more interesting observations on two out of several families born in Petri-dish cultures of *Navicula mutica*. In both of these the parent shell was incompletely emptied—but in others all the protoplasm came out as it probably does in the wild, leaving a very clean white shell behind. Such shells often have holes in the wall of the terminal chamber, apparently made by the issuing protoplasm. The diatom cultures were of course renewed as required, and larger vessels used as the young grew up.

The largest Petri-dish brood (N 4) numbered some 150 young. On 3 and 4 February 1939 a *Polystomella* measuring a little over 1·5 mm., which had been feeding on *Navicula*[2] in sea water since 17 January on the laboratory bench at temperatures of 55–60° F., was seen under the surface film, supported by a large number of long pseudopodia. On 7 February it was still at the surface, with almost all its protoplasm outside and divided up into the family of two-chambered young, which were engaged in collecting diatoms and ? bacteria out of the surface film into their pseudopodia.

8 February. Many young have a third chamber.
9 February. Young spreading over the surface film. Some with four chambers.
10 February. Four to five chambers. Renewed the sea water carefully with a pipette.
11 February. Put a few young on the bottom of the dish where there was more food.
18 February. Parent has fallen to the bottom. The brown protoplasm remaining in the last few chambers is putting out pseudopodia. Wall of terminal chamber seen to be damaged. Some young with six chambers.

[1] J. J. Lister studied this method of reproduction in *Polystomella* from the English Channel in the months of May, June, and July.

[2] Grown in Erdschreiber, which was replaced by sterile sea water when the Foraminifera were introduced.

23 February. 4½ chambers of parent shell seen to be full of protoplasm. Young on bottom with eight to nine chambers. On surface where food is scarcer, six to seven chambers.

26 February. Young on bottom much better grown than at surface, where diatoms are very scarce.

28 February. Young on bottom with ten to eleven chambers.

3 March. Parent shell with five to six chambers full.

4 March. Young up to one dozen chambers. Some which started with irregular growth now seen to be growing regularly.

7 March. Biggest young with fifteen to sixteen chambers.

14 March. Cannot now count chambers of big young in shell. Parent shell with protoplasm in 8½ chambers.

8 April. Young up to over 0·5 mm. Fixed some, about one dozen chambers. Diatoms not very good now. On 7 March parent and some of the best young were transferred to a fresh culture of *Navicula* in Erdschreiber. These young now reach 740 μ. Parent shell has protoplasm in almost all exposed chambers.

30 April. Most young about 1 mm. across, one is 1·2 mm. One small, with only seven to eight chambers ('dwarf'). Young look well, but parent very pale and starved looking. Diatoms going off.

7 May. Taken to cooler room—at 13° C.

28 May. Some young up to 1·3 mm., with about forty chambers. Parent probably died about now.

14 June. Dwarf shell empty—about 400 μ.

23 August. Fixed sixteen young, brown in colour, and one very pale with greatly reduced protoplasm, measuring 1·1–1·5 mm.; and two smaller pale ones, also degenerate and measuring under 1 mm.

Another brood (N 2) was born in a similar culture of *N. mutica*, but kept in Erdschreiber at 55–60° F., between 17 and 23 February. The parent shell measured about 1·6 mm. across and had been in the culture since 16 January. In the interval its colour had darkened to a very rich brown shade, especially near the terminal chamber, and it had probably grown one or two new chambers. It was found up the side of the dish on 23 February, a good deal of the protoplasm remaining in the shell and part of that which had emerged undivided. Some healthy looking young were close by—but only thirty-five were ever recovered in this culture. The diatoms were especially good.

7 March. Parent shell has one to two very irregular new chambers.

14 March. A few young have fifteen to sixteen chambers, one only two to three. Many are abnormal and there are some empty shells.

16 March. Parent shell now has about eighteen chambers full of very dark protoplasm.

7 April. Parent shell now has protoplasm in all visible chambers.

30 April. Average diameter of young shells about 800 μ. There were twenty-six good young and nine very deformed. Some fixed in Susa showed about twenty-six chambers, also that individuals which appeared to have begun life as monsters came to grow quite regularly after a time, even when two nuclei were present (one pearshaped initial chamber), presumably owing to imperfect division of the parent protoplasm at schizogony.

27 May. There are twenty-five young now, up to 1·1 mm. across.

28 May. Put in cooler room, at 13° C., but kept at various temperatures (say 13–17° C.), during the summer months.

23 August. Fixed the parent. The protoplasm was a very dark colour, but the shell was highly irregular near the terminal chambers. In section the whole series of chambers was found to contain protoplasm, with about a dozen nuclei and a few shreds of chromidium. It is interesting to observe that the solitary nuclei which do not appear to have undergone recent division are quite full of small nucleoli, looking like the nuclei usually characteristic of a megalospheric form.

2 September. Young now over 1 mm. in diameter, in many cases with some irregularity towards the end of the series of chambers. Rather pale.

23 September. Taken to Glasgow in cultures of *Navicula mutica* in jam pots, and kept there at about 15–16° C. The cultures became rather poor and the *Polystomella* paler.

8 November. Moved to better cultures and improved in colour.

21 November. The *Polystomella* in about 2 l. of Erdschreiber in a large glass basin grown with *Navicula mutica*, were placed in a sink with running cold water[1] at a temperature of 11·5° C. (sea temperatures recorded at Plymouth Pier in Nov. 1939 about 13·5° C.). *Polystomella* now quite a good colour, measure up to 1·3 mm.

15 December. Temperature down to 9–10° C. (sea temperature at Plymouth fell to 10° C. by the end of the year 1939). Careful inspection showed the *Polystomella* of a good colour, but no evidence of recent growth.

18 January 1940. *Polystomella* inspected almost daily since 8 January, still brown.

20 January. One *Polystomella* looks pale. Temperature in the sink this month about 8° C.

21 January. Temperature down to 6·0° C.—very cold weather (sea temperatures at Plymouth down to about 7·5° C. by the end of January 1939). Basin was broken, and had to be left so that the culture was at about 10° C.

9 February. Two *Polystomella* now look almost white, one with brown patches.[2]

14 February. Twenty-three brown *Polystomella*, and two almost white ones which look dead.

17 February. Two very pale *Polystomella* crushed, and the protoplasm seen to be divided up into spheres of 4 μ to two or three times as big, with shining inclusions. One more *Polystomella* looks pale, the rest seem to be feeding well.

23 February. Another *Polystomella* 'white', seen next day to have brown patches on its lower side, in the fossettes.

26 February. Pale *Polystomella* of the 17th is colouring up again. 'White' one of 23rd crushed and found to contain dead rounded up flagellispores with shining inclusions. The brown patches seen to be excretory granules. Temperature this month varying 1–2° on either side of 10° C. (sea at Plymouth in January 1939 about 8° C.).

9 March. Temperature 9° C. Four 'white' *Polystomella* seen: (1) First seen to be white on 7 March and almost certainly not so the previous day. About noon the shell was observed to be full of active flagellispores, with some excretory granules. Examined at intervals all day, but no emergence seen. Next morning the activity had ceased—the little flagellates apparently having died in the shell. (2) Crushed on 10 March, cytoplasm dead, unsegmented. (3) Crushed on 10 March, cytoplasm dead, mostly segmented. (4) Crushed on 10 March, cytoplasm dead, segmented into spheres and distorted flagellispores, both showing some activity. No more 'white' *Polystomella* were seen.

19 March. Left eighteen young in culture, three rather pale, and went to Plymouth.

[1] We are fortunate in Glasgow in having a plentiful supply of water from the Trossachs which runs very cold all the year round, and shows a seasonal variation in temperature which happens to be only a little greater than the shallow water temperatures recorded from the pier at Plymouth.

[2] The significance of some details now given will appear in Pt III of this paper.

The temperature in the culture slowly rose from 9·5 to 11° C. by the time of my return (sea at Plymouth only up to about 10° C. at this date in 1939).

21 April. Eighteen *Polystomella*, no empty shells; two or three pale, but some very brown indeed in places.

30 April. Crushed three pale *Polystomella*; all dead or degenerate. The *Navicula* cultures became unsatisfactory and the *Polystomella* were transferred to cultures of other diatoms.

18 May. They were still feeding and mostly a good colour. Temperature about 13° C. (sea at Plymouth up to about 13·5° C. by the end of May 1939).

29 June. Fourteen *Polystomella* apparently still feeding, but pale or patchy in colour. Hot weather. Temperature up to 19° C. (sea at Plymouth in June 1939 about 16·5° C.).

12 July. All very pale. Left to feed in a good culture of mixed diatoms during vacation. Temperatures probably about 16–17° C. (sea temperatures recorded at Plymouth Pier July–August 1939 about 16–17° C.).

14 September. Culture now pale and patchy, though there are remains of a good growth. *Polystomella* found: six of a good colour, 1–1·5 mm.; nine very pale; two greenish, look dead. Some of the shells were preserved as dry mounts, five crushed. Contents of two pale and two greenish ones very unhealthy or dead.

These culture records show how rapidly the young megalospheric *Polystomella* may grow. Lister noted that two chambers may be formed on the first day (Notebooks, Vol. II, p. 116) and another two by the end of the second day (1903, p. 69). Actually an average rate of a new chamber almost every second day was maintained for about 3 months, by which time the young measured a millimetre or so across the shell and had some forty chambers. In June 1939 some young *Polystomella* from the sea, measuring about 400μ, formed another new chamber every other day with great regularity up to a period of 10 days in cultures of *Navicula mutica*. In July a similar experiment indicated that there was some falling off in the amount of food taken and in the rate of growth in *Polystomella* which already measured about 1 mm. Probably the average number of chambers finally attained is somewhere between forty-five and fifty; although one large megalospheric shell collected in January to keep in culture measured 1·8 mm. and was found to have contained sixty-seven chambers at the time of its collection.

No suggestion of a repeated schizogony was ever seen in my cultures.

The history of culture N 2 shows that at the end of a year some of the *Polystomella* attempted to sporulate. This seemed most surprising after so many vicissitudes in the course of their lives, and showed that it is not difficult[1] to keep these Foraminifera in cultures up to this critical stage, which as already noted will be the subject of further discussion in a later part of this series.

It is time now to refer to an unexpected imperfection in the results obtained in my cultures. In both the grown *Polystomella* taken from the sea in January 1939 and of the young reared from them at Plymouth it was found that the shells tended to become abnormally heavy, apparently owing to an extraordinary secondary deposit of shell substance which tends to fill up all the

[1] They might have done even better if they had begun life in larger vessels, for instance, or if the temperature had been more consistently controlled. It is hoped to repeat the rearing of a megalospheric family under better conditions when supplies again become freely available.

depressions, thickening the prominences and gradually obliterating the normal shell pattern. One such case is illustrated in Text-fig. 8, drawn from a sketch in a laboratory notebook in August 1939. This *Polystomella* had lived in *Navicula mutica* cultures since 17 January. It appeared as if the openings in the shell might be occluded, but several of the vertical canals opening on the umbo could still be seen and pseudopodia were put out while the sketch was being made. The same sort of disfigurement was seen in some of the young after a few months, and became worse the longer they lived in the cultures; the shells were thick from one side to the other, and did not usually reach as large a diameter as their parents, or as many wild megalospheric specimens. This general thickening is a different phenomenon from other shell abnormalities such as:

Text-fig. 8. *Polystomella* with abnormally thickened shell, after some months in culture; cf. Text-fig. 4 *a*. Some of the young reared in cultures into their second year became much worse than this.

(1) The irregular growth which often occurs in very young megalospheric offspring of schizogony, especially perhaps in cultures but not exclusively there, and which as we have seen may right itself after the formation of a few irregular chambers.

(2) The real monsters which develop when, e.g. two young grow as a twin, two initial chambers being formed.[1] Possibly such a beginning may lead to grown-up monstrous shells like those depicted by Schultze (1854, p. 30, pl. v), with more than one whorl of chambers. Other abnormal shells may also occur in the sea, e.g. with the chambers taking on a rectilinear arrangement at a certain stage (see Millett, 1904, p. 604, pl. xi) and cf. *Ozawaia* Cushman (Cushman, 1933).

(3) Shells with small irregular new chambers, such as are formed after injury to the more or less naked protoplasm at an early stage of the process of their formation.

The thickening is obviously due to some cultural condition, and at present it is not possible to account for it. Heron-Allen (1914, p. 262) relates how he got extra ridges, etc., on *Massilina* shells in cultures when he replaced the water of evaporation with hard water from his well. But thanks to Dr J. D. Robertson, of this Department, who very kindly made the necessary estimations, I can say that my culture media only contained about the average amount of calcium in sea water. Unused medium (Bearsden earth extract + Clyde sea water) contained 409 mg./l.; while after nearly 4 months

[1] Quite regular adult megalospheric specimens with more than a single nucleus occur in the sea (see also Lister, 1895, p. 425). But these have only a single initial chamber, so that if they indicate twinning it is here more complete from the outset (see also Heron-Allen, 1914, p. 249).

use by a culture of diatoms and *Polystomella*, another sample (Plymouth earth extract + Clyde sea water) contained 384 mg./l.; the salinity of the two samples being within 1 % of each other, indicated that there had been no appreciable evaporation in the used medium.

Another peculiarity of the several hundreds of young I saw in my cultures was that not one ever showed the least sign of spines on the keel at the periphery of the shell, such as are shown in three- to four-chambered young by Lister (1903, p. 69) and are commonly seen on shells of small size (under 1 mm.) from the sea usually said to be young *Polystomella* of various species (see Williamson, 1849, p. 163, on young *P. crispa* 'when obtained from deep water'; Schultze, 1854, Taf. v, on young *P. strigilata*; Brady, 1884, on young *P.? macella*, and on *P. imperatrix* at all ages). Such small spiny shells are not infrequently present in the Drake's Island collections (down to about 5 fathoms) and are very common amongst the small *Polystomella* found in shallow *Corallina* pools on the shores of the Firth of Clyde (at Millport).

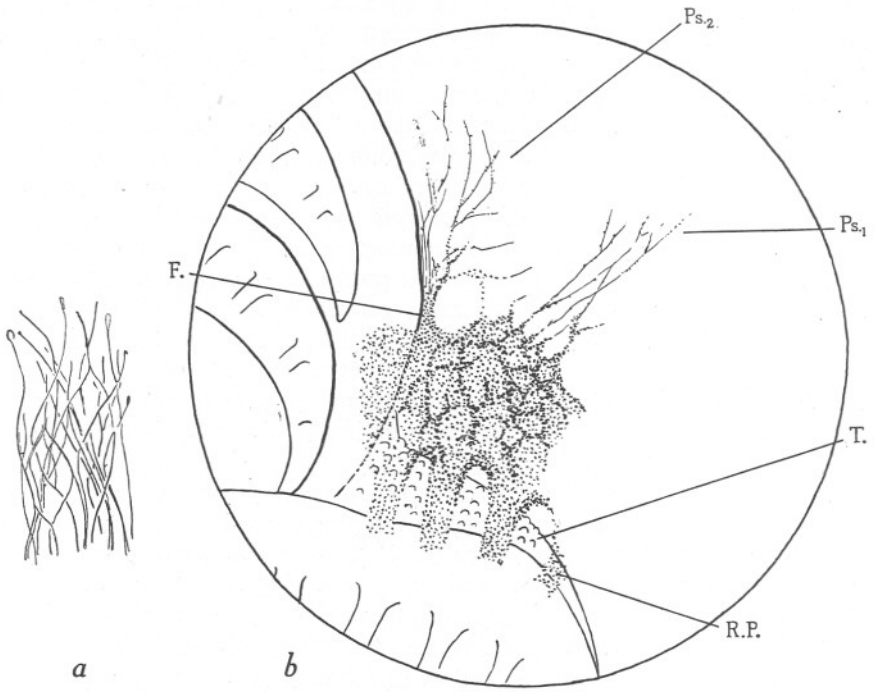
Regarding the actual emergence of the protoplasm and its subsequent fission, my observations, as far as they go, confirm the account given by Lister. As he reports the 'premonitory halo' of numerous colourless pseudopodia (1903, fig. 10, p. 67) is as a rule developed early in the day, between 6 and 10 a.m., the *Polystomella* often having crawled up the side of the culture vessel beforehand. Just as when a portion of the protoplasm emerges to form a new chamber, so here the pseudopodia are soon followed by the brown protoplasmic body, which appears to leave the shell by way of the canal openings near the last chamber[1] and probably by the terminal openings also. In some cases, as has already been mentioned, additional large openings are made in the wall of the terminal chamber as described in *Discorbina* (see Schlumberger, 1896) and *Peneroplis* (Winter, 1907, p. 23). As already noted Lister found small nuclei in his reproducing microspheric *Polystomella*; these went down to about 7μ in diameter (1903, p. 70). In my preparations there are none so small, all the nuclei being well over 10μ. In a few young of two to three chambers they measured 15–20μ, and at a dozen chambers about 30μ. They show nucleoli from the beginning; and indeed, as compared with a collection of young from the sea, a family likeness may be observed in the size, etc., of the nucleoli in a brood of young, as well as in the appearance of the chromidial mass, which may be very compact or more or less diffuse, scanty or plentiful. This is of course due to the protoplasmic structures being taken over from the parent. I am not sure whether the size of the initial chamber is more uniform within a family or not—there is evidence in my preparations of the measurements being affected by pressure of the cover-slip, and special observations would be necessary to determine this point. Retral processes appear on the second chamber of the young (see Text-fig. 1, F, A); the nucleus leaves the

[1] In his Laboratory Notebooks (Vol. II, p. 131) Lister says that during emergence of the protoplasm he found the small nuclei, measuring 7–10μ in diameter, unevenly distributed in the cytoplasm, some in the canal system.

initial chamber when there are about a dozen chambers and wanders out to take up its position usually a little behind the middle chamber of the series. The chromidial masses have entirely disappeared soon after this stage, and the nucleus now has the numerous nucleoli usually present in the megalospheric phase.

Formation of a New Chamber

Some observations were made on the formation of a new chamber. An unusually dense fan-shaped mass of closely set anastomosing pseudopodia make their appearance, radiating out from the terminal apertures of the last chamber of the shell (see Text-fig. 9*a*). Beyond them bundles of ordinary



Text-fig. 9. *a*, bundle of special pseudopodia emerging from pores of shell just above the foramina and at sides of last chamber where new chamber will lie, 12.30 a.m. Zeiss, $\frac{1}{6}$ in., 4. *b*, detail of new chamber in process of formation. Zeiss binocular, ×300; 11.0 p.m.–midnight. *F.*, cytoplasm in foramen of new chamber, i.e. terminal aperture; *Ps.*1, pseudopodia, gone at 11.30 p.m. leaving cytoplasm with clean outline, ? shell appearing. *Ps.*2, pseudopodia still lively at 11.45 p.m.; *R.P.*, cytoplasmic retral process, 11.30 p.m.; *T.*, tubercles on terminal face of last old chamber which will form part of the new last septum.

pseudopodia may reach out farther to collect any material that may be available (diatoms, excretory granules, sand, etc.) to make into a 'face-mask', inside which the new chamber will be formed. But the process can go on

without this cover, as in the case of a specially cleaned *Polystomella* under irrigation, and then the details are more clearly seen. Activity usually begins in the late afternoon or early evening, the protoplasm, which may be withdrawn at the time from the last chamber, passing out in the form of pseudopodia which flow through the successive sets of foramina, and also emerge at the fossettes and pores at the sides of the last chamber which will be partly covered by the new one. Some of the special pseudopodia after a time begin to arch over in a reticulum which outlines the cavity of the future chamber, usually about 10 p.m. to midnight, the 'face-mask', when present, being pushed away to the outside. They gradually swell at their bases and merge into one another there, whilst a fluid wells out amongst them and comes to fill up the space they enclose under their extremities with a uniformly granular mass of colourless protoplasm in which the pseudopodial streams fade out and ultimately disappear into the general circulation of the mass (see Text-fig. 9*b*). This comes to have a clear-cut surface, fashioned in the shape of the new cavity even to the retral processes and the projections at the future foramina, as shown in the figure. The longer and more active pseudopodia which normally collect the 'face-mask' material have disappeared, and the short pseudopodia remaining on the surface of the protoplasmic mass now appear stiff, with very sluggish movements. They may come and go, as the shell is deposited on the mass; it is impossible to know how much of their variation under observation is due to the unnatural illumination, etc., but in any case the shell seems to be porous from the beginning, and therefore bathed in protoplasm which lays it down initially and may continue to add to it (and at times to reabsorb it) throughout life. As soon as the surface is available a collection of shining granules may be seen there which gradually form a thin layer of shell. This seems to be laid down in patches like the pieces of a jigsaw puzzle, which unite, losing their separate outlines more or less completely as the shell thickens. After a time the characteristic tubercles are formed on the outside and the keel is laid down at the periphery. During the next day the *Polystomella* remains immobile, while the shell is deposited. There is a flow of brown protoplasm into the now penultimate chamber and sometimes into the base of the new one. Then the colourless mass there becomes vacuolated and may be withdrawn altogether, leaving the new chamber empty for a time, as is usual during the greater part of the life of a *Polystomella*. Pseudopodia emerge, the 'face-mask' is cast off, and the *Polystomella* moves away to begin feeding again some 24 hr. after the emergence of the protoplasmic mass. Although a new chamber may occasionally be formed at other times of day, this usually happens early in the night as described; and therefore it would perhaps be advisable when it is necessary to move *Polystomella* in cultures to do so late in the afternoon, say 5–6 p.m., when there is least chance of spoiling a new chamber with a shell too thin to stand being handled. Even if this is injured, however, or if an irregular chamber is formed for any reason, there may be restitution of the normal shape in the course of one or a few additions if circumstances are favourable.

Earlier observations on the development of new chambers are recorded for *Polystomella*, *Rotalia*, and *Miliola*, by Schultze (1854, p. 30); for *Peneroplis* by Winter (1907, p. 23); for *Quinqueloculina* by Hofker (1930*a*, p. 384); and for *Discorbina* by le Calvez (1938, p. 266). Excepting that I have seen nothing in my *Polystomella* to suggest that a new chamber increases in size after it is provided with a shell (as Schultze maintains), all the accounts agree so far as they coincide.

Schultze (1854) comes to the conclusion that *Polystomella strigilata* (also some Rotalidae) can live for several years, since he was able to keep them alive in captivity for a period of 9 months. From my experience this does not seem likely; I would rather agree with Winter's suggestion of senility ('Peneroplengreise', 1907, p. 19) in individuals which pass the sexual breeding season of their contemporaries without undergoing reproduction. The microspheric form usually appears as a comparatively youthful organism, even when it reproduces; but the megalospheric phase when it is grown up tends to become paler, grows much less, and is generally less active. Although a few may survive the season for sporulation it seems very doubtful that they ever again show great activity. If we regard the two generations as making up the life cycle then, it normally occupies a period of about 2 years.

Part III. Sporulation. Development and Emergence of the Flagellate Swarm Spores,[1] their Form and Behaviour, and attempts to obtain their Further Development

Development and Emergence of the Flagellate Swarm Spores

Living Material. Sporulation in *Polystomella* comprises the development and emission by the mature megalospheric stage of very numerous flagellate swarm spores.

All through the winter months it is possible to find undeveloped megalospheric and microspheric individuals down to twenty or thirty chambers or less. These may become pale and shrunken inside their shells during the lean months and many of them probably die. Several small *Polystomella*, somewhat under 1 mm. in diameter, of a good colour, were collected in January 1939 and placed in cultures of *Navicula*, where they fed well and grew new chambers. One reproduced asexually late in April, the other eight included one microspheric (which grew to 1·5 mm. across) and seven megalospheric individuals. But the majority of the megalospheric forms are fully developed by the onset of winter; they will feed and grow a few more chambers when food is available, but from January on more and more of them come to an end in sporulation. The brown colour fades away in a short space of time, a day or two, or sometimes overnight. Meanwhile especially long pseudopodia are put

[1] Known as *zoospores* or *flagellispores* (French)=*flagellospores* (German). The word *flagellula* is to be avoided. It has been criticized as an ill-coined word which does not really mean *a small flagellate* as intended, but *a small flagellum*.

out, often in a long curved bundle which may reach right across an ordinary Petri dish, and large quantities of xanthosomes are shed, so that the pseudopodia may look quite yellow. (This is not, however, a large factor in the loss of colour which is primarily due to an alteration of the chromatophores.)[1] The *Polystomella* walks away from the yellow tracks by means of ordinary colourless pseudopodia. The creature has become very sticky by secretion of a great deal of mucus (see p. 625). The pseudopodia are withdrawn, and the protoplasm in the shell takes on an almost translucent character, while larger or smaller masses of brown excretory granules remaining in the shell often become visible on the under-side—indicating presumably that fission of the cytoplasm has taken place, the heavy, coloured, xanthosomes falling down below the bodies of the developing flagellispores (cf. *Gromia*, Jepps, 1926). A *Polystomella* at this stage is a conspicuous object of a dazzling whiteness which gives it a remarkably plump appearance. The flagellispores usually emerge during the following night, mostly shortly after midnight. They may be seen in amazingly rapid motion inside the shell just before emergence if a thin place can be found and suitably illuminated under the microscope (Zeiss eyepiece no. 4, objective A; × about 100[2]). Presently they all rush out in a great cloud, or they may swim out in a smaller steady stream in the course of several hours, leaving the shell by the foramina and probably by the canal openings also. As a rule shells do not appear to be extensively damaged after sporulation, although some of the usual openings may be enlarged and the little flagellates naturally take advantage of any extra exits that may be available. The shell is left absolutely empty and clean after a good sporulation, apart from small collections of granules, mainly xanthosomes, which may remain in some of the empty chambers; such shells showing brown patches on the side which happens to lie below are recognizable in the collections from the sea for a long time afterwards.

In *Peneroplis* there is said to be a difference in the habit of the two phases; 'Wenn die Agamonten älter werden, hälten sie sich mehr am Boden...die Gamonten steigen höher, was im Reifestadium mir besonders characteristisch schien' (Winter, 1907, p. 20). Nothing of the kind has been detected in *Polystomella*, and it is not possible to say whether there is any special tendency for this foraminiferan to climb up to a higher place before sporulation. Certainly some of those which did so in culture vessels fell off when all the protoplasm retreated inside the shell, although others remained attached by the tough mucous trail of the long cleansing pseudopodia. Lister wrote in his Notebooks (1892–1905, Vol. II), in June 1895, that he had probably failed to find 'zoospores' in 1894 because he had only examined *Polystomella* climbing on the walls of his glass vessels, and these with 'zoospores' would have fallen down

[1] It is hoped to describe this more fully in a later part of these studies.

[2] A little care may be necessary to distinguish between this dance of the flagellispores and a somewhat similar commotion in the shell due to an invasion of a more or less decayed *Polystomella* by much smaller motile bacteria.

when the pseudopodia were withdrawn. Nor is it known exactly when they stop feeding. A few *Polystomella* sporulated in Petri-dish cultures of diatoms, and it was here that the earliest observations were made which gave the clue to the appearance of a sporulating individual. Four healthy brown *Polystomella* (1·3–1·5 mm. across), collected at Drake's Island on 13 January 1939, were cleaned and left in a good culture of *Navicula mutica*, the earth extract medium being replaced by sterile sea water, at a low temperature (10° C. or less). Up to 20 January, no note of any change of colour was made, but on 21 January, one was seen to be lying in the middle of the dish on 'good pasture' with 'many pseudopodia', and 'very white' in colour. Next day the pseudopodia had vanished, but the shell had a light covering of *Navicula*. By 24 January, the temperature being noted as 6° C., it was seen to be empty. On the other hand, one or two other *Polystomella*, which sporulated in this set of cultures later on, had fed and actually grown a few new chambers first.

Many of the details given above were recorded from *Polystomella* taken from the sea, cleaned, and kept in Petri dishes of sea water at various temperatures, and one or two lots kept in the laboratory under other conditions, during the sporulation season.

Although collections had been brought in from Drake's Island at short intervals throughout the earlier winter months, and sometimes kept for a day or two in Petri dishes before being used in experiments with various fixatives, no white *Polystomella* were noticed in them until 15 March. It is probable that they would not have been picked out before their significance was known, and the material was not kept before examination for so long an interval as is usually required for brown *Polystomella* to complete the process of sporulation after capture. It was, however, noted that microspheric forms seemed to be 'very scarce' in the collection of 25 January; and although about 380 *Polystomella* collected on 1 February, some of which proceeded to sporulation in the laboratory 3 days after capture, gave about 6% of microspheric specimens, a certain amount of selection had in fact been made by picking out those of a large size, so that in all probability the proportion was actually but little, if at all, increased (see p. 635). It is unlikely therefore that very much sporulation had occurred before the end of January. The sporulation season seems to fall during the coldest weeks of the year,[1] possibly reaching its height soon after the vernal rise of temperature sets in. According to Harvey (1928) the lowest winter temperatures for 'inshore water' of the English Channel were recorded (in 1924) about the middle of February, and for 'surface water' about a month later. In 1939 the temperatures posted on Plymouth Hoe from civic observations at the pier fell to their lowest (45–47° F.) during February—so possibly in this year the 'inshore water' where *Polystomella* lives, also passed through

[1] This is probably what Schaudinn meant when he said 'Mit dem Eintreten der kalten Jahreszeit merkte ich dass viele grosse *Polystomellen* eines Kulturglases nahe daran waren die Flagellosporen zu bilden, d.h. . . . hatten sogar schon die Sporenkerne gebildet' (1903, p. 500). Le Calvez (1938, p. 216) mentions that he found over 75% of the *Polystomella crispa* living on the *Lithothamnion* at Banyuls to be microspheric 'pendant les mois froids'.

its coldest phase somewhat earlier than in 1924. Miss M. F. Mare, who was engaged on planktonic observations at Plymouth in 1939, was kind enough to inform me that the spring diatom increase went steadily forward from about 14 February. Sporulation thus began shortly before the diatom increase was detected, when the *Polystomella* were at the end of their season of scarce food. When it was first observed the hours of daylight had been increasing for a few weeks since the shortest day of winter.

When it was discovered that a definite season of sporulation was going on, it was decided to seize the opportunity of making a serious attempt to carry the life cycle through this phase, which it was recognized would probably prove one of considerable difficulty if *Polystomella* were found to fall into line with other organisms previously investigated, such as *Gromia oviformis*, for example (Jepps, 1926). The following observations on various factors which might affect the formation of the flagellispores are therefore to be regarded as incidental to the chief object of investigation at the time; and the conclusions on the whole tentative, since so many of the experiments were on a small scale and often not repeated.

When sporulation was first observed in the collected material, the catches were still being brought into the laboratory and washed with filtered outside sea water (see p. 620) which had been standing there, so that the *Polystomella* were warmed up say from about 8° C. in the sea to laboratory temperatures, varying roughly about 13–15° C. It is possible that this stimulated some of them to sporulation, for it occurred in five catches from 25 January to 8 March in a fair proportion (up to 20 %) of the megalospheric *Polystomella*, in each case 3–4 days after capture. In one outstanding experiment 'about 100' brown *Polystomella* were washed well in the laboratory, and then placed in a glass basin containing a substratum of sand and a growth of diatoms, etc., accumulated during several weeks in the sea-water circulation of the aquarium, the temperature remaining between 10 and 11° C. After 3 days some of them were seen to have active flagellispores in their shells, and after 8 days it was found that ninety-seven out of 125 individuals had sporulated, leaving empty shells behind. Of the remainder two seemed dead, one was microspheric and twenty-five were megalospheric. This is the highest proportion of sporulating individuals out of a brown sample that ever occurred in my experiments. I do not think this result is due to the food provided in the substratum; in any case no such increase was observed when *Polystomella* had a few days earlier been placed in a Petri-dish culture of *Navicula* kept in the laboratory; nor to the lower temperature of the circulating water, since a series of samples of the same catch in Petri dishes and in another basin of similar dimensions to the above gave no more than 20 % of sporulation in a total of some 350 *Polystomella* kept at various temperatures between 10 and 15° C. after their washing at laboratory temperature. It may be provisionally attributed to the fact that the water was in circulation in this basin, as it was not in the second basin referred to above, which stood alongside the first and contained similar water.

It would be worth testing this suggestion when the opportunity again arises. Føyn (1936*b*, p. 17) put his *Discorbina* 'unter fliessendes Seewasser zur Schwärmerbildung' with success. It should be noted, however, that a previous lot of *Polystomella* collected on 1 February and treated, so far as is known, in the same way had failed altogether to sporulate in the same basin in a week. The only possible difference seems to be that they might by some chance have missed the stimulus (if it exists) of an appreciable rise of temperature beforehand. A small batch of one dozen megalospheric individuals had given two in sporulation in this basin 3 days after their capture on 25 January, so that this difference is not so likely to be a question of the advancing season.

After 8 March, with a view to avoiding unnatural conditions so far as possible, care was taken not to subject the *Polystomella* to a rise of temperature before sporulation. By getting them early in the day, and washing them in a more or less open antechamber to the constant temperature rooms it was possible to maintain their environment at about 10° C. On 15 March white *Polystomella* were for the first time found in the catch. Of seventeen such individuals thirteen sporulated during the following night, only one or two having a few flagellispores left in their shells by 10 o'clock next morning. The remaining four went translucent, but were fixed without sporulating on 21 March, the filtered outside water having been changed almost daily; only one was found to be microspheric. Of the brown *Polystomella*:

60–70 kept in a Petri dish at 10° C. Gave 3 sporulating during the night 16/17 March.
1 sporulating during the night 17/18 March.

60–70 kept in a Petri dish at 13° C. Gave 1 sporulated during 26 hr. from 21 to 22 March.[1]

(These two lots were in filtered outside water changed once a day.)

100 kept in a basin at 11° C. Gave 1 sporulating during the night 16/17 March.

(This lot was in circulation water. The *Polystomella* which sporulated had gone white by the morning of 16 March.)

It is suggested that the falling off in numbers of sporulating *Polystomella* as compared with the last catch might be due to the omission of the warming up stimulus; as also perhaps their more scattered distribution in time, since on this supposition only those underwent sporulation which would have done so if left in the sea.

By the date of the next collection, 21 March, the season was more advanced and greater numbers sporulated in spite of this omission. It was estimated that about 10 % of the living *Polystomella* in this catch were already white when brought in. The results of experiments with this collection are shown in Table II. The catch was divided into two portions, one of which was placed at approximately each of the temperatures shown in column 1, in filtered outside sea water. After washing the *Polystomella*, the water of those marked + in column 3 was changed daily. The last column shows the total number which had sporulated by 26 March or, in some of the experiments, by 27 March.

[1] Between 9.30 a.m. on 21 March 1939 and 11.30 a.m. on 22 March 1939.

These experiments suggest that once *Polystomella* has reached the 'translucent' stage the process of sporulation is not as a rule disturbed by fishing it out of the sea, even when it is placed in very much warmer surroundings. It is shown that 'white' *Polystomella* which have not reached so advanced a stage may not sporulate any sooner than those which are still 'brown' when caught. Of these it seems that a considerably smaller number may go on to sporulation

TABLE II

° C.	*Polystomella* brought in at 4.15 p.m. 21 March	Water changed	Result	Total sporulated
10	27 'white': 16 'translucent'	0	All finished sporulation before noon on 22 March	16/16
	11 'cream'	0	1 sporulating at noon on 22 March 2 sporulated 22–23 March 1 sporulated at 8.45 p.m. on 24 March 3 sporulated 24–25 March	7/11
	200 'brown': 100	+	Over 50 sporulated during the night 24–25 March, two by 9.30 p.m.	50/100
	100	0	38 sporulated during the night 24–25 March, none by 8.45 p.m.	38/100
14–20	22 'white': 12 'translucent'	0	9 sporulating at 2.30 a.m. on 22 March 2 more early on 22 March	11/12
	10 'cream'	0	3 sporulated 24–25 March	3/10
	200 'brown': 100	+	3 sporulating by 10 p.m. on 24 March	3/100
	100	0	3 sporulated during the night 24–25 March, none by 10 p.m.	3/100

if they are kept at the higher temperatures all the time. Something of the kind was hinted at in the few comparable sets of *Polystomella* in the earlier series which were given a warm up on catching and then kept at various temperatures. The small numbers placed in *Navicula* or *Synura* cultures in Petri dishes earlier in the year (see pp. 620 and 636) indicated the same thing. These were washed in the laboratory at room temperature, and then some were placed in a constant temperature room which was cooler. Out of twenty-five known megalospheric forms kept in the laboratory, only two attempted to sporulate; whereas out of twenty-one kept between 10 and 13° C. nine made the attempt. It is interesting to note that the dates of these attempts were spread over the months as shown in Table III, *L* representing cases occurring in the laboratory, and *C* those in the low-temperature room.

TABLE III

	January	February	March	April	May
	C	*C*	*C*	*L*	*C*
	—	*C*	*C*	*L*	—
	—	—	*C*	*C*	—
	—	—	*C*	—	—
Totals	1	2	4	3	1

The *Polystomella* in any one culture showed no tendency to sporulate simultaneously, and whether the sea water was changed each day or not seemed to make no difference to the number undergoing sporulation.

Table II illustrates the fact that the emission of flagellispores by *Polystomella* collected while still brown most often takes place some $3\frac{1}{2}$ days after their capture, whether they are subsequently kept at laboratory temperatures or in a cold room. Comparatively few individuals sporulated earlier or later. This was found to be true for most of the catches,[1] though for some unknown reason in one or two lots the process went rather faster or a little more slowly. The same thing was found to hold for the few observations it was possible to make at Plymouth in 1940 with *Polystomella* from the 'White Patch' ground. Lister (1895, p. 426) refers to an instance of sporulation 4 days after capture, giving some description of the process. The relative constancy of this interval and the fact that such a large proportion of the catch may sporulate almost simultaneously seem to indicate that something involved in the catching and subsequent treatment of the *Polystomella* has an effect on sporulation in individuals which have reached a certain stage of ripeness,[2] a phenomenon which is probably not peculiar here. Also after the initiation of the process it was found that the disturbance occasioned by examination at an advanced stage might precipitate the emission of the flagellispores. On 19 April a 'cream' *Polystomella* was taken from the catch and isolated in a small Petri dish of earth extract medium at a temperature of about 12° C. At 5.30 p.m. it was still 'cream', and through a rather thin shell it was plainly seen that the protoplasm was undivided. At 9.0 p.m. it was unchanged, and it was then noted that two small rounded masses of protoplasm which had oozed out near the keel of the shell still showed a few brown chromatophores near their centres. There were a few short pseudopodia out. At 11.0 p.m. the *Polystomella* was again put under the microscope. The extruded masses were at once seen to have lost their clear-cut outlines, and their surfaces to be heaving rapidly about as they divided up into smaller masses of irregular shape and then into little spheres with shining inclusions. No brown chromatophores were any longer visible. Flagella broke out before the small spheres (roughly 4μ in diameter) finally became separate. The protoplasm inside the shell was following suit, beginning at the damaged place, and by 2 a.m. the emergence of the flagellispores was in full swing. It has been repeatedly observed that great crowds of these suddenly came out when *Polystomella* which were due to sporulate were put under the microscope for examination, especially during the critical hours about midnight and soon after.[3]

[1] Apart from specimens which become established in cultures.

[2] E.g. it either retards or expedites the process.

[3] A similar experience was had with a large spherical *Gromia oviformis*, about 2 mm. in diameter, collected at Millport on 24 November 1939, and placed in a diatom culture at Glasgow next day. On 4 December there was a whitish film over the brown shell contents; and at about 5 p.m. some protoplasmic masses which had been extruded were divided up to form the uniflagellate swarm spores of this species. There was not much change by 8.0 p.m.

It was noticed quite early in this investigation that a large proportion of the shells were emptied say between 9.0 p.m. and 9 a.m., whether they had been collected before or after the brown colour of their contents had disappeared; so, in order to have a good supply of flagellispores, sporulating *Polystomella* were examined at intervals throughout the night, usually being left in darkness between the examinations. It was thus discovered that emergence of the flagellispores began in the majority at about 2 a.m., often continuing until daybreak, although a few individuals might be active an hour or two before midnight, and a few would not begin until later in the morning of the next day. The only variation in treatment which might possibly have advanced sporulation in my experiments was: (1) changing the sea water earlier in the day, or (2) placing the *Polystomella* in a diatom culture.

Both procedures might have something of the effect of a circulation of the medium, which as we have seen may perhaps facilitate sporulation. Le Calvez (1938, p. 205), after observing that the emission of flagellispores in *Iridia lucida* 'se produit à une heure avancée de la nuit', adds 'J'ai constaté cette sortie nocturne chez presque tous les Foraminifères'. Is the nocturnal habit an indication that darkness itself may also favour the emission of flagellispores? Føyn (1936*b*, p. 17) placed his *Discorbina* 'ins Dunkel' when he desired them to sporulate. On the other hand, flagellispores are quite often seen to be active and emergence to take place during the hours of daylight in the laboratory.

One or two collections taken later than 21 March in 1939 gave no results of special interest here excepting perhaps that of 26 April, which illustrates how the two forms of reproduction are going on simultaneously at this season. The temperature of the sea was recorded as about 10·5° C.; the *Polystomella* were washed at about 12° C. and left at 10° C. Microspheric forms were about as common as megalospheric. On 27 April there were sorted out twelve dark brown individuals showing the microspheric 'figure' (see p. 634) and seventeen paler brown ones. These were placed in well grown diatom cultures, and gave the results shown in Table IV.

TABLE IV

Dark brown 'Microspheric' *Polystomella*		Mid-brown *Polystomella*	Pale brown *Polystomella*
6 kept at 10° C.	6 kept at 13° C.	10 kept at 13° C.	7 kept at 13° C.
Schizogony in 5 by 22 May	Schizogony in 4 by 22 May	Schizogony in 1 by 5 May	Schizogony in 0 by 5 May
1 megalospheric	1 sporulated on 6 May	6–8 sporulated by 5 May	6 sporulated by 5 May
		1 megalospheric	1 megalospheric

One *Polystomella* was observed to sporulate as late as 28 June, but most of the megalospheric individuals surviving so long showed little activity.

until the *Gromia* was pushed with a dissecting needle, when it suddenly became quite white, and then appeared to start 'steaming'—the 'steam' gradually disappearing in crowds of dispersing flagellispores. The steaming continued at 1.0 a.m. By 9.30 next morning the shell was empty except for the mass of stercomata and xanthosomes left behind by the departed protoplasm, and the Petri dish was swarming with the still active flagellispores.

Lister seems to have made his observations on schizogony in smaller *Polystomella* than those I studied at Plymouth (see p. 634), the smaller sizes given by him for the nuclei in the megalospheric young (see p. 641) being perhaps referable to this fact. The figures he gives for the megalospheric form indicate that they all comprised fewer chambers than my large mature specimens. The highest number of chambers mentioned by Lister is forty-one (1895, table on p. 423, and cf. p. 639 of this paper), and the average number of chambers in twenty-seven reproducing individuals collected March–May 1895 is 30·5 (Notebooks, Vol. 1); whilst here and elsewhere (1895, p. 426) he refers to specimens of seventeen and eighteen chambers respectively which were in course of forming flagellate spores. I also obtained sporulation (but not schizogony) in small specimens, down to about 25–30[1] chambers at any rate. They were noted in the Drake's Island material in March and April 1939; and were much more common in the same months the following year in the collections from 'White Patch'.

I can only suggest that these *Polystomella* happened to come from less favourable grounds than the fine large specimens I have been fortunate enough to be supplied with at Plymouth since 1938. The frequent presence of spines on Lister's small specimens,[2] and the shape of some of his figures are reminiscent of the small *Polystomella* occurring at Millport (see footnote to p. 620) for example, which are probably ill-nourished and subjected to great and disturbing variations in their shallow habitat. An interesting possibility is that the small sporulating individuals may have been produced late in the season of the previous year and so may have missed the time of plentiful food—reproducing nevertheless at about the same date as their better fed and better grown elders of the same generation.

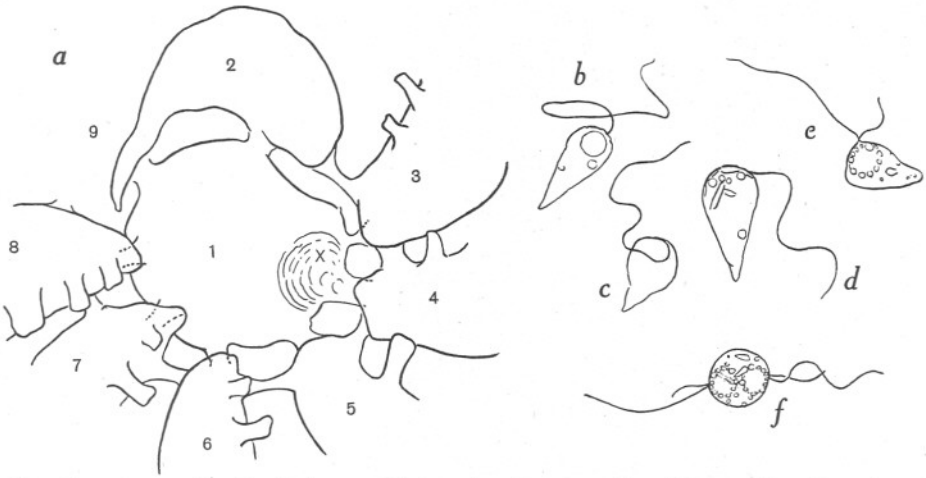
Fixed Material. The first sign of approaching sporulation seen in fixed material is the opening up of extra channels of communication between the chambers, as indicated by Lister (1895, p. 425 and pl. 8, fig. 32*a*; also 1903, p. 71). This condition was found from January onwards in specimens fixed after 24 hr. in the laboratory—which might have brought on the process as suggested on p. 647. It happened that no *Polystomella* was fixed the same day as it was collected until late in March 1939, and by that time freshly caught brown specimens after decalcification showed cytoplasmic bridges growing in from the innermost whorl of chambers to make a connexion with the large central initial chamber (see Text-fig. 10*a*). The same thing was seen in *Polystomella* freshly caught at the 'White Patch' ground at the end of February 1940, when the whole population looked rather pale and starved. In some of these modified specimens the large nucleus has disappeared, and I can see no sign of any nucleus at all, either in whole mounts or in sections.

It is instructive to compare two lots of *Polystomella* collected towards the end of March 1939, i.e. probably at about the height of the sporulation season.

[1] Estimated from the number of chambers exposed in the outermost whorl.

[2] Also seen on a certain number of the smaller specimens from Drake's Island (see p. 641.)

A sample of the catch on 24 March was cleaned and left in a small basin of filtered outside sea water at 9–10° C. Some representative specimens were taken out and fixed each day in Zenker at 40° C. Some of the catch of 30 March, on the other hand, was carefully sorted, at similar temperatures, into five categories by colour, viz. 'dark brown', 'mid-brown', 'pale brown', 'golden', and 'pale cream', which were fixed separately in Zenker as before. From whole mounts and sections of this material a rough time-table of the stages in sporulation may be constructed which agrees quite well with other more casual and fragmentary observations, and was confirmed also by a parallel set of samples from the collection of 30 March, which was allowed to



Text-fig. 10. *a*, sketch of the central cytoplasmic parts of a *Polystomella* collected on 13 January, and fixed 14 January. Zenker at 40° C. Boraxcarmine. Whole mount. 1–8, first eight chambers; 9, position of chamber 9. At *X* is an inrush of cytoplasm, due to fixation, from chamber 4 to the initial chamber (1) through one of the median secondary openings heralding sporulation. *b*, *c*, *d*, sketches illustrating living flagellispores of *P. crispa*. Only one flagellum was seen during life. *e*, the same after exposure to osmic vapour; both flagella are now visible, but there has been some shortening of the body. *f*, a motile sphere, also after osmic vapour, showing a stage in fission.

sporulate in Petri dishes. It is of course not to be expected that every sporulating *Polystomella* will adhere rigidly to any time-table.

It is not known how long before flagellispore formation one may find the opening up of the extra channels through the shell—but they appear to increase very fast during the last 48 hr. Not only does the initial chamber acquire median bridges to all the adjacent chambers at their inner ends, but these chambers come to communicate much more freely with one another, involving the canal system, which broadens out, forming wide sinuses in places, as may be clearly shown up when the system is full of xanthosomes on their way out of the shell: the foraminal openings also may become greatly enlarged. A *Polystomella* captured brown at this time of year may begin to

look pale on the second day after capture, the brown colour may all be gone by the morning of the third day, and the special extrusion of xanthosomes over. There is often a distinctly yellowish phase, 'golden' in my series, after which the shell contents come to look quite white and ultimately translucent in the course of the third day, immediately before the emergence of the swarm spores during the following night. The large nucleus of the parent megalospheric individual disappears as a rule during the time the brown colour is going. In my preparations it is still present in three out of every four individuals at the 'golden' stage, but in hardly any which were classified as 'white' or 'cream'. It might be mentioned here that the old nucleus seems to be slightly enlarged and rather empty of nucleoli towards the end of its existence; but I have seen no stages in its dissolution. There is apparently an interval during which I can find no nucleus; and then small nuclei, only a few μ across, make their appearance, scattered evenly through the cytoplasm, seeming to increase in numbers until the whole animal is stuffed full of them. One has the impression that they become a little smaller as they increase in numbers as if they were multiplied by some kind of division. Finally, as already described, the cytoplasm undergoes a multiple fission. In sections it begins to look like a fine reticulum with knots in its meshes enclosing one or several of the small nuclei surrounded by darkly staining granules and eosinophil bodies; while, in between, are intensely staining strands which in places form the walls of vacuoles. At these knots rounded bodies gradually concentrate, each of which seems ultimately to undergo two rapid divisions to form the individual flagellispores immediately before they emerge from the shell.

Lister (1895, p. 425) gives an account of his observations on sporulation in *P. crispa*, with illustrations of various stages in pl. 8. I have confirmed the main facts, but I cannot agree with his interpretation of some of the details. The latter are extremely difficult to understand in such a large mass of protoplasm full of granules and other inclusions of various kinds. Fixation also seems unusually imperfect at this stage, owing perhaps to the great amount of mucus which is present. My clearest preparations showing small nuclei were made from *Polystomella* fixed in osmic vapour for 10–15 min., followed by hot corrosive acetic as described on p. 616. I have nothing at present to say about the mode of disappearance of the large nucleus, nor concerning the origin of the small ones. Though I confess I have sometimes not been certain of the presence or absence of the latter, I have never suspected their presence as long as the large nucleus could be seen. They show up at later stages as red or blue spots after picrocarmine or Ehrlich's haematoxylin respectively.

Form and Behaviour of the Swarm Spores

The ultimate divisions giving rise to the flagellispores take place inside the shell as a rule. There is the usual kind of variation in the details of the successive fissions—a coarsely reticular appearance of the cytoplasm having been noted in some cases at a rather late stage; in others, spheres of different

dimensions separate off and divide up afterwards to the small swarm spores. These are usually fully formed and very active when they leave the shell, although after emergence figures showing that the last two fissions have not been completed are not infrequently seen (see Text-fig. 10). Pairs of flagellispores are thus not uncommon.

When swimming freely the swarm spores are more or less carrot-shaped bodies, about 6–8 μ long (see Text-fig. 10 *b–d*), proceeding with the blunt end foremost and swinging round in a wide spiral as each little flagellate rotates on its axis under the influence of the rather thick longer flagellum which appears to precede it, but is rarely seen in action owing to its rapid motion of considerable amplitude. This flagellum reaches a length about three times that of the body or even more, and the shorter second flagellum which is invisible during active progression is about body length. (See osmic preparations in Text-fig. 10 *e,f*). One gets the impression that it may be carried relatively straight ahead. The whole movement seems well calculated for the sweeping of as large an area as possible by the longer flagellum; as also is the way in which the swarm spores spread out evenly through all the available water, even in the larger vessels, showing no orientation with regard to light, gravity, etc. They swim actively, with very occasional short rests on the bottom, for some hours, say 8–10 under ordinary conditions in the laboratory,[1] and then settle down on the bottom, permanently rounded up in the form of little spheres measuring 4–5 μ across. These gradually fade away, losing their flagella, though their ghosts may be seen for some days, picked out by the shining inclusions which may remain in them. Of these inclusions two kinds have been distinguished: refractile angular bodies of a somewhat crystalline appearance, insoluble in hydrochloric acid and in sodium hydroxide solution, of which half a dozen may be present (Pl. V, fig. 2); and secondly, a small number of less shining discoid bodies which appear to lie flat under the pellicle near the anterior end of the body, soluble in sodium hydroxide, staining blue with Nile blue hydrochloride, and perhaps with methyl green and acetic acid. In osmic acid, although nothing darkens conspicuously, the flagella are well demonstrated. Iodine solutions are useless for showing up the structure.

The swarm spores may go on swimming for half an hour or so under the microscope, especially if uncovered and not too brightly illuminated or overheated. But sooner or later they begin to shorten, and finally round up and become ghostly as described above. In fixed and stained preparations there is a strong tendency to assume the spherical shape, possibly owing to swelling. Figures of selected specimens are given in Pl. V, figs. 2 and 3. The vacuoles, which in some preparations contain eosinophil bodies of various sizes, seem to arise as the swelling comes on.

[1] But see Exp. 16 (p. 660), when on 31 March 1940 some were still swimming in apparently good form after about 50 hr. in earth extract medium, at 8–10° C., in the dark excepting for three short intervals when they were under microscopic observation.

J. J. Lister (Notebooks, Vol. II, p. 10, dated 4 June 1893[1]) describes a *Polystomella* he had collected the previous day on the shore at Highcliffe (Hants), and which when he crushed it yielded 'zoospores', 'yellow masses' (=excretory granules) and 'algae in pairs' (=foraminal plugs). He did not study the zoospores in detail, stating (1906, p. 8) that he had not even counted the flagella. It seems, however, from other passages in his Notebooks that he believed there were two, and that they were perhaps unequal (see also 1895, p. 426, pl. 8). Other references to these swarm spores in the literature are made first by Schaudinn as already noticed (see pp. 610–646 and Text-fig. 1 D, which is either very diagrammatic or shows a rounded-up form); secondly by Kofoid (1934), who gives additional support to the hypothesis that they are flagellate parasites because Myers (1935*a*) had shown the products of sporulation in another foraminiferan (*Patellina*) to be amoebulae; and thirdly by le Calvez (1938, p. 300), who says in table I that they measure $1{\cdot}8 \times 3\mu$, and bear two flagella respectively 12 and 4μ in length. This last reference is difficult to explain.

Attempts to Trace the further Development of the Swarm Spores

Apart from the casual observation of swarms of the flagellate spores coming from a single *Polystomella* or from two or more individuals, some half-dozen special sets of observations were recorded when the swarm spores were watched under a Greenough binocular microscope for considerable intervals of time immediately after their liberation or a few hours later. Samples of the flagellispores from single individuals were studied swimming freely in watch glasses, and mixtures were made from these in various ways; in Petri dishes also mixed swarm spores from two to four *Polystomella* were watched, at laboratory temperatures or down to 10° C., in filtered outside water or in earth extract medium. In one case the dishes were kept in the dark when not under observation. In no instance was any sign of fusion detected, either amongst these still swimming freely in the water or amongst those which had settled down on the bottom of the dish.

Single flagellispores were seen to tumble about one spot for a while now and then; and sometimes two would tumble about together, thus appearing to be playing around one another, but they always parted company in the end. Finally, as already noted, the swarm spores ceased to move and degenerated, without ever showing the least tendency to become amoeboid.

It might be said that under microscopic observation, even with a Greenough binocular, conditions might be against the performance of syngamy. The series of experiments now to be described, where the swarm spores were not subjected to examination until some time had elapsed, afford no reason to assume that it commonly occurred here either.

[1] It is not possible to verify my references to Lister's Notebooks at the present time of disturbance as they are stored in a place of comparative safety. The references given are taken from my own notes made at the British Museum some years ago.

SERIES I

This series (Exps. 1–8) was set up with *Polystomella crispa* (P.),[1] from the Drake's Island ground. They were brought into the laboratory and washed with filtered outside sea water (F.O.S.W.) at laboratory temperatures (about 13° C.). Unless otherwise stated they were kept in about 25 c.c. F.O.S.W. in Petri dishes (P-dishes). 'Big basins' measured 9–10 × 4–5 in. and held about 2 l. of medium. Cover-slips (with or without a culture of *Navicula mutica* var. (N.) growing on them) were suspended in the latter on sewing cotton tied to a cross-thread by a fisherman's bend so that they could easily be removed for microscopic examination and replaced: or they were floated on the surface of the medium or placed on the bottom of the dish, sometimes before a culture of N. was grown therein. Earth extract medium (E.) or sterile sea water (S.S.W.) prepared as described in footnote 2 on p. 660 were also used. N. cultures were always grown in E., in P-dishes or in big basins. Sometimes P-dish cultures were placed, uncovered, in big basins of sea water, thus providing a variety of more or less well-stocked feeding grounds.

Experiment 1. Set up during the morning of 4 February 1939. Out of about 100 P. collected 1 February, three shells were found empty, active swarm spores in the dish.[2] Sea water not changed since 2 February. Other P. were removed. On 5 February added 1–2 c.c. of S.S.W. with swarm spores from Exp. 2. Sea water changed[3] for F.O.S.W. at 4.15 p.m. on 4 February, and renewed 5 and 7 February. Temp. about 13° C. Subsequent examinations 7 February (ghosts of flagellispores about 5 μ in diameter seen)[4] and 12 February.

Experiment 2. Set up 11.0 a.m. on 5 February. Two more P. from same lot looked white; washed and left in S.S.W. on 4 February. One showed active swarm spores at 3.30 p.m., both empty next morning. S.S.W. replaced by F.O.S.W., changed 7 February. Temp. about 13° C. Subsequent examinations 7, 12 February.

Experiment 3. Began at 11.30 a.m. on 4 March 1939. In dish of 30 P. collected 28 February and 1 March two found empty. Flagellispores pipetted into N. culture in P-dish at 11.30 a.m., 4.0 p.m., 9.30 p.m., and 5 March at 9.45 a.m. Temp. about 13° C. Subsequent examinations 7, 10, 18 March.

Experiment 4. Set up at 9.45 a.m. on 5 March, from same dish, in which about a dozen P. have now sporulated. Some of the deposit and ten empty shells pipetted into big basin F.O.S.W. with P-dish culture N., and with cover-slips lying on the bottom. On 12 March added 4 P. containing active flagellispores from dish of fifty collected 8 March, two at 2.0 a.m. and two at 4.40 a.m. Cover-slips suspended in basin at 10.0 a.m. Temp. 13° C. throughout. Subsequent examinations 12, 15, 17, 18 March.

Experiment 5. Set up 2.0–5.30 a.m. on 12 March 1939, with 75 P. collected 8 March, and kept at 10° C. in sea water renewed 10 and 11 March 1939. Fourteen P. white and removed to S.S.W. at 2.0 a.m. on 12 March. About ten of these with active swarm spores later transferred to big basin F.O.S.W., with P-dish culture N. and suspended cover-slips. Temp. 10° C. Subsequent examinations 12, 15, 17 March, 10 April.

Experiment 6. Set up at 2.30 a.m. on 12 March 1939, with 85 P. from same collection, kept at 13° C., otherwise treated as in Exp. 5. At 2.30 a.m. on 12 March six with flagellispores were transferred to similar basin. Temp. 13° C. Subsequent examinations 12, 15, 17 March, 17 April.

[1] See p. 613.

[2] Presumably emerged during the preceding night.

[3] By carefully pouring or pipetting it off, causing as little disturbance as possible to remaining shells, or to flagellispores rounded up on the bottom.

[4] This will not again be mentioned; it is an indication that fairly high magnifications were used in making examinations of material from all the available surfaces in the cultures.

Experiment 7. Dated 10.30 a.m. on 9 March 1939, when 'about 100 P.' from same collection were placed in basin A under the laboratory sea-water circulation system, with good growth of diatoms, etc. (see p. 647). At 4.0 a.m. on 12 March a sample of 4 P. was inspected and all had active swarm spores in shells. On 16 March ninety-seven shells out of 125 were found to be empty. Temp. after first washing 8–11° C. Subsequent examinations 15 March, 23 April.

Experiment 8. At the same time another '100 P.' were placed in basin B of filtered circulation sea water, with N. cultures in P-dishes on bottom. On 12 March 1939 there were seventeen empty shells out of 112. Temp. as in Exp. 7. Subsequent examinations 15, 17, 18 March.

SERIES II

In this series (Exps. 9–15) the *Polystomella* were not warmed before sporulation excepting where stated. Collected from Drake's Island ground.

Experiment 9. Set up at 2.0 a.m. on 22 March 1939.

(1) Three 'translucent' P. from dish of twenty-two white P., collected 21 March, washed at about 20° C., and kept at 14° C., were transferred to P-dish culture N. at 2.0 a.m., with active flagellispores emerging. Shells almost empty at 4.0 a.m. Temp. 14° C. Subsequent examinations 11.0 a.m. on 22 March, 9 April.

(2) Five similar P. kept separately in watch glasses A–E of F.O.S.W., in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m. (no movement now) on 22 March and on 23 March.

(3) Mixed swarm spores from watch glasses A+B+C+D in P-dish culture N. Examined 11.0 a.m. on 22 March, 9 April.

(4) Mixed swarm spores from watch glasses A+B, B+D, A+D, in three separate watch-glasses in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m., on 22 March.

Experiment 10. Set up at 3.30 a.m. on 22 March 1939. Three translucent P. from stock dish of twenty-seven white P. similar to last, but kept throughout at 10° C., with active flagellispores in shells, put into P-dish culture N. At 4.0 a.m. added some flagellispores from eight more P. which had become active in stock dish. Examined 11.0 a.m. on 22 March, 9 April.

Experiment 11. Set up at 8.30 p.m. on 27 March 1939. Out of 'about 100 P.' collected 24 March 1939, and kept at 9–10° C. in small basin, four emitting flagellispores were placed in 400 c.c. F.O.S.W. in flask in which there was a little brown algal growth.

(1) Some of this suspension was kept in P-dish F.O.S.W. at 9–10° C. Examined 11.0 p.m. on 27 March and on 28, 29, 31 March, 9 April.

(2) At 10.15 p.m. on 27 March 1939 flask emptied into big basin with two P-dish cultures N., and suspended cover-slips. Kept at 9–10° C. Subsequent examinations 28, 29, 31 March, 10, 16 April. Temp. rose to 20° C., one day[1] and fell again to 11–13° C. From 22 April kept at 13° C. See Exp. 15 for further examinations.

Experiment 12. Dated 9.50 a.m. on 1 April 1939. Two P. out of dish of three pale brown P., collected 30 March, emptied overnight. Temp. 9–10° C. throughout. Subsequent examinations 3, 4 April.

Experiment 13. Set up in the morning of 3 April 1939. One P. sporulated overnight in each of two dishes containing three and thirteen mid-brown P. respectively, collected 30 March. Swarm spores were mixed:

[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

(1) '1 volume' from each, in each of four watch-glasses in damp chamber. Temp. 9–10° C. Examined 1.0 p.m. on 3 April and on 4 April.

(2) '2 volumes' from each, into P-dish culture N. Kept at 9–10° C. Examined 3.30 p.m. on 3 April (but very small organisms would hardly have been distinguished in the body of the culture), 9, 16 April. Temp. rose to 20° C.[1]

(3) '2 volumes' from each, in P-dish F.O.S.W. Kept at 9–10° C. Examined 1.0 p.m. on 3 March and on 4, 5, 9 April.

(4) '2 volumes' from each, to each other in original dishes. Kept at 9–10° C., behind a screen to shade from direct light from window. Examined 1.0 p.m., 8.30 p.m. on 3 April and on 4, 5, 9 April.

Cover-slips were floated on top of medium in P-dishes (2), (3), (4).

Experiment 14. Set up at 2.10 a.m. on 20 April 1939. Pale P., collected 19 April, kept in separate P-dishes E., under black paper, at 12–14° C.[1] Three had swarm spores emerging at 2.10 a.m. on 20 April, viz. dishes A, B, and 2. Examined these dishes 5.0 a.m., 11.0 a.m., 9.30 p.m., on 20 April and on 21 April.

(1) The three shells and some emerged swarm spores from A+B+2 pipetted into P-dish E., and then to big basin E. with P-dish culture of N., suspended and floating cover-slips. Kept about 12° C. Examined 21 April, 6 May (E. replaced with F.O.S.W. on 31 May, and basin used as pasture for freshly caught P.), 24 June.

(2) Swarm spores from A+B+2 pipetted into P-dish E, kept under black paper, at about 12·5° C. Examined 5.0 a.m., 6.0 a.m., 11.0 a.m., 6.0 p.m. on 20 April and on 21, 22, 23, 24 (changed E.), 26 April.

(3) Swarm spores from A+B+2 pipetted into P-dish culture N. in E. Temp. about 12·5° C. Examined 6.0 p.m. on 20 April (obscured by *Bodo*), and on 30 April.

(4) Mixed swarm spores from A+2, B+2, A+B, respectively on slides at 2.50 a.m. Temp. 12·5° C. Examined 3.0 a.m., 5.0 a.m., on 20 April (flagellispores rounded up and motionless).

(5) Mixed swarm spores at 11.0 a.m. from A+B+2 in a fresh P-dish E. Temp. 12·5° C. Examined 6.0 p.m. on 20 April and on 21, 22 (changed E.), 23, 24 (changed E.), 26 April.

(6) Mixed swarm spores at 11.0 a.m. from A+B, A+2, B+2, A+B+2, respectively in four separate watch glasses in damp chamber. Temp. 12–14° C. Examined 9.0 p.m. on 20 April and on 21 April.

Experiment 15. Dated 9.30 a.m. on 28 April 1939. Out of seventeen rather pale P., collected 26 April, and put into big basin used in Exp. 11, after removing four empty shells, on 27 April, four were found empty.[2] Temp. 13° C. Subsequent examinations 6, 22 May.

SERIES III

In this series (Exps. 16–18) the *Polystomella* were collected at the 'White Patch' ground.

Experiment 16. Set up at 6.0 a.m. on 29 March 1940. Room darkened 28 March to 12.30 p.m. on 30 March. From seventeen white P., of various sizes, collected 28 March, washed F.O.S.W. at 5·0° C., kept in two P-dishes E. (containing 4 and 13 P. respectively) at 8–10° C., flagellispores began to emerge at 1.30 a.m. on 29 March.

(1) At 6.0 a.m. 13 P., some from each P-dish, with swarm spores, put into N. culture grown in big basin, cover-slips suspended, kept at 9·0° C. Examined 1.0 p.m., on 29 March (seven shells quite empty), and on 31 March, 4 April. And see Exp. 17.

[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

[2] Presumably emptied overnight.

(2) P-dish of E. which had contained thirteen white P. and had many flagellispores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March (a few carrot-shaped flagellispores still swimming),[1] 4, 10 April.

(3) P-dish which had contained four white P. and had few swarm spores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March.

Experiment 17. Set up at 11.30 p.m. on 8 April 1940. Out of about thirty brown P. collected 5 April, and kept in two P-dish cultures N., i.e. food available, at 8–10° C., three from one dish and one from the other with active swarm spores put into watch-glass, and half an hour later transferred to big basin used in Exp. 16 (1). Temp. 8–10° C. Subsequent examinations 11 April, 18 July (temp. now 13·5° C.).

Experiment 18. Dated noon on 9 April 1940. In dish of about thirty brown P., from same collection, kept in E. with no food, three small P., i.e. not over 1 mm. in diameter, emptied since 3.30 a.m. Temp. 8–10° C. Examined 10 (found small shell, see p. 610), 11 (removed other P. and put in a little N. culture), 12 April.

The series of experiments is, as will be seen, incomplete, but a fair range of conditions is covered. No further development was obtained in any, excepting perhaps the last which produced the small shell described on p. 610 (see Text-fig. 2). Nor was any later stage ever seen in the cultures of *Polystomella* started in January 1939 in diatom cultures (p. 649), where, however, it happened that there never were swarm spores from more than a single *Polystomella* at any one time. It was estimated that a single parent *Polystomella* of moderate size might produce swarm spores to the order of some 500,000; and when one considers the relatively small number of microspheric individuals developed in nature from the large proportion of megalospheric *Polystomella* in the ordinary population, which themselves originate in broods of less than 200 from each of the former, one should perhaps not be surprised at a very low measure of success in experimental cultures, even if it takes two swarm spores to produce one microspheric animal. Their natural rarity does seem to suggest some special difficulty in their development; and perhaps one may conclude from these experiments that it is probably not simply any difficulty in one swarm spore making contact with another. It might be that union can only occur between very special pairs of flagellispores, or that some very special circumstance is necessary for their further development, with or without fusion. It may be that this difficulty is not confined to *Polystomella* (see p. 611, and cf. Rhumbler, 1909, p. 325). It would therefore be well worth while to make further attempts to discover what it is.

In the meantime attention might be drawn to the following points:

(1) *Medium.* Gross (1934) reports a striking success in the culture and induction of syngamy amongst the flagellate gametes of *Noctiluca* (Cystoflagellata) in earth extract medium, a success he had failed to achieve in sea water. In my experiments sea water of various kinds[2] was used; and also earth

[1] After an unusually long interval, since all shells had been removed at 6.0 a.m. on 29 March (see p. 655).

[2] Viz. sterile sea water, i.e. boiled and kept in the laboratory for at least a month before use; outside sea water (see p. 620), filtered through a single filter paper; and sea water circulating in the aquarium tanks, which, however, since the time of Lister (1903) is known not to be satisfactory for certain stages in the development of *Polystomella*.

extract medium, the only (doubtful) success being obtained when the latter was used throughout sporulation and subsequently (Exp. 18). In this case no diatoms were present until late in the experiment, which almost certainly means that the *p*H was about 8·0. In the *p*H determinations made now and then values above 8·5 were never obtained, even in Petri-dish cultures of *Navicula* so long as they remained healthy. Nevertheless, the *p*H was always a little higher in the presence of diatoms, and in view of the results of Gross (1934) even this small rise may make a difference. The unusual survival of flagellispores noted in Exp. 16 occurred in similar circumstances.

(2) *Food.* It is hardly possible that this is important during sporulation and the earliest succeeding stages—excepting perhaps in so far as its presence affects the *p*H. In the experiments there are several instances where the flagellispores had hours together in plain sea water or earth extract medium beforehand, even if they were later placed with diatom cultures. These it was hoped would have supplied a sufficiency of suitable food of various sizes for the earliest stages, beginning with such small organisms as the bacterial growths which were always present. In other cases sporulation was at least completed in a diatom culture.

(3) *Temperature.* The experiments were carried out at various temperatures, Exp. 18 at the lowest of all (under 10° C.). It is believed that sporulation is best carried out at such temperatures (see p. 649); and arguing from natural conditions in the sea it may be expected that the early subsequent development is at any rate possible at similar low temperatures. It does not follow, however, that it is readiest under these conditions, as is indicated by the work of Maclagan (1932) on *Smynthurus* (Collembola), in which it was shown that, though this insect lays the largest number of eggs at about 7° C., development proceeds best at about 16° C.

(4) *Concentration of Swarm Spores.* This may be important in more ways than one. Apart from the well-known facts that any kind of development may be rendered abnormal, or may cease altogether, when the organisms are too thick on the ground, and that if it is a case of syngamy a certain minimum concentration is on the other hand necessary, it has been suggested that facilitating substances may be passed into the medium by one or both of the partners in such fusion (see, e.g., *Nature*, Vol. 143, 17 June 1939, p. 1036, referring to the work of Moewus, Kuhn and others). Such substances must be present in the medium in certain concentrations.

No attempt was made to count the flagellispores per unit volume, but a good range of concentrations was in fact used in the course of the experiments. Renewing the medium at various stages might also have an effect in this respect, and here again there was considerable variation in the procedure.

(5) *Light.* Light as is well known has many unexplained affects on living organisms. Reference to some of these is made in *Nature* (loc. cit.), where the liberation of the special sensitizing substances in the presence or absence of light is noticed in connexion with syngamy in *Chlamydomonas* (Phytomonad-

ina) and in fishes respectively. No special arrangements with regard to light were made excepting where mentioned in Exps. 13, 14, 16.

(6) *Maturing of the Flagellispores.* This might have been affected by the disturbance suffered by the *Polystomella* taken from the sea and subjected to experiment. Possible effects would perhaps have been varied by the use of flagellispores produced: (*a*) During the night following capture (Exps. 9, 10, 14, 16), (*b*) two to four days after capture (Exps. 1–8, 11, 12, 13, 15, 17, 18); or (*c*) by *Polystomella* which had been for some time in culture. As already explained swarm spores from more than one such *Polystomella* were never available at any one time, so that syngamy might here have been out of the question for a different reason.

It might also be that the flagellispores are only capable of syngamy during a limited period of time after they are liberated. Some attempt to circumvent such a difficulty was made by mixing the swarm spores at various intervals after emergence, and in other experiments allowing them actually to come out more or less simultaneously from two or more *Polystomella* previously placed together in the same dish.

(7) *Selective Mating of the Presumptive Gametes.* It has been established in some of the lower vegetable organisms amongst the algae and fungi that syngamy only takes place between the products of certain pairs of parents, though these may not differ in any visible characteristics. American investigators have recently claimed that there are distinct 'mating types' amongst the Ciliata, only some of which are interfertile (see Jennings, 1939). Whatever the explanation of this, if similar barriers exist in *Polystomella*, it was hoped that they might have been overcome by using as many parents as possible in the experiments. Although only two or three were usually available, flagellispores from 'about a dozen' (Exp. 4), 'about ten' (Exp. 5), six (Exp. 6), and seven (Exp. 16), were used in others. In Exps. 9 and 14 mixed flagellispores from different individual pairs were observed, and no difference in behaviour noted.

Because these experiments are only a beginning, and have not as yet given any positive result, it is thought worth while to describe them in some detail, in the belief that when this line of inquiry is resumed, perhaps by another investigator, they may provide a starting point a little further on than was available in 1938.

Summary

Part I. After a brief statement of the present unsatisfactory position regarding the life history of *Polystomella crispa*, an account is given of experiments with various fixatives on *P. crispa*, collected near Drake's Island, Plymouth. A method is described for the cultivation of *Polystomella* in diatom cultures, especially with *Navicula mutica* var., with which a flagellate (*Bodo* sp.) was constantly present. The identification of individual Foraminifera, and determination of growth, were often possible by some irregularity in the shell, or by noting the attachment of one or more stalked egg cocoons of an unknown

turbellarian worm. The feeding of *Polystomella* on diatoms seized by the pseudopodia outside the shell is described; the structures previously taken for ingested algal cells inside the shell are shown to be foraminal plugs ('bouchons' of le Calvez) which are discarded from time to time and thrown out of the shell along with the excretory granules (xanthosomes), mostly via the canal system.

Part II. Notes are given on the microspheric form and its reproduction, through the spring and summer, by schizogony. The rearing of two broods in laboratory cultures is described, with an account of the formation of a new chamber to the shell. It is concluded that a complete life cycle consisting of one microspheric and one megalospheric phase occupies a period of about two years.

Part III. Sporulation of the megalospheric form is described as seen during life; and various factors are discussed which might affect the process. The relatively small size of the reproducing *Polystomella* with which Lister worked is ascribed to their having lived under less favourable conditions. From a study of stained preparations, certain details are added concerning the opening up of the shell, presumably to facilitate the eventual escape of the flagellate swarm spores; also concerning the accompanying cytoplasmic and nuclear changes. Some account is given of the form and structure of the swarm spores, both alive and in permanent preparations. Experiments are described which constitute an attempt to carry the life cycle beyond the stage of sporulation. They are so far unsuccessful, with one possible exception, under all the various experimental conditions which are briefly discussed in their turn.

REFERENCES

Page references to the works of F. Schaudinn are all given for his collected *Arbeiten* (Hamburg and Leipzig, 1911), which is easily available, and usually more convenient than the various journals in which they originally appeared.

BRADY, H. B., 1884. *Report on the Foraminifera dredged by H.M.S. 'Challenger' during the years 1873–6.* Challenger Reports, Vol. IX, Zoology.

BÜTSCHLI, O., 1894. *Protoplasm and Microscopic Foams.* Translated by E. A. Minchin. London, p. 94.

CALVEZ, J. LE, 1938. Recherches sur les Foraminifères. *Arch. Zool. Expr. Gén.*, T. 80, p. 163.

CARPENTER, W. B., 1860. Researches on Foraminifera, 4th series. *Phil. Trans. Roy. Soc. London*, Vol. 150, p. 535.

CUSHMAN, JOSEPH A., 1933. *Foraminifera; their Classification and Economic Use*, 2nd ed.; also illustrated key to the same. Sharon, Mass.

DUJARDIN, F., 1835. Observations nouvelles sur les prétendus Céphalopodes microscopiques. *Ann. Sci. Nat.*, Sér. 2, T. III, Zoologie.

—— 1841. *Histoire Naturelle des Zoophytes.* Paris.

EHRENBERG, H., 1839. Über noch jetzt zahlreich lebende Thierarten der Kreidebildung und der Organismus der Polythalamien. *Abhandl. d. Akad. der Wiss. zu Berlin*, p. 18.

FØYN, BJØRN, 1936*a*. Über die Kernverhältnisse der Foraminifere *Myxotheca arenilega* Schaudinn. *Arch. für Protistenk.*, Bd. 87, p. 272.

FØYN, BJØRN, 1936*b*. Foraminiferenstudien. I. Der Lebenszyklus von *Discorbina villardeboana*. *Bergens Mus. Årb., Naturv. rekke*, No. 2.

—— 1937. Foraminiferenstudien. II. Zur Kenntniss der sexuellen Fortpflanzung und der Entwicklung der Gamonten von *Discorbina villardeboana*. *Bergens Mus. Årb., Naturv. rekke*, No. 5.

GATENBY, J. BRONTË, 1937. *Biological Laboratory Technique*. London.

GATENBY, J. BRONTË & COWDRY, E. V., 1928. Bolles Lee's *Microtomist's vade-mecum*, 9th ed. London.

GROSS, F., 1934. Zur Biologie und Entwicklungsgeschichte von *Noctiluca miliaris*. *Arch. für Protistenk.*, Bd. 83, p. 178.

—— 1937. Notes on the culture of some marine plankton organisms. *Journ. Mar. Biol. Assoc.*, Vol. XXI, p. 753.

HARVEY, H. W., 1928. *Biological Chemistry and Physics of Sea Water*. Cambridge Comparative Physics Series.

HERON-ALLEN, E., 1914. Contribution to the bionomics and reproductive processes of the Foraminifera. *Phil. Trans. Roy. Soc. London*, B, Vol. 206, p. 227.

—— 1930. The further and final researches of Joseph Jackson Lister upon the reproductive processes of *Polystomella crispa* (Linné). *Publication No.* 3067, *Smithsonian Institution, Washington*.

HOFKER, J., 1930*a*. Notizen über die Foraminiferen des Golfes von Neapel. *Publ. Staz. Zool. di Napoli*, Vol. X, Fasc. 3, p. 365.

—— 1930*b*. Die Fortpflanzung der Foraminiferen. *Ann. Protistologie*, Vol. III, p. 25.

JENNINGS, H. S., 1939. Mating in *Paramecium*. *Genetics*, Vol. 24, p. 202.

JEPPS, MARGARET W., 1926. Contribution to the study of *Gromia oviformis* Duj. *Quart. Journ. Micr. Sci.*, Vol. 70, p. 701.

KERR, J. GRAHAM, 1921. *Zoology for Medical Students*. London.

KLEBS, G., 1892. Flagellatenstudien. I. *Zeitschr. f. wiss. Zool.*, Bd. LV, p. 265.

KOFOID, C. A., 1934. An interpretation of the conflicting views as to the life cycle of the Foraminifera. *Science*, Vol. 79, p. 436.

LANG, A., 1901. *Lehrbuch der vergleichenden Anatomie der wirbellosen Tiere, Lief. II, Protozoa*. 2nd ed. Jena.

LISTER, J. J., 1892–1905. *Laboratory Notebooks*, Vols. I and II. In Heron-Allen and Earland collection of the British Museum of Natural History, London.

—— 1895. Contributions to the life-history of the Foraminifera. *Phil. Trans. Roy. Soc. London*, B, Vol. 186, p. 401.

—— 1903. *The Foraminifera*, in *A Treatise of Zoology*, edited by E. Ray Lankester, Pt. I, Fasc. 2. London.

—— 1906. The life-history of the Foraminifera. *Rep. Brit. Assoc. Av. Sci.*, Section D.

—— 1907. The Foraminifera. *Proc. Roy. Inst. Great Britain*, Vol. XVIII, p. 459.

LOWNDES, A. G., 1935. The twin polygraph and strobograph. *Nature*, Vol. 135, p. 1006.

MACLAGAN, D. STEWART, 1932. An ecological study of the Lucerne Flea. *Bull. Ent. Res.*, Vol. 23, p. 101.

MARINE BIOLOGICAL ASSOCIATION, 1931. *Plymouth Marine Fauna*, 2nd ed.

MEEK, F. B. & HAYDEN, F. V., 1864. Palaeontology of the Upper Missouri. *Smithsonian Contributions to Knowledge*, No. 172, p. 13.

MILLET, F. W., 1904. Report on the recent Foraminifera of the Malay Archipelago. Pt. XVII. *Journ. Roy. Micr. Soc.*, p. 597.

MYERS, EARL HAMLET, 1935*a*. The life-history of *Patellina corrugata*. *Bull. Scripps Inst. Oceanography*. Technical series, Vol. 3, No. 15, p. 355.

—— 1935*b*. Morphogenesis of the test and the biological significance of dimorphism in the Foraminifer *Patellina corrugata* Williamson. *Bull. Scripps Inst. Oceanography*. Technical series, No. 16, p. 393.

MYERS, EARL HAMLET, 1936. The life-cycle of *Spirillina vivipara*. *Journ. Roy. Micr. Soc.*, Vol. LVI, p. 120.

OVEY, C. D., 1938. Difficulties in establishing relationships in the Foraminifera. *Proc. Geologists' Assoc.*, Vol. XLIX, p. 160.

RHUMBLER, L., 1900. Foraminiferen, in *Nordisches-Plankton*, Erste Lief, Teil XIV. Kiel and Leipzig.

—— 1909. Die Foraminiferen der Plankton-Expedition, Pt. I. *Ergebnisse der Plankton-Expedition der Humboldt-Stiftung*, Bd. III.

ROMEIS, B., 1928. *Taschenbuch der microscopischen Technik*, 12th ed. Munich and Berlin.

SAEDELEER, H. DE, 1932. Recherches sur les pseudopodes des Rhizopodes testacés *Arch. Zool. Expér. Gén.*, T. 74, p. 597.

SANDON, H., 1934. Pseudopodial movements of Foraminifera. *Nature*, Vol. 133, p. 761.

SCHAUDINN, F., 1893. *Myxotheca arenilega* n.g., n.sp., ein neuer mariner Rhizopode. *Arbeiten*, p. 31.

—— 1894*a*. Über die systematischen Stellung und Fortpflanzung von *Hyalopus* n.g. (*Gromia dujardinii* Schultze). *Arbeiten*, p. 45.

—— 1894*b*. Die Fortpflanzung der Foraminiferen und eine neue Art der Kernvermehrung. *Arbeiten*, p. 41.

—— 1895*a*. Über den Dimorphismus der Foraminiferen. *Arbeiten*, p. 57.

—— 1895*b*. Untersuchungen an Foraminiferen. I. *Calcituba polymorpha*. *Arbeiten*, p. 1.

—— 1899. Untersuchungen über den Generationswechsel von *Trichosphaerium sieboldii* Schn. *Arbeiten*, p. 150.

—— 1903. Untersuchungen über die Fortpflanzung einiger Rhizopoden. I. *Polystomella crispa*. *Arbeiten*, p. 499.

SCHLUMBERGER, CH., 1896. La plastogamie dans les Foraminifères. Abstracted in *Zool. Centralbl.*, Bd. III, p. 684.

SCHMIDT, W. J., 1937. Über den Feinbau der Filopodien insbesondere ihre Doppelbrechung bei *Miliola*. *Protoplasm*, Bd. 27, p. 587.

SCHULTZE, MAX S., 1854. *Über den Organismus der Polythalamien*. Leipzig.

SCHULZE, F. E., 1877. Rhizopodienstudien. VI. *Arch. für mikr. Anat.*, Bd. 13, p. 9.

TAN SIN HOK, 1935. Zur Theorie des Trimorphismus und zum initial Polymorphismus der Foraminiferen. *Natuurk. Tijdschr. Ned. Ind.*, Vol. XCV, p. 171.

VERWORN, M., 1889. Psycho-physiologische Protistenstudien. Jena.

WILHELMI, J., 1909. Tricladen. *Fauna und Flora des Golfes von Neapel*. Mongr. 32.

WILLIAMSON, W. C., 1849. On the structure of the shell and soft animal of *Polystomella crispa*. *Trans. Micr. Soc.*, Vol. II.

—— 1852. On the minute structure of the calcareous shells of some recent species of Foraminifera. *Trans. Micr. Soc.*, Vol. III.

WINTER, F. W., 1907. Zur Kenntniss der Thalamophoren. *Arch. für Protistenk.*, Bd. X, p. 1.

YOUNG, J. Z., 1935. Osmotic pressure of fixing solutions. *Nature*, Vol. 135, p. 823

EXPLANATION OF PLATES IV–V

I am indebted to the Carnegie Trust for a grant towards the cost of the coloured figures in Plates IV and V.

PLATE IV

Painted by Miss Cecily Brown Kelly.

Fig. 1. A living *Polystomella crispa* from Plymouth.

Fig. 2. Empty shell of same to show terminal face of last chamber. *Ch.*, chambers of the shell, the cavities of which are in communication by the foramina; *for.*, foramina in front of last chamber, which form the terminal apertures of the shell; *foss.*, fossette (d'Orbigny); *K.*, keel of the shell; *ps.* pseudopodia arising from protoplasm inside and outside the shell; *ret.pr.*, ridges over the retral processes of the chambers, between the fossettes; *sep.*, ridges over the septa between the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

PLATE V

Fig. 1. *Bodo* sp. *Corr.ac.*, iron haematoxylin. This fixative gave better results with this flagellate than did Schaudinn's mixture, Bouin, or osmic vapour and alcohol. A fair number of nuclei in the preparation show the large karyosome and peripheral granules characteristic of the genus, and in many cases the kinetoplast does not stain uniformly, sometimes appearing as a thick-walled empty sack, especially after hot Schaudinn. *N.*, nucleus; *K.*, kinetoplast.

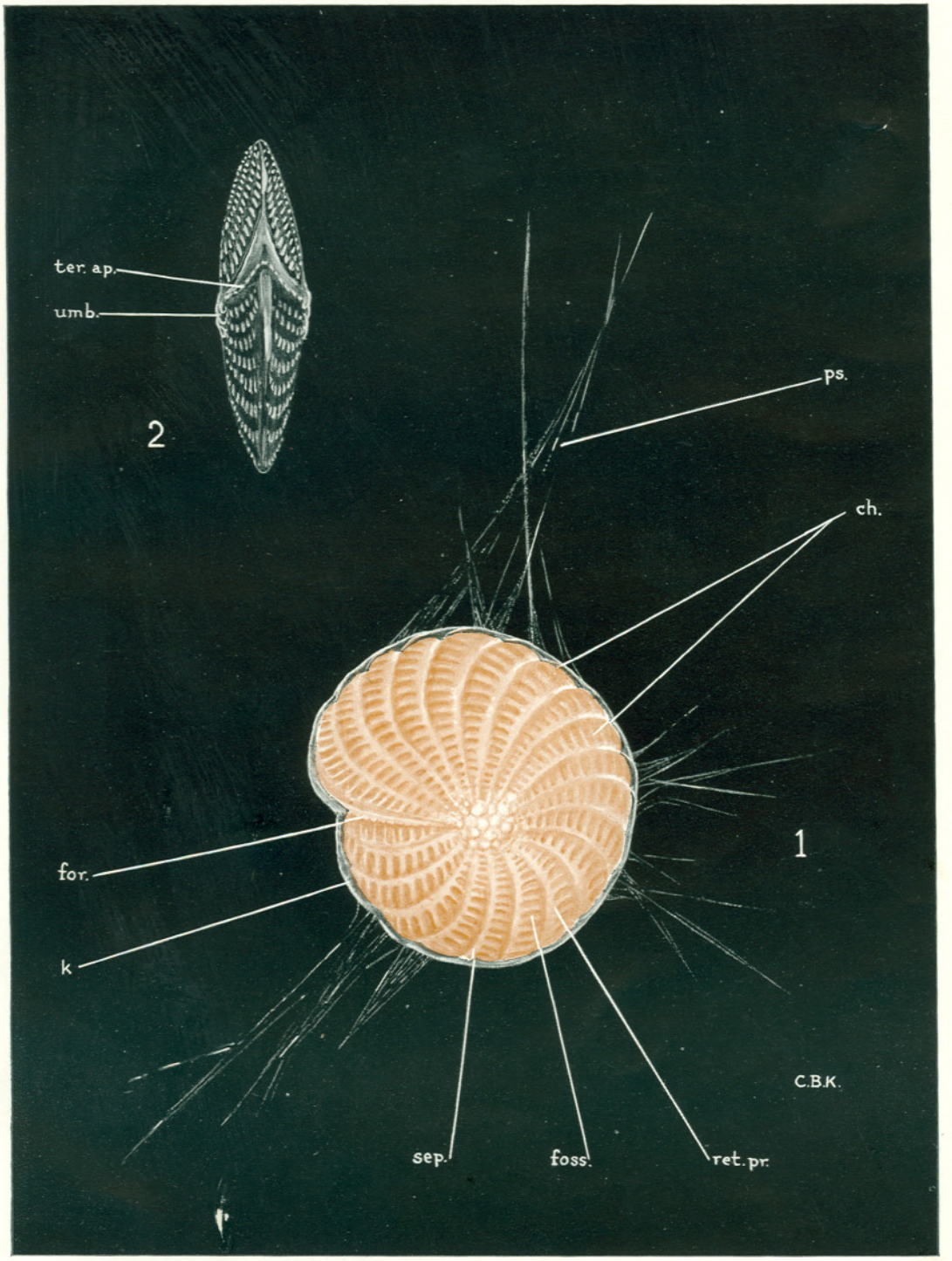
Figs. 2, 3. Flagellispores of *Polystomella crispa* stained with iron haematoxylin and eosin, after fixation in osmic vapour and alcohol and in Schaudinn's fluid respectively. In Fig. 3 the longer flagellum was about five times as long as the body. *cryst.*, refractile inclusion; *N.*, nucleus; *eos.*, eosinophil body in vacuole.

Figs. 4–6. Illustrating three successive stages in the feeding of *Polystomella*, as described in the text on p. 629.

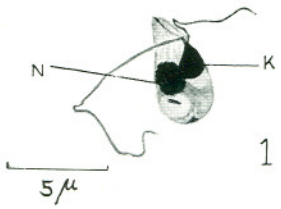
Fig. 7. Part of Fig. 5 at a higher magnification, showing undigested diatoms, and excretory granules (xanthosomes).

Fig. 8. Part of a similar cast feeding cyst showing frustules of a naviculoid diatom and excretory granules.

Figs. 9, 10, 11. Pseudopodia of *Polystomella*. From film made by A. G. Lowndes. Zeiss obj. A, C, D, resp., eyepiece × 10. The granules are well shown in Fig. 11, anastomoses in Fig. 9 and 'webs' in Fig. 10. The black segments at the margins are the edges of the shells.



Polystomella crispa L.



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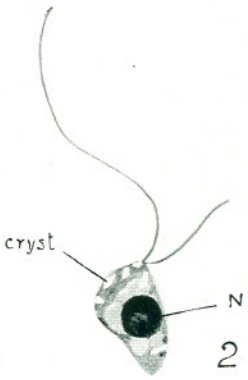

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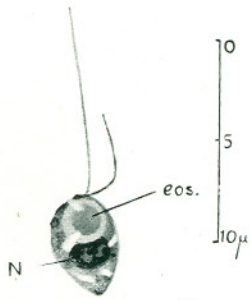

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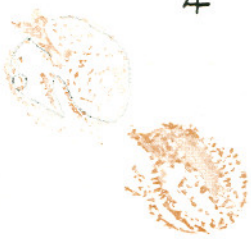

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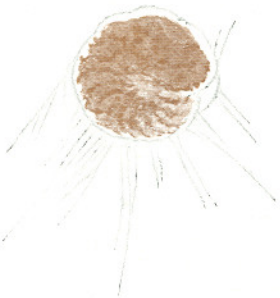

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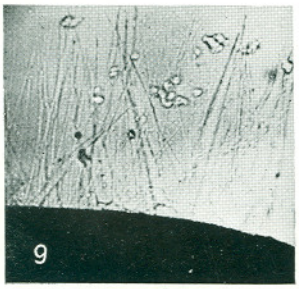

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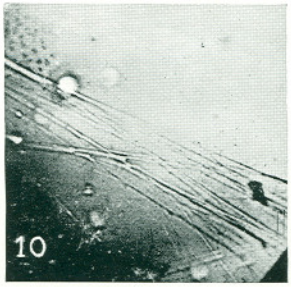

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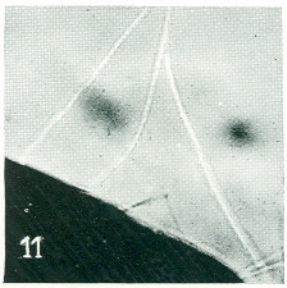

11

Polystomella crispa L.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Mechanism of the Semicircular Canal. A Study of the Responses of Single-fibre Preparations to Angular Accelerations and to Rotation at Constant Speed

By O. Lowenstein and A. Sand

Proc. Roy. Soc., B, Vol. 129, 1940, *pp.* 256–75

Single-fibre responses from the horizontal ampulla of the isolated labyrinth of *Raja* have been recorded by the oscillographic method with the aim of a quantitative analysis of the responses of this sense organ. The rate of increase or decrease of discharge above or below the level of a spontaneous resting rhythm is a linear function of the rate of angular acceleration. The threshold acceleration first producing a noticeable response from the organ is in the region of 3° per sec.^{2}

During prolonged rotation of constant speed the frequency of discharge, having attained a maximum or minimum according to the nature of the initial acceleration, gradually returns to the spontaneous value over a period of about 20–30 sec. These results when interpreted in terms of the physical properties of the cupula terminalis account adequately for the time relations of the rotatory nystagmus and after-nystagmus of the eyes. O.L.

The Individual and Integrated Activity of the Semicircular Canals of the Elasmobranch Labyrinth

By O. Lowenstein and A. Sand

J. Physiol., Vol. 99, 1940, *pp.* 89–101

The individual responses of the ampullary end organs in the three semicircular canals of the isolated labyrinth of *Raja* have been investigated by the oscillographic method, with the aim of ascertaining the mode of collaboration of individual sense endings during rotation and tilting about the vertical axis and the longitudinal and transverse horizontal axes. When the labyrinth is at rest there is a spontaneous discharge of impulses from each ampulla which is increased or inhibited when the labyrinth is rotated or tilted. The horizontal canals respond to rotation about the vertical axis, but not to rotation about the two horizontal axes, whereas the anterior and posterior vertical canals respond to rotation about all three axes. The six canals of the two labyrinths collaborate in pairs exhibiting lateral, transverse and diagonal

synergy according to the nature of the rotation. The integrated action of the canals during rotation about the three axes was analysed in relation to the eye-muscle reflexes evoked by these rotations. O.L.

Osmotic Relations of some Metazoan Parasites

By N. Kesava Panikkar and Nora G. Sproston

Parasitology, Vol. xxxiii, 1941, *pp.* 214–23

The osmotic behaviour of three parasites in normal and experimental media has been studied with a view to understanding the relationship with their hosts. *Angusticaecum* sp., a nematode from the intestine of the tortoise, is hypertonic in media of very low concentrations (1·1–1·3 % NaCl in tap water), but becomes isotonic in sea water and slightly hypertonic in 50 % sea water. Ligaturing experiments show that its cuticle is permeable to water and probably to salts. *Lernaeocera branchialis*, a blood-feeding copepod from *Gadus* spp., is hypotonic to the surrounding sea water so long as it remains attached to its host, its blood showing an osmotic pressure equivalent to 2·0–2·8 % NaCl. Isotonicity with the medium is established when the parasite is excised and kept alive. Hypotonicity of *Lernaeocera* is probably caused by the low osmotic pressure of the blood of its host (1·443 % NaCl in *Gadus pollachius*), to which it is permanently attached. *Bopyrus squillarum*, a blood-sucking isopod from *Leander serratus*, is isotonic or slightly hypotonic to sea water, the tendency towards hypotonicity being probably the result of the hypotonic nature of the blood of the host. The osmotic properties of *Lernaeocera* and *Bopyrus* would suggest their ability to survive in dilute sea water, a fact which is supported by their occurrence on hosts living in inshore or estuarine habitats. N.K.P. and N.G.S.

A Comparative Study of the Effects of Ions on Whole Nerve and Isolated Single Nerve Fibre Preparations of Crustacean Neuromuscular Systems

By Talbot Howe Waterman

J. Cell. Comp. Physiol., Vol. 18, 1941, *pp.* 109–26

A comparative study of the effects of ions on the perfused neuromuscular system of three decapod crustaceans has been made. The contractile responses of the flexor dactyl muscle to stimulation of the whole leg nerve (in *Panulirus*, *Maia*, and *Cambarus*) and to stimulation of isolated single motor nerve fibres (in *Cambarus*) have been isometrically recorded. The tension developed in response to brief tetanizing stimulation varied inversely with the amount of magnesium in the perfusion fluid, being greatest when none of this ion was

present and approaching zero when four to five times the normal amount were perfused.

Changes in perfusion fluid calcium content usually gave rather irregular results, but some single nerve fibre experiments suggested that this ion had qualitatively different effects on the slow and the fast neuromuscular systems. Increased potassium led to decreased contractile responses in whole nerve preparations and single nerve fibre preparations of the slow closer system, but to markedly increased contractile responses in single nerve fibre preparations of the fast closer system. Exactly comparable results were found when both the slow and the fast motor closer motor fibres were prepared and alternately stimulated in the same nerve muscle preparation. Evidence is cited suggesting that these potassium effects occurred at the myoneural junction in some of the steps involved in neuromuscular transmission.

T.H.W.

The Action of Potassium on Echinoderm, Molluscan and Crustacean Muscle

By G. P. Wells

J. Exp. Biol., Vol. 18, 1942, *pp.* 213–22

The effects of various potassium concentrations on the cloacal complex of *Cucumaria elongata*, and on the hearts of *Aplysia punctata*, *Helix pomatia* and *Carcinus maenas* are described. It is pointed out that there exists a fundamental similarity between many, and perhaps all, types of rhythmic muscle, as regards their responses to changes in potassium concentration.

Data on calcium : potassium antagonism and ammonium : potassium parallelism in the *Helix* heart are also presented; in connexion with the former, there is a brief discussion of the physiological differences between marine and other animals.

G.P.W.

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 of this *Journal*.

The Laboratory is open throughout the year and its work is carried out under the supervision of a Resident 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 in September, and marine animals and plants are supplied to educational institutions.

Research work at sea is undertaken by the steam drifter "Salpa" and by a motor boat, which also collect the specimens required in the Laboratory.

TERMS OF MEMBERSHIP

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Annual Members	per annum	1	1	0
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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.; and 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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