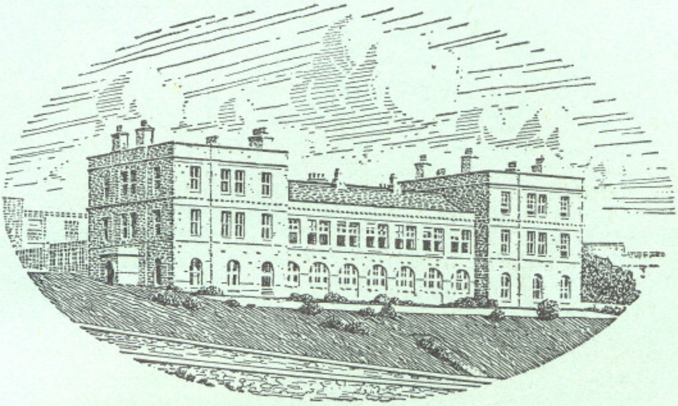
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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OBSERVATIONS ON THE BIOLOGY AND POST-EMBRYONIC DEVELOPMENT OF *IDOTEA VIRIDIS* (SLABBER) (ISOPODA, VALVIFERA) FROM NEW ENGLAND CREEK, SOUTH-EAST ESSEX

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(Text-figs. 1–14)

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INTRODUCTION

In the course of an ecological survey of a saline lagoon, New England Creek in south-east Essex (Howes, 1939), it was found that one of the dominant species of animals present was a form, possibly a local race, of *Idotea viridis*. New England Creek runs from the Yokefleet, a branch of the River Roach, on to the Maplin Sands. It was dammed at both ends in 1925 while still full of water, and by 1934 had become a lagoon containing approximately 250,000 cu. m. of water of lower salinity than, and of a slightly different composition from, sea water (Howes, 1939). The lagoon was visited at regular intervals, usually about every four weeks, throughout 1934, 1935 and 1936, samples of the water being taken for analysis and specimens of the fauna collected at each visit. Further, a standard haul was made with a plankton net on each occasion. The *I. viridis* proved to be an active swimmer and, apart from specimens collected by hand, over eight thousand in all were taken in the plankton alone. Thus a collection was made which included all stages in the post-embryonic development of this species and, since it was well preserved, provided excellent material for studying its biology.

The species *I. viridis* appears to contain animals showing a wide range of morphological variation, especially between individuals collected from different localities, i.e. there appear to be well-defined local races of which the New England Creek form may be one. Since the latter does not exactly tally with the earlier accounts of *I. viridis*, a detailed description was deemed advisable before discussing its biology. After this description, the paper deals first with the annual change in numbers of *I. viridis*, the number of eggs in the brood pouch and the distribution of different size groups in the plankton hauls throughout 1936. The nature of these phenomena may be peculiar to this race. Following this, the newly hatched animal is described, and then the change in shape of the body, the coxal plates and of certain appendages, as the animal develops into the adult. Although the material suffers from being derived from a form of *I. viridis* that may not be common in England, which may, indeed, be confined to the lagoon, New England Creek and to dykes draining into that creek, nevertheless it is believed that the results of this latter investigation recorded below are, in a modified degree, applicable to the Idoteidae as a whole. Idoteids appear rarely to be found together in large numbers, and a series of individuals, representing every instar from hatching to maturity, is so difficult to obtain that little is known of their development.

METHODS AND NOMENCLATURE

The animals were collected by hand and with nets, the majority with a plankton net. They were then killed and fixed by the addition of sufficient formalin to the water in which they were, to produce a final concentration of approximately 5 %. Fixed animals were used for all measurements recorded. The animal was placed on a well slide in water, lightly pressed flat by means of a cover-slip or piece of slide, lighted from above and measured to the nearest $\frac{1}{100}$ mm. by means of a travelling microscope at a magnification of 20 diameters; for animals of the first and second instars, an eyepiece micrometer was substituted for the travelling microscope. Apart from errors inherent in the method owing to distortion on fixation, measurements made of the same animal on different occasions showed that an accuracy of well within ± 2 % was usually attained.

For morphological studies, animals or appendages were mounted untreated in Canada balsam or Gurr's water mounting medium: specimens were also heated over a boiling water bath in 2 % caustic soda and the chitinous skeleton obtained then mounted in Canada balsam. In regard to the nomenclature of the three main divisions of the body, I have followed Calman (1909) since there seems to be no reason to suppose that they are other than homologous with the corresponding regions of the Malacostraca as a whole, and have called them, cephalon, thorax and abdomen, discarding the arachnidan terms used by Sars (1899) and Collinge (1917), namely cephalon, mesosome and metasome, respectively. Following the nomenclature customary in Isopoda, the first free thoracic segment is referred to as the first thoracic segment and the remaining segments numbered accordingly. Needham (1937), to whose work considerable reference is made, calls the first *free* thoracic somite the second thoracic segment. In the light of more recent work, Collinge's "lateral clefts" of the cephalon are referred to as "occipital grooves" and that part of the cephalon lying behind them as the "maxillipedal somite" (Jackson, 1926). The names of the joints of the maxillipedes are taken from Hansen (1925), the lateral half of Collinge's (1918) divided coxopodite then becoming the proximal epipodite and the median half the coxa proper. The use of the term "fused dactylus and carpo-propus" for the distal joint of the maxillipedal palp may be quite unjustified, but since it was necessary to refer to individual joints by name, I have taken the liberty of thus modifying Hansen's description of the maxillipede of *Glyptonotus*; it is not claimed that this is a correct homology, but the knee is definitely situated between the two distal joints in the species described above. All the illustrations in this paper are made from individual specimens and no attempt has been made to produce generalized diagrams, hence slight variations in shape are to be expected. It should be noticed that there are considerable variations in size in each instar.

THE ADULT *IDOTEA VIRIDIS* (SLABBER) FROM NEW ENGLAND CREEK

Body narrow, elongated (Fig. 1), about 4·6 times as long as broad; dorsal surface moderately convex, surface either smooth or pitted. *Cephalon* (Fig. 2*a*) wider than long, ratio breadth/length, males 1·45 ± 0·12 (120 specimens), females 1·48 ± 0·12 (45 specimens); anterior margin slightly emarginate, posterior margin straight; lateral margin with slight fissure at point of demarcation of the maxillipedal somite from rest of cephalon; fissure continued as strongly-marked occipital groove, extending one-quarter of width of cephalon inwards, not visible in mid-line; maxillipedal somite about one-fifth length of cephalon, lateral borders tapering to an antero-lateral blunt point faintly divided by groove. Eyes large, length about one-third cephalon, situated dorso-laterally, posterior margin on median transverse line of cephalon. *Antennulae* (Fig. 2*b*) extending well beyond third peduncular joint of antenna, first joint expanded, irregularly angular, second joint nearly as long but narrower, third joint slightly elongated; flagellum (Fig. 2*c*) single-jointed, clavate, lateral angle acute, antero-medial surface rounded with three or four setae and eight (one isolated lateral, three pairs and one single median) spindle-shaped, pedunculate aesthetascs (Fig. 2*d*), each with truncate distal tip where pore opens to exterior. *Antennae* (Fig. 2*e*) elongated, first peduncular joint very short, with dorsal forwardly directed rounded angle, second longer, incised laterally with dorsal forwardly directed obtuse point, third scarcely longer than second, concave distally on both dorsal and ventral surfaces with median and lateral anterior projections, fourth longer than third with dorsal forwardly directed obtuse point and single antero-lateral spine, fifth joint longer and narrower than fourth; flagellum about one and a quarter times length of peduncle with 11 to 13 joints in fully grown males, first joint somewhat elongated, terminal style (Fig. 2*f*) almost cylindrical, half length of preceding segment. *Maxillulae* (Fig. 2*g*, *h*): outer lobe terminating in eleven curved spines, six outer (four or five toothed), five inner (three toothed, two median slender and untoothed); inner lobe terminally rounded with three setose spines and small outer spine. *Maxillae* (Fig. 2*j*) consisting of two laminar setose lobes, outer bipartite lobe with long ctenate setae, inner lobe with row of setae, three innermost slightly stouter and markedly plumose. *Maxilli-*

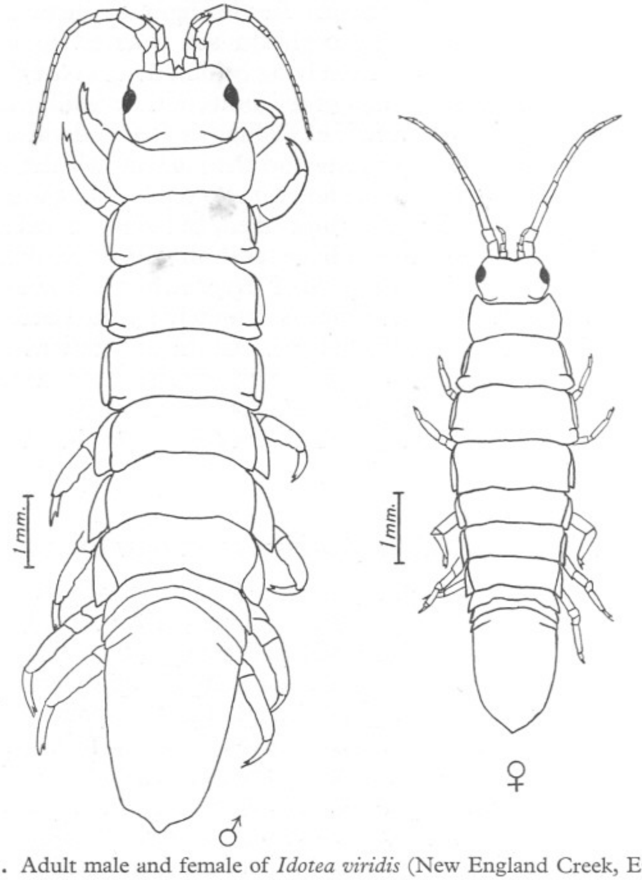

Fig. 1. Adult male and female of *Idotea viridis* (New England Creek, Essex).

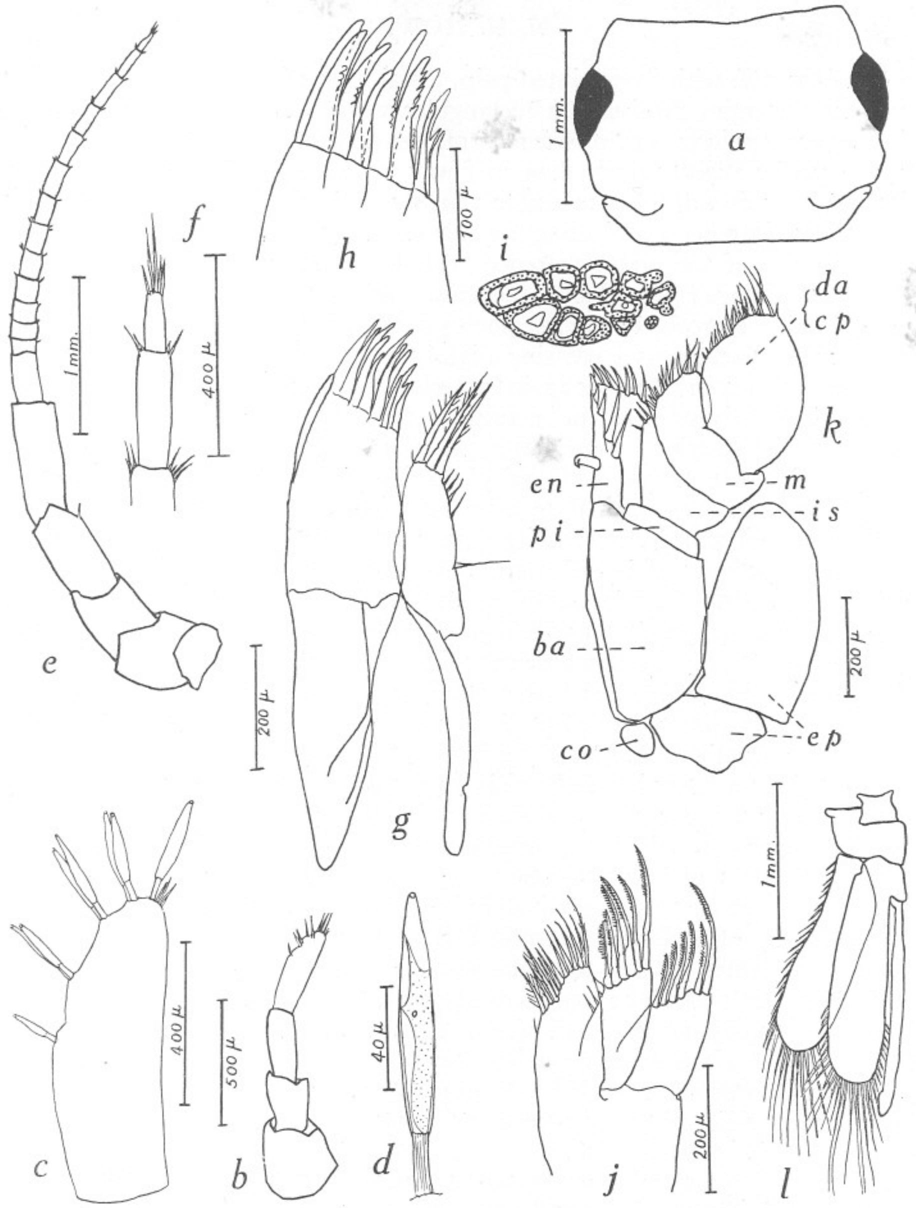

Fig. 2. *Idotea viridis* (New England Creek, Essex). *a*, cephalon, dorsal view, 12·5 mm. ♂; *b*, right antennula, dorsal view, 11 mm. ♂; *c*, flagellum of right antennula, artificially flattened to show distribution of aesthetascs; *d*, aesthetasc; *e*, right antenna, dorsal view, 11 mm. ♂; *f*, terminal setose style of right antenna; *g*, right maxillula, ventral view, 13 mm. ♂; *h*, tip of outer lobe of right maxillula; *i*, oblique section through maxillula passing transversely through bases of spines, inner side of lobe to the right, dorsal surface at top; *j*, left maxilla, ventral view, 13 mm. ♂; *k*, right maxillipede, ventral view, 13 mm. ♂; *ba*, basis; *co*, coxa; *cp*, *da*, carpo-propus, dactylus; *en*, endite; *ep*, epipodites; *is*, ischium; *m*, merus; *pi*, preischium; *l* ,second right pleopod, ventral view, 14 mm. ♂. (In all cases the appendages are described in their *anatomical* position.)

pedes (Fig. 2*k*) with four-jointed palp, distal joint of palp with marked nick on inner margin, pre-ischium rectangular when viewed from above; basis large, nearly as long as palp; size ratio of coxa and proximal epipodite variable; tip of endite slightly more than half way up inner border of merus, inner border bent dorsally, hence endite roughly L-shaped in cross-section, with single coupling hook projecting inwards at angle; width of dorsally-directed portion half of transverse flattened region. First, fifth, sixth and seventh segments of *thorax* (Fig. 1) shorter than second, third and fourth; first shortest and seventh scarcely longer; segments two, three and four approximately same length; segments increasing slightly in width up to segments five and six, segment seven narrower again; anterior angles of pleuron of first thoracic segment sharply pointed, projecting laterally beyond borders of head and forward well anterior to occipital groove; in adult males this segment about 20 % wider than cephalon. Coxal plates (Fig. 3*e*, *f*) in two groups, those of segments two, three and four showing only a narrow projection beyond lateral pleural margin, occupying from under three-quarters to over nine-tenths respectively of lateral margins of segments, visible right up to and round antero-lateral angles; coxal plates of segments five, six and seven wide, almost triangular, projecting well beyond lateral borders of segment throughout length; coxal plate of segment seven with backwardly projecting acute angle. In segments two, three and four, a small area of pleuron separated from rest by a medially directed transverse slit visible from below; in segments two and three remains of pleuron projecting to lateral border of coxal plates, separated from latter by slight nick; in segment four ending more medially and in segment five scarcely visible. Remaining thoracic appendages (Fig. 3*a*, *b*) similar to those of other members of genus. *Abdomen* (Figs. 1, 3*d*) with two short segments and antero-transverse grooves indicating coalesced third, forming about 33·5 % of total body length (anterior border of cephalon to posterior tip of abdomen); terminal fused segment moderately long, 84 % of total abdomen length; total abdomen in intact animal about 1·9 times as long as wide; posterior margin convex with single median tooth and lateral corners forming an angle of about 110°. *Appendix masculinus* of *second pleopods* (Fig. 2*l*) extending well beyond end of endopodite. *Uropoda* (Fig. 3*c*) flattened, elongated, rounded anteriorly, slightly broader in middle than at ends, endopodite obtusely truncated; setose style arising from basal plate reaches to tip of endopodite.

Length of animal: adult males, 12–14 mm.; ovigerous females, 5·5–10·5 mm.

Colour: background generally brown, but not rarely white or with white patches, with variously distributed melanophores giving a large range of different patterns, most commonly with an even distribution. Background remains brown in alcohol.

Distribution: New England Creek and dykes draining into it, near Great Wakering, south-east Essex. This environment has been described in some detail elsewhere (Howes, 1939). The species is very common indeed and it is calculated that the creek contains of the order of two and a quarter million individuals when peak numbers are present (Fig. 4).

It is strange that no reference is anywhere made to the fact that the endite of the maxillipede is bent upwards so as to have the inner surfaces in both a horizontal and a vertical plane, thus making the joint L-shaped or triangular in cross-section, with the coupling hook arising from the angle thus made. This condition is probably widespread in the Idoteidae and certainly occurs in *I. baltica*, *I. neglecta*, *I. emarginata*, *I. granulosa*, and *I. linearis*, although often to a much less marked degree than in *I. viridis*.

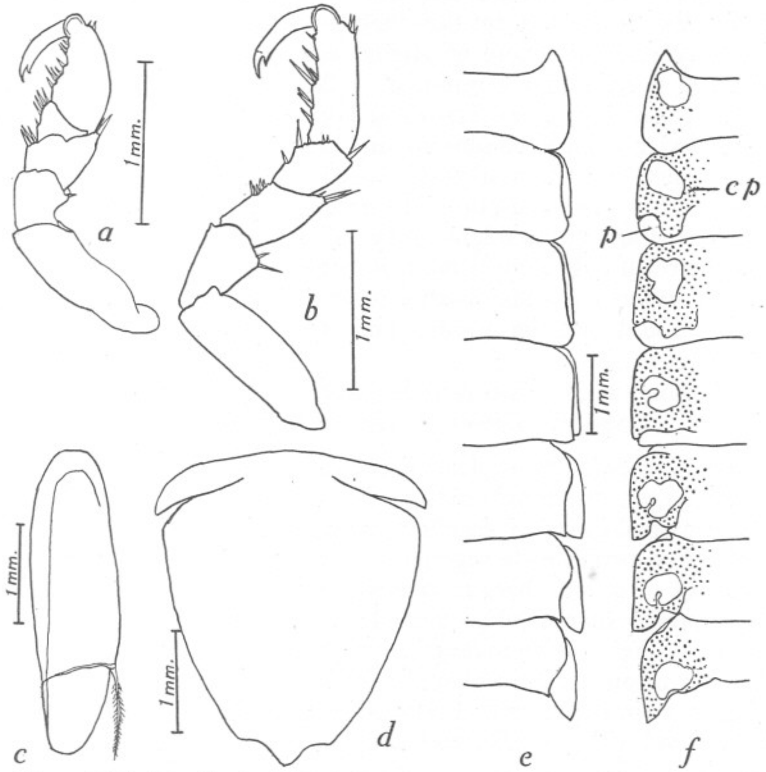

Fig. 3. *Idotea viridis* (New England Creek, Essex). *a*, anterior view of first pereiopod, 11 mm. ♂; *b*, anterior view of seventh pereiopod, 11 mm. ♂; *c*, left uropod, ventral, of 12 mm. ♂; *d*, dorsal view of fused segments of abdomen of 12 mm. ♂ (prepared by dissecting out pleopods and soft tissues and pressing abdomen flat); *e*, dorsal view, *f*, ventral view, of coxal plates of 13·4 mm. ♂ (preparations made after treatment with caustic soda. Ventral view, coxal plates are dotted); *cp*, coxal plate; *p*, pleuron. (In all cases the appendages are described in their *anatomical* position.)

The aesthetascs of the antennules do not seem to have been observed, but experience has shown that they have the appearance of setae when the appendage is mounted in either Canada balsam or in water mounting medium;

and in marine forms they are often so covered by ectoparasitic Peritrichida as to be completely obscured. In water their structure can more easily be made out and it was possible to study them in some detail in the *I. viridis* from New England Creek, where they were scarcely ever parasitized. They closely resemble the "olfactory setae" or "Riechstäbchen" described by Scourfield (1896, 1905) in Cladocera and other forms, being shaped like those of, e.g., *Daphnia magna*, except that they are not divided transversely and are stalked. They resemble those of *Asellus aquaticus* in being divided into a hyaline distal portion and a proximal stalk with strongly chitinized walls. These aesthetascs have been examined both with ultra-violet and red and blue light under critical conditions and in the position occupied by the "terminal pellet" on the aesthetascs of Cladocera (Scourfield, 1905) there is in this species a tiny open pore. The arrangement of the aesthetascs on the flagellum of the antennule might prove to be a useful secondary systematic character, since, if fully grown animals were compared, it was found to be constant. It suffers from the disadvantages that the organs are very liable to be parasitized and that the number and arrangement alters from instar to instar.

Idotea viridis *from New England Creek compared with* I. viridis *(Slabber) as described by Sars* (1899)

Body form somewhat more slender, cephalon wider, frontal margin similar. Eyes appear larger. Antennule similar. Joints of antennary peduncle differing in detail, proximal joints of flagellum shorter and style of flagellum shorter in proportion to penultimate segment. Coxal plates only occupying anterior three-quarters of lateral margins of segments two, three and four and not reaching posterior margin of segment five. Legs similar, joints of P 1 about 25 % broader in proportion to length, P 7 carpus markedly shorter than merus, again broader in proportion to length. Abdomen similar. Stylet of pleopod 2 projecting to a similar extent. Endopodite of uropod more tapering; tip of setose style level with posterior end of uropod.

I. viridis *from New England Creek compared with* I. viridis *(Slabber) as described by Collinge* (1917)

Body shape similar, cephalon proportionately wider, eyes larger. Basal joints of antennules differing in shape and proportions. Antennae: style shorter. Maxillulae: eleven spines (seven or eight, toothed) instead of nine (three, toothed). Characteristically, two median spines slender and untoothed. Except for first, segments of thorax almost equal in length. Coxal plates of segments two, three and four similar but narrower, of segments five, six and seven not contiguous and narrower. Maxillipedes similar; other thoracic appendages, P 1 slightly stouter in proportion, propus of P 7 very much stouter, carpus shorter than merus. Abdomen similar. Endopodite of uropod more tapering, setose style reaching posterior tip of endopodite.

It thus appears that *I. viridis* from New England Creek differs from those described by Sars and by Collinge in many particulars, especially in the size of the eyes, the shape of the style of the antenna, the size of the coxal plates as measured by the lateral expansions visible from the dorsal surface, the build of the peraeopods, the shape of the endopodite of the uropod and the length of the setose style. It further differs from Collinge's description in that the outer lobe of the maxilla terminates in eleven spines instead of nine. But these are characters which appear to vary more or less independently of each other throughout the species. Mr G. M. Spooner kindly lent me his collection of *I. viridis*, which contained specimens from nine different localities, six in the Tamar Estuary, two near Weymouth and one from Holbeach on the Wash, and my collection includes animals from three different habitats near Great Wakering, Essex. On the whole, animals obtained from the same locality closely resemble one another, apart from age and sex differences, but may differ from the *I. viridis* described by Sars, by Collinge or that from New England Creek in some or all of the characters enumerated above, except that every adult male from the two collections had eleven spines on the outer lobe of the maxilla. This last character is not likely to prove of diagnostic value since it occurs commonly throughout the genus (Collinge, 1917).

There remain three characters which serve to differentiate the species *viridis* from other members of the genus *Idotea* and yet include in that species the animals described by Sars and by Collinge as *I. viridis*, together with the twelve forms mentioned above. These are, in the adult male: (*a*) body slimly built, with lateral margins almost parallel and with the widest region in the fifth and sixth thoracic segments rather than in the third or fourth as in other species; (*b*) cephalon with overall breadth only about 1·5 times overall length; (*c*) abdomen slightly tapering posteriorly, terminating in a single median tooth; obtuse postero-lateral corners very distinctly marked but never projecting backwards as lateral teeth.

This scheme closely follows the points stressed by Sars (1899, p. 84) and it is the basis upon which the *Idotea* collected in New England Creek are considered to be *I. viridis*.

SEASONAL CHANGES IN NUMBERS

New England Creek runs from the Yokefleet, a branch of the River Roach, on to the Maplin Sands. It was dammed in 1925 and a body of water was enclosed with the intention of draining the creek later on. This has not been done, but the salinity of the water has become reduced and a great many chemical changes have taken place. A fairly detailed study of these changes and of the fauna and flora has been made and is published elsewhere (Howes, 1939). The creek in 1934–6 was about 1½ miles long, had a maximum depth of 3½ m. and a surface area of 144,000–170,000 sq. m., and contained between

230,000 and 299,000 cu. m. of water. The predominant plant species were *Ruppia maritima* and *Chaetomorpha linum*, the former occurring in large quantities and reaching the surface.

Idotea viridis was very common, and was found swimming and crawling on the stems of *Ruppia*. Observations suggest that it eats polyps of *Membranipora* and of coelenterates, though any dead animal in the creek always had a large number of specimens crawling on it. In cold weather the numbers of *Idotea viridis* appear to be very much reduced and only a few specimens can be found crawling in weed near the bottom.

The creek was visited once every four weeks, and on each visit a haul was made with a silk plankton net, 100 strands to 1 in., over a measured length of 1325 m. Details of this haul and a discussion of its value as a quantitative method are given in an earlier paper (Howes, 1939) and it suffices here to state that approximately 100 cu. m. of water were filtered. At the completion of each haul, not only were the contents of the bucket preserved, but the silk was carefully examined, all visible adhering organisms removed with a camel hair brush and added to the catch in the bucket. The number in each catch represents both swimming animals and others swept off surface weed by the net. Results obtained by counting the number of *Idotea* in each catch for the years 1934, 1935, 1936 are given in Fig. 4.

The interest of this diagram lies in the paucity in numbers of *I. viridis* in the first five months of the year, the very rapid rise to a maximum in late June and in July and the way in which the numbers remain comparatively high until the end of October, then fall off rapidly, rising again in December. It is believed that the lateness of the attainment of maximum numbers is mainly due to the carnivorous nature of the New England Creek race and that no considerable reproduction occurs until a plentiful supply of coelenterates and especially of *Membranipora crustulenta* var. *fossaria* is available. Observation has shown that these forms become common about May and that the *Membranipora*, at least, continues to be present in quantity until October. The significance of the rise in December is not understood. The method of collecting nekton forms by means of a plankton net towed just below the surface is obviously open to criticism and it is important to enquire how far the picture presented by these data really represents the behaviour of the population in the creek. It is known that all sizes of *Idotea*, including ovigerous females, are taken in the hauls (Fig. 5), so that the result cannot be entirely due to certain instars retiring beyond the reach of the net. Further, a visit to the creek in late summer or early autumn impresses one with the extraordinary plentifulness of *I. viridis*; on the other hand, in late winter or early spring it is possible to spend a whole day collecting and not see a single specimen unless they are very specially looked for; even dragging weed from the depths will only yield an occasional individual. It can therefore be concluded that Fig. 4 is a fair rough representation of the behaviour of the population as a whole. It can be calculated that at the peak periods in 1934 and 1936 the

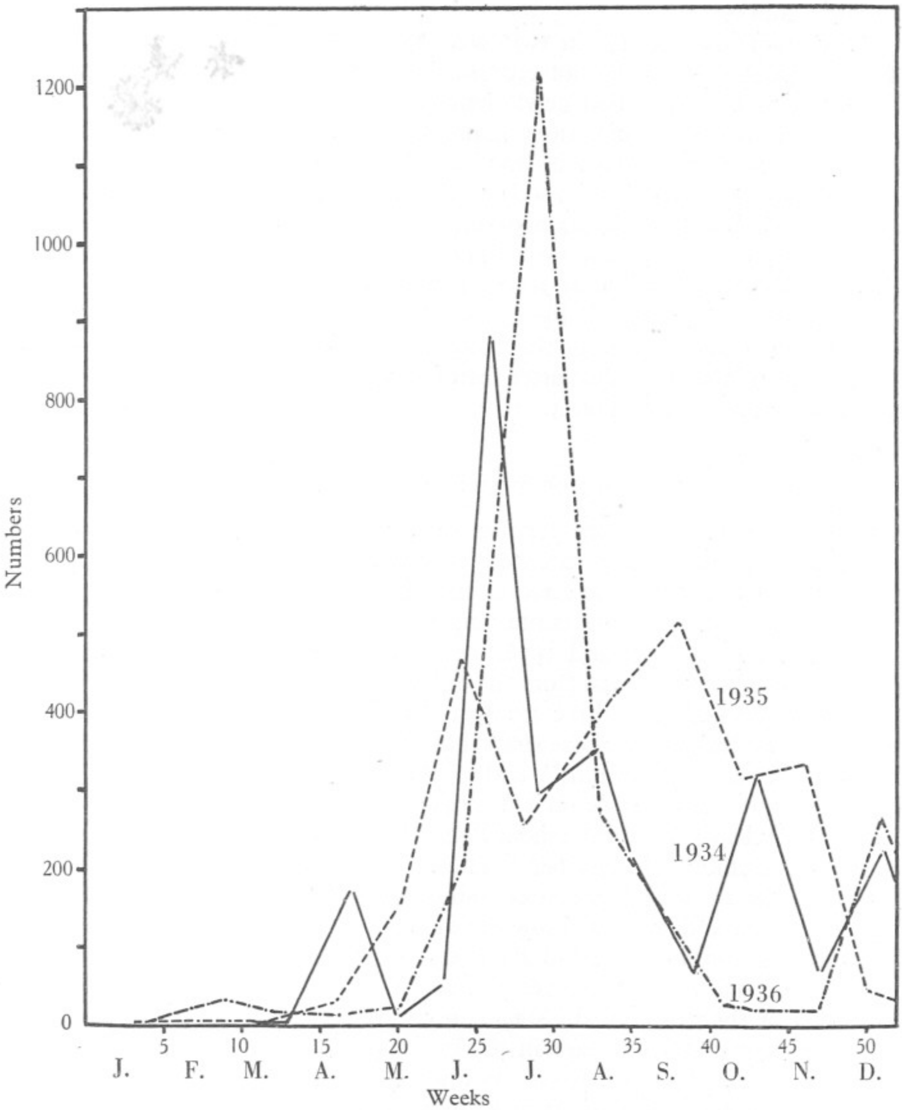

Fig. 4. Curves showing the annual change in numbers of *Idotea viridis* (all sizes) in 100 cu. m. of water, from New England Creek, Essex, for the years 1934, 1935 and 1936, in which the totals were 2656, 2339 and 3127 respectively. (—— 1934; – – – 1935; – · – · – 1936.)

total number of *I. viridis* in New England Creek was of the order of two and a quarter million.

All the idoteids collected in 1936 were measured and have been segregated into size groups, which do not represent instars. These results are shown in Fig. 5. It will be seen that newly hatched individuals (1·50–1·99 mm. long) first appear in May, reach their maximum in July, disappear in October and November and reappear in small numbers in December, suggesting that *I. viridis* has a second late brood, as do many marine plankton forms; the figure also shows that animals between 2·00 and 5·99 mm. in length represent the greater part of the plankton collections. This is taken to mean that there is a considerable mortality of young forms, especially at that ecdysis which precedes sexual maturity (Table I) and possibly that the older forms tend to some extent to remain nearer the bottom. For the reasons presented above, it is not considered that this latter factor involves any fundamental alteration in the data presented in Fig. 4.

NUMBER OF YOUNG IN BROOD POUCH

In order to determine the fertility of *I. viridis*, the numbers of embryos in the brood pouches were counted. Preliminary investigation showed that if ovigerous females were selected as such from a collection, there was always a tendency to choose those containing large numbers of eggs, hence every female collected in 1935 and 1936 has been examined and, if ovigerous, the embryos have been removed from the brood pouch and counted. The embryos have been divided into three groups, (*a*) the spherical or semi-spherical, referred to as "eggs", (*b*) those shaped as an oblate spheroid and with obvious mesodermal bands, "medium", and (*c*) those with appendages, "advanced". The results of counts made on 121 ovigerous females are shown in Fig. 6.

It would clearly be most misleading to express these results in terms of an average number of eggs per female, since there is a large number (41) of females whose brood-pouch contained 1–6 embryos, mostly in the "advanced" stage, and the remaining females fall, with six exceptions, into four main groups, containing round about 9, 13, 17 and 23 embryos respectively. The exceptional animals had 26, 31, 33, 34 and 46 embryos. There is some tendency for the "advanced" stages to be grouped on the left, i.e. for there to be few animals of this stage in the brood-pouch and for the "eggs" to be on the right. The data available do not justify an attempt fully to explain these facts, but they may be partially accounted for. Observations show that there is some difference in the stage of development of embryos within the marsupium, e.g. while the majority are ready to hatch, three or four may be at an earlier stage. It is possible that these latter may be retained after the others have left the pouch, in this way accounting for the females with 2–6 embryos. Jancke (1924) showed that in *Asellus aquaticus*, the number of fertilized eggs was twice reduced by expulsion, first, of early embryos and later, of more

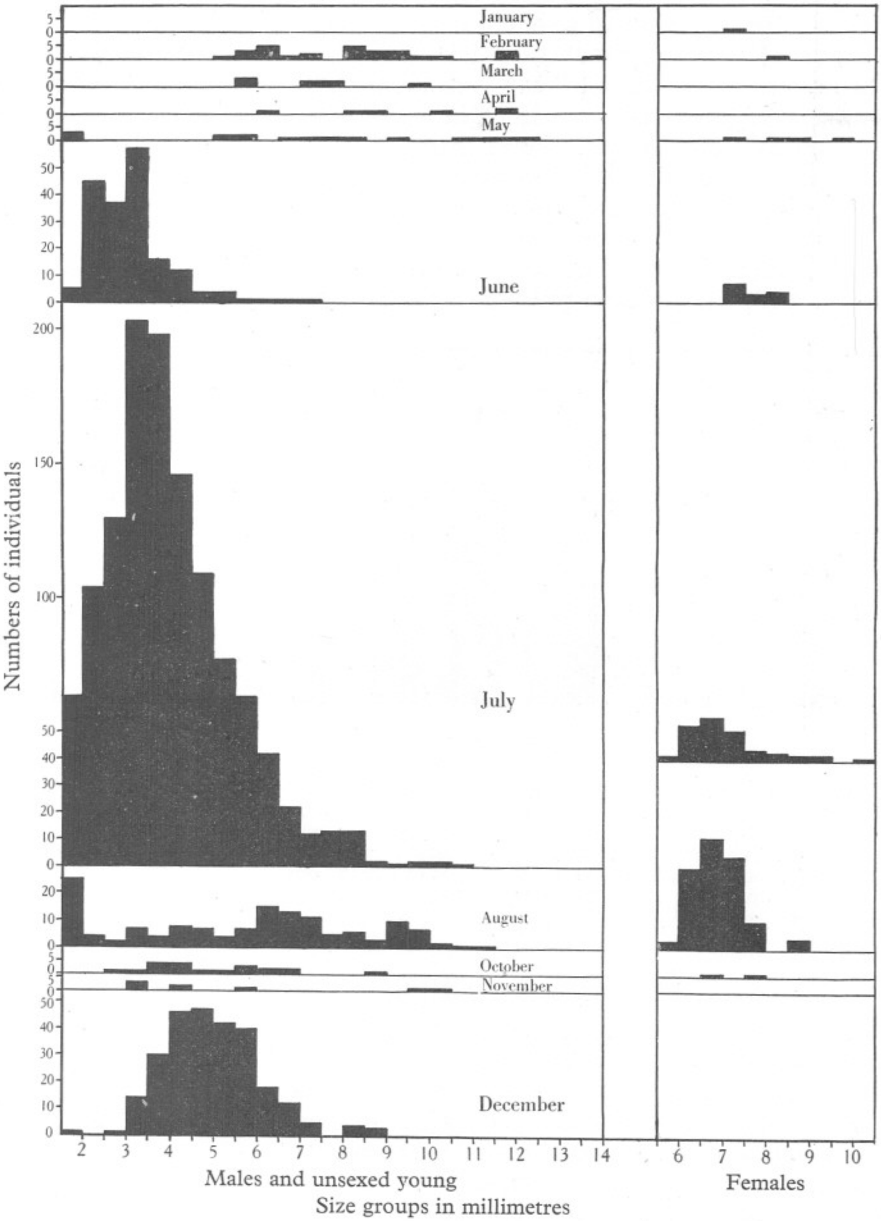

Fig. 5. Size-composition of plankton catches of *Idotea viridis* in New England Creek throughout 1936.

developed embryos; 150 fertilized eggs eventually giving rise to a brood of about 8. He also observed a similar phenomenon in *Idotea viridis*, according to Zimmer (1927), viz. a reduction of 30–40 eggs to 4–6 young ready to hatch. The decrease in "advanced" and "medium" embryos from left to right in the figure suggests the possibility that a similar process also takes place in *I. viridis* in New England Creek. Females with only one embryo in the marsupium occurred only in August and September; it is not known whether this is again part of the phenomenon discussed above, or whether the embryo was retained in the pouch, there to pass the winter. The grouping of the remaining females is not correlated either with size of mother or with the season: it is possible that a genetical factor may be operating.

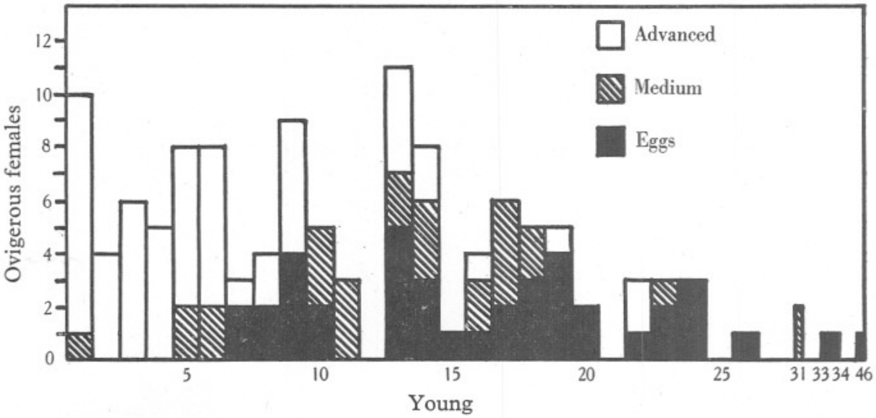

Fig. 6. Diagram showing variation in number of young in brood pouch of ovigerous *Idotea viridis*. The base line is divided into number of young in brood pouch and the values of the ordinates are proportional to the number of females containing a given number of eggs. The young are grouped into "eggs", "medium" and "advanced". The four columns on the right, which are half the width of the other columns, represent animals with exceptionally large numbers of young in the brood pouch. The total number of females was 121.

THE NEWLY HATCHED *IDOTEA VIRIDIS*

The material upon which this description is based was collected in the plankton, but the fact that these really were newly hatched animals was confirmed by examination of young from brood-pouches.

I. viridis, when just hatched (Fig. 7*a*), is between 1·5 and 2·4 mm. long, mean length 1·8 mm. Cephalon relatively enormous, body tapering posteriorly. Flagellum of antennule consisting of two segments. Thorax of six large segments each bearing a pair of legs and one limbless posterior small segment; this is characteristic of all isopod young at this stage (Calman, 1909). Coxal plates relatively broad, visible on all thoracic segments including first and rudimentary seventh; convex laterally, large and very conspicuous on thoracic

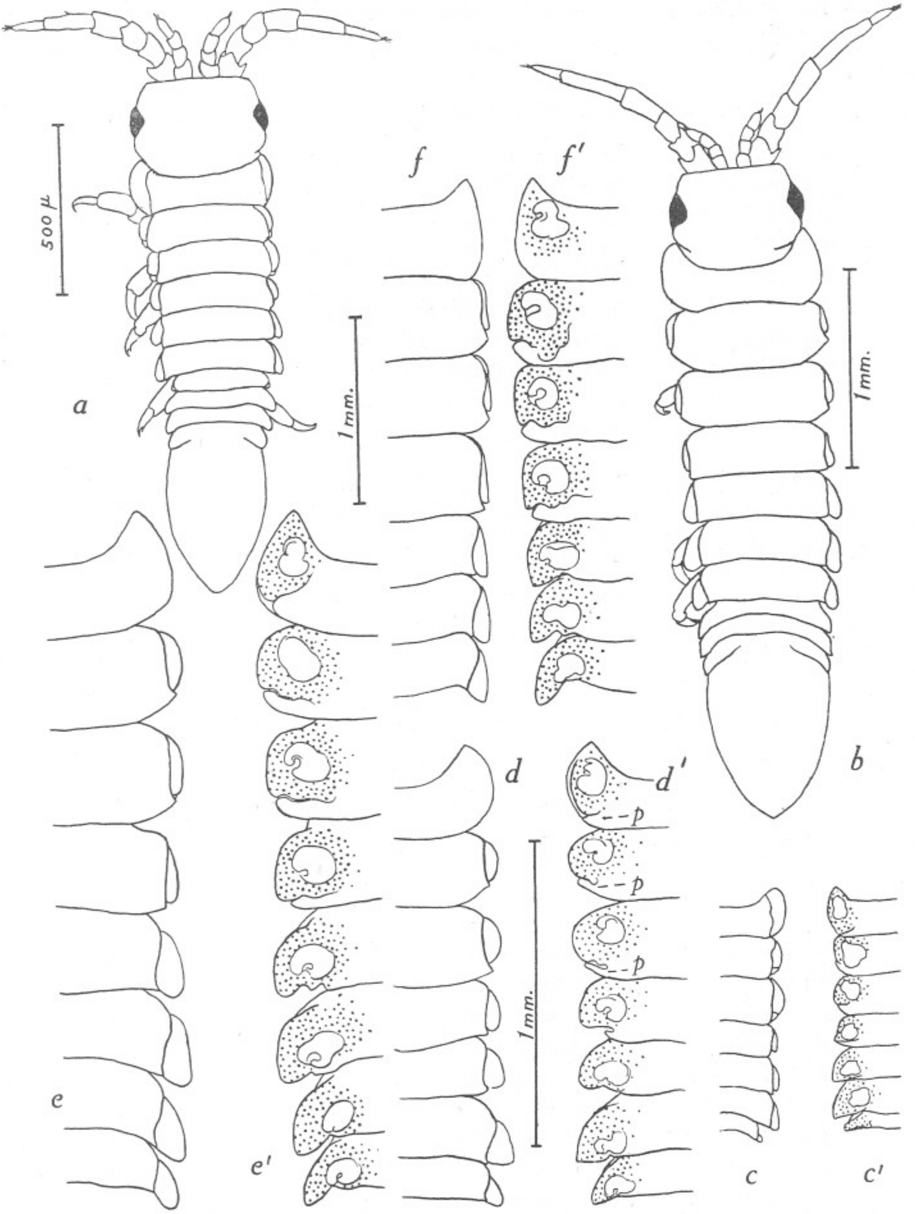

Fig. 7. *a*, newly hatched *Idotea viridis*; *b*, *I. viridis* in instar II; *c*, *c′*, *d*, *d′*, *e*, *e′*, dorsal and ventral views respectively of coxal plates in instars I, II and III (all drawn to the same scale); *f*, *f′*, dorsal and ventral views of coxal plates of instar IV; *p*, pleuron.

segment one, diminishing in size to segment six, minute on segment seven. Abdomen with two short segments and antero-transverse groove indicating coalesced third, as in adult.

THE ECDYSES BETWEEN HATCHING AND MATURITY

Several attempt at rearing *I. viridis* from New England Creek in the laboratory were made, but, although adults lived for some weeks in captivity, they all died without growing or producing young, probably because it was impossible to maintain supplies of suitable food. Hence it was necessary to try to determine the number of ecdyses from the preserved material. It was found that if the animals were classified according to the number of joints of the flagellum of the antenna, there was marked correlation between this quantity and the length of the animal, but with considerable overlapping in size between adjacent classes and, in certain cases, e.g. forms with eight, nine and ten joints, almost complete overlap. Where the length of a number of animals extended over the same range, even if there were differences in the number of joints of the flagellum, they were taken as one class. It was then found that these classes could be arranged serially so that each group represented a different instar, saving that the first group included animals covering an exceptionally wide size range and with obvious morphological differences. This group will be dealt with later. That the other groups did, in fact, represent instars, was confirmed as follows: all the animals in a group were examined and those about to moult, i.e. with the skeleton of the coming instar visible inside the existing skeleton, were segregated. The new antennae could be seen inside the old antennae of these forms and hence the coming

TABLE I. INSTARS OF *I. VIRIDIS*

Ratio of mean lengths of animals in instar to that of animals in preceding instar "Przibram quotient"	Instar no.	Number of segments in flagellum of antenna	Mean length of animals in instar mm.	Limits of length of animals in instar mm.	Notes
	1	2	1·8	1·5–2·4	Newly hatched
1·44	2	2	2·6	2·1–3·6	
1·38	3	3	3·6	3·0–4·5	
1·25	4	4 or 5	4·5	3·8–5·6	
1·31	5	6 or 7	5·9	4·9–7·5	At this stage some ♀♀ become mature
1·26	6	8, 9 or 10	7·4	6·1–8·5	At this stage rest of ♀♀ become mature, ♂♂ have *appendix masculinus*
1·36	7	11, 12 or 13	10·1	8·4–12·7	Males show sudden widening of 6th thoracic segment

change in the number of joints determined; it was found that the smallest animals in group one, which had only six thoracic peraeopods, could have the same number (two) of segments in the flagellum in the following, as in the present, instar. This group was therefore separated into instars on the basis of the number of thoracic legs. It could be seen that animals of the second instar added one joint to the flagellum; of the third, one or two; fourth, one or two; fifth and sixth, two or three (Fig. 8): on this basis Table I was constructed.

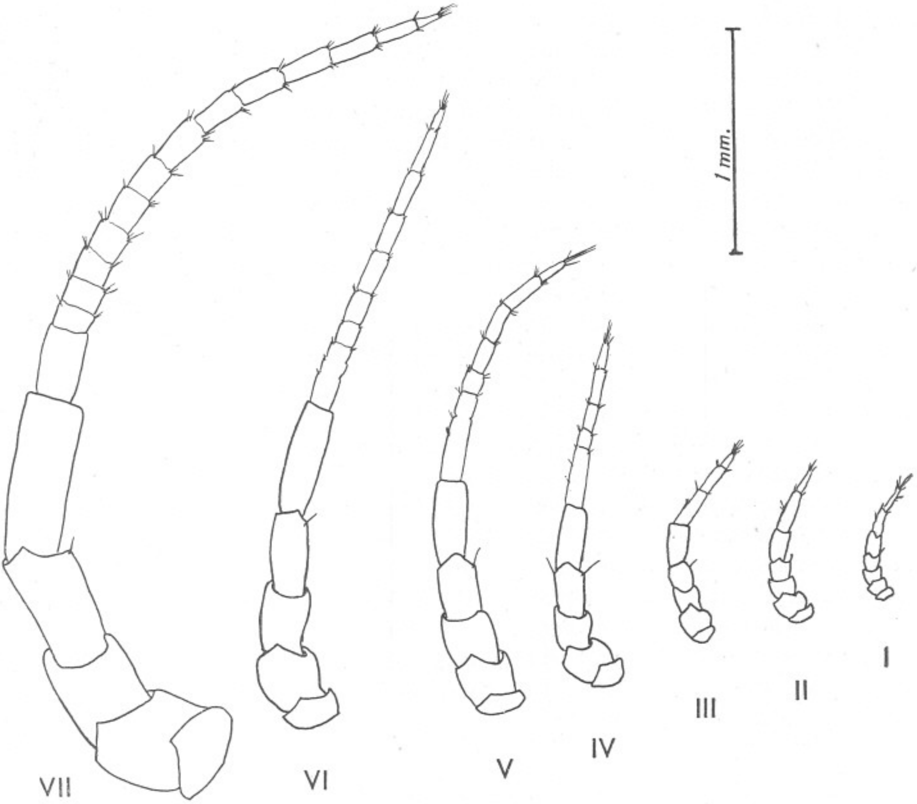

Fig. 8. Antennae of different instars of *Idotea viridis* showing increase in number of joints of flagellum. Newly hatched animal on right. The numbers below the appendages indicate the instar to which the animal belongs.

The lengths of twenty animals in each instar were measured; results so obtained are given in the table.

Normally there are six moults after hatching before the male *I. viridis* from New England Creek stops growing, and rarely there may be a seventh, after which the animal has fourteen or fifteen joints on the flagellum of the antenna and reaches a length of nearly 14 mm. Animals of this size were extremely rare. Usually, in the sixth instar the male has the *appendix masculinus* of the 2nd pleopod in the adult condition; females appear to become ovigerous in the fifth instar but, in any sample, females in this and the sixth instar occur in approximately equal numbers. The evidence suggests that the females moult with increase in size once after attaining maturity, but the possibility of females becoming mature in either the fifth or the sixth instar is not completely excluded. Only one female having the number of joints of the flagellum of the antenna corresponding to that in the seventh instar of the male has been found, although several hundred females have been examined. It is therefore concluded that if the females moult before each brood, as in the majority of isopods, female *I. viridis* from New England Creek usually do not change their bodily dimensions after the sixth instar. It seems unlikely that females would use the same brood pouch for successive clusters of eggs, especially since moulting is so commonly bound up with the opening of the vagina and hence with fertilization. No females with torn marsupia were ever found although in many the brood pouch was quite empty. Probably the females retire to the depths of the creek to moult. Alternatively, the females may have but one or two broods and then die. The data available do not permit of a decision being made on these points.

The fact that the great majority of the females never grow after reaching the sixth instar, while the males undergo a further moult, explains why the male of a copulating pair is always larger than his mate.

The "Przibram quotient", i.e. the ratio between the length of an animal at a given instar and that at the preceding instar, has been calculated. According to Przibram's (1931) theory, arthropods double in weight from instar to instar and hence, assuming that the body does not alter in form, the corresponding ratio for increase in length should be $\sqrt[3]{2}$, i.e. 1·26. The figures given suggest that this "law of geometrical progression" may be applicable to the growth of *I. viridis*.

CHANGES IN BODILY FORM

The post-embryonic changes in bodily form and in the shape of some of the appendages of *I. viridis* have been investigated. There is considerable change both in the shape and proportions of different parts from instar to instar and, in this paper, only those parts showing the most marked and what are believed to be taxonomically the most significant changes, are dealt with. These changes are described in two ways: (*a*) where no irregular alteration in shape is involved, a series of measurements has been made and plotted on a double logarithmic grid to test for simple allometry (Huxley & Teissier, 1936); (*b*) where changes in shape not easily susceptible to measurement, or in number of joints, etc. do occur, e.g. uropods or antennae, these have been illustrated serially in Figs. 7, 8 and 13 and are described in the text.

Allometry

Measurements were made on 141 unsexed and male *I. viridis* and on 45 females. Females were only distinguished from the fifth instar onwards. The dimensions selected for discussion are illustrated in Fig. 9; actually many more dimensions were measured, but, since the object was primarily to facilitate the description of morphological changes rather than to discuss "relative growth", they have been omitted.

The dimensions taken (Fig. 9) are, the greatest breadth of the cephalon and of the third and sixth thoracic segments, including the coxal plates, the length of the seventh thoracic segment and the length of the abdomen: the first three are compared with the total length as the standard, measured from a line drawn transversely between the tips of the antero-lateral projections of the cephalon to the posterior tip of the abdomen, the fourth with the total length minus the length of the seventh thoracic segment, and the fifth with the length of cephalon plus thorax only. "The law of simple allometry can be expressed by a formula of the type, $y = bx^{\alpha}$, where y is the part, x the standard or whole, and b and α are constants" (Huxley & Teissier 1936, p. 780) so that if simple allometry is acting, the graph of $\log y/\log x$, is a straight line (Huxley, 1932).

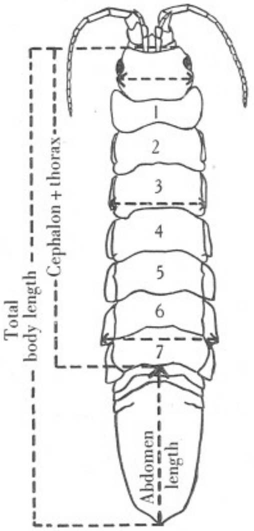

Fig. 9. *Idotea viridis.* Dimensions employed in discussion on allometry.

In this paper, the three standard dimensions described above are taken as x and the other five as the compared dimension y.

The best fitting line to the plot of the logarithms of x and y has been calculated from the logarithms of the measurements by the method of least squares. No attempt has been made to group the data since it was difficult to decide upon a suitable basis of classification. Grouping according to size would cause animals from different instars to be placed in the same class and grouping in instars seemed unwise owing to the considerable overlap between adjacent instars (Table I). The values obtained for α, the equilibrium constant or growth constant (Huxley & Teissier, 1936) only are given since the constant b depends on the units of measurement employed and has been altered in the graphs (Figs. 10, 11), to avoid superimposing them. In the animals examined, the body length ranged from 1·6 to 13·4 mm.

The graphs of $\log y/\log x$ (Figs. 10, 11) do appear to fall reasonably well on a straight line and it is therefore concluded that simple allometry is at work and that the "growth constant" α may justifiably be calculated from the data.

Sixth thoracic segment (Fig. 10B).

In adult males the body is at its broadest in the region of the fifth and sixth thoracic segment, which rarely differ significantly in breadth. The "growth constant" of the breadth of this segment, body length being used for comparison, therefore gives valuable information as to whether the animal as a whole becomes slimmer or broader during growth: further it is chosen in

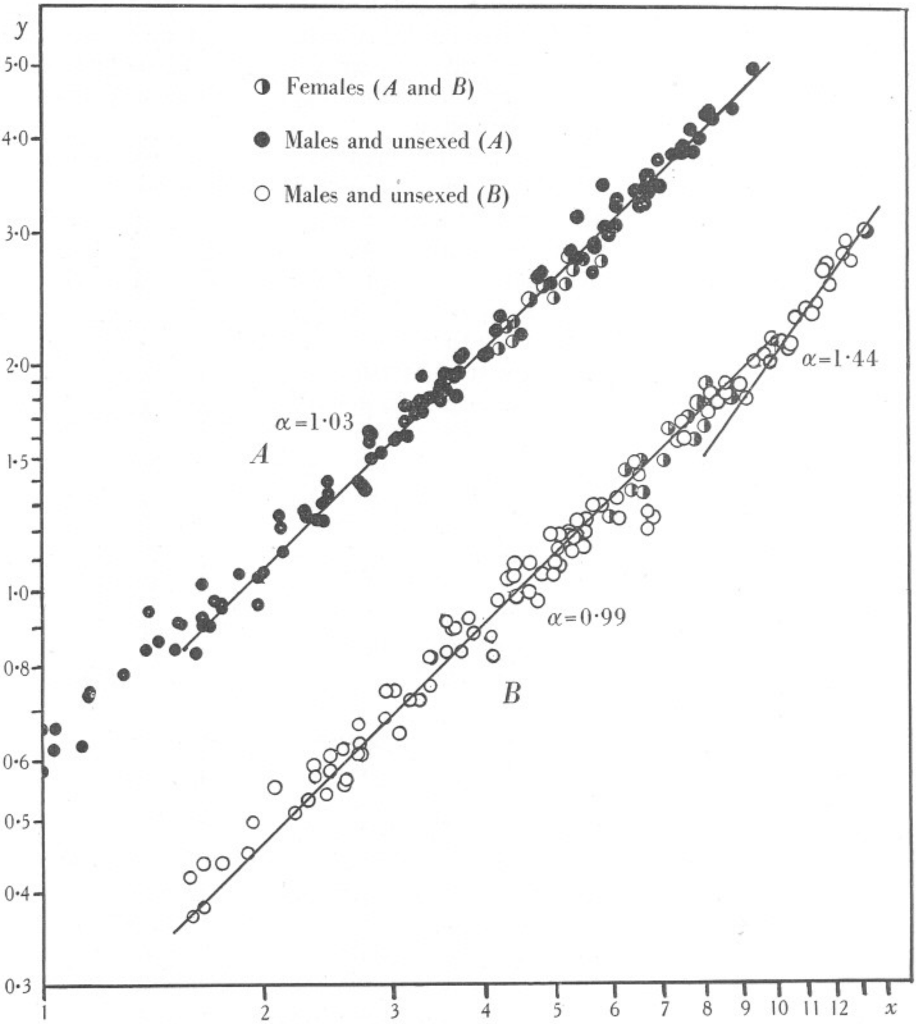

Fig. 10. A (upper line); length in millimetres of abdomen (y) plotted against length of cephalon + thorax (x). B (lower line); breadth in millimetres of 6th thoracic segment (y) plotted against total body length (x). Both double logarithmic plots.

preference to the fifth segment because, in the females, it is less likely to be influenced by the broadening of the more anterior segments concomitant with the formation of a marsupium. It is to be doubted whether the value of α, 0·994, differs significantly from 1, so that up to and including the sixth instar, the sixth thoracic segment in the unsexed animals and in males and females grows isometrically with the body length and the overall bodily dimensions are unaltered. In the seventh instar the body becomes stouter, since the constant is now 1·44. It is interesting that the sixth thoracic segment in the female behaves as that of the male and is uninfluenced by the formation of the brood pouch.

TABLE II. VALUES OF α

x	y	α	Notes
Total body length	Cephalon breadth	0·83	
Total body length	Breadth of 6th thoracic segment	1·44	Instar VII
		0·99	Instars I–VI inclusive
Total body length	Breadth of 3rd thoracic segment	0·88	Males and unsexed
		2·41	Females, instars V? and VI
Total body length minus length of 7th thoracic segment	Length of 7th thoracic segment	2·03	Instar VII
		1·15	Instars III–VI inclusive
		irregular	Instars I and II
Length of cephalon plus thorax	Length of abdomen	1·03	Instars II–VII Animals in instar I appear to deviate from line

Third thoracic segment (Fig. 11A).

The third thoracic segment is the widest in the adult female. The widening appears to take place at the ecdyses between the fourth and fifth, and fifth and sixth instars, and if the line is produced back to meet that for unsexed animals and males, it cuts the latter at a point equivalent to a body length of 6·3 mm. This is fairly close to the mean length (5·9 mm., with a range of from 4·9 to 7·5 mm.) of animals in the fifth instar, and suggests a sudden rather than a gradual response to the developing gonads. The "growth constant" (0·88) of this segment for unsexed animals and for males shows that it becomes proportionately narrower with increasing age, so that the widest region of the thorax moves back to the sixth thoracic segment.

Seventh thoracic segment.

The seventh thoracic segment of the newly hatched animal is very small: at the first moult it doubles in length and then thereafter up to and including the sixth instar has a growth constant of 1·15 compared with the total length minus the seventh thoracic segment. In the seventh instar the constant rises to 2·03.

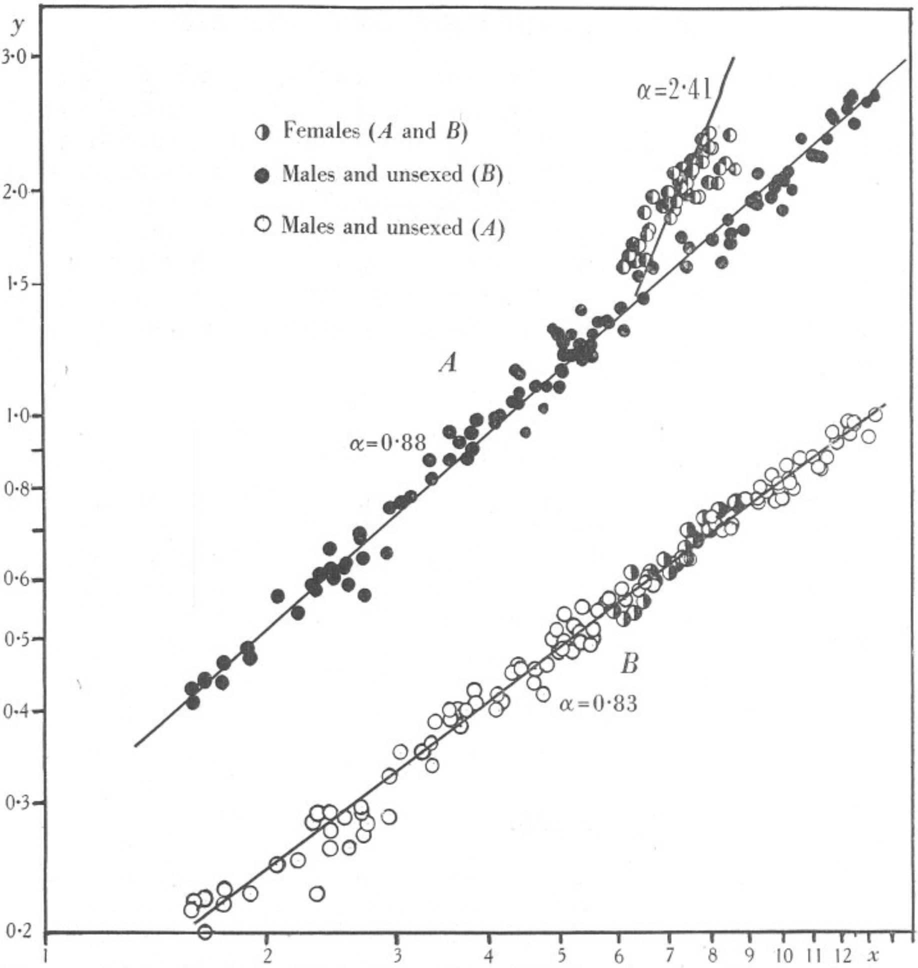

Fig. 11. A (upper line); breadth in millimetres of third thoracic segment (y) plotted against total body length (x). B (lower line); breadth of cephalon/2 (y) plotted against total body length (x). Both double logarithmic plots.

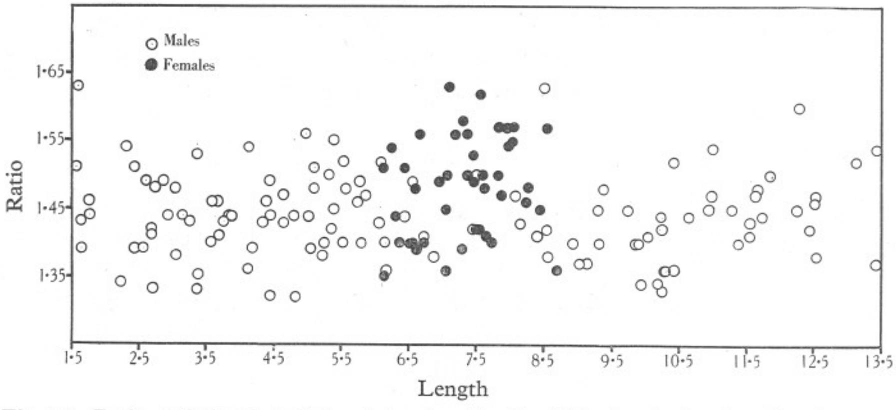

Fig. 12. Ratio cephalon breadth/cephalon length of individual animals plotted against total body length (anterior border of cephalon to posterior tip of animal) in millimetres, showing that the ratio does not change as the animal becomes bigger.

Cephalon (Figs. 11B, 12).

The cephalon becomes proportionately narrower and shorter with age compared with the rest of the body but the ratio length/breadth of its overall dimensions does not change although its anterior margin becomes convex anteriorly and there is some change in outline. The relatively enormous cephalon of the newly hatched *Idotea* is shown in Fig. 7*a*.

Abdomen (Figs. 10A, 13).

The proportion of abdomen to thorax plus cephalon does not change significantly after the second ecdysis. There is some evidence from the graph that in the first two instars the growth constant is less than 1 and while both the number of points and their wide scatter make it difficult to come to any definite conclusion, it is clear that this is correlated with the doubling in length of the seventh thoracic segment during the first post-embryonic moult. Following this, the abdomen grows isometrically with the cephalon plus thorax. Since this is so, and since the growth constant of the cephalon is 0·83, it follows that the thorax (morphologically the second to eighth thoracic segments) occupies an increasing proportion of the total length as the animal becomes older. The ratio breadth/length of the abdomen remains constant at approximately 1·9 throughout the whole post-embryonic period, hence the same growth constant is applicable to its breadth as to its length.

CHANGES IN SHAPE

Seventh thoracic segment (Fig. 7*c*, *d*, *e*, *f*; Table II).

I. viridis is hatched with a small, limbless seventh thoracic segment, as is usual in isopods. In instar II this segment becomes proportionately larger in all dimensions, doubling in length, although the total length of the animal is only 1·44 times as long as in instar I, and short limbs are present. It continues to grow at a rapid rate, increasing in size proportionately to the other segments at each instar until in instar V it is only a very little shorter than the longest thoracic segment. In the last instar it again shows a sudden proportionate increase in length.

Coxal plates (Fig. 7*c*, *c′*, *d*, *d′*, *e*, *e′*, *f*, *f′*).

As has been stated above, *I. viridis* is hatched with exceptionally large coxal plates (Fig. 7*a*, *c*, *c′*) and it is especially noteworthy that not only do they occur on the six posterior thoracic segments but that they are large and conspicuous on the first. In the second instar, the coxal plates of segment one are no longer visible from the dorsal surface but their lateral boundaries can still be seen on the ventral surface (Fig. 7*d′*). It is believed that they are still present in all later instars but fuse with the ventral side of the segment

(Fig. 7*e′*, *f′*, shaded area on first thoracic segment). The lateral expansions of the first thoracic segment would therefore be the pleura of that segment which have not been replaced by the coxal plates (Calman, 1909, p. 202). Clearly it is impossible to follow the behaviour of these coxal plates without histological examination. The coxal plates on thoracic segments two, three and four of animals of instar I are comparatively large; in the second instar they have increased proportionately in size and have become very convex laterally, but do not occupy the whole of the lateral margin of the segment (Fig. 7*d*). The pleura are small (Fig. 7*d′*). In instar III (Fig. 7*e*, *e′*) the coxal plates have become flattened and have diminished in size relative to their segments and the pleura have grown out laterally behind them. In instar IV (Fig. 7*f*, *f′*) the coxal plates have become more flattened, have extended to the anterior border of the segment, and the pleura are now flush with their outer borders. This is the adult condition and no further changes of any magnitude occur. The whole process can be summarized as initial growth of the coxal plates followed by a change in shape accompanied or possibly induced by later growth of the pleura. On segments five, six and seven the same process occurs except that there is no late growth of the pleura.

Antennule.

The changes in shape of the antennule of the successive instars are illustrated in Fig. 13*a*, I–VII. Apart from the gradual assumption of the adult form, the most obvious changes are in the flagellum. In instars I and II this latter is conical and bears only one aesthetasc. In instars III, IV, V and VI it becomes first of all cylindrical and then rounded at its antero-medial border; at the same time the number of aesthetascs increases from 2 to 3, 3 to 4 and 4 to 6 respectively. In the last instar the antero-medial margin becomes sloped and there are eight aesthetascs arranged in a series along the slope. The change in shape of the flagellum along its antero-medial border appears to be due largely to the increase in the number of aesthetascs. Round the base of each of these the exoskeleton appears to become flattened and hence when they cease to be confined to the tip of the flagellum and spread along its antero-medial margin, this becomes terraced.

Antenna (Fig. 8).

The changes in the number of joints of the flagellum of the antenna have already been discussed. The peduncle chiefly shows a thickening of the joints.

In the female, both antennule and antenna have the form characteristic of the sixth instar.

Maxillula.

The first maxilla possesses its characteristic number of spines from the first instar onwards but at first they may be smooth and they may not achieve the toothed condition characteristic of the adult until the fifth or sixth instar. This is variable.

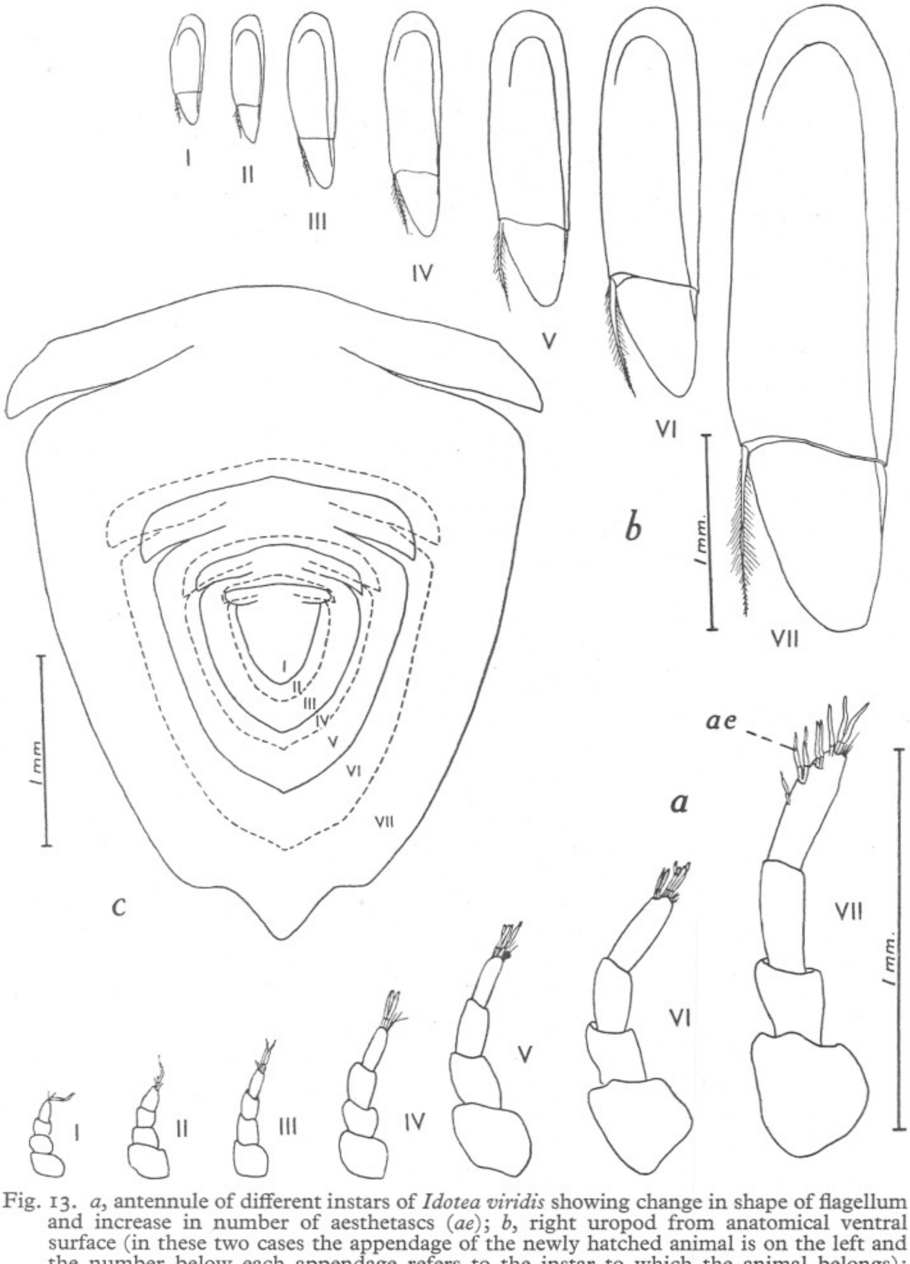

Fig. 13. *a*, antennule of different instars of *Idotea viridis* showing change in shape of flagellum and increase in number of aesthetascs (*ae*); *b*, right uropod from anatomical ventral surface (in these two cases the appendage of the newly hatched animal is on the left and the number below each appendage refers to the instar to which the animal belongs); *c*, abdomina of different instars, numbers indicating the instar, newly hatched animal in centre. The boundaries of the abdomina of alternate instars are dotted for clarity. The preparations were made by dissecting out pleopods and soft tissues and pressing abdomen flat.

Abdomen (Fig. 13*c*, I–VII).

The shape of the posterior part of the abdomen varies from instar to instar, as shown in the figure. The posterior tooth appears first in the fourth instar, the postero-lateral angles in the sixth instar and only in the seventh is the characteristic adult form assumed. The shape of the exoskeleton of the female abdomen is characteristically that of the sixth instar but the arrangement of pigment and of melanophores is usually such as to emphasize the postero-lateral angles.

Uropods.

Since these fold over on the ventral surface of the abdomen, forming the floor of a box enclosing the pleopods, it is to be expected that their changes in shape are correlated with those of the posterior fused segments of the abdomen. This is indeed the case (Fig. 13*b*, I–VII). The flattened posterior border of the uropods in instar VII is correlated with the fact that with the full development of the posterior tooth and lateral angles of the abdomen, the uropods do not form a floor on the ventral surface of the tooth, but run straight across from the transverse straight portion of the postero-lateral angles.

DISCUSSION

The data on the biology of *Idotea viridis* from New England Creek have been briefly discussed above and since they are not necessarily applicable to *I. viridis* from other habitats or to the genus in general, will not be further dealt with.

Relative growth in an isopod, *Asellus aquaticus*, has recently been investigated in detail by Needham (1937) and it is of interest to see how far his results aid in the interpretation of the less extensive data obtained with *Idotea viridis* and how far the relative growth of the two animals is similar. The data given here are for post-embryonic development only and would therefore fall during Needham's "later period of simple allometry" but it seems possible that persistent rhythmic variations in α occur in *I. viridis* as also in this period in *Asellus aquaticus* (Figs. 10, 11). The values of α in *Idotea viridis* compared with those obtained by Needham (Table II, p. 293) in *Asellus aquaticus* are as follows:

		I. viridis	*A. aquaticus* (Needham, 1937)
Cephalon breadth	Males and females	0·83	0·80
3rd thoracic segment breadth	Males and unsexed	0·88	1·06
	Females, instars V? and VI	2·41	1·15
6th thoracic segment breadth	Males, instars I–VI inclusive	0·99	1·06
	Instar VII	1·44	
	Females, instars I–VI	as for males	1·01
Abdomen width		1·00 (approx.)	1·00

On the whole there appears to be a general similarity in the results obtained with the two animals. The cephalon (head plus morphological 1st thoracic segment) breadth is negatively allometric in both species and the values of the growth constant surprisingly close. On the other hand, *Idotea viridis* becomes slightly slimmer or retains nearly a constant build up to and including the sixth instar, while *Asellus aquaticus* becomes slightly stouter.

There is considerable evidence that the changes in regional growth rates follow the same plan in both animals. The broadest region moves back in *Idotea viridis*, as in *Asellus aquaticus*, and similarly the sixth thoracic segment becomes the broadest in adult males, while in the females the broadest region moves forward at maturity to the third thoracic segment. Needham's (1937, p. 306) discussion on the question of the occurrence of a "metamorphic moult" has important bearings on the suddenness of the change in growth rate of the third thoracic segment in the female *Idotea viridis*. It does appear that in *I. viridis* there is in fact a "metamorphic moult", between the fourth and fifth instars, but the number of points above the line between total body lengths of 5 and 6 mm. (Fig. 11 A) suggest that the growth centre may neither move forward so rapidly as the diagram suggests, nor may the growth constant for the third thoracic segment be so high. This appears to be in agreement with the findings of Needham, but, since this worker does not claim that his groups correspond to instars, it is difficult to find an exact parallel in the two cases. There seems to be no sign of a corresponding sudden widening of the third thoracic segment in the male *I. viridis*, although there is in *Asellus aquaticus*, and Needham's fig. 4 gives no evidence of the sudden widening of the sixth thoracic segment in the male *A. aquaticus* such as occurs in *Idotea viridis*. Sudden changes in bodily form as the gonads mature appear to be characteristic of *I. viridis*.

As in *Asellus aquaticus* (Needham, 1937, p. 310), the difference in shape of the sexually mature *Idotea viridis* is correlated with their breeding habits. The brood pouch is developed in the region of the anterior thoracic segments, hence their wideness, and similarly the relative breadth of the fifth and sixth thoracic segments in the male is probably correlated with his carrying the female underneath his body while mating.

The data on relative growth show that the form of *I. viridis* does change from instar to instar and it is therefore not to be expected that if the volume is doubled at each moult the "Przibram quotient" will equal 1·26 in every case. Table I shows that in the earliest moults when the seventh thoracic segment is increasing rapidly, the quotient exceeds 1·26, in other words the animal lengthens in proportion to its volume; for the moults between the third and fourth, fourth and fifth, and fifth and sixth instars when other data show that there is little change in form, the quotient is very close to 1·26; while when the seventh thoracic segment again suddenly increases in length (Table II) between the sixth and seventh instar of the males, the quotient again rises. The values given in Table I, taken in conjunction with the other data, strongly suggest that the "law of geometrical progression" is applicable to the growth of *I. viridis*. There appear to be no similar data available for other isopods with which these results can be compared.

The changes which, while not easily lending themselves to measurement, are presumably caused by differential growth rates similar to those discussed above, are striking, especially those of the coxal plates (Fig. 7*c*–*f*′). *I. viridis* is hatched with large coxal plates on all the thoracic segments, except the maxillipedal, including the anatomical first, on which they are large and conspicuous. The coxal plate on the first thoracic segment later becomes indistinguishably fused with the pleuron in the adult. As growth proceeds, the relative size of the coxal plates is reduced, partly maybe owing to the proportionate increase of soft parts relative to exoskeleton setting up mechanical stresses which tend to pull them below the pleura, but also, at any rate in segments two, three and four, by the late growth of the pleura. Since in the Asellota the coxopodite is still movably articulated with the body (Calman, 1909), it might be expected that the fusion of the coxopodite with the body, and hence the formation of coxal plates, took place late in the phylogeny of the Isopoda and that the coxal plates would appear late in ontogeny. If so, apparently the evolutionary process has become reversed and, while originally lateral growth of the pleura preceded growth of the coxopodite, it now takes place the other way round.

The increase in the number of joints in either the antennule or the antenna during growth is probably of widespread occurrence in Crustacea where one of these appendages is very long. Schellenberg (1938) has recorded an increase in the number of joints of the outer flagellum of the antennule of *Niphargus tatrensis* var. *aggtelekiensis* similar to that found in the antenna of *Idotea viridis*. The changes in the antennule and maxillula are of the type that might be expected to occur during growth and are regular throughout. On the other hand, the abdomen and hence the uropods assume their final characters somewhat suddenly. Bate & Westwood (1868, vol. II, p. 381) observed the "comparative paucity of the articulations of the lower antennae in young individuals" of *I. tricuspidata* (now *I. baltica* (Pallas)) and that animals from the British Museum collection exhibited "variations in the form of the terminal segment of the body, with the length and size of the respective specimens". Again, Tattersall (1906, p. 48) in discussing *I. baltica* states that "Young examples of this species are difficult to distinguish from such species as *I. pelagica*, *I. granulosa* and *I. viridis*, in which the telson has a very similar shape". On the other hand, Collinge (1917, p. 728) states that he found very few variations in the form of the antennae, apart from the number of joints in the flagellum, or in the shape of the terminal segment of the body, and Sars (1899, p. 81) found the terminal segment in *I. baltica* "to be pretty constant even in very young specimens". No data on the development of an idoteid other than those given in this paper appear to be available, but, on the grounds that positive evidence outweighs negative evidence, there is reason to believe that changes in shape of the abdomen occur during growth in *I. baltica* as they do in *I. viridis*, to which species the observations of Bate & Westwood are decidedly applicable since the shape of the abdomen changes considerably during growth (Fig. 13). How far these observations can be further extended to other species remains to be seen.

Huxley lays great stress on the important bearing of allometry on taxonomy and states (1932, p. 204) "systematists are agreed that mere size differences may have no taxonomic significance...but they usually attach much greater importance to differences in the *percentage* size of parts. Our studies of heterogony (i.e. allometry), however, make it obvious that such differences may have precisely as little taxonomic significance as those in absolute size." If one includes under allometry all the changes observed during the post-embryonic development of *I. viridis*, the relevance of Huxley's remarks is at once apparent. Collinge (1917, p. 737) uses body shape, width of coxal plates and shape of abdomen in his key to the British species of the genus *Idotea* and in this paper it has been suggested that the first and the last of these be employed as diagnostic characters of *I. viridis*: it has, however, been shown above that not one of these characters is constant throughout the whole post-embryonic life of *I. viridis* and, since little is known of the early instars of closely related forms, it may prove difficult to distinguish between them. Further, additional characters such as the shape of, and number of aesthetascs on, the flagellum of the antennule vary from instar to instar, as do the number of joints in the flagellum of the antenna. Only the ratio breadth/length of the cephalon and the number of spines on the outer lobe of the maxillula emerge as constant throughout the whole period.

It is quite easy to see that if males and females of *I. viridis* became sexually mature either one instar earlier or later than they do in New England Creek, they would differ from the New England Creek adults in general appearance or "habitus", in the proportions of many parts and in the shape of the abdomen. There is evidence that a process of this nature does occur. Adult males of *I. viridis* from different localities may vary in body length from 9 to 23 mm., although 12 mm. appears to be the commonest length (Collinge, 1917, p. 729).

The change in shape of the abdomen has been investigated in specimens from Mr Spooner's and my collections (Fig. 14); the results show that in small adult males, while the postero-lateral angles of the abdomen do appear to be considerably less marked than in larger forms, the median tooth is much less affected, although in the smaller forms (*a* and *b*) there is some tendency for the abdomen to be similar in shape to that of the fourth and fifth instars of the New England Creek form. It has proved to be possible to arrange the abdomens in series according to the development of the postero-lateral angles and a comparison between Figs. 13 and 14 shows that this follows fairly closely the ontogenetic sequence. The correlation between size and shape is not very clearly marked but close correlation is not to be expected

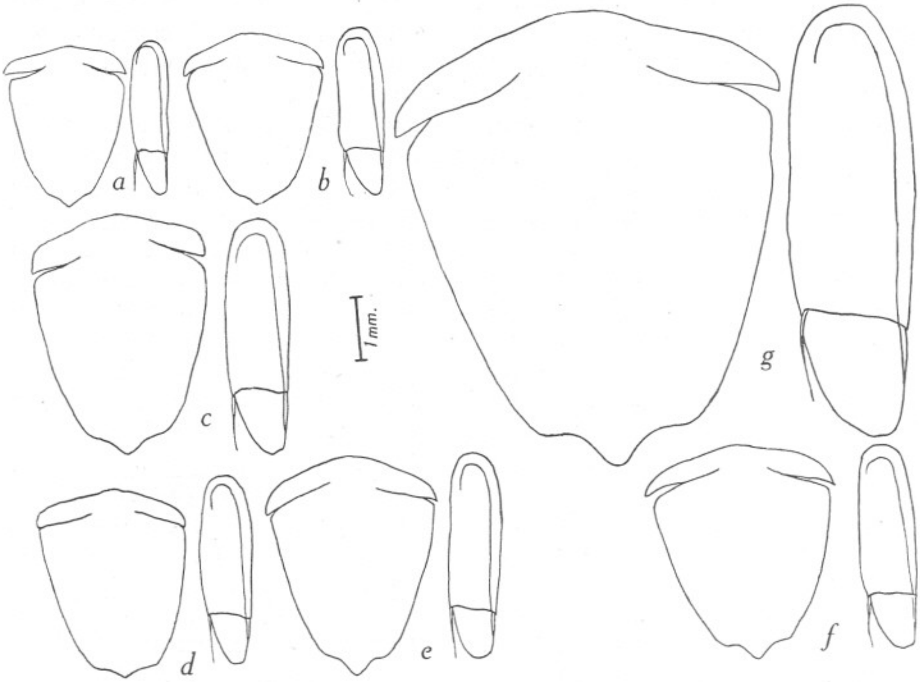

Fig. 14. Abdomens of adult male *Idotea viridis* from various localities arranged in series to illustrate the different degrees of development of the postero-lateral angles. Right uropods showing correlated changes in shape are included. The abdomens have been prepared by flattening after removal of pleopods and tissues. In the following table, when animals from two localities are entered under the same letter, the first only has been drawn, since the abdomens from males from both sources are similar.

Abdomen and uropod.	Source	Length of mature ♂ mm.	Collected by
a	Wivelscombe, High Marsh, Tamar	9·0	G. I. Crawford
b	Ernesettle Flat, Tamar	9·5	G. M. Spooner
	Langton Herring, Fleet	11·0	,,
c	Zostera pools, Maplin Sands, Essex	12·0	N. H. Howes
	Brackish pool inside sea wall, Holbeach, the Wash	14·6	G.M.S.
d	Lodmoor, Weymouth	10·0	,,
e	Thorn Point Causeway, Tamar	11·4	,,
f	New England Creek, Gt Wakering, Essex	12·0	N.H.H.
g	Brackish pool inside sea wall, near Cupid's Corner, Gt Wakering, Essex	22·2	,,

The localities in the Tamar Estuary mentioned above will be found in a map given by Hartley & Spooner (1938).

since comparatively few specimens were available and the range of size in each instar is considerable (Table I). It must be concluded that although the attainment of sexual maturity at a small size, or at an earlier instar than usual, i.e. paedogenesis, causes considerable difference in abdomen shape, other factors, possibly those effecting the precocious onset of sexual maturity, have also some influence on bodily form, for instance, by inducing the early appearance of the median tooth without altering the time of appearance of the postero-lateral angles.

I have pleasure in acknowledging my indebtedness to Prof. H. G. Jackson for much advice, to Mr G. M. Spooner for suggestions and for the loan of much material, to Prof. D. M. S. Watson, F.R.S. for his interest and encouragement and to Mr F. J. Aumonier for technical assistance in the examination of the aesthetascs. The collecting apparatus used in this work was obtained through a grant from the Government Grants Committee of the Royal Society.

SUMMARY

A local race of *Idotea viridis* from south-east Essex is described and its classification as *I. viridis* discussed. This animal is a nektonic form and so far has only been found in New England Creek in south-east Essex. The biology and post-embryonic development of this race is described. Quantitative samples were taken of the animals present in the creek in 1934, 1935 and 1936. *I. viridis* was rare in the first five months of the year; numbers rose to a maximum in late June and July and remained comparatively high until the end of October.

The number of young in the brood pouch is very variable. There is some evidence that the number of fertilized eggs was reduced by the expulsion of embryos before their development was complete. The newly hatched *I. viridis* is described.

There are six ecdyses between hatching and maturity in the males and four or five in the females. The "Przibram quotient" has been calculated between successive instars and there is evidence that the "law of geometrical progression" may be applicable to growth in *I. viridis*.

Changes in external form during post-embryonic development have been investigated by studying the allometry. Changes in shape not susceptible to measurement are also described. The shape of, and number of aesthetascs on, the flagellum of the antennule change from instar to instar. The number of joints on the flagellum of the antenna also increases progressively. The shape of the abdomen changes and the adult form is only achieved in the last instar of the males. The number of spines on the outer lobe of the maxillula and the ratio breadth/length of the cephalon remain constant throughout the whole post-embryonic development.

The taxonomic significance of the changes in shape and in proportions is discussed with reference to collections of *I. viridis* made in other parts of England.

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NOTES ON THE BIONOMICS OF THE TUBE-BUILDING AMPHIPOD, *LEPTOCHEIRUS PILOSUS* ZADDACH

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(Text-figs. 1–3)

CONTENTS

INTRODUCTION

It is well known that amphipods belonging to several different families live habitually in nests or burrows built for this purpose. These tubicolous species are all more or less adapted for the construction of tubes, while many have other modifications associated with their feeding habits, which are necessarily rather different from those of the more numerous free swimming species. An inquiry into the natural history of the small brackish water amphipod, *Leptocheirus pilosus* Zaddach, disclosed habits that appear somewhat different from those of the other tubicolous species which have been investigated.

L. pilosus has been recorded from brackish or almost fresh water on most of the coasts of Europe, from the Baltic to the Mediterranean. In England the records are sparse, but the species seems fairly widespread, and probably occurs in most of the estuaries of the British Isles. The whole of the material used in this work was obtained from a sluice pond at Keyhaven near Lymington in Hampshire, which, in September, contained about one-quarter sea water, but which is probably subject to considerable variations. The animal was abundant here, together with the other characteristic brackish water amphipods, *Corophium insidiosum* Crawford and *Gammarus zaddachi* Sexton. It can be kept in the laboratory without much difficulty in shallow dishes containing the alga *Chondrus crispus*, among which it lives, together with a quantity of the detritus from the bottom of the pond. The water in these dishes should not be more than 2 or 3 in. in depth, and overcrowding must be avoided, as the amphipod will only live in well oxygenated water.

Leptocheirus belongs to the family Photidae, the majority of which are known to be tubicolous. It is rather smaller than most of the Amphipoda, rarely exceeding 4 mm. in length, while the characteristic lateral compression of the body has, to a considerable extent, been lost. In a fully grown male the distance between the lower edges of the coxal plates of each side of the body is equal to about a third of the length from head to telson.

When alive the animal is a dark reddish brown on top, while ventrally it is much paler. This colour is due to the presence of a number of large much-branched pigment cells, dark red in colour, situated on the back, the mesosome, and the upper part of the coxal plates. They are fewer on the metasome and urosome, and entirely absent from the appendages, with the exception of the posterior margin of the basal (1st) joints of the last three pairs of peraeopods. These pigment spots are of the type observed by Holmes (1901) in *Amphithöe longimana* Smith, except that the large pale green cells found in this animal are not present in *Leptocheirus pilosus*. Besides the pigment cells the animal is coloured by the gut, which is usually black and opaque; by the gonads, especially prominent in the female, which are yellow or orange in colour; by the eggs, which are dark brown while being carried in the brood pouch; and, in most, by an opaque white patch, which appears to be in the chitin, extending from behind the head to the third or fourth segment of the mesosome. The eye is black.

There is a certain amount of variation in colour among individuals, and they appear to possess the power of altering their colour slightly, but to an even smaller extent than does *Amphithöe longimana*. Several animals were kept in a dish containing part of the thallus of *Ulva* in clear water. After three days the pigment cells were still expanded, although since the gut was empty, the animals were rather paler than at the beginning of the experiment. On the fourth day they all died, probably from lack of suitable food. After death, and particularly in specimens preserved in alcohol, the colour fades considerably, though the pigment cells appear to remain uncontracted. When out of water the body, and especially the coxal plates, has a red and green iridescence. The chitin of the exoskeleton is hydrofuge, and an animal which has become caught in the surface film is unable to break it. This is perhaps due to the mucus secreted from the dermal glands on the coxal plates.

Morphologically the whole genus has been described in detail by Sexton (1911).

THE CEMENT GLANDS

The presence of glandular tissue in the first and second pairs of peraeopods* of many amphipods was first recognized by Smith (1874), who suggested that they secreted the cement used in tube building. He also noticed that they communicated to the exterior through a pore at or near the tip of the

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* In this paper the nomenclature of Stebbing (1906) is followed, and the first pair of peraeopods is accordingly that immediately posterior to the gnathopods, and attached to the third free segment of the body.

claw. Calman (1909) states that dermal glands are found in the Ampeliscidae on most of the appendages, and in the Talitridae scattered all over the body. Claus (1879) found unicellular glands scattered all over the body of *Phronima*. Holmes (1901) and Skutch (1926) both describe glands in the first and second peraeopods of two tubicolous species of *Amphithöe*, and have observed a silken thread emerging from a pore in the tip of the claw. Neither of them describe any dermal glands in other parts of the body.

The glands in the peraeopods of *Leptocheirus pilosus* are shown very well when stained in haematoxylin, and cleared (Fig. 1*a*). The two main glands are in the basal (1st) joint, running its whole length, one on each side of the muscle. There is also a smaller one behind these two. They join together distally and pass through the ischial (2nd) joint, where they are slightly expanded, into the meral (3rd) joint in whose proximal end they are again expanded. Besides these there are two smaller pieces of glandular tissue in the distally dilated ends of the meral (3rd) and carpal (4th) joints. All these glands connect (probably by some sort of a duct, though none can be seen) with the canal which runs along the upper edge of the claw, to open at a pore in its truncated tip.

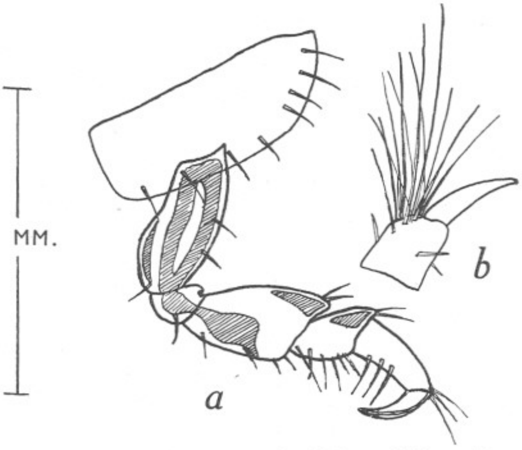

Fig. 1. *a*, Second peraeopod of *Leptocheirus pilosus*; glandular tissue shaded. *b*, Distal end of palmar joint, and claw, of peraeopod seven.

Besides these glands in the first and second pairs of peraeopods, dermal glands are found scattered over the body, being especially numerous on the coxal plates. These glands probably secrete a mucus cement in the same way as do the similar glands in the Talitridae.

THE CONSTRUCTION OF THE TUBE

Three different types of amphipod tube have been described, and that of *Leptocheirus pilosus* belongs to a fourth.

The first type is the Sand Burrow, and the least specialized sand burrows are built by members of the family Talitridae. These have dermal mucus glands scattered all over their bodies and as the animal burrows, the sand grains lining the burrow are probably smeared by this mucus, and hence stick together, preventing the walls of the burrow from caving in. Reid (1938) has described the digging habits of *Talorchestia deshayesii* (Audouin), and in this species at any rate the burrow is not permanent, but is filled up immediately behind the animal. Hence the mucus secreted on to the sides of the burrow can never harden into a permanent cemented wall.

Hunt (1925) gives a brief account of the tube of *Ampelisca*, which is of rather a more permanent nature. The animal burrows into the sand, and curls up just below the surface, with the head and antennae projecting out of the entrance. A cement is secreted from the dermal glands, which are on all the legs, and this becomes mixed with the grains on the inside of the burrow, eventually hardening into a purse-like tube in which the animal lives. No form of silk is produced and indeed it would scarcely be possible for the sand grains to be bound up by a silken thread. A similar type of semi-permanent sand burrow has been described in *Microprotopus maculatus* Norman by Schellenberg (1929). These tubes are still burrows, being supported on all sides by the surrounding sand.

The second type is the Mud Burrow, as described by Hart (1930) in *Corophium volutator* Pallas. The wall of the U-shaped burrow is strengthened and prevented from falling in by a secretion of mucus from the glands, which is mixed with the mud as the animal burrows through it. This ultimately hardens. Here again no silken thread was observed. This burrow is semi-permanent and is kept open while it is inhabited. Hart also notes that under some circumstances *Corophium volutator* can build a clumsy and fragile tube of mud and algal fragments, instead of the more usual burrow below the surface of the mud. In *C. insidiosum* I have noticed that a similar tube is the one normally used. This species does not appear to burrow.

The third type is a true tube built of comparatively large pieces of material, dead vegetation, living algal filaments and small stones, all bound together by strands of the so-called "amphipod silk". This type of nest was found in two species of *Amphithöe* by Holmes (1901) and Skutch (1926), and is probably the most highly specialized. The only cement glands are those in the first and second pairs of peraeopods, and from the pores in the claws of these legs is spun a definite silken thread, which is fixed to the nest-building materials while still adhesive, later hardening. The finished nest consists of a bundle of algal filaments, etc., bound together by silk, down the middle of which runs the cylindrical tube lined by a silken web.

The tube of *Leptocheirus pilosus* belongs to a fourth type, and, as far as specialization is concerned, lies between those of *Corophium* and *Amphithöe*. *Leptocheirus pilosus* belongs to a family which is probably related to the Amphithoidae, though its members are in general not so specialized for a tubicolous existence.

This tube is a flat blister-like capsule fixed to the smooth surface of the thallus of *Chondrus crispus*, or to the upper sides of smooth stones. If the animal is kept in a glass vessel it will build its tube against the glass. The capsule is slightly longer than the animal which lives in it and rather more than half as broad as long. It is open at both ends, and the area of the mouth is considerably less than that of a cross-section through the tube in the middle, leaving an exit just large enough to allow the animal to get out. The wall of the tube is built of microscopic particles of detritus, mud and sometimes a very few short lengths of red or green algal filaments seldom more than five cells long. These are all cemented together into a relatively tough, thin and flexible wall fixed to the surface by its edges. The inner wall of the tube is formed by the bare surface against which it is built, and there is no lining of mud on this side. Thus if the tube is built against a piece of glass, the animal in the tube can be observed under natural conditions through the glass, which would not be possible with any of the other amphipods which have been examined. There is no question of a tube of long algal filaments bound together by "amphipod silk", as is found in *Amphithöe*.

When *Leptocheirus pilosus* is put into a vessel containing some of the detritus from the bottom of its pond, it will swim about for five minutes or so immediately above the bottom, trailing its last three pairs of peraeopods in the mud. Detritus becomes entangled in the long hairs on the distal ends of the palmar (5th) joints of these limbs (Fig. 1 *b*), and is probably mixed with mucus, which covers the body. It is not, however, secreted from any pore in the claws of these posterior peraeopods. Soon the animal has a short trail of particles, perhaps a millimetre long, attached to these setae. It then settles down, usually in an angle of the dish where the mud lies thickest, and clears a patch for itself by the powerful beating of the pleopods. Next it lies on its side and begins to build the tube, starting with the trails of mud that have been collected on the posterior peraeopods. These are manipulated by the two anterior pairs of peraeopods and also by the gnathopods, and are fixed to the glass by a mucus cement secreted from the pore at the tip of the claw. No silken thread can be seen emerging from this pore, as in *Amphithöe*.

While building the nest the animal frequently turns through 180° to face the other way and goes on building on the other side, until soon it lies within a belt of mud particles cemented loosely together, and fixed above and below its body to the glass. Meanwhile the pleopods are beating strongly in metachronal rhythm, creating a steady current. The flow is from head to telson below the body of the animal, down the space enclosed on either side by the deep coxal plates which are further apart than in most amphipods.

Any particles of suspended detritus near the head of the animal are drawn in under its body and caught in the sieve setae on the second gnathopod, in the same way as is the food, as described further on. These particles are combed off by the claw and palmar joint of the same gnathopod and are worked into the incipient tube by the first and second peraeopods. The animal is continually somersaulting round to face in the opposite direction, and the particles caught are thus packed on to the tube at each end alternately. This packing is done by the first and second pairs of peraeopods which are especially adapted for the secretion of cement from their hollow claws. Since the particles are worked by these claws, they will be well covered with cement which later hardens, sticking them together.

Under natural conditions the water in the pond is not still, either flowing out rapidly through the sluice, or on the flood tide, coming into the pond from the river at the other end, or leaking back through the faulty sluice gates. It therefore carries a considerable amount of suspended matter which would normally soon sink to the bottom, and hence there is an adequate supply of suitable building material in the water flowing over the *Chondrus* plants which grow, several inches above the bottom, on the stone walls leading up to the sluice gates. It is here that *Leptocheirus pilosus* lives and builds its nests. It will not live in foul black mud and does not build its nest on the undersides of stones lying in the mud, neither does it burrow.

The time required to complete the tube depends on the amount of building material available. With an adequate supply it is usually finished in about half an hour.

A newly made tube is comparatively loose and fragile, especially on the outside, where the particles of detritus are not firmly cemented together. More suspended particles are continually sieved out of the respiratory stream by the setae on the second pair of gnathopods, and these, together with the faeces, are worked into the wall from the inside by the anterior peraeopods. More cement is all the time being secreted on to the lining of the tube, both from the peraeopods, and from the dermal glands in the coxal plates which are in contact with different parts of the tube whenever the animal changes its position. The loose outer layer is soon washed away by the stream which, at some states of the tide, flows rapidly over it. Thus the finished tube, which may take a day or two to build, consists of the lining of the original one, composed of small particles of mud, tightly packed and cemented together into a tough and flexible wall well able to withstand the ebb tide which may flow over it with considerable force. It is not called upon to survive wave action.

This tube may be compared to the lining of the burrow of *Ampelisca* or *Corophium*, which is above the surface of, and not supported by the surrounding mud or sand. It is stronger than the lining of a burrow, though still composed of mud particles packed and cemented together by the dermal glands. No silken web is developed and for this reason the tube is more primitive than that of *Amphithöe*.

A series of experiments was conducted to find what use was made of different building materials. Four types were investigated.

(1) Natural detritus. The tubes were built in under half an hour.

(2) Detritus dried by combustion. As above.

(3) *Ulva* thalli. Where a large piece was provided the animals would, after several hours, come to rest in the wrinkles on the surface. Where there were many small shreds of one square millimetre or so, they would be collected together in a corner of the dish, the animal lying underneath the

pile. After a day or more some of the pieces are very loosely bound together in a thin mucus film.

(4) Silver sand. The specimens, after several hours, settle down on the sand and loosely cover themselves with grains. After a day the sand grains cohere very loosely, and if the "tube" is detached from the glass it can be towed about in the water by a pair of forceps without coming to pieces. It will not support its own weight out of the water. When examined under the microscope the sand grains are seen to be held together on a net of very fine, irregular and anastomosing threads. These are probably secreted from the claw ducts on the 1st and 2nd peraeopods alone, and the fragility of the structure is due to the fact that the grains are too large to be cemented together by the secretions from the coxal plates.

If the first and second pairs of peraeopods are removed from an animal it will live for several days, but is unable to build a tube. The glands on these legs are therefore essential for the building of the tube, and the first loose covering is probably cemented by mucus from these alone. Only at a later stage, in the formation of the tough permanent wall, are all the cement glands used to bind all the particles firmly together. As a control, some specimens were observed, from which the fourth and fifth pairs of peraeopods, which carry no cement glands, had been removed. These were able to construct normal tubes, and it follows that the shock of removing four legs would not be sufficient to account for the inability of the specimens in the previous experiment to build their tubes.

Position in the Tube

If the bottom of a glass dish is covered with microscope cover-slips, and the dish is then filled with muddy water and a number of *Leptocheirus pilosus*, the animals will soon have built their nests on the cover-slips which may then be turned over, the four corners being supported with plasticine. The animal can now be observed in its tube under conditions which are much more natural than would be possible with most other tubicolous amphipods, which surround themselves with complete cylindrical walls of opaque materials.

L. pilosus lies with its head just inside the mouth of the tube, with the antennules waving about in the water outside. The antennae are generally held motionless, and pointing downwards. In the female the meral and carpal joints of the first gnathopod are horizontal, and at right angles to the first two joints. The gnathopods are small, and are not crossed. They are in constant movement and help to convey food to the maxillipeds and thence to the mouth. Even when there is no food, the claw is continually opening and shutting. The first gnathopods of the male, which are very long, are tightly crossed under the head (Fig. 3). The palm often projects just out of the mouth of the tube while the limb is practically still. The claw and palm are too far from the mouth to be of any assistance in feeding. They are indeed a hindrance to the animal when changing its position in the tube, and I know of no other genus of Amphipoda which carry their gnathopods in this way. No suggestion is put forward to explain the reason, if any, for this position. In other respects the position in the tube is the same for both sexes (Fig. 2). The body is arched, the mesosome being above the head, while the urosome is held vertically downwards, with the uropods touching the floor of the tube. The peduncle of the first pair of uropods is at an angle to the others, and its rami point forwards. The first two pairs of peraeopods have their claws and carpal joints resting on the floor of the tube, and are never at rest, always stamping at it. The second gnathopod has its first two joints vertical, with the remainder horizontal. Its position is more fully described below. The last three pairs of peraeopods are bent back over the metasome, in the position characteristic of so many other amphipods, with their claws hooked into the wall of the tube. About once every thirty seconds, or as often as the animal is alarmed, the metasome is still further arched, withdrawing the head, while the peraeopods push against the tube, causing the animal to turn a neat backwards somersault so that its head appears at the other entrance. The same reversal of position has been described in *Amphithöe*.

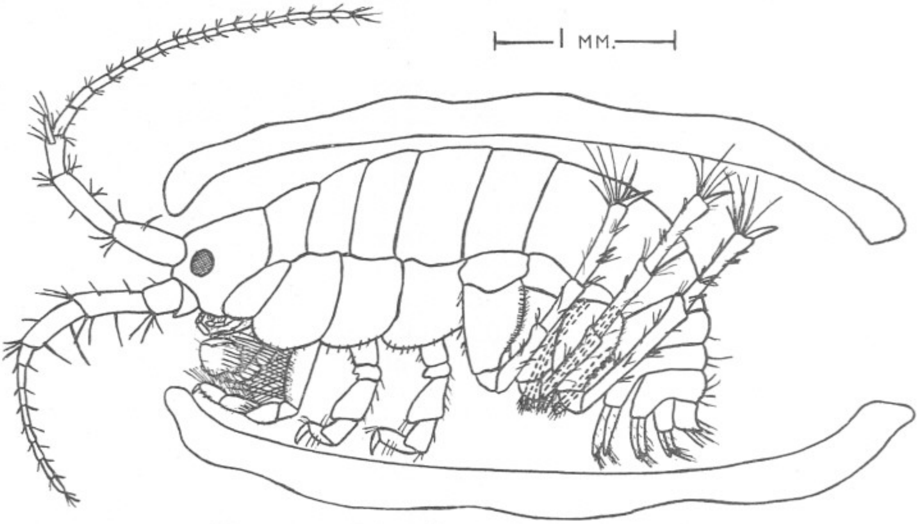

Fig. 2. *Leptocheirus pilosus* ♂. Position in the tube.

FEEDING HABITS

Hart (1930) and others have recognized three types of amphipod feeding:

(1) Biting, e.g. *Talitrus*, *Lysianassa*, *Amphithöe*, etc.
(2) Selective deposit feeding, e.g. *Corophium*.
(3) Filter feeding, e.g. Haustoriidae.

Leptocheirus pilosus belongs to the second class. Its mouthparts are in all essentials similar to the biting mouthparts of *Amphithöe*. The mandibles have molar processes, and the other parts do not have the specialized fringes of pinnate setae described by Dennell (1933) in *Haustorius arenarius* which is a true filter feeder. Any feeding current is not produced by the mouthparts. A stream of water is, however, induced to flow from the head to the telson by the beating of the pleopods. Since the animal is in a tube, the water cannot flow in at the sides and a strong current through the entrance is thus produced. Any solid particles are sieved (this is a better word than filtered) out of the stream by a special arrangement of setae on the second gnathopod.

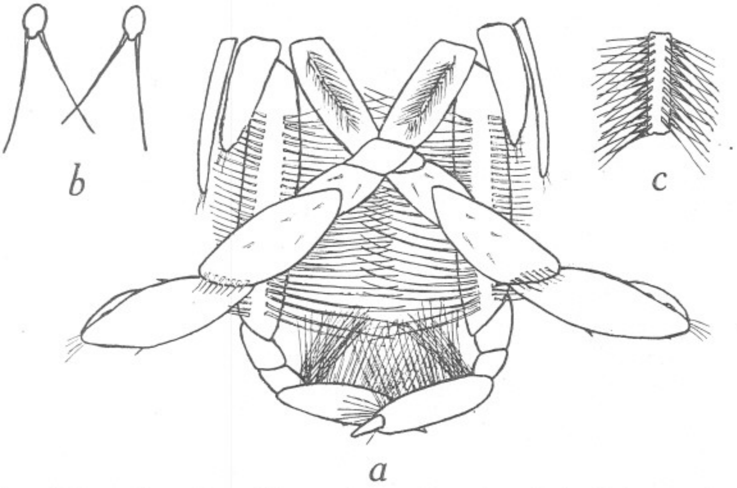

Fig. 3. *Leptocheirus pilosus* ♂. *a*, First and second gnathopods in their normal positions seen from in front, to show the arrangement of sieve setae. *b*, Diagram of a transverse section through the basal joint of the second pair of gnathopods, showing the two rows of sieve setae. *c*, Part of a sieve seta. Magnified.

On the anterior face of the basal (1st) joint of this limb there are two rows of very long feathery setae. The outer row is parallel with the walls of the tube, while the inner points inwards at an angle of about 30° (Fig. 3*b*). The ends of the setae of this inner row cross in the middle with those on the opposite gnathopod forming a fine sieve through which the stream flowing down the tube must pass. There are single rows of similar setae projecting upwards and inwards from the horizontal meral and carpal joints, and there is thus an enclosed space behind the first pair of gnathopods and under the mouth. This is closed posteriorly by the two inner rows on the basal joints, laterally by the outer rows on these joints, and dorsally and ventrally by the head, and setae of the meral and carpal joints respectively. Any solid suspended matter in the food stream is caught in this space. Periodically the second gnathopod is bent at the ischial joint, and the distal segments are lifted up into the space between the two rows of setae on the basipodite. Any particles caught in these setae are combed off into the enclosed space by those on the distal joints of the limb. The detritus in this space is turned over by the maxillipeds, assisted by the first pair of gnathopods in the female, and any suitable particles are transferred to the mouth. This method of food collection, for which the term sieve feeding is proposed, is quite different from the filter feeding of *Haustorius arenarius* and many other animals. In the latter, water having various food particles in suspension is drawn through the mouth appendages, which are highly specialized, both creating the feeding current and performing the filtration. In the sieve feeder the creation of the feeding current and the extraction of food particles from it are performed by appendages other than those of the head, and the food materials so collected are offered to the mouthparts as a loosely concentrated mass of detritus, in much the same state as is the food of any other selective deposit feeder. The mouthparts are in no way specialized for filter feeding and a sieve feeder, such as *Leptocheirus* could not be classed with *Haustorius*, but rather with *Corophium* and the other selective deposit feeders.

Besides sieve feeding *Leptocheirus pilosus* can also behave as a normal selective deposit feeder. If any large mass of detritus is perceived near the mouth of the tube by the sensory antennules, the animal will partially emerge from the tube, grasp at it with the antennae, which have long and stout setae on the ventral sides of the peduncular joints, and draw it into the mouth of the tube. In neither sex are the gnathopods used to grab at food, as in *Amphithöe*. This method of collecting food was often seen in animals in the tubes on the bottom of a glass dish, where there was plenty of detritus lying round the nest. It is probably used by the occupants of nests on flat stones near the bottom of the pond, but when the tubes are on *Chondrus crispus* some distance above the bottom, large masses of food which could be taken in by the antennae would not be common, and it is probable that the sieve method is then the most usual.

Leptocheirus pilosus never gnaws at such algae as *Ulva* or *Enteromorpha*, as does *Amphithöe*. It will die if it is put in a dish containing nothing but such an alga. The contents of the gut consist entirely of very small particles, diatoms and very small lengths of algal filaments being recognizable.

The feeding habits of *Leptocheirus pilosus* are thus entirely different from those observed by Holmes and Skutch for *Amphithöe*, which has no adaptations for sieve feeding, and is often carnivorous. Hart describes a very much more simplified form of sieve feeding in *Corophium volutator*, which possesses a single row of setae on the distal joints of the first pair of gnathopods; but *Corophium* is primarily a true selective deposit feeder, and does not use these setae to any great extent.

Most of the material which enters the space in front of the sieve setae is not used as food. Part of it is worked into the walls of the tube by the first and second peraeopods. The rest is left behind when the animal somersaults round, and is then kicked out of the entrance of the tube by the urosome, which appears at this end. No solid particles are allowed to get past the second gnathopods, and so the tube is kept clear of particles which might, in very dirty water, block it up. The faeces are disposed of in the same way.

The tubes on the *Chondrus* fronds are exposed for several hours at each tide. The animals remain in them, and water is held in the tube by capillarity. When thus exposed the pleopods beat gently, keeping up a circulation of the water in the nest, which is thus able to aerate the branchial lamellae with the oxygen which presumably diffuses into the water at each end of the tube. The antennae and antennules are withdrawn, and the animal no longer performs its frequent changes of position. If it is removed from its tube when out of the water it is not able to break the surface film when the *Chondrus* plant is reimmersed. Under natural conditions however, it remains in its tube, and is thus never out of water.

Leptocheirus pilosus is able to swim quite efficiently, unlike *Amphithöe* which, if removed from its nest, will lie on the bottom, grasping at any solid object which is there. *Leptocheirus pilosus* will usually, however, enter a vacant tube, or build a new one as soon as possible. It may eject another individual from its tube, if it can enter the tube from behind before the occupant has time to reverse its position and face the intruder. If it can get in from behind, the rightful owner is easily pushed out. It cannot, however, be ejected if it is facing the intruder. *L. pilosus* will seldom leave its tube spontaneously, though a few individuals can be caught if a net is drawn gently over the *Chondrus* plants. Since any disturbance causes the animal to retreat further into its tube, these few must probably have been swimming about freely.

Breeding Habits and Life History

No attempt to go very deeply into this question was made, and further work on the breeding habits of amphipods in general is contemplated. In the meantime the few observations which were made may be recorded.

Ovigerous females were obtained from the beginning of April till September inclusive. These on the average carried eleven eggs each, and very few individuals were found with more or less than this number. The number is just half that given by Chevreux, but this may be due to climatic or other factors.

There was a great preponderance of females over males during the whole time the species was under observation, and the relative numbers did not appear to fluctuate much. A collection of fifty-eight made in August contained seven males and thirty-six females, with fifteen too young for their sex to be recognized.

Two adult individuals were never observed together in one nest, and indeed there would not be enough room in any ordinary one. According to Skutch a male and female *Amphithöe rubricata* are often found together. Only one copulating pair was observed, and this was outside the nest. It was examined under a hand lens, and the male seemed to be holding his mate with the second gnathopods and anterior peraeopods. The first gnathopods were crossed under the body in the usual position, and were not used in the clasp. The peculiar carriage of these limbs does not seem to be explicable as a clasping adaptation. The pair separated when being transferred to another dish for further observation under the microscope, and this would seem to cast some doubt on whether they were really copulating, since the clasp is usually extremely tenacious.

In some species of Amphipoda, *Haustorius arenarius* investigated by Dennell (1933) and *Corophium bonelli*, by Crawford (1937) and a few others, the male is unknown, and reproduction is presumably parthenogenetic. No experiments were undertaken to determine whether this was so with *Leptocheirus pilosus*, either as a general rule or occasionally, but the single and rather doubtful observation of a copulating pair, together with the disproportionate sex ratio suggest that parthenogenesis may occur. In most amphipods clasping pairs are very noticeable throughout the breeding season.

As in most species the young remain in the brood pouch until after the first moult, while they remain in the tube of the mother until the second, and possibly later. On leaving they soon construct their own tubes. Moulting is normal, the animal emerging from a dorsal crack just behind the head and leaving the old skin otherwise unbroken. The moult always occurs outside a tube, and if the animal has grown during the process it probably builds a completely new tube.

Conclusion

In its general behaviour *Leptocheirus pilosus* occupies a position between *Corophium volutator* and *Amphithöe rubricata*. It is able to swim efficiently, unlike *A. rubricata*, but it appears that it does not habitually leave the nest, as does *Corophium volutator*. The nest is a true one, not a mere burrow in the mud, but in its cementing it resembles the burrow of *C. volutator*. The secretion of silken threads for the construction of the nest is not found, and the cement glands are not confined to the first two peraeopods, as in *Amphithöe rubricata*.

The sieve-feeding mechanism is an interesting adaptation to the tubicolous existence, which does not appear previously to have been recorded in the Amphipoda. In this respect *Leptocheirus pilosus* is probably more highly specialized than are the other tube-building forms. *Amphithöe* has both pairs of gnathopods grasping and subequal, and seizes the food with them in the same way as do such free swimming genera as *Gammarus*. It must partly emerge from its tube in order to grasp at passing food masses, and has no means of feeding while it is inside the tube. *Corophium volutator* has a single row of primitive sieving setae on the posterior border of its first gnathopod, which are analogous to the double row on the anterior border of the second gnathopod of *Leptocheirus pilosus*. It obtains its food more frequently, however, in the normal manner of other detritus feeders, and its sieving mechanism is not so highly developed as that of *Leptocheirus pilosus*.

This sieve feeding is analogous to the true filter feeding of *Haustorius arenarius* (Dennell, 1933), and the mysids. In these the collection of food particles, and creation of the food current, are both performed by specialized mouthparts, while in *Leptocheirus pilosus* this is done by the appendages of the meso- and metasome, and the mouthparts are of the unspecialized biting type. Since the animal lives in a tube, the current produced by the pleopods is bound to flow down the tube and over the sieving setae; it cannot get in at the sides, nor from underneath behind the second gnathopod, as would happen if there were no tube. It can therefore do its sieving on a larger scale than can the mysids or *Haustorius arenarius*, whose food current is of a much smaller capacity, and is produced by the mouthparts in an enclosed space which, since there is no tube, can only be provided by a fold of the carapace, or by the walls of pinnate setae through which water cannot easily pass. No comments will be made on the breeding habits of *Leptocheirus pilosus* until further experimental evidence has been obtained.

I wish to express my thanks to Mr D. M. Reid, for his valued help and criticism of this work.

SUMMARY

A study made of the adaptations to tubicolous life found in the small brackish water Photid Amphipod, *Leptocheirus pilosus* Zaddach, revealed several interesting points.

The presence of cement glands in many amphipods has been recognized by several previous authors. In *L. pilosus* these glands are found mainly in the upper joints of the first two pairs of peraeopods, opening to the exterior through a common duct in the claw. Unicellular glands are also found all over the body, particularly on the coxal plates.

The tubes of amphipods are of four main types:

(1) Sand burrow, e.g. Talitridae, *Ampelisca*.

(2) Mud burrow, e.g. *Corophium volutator*.

(3) Homogeneous leathery tube, e.g. *Leptocheirus pilosus*.

(4) Nest of algal fragments, bound together by "amphipod silk", e.g. *Amphithöe rubricata* and *A. longimana*.

The third type has not previously been described. It consists of a short, flat blister-like capsule fixed to a flat surface, usually the alga *Chondrus crispus*, and is built of minute particles of detritus cemented firmly together with a secretion from the dermal glands. Experiments showed that *Leptocheirus pilosus* could rapidly make a normal tube, both from natural and combusted detritus. If supplied only with fragments of *Ulva*, or sand grains, a very fragile tube was eventually constructed, the parts being fixed together by weak strands of mucus. No "amphipod silk" was used. An animal from

which the 1st and 2nd pairs of gland-bearing peraeopods have been removed is unable to build a tube.

Leptocheirus pilosus collects its food by the method of sieving. A stream of water, created by the pleopods, flows down the tube and any solid particles suspended in it are collected by an arrangement of long setae on the second pair of gnathopods. From the mass of detritus thus collected, suitable food particles are selected by the mouthparts. The remainder is incorporated into the wall of the tube from the inside. This method of feeding is of the selective deposit type, and does not resemble the filter feeding found in some species. The animal is able also to draw into the tube masses of detritus with the antennae, but it does not gnaw at large pieces of an alga, nor is it carnivorous.

From the scanty observations made, the breeding habits of the species do not appear to be normal. There is a great preponderance of females and the occurrence of copulation is doubtful. The peculiar carriage of the well developed first pair of gnathopods by the male does not appear to be an adaptation for pairing, and no explanation for it is put forward. It is tentatively suggested that the species may be parthenogenetic.

The young remain in the mother's brood pouch until after the first moult, and remain in her tube for some time after this. On leaving it they forthwith build tubes of their own.

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SOME NEW SAND-DWELLING COPEPODS

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(Text-figs. 1–7)

The copepods described in this paper come from two sources, the Firth of Clyde and the St Lawrence River. Several new species were collected from Balloch Bay on the Greater Cumbrae in 1935 and 1936 during an attempt to obtain some of the early species described from Scottish waters. The remainder were collected while working at the Biological Station at Trois Pistoles, Quebec, during a visit to Canada in 1937. The environment in Balloch Bay is composed of a fine muddy sand, strewn with boulders. The bay is on the eastern side of the island and therefore in a sheltered position. A description is given in Appendix III of *The Biology of the Sea Shore*, by Flattely & Walton (1922). The conditions under which the Canadian material was found will be dealt with in a later paper describing the remainder of the collection from the St Lawrence; it will be sufficient here to state that the conditions are essentially marine.

Copepods from three allied genera are included here: *Paramesochra* T. Scott, 1892; *Leptopsyllus* T. Scott, 1894; and *Remanea* Klie, 1929. The first two have for some time been in a state of considerable confusion and it has been a matter of some doubt into which genus the various species should be placed. Several efforts have been made to clarify the position (notably by Klie, 1929 and Monard, 1935) but the more recent work of Kunz (1938) has produced a clear separation based on the presence or absence of an endopod on the second leg. When the variation in the structure of the endopods of the second, third and fourth legs is studied in these two genera it becomes apparent that such a separation is far from natural, but it will suffice until a thorough revision of these and allied genera has been made. In an earlier paper (1935) I suggested that the genus *Emertonia* of Wilson (1932) was in reality a synonym of *Leptopsyllus*. Kunz (1938) points out that it has enough distinct characters to render it a valid genus and with this opinion I must for the present agree.

Klie (1929) included *Leptopsyllus* and *Paramesochra* in a single genus and for this view there is much support since there is a considerable overlapping between the two. Moreover, *Leptopsyllus littoralis* n.sp. is intermediate between *Emertonia* and *Leptopsyllus*, resembling the former in the possession of a 1-segmented exopod in the first leg but differing from it in the absence of endopods on legs 2–4.

Included in this paper are keys to the genera *Paramesochra* and *Leptopsyllus* based on those of Kunz, and extended to include the new species here described.

The following abbreviations have been used in the figures:

a.1.	first antenna.	*a*.2.	second antenna.	*c.r.*	caudal rami.
g.a.	genital area (♀).	*U.*	urosome.	*md.*	mandible.
mxl.	maxillule.	*mx.*	maxilla.	*mxp.*	maxilliped.

I, II, III, IV, V, and VI. Legs 1–6.

Genus Leptopsyllus T. Scott, 1894

As stated above, this genus has now been modified and includes only those forms which lack endopods on the second legs.

KEY TO THE SPECIES

1.	Third endopod absent ...	2
	Third endopod present, 1-segmented ...	*arcticus* (Lang) 1936
2.	Fourth endopod absent	3
	Fourth endopod present, 1- or 2-segmented ...	4
3.	First exopod 1-segmented	*littoralis* n.sp.
	First exopod 2-segmented	*spinipes* n.sp.
4.	Fourth endopod 1-segmented ...	*paratypicus* n.sp.
	Fourth endopod 2-segmented ...	*typicus* T. Scott, 1894

Leptopsyllus littoralis n.sp. (Fig. 1).

Occurrence. Balloch Bay, Firth of Clyde; washed from sand at about half-tide level, at a depth of between 7 and 10 cm.

Female. Length 0·48 mm. Body tapering only slightly, somewhat constricted between segments. First antenna 9-segmented, the first two segments the largest; second antenna with the usual 3-segmented endopod and 1-segmented exopod, the latter with two terminal and two lateral setae. Mouth parts of generic type. First leg with 1-segmented exopod and 2-segmented endopod; exopod bearing four terminal and one lateral setae, with numerous small marginal setae; endopod with two large and one small setae at end of distal segment. Legs 2–4 all with 3-segmented exopods and no endopods. Fifth legs with distal segments fused with basal segment, the basal segments of opposite sides being fused in the mid-line and expanded into the usual lamella, which overlaps the genital area and is without setae; distal segments prolonged into spines and bearing four setae of which three are situated outside the spine. Caudal rami twice as long as wide and about two-thirds as long as anal segment. Egg-sac single with five or six spherical eggs carried one behind the other.

Male. Length 0·41 mm. First antenna 6-segmented; legs 1–4 almost iden-

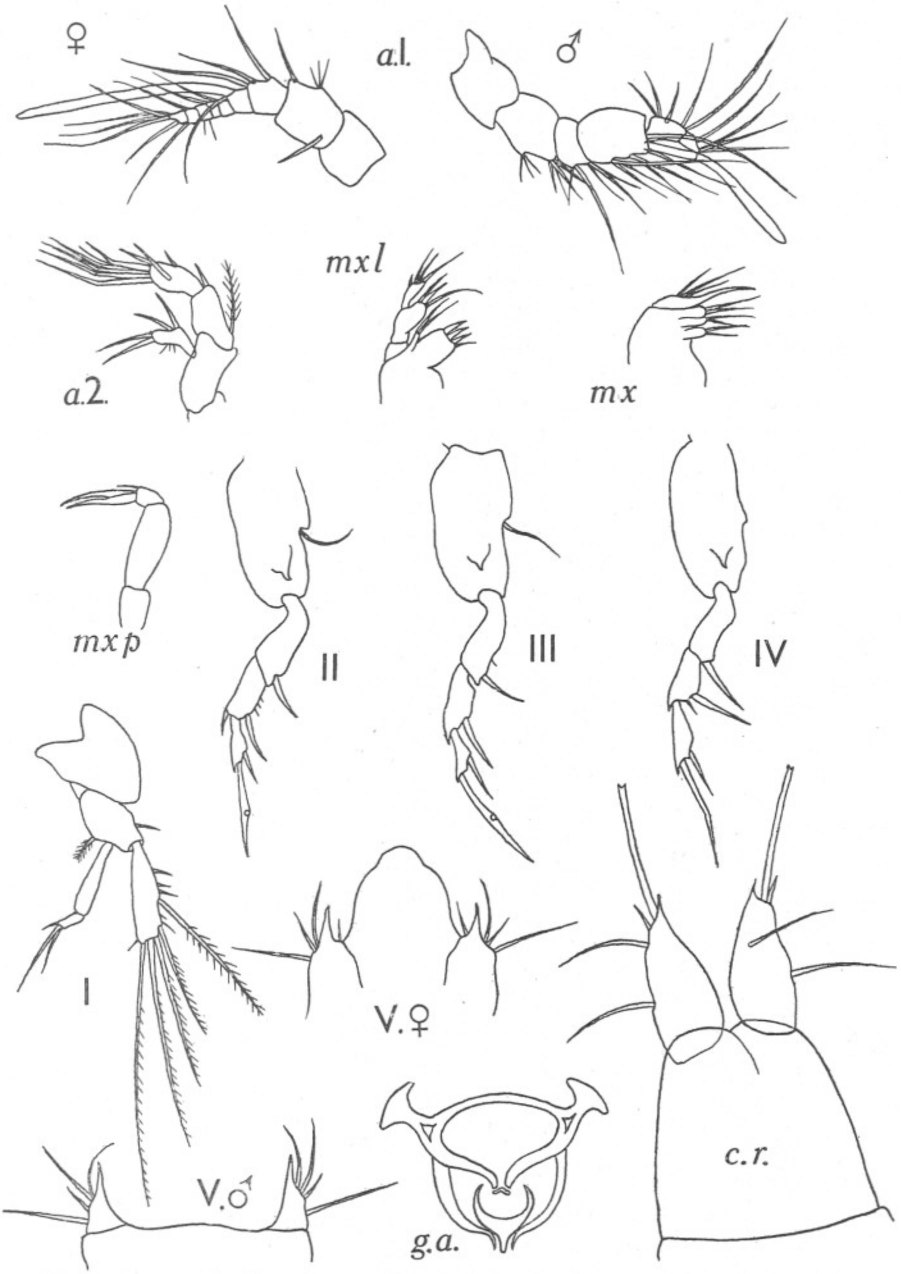

Fig. 1. *Leptopsyllus littoralis* n.sp. The figures are all drawn to the same scale.

tical with those of the female; 5th legs similar to those of female but without the large basal expansion.

This species resembles *Emertonia gracilis* Wilson (1932) in the structure of the first pair of legs, though the proportions of the segments of the endopod differ; it also differs from this and from previously described species of *Leptopsyllus* in lacking endopods on three pairs of legs, the second, third and fourth, and in the structure of the fifth legs.

Leptopsyllus spinipes n.sp. (Fig. 2).

Occurrence. Balloch Bay, Firth of Clyde; washed from sand near low-water springs, down to a depth of 10 cm.

Female. Length 0·43 mm. Body of usual form, moderately constricted between segments; urosome 4-segmented. Rostrum small; first antenna 7-segmented, the basal segment the largest; second antenna with 1-segmented exopod bearing two terminal and one lateral seta. Mouth parts of the usual structure. First legs with 2-segmented rami, endopod longer than exopod and tipped with two setae. Legs 2–4 with 3-segmented exopods and no endopods. Fifth legs of opposite sides fused in mid-line but basal segment not greatly expanded and ending in three pointed lobes; distal segments distinct, with two setae. Caudal rami as wide as long, subtriangular in shape, forming flattened plates armed with several short spines and setae.

Male. Length 0·36 mm. Body similar to that of female; urosome 5-segmented. First antenna 7-segmented, the 3rd segment bearing a relatively large curved spine. Legs 1–4 identical with those of female; fifth legs without inner basal expansion, distal segments with three setae; sixth legs with small basal expansion. Caudal rami somewhat longer than in female (half as long again as wide) and with fairly long terminal setae.

This species resembles *L. littoralis* in the absence of 2nd, 3rd and 4th endopods, but differs in the structure of 1st and 5th legs and caudal rami. The caudal rami of the male of this species are rather different from those of the female, which were probably slightly compressed while being drawn.

Leptopsyllus paratypicus n.sp. (Fig. 3).

Occurrence. Balloch Bay, Firth of Clyde; washed from sand.

Female. Unknown.

Male. Length 0·36 mm. Body of usual form, with 5-segmented urosome. First antenna 5-segmented, bearing aesthetascs on 4th and 5th segments; 2nd antennae with 1-segmented exopods bearing three terminal and one lateral seta. First legs with 2-segmented rami, the endopods slightly longer than the exopods. Second and third legs alike, with 3-segmented exopods and no endopods. Fourth legs with 3-segmented exopods and 1-segmented endopods, armed with a single large terminal spine. Fifth legs with basal segments of opposite sides fused in mid-line but not expanded; distal segments

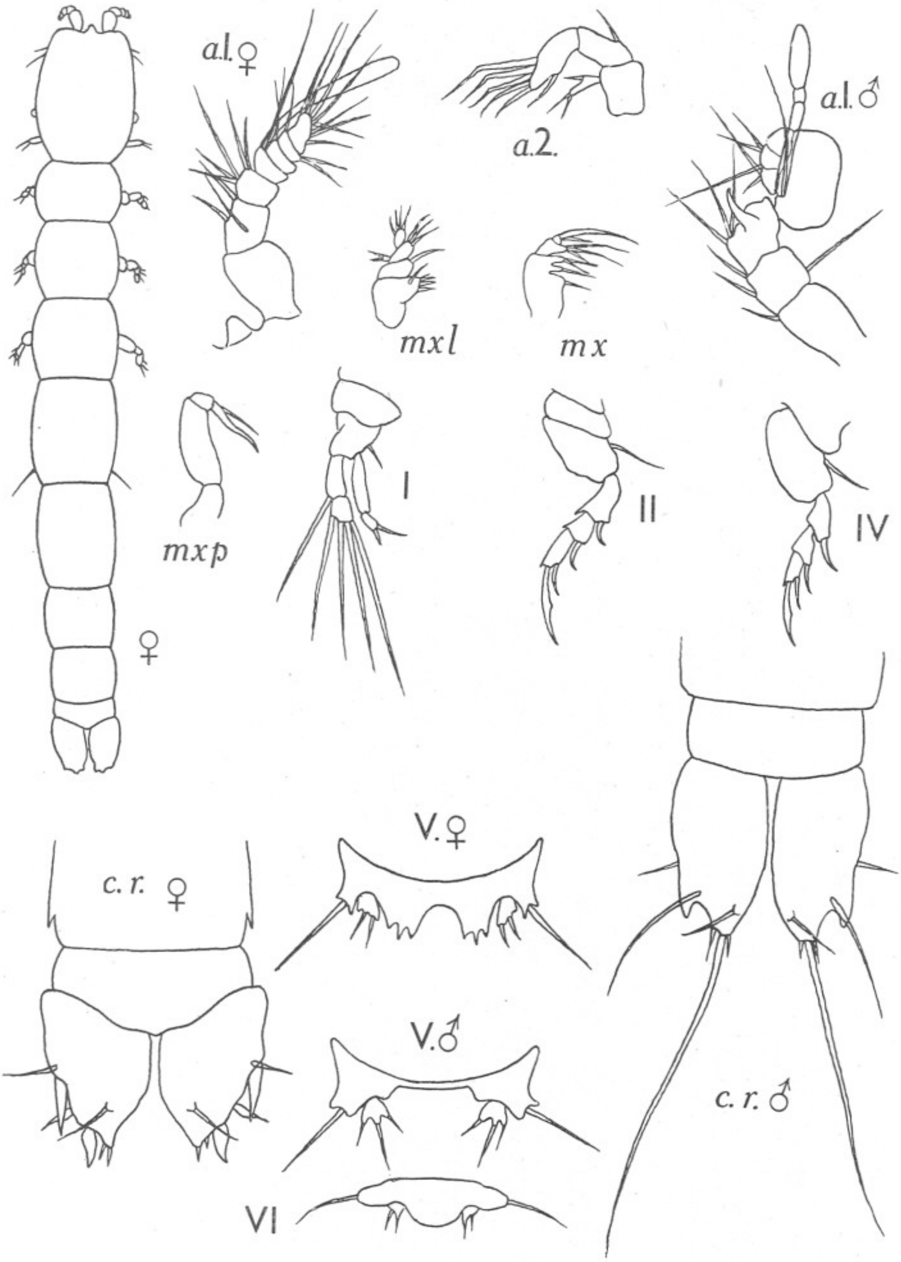

Fig. 2. *Leptopsyllus spinipes* n.sp. The figures are all drawn to the same scale except the dorsal view of the female which is less highly magnified.

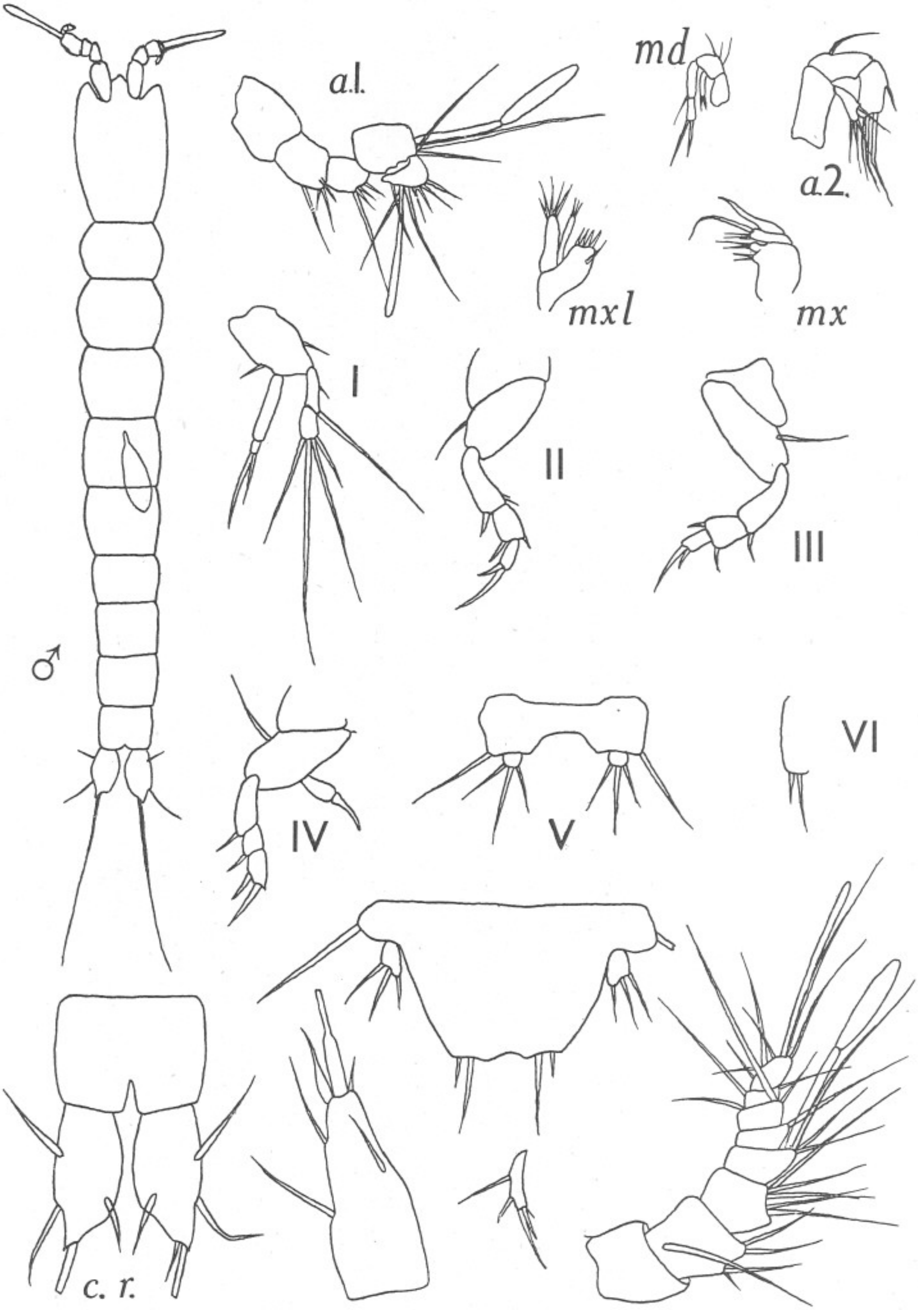

Fig. 3. *Leptopsyllus paratypicus* n.sp. Male only. The unlabelled figures are those of the first antenna, exopod of the second antenna, fifth legs, and caudal ramus of the female of *Paramesochra minor* (T. & A. Scott). All to the same scale.

with three long setae. Caudal rami twice as long as wide and a little longer than the anal segment, armed with one long and one short terminal seta and three other setae.

This species resembles *L. typicus*, of which only the female has been described, in having no endopods on the 2nd and 3rd legs but differs from it in having only 1-segmented endopods on the 4th legs and in the armature of the caudal rami. The great difference in size (0·74 and 0·36 mm.) also rules out the possibility of this being the male of *L. typicus*.

Genus Paramesochra T. Scott, 1892

Key to the Species

1.	Fourth exopod 2-segmented ...	**2**
	Fourth exopod 3-segmented ...	**4**
2.	Second exopod 3-segmented ...	**3**
	Second exopod 2-segmented ...	*laurentica* n.sp.
3.	Second endopod 2-segmented ...	*intermedia* (T. & A. Scott) 1895*a*
	Second endopod 1-segmented ...	*minuta* n.sp.
4.	Basal segment of 1st antenna strongly hooked distally	*dubia* T. Scott, 1892
	Basal segment of 1st antenna not hooked ...	**5**
5.	Anal segment with lateral backwardly projecting points ...	*pterocaudata* Kunz, 1937
	Anal segment without such points ...	**6**
6.	Fourth endopod 1-segmented ...	**7**
	Fourth endopod 2-segmented ...	**11**
7.	Caudal rami without inner setae ...	**8**
	Caudal rami with an inner dorsal seta ...	**9**
8.	Caudal ramus with a stout spine at outer distal corner	*holsatica* Klie, 1929
	Caudal ramus with plumose seta in place of spine ...	*pygmaea* n.sp.
9.	First endopod 1-segmented ...	*constricta* (Nicholls) 1935
	First endopod 2-segmented ...	**10**
10.	First endopod with large curved spine and one seta on terminal segment which is one-quarter of basal segment; size 0·40–0·46 mm. (♂ only) ...	*coelebs* (Monard) 1935
	First endopod with two spines on terminal segment which is one-third of basal segment; size 0·70 mm. (♂ used for comparison) ...	*major* n.sp.
11.	Caudal ramus prolonged into a point posteriorly; end segment of 4th exopod with three setae; basal expansion of 5th leg tapering to a point ...	*acutata* Klie, 1934
	Caudal ramus not pointed posteriorly; end segment of 4th exopod with two setae; basal expansion of 5th legs rounded, with two setae ...	**12**
12.	Basal segment of 4th endopod without setae ...	**13**
	Basal segment of 4th endopod with an inner seta ...	**15**
13.	Second endopod without setae ...	**14**
	Second endopod tipped with one seta	*herdmani* (Thompson & Scott) 1900

14\. Caudal rami three times as long as wide; 1st exopod as long as whole endopod; size about 0·63 mm. ... *robertsoni* (T. & A. Scott) 1895*b*
Caudal rami 2–2½ times as long as wide; 1st exopod no longer than basal endopod; size 0·33–0·46 mm.... ... ... *minor* (T. & A. Scott) 1895*b*

15\. Basal segment of 3rd endopod with one inner seta; end segments of 5th legs rounded, bearing setae only ... ... *similis* Kunz, 1937
Basal segment of 3rd endopod without setae; end segment of 5th leg tapering to end in a large spine ... ... *helgolandica* Kunz, 1937

Paramesochra minor (T. & A. Scott) 1895*b* (see Fig. 3).

Occurrence. Trois Pistoles, St Lawrence River; washed from coarse sand near seaward end of breakwater, at a depth of between 5 and 8 cm.

Distribution. Musselburgh, Firth of Forth; Kames Bay, Millport, Firth of Clyde; Kiel Bay, Germany.

A few specimens of this copepod were obtained near the breakwater at Trois Pistoles, Quebec, which differ in only small points from previous descriptions. The first antennae are 8-segmented in the female; the exopod of the 2nd antenna has three terminal and two lateral setae; the general proportions of the 5th legs are similar but the shape of the basal expansion is not quite the same; and the setae of the caudal rami do not quite correspond. These small differences do not justify a new species. It is remarkable that this species should be so widely distributed when it is customary among these sand-dwellers to find entirely different species in adjacent bays.

Paramesochra pygmaea n.sp. (Fig. 4).

Occurrence. Balloch Bay, Firth of Clyde; washed from sand near low-water springs, down to a depth of 10 cm.

Female. Length 0·25 mm. Body distinctly tapering posteriorly, somewhat constricted intersegmentally in the metasome; urosome 4-segmented. First antenna 7-segmented, basal segment the largest; 2nd antenna with 1-segmented exopod armed with one terminal and two lateral setae. First legs with 2-segmented rami; endopod nearly half as long again as exopod; endopod with two terminal setae, exopod with four terminal setae. Second and third legs identical, with 3-segmented exopods and 1-segmented endopods, the latter somewhat swollen distally and bearing each a single small terminal plumose seta; distal exopod segment bearing three spines and one plumose seta. Fourth legs similar to 2nd and 3rd but lacking the terminal plumose seta on the exopods. Basal expansions of 5th legs large and almost completely fused in the mid-line, with two terminal setae on each side; distal segments with five setae. Caudal rami slightly more than twice as long as wide and about half as long again as anal segment, bearing three unequal setae terminally and one lateral seta.

Male. Length 0·24 mm. Body similar to that of the female but with 5-segmented urosome. First antenna 5-segmented, with a short stout aesthetasc on the 4th segment. Legs 1–4 identical with those of female; 5th legs with basal expansion scarcely extending beyond end of distal segments and without terminal setae; distal segment with one long spine and three short setae.

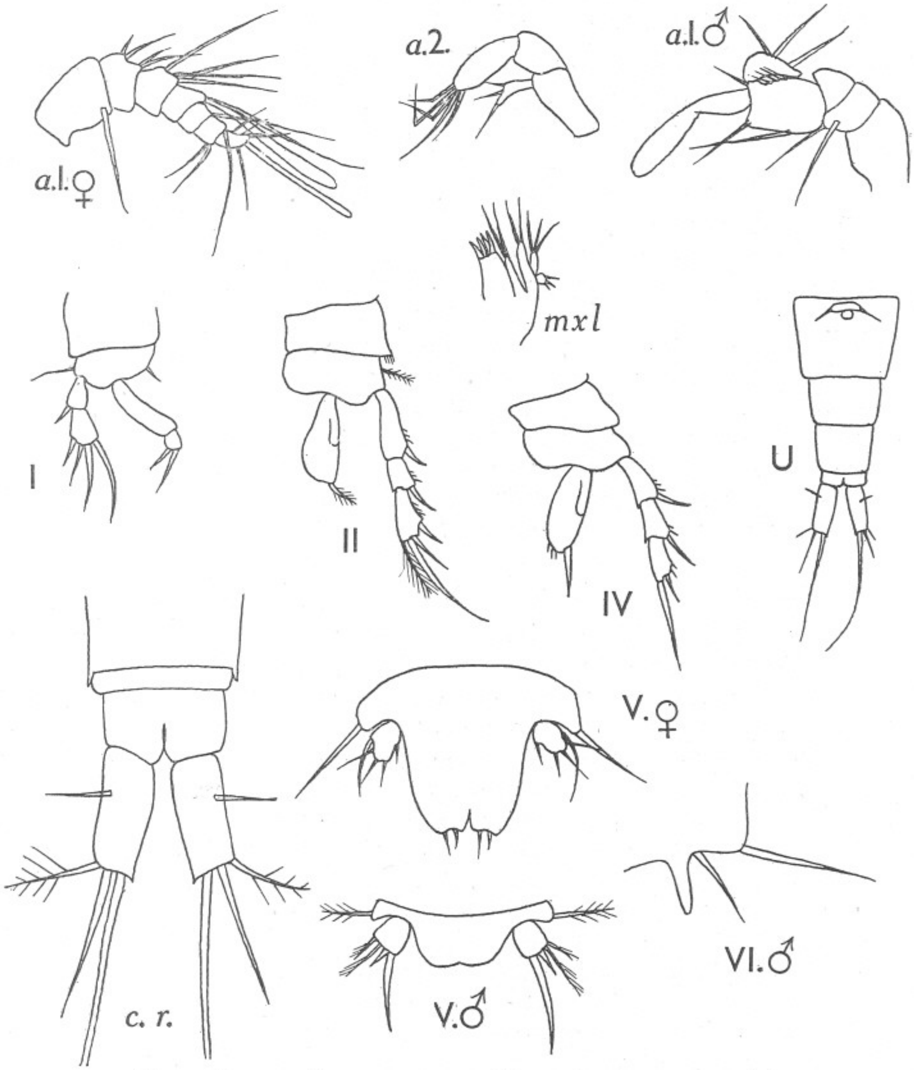

Fig. 4. *Paramesochra pygmaea* n.sp. Except for the urosome of the female the drawings are all to the same scale.

This species resembles *P. holsatica* in the general structure of its legs and in its small size, but can be distinguished easily by the presence of a small plumose seta at the outer corner of the caudal ramus in place of the stout spine in *holsatica*.

Paramesochra minuta n.sp. (Fig. 5).

Occurrence. Balloch Bay, Firth of Clyde; washed from sand.

Female. Length 0·38 mm. Body not tapering very much; urosome 4-segmented, anal segment very short. First antenna 8-segmented; exopod of 2nd antenna 1-segmented with two terminal and one lateral seta. First legs with 2-segmented rami about equal in length or the exopod slightly the longer. Legs 2 and 3 with 3-segmented exopods and 1-segmented endopods, the latter somewhat narrowed distally and bearing only three short hairs. Fourth legs with 2-segmented exopods and 1-segmented endopods, not constricted terminally but bearing a single large spine. Fifth legs with very large basal expansions entirely fused and semi-circular in outline, with one seta on each side; distal segments very small and fused with basal segments, distinguishable only as small knobs each bearing two setae. Caudal rami twice as long as wide and about half as long again as the anal segment.

Male. Unknown.

This species resembles *P. intermedia* in several particulars but differs in size, in the structure of the 2nd endopods and of the 5th legs.

Paramesochra major n.sp. (Fig. 6).

Occurrence. Baie de Mille Vaches, St Lawrence River; washed from very coarse sand taken by grab in a depth of 8 m.; numerous specimens.

Female. Length 0·80 mm. Body with metasome wider than urosome, tapering gradually posteriorly; urosome 4-segmented. First antenna 9-segmented, basal segment the largest. Second antenna with 1-segmented exopod bearing one terminal and two lateral setae and two small hairs. First legs with 2-segmented rami, the endopod about twice as long as the exopod and with its distal segment not always clearly separated from the basal segment, the latter about five times as long as the end segment. Legs 2–4 with 3-segmented exopods and 1-segmented endopods. The distal segment of the 2nd exopod with three spines and one plumose seta, of 3rd exopod with two spines and one plumose seta (the proximal spine being absent) and of 4th exopod with two spines only. Second and third endopods rounded, with one terminal plumose seta; 4th endopod tapering, with one stout spine. Fifth legs with basal expansions fused for two-thirds of their length, bearing two setae; distal segments small, with three setae. Caudal rami three times as long as wide and twice as long as anal segment. Egg-sac single, with 9–10 eggs.

Male. Length 0·70 mm. Body similar to that of female; urosome 5-segmented. First antenna 6-segmented with a short stout aesthetasc on 4th segment. First leg as in female but with basal endopod segment only three times as long as distal segment. Second and third legs similar and like 2nd leg

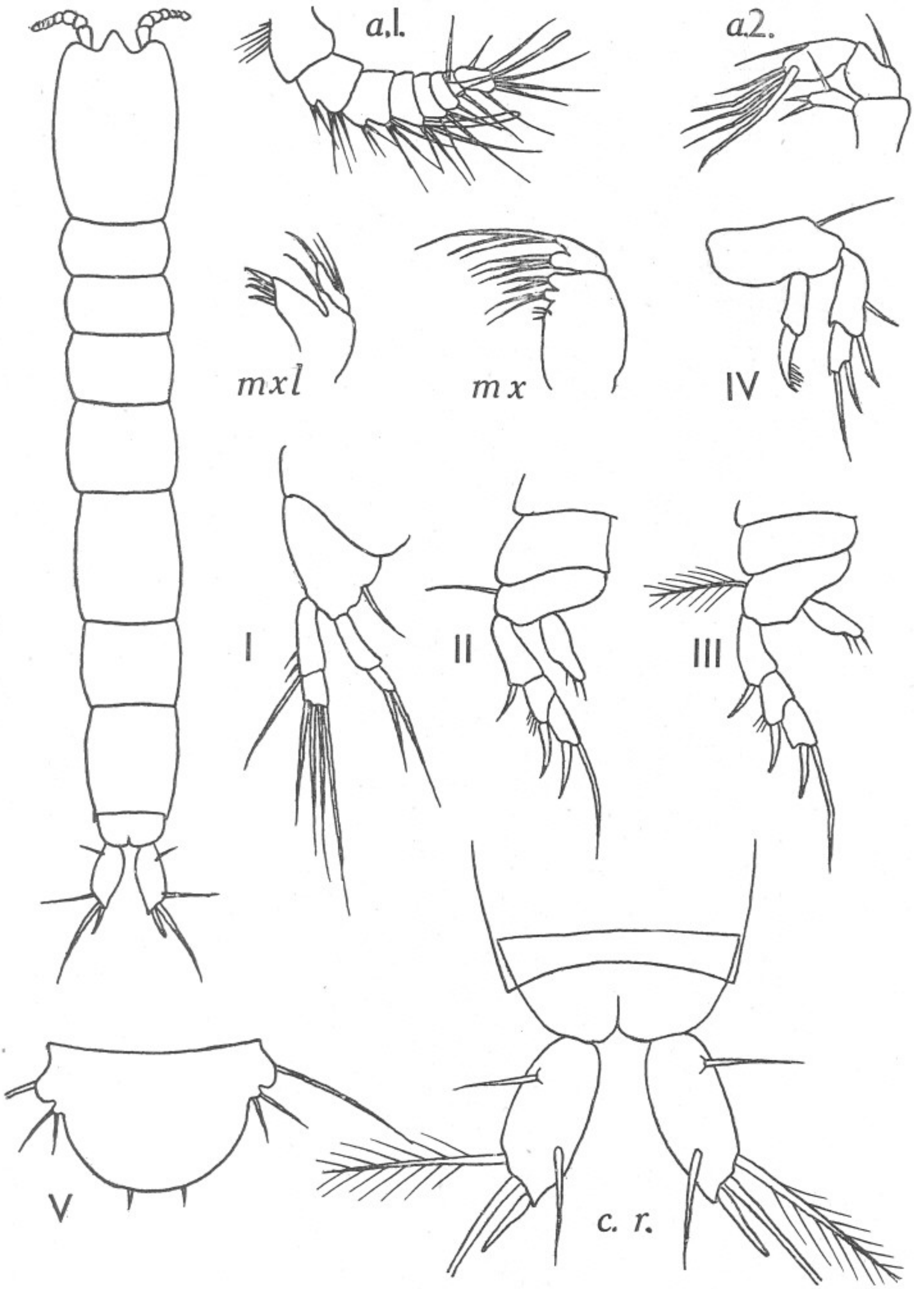

Fig. 5. *Paramesochra minuta* n.sp. Female only. All to the same scale except for the dorsal view of the adult.

Fig. 6. *Paramesochra major* n.sp. All figures drawn to the same scale.

of female. Fourth legs like those of female. Fifth legs with small basal expansion, without setae; distal segment with four setae. Caudal rami like those of female.

This species is the largest yet described belonging to this genus, though *Leptopsyllus typicus* is nearly as large, and although resembling *Paramesochra coelebs* (only the male of which is known) is quite easily separated by the difference in the 1st legs and in the size.

Paramesochra laurentica n.sp. (Fig. 7).

Occurrence. Baie de Mille Vaches, St Lawrence River; washed from coarse sand taken by grab from 8 m. One specimen only.

Female. Length not known. First antenna 7-segmented, the 3rd the largest. Second antenna apparently without an exopod but this may have been lost in dissection. First leg with 2-segmented rami, endopod nearly twice as long as exopod. Legs 2–4 with 2-segmented exopods and 1-segmented endopods, the latter with a single seta in 2nd and 3rd legs and with a single stout spine in 4th legs. Distal segments of 2nd and 3rd exopods each with four spines and one plumose seta; 4th exopod with two spines and one plumose seta. Fifth legs of the usual pattern, basal expansion with two setae on each side; distal segment with three setae. Caudal rami little more than twice as long as wide, with three unequal setae.

Male. Unknown.

This species differs from all other species so far described in the presence of 2-segmented exopods in legs 1–4.

Genus Remanea Klie, 1929

This genus was described by Klie to receive a new copepod obtained in Kiel Bay. While it differs from *Paramesochra* and *Leptopsyllus* in the presence of a 3-segmented 1st exopod and the 2-segmented exopod of the 2nd antenna, it resembles many species of *Paramesochra* in its shape and in the 5th legs which are fused for part of their length. It appears to me to be a related genus.

Remanea arenicola Klie, 1929.

Occurrence. Fintry Bay, Isle of Cumbrae, Firth of Clyde; washed from fairly coarse sand near low-water mark.

Distribution. Kiel Bay, in coarse sand.

Several specimens of this copepod were taken in 1935 from the relatively coarse sand of Fintry Bay on the west shore of the Cumbrae, exposed to rough weather. These specimens are very slightly larger than those found by Klie (female 0·38 mm.), but that is the only difference.

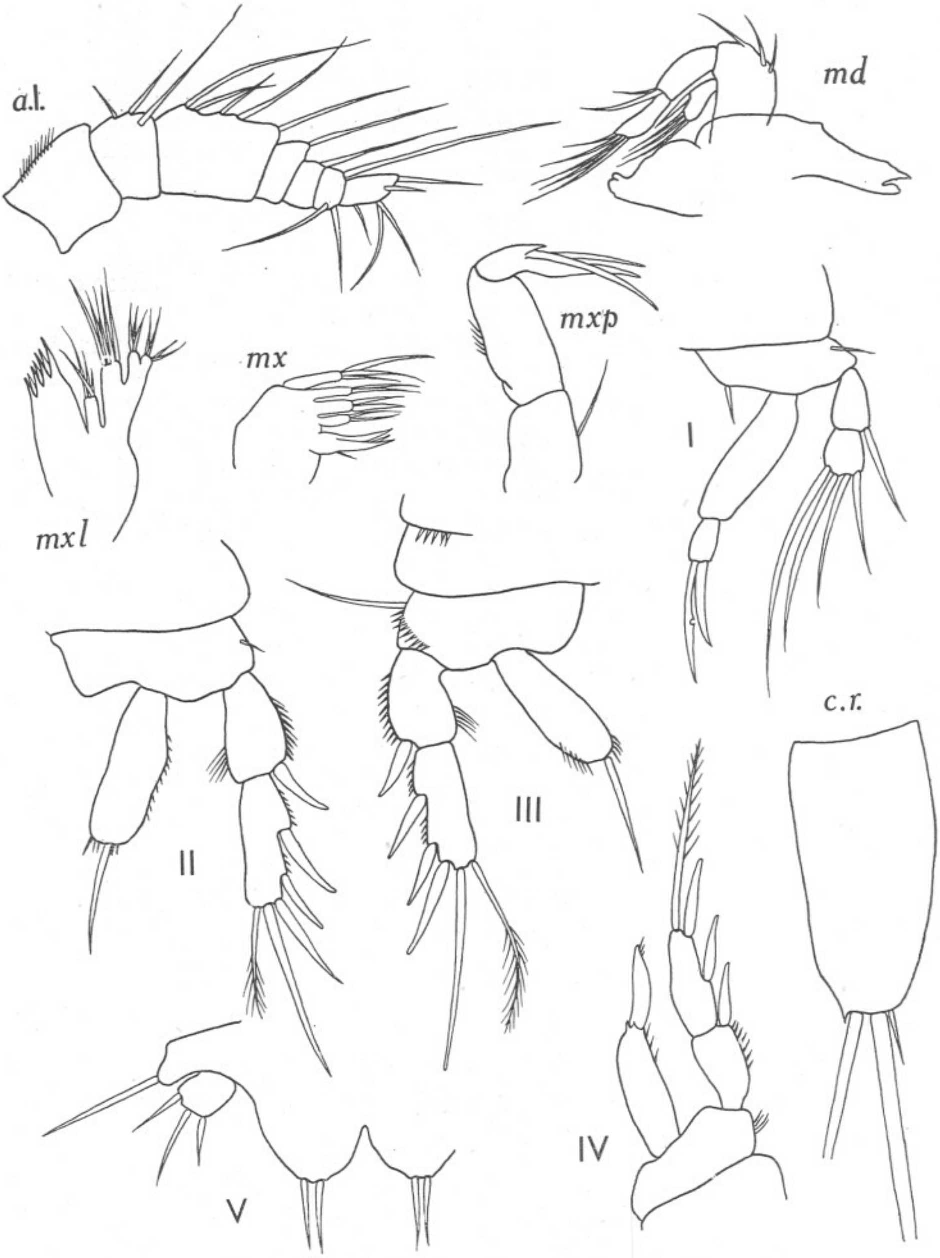

Fig. 7. *Paramesochra laurentica* n.sp. Female only.
All figures drawn to the same scale.

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THE HYDROID OF THE MEDUSA *DIPURENA HALTERATA* (FORBES)

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(Text-figs. 1–3)

Although the medusa *Dipurena halterata* (Forbes, 1846) is well known, and has been figured by a number of authors (e.g. Forbes, 1848; Browne, 1897), the hydroid of this peculiar species has remained unknown. This medusa is rather scarce at Plymouth (Russell, 1938). On May 3 1938 I had the good fortune to find at Plymouth a small hydroid of the *Syncoryne* type which later liberated young medusae, and these proved to be young specimens of *Dipurena halterata*.

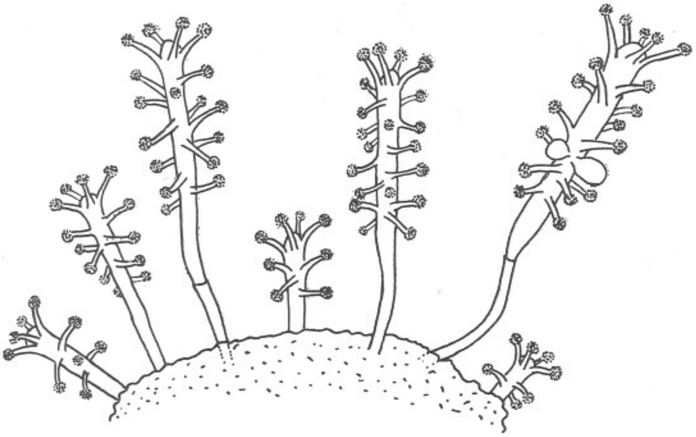

Fig. 1. Portion of colony of *Dipurena halterata*; Plymouth, 4. v. 38.

The hydroid was found growing on and in a sponge, *Chalina montagui* (Bowerbank), attached to a piece of *Lepralia foliacea* (Ellis & Solander), dredged near the Mewstone. Another piece of sponge from the same locality carried a young colony of the hydroid.

The Hydroid. The colony was small, with a total height rarely exceeding 2·5 mm. (Fig. 1). At first sight the polyps appeared to be very similar to those of *Zanclea implexa* (Alder). The polyps had the brownish or reddish colour common in species of *Syncoryne*. There were no stolons on the surface of the sponge, but there were many branching stolons deeply buried in the substance of the sponge. In the fully developed polyps the stems, covered by a thin non-annulated perisarc, reached a height of 0·5–0·8 mm. above the surface of the sponge. Young polyps on the surface were sessile with no visible hydrocaulus. Hand sections kindly prepared by Mr L. R. Crawshay, who identified the sponge, revealed that the young hydranths began their development deep down in the substance of the sponge and later forced their way to the surface. These buried rudimentary polyps possessed short tentacles with capitate heads which later, on reaching the surface, developed into typical tentacles. The sponge itself appeared to be in an emaciated condition, but whether this was due to the presence of the hydroid was impossible to determine because of the lack of adequate material. A detailed study of this association might prove well worth while.

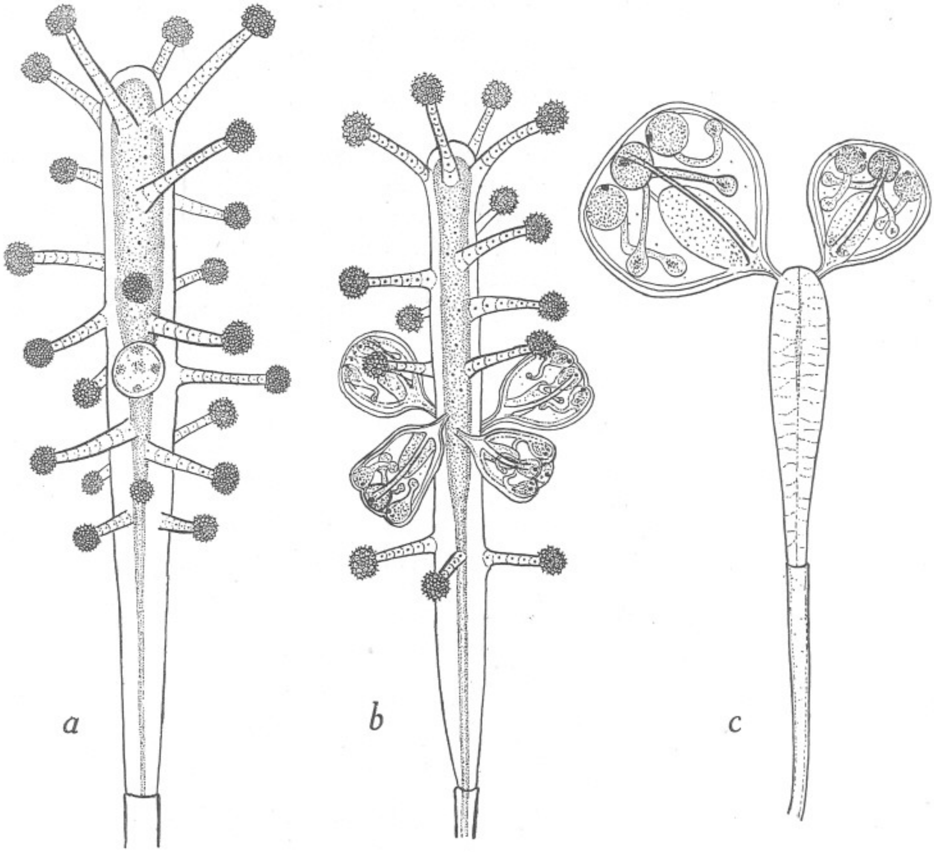

Fig. 2. *Dipurena halterata*; *a*, fully developed hydranth with a young medusa bud, 5. v. 38; *b*, hydranth with four medusa buds, 12. v. 38; *c*, hydranth reduced to a blastostyle, 18. v. 38; all from Plymouth.

The fully developed hydranths were 1·0–1·7 mm. in length with a diameter of 0·14–0·15 mm. The tentacles were all capitate, 17–24 in number. The distal end of the hydranths had four or five tentacles more or less in a whorl around the mouth. The other tentacles were irregularly scattered over the anterior and middle parts of the hydranth while the posterior part carried no tentacles (Fig. 2*a*). These tentacles were longer than the diameter of the hydranth and had capitate heads 0·06–0·08 mm. in diameter.

Medusa buds were borne on the hydranth a little behind the middle region of the body (Fig. 2*b*). Fully developed buds were covered by a thin transparent perisarc and were 0·40–0·45 mm. in diameter. A single hydranth had one to three medusa buds on it at the same time. These medusa-bearing hydranths usually became reduced to blastostyles devoid of tentacles (Fig. 2*c*).

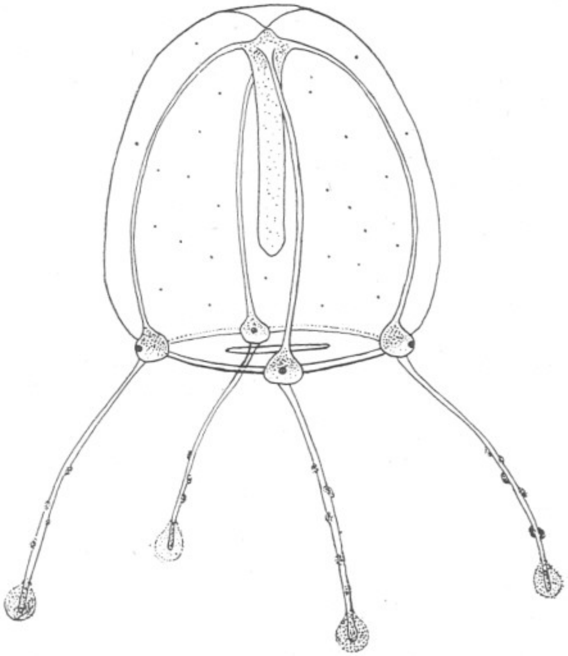

Fig. 3. Newly liberated medusa of *Dipurena halterata* obtained from the hydroid; Plymouth, 6. v. 38.

THE MEDUSA. The newly liberated medusa was bell-shaped, a little higher than wide, with a height of 1·5–1·6 mm. and a diameter of 1·3–1·4 mm. (Fig. 3). The umbrella had a fairly thin jelly with a few scattered nematocysts on its exumbrellar surface. The velum was broad. The stomach was cylindrical, reaching to about two-thirds of the height of the subumbrellar cavity. The mouth was simple without lips. The radial canals and ring canal were very narrow. There were four perradial tentacles arising from distinct bulbs each with a prominent black ocellus. The ends of the tentacles were swollen to form distinct knobs armed with batteries of nematocysts. The distal half of the tentacles also carried small irregularly placed batteries of nematocysts. The colour of the manubrium was a pale green, while the tentacle bulbs and the endoderm of the capitate ends of the tentacles were brick red in colour. At this stage there was no green colour in the tentacle bulbs, it appeared later, however, in the specimens which I reared.

One reared specimen reached a bell height of 2·8 mm. and a diameter of 2·5 mm. At this stage the linear swellings on the radial canals had developed. These were regarded by Forbes (1848) as ovaries, but it has since been shown by Haeckel (1879) and Browne (1897) that the gonads develop on the manubrium. The exact nature of these swellings is still unknown. Similar swellings have been observed by Russell & Rees (1936) on the radial canals of *Zanclea*. The terminal knobs on the tentacles were now much bigger than in the newly liberated medusa and had a diameter of 0·16–0·22 mm. The irregularly placed batteries of nematocysts of the young medusa were now developing into complete rings of nematocysts. The best developed tentacle possessed three complete rings and one incomplete ring of nematocysts as well as the terminal knob.

During July and August 1938 I collected many specimens of *Dipurena halterata* in various stages of development from the plankton at Valentia Harbour, Co. Kerry. A comparison of the young stages from Valentia with those reared from the hydroid at Plymouth confirms earlier observations made in this paper that the hydroid and its young medusa are stages in the life history of *D. halterata*.

Although the hydroid is of the *Syncoryne* type it cannot be referred to *Syncoryne* because the characters of the medusa alone are sufficient to warrant the generic distinction of the species from *Syncoryne*. It is therefore proposed to retain the name *Dipurena halterata* for both hydroid and medusa.

I am indebted to the Royal Society for a grant to cover my expenses at Valentia Island.

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ON THE NEMATOCYSTS OF HYDROMEDUSAE. II

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(Text-figs. 1–4)

In a previous publication (Russell, 1938) I described the nematocysts in twenty species of Hydromedusae. The present report gives observations on five more species among which are three species of *Aequorea*. Among the medusae there is perhaps no genus in which the identity of the different species has been so confused as that of *Aequorea*. This genus is characterized by a large number of radial canals, and the specific distinctions are chiefly based on the number of these canals and the ratio that this number bears to the number of marginal tentacles. But in any one species neither are constant in number, and indeed both may show considerable variation in either direction. This is perhaps only to be expected since the character is of a meristic nature and must be dependent upon the rate of growth of the individual and the size to which it grows under different conditions. The type of the genus as selected by Haeckel (1879) is *A. forskalea* Péron & Lesueur. This name was given by Péron & Lesueur (1809) to a medusa described and figured by Forskål (1775, p. 110 and 1776, pl. xxxii) as *Medusa aequorea*. Some authors (e.g. Bigelow, 1913, p. 38) have retained the specific name *aequorea*, but this name cannot be kept since it had been used previously both by Linnaeus* and by Löfling (1858) for species which were inadequately described. Forskål's drawing is excellent and leaves no doubt as to which species he had. The usual number of radial canals is about 60–80 (cf. Claus, 1883; and Fraser, 1916). In his description, however, Forskål says that there are 129 canals, but he also states that these are paired, and we are probably right in assuming, as Claus (1883) did, that he was referring to the lamellae of the gonads and that the radial canals were really half this number. The medusa figured by Forskål has 104 radial canals and 105 tentacles. This is slightly more than is usual, but occasional large specimens may be found with 90–100 or slightly more canals. The species was excellently described in full detail and in all its stages by Claus (1883) as *A. forskalea*, and this is an additional argument for keeping that name.

A. forskalea forms an excellent type for the genus, for its characters can be

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* The description given by Linnaeus in *Systema naturae*, 10th edition, 1758, p. 659, refers to Loefling's medusa. It may be questioned whether this medusa belongs to the same genus. I have not seen Loefling's original description, but Péron & Lesueur (1809) give for it "point de bras", i.e. "no mouth lips"; this probably fits the genus *Aequorea* better than any other.

regarded as forming a central point from which other species depart in different directions. Ideally it is a species in which the numbers of radial canals, mouth lips, and marginal tentacles are equal. But owing to slightly different rates of growth and development of the three characters this degree of perfection is rarely exactly attained. Thus the tentacles may be fewer or more than the radial canals, but they are hardly ever less than half or more than twice the number of canals. The tentacles also do not always appear on the umbrella margin opposite the radial canals, probably because, if the tentacle happens to be the first of the two to develop, the corresponding radial canal does not always grow along the exact line necessary to reach the ring canal opposite the tentacle.

Bearing these facts in mind the identification of this species becomes much easier, and I have as a result found little difficulty in distinguishing it from two other species which occur off Plymouth. These are *A. vitrina* Gosse, whose number of tentacles far exceeds that of the radial canals, and *A. pensilis* (Modeer), whose number of radial canals far exceeds that of the tentacles.

An examination of the nematocysts of these three species has confirmed their separation.

The other two species whose nematocysts are described in this paper are the siphonophores *Agalma elegans* (M. Sars) and *Velella velella* (L.). The nematocysts of *Aequorea forskalea* and *A. vitrina* were examined on medusae preserved in formalin and sea water. For the other three species observations were made on fresh material.

LEPTOMEDUSAE

Aequorea forskalea Péron & Lesueur

I am indebted to Mr P. G. Corbin for a collection of many *A. forskalea* taken in the mouth of the English Channel in July 1937. These specimens were between 45 and 100 mm. in diameter; they had 64–99 radial canals and 51–79 tentacles. The ratio of the diameter of the stomach to that of the umbrella varied approximately from 1 : 1·8 to 1 : 2·4.

The nematocysts, both in the marginal tentacles and the mouth lips were of two kinds: basitrichous haplonemes* and atrichous haplonemes (Fig. 1 *a–c*).

Basitriches: $12–18 \times 2{\cdot}5–4\,\mu$ undischarged.

Atriches: $17–34 \times 7–17\,\mu$ undischarged.

Both types of nematocyst showed considerable variation in size from one specimen to another. This was especially noticeable in the large oval atriches, which tended to fall into two size groups 17–22 and $26–34\,\mu$ long, both sizes being present in a single specimen, but only the smaller size occurring in the mouth lips.

My results thus differ from those of Weill (1934, p. 458) who states that

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* I cannot be sure whether these nematocysts, in this and the next two species of *Aequorea*, are basitrichous haplonemes or microbasic mastigophores (see Russell, 1938, p. 146).

basitriches are not present in the mouth lips. He gives the size of the basitriches as 15 μ long and that of the atriches as 15–20 μ.

Now that I have seen so many specimens of *A. forskalea* I think there can be little doubt that *A. forbesiana* of Gosse (1853) is the same species.

Aequorea vitrina Gosse

I have seen four specimens of *A. vitrina*, whose characters were as follows:

Diameter of umbrella mm.	Diameter of stomach mm.	No. of radial canals	No. of tentacles	No. of mouth lips
55	25	76	*ca.* 304	..
75	37	68	*ca.* 275	..
80	40	68	>300	*ca.* 39
90	40	90	*ca.* 600	*ca.* 40

The nematocysts both in the marginal tentacles and in the mouth lips were of two kinds: basitrichous haplonemes and atrichous haplonemes (Fig. 1 *d*, *e*).

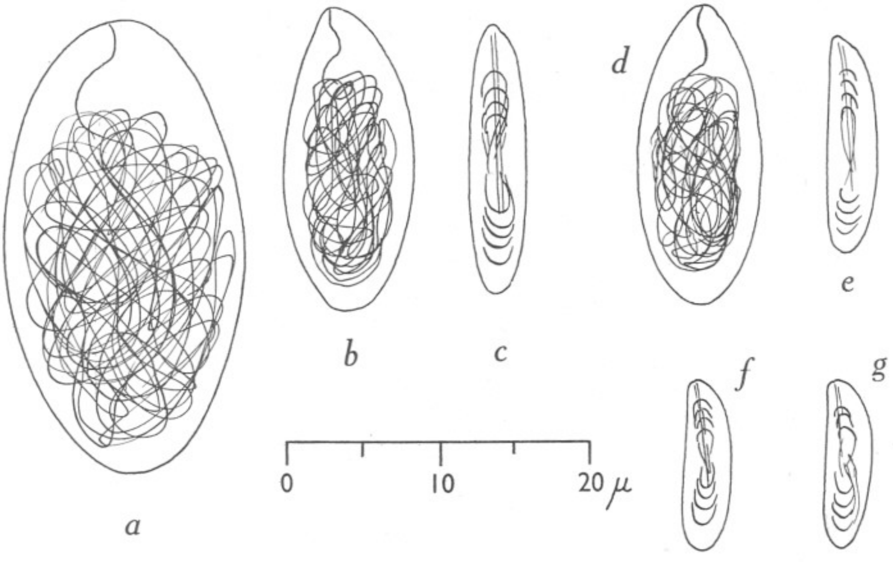

Fig. 1. *a–c*, *Aequorea forskalea*; *a*, *b*, atrichous haplonemes; *c*, basitrichous haplonemes; *d*, *e*, *A. vitrina*; *d*, atrichous haploneme; *e*, basitrichous haploneme; *f*, *A. pensilis*, basitrichous haploneme; *g*, ? *A. pensilis*, basitrichous haploneme. × *ca.* 2000.

Basitriches: 10–15 × 2·5–3 μ undischarged.

Atriches: 19–22 × 7·5–8·5 μ undischarged.

The very large atrichous haplonemes, characteristic of *A. forskalea* were absent.

The number of mouth lips, estimated on the basis of the number of transparent lines of large endodermal cells running down the stomach wall, appears to be usually in the neighbourhood of half or less than half the number of radial canals in *A. vitrina*, whereas in *A. forskalea* they are of more nearly the same number as the radial canals.

The specimens described by Hartlaub (1894) as *A. forskalea* are clearly *A. vitrina*.

Evans & Ashworth (1909) recorded a number of medusae from the Firth of Forth, which were identified by E. T. Browne as *A. norvegica* Browne. I have found one of these specimens in Mr Browne's collection. Its nematocysts were of the same types and approximately the same size as those of *A. vitrina*.

Basitriches: 12–13 × 3 μ undischarged.

Atriches: 18–20 × 7·5–8 μ undischarged.

The numbers of mouth lips both in the medusae described by Evans & Ashworth and in the type specimen of *A. norvegica* described by Browne (1903) also agree with those of *A. vitrina*. I am therefore inclined to agree with the suggestion made by Kramp (1933) that *A. norvegica* and *A. vitrina* are the same species. There is, however, an indication that the medusa does not grow to so large a size in these waters as it does farther north.

A. vitrina evidently comes very close to *A. coerulescens* (Brandt) as described by Bigelow (1909). In fact if it were not for the deep bluish black pigmentation in the tentacle bulbs, which appears to be so characteristic of *A. coerulescens*, there does not seem to be any character by which they can be distinguished. Bigelow's photograph of that species (1909, pl. 4, fig. 4) might well be a typical *A. vitrina*. An examination of the nematocysts of *A. coerulescens* might be helpful.

On the American side of the Atlantic *A. vitrina* appears to be represented by *A. albida* A. Agassiz.

Both *A. forskalea* and *A. pensilis* are present in the Atlantic and the Pacific, and it seems reasonable to suppose that eventually *A. vitrina* may be shown to have a similar distribution by regarding *A. coerulescens* and *A. albida* as geographical races. We need, however, more detailed observations on the numerical characters of these species.

Aequorea pensilis (Modeer)

In September 1938 a number of medusae were collected off Plymouth which are undoubtedly *A. pensilis*. The nematocysts, both in the marginal tentacles and in the mouth lips, were only of one kind, basitrichous haplonemes (Fig. 1*f*).

Basitriches: 9–13 × 2·5–3 μ undischarged.

The medusae were thus immediately distinguishable from the two preceding species of *Aequorea* by the absence of large oval atrichous haplonemes.

The occurrence of *A. pensilis* off Plymouth was somewhat unexpected and it is necessary to discuss these specimens in some detail. Full details of the numerical characters are given in Table I. It will be seen that these characters agree well with those in the table drawn up by Bigelow (1919, p. 312).

Bigelow gives the ratio of radial canals to tentacles as between 10 : 1 and 12 : 1 for specimens of 60 mm. diameter and less. The Plymouth specimens, over a much larger range of numbers, show a ratio of 3·5 : 1 to 13 : 1. It is, however, to be observed that this ratio changes with the size of the medusa. In specimens under 40 mm. in diameter it varies between 8 : 1 and 13 : 1. Above this size the number of tentacles increases owing to the development of tentacles on some of the marginal bulbs. The ratio of the stomach diameter to that of the umbrella varies between 1 : 1·3 and 1 : 2·0, thus agreeing closely with the corresponding ratios of 1 : 1·4 to 1 : 2·0 derived from Bigelow's table. In all specimens the central mass of jelly was very thick, and in the form of a plano-convex lens, and anastomosis of the radial canals was frequent. The gonads were yellowish green overlain by bluish grey pigment: the green colouration disappeared after preservation.

TABLE I. *AEQUOREA PENSILIS* CAUGHT OFF PLYMOUTH, SEPTEMBER 1938

Diameter of umbrella mm.	No. of radial canals	No. of tentacles	Ratio of radial canals : tentacles	Diameter of stomach mm.	Ratio of diameter of stomach : umbrella
16	129	11	13 : 1	10	1 : 1·6
18	124	10	12 : 1	10	1 : 1·8
20	>117	13	..	15	1 : 1·3
20	128	16	8 : 1	15	1 : 1·3
20	122	11	11 : 1	13	1 : 1·5
22	137	12	11 : 1	14	1 : 1·6
22	121	12	10 : 1	15	1 : 1·5
25	127	12	11 : 1	17	1 : 1·5
27	>111	15	..	16	1 : 1·7
29	>126	16	..	18	1 : 1·6
30	>118	>16	..	18	1 : 1·7
30	146	13	11 : 1	16	1 : 1·9
30	139	15	9 : 1	17	1 : 1·8
30	130	12	11 : 1	15	1 : 2·0
32	125	15	8 : 1	18	1 : 1·8
35	127	16	8 : 1	19	1 : 1·8
35	148	15	10 : 1	25	1 : 1·4
37	146	11	13 : 1	19	1 : 1·9
37	123	>14	..	20	1 : 1·9
40	151	16	9 : 1	20	1 : 2·0
45	140	..	..	24	1 : 1·9
45	166	17	10 : 1	27	1 : 1·7
45	154	22	7 : 1	30	1 : 1·5
50	138	24	6 : 1	28	1 : 1·8
55	127	22	6 : 1	30	1 : 1·8
60	126	>21	..	30	1 : 2·0
60	140	34	4 : 1	38	1 : 1·6
60	144	41*	3·5 : 1	37	1 : 1·6
60	129	24	5 : 1	..	..
65	138	22	6 : 1	40	1 : 1·6
80	*ca.* 175†	..	..	55	1 : 1·5

* 18 large. † 85–90 in half—remainder damaged.

While in the above characters the specimens agree with *A. pensilis*, the structure of the tentacle bases is exactly like that of *A. macrodactyla* (Brandt); they are conical and laterally expanded, and clasp the umbrella margin and have a slight median spur (Fig. 2*c*, *d*). The median spur is prolonged downwards over the abaxial surface of the tentacle bulb as a median ridge. But the true condition of spur and ridge is only seen when the specimens are perfectly preserved. In many specimens the tentacle bulbs had become distorted and merely appeared as rather large laterally expanded swellings as has been described by various authors for *A. pensilis*. The tentacles are irregularly distributed round the umbrella margin. The number of marginal warts between adjacent tentacles is variable; usually there are about seven to nine. The marginal vesicles are extremely numerous and may exceed forty between two adjacent tentacles; each contains 2–5 concretions.

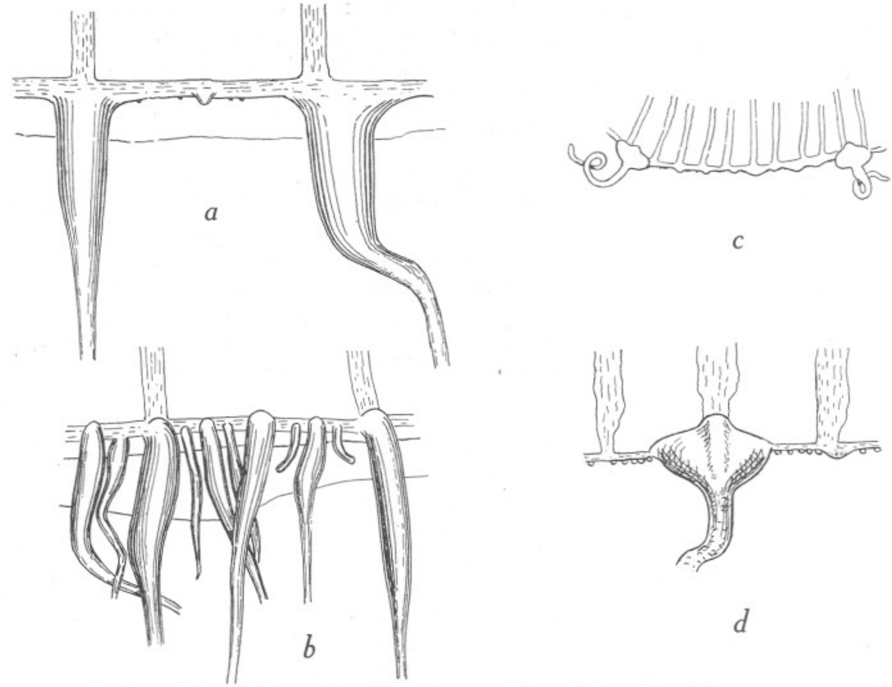

Fig. 2. Portions of umbrella margin of: *a*, *Aequorea forskalea*; *b*, *A. vitrina*; *c*, *d*, *A. pensilis*. (*a*, *b*, and *d* approximately to same scale; *c*, less enlarged.)

In many specimens it was impossible to find any excretory pores or papillae; in some, small slits without papillae could be found, while in others the papillae were quite distinct. The above three conditions might be found in the same specimen.

The Plymouth specimens thus appear to combine the characters of the two species *A. pensilis* and *A. macrodactyla*, except in so far as the number of radial canals is concerned; for *A. macrodactyla* is generally considered to have far fewer radial canals. Among the Plymouth medusae there was, however, one specimen (not included in Table I) which had only 63 radial canals. It was 27 mm. in diameter and had ten tentacles; the diameter of the stomach was 13 mm. In all other respects it was identical with the other specimens, and had the same single type of nematocyst. The occurrence of this one specimen among the others raises the question whether at times *A. pensilis* may have only half the normal number of radial canals and whether therefore *A. pensilis* and *A. macrodactyla* are really separate species. I will return to this later.

A. pensilis has not been recorded from the North Atlantic before. It is possible that the specimen so well figured by Forskål (1776) came from the Mediterranean, but he makes no mention of its origin and it may just as well have come from the Red Sea. But a search in the literature shows the occurrence of medusae which are probably *A. pensilis* recorded under other names.

The medusae referred to by Claus (1883, p. 80) as *A. discus* are most probably *A. pensilis*. Claus, who had seen so many typical *A. forskalea*, did not hesitate to regard this as a distinct species on account of its disproportionate number of radial canals. A summary of Claus's table for the characters of *A. discus* is given in Table II.

Table II

Diameter of umbrella mm.	No. of radial canals	No. of tentacles
7–8	59	8
12	95	8
15	112	8
20	94	16
30	93	16+

Browne (1897) describes six specimens from Valencia under the name of *forskalea*. While the largest of these is clearly *A. forskalea*, being 160 mm. in diameter and having 88 radial canals and 56 tentacles, the remaining five are just as clearly *A. pensilis*. Table III gives a summary of Browne's observation on these specimens.

Table III

Diameter of umbrella mm.	Diameter of stomach mm.	No. of radial canals	No. of tentacles
25	13	140	7
30	..	114	8
55	26	145	16
60	33	180	28
135	55	153	43

I have found a specimen in Mr Browne's collection taken on the same date as those mentioned above and it agrees with the Plymouth specimens in having only one type of nematocyst.

There can thus be little doubt that *A. pensilis* is a fairly common and widespread medusa, and as such it might be expected to show variation from place to place. We now have quite a number of observations for comparison and in Fig. 3 I have plotted the numbers of tentacles against the numbers of radial canals for most of the specimens recorded in the literature. These include the following specimens: those listed by Bigelow (1919, pp. 312, 314) as *A. pensilis** and *A. macrodactyla*; those recorded by Vanhöffen (1911, p. 232) as *Mesonema coelum pensile*; the Plymouth specimens recorded in this paper; and those recorded as *Aequorea discus* by Claus (1883, p. 80) and as *Polycanna forskalea* by Browne (1897, p. 829). The figure shows that there is a tendency towards two distinct groupings into *Aequorea macrodactyla* and *A. pensilis*. Yet this distinction does not seem to be sufficiently clear cut to warrant their separation into two species. It is preferable to regard them all as belonging to one species, *A. pensilis*, with a variety, *macrodactyla*, in which only half the full number of radial canals is developed. The occurrence of only one specimen of the latter type among the many specimens caught off Plymouth would seem to favour this view. It is possible that in some localities one or the other variety might predominate. This splitting of the one species into two varieties is also more convenient, since, with increasing observations, specimens are sure to be found on the border line between the two, whose identity would be uncertain if the two species were kept distinct.

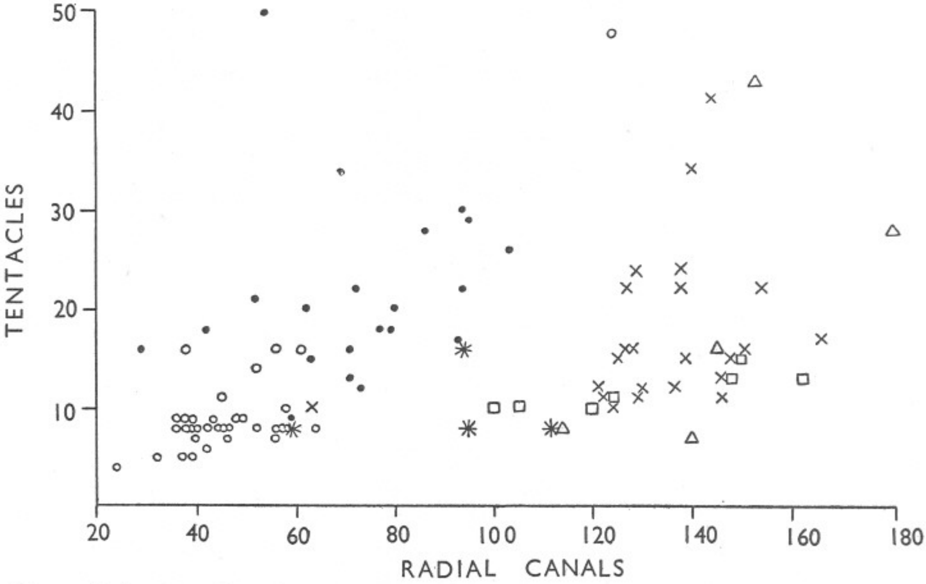

Fig. 3. Ratio of number of marginal tentacles to number of radial canals of *Aequorea pensilis* from the following sources. □, *A. pensilis*, and ●, *A. macrodactyla* from Bigelow (1919, pp. 312, 314); ○, *Mesonema coelum pensile* of Vanhöffen (1911, p. 232); ×, *A. pensilis* from Plymouth, recorded in this paper; *, *A. discus* of Claus (1883, p. 80); and △, *Polycanna forskalea* of Browne (1897, p. 829).

Mention should be made here of another specimen of *Aequorea*. On

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* Excluding the two of Maas (1905) with *ca.* 200 and 250 canals.

October 4 1932 the weekly oblique haul with the 2 m. ring trawl contained fragments of a large medusa. Fragments of about five-sixths of this specimen were present and it must have been about 180 mm. in diameter. There were 114 radial canals, making a probable total of about 140, and the tentacles were about two-thirds the number of radial canals and very irregularly distributed. The gonads were bluish grey in colour. The tentacle bulbs appeared more like those of *A. forskalea*, but they had become very much squashed in preservation. On examining the nematocysts, it was found that both in the tentacles and mouth lips only one type was present, namely basitrichous haplonemes.

Basitriches: 10–11 × 2·5–3 μ undischarged (Fig. 1*g*).

The large atrichous haplonemes so typical of *A. forskalea* were absent. I hesitate to identify this medusa for certain, but from the number of radial canals and the type of nematocyst it seems possible that it may have been a very fully developed *A. pensilis* in which the number of tentacles has increased considerably. The specimen appears to agree with that described by Forbes (1851) from the Minch as *A. forskalea*. His specimen, *ca.* 130 mm. (5 in.) in diameter, had 136 radial canals and *ca.* 68 large tentacles, with 68 small tentacles and 136 marginal bulbs.

ON THE IDENTIFICATION OF THE THREE PLYMOUTH SPECIES OF *AEQUOREA*

The distinguishing characters of the three species, *A. forskalea*, *A. vitrina*, and *A. pensilis* including var. *macrodactyla*, in their adult stages, can now be defined as follows.

	forskalea	*vitrina*	*pensilis*
No. of radial canals	Usually 60–80 rarely exceeding 100	60–90	*ca.* 120–180 or half this number
No. of tentacles	*ca.* 40–85, usually approximating to number of radial canals, rarely less than half or more than twice	*ca.* 300 or more; always exceeding number of radial canals by more than three times	Usually less than, often much less than, one-third of the number of radial canals
Tentacle bases	Elongated and conical (Fig. 2*a*)	Elongated, and slightly laterally compressed (Fig. 2*b*)	Conical, laterally expanded, often with median spur (Fig. 2*c*, *d*)
Mouth lips	Approximating to number of radial canals*	Usually about half the number of radial canals	Approximating to number of radial canals†
$\frac{\text{Umbrella diameter}}{\text{stomach diameter}}$	*ca.* 1·8–2·4	*ca.* 1·8–3·0	*ca.* 1·3–2·0
Nematocysts	Two kinds	Two kinds	One kind

* Fraser (1916) states that the mouth lips are never more than half the number of radial canals in specimens from Departure Bay in the Pacific. This may be a geographical variation.

† Maas (1905) gives figures in the neighbourhood of half the number of radial canals: his drawing (pl. viii, fig. 54), however, shows approximately equal numbers.

The ratio of the number of tentacles to the number of radial canals is also a sure guide for the identification of the young stages of the three species. Some difficulty may, however, arise in separating the young of the *macrodactyla* variety of *A. pensilis* from the young of *A. forskalea*. But herein lies the value of a study of the nematocysts, for it is only necessary to crush a tentacle under a cover glass, or take a piece of mouth lip, and examination under the microscope with a $\frac{1}{6}$ in. objective will disclose at once the presence or absence of the conspicuous atrichous haplonemes characteristic of *A. forskalea*. An additional diagnostic character for young stages appears to lie in the number of marginal vesicles. In *A. forskalea* there are only one or two marginal vesicles between adjacent marginal bulbs and tentacles, whereas in *A. pensilis* they are much more numerous.

Of the three species *A. vitrina* appears to be the most northerly in its distribution and *A. pensilis* is perhaps most confined to warm water.

It is not without interest to note here that, among the Leptomedusae that I have examined so far, I have only found one other species besides *A. pensilis* in which I could see no atrichous haplonemes. This was *Phialella cymbaloides*. It suggests the possibility that *Aequorea pensilis* and the other two species of *Aequorea* may have evolved separately.

SIPHONOPHORA

Agalma elegans (M. Sars)

Although the identity and synonymy of *A. elegans* (M. Sars) is well known it will be as well to state here that the species examined was that with the tricornuate tentillae, since Sars described two species under the one name.

There were four kinds of nematocysts. Large microbasic mastigophores and anisorhize homotrichous haplonemes, in the batteries of the tentillae; desmonemes in the two terminal filaments of a tentilla; and microbasic euryteles situated at the ends of the tentacles and in two small groups on either side of the velar openings of the nectophores (Fig. 4*a*–*h*).

Mastigophores: 185–205 × 25–28 μ undischarged.

The thread was beset along its whole length with three rows of stout barbs.

The barbs appear to be set in three parallel longitudinal rows and not spirally (cf. Schneider, 1900, p. 101).

Anisorhize: up to *ca.* 75 × 6–7 μ undischarged.

The smallest nematocysts of this type were *ca.* 22 × 4 μ.

Desmonemes: 6–7 × 6 μ undischarged.

Euryteles: 90–105 × 30–35 μ undischarged; *ca.* 70–23 μ discharged.

These large euryteles had three basal stylets similar to those of stenoteles.

In addition to the above nematocysts there were large numbers of "anacrophores" in the terminal filaments and distal ends of the spiral nematocyst bands. These ranged in size from 7 × 2 to 20 × 5 μ undischarged. In a previous publication (Russell, 1938) I suggested that these may be stages in the develop-

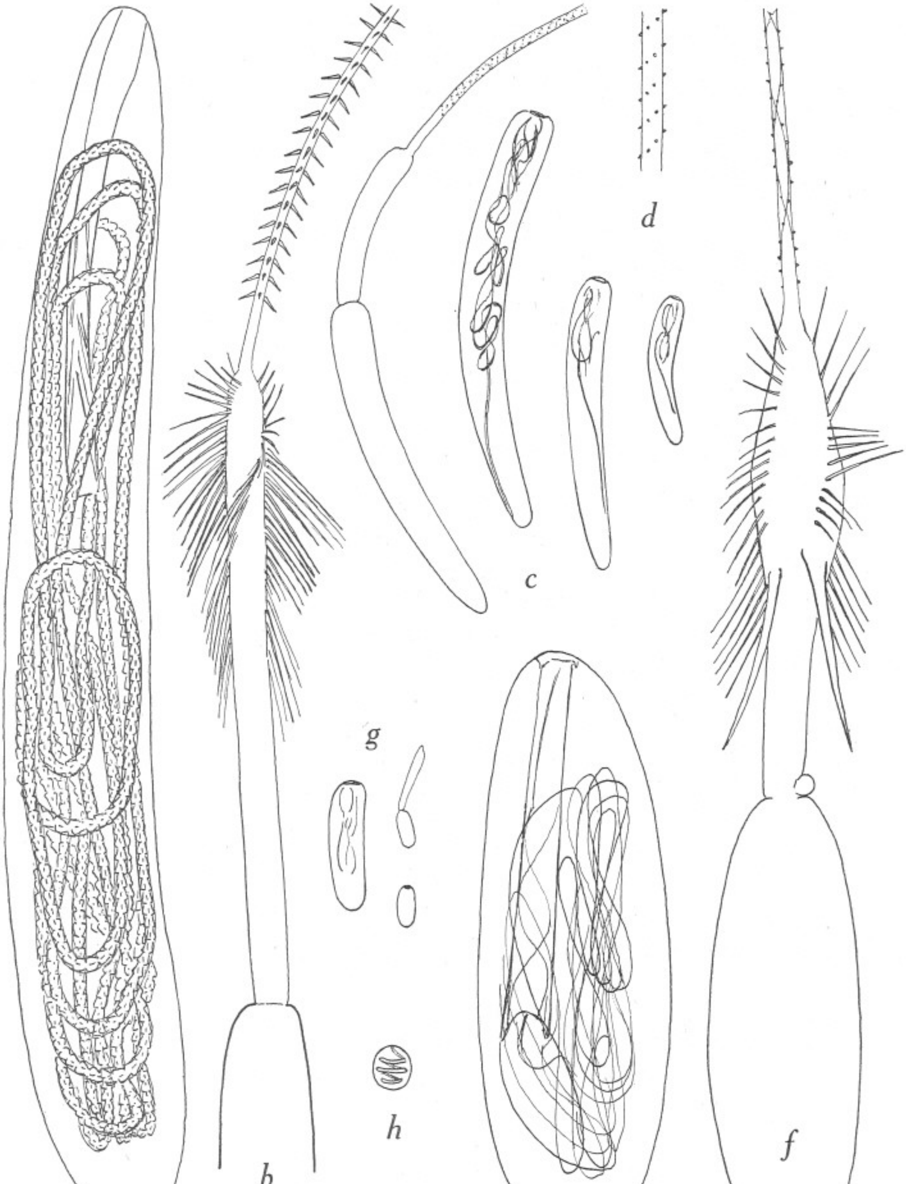

Fig. 4. *Agalma elegans*: *a*, *b*, microbasic mastigophore; *c*, anisorhize haplonemes of different sizes, with enlarged portion of thread, *d*; *e*, *f*, eurytele; *g*, "anacrophores"; *h*, desmoneme. × *ca*. 850.

ment of the desmonemes and anisorhize haplonemes. After seeing the nematocysts of *Agalma elegans* I am inclined to modify this suggestion and say that they are anisorhize haplonemes in an arrested state of development. Among the anisorhize haplonemes a complete range in size may be found in any one battery from the smallest "anacrophore" upwards. The "anacrophores" up to a length of about 20 μ are straight with rounded ends, while the smallest anisorhize haplonemes down to a length of about 27 μ are tending towards this shape. It is also noticeable that the smaller the anisorhize the shorter is its thread. There does, however, usually appear to be a break in the series between about 20 and 27 μ.

Velella velella (L.)

In October 1938 a small specimen of *Velella*, *ca.* 15 mm. long, was caught in the plankton. There were two kinds of nematocysts: stenoteles and atrichous haplonemes.

Stenoteles: these were of two sizes, 18–21 × 14–16 μ and 13–15 × 10–11 μ undischarged.

Atriches: 8–9 × 4·5 μ undischarged.

The specimen when examined was in a very decomposed condition and the armature of the discharged stenoteles always broke away and could not be made out in detail.

These nematocysts agree fairly closely with the sizes given by Weill (1934, p. 516) for specimens from Samoa preserved in alcohol in the British Museum. This is additional evidence in favour of the conclusion of Bigelow & Sears (1937) that the Atlantic and Pacific *Velella* are the same species.

SUMMARY

The nematocysts of the three species of Leptomedusae, *Aequorea forskalea*, *A. vitrina*, and *A. pensilis*, and of the two siphonophores *Agalma elegans* and *Velella velella* are described.

The specific characters of the three species of *Aequorea* are discussed and it is shown that *A. pensilis* is probably the same species as *A. macrodactyla*.

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SEASONAL VARIATIONS IN THE FAT CONTENT OF THE FLOUNDER, *PLEURONECTES FLESUS* L.

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(Text-figs. 1–5)

CONTENTS

INTRODUCTION

In only a few fishes has any attempt been made to trace variations in the fat content from season to season and during the spawning period. Of the economically important herring, however, a great many seasonal fat analyses have been made, and more than one species of salmon has received attention from this point of view. So far as I am aware little has been done along similar lines for any of the bottom-dwelling flat fishes. When, therefore, there arose an opportunity of examining a plentiful supply of flounders, about which a good deal of other relevant information would be available, it was decided to concentrate on this species. The flounders in question were obtained by Mr P. H. T. Hartley during his investigations into the fisheries of the Tamar and Lynher estuaries. I am greatly indebted to him for his kindness in passing them on to me after he had weighed and measured them, noted the sex and maturity and removed the stomach of each fish. He subsequently supplied me with copies of his records which have proved invaluable.

METHODS

The fat content of a fish has sometimes been estimated approximately by visual judgement, as in assessing the quality of herrings by the amount of intestinal fat (ister) seen on opening the body cavity (Hjort, 1914; Bjerkan, 1917). Occasionally judgement has been made from frozen sections cut from selected regions of the body and stained by one of the fat stains. This was the method mainly employed by Greene in his important work on the storage of fat in the muscular tissue of the king salmon, *Oncorhynchus tschawytscha*. Some investigators have preferred the method of chemical analysis; in this connexion can be mentioned the work of Johnstone (1915, 1918) and of Bruce (1924) on the Manx herring. Johnstone partly confirmed his results by cutting paraffin sections of portions of the muscular tissue, thereby demonstrating variation in the quantity of adipose tissue present. As it is not usually the practice to analyse chemically the whole of one fish, and it is always difficult if not impractical to deal with large numbers in this way, the chemical method may be no more efficient in demonstrating seasonal or ontogenetic variations in fat content than is the histological method, provided that the species treated in the latter way is one which gives a clear picture of the fat when cut and stained. On the other hand, the histological method can demonstrate only fat which is in such a form as to be readily stainable, and it does not allow of the calculation of calorific values.

In the present work histological technique has been used throughout. It was early realized that the cutting, staining and mounting of frozen sections takes up too much time to allow a really large number of fish to be examined. A much simpler technique was therefore devised and one that gave a better fat picture for each fish. The method consisted of cutting a slice 1–2 cm. thick across the fish at its widest part midway between head and tail. Prior to cutting, the fish were hardened in 10 % formalin for at least several days. The slice was rinsed in 50 % alcohol and immersed in a solution of Scarlet Red (Sudan IV) in equal parts of 70 % alcohol and acetone contained in a tightly stoppered jar. After 20 min. to half-an-hour it was removed from the stain, washed in 50 % alcohol and then preserved in 5 % formalin. For the purpose of preservation formalin is not ideal and does not keep the sections in as good a condition as does glycerine. It will, however, keep them for a few weeks and sometimes months without much deterioration of the staining, and the slices can always be restained to some extent. When large numbers of such slices of fish have to be preserved glycerine is almost prohibitive on account of its cost. I have not noticed any difference in result between neutral and non-neutral formalin.

The method enabled fairly large samples of fish to be handled expeditiously, for a number of slices could be stained at one time. It was found unnecessary to take more than one slice from each fish. A series of slices down a fish all gave similar fat pictures.

The appearance of typical slices is illustrated in Fig. 1. Various regions would stain bright red and be large or small in area according to the amount of fat and adipose tissue present. In a fat fish areas roughly triangular in shape showed up distinctly among the muscles of the bordering fins. A bone, sliced across, generally showed more or less fat in and around it. The picture varied a little, of course, with the level of the cut, whether, for instance, it went

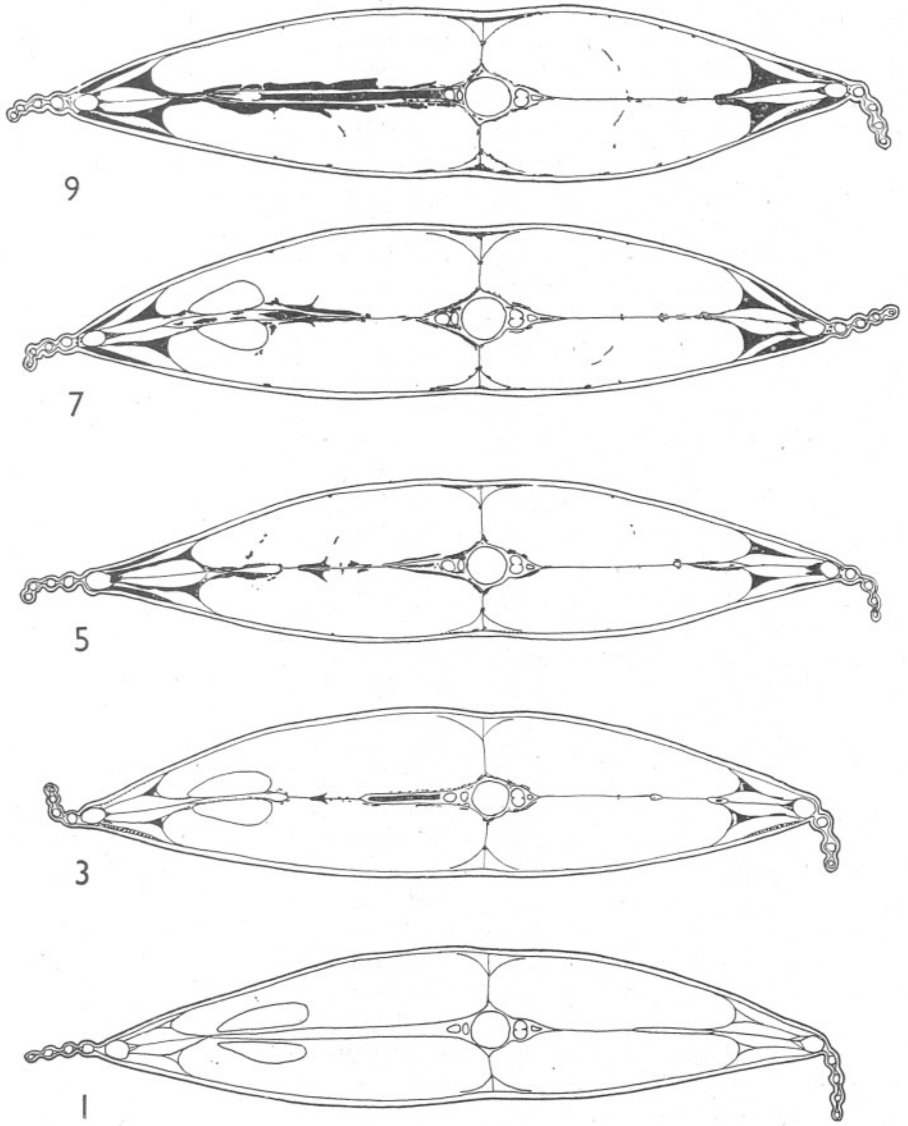

Fig. 1. Diagrammatic representations of transverse slices of the flounder midway between head and tail to show the relative amounts of fat in the five main arbitrary degrees as follows: 1, none; 3, little; 5, medium; 7, fat; 9, very fat. In this figure degrees 1, 3 and 7 are illustrated by female fish, 5 and 9 by male fish. The fish selected as typical for the purposes of this illustration were all about 20 cm. long. The figures are drawn by projection in an episcope, the fat which is stained deep red in the actual specimens being here shown as black.

through the middle of a vertebra or passed between two of them. Variation in the level of the cut, however, never affected the general appearance of fatness; it did not expose so much more or so much less fat as to make a fish appear fatter or leaner than it would if sliced in another plane. The two faces of a slice always corresponded closely although they would rarely be cut at the same vertebral level. In judging fatness both faces of a slice were taken into consideration. After some experience it became easy to allot each fish to one of nine arbitrary degrees of fatness. Five primary degrees were chosen, none (degree 1), little (3), medium (5), fat (7), and very fat (9); between these are the intermediate degrees 2, 4, 6 and 8. The five primary degrees are diagrammatically illustrated in Fig. 1 drawn from actual slices selected as typical, and optically projected by an episcope so that the outlines of the fat areas could be accurately traced. These drawings, together with actual type specimens, were used for comparison when assessing a sample of newly stained slices.

The accuracy of the method just outlined was checked from time to time by reassessing samples of fish the original records of which were not remembered. It always happened that not less than 80 % of the fishes comprising the sample were given the same degree as before; the remainder would be placed one degree higher or lower, about half of them higher and about half of them lower. Very occasionally an odd fish would be moved two degrees up or down the scale of nine. Thus the method was well within the limits of error which could be allowed.

The main results based on the large slices were supplemented by cutting frozen sections from as many fish as possible. Large pieces of tissue were removed from the pigmented upper side half-way between head and tail. The pieces were generally obtained from large slices which had been previously cut and stained. The tissue was taken in the region of the lateral septum, extending some way on either side and down to the vertebral column. From each piece of tissue generally three, sometimes more sections were stained and mounted. Some were also cut from the region of the dorsal and anal fins after removal of the bones.

For cutting, the tissue was surrounded by an aqueous solution of gelatine (5 %) and the sections allowed to fall into a dish of the same solution kept just melted by gentle heat. Selected sections were stranded on clean slides, cooled and hardened first in formalin vapour and then in 5 % formalin. The sections thus stuck to the slides were stained in Scarlet Red dissolved in equal parts of 70 % alcohol and acetone. The sections were permanently mounted in glycerine sealed by glycerine jelly and "Murrayite". They have kept very well to date.

The sections were cut 150μ thick. Thinner sections were useless as the fat cells are relatively large and most of the fat is lost when their membranes are ruptured. By cutting thick sections fat loss was relatively reduced. The sections were thin enough for easy observation by transmitted light.

Distribution of the Adipose Tissue

In this paper attention is confined to the fat and adipose tissues in and around the edible muscular portions of the flounder, and to some extent of its skeleton. The head and viscera are neglected.

Adipose tissue is developed in definite regions. The largest and most striking are clearly visible in a stained slice across the body of the fish; they are the fatty regions of the dorsal and ventral unpaired fins. The muscles to the fin rays are packed around with fatty tissue filling up the spaces which might otherwise be left between them. In a skinned fish which has been preserved in formalin, or in one that has been boiled, this adipose tissue is distinguishable from the whiter muscles by its yellow brown colour. This colouration seems to have misled Stirling (1886) into describing the tissue as red muscle, both in the plaice and in the flounder. In really fat fish much fat is deposited in these regions, padding out the edges of the fish so that they feel plump between the fingers. On the other hand a lean fish, particularly one which has lost all its fat at spawning, feels thin and even looks emaciated where the median fins join the body. In such a fish the adipose tissue is in a soft and collapsed condition owing to the withdrawal of the fat it formerly held.

There is generally a little fat between the skin and underlying muscles. Where the lateral septum joins the skin there is often a considerable accumulation which in transverse section (Fig. 2 A) frequently appears as a roughly triangular area bisected by the septum itself. There are similar deposits of fat where the myocommata reach the skin (Fig. 2 B). Adipose tissue extends down the myocommata in the region of the superficial lateral muscle and is often well developed where the myocommata join the lateral septum. Where the myocommata pass through the deep lateral muscle adipose tissue is generally absent, but in fat fish a little fat is deposited here and there.

The skeleton is a fat depot of some importance, particularly the vertebral column. The spongy parts of the vertebrae and their hollows are frequently heavily laden with fat, and so are the grooves and canals of the neural and haemal spines. Adipose tissue is often present around the bones. The bones of the skull may also be laden with fat.

Groups of fat globules are present in the skin among the chromatophores, but the quantity of fat so stored is very small in comparison with that in the depots already described.

Fat is distributed in a closely similar manner in many flat-fishes. Several specimens of each of the following species have been examined. *Pleuronectes platessa*; *P. limanda*; *P. microcephalus*; *Rhombus laevis*; *Lepidorhombus whiff*; *Arnoglossus laterna*; *A. imperialis*; *Solea vulgaris*. It is also very similarly distributed in *Zeus faber*.

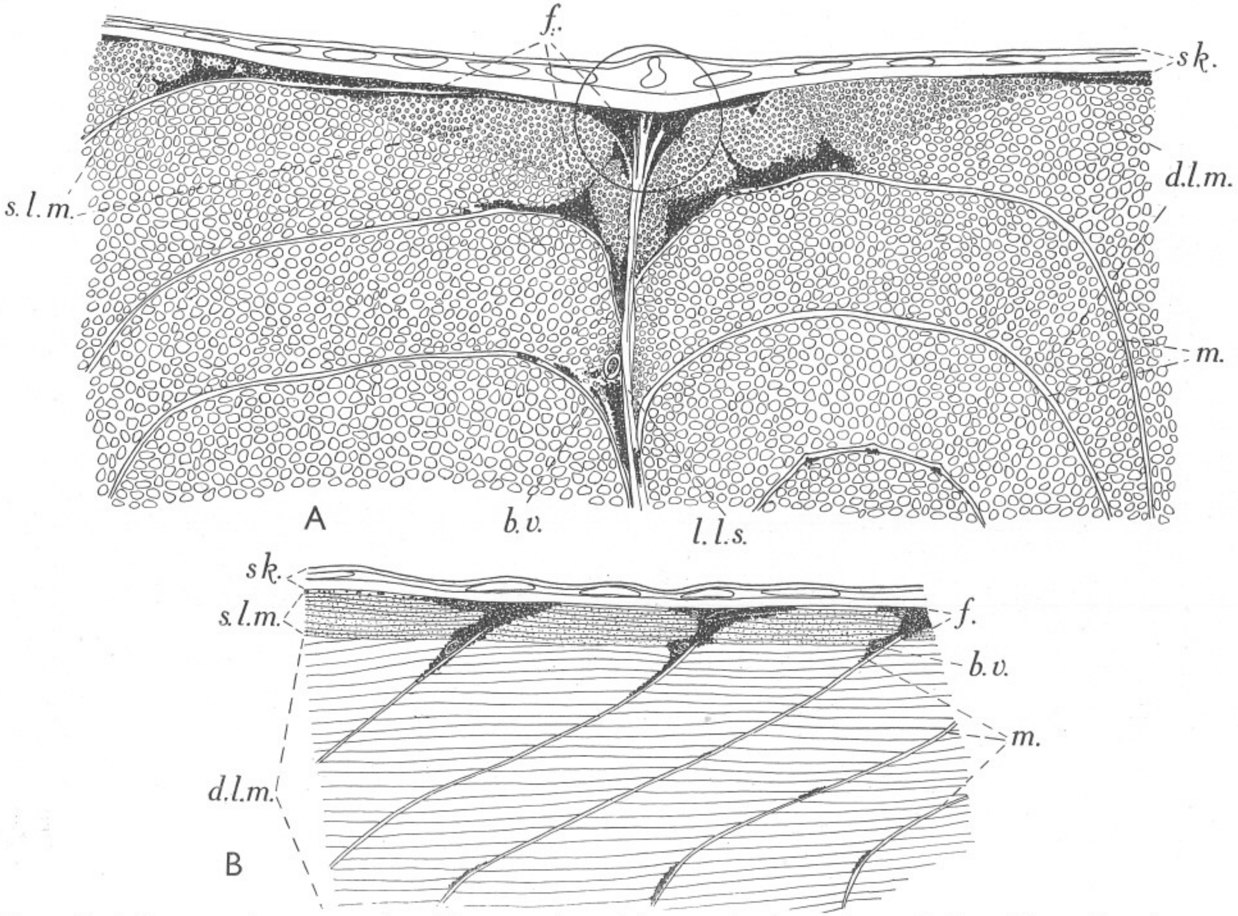

Fig. 2. Semi-diagrammatic representations of frozen sections of the muscular tissues close to the lateral line, midway between head and tail, on the pigmented side of the flounder. × 5. Fat shown in black. The circle indicates the region shown in greater detail in Fig. 5. A. Transverse section from a female caught at sea in February 1937. The fish was 35·7 cm. in length and was placed in fatness degree 8. Intramuscular fat was very plentiful in the superficial lateral muscle. B. Longitudinal section some little distance from the lateral line septum. This is from a male caught at sea in February 1938. The fish was 28·8 cm. long and was placed in fatness category 6. Intramuscular fat was very plentiful in the superficial lateral muscle. *b.v.* blood vessel; *d.l.m.* deep lateral muscle (musculus lateralis profundus); *f.* fat in adipose tissue; *l.l.s.* lateral line septum; *m.* myocommata; *sk.* skin; *s.l.m.* superficial lateral muscle (musculus lateralis superficialis).

IMMATURE FLOUNDERS IN THE ESTUARIES

The growth and life history of the flounder in the Tamar and Lynher estuaries is discussed by Hartley (1939). The immature fish feed and grow in the estuaries for several years before they go down to the sea to spawn. These immature fish formed the bulk numerically of the catches investigated. The fatness of these immature fish was followed from February 1937 to February 1938, over five hundred fish being examined during this period. In addition some small exploratory samples were examined prior to February 1937; as far as they go they fit into the sequence here described. The results for males and females are combined since no significant difference was found between them.

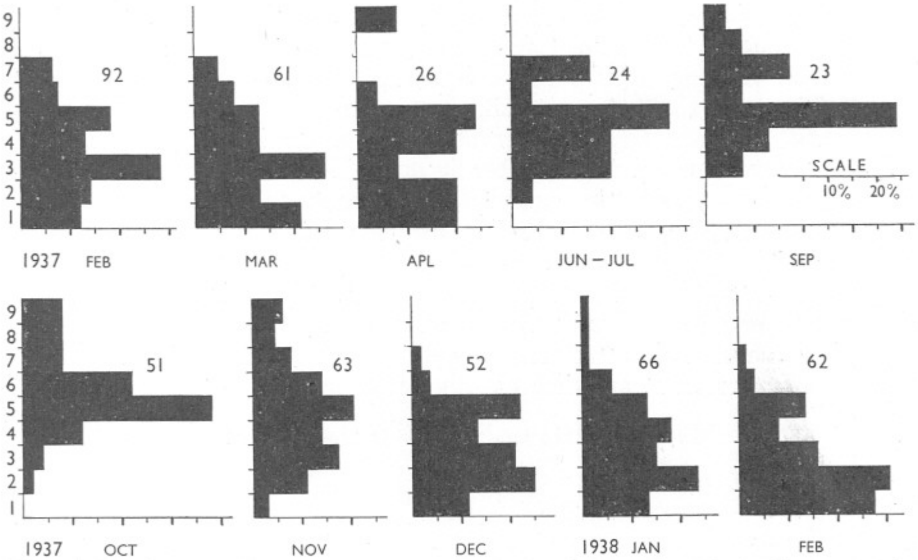

Fig. 3. The fatness of immature flounders caught in the estuaries. The ordinates are the arbitrary degrees of fatness explained on p. 364 and illustrated in Fig. 1. The abscissae are percentages. The number inserted in each figure gives the actual number of fish on which it is based.

The monthly results are given in the form of diagrams (Fig. 3). The fish were sliced and stained by the method already described. The diagrams give the percentage number in each fatness group. The number of fish on which each diagram is based is indicated in the figure.

It is at once obvious that in February 1937 the majority of the flounders had a medium (degree 5) or less than a medium amount of fat in their tissues; 12 % had none. By March the percentage with none had increased to rather more than twenty-one and the sample as a whole was a little less fat than in the preceding month, although the difference is but slight; allowing for the experimental error inherent in the method it is perhaps not significant. The first week in April showed little change; the sample was a small one in which two large immature but very fat females probably give an undue prominence to degree 9 in the diagram. As explained by Hartley (1939), it was difficult to catch flounders during the summer and the samples taken then were all small. Only one fish was caught during May; it was of medium fatness (degree 5). The immature fish of June and July are combined in the one diagram. It is notable that in no fish caught during these months was fat entirely absent, while the general fatness of the sample was higher than before. In August a very few fish were caught but it is worth recording that of the four immature ones obtained three were fat (degree 7) and one medium to fat (degree 6). In September and October immature flounders reached their maximum fatness for the year. It is apparent from the diagrams that the majority were of medium fatness (degree 5) and over, a striking contrast to the condition of February, March and April fish. In November fatness was on the decline, and once again a few fish were found with no visible fat at all. December and January fish were on the whole definitely lean, while in February 1938 the leanest sample of the series was obtained, over half the fish having either no fat at all or very little indeed.

It is possible to work out a mean degree of fatness for each monthly sample: these means are presented in the first column of Table I. Again it will be seen that the immature fish fatten from March to September and October when the maximum is reached and then steadily lose fat until the following February.

TABLE I. MEAN DEGREES OF FATNESS OF IMMATURE FLOUNDERS

		Total fish	Fish under 16 cm.	Fish over 16 cm. and under 20 cm.	Fish over 20 cm.
1937	Feb.	3·6 (92)	3·1 (33)	3·8 (42)	3·9 (17)
	Mar.	3·3 (61)	2·9 (41)	3·5 (28)	4·3 (18)
	Apr.	3·7 (26)			
	May	. .	. .	. .	. .
	June	4·2 (24)	3·9 (9)	5·0 (7)	5·1 (8)
	July				
	Aug.	. .	. .	. .	. .
	Sept.	5·6 (23)	5·0 (23)	6·0 (34)	5·6 (17)
	Oct.	5·6 (51)			
	Nov.	4·7 (63)	4·3 (6)	5·1 (36)	4·2 (21)
	Dec.	3·3 (52)	3·0 (63)	3·5 (30)	3·9 (25)
1938	Jan.	3·3 (66)			
	Feb.	2·6 (62)	2·5 (35)	2·6 (10)	3·0 (17)

The numbers in brackets give the actual number of fish on which each mean is based.

The size range of the immature fish was considerable. Most of them were over 10 cm., but less than 20 cm. in length. A few were less than 10 cm. long. Of those which exceeded 20 cm. only a very small number were longer than 25 cm., the largest of all generally being female.

In order to ascertain whether fatness varies to any extent with size, the fish have been grouped into three classes according to length. The limits of each size group are somewhat arbitrarily selected, but a limit at 16 cm. divides almost equally the yearly total of fish less than 20 cm. long, although it will be seen from Table I that in any one sample there are usually more of one length class than of the other. It is not as a rule until they are longer than 20 cm. that flounders mature and go down to the sea. The mean degree of fatness was calculated for each size group for each month, or for two months combined. These figures are given in Table I.

It will be observed that though there is little to choose between the relative fatness of the three size groups, the under 16 cm. group are consistently leaner than the 16–20 cm. fish, and in all but one month (November) leaner than the fish over 20 cm. The latter have a slightly higher mean than the 16–20 cm. fish except in the combined September and October groups and in November. Although the significance of this difference is uncertain as the numbers of fish were small, there is nevertheless the possibility that a proportion of the fish over 20 cm., which would previously have been ranked as immature, are in the period September to November sufficiently far advanced in maturity to be then recognized as maturing flounders and would therefore no longer be counted among the immatures. Such fish would be likely to be among the fattest in the sample and their removal would bring about a lowering of the mean.

On one point the means for the three length groups are quite definite; they demonstrate conclusively that the annual cycle of fatness in immature fish over 10 cm. long takes place irrespective of size.

MATURING AND SPAWNING FLOUNDERS

Maturing and spawning flounders leave the Tamar and Lynher estuaries early in the year and by the end of February only immature fish remain. It is possible to distinguish the ripening fish, or at least some of them, during the preceding summer: in the later months of the year this can be done with greater certainty as the gonads become more swollen. Ripening estuarine fish are always relatively large, the females generally exceeding 25 cm. in length, though the males are often smaller than this. The fatness records for maturing estuarine flounders are presented graphically in Fig. 4 *a–c*, and the means based on the same figure are given in Table II. As the total number

TABLE II. MEAN DEGREES OF FATNESS OF MATURING AND MATURE FLOUNDERS

	Mean degree of fatness	No. of fish
Estuarine fish:		
June, July and August 1937	7·0	11
November and December 1936 and 1937	7·0	30
January and February 1937 and 1938 ...	6·1	33
Sea fish:		
February 1937 and 1938	6·5	75
March 1937 and 1938	4·7	62
April 1937 and 1938	2·4	29
Females returned to the estuaries after spawning:		
May–August 1937	6·4	7

of fish was rather small two years are combined. There was no marked difference between corresponding months of the two years when each year was considered separately.

If the fatness of these ripening fish be compared with that of immature flounders for the corresponding seasons it will be seen that they are much fatter. The mean for the summer months is 7·0, a figure never reached by the immature fish. It is conceivable that this may be exceeded during September and October at which time immature fish are fattest. Unfortunately all large fish caught during those months were required for marking experiments and there are therefore no fat records available. The mean is, however, still 7·0 during the last two months of the year, a period when immature fish rapidly lose fat. By January and February, however, there has been a slight decrease in the fatness of those maturing fish which have not yet moved down to the sea.

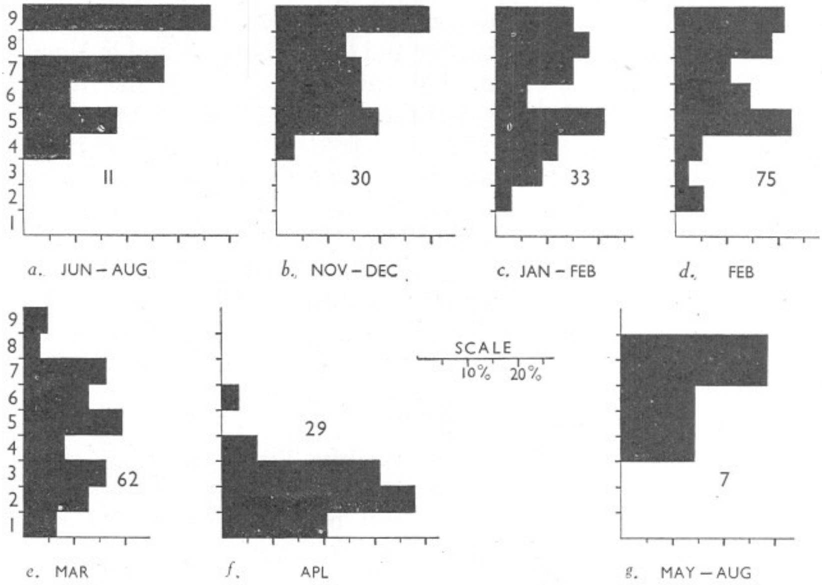

Fig. 4. The fatness of maturing and spawning flounders. *a–c*, fish caught in the estuaries; *d–f*, fish caught at sea; *g*, females caught in the estuaries after spawning. *a* is based on fish caught in 1937 only; *b* on fish caught in 1936 and 1937; *c–f* on fish caught in 1937 and 1938; *g*, on fish caught from May 29 to August 9, 1937. The scales are as in Fig. 3.

A few flounders were caught at sea during January, but it was not until February that they were at all numerous in the trawl. While some of these would have come from the estuaries a short time before capture, it is possible that some had been in the sea since at least the preceding season. Unfortunately we know nothing of the life of such fish in the sea; as Hartley (1939) points out, flounders are rarely taken in the trawl out of the breeding season.

Ripe and ripening sea-fish during February generally have plenty of fat in their tissues although a few are quite lean. The mean is high (6·5), for these fish as a whole are relatively fatter than are immature fish at their best during the late summer months, while they are, of course, very much fatter than are immature fish during February. This fat is, however, rapidly used up during the spawning period so that in April, by which time the fish are quite ripe or have already spawned, sea flounders are very lean indeed. The loss of fat is so rapid that the adipose tissue presents a collapsed appearance, sections showing crumpled cell walls enclosing no fat at all or only a few droplets. It will be noticed that in two months the figure for mean fatness falls from 6·5 to 2·4, whereas in immature fish it takes at least four months for the mean to fall through the smaller range of 5·6–2·6.

It would be instructive to find out if possible whether the loss of fat during the spawning period is at all accelerated during the presumably strenuous act of shedding eggs or sperm. The figures denoting mean fatness for February, March and April (Table II) include partly spent and spent fish. If we work out the mean fatness of these spawning and just spawned fish we get the following figures, the number in brackets indicating as usual the actual number of fish on which each mean is based: February, 5 (1); March, 4 (18); April, 2·4 (9). Ignoring February when only one spent fish was obtained it will be seen that while the figure for March is appreciably lower than the mean for all March sea fish combined, that for April is exactly the same. From this last result it seems likely that the decrease in fatness is a process not particularly accelerated by exhaustion during spawning. The lower mean for spent and partly spent fish in March may perhaps be simply due to these fish being a little in advance of the others in maturity, or as the difference is small it may not actually be significant.

Recovery of Spent Fish

Relatively few spent fish are caught in the sea, and these are nearly all obtained in March and April. After April they are not caught at all when trawling at sea, perhaps because they move too close inshore (Hartley 1939). Some of them perhaps die shortly after spawning. A few females, but apparently no males, make their way back to the estuaries and may there be caught in the tuck-nets, when they are easily recognized as spent by the condition of the gonad. Seven such females have been examined; they were caught on different dates between and including May 29 and August 9, 1937. Their mean fatness was high (see Table II and Fig. 4 *g*); the loss at spawning had evidently been made good rapidly. The histological difference between their tissues and those of ripe fish is dealt with below.

THE REGION OF THE SUPERFICIAL LATERAL MUSCLE

Frozen sections of the superficial lateral muscle region of the dark side halfway between head and tail were cut from one hundred and thirty-eight flounders selected as typical specimens of both sexes caught at all seasons of the year, both mature and immature fish. These were sufficient to give some idea of the histological distribution of the fat in this important region and of its seasonal changes.

The distribution of the subcutaneous fat and that on the myocommata as seen in these sections has already been described (p. 365). The fat is present in the form of liquid globules each enclosed within a fine membrane, the wall of the fat-storing cell. When the cell is fully loaded the wall is difficult to distinguish, but when the cell loses fat, as during spawning, it is more readily visible (Fig. 5 *c*). Large globules may be as much as 125 μ in diameter, but most of them are of various sizes smaller than this. In very fat fish the globules all tend to be large (Fig. 5 *a*) and are so closely packed as to be distorted by pressure against one another. In leaner fish the globules are on the whole smaller and not so numerous, but nevertheless they may be closely packed and distorted (Fig. 5 *b*), the difference in the amount of fat between a lean fish and a fat one being due as a rule mainly to the smaller amount of adipose tissue in the former and not so much to a wider spacing of the globules (compare *a* and *b* in Fig. 5). This is particularly true at the season of maximum fatness, but it does not hold good for fish which have recently lost much fat from their tissues. At the end of the spawning season the globules are often spaced apart and, because they are no longer pressed against one another, they round up within their cells (Fig. 5 *c*). In fish which have suffered severe fat loss globules are not only much reduced in size, some being minute, but they are often rather widely spaced apart due to the complete absorption of many others (Fig. 5 *e*). The absorption of the fat brings about a collapse of the adipose tissue cells which are crushed and crumpled between skin and muscles. Immature

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Fig. 5. Semi-diagrammatic representations of the muscular and fatty tissues cut transversely near the junction of the lateral line septum with the skin. The area shown is indicated approximately by the circle in Fig. 2 A. The sections were stained in scarlet red and mounted in glycerine. For full explanation see text. *a*, from a ripening male. Tamar estuary November 1937. Length 21·5 cm. Fatness degree 9. A moderate amount of intramuscular fat was present but is not shown in the figure. × 22; *b*, from a ripening male. Tamar estuary November 1937. Length 21·0 cm. Fatness degree 5. A little intramuscular fat was present but is not shown in the figure. × 22; *c*, from a ripe female, caught at sea February 1938. Length 36·6 cm. Fatness degree 5, showing evidence of considerable fat absorption. Intramuscular fat was plentiful in most fibres of the superficial lateral muscle. A few clear fibres are seen. × 92; *d*, a few fibres of the superficial lateral muscle showing arrangement of the intramuscular fat droplets. From a ripening female caught at sea in February 1937. Length 35·7 cm. Fatness degree 8. × 350; *e*, from a ripe female caught at sea in March 1937. Length 34·9 cm. Fatness degree 5. Fat globules small and scattered, evidence of much absorption having taken place. A considerable amount of intramuscular fat was present but is not shown in the figure. × 14; *f*, from a female which had returned to the Tamar estuary after spawning. Caught June 1937. Length 34·7 cm. Fatness degree 7. No intramuscular fat was present. × 14.

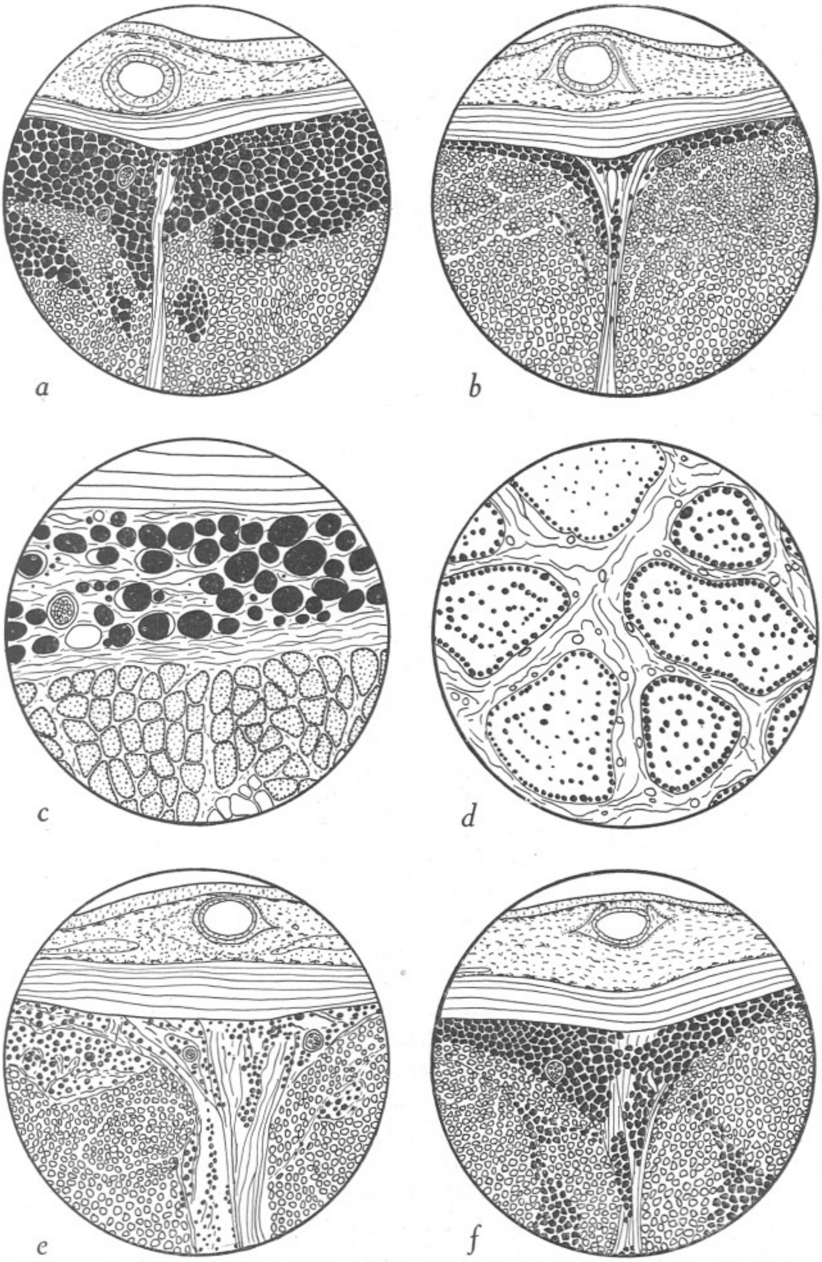

Fig. 5.

estuarine fish at the end of the winter show similar evidence of fat reduction, but to a less extent, the globules then not being on the whole quite so large and tightly packed as in September and October. In some individuals during February and March the globules are tiny, rounded and well separated. Thus the seasonal and spawning variations in general fatness can be followed, as might perhaps have been expected, in the histology of the adipose tissue of a particular body region.

Although histological investigation has been concerned chiefly with the superficial lateral muscle region, sections of the fat reservoirs between the dorsal and anal fin muscles have been cut from a few individuals. The condition of these depots appears to correspond exactly with that of the adipose tissue under the skin. In fish which have lost much fat during the spawning period the fat globules are all very small, rounded and widely scattered among empty and crumpled cells, just as they are in the mid-lateral region. It has already been remarked that loss of most of the fat in this region of a formerly fat flounder can be detected in the entire fish (p. 365). The withdrawal of much of the fat leaves the tissue readily compressible between finger and thumb. This is still more easily observed while handling large slices across the middle of the fish.

Fat is withdrawn from the skeleton about the same time as from the other depots. On account of the difficulty of sectioning this region it has not been examined in histological detail.

At certain times and in certain individuals, fat droplets appear inside most of the fibres forming the superficial lateral muscle. In the king salmon (Greene, 1914) this muscle similarly carries drops of intramuscular fat, only in much larger quantity. This is in addition to a large quantity of intermuscular fat between the fibres; the total amount of fat of both types being relatively enormous. In the flounder there is no intermuscular fat in either the superficial or deep lateral muscles, but the presence of intramuscular fat droplets at certain times is very interesting and merits attention. So far as I am aware, such intramuscular fat has been described only for the salmon. I have, however, seen fat droplets in large numbers in the fibres of the superficial lateral muscle of quite small specimens of the hake, *Merluccius merluccius* (L.). They do not seem to occur in the much fatter herring, but doubtless other fish will be found to possess them.

Intramuscular fat droplets are most strongly developed in sea flounders during the spawning period. The droplets are generally no more than 2 or 3 μ in diameter and are frequently less, but occasionally in a few fibres, perhaps in different parts of the same fibre, they reach a larger size and may be as much as 7 or 8 μ across; rarely they are larger still. A transverse section of a fibre (Fig. 5 *d*) generally shows a ring of droplets immediately under the sarcolemma with others scattered rather uniformly in the main body of the fibre itself. Both in size and distribution within the fibre they correspond closely to the description Greene has given for the king salmon.

During summer months no intramuscular fat is visible in the majority of estuarine fish, both maturing and immature. The cytoplasm of the muscle also takes up little or no stain, being clear and almost colourless in appearance in the preparations. In the autumn, however, this muscle sometimes stains pale pink with Scarlet Red and in some fish intramuscular fat droplets are to be seen. These droplets are very pale in colour, suggesting that when they first appear they contain little true fat. During the winter immature fish vary in condition; in some the cytoplasm is unstained, such fish having as a rule no intramuscular fat or at most very little. In others the cytoplasm stains pink and these fish generally have intramuscular fat droplets, occasionally in abundance. Maturing fish at this time nearly all show a pink staining of the cytoplasm and have fat droplets in the fibres, in a few individuals these are in large numbers. The concentration of intramuscular fat in the immature fish shows little or no increase beyond the winter condition, but in mature fish from the sea during February and March much of it is found, the droplets staining much more deeply at this time than before. The cytoplasm too is generally stained pink, often quite deeply. Only exceptionally in such fish is there no intramuscular fat. This condition of strongly stained cytoplasm and much intramuscular fat persists until after spawning. Even in fish which have lost most of the fat from their adipose tissue the superficial lateral muscle stains quite deeply and contains fat droplets in abundance. This fat is evidently not reduced in quantity while that from the adipose tissue is being used up. It is nevertheless soon lost; females which have returned to the estuaries after spawning show clear unstained cytoplasm (like most other summer fish) and no intramuscular fat—or at most only a trace. These latter fish are generally fairly fat, their adipose tissues being well laden with large globules closely crowded together (Fig. 5*f*). The absence of fat from their superficial lateral muscle fibres is therefore not due to lack of fatty materials at that time. The immature fish in the estuaries also lose their intramuscular fat about the same time.

The foregoing description of the intramuscular fat cycle is only a brief sketch of changes followed with the aid of a single stain; an insufficient number of fish have been sectioned for a complete account to be given. Microchemical investigation is desirable before attempting seriously to discuss its implications. It may be noticed, however, that while this intramuscular fat is most strongly developed in mature sea fish, and might possibly be related to the maturation of the gonads, it also appears in immature fish, although to a less extent, about the same time. Hartley (1939) has shown that there is sometimes to be observed a spurious ripening among immature flounders. The presence of intramuscular fat can also, of course, be correlated with temperature, and perhaps with other conditions of the environment as well.

DISCUSSION

We have seen that immature flounders are fattest at the end of the summer and leanest at the end of the winter. This refers to the population as a whole, for at any one time some fish are much fatter than others of the same size and sex. Sex appears to have little effect on general fatness, while there is only a slight tendency to greater fatness with increase in size. No doubt some individual flounders are naturally lean while others have an equally natural tendency to store fat in their tissues; this is probably to some extent independent of the amount of food consumed. It seems legitimate to assume, however, that after a period of intensified feeding lean fish will be fatter than usual and naturally fat fish very fat. During periods of starvation both types of fish may be expected to use up some or all of their fat reserves. With this idea in mind we can correlate seasonal variation in fatness with feeding.

Hartley has examined the stomach contents of the flounders used in these fatness determinations and he has shown (1939) that the immature fish have a marked period of fasting during the winter and that this fast is not completely ended until probably April. This period when little food is taken is that during which immature flounders lose much of their autumn fat. In the winter of 1937–8 Hartley found that the fast began earlier than in the preceding year, and to this may perhaps be correlated the greater leanness in February 1938 as compared with February 1937. During the summer and until September feeding is most intense; it is at the end of this period that the immature fish are fattest.

Maturing and mature flounders behave differently for they continue to feed throughout the fasting period of the immature fish, only ceasing to take food during the spawning season in the sea. To these habits again fat content may be correlated, maturing flounders remain very fat until the end of the year and then show a rapid loss while breeding. During spawning a great deal of energy is probably expended in muscular and other activities; abstention from food at this time will cause a heavy demand upon the fat reserves, and, as we have seen, they are depleted very rapidly.

It might be expected that in maturing fish fat would be withdrawn from the tissues during maturation of the gonads, which in the female especially accumulate a great quantity of fatty substances. The fat for the gonads, however, may come from the visceral fat which has not been investigated during the present work. It should be remembered too that the fish are continually feeding while the gonads are filling. At any rate the gonads reach their full size before there is any loss of fat from the muscular regions. This is in agreement with what is known of the herring where it appears not improbable "that the metabolic demands of the growing gonads are met, in the first instance by the fatty reserves of the liver, while at a later stage the stores of mesenterial fat may be called upon. There is no evidence that the fat of the muscular tissue undergoes depletion, until shortly before the time of spawning" (Bruce, 1924, p. 483). Johnstone (1918) found that in the herring maturation of the gonads is accompanied by an increased loading of the subdermal and intermuscular connective tissues with fat, and that this loading does not decrease until a short time before spawning and "after the major part of increase in mass of the gonads has taken place". As is well known, herring do not feed while spawning. More recently Channon and El Saby (1932) have shown that in maturing herring the muscle fatty acids continue to increase in amount after those of the liver and mesentery have begun to decrease.

Johnstone (1915, 1918) emphasized the importance of temperature as a factor in influencing variations undergone by the tissue fat. He showed the close correspondence between sea-temperature and rise and fall of fat content, and argued that temperature must be a factor in addition to the sexual cycle. Thus Clyde winter-spawning herring maturing in cooling water have a considerably lower maximum fat content than the Manx autumn spawners. He had little to say about feeding. The close correlation between fatness and feeding is discussed in a recent paper by Lovern & Wood (1937). The facts are strongly reminiscent of those already pointed out for the flounder.

The effect of seasonal temperature variations on the flounder is probably reflected in the intensity of the feeding of immature fish. It is while the temperature is rising and during the period when it is presumably maximal that feeding is most intense, with a corresponding effect on the fat reserves. The winter fast begins while the temperature is falling. Maturing fish, on the other hand, continue to feed while the temperature is falling and any effect on them is not apparent.

With the immature flounder can be compared the immature plaice. Johnstone (1918) records that in plaice less than 30 cm. long the weight per unit of length varies regularly with the sea-temperature, the plaice being heaviest for their length when the sea-temperature is maximal and lightest when it is minimal. Hartley (1939) has worked out a similar condition factor for the flounder and the same relation is apparent although he does not specially distinguish between immature and mature fish. Hartley's condition factors correspond fairly closely with the figures for mean fatness if due allowance be made for the fact that they are not tabulated on quite the same basis.

Bruce (1924) found that the fat content of herring muscle at any given stage of maturity is increased by age. Lovern (1938) has recently stated that the fat content of the freshwater eel "increases almost linearly with length, at any rate between certain limits". Bruce's and Lovern's findings are based on chemical analyses. It has been seen that in the immature flounder a size increase also brings about a slight general fattening as measured histologically. An insufficient number of mature fish are available for a good comparison to be made between their smaller and larger sizes. The figures that are available suggest that there is very little tendency to increased fatness with size.

Some further comparisons of the intramuscular fat of the flounder with that of the king salmon are of interest. It will be remembered that in the flounder fat in the fibres of the superficial lateral muscle is apparently not drawn upon while that of the normal adipose tissue is removed during the last stages of maturity (p. 375); it disappears last of all. In the salmon (Greene, 1914) there is a very large load of intramuscular fat at the beginning of the long migration; this is gradually used up during the journey but is not completely removed and even at death a considerable quantity is still present. The deposition of fat in the superficial lateral muscle of the salmon begins in embryonic life and increases in amount up to the time of cessation of feeding. The deep lateral muscle on the other hand does not show fat droplets inside its fibres until the salmon ceases to feed early in the journey to the spawning grounds. These droplets thus appear at a maturity stage corresponding very roughly with that in the flounder at which the intramuscular fat develops in the superficial but not the deep lateral muscle. The droplets in the deep lateral muscle of the salmon maintain a relatively uniform distribution in the fibres until death. Greene infers that the fat in the fibres is utilized in the production of muscular energy expended during the migratory journey up river. He suggests that this intramuscular fat is maintained at its uniform distribution by a continual replenishment from the intermuscular fat depots and those of the adipose connective tissues which are depleted during the migration. He argues that with the cessation of feeding the products of digestion no longer reach the active musculature, and that there is then thrown into the deep muscle fibres a supply of fat adequate to the energy needs. Reference should be made to Greene's own paper for details of his hypothesis to which he devotes much space.

Comparing salmon and flounder it is evident that the superficial lateral muscle has a much greater potentiality to the deposition of fat droplets in its fibres than has the deep lateral muscle. In the salmon it early begins the accumulation of what eventually becomes a great store of fat; the deep muscle on the other hand does not have an intramuscular loading until very much later and it is never as great as that of the superficial muscle at its maximum. In the flounder the superficial lateral muscle is the only one of the two lateral muscles ever to acquire droplets of fat in its fibres. They are most strongly developed in mature fish, especially after cessation of feeding. They also occur in some immature fish and it may be significant that it is during or just after the winter fast that they are most numerous. In all classes of flounder during the summer when they are intensively feeding these droplets are almost entirely or quite absent. It may be that in all fishes the superficial lateral muscle has a greater tendency to store fat than has the deep lateral muscle. The fat may not always be detectable by histological techniques; it may be in a form recognizable only by chemical extraction. The fact that no droplets are to be seen in the fibres of any particular fish does not necessarily mean that there is no fat there at all. A seasonable comparison between superficial and deep lateral muscles of many types of fishes carried out biochemically and checked histologically might yield interesting results.

SUMMARY

In the flounder adipose tissue forms a padding between the muscles to the dorsal and ventral fins. A little is present under the skin, more especially where the lateral line septa and the myocommata meet the skin. In addition a quantity of fat is associated with the skeleton.

Immature flounders living in the estuaries of the Tamar and Lynher are fattest after intense feeding during the summer, and leanest after the winter fast.

Maturing flounders have a high fat content before going to sea to spawn. While in the sea during the spawning season, February to April, very little feeding takes place and there is a rapid drop in fat content. Spent fish are very lean.

Those females which return to the estuaries after spawning rapidly make good their fat loss.

There is considerable individual variation in fatness irrespective of seasonal and spawning variations.

Large fish tend to be slightly fatter relatively than small. General fatness does not appear to be affected by sex.

In some individuals the fibres of the superficial lateral muscle contain numerous fat droplets during the autumn and winter. They are not present as a rule during the summer. These intracellular droplets are a striking histological feature of mature sea fish during the spawning season.

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ON THE SEASONAL ABUNDANCE OF YOUNG FISH. VI. THE YEAR 1938

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(Text-figs. 1–3)

The present report continues the series of observations on the seasonal abundance of the pelagic young of teleostean fish in the plankton off Plymouth (Russell, 1938*b*), and gives the results for the year 1938. These results are published in the same form as in previous reports. The dates on which collections were made with the 2 m. stramin ringtrawl are given in Table I, and the monthly average catches of the young fish per half-hour oblique haul in Table II. In Fig. 1 is given the curve for the average catches for each fortnight of all young fish, excluding clupeids, and superimposed upon this the corresponding curve for the average catches over the period 1930–4 inclusive.

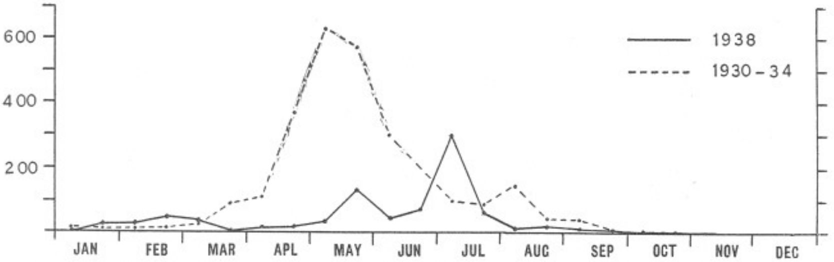

Fig. 1. Curves showing the average catches in half-hour oblique hauls with the 2 m. ringtrawl for each fortnight for all young fish, excluding clupeids, in 1938 (——) and the same averaged over the period 1930–4 inclusive (-----).

The results are practically a repetition of those for 1936 and 1937. The complete absence of the peak for the young of spring spawners was repeated in 1938, and if anything their numbers were even lower than in the two previous years. The sum of the monthly average catches of those post-larvae which show maximal abundance in the months June to October inclusive, excluding clupeids, was 135 in 1938, as against 174 in 1937 and 115 in 1936. The 1938 figures should perhaps have been slightly lower as they were heavily weighted by one exceptionally heavy catch on July 5. The numbers in this catch were rather remarkable, there being 2525 clupeids and 502 other fish, while the remaining July catches gave figures of 180, 94, and 80 for all fish including clupeids. There were no *Sagitta elegans* in the catch on July 5, nor were there any indications from the plankton that the water might have been of different origin on that day as compared with other weeks in June and July. But the whole plankton catch was itself unusually large on July 5 and we can only conclude that the plankton had been collected together by some unusual condition of tidal or other currents. Lest this anomaly should cast doubt on the general picture based on these single weekly hauls it should be pointed out that during each week ringtrawl collections are made for other purposes. These collections have always confirmed the results of the routine observations.

The general poverty of young fish in 1938 again shows correlation with the low phosphorus content of the water in the previous winter, which was −16% below the mean (Cooper, 1938, p. 187).

The sums of the average monthly catches of the more important species for the year 1938 divided by the corresponding average sums for the period 1930–4 inclusive are given below. In the second column are the figures for the best year divided by the worst year from 1930 to 1938 inclusive.

	$\frac{1938}{\text{Av. 1930–4}}$	$\frac{\text{Best}}{\text{Worst}}$
G. merlangus	0·08	23·6 ('32/'36)
G. minutus	0·13	40·3 ('32/'36)
Onos spp.	1·21	13·0 ('30/'35)
Arnoglossus sp.	0·51	11·5 ('31/'34)
S. norvegicus	0·05	28·7 ('32/'38)
P. limanda	0·24	14·0 ('31/'35)
P. microcephalus	<0·04	>41·0 ('32/'38)
S. variegata	0·20	7·6 ('32/'38)
Callionymus sp.	0·14	7·7 ('30/'38)
S. scombrus	1·54	11·2 ('30/'35)
Gobiid sp.	0·05	119·0 ('30/'37)

The year 1938 has thus been the worst during the period 1930–8 for *S. norvegicus*, *S. variegata*, and *Callionymus*.

The decrease in the numbers of *Callionymus*, which was commented on in the report for the year 1937, has been continued, these having fallen from an average of 548 for the period 1930–4 to only 76 in 1938. The first column in the above table shows very clearly the decrease in the numbers of those species which were of considerable importance in the young fish population in the years 1930 to 1934. Except for *Onos*, *Arnoglossus*, and *Scomber*, all species are now less than one-quarter as numerous as they were in the period 1930–4, and, of these, half have been reduced to less than one-tenth.

If the number of young of summer spawners (135) be subtracted from the total young fish (450), we are left with 315 for the remaining young fish most of which are the young of spring spawners. This is the lowest number yet recorded and is less than one-quarter of the 1395 specimens recorded in 1931.

There are indications that this decline in production is now being shown in the stocks of adult fish.

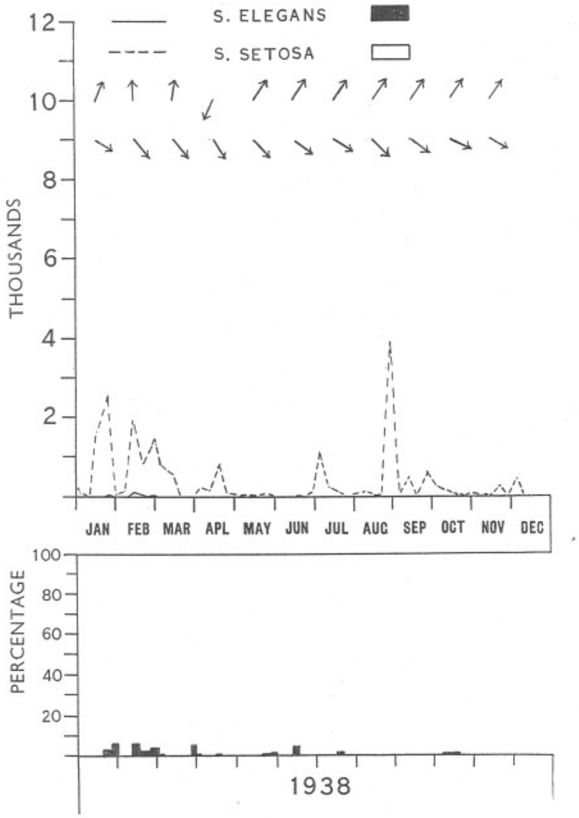

Fig. 2. Above, curves showing the actual abundance of *Sagitta elegans* (——) and *S. setosa* (-----) in half-hour oblique hauls with the 2 m. ringtrawl during the year 1938. Below, the percentage composition of the *Sagitta* populations during the same period: *S. elegans*, black; *S. setosa*, white. At the top of the diagram the arrows indicate the mean directions (true) of the flow of water past the Varne Lightship (above) and the Royal Sovereign Lightship (below) from data kindly supplied by Dr J. N. Carruthers.

As regards the plankton the conditions during 1938 were exceptional (Figs. 2, 3). There has been an almost complete absence of *Sagitta elegans*, and *S. setosa* has predominated since August 1937. This is the longest consecutive period that there has been of this nature since detailed observations were started in 1930. At the same time the numbers of *S. setosa* have been unusually low, and there has been a marked scarcity of plankton animals of all kinds in the ringtrawl catches. The total number of medusae of all species,

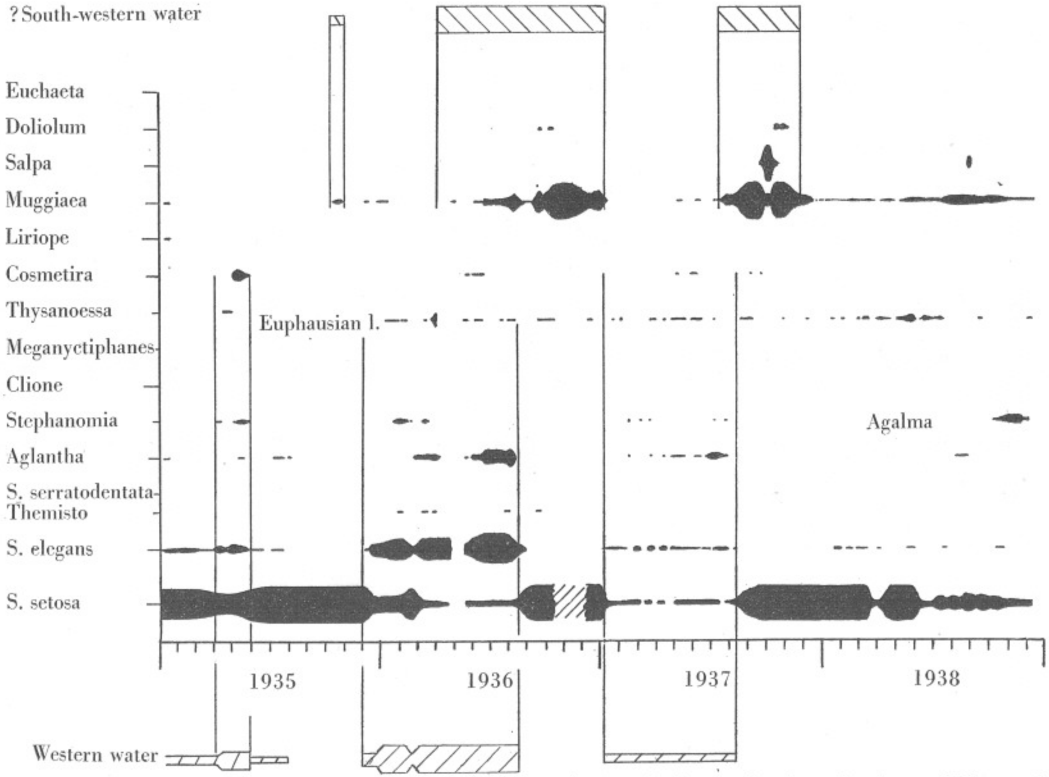

Fig. 3. Diagram showing the occurrence of the various plankton indicators in the collections off Plymouth during the years 1935 to 1938 inclusive. (Continued from Russell, 1938*b*, p. 497, Fig. 4.) The *Muggiaea* species were *M. kochi* up to January 1937, and thereafter *M. atlantica*, the salps were *S. fusiformis*, and the doliolids *D. nationalis*.

for instance, was only 2111. This is the lowest for any year since records started in 1930 (Russell, 1938*a*). It is interesting to record that the siphonophore *Agalma elegans* appeared in the catches in October and remained until the beginning of December. This is the first time that this species has been recorded from Plymouth. The species has not been accompanied by *Sagitta elegans* and it seems probable that it has been brought into the area by wind-driven surface water. It is also noteworthy that *Muggiaea atlantica* has occurred in the catches in every month of the year, although in rather low numbers.

The occurrence of many pilchard eggs was again a prominent feature. They were present from April to December, their approximate numbers, in the catches in which they occurred, being as follows. April 4 (60), 11 (160), 19 (1240), 24 (1220); May 2 (8910), 9 (3300), 17 (1700), 23 (920), 30 (1580); June 7 (2280), 14 (5540), 21 (14,120), 28 (18,180); July 5 (28,340), 11 (1380), 18 (3580), 21 (2860); August 8 (1400), 22 (30); September 6 (20), 12 (630), 19 (680), 27 (1760); October 3 (960), 10 (870), 17 (60), 25 (480); November 1 (310), 7 (1920), 14 (10), 28 (1); December 5 (1).

The pilchard eggs occurred almost to the exclusion of the eggs of other fish during this period.

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Table I. Dates on which Collections were made, 1938

All 2 miles east of Eddystone unless otherwise stated

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
5	7	4*	4	2	7	5	8	6	3	1	5
10†	14	14	11	9*	14	11	15	12	10	7	9‡
17	21	18	19	17§	21	18	22	19	17	14	..
25	28	23	24	23	28*	21‖	29	27	25	21	..
31*	..	28	..	30	..	..	..	..	..	28	..

* L 4–L 5.
† Off Rame Head.
‡ L 3–L 4.
§ 4 miles W.S.W. of Rame Head.
‖ West of Rame Head.

TABLE II. MONTHLY AVERAGE CATCHES OF POST-LARVAE PER HALF-HOUR

Oblique hauls with 2-m. ringtrawl, 1938

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Σ
Total young fish	24	107	22	23	149	169	845	27	12	10	11	1	1400
Ditto, less Clupeids	14	38	18	16	94	62	182	16	8	2	+	..	450
All Clupeid spp.	9	69	4	7	55	107	663	11	4	8	10	1	948
Clupea harengus	1	..	..	..	..	..	..	..	..	..	..	..	1
Gadus pollachius	..	1	..	+	3	+	..	..	..	..	..	..	4
Gadus merlangus	..	3	+	2	4	7	+	..	..	..	..	..	16
Gadus minutus	..	7	..	5	+	..	..	..	..	..	..	..	12
Gadus luscus	3	7	..	..	..	+	+	+	..	+	..	..	10
Gadus callarius	..	..	..	..	..	..	..	..	..	..	..	..	..
Onos spp.	..	..	..	+	56	4	3	+	..	..	..	..	63
Molva molva	..	..	..	..	..	..	..	..	..	..	..	..	..
Merluccius merluccius	..	..	..	..	..	..	..	+	..	..	..	..	+
Raniceps raninus	..	..	..	..	..	..	..	..	..	..	..	..	..
Capros aper	..	..	..	..	..	..	..	..	..	..	..	..	..
Zeus faber	..	..	..	..	..	..	..	..	..	..	..	..	..
Arnoglossus sp.	..	..	..	..	..	+	14	2	1	+	..	..	17
Rhombus laevis	..	..	..	..	+	..	..	..	..	..	..	..	+
Rhombus maximus	..	..	..	..	..	..	..	..	..	..	..	..	..
Scophthalmus norvegicus	..	..	..	+	1	1	1	+	..	..	..	..	3
Zeugopterus punctatus	..	..	..	..	..	..	+	..	..	..	..	..	+
Zeugopterus unimaculatus	..	..	..	..	..	..	..	..	..	..	..	..	..
Pleuronectes limanda	..	..	..	2	10	1	+	..	..	..	..	..	13
Pleuronectes flesus	..	..	..	..	..	..	..	..	..	..	..	..	..
Pleuronectes microcephalus	..	..	..	..	+	..	+	..	..	..	..	..	+
Solea vulgaris	..	1	+	1	+	..	..	..	..	..	..	..	2
Solea variegata	..	..	..	1	1	3	12	..	..	..	..	..	17
Solea lascaris	..	..	..	..	..	..	+	..	+	..	..	..	+
Solea lutea	..	..	..	..	..	1	..	..	..	..	..	..	1
Serranus cabrilla	..	..	..	..	..	..	..	..	..	..	..	..	..
Caranx trachurus	..	..	..	..	..	..	..	4	4	+	..	..	8
Mullus surmulletus	..	..	..	..	..	..	..	..	..	..	..	..	..
Morone labrax	..	..	..	..	..	..	..	..	..	..	..	..	..
Ammodytes sp.	12	19	16	1	1	1	5	+	1	1	..	..	57
Ammodytes lanceolatus	..	..	..	2	3	5	27	2	..	..	..	..	39
Cepola rubescens	..	..	..	..	..	..	..	..	..	..	..	..	..
Callionymus sp.	..	..	..	3	13	18	38	3	1	+	..	..	76
Labrus bergylta	..	..	..	..	..	2	2	..	..	..	..	..	4
Labrus mixtus	..	..	..	..	..	..	1	..	..	..	..	..	1
Ctenolabrus rupestris	..	..	..	..	..	4	13	+	..	..	..	..	17
Crenilabrus melops	..	..	..	..	..	1	3	..	..	..	..	..	4
Centrolabrus exoletus	..	..	..	..	..	..	+	..	..	..	..	..	+
Trachinus vipera	..	..	..	..	..	..	1	+	+	..	..	..	1
Scomber scombrus	..	..	..	..	..	7	26	1	..	..	..	..	34
Gobius spp.	..	..	..	..	+	..	2	..	..	..	..	..	2
Lebetus scorpioides	..	..	..	..	..	+	+	..	..	+	..	..	+
Blennius ocellaris	..	..	..	..	..	..	..	..	..	..	..	..	..
Blennius pholis	..	..	..	..	..	2	1	..	..	..	..	..	3
Blennius gattorugine	..	..	..	..	..	7	31	2	1	..	..	..	41
Chirolophis galerita	..	1	+	..	..	..	..	..	..	..	..	..	1
Agonus cataphractus	..	..	..	..	..	..	..	..	..	..	..	..	..
Trigla spp.	..	..	..	..	1	..	1	..	..	..	..	..	2
Cottus spp.	..	1	1	1	..	..	..	..	..	..	..	..	3
Liparis montagui	..	..	..	..	..	..	..	..	..	..	..	..	..
Lepadogaster bimaculatus	..	..	..	..	..	..	..	..	..	..	..	..	..
Lophius piscatorius	..	..	..	..	..	..	..	..	..	..	..	..	..
Pipe fish	..	..	..	..	..	..	..	..	..	..	+	..	+

PHOSPHORUS, NITROGEN, IRON AND MANGANESE IN MARINE ZOOPLANKTON

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In 1936 an investigation of the inorganic constituents of certain marine zooplankton organisms was started but had temporarily to be suspended. The results obtained are presented as an interim report.

Preparation of Material and Methods of Analysis

Nauplii of the cirripede crustacean *Balanus balanoides* (sample 2) were extracted from the mantle cavities of adults on February 20 1936, by Dr H. B. Moore. Only specimens able to swim towards the light were used for analysis. These were concentrated by centrifuging, placed on a no. 50 Whatman filter, washed with 1 ml. of sea water and dried under suction before transferring to a small tared porcelain crucible. Prepared in this way the sample must contain a little sea water which will slightly affect both wet and dry (105° C.) weights. The elementary analyses reported in Table I were all made on the same dried material and are comparable. Since parallel counts of the nauplii showed wide variations, the numbers of animals attributed to the sample and the wet and dry weights of single organisms are approximate only.

Samples 9–13, from a 2 m. ring-trawl catch taken two miles off Revelstoke Point on June 5 1936, were all picked out alive with stainless steel forceps, measured, and transferred to tared glass evaporating dishes by Mr F. S. Russell. The post-larvae of the fish *Callionymus lyra* and the mature chaetognath, *Sagitta elegans*, had the following measurements:

	C. lyra	*S. elegans*
No. of animals ...	32	125
Length (mm.):		
Range	5–9	9–18
Median	7	14

Fifty ctenophores, *Pleurobrachia* (sample 11), all lay between 7 and 10 mm. in height. A further 225 animals (sample 16*a*) were taken from another catch on September 28 1936, for manganese analysis.

After removal of excess sea water by filter paper, the samples were weighed wet and then dried to constant weight at 105–110° C. Aliquot parts of dried material were weighed out on a Kuhlmann micro-balance for carbon analysis by Pregl's method, for micro-Kjeldahl nitrogen determinations by the method of Parnas and Wagner and for wet digestion for analyses of phosphorus and

TABLE I

Sample no.	Organism	Date of capture 1936	No. of animals analysed	Wet weight of one animal mg.	Dry weight of one animal mg.	Dry weight / Wet weight %	Percentage composition based on dry weight			
							P	N	Fe	Mn
2	*Balanus balanoides* nauplii*	20. ii	102,000	0·0161	0·000915	5·67	1·7	9·7	0·12	<0·00003
9	*Callionymus lyra* post-larvae	5. vi	32	8·72	1·23	14·1	1·60	8·24	0·031	..
10	*Sagitta elegans* mature	5. vi	125	5·71	0·530	9·29	0·945	9·24	0·042	..
11	*Pleurobrachia pileus*†	5. vi	50	115	5·09	4·43	0·230	1·34	0·0050	..
11	,, ,, (ash free)	5. vi	50	..	..	..	0·630	3·68	0·0137	..
16*a*	,, ,,	2. x	225	481	19·6	4·07	..	..	..	<0·00005
12	Portunid megalopas	5. vi	15	4·47	0·784	17·5	1·14	6·32	0·077	..
13	Portunid zoeas and Crangonid larvae	5. vi	34	3·27	0·519	17·5	1·16	7·22	0·035	..
14	Portunid zoeas and Crangonid larvae	5. vi	20	1·77	0·301	17·0	..	..	0·093	..

* C 43·1 %, H 9·2 %, ash 16·3 %, chitin <2 % on dry weight.

† Ash at dull red heat 63·57 %.

Sample no.	Organism	Content of one animal μg.				Ratios in mg.-atoms	
		P	N	Fe	Mn	N/P	Fe/P
2	*Balanus balanoides* nauplii	0·016	0·89	0·0011	<0·00003	12·6	0·039
9	*Callionymus lyra* post-larvae	19·6	101	0·38	..	11·4	0·012
10	*Sagitta elegans* mature	5·02	49·0	0·22	..	21·6	0·025
11 and 16*a*	*Pleurobrachia pileus*	11·7	68·0	0·25	<0·009	12·8	0·012
12	Portunid megalopas	8·93	49·5	0·60	..	12·2	0·038
13	Portunid zoeas and Crangonid larvae	6·05	37·4	0·18	..	13·7	0·017
14	Portunid zoeas and Crangonid larvae	..	..	0·28	..	..	..

iron (Cooper, 1935*a*, *b*). In all the samples copper was determined by the diethyldithiocarbamate method. The amounts found were always greater than records for similar animals in the literature. The detailed results are withheld until the method can be re-examined.

Manganese determinations, requiring a large amount of material, were made only on *Pleurobrachia* and on *Balanus* nauplii. *Pleurobrachia* (225 animals; 108 g. wet weight) were digested with 5 ml. conc. sulphuric acid and perhydrol drop by drop. Some green matter remaining was destroyed with potassium persulphate. The solution was diluted and filtered through sintered glass and the manganese determined by the method of Willard and Greathouse (Yoe, 1928, p. 273) in which potassium periodate is employed to oxidize manganese to permanganate.

DISCUSSION OF RESULTS

The presence of iron and copper in all the samples (Table I) confirms Fox & Ramage's report (1931) of their ubiquity in marine invertebrates and suggests further that the occurrence of both in sea water ought to show considerable seasonal fluctuations. The analyses of iron in the plankton indicator, *Sagitta elegans*, differ strikingly from those made two years earlier on *S. setosa* (Cooper, 1935*b*).

	Date of capture	No. in sample	Average length mm.	Content of one animal (μg.)		Ratio Fe/P mg.-atoms
				P	Fe	
*Sagitta setosa**	24. v. 34	6	15	5·3	6·8	0·72
Sagitta elegans	5. vi. 36	125	13·9	5·02	0·22	0·025

* No record has been kept of the condition of the forceps used for picking out *S. setosa* from the 1934 catch. These might have been rusty.

The contents of phosphorus, nitrogen and iron in *Pleurobrachia*, even after recalculation to an ash-free basis, are all considerably lower than those of other animals examined, suggesting that its gelatinous structure must consist of carbohydrate or fatty material and not of protein. *Pleurobrachia* contained less than 2×10^{-6} % of their wet weight as manganese ($< 5 \times 10^{-5}$ %

TABLE II. SEASONAL CHANGES IN IRON AND PHOSPHORUS CONTENT OF PLANKTON

Catches taken with the quantitative net in the English Channel at Station L 4 between the surface and 45 m.

Date 1935	Content in plankton from a cubic metre of sea water		Ratio Fe/P	
	mg. Fe	mg. P	wt./wt.	mg.-atoms
April 3	0·73	0·602	1·20	0·68
April 23	0·96	1·16	0·82	0·46
May 8	0·85	0·64	1·33	0·74

of dry weight). Since sea water contains from $0 \cdot 1$ to 1×10^{-6} % Mn (Thompson & Wilson, 1935), this animal evidently effects no considerable concentration of manganese from sea water. *Balanus* nauplii yielded a similar result.

Table II records some analyses of iron and phosphorus in mixed plankton in the spring of 1935 which confirm the picture already found in 1934 (Cooper, 1935*b*, table IV).

I wish to express my indebtedness to Mr F. S. Russell, who provided all but one of the zooplankton samples and measured them for me, and to Dr H. B. Moore, who prepared a very clean sample consisting only of lively *Balanus* nauplii.

SUMMARY

Analyses of phosphorus, nitrogen, iron and manganese are recorded for *Balanus* nauplii (a cirripede crustacean), post-larval *Callionymus* (a teleostean fish), mature *Sagitta elegans* (a chaetognath), *Pleurobrachia* (a ctenophore) and for crab zoeas and megalopas (Table I). A sample of *Sagitta setosa* in 1934 contained much more iron than that in *S. elegans* here reported. The ctenophore, *Pleurobrachia*, is relatively poor in phosphorus, nitrogen and iron and its ash-free protoplasm must evidently be rich in either fats or carbohydrates. Neither *Pleurobrachia* nor *Balanus* nauplii effect any appreciable concentration of manganese from sea water.

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THE ECOLOGY OF THE TAMAR ESTUARY

V. UNDER-WATER ILLUMINATION. REVISION OF DATA FOR RED LIGHT

By L. H. N. Cooper, D.Sc. and A. Milne, M.A., Ph.D.

From the Plymouth Laboratory

When the Pulfrich photometer is used to measure absorption of light by a turbid estuarine or sea water, the true extinction coefficient for light of a given wave-length

$$\mu_{SW} = \mu_M + \mu_{DW},$$

where μ_M is the extinction coefficient as measured and μ_{DW} is the extinction coefficient of the distilled water used in the comparison tube of the photometer. When our paper on under-water illumination in the Tamar Estuary was prepared (Cooper & Milne, 1938), we knew of no modern data on the extinction coefficient of distilled water in the deep red, except those of Lange & Schusterius (1932) which we unjustifiably rejected. James (1938) has since published exhaustive data of his own and gives, in full, results obtained by Prof. J. R. Collins and Dr Ernst Ganz. These leave no doubt that between 700 and 760 mμ the coefficient rises very steeply from about 0·6 to 2·6, and that the distilled water correction which we applied to our results was in serious error. This spectral region is also one in which visual sensitivity is decreasing rapidly. These two factors much favour the transmission and visual perception of light of shorter wave-length. However, the two red filters, S 72 and S 75, equally strongly favour the transmission of light of longer wave-length. Average extinction coefficients cannot be evaluated by simple inspection. Using the data of James, of Collins and of Ganz we have worked them out for the conditions of our experiments.

EFFECTIVE EXTINCTION COEFFICIENT OF DISTILLED WATER MEASURED BY THE RED FILTERS OF THE PULFRICH PHOTOMETER

Consider a light source of known energy distribution and let J_λ represent the relative energy at a given wave-length. After passing through a filter having a transmission c_λ, the relative energy will be reduced to $c_\lambda J_\lambda$. If V_λ represents relative visibility (i.e. the relation between luminous sensation as perceived by the eye and radiant energy), the visual sensation produced by this transmitted light will be $c_\lambda J_\lambda V_\lambda$. The transmission coefficients of the filters expressed as percentages have been supplied by Messrs Carl Zeiss (cf. Cooper & Milne, 1938, Fig. 1). Relative visibility has been taken from

Smithsonian Physical Tables (1933, 8th revised ed.), Table 346; $c_\lambda J_\lambda V_\lambda$ may be plotted against wave-length, and the area $\int_{\lambda_1}^{\lambda_2} c_\lambda J_\lambda V_\lambda \, d\lambda$ enclosed by the curve and the wave-length axis will provide a measure of the light sensation perceived by the observer's eye. It may be evaluated most conveniently with a planimeter.

Since in our work we used 0·25 m. absorption tubes, we have also calculated the relative energy of a narrow wave band, having extinction coefficient μ_λ, after passing through 0·25 m. of distilled water, $J_\lambda e^{-0\cdot 25\mu_\lambda}$. This intensity also may be transmitted by the spectral filter and perceived by the eye so that the measure of light sensation will be $c_\lambda J_\lambda e^{-0\cdot 25\mu_\lambda} V_\lambda$. This may be plotted on the same graph as $c_\lambda J_\lambda V_\lambda$. The ratio of the areas suffices to evaluate the logarithmic term in the expression

$$0\cdot 25\mu_A = 2\cdot 303 \log_{10} \frac{\int_{\lambda_1}^{\lambda_2} c_\lambda J_\lambda V_\lambda \, d\lambda}{\int_{\lambda_1}^{\lambda_2} c_\lambda J_\lambda e^{-0\cdot 25\mu_\lambda} V_\lambda \, d\lambda}.$$

The value obtained for μ_A is the effective extinction coefficient of distilled water for a given filter, which is required as our correction factor. It applies only for 0·25 m. tubes.

Since we do not know the spectral energy distribution of the Nitra lamp supplied with the Pulfrich photometer, we have calculated the effective extinction coefficient, first from Abbot's mean noon sunlight data (Seventh Internat. Congress of Photography, 1929), secondly for light having an equal energy spectrum over the range of wave-length considered, and thirdly for a light source having a colour temperature of 2800° K. (Table I). Considerable latitude in the colour composition of the light source may be tolerated. Much more important are the great variations of the extinction coefficients based on the determinations of different observers and of relative visibility with wave-length. Furthermore, in this region considerable personal idiosyncracy in relative visibility is to be expected.

James (1938, p. 37) discusses the variable coefficients for distilled water found by different observers. These cannot be attributed to errors of measurement and must be due to some variable property of distilled water itself, possibly minute traces of impurities or variable degree of association of the water molecules dependent on the previous history of the water. Whatever the cause, any ordinary laboratory sample of distilled water is likely to show variability at least as great. Below 600 mμ the coefficient is small compared with the measurements on a fairly turbid estuarine water, so that some degree of uncertainty is of little consequence. Above 650 mμ matters are very different. The best we can do is to apply an average effective extinction coefficient as correction factor, as suggested in Table I.

In relatively transparent ocean water, accurate measurement of the extinction coefficients for blue, green and yellow light requires a water column much longer than can conveniently be accommodated by the Pulfrich photometer. Even if this difficulty could be overcome, the correction terms for the absorption of light by ordinary laboratory samples of distilled water remain too uncertain for the method there to be of much value. Its utility is confined to fairly turbid inshore or estuarine waters.

TABLE I. EFFECTIVE EXTINCTION COEFFICIENT FOR RED LIGHT

Transmitted by 0·25 m. of distilled water and measured with the filters of the Pulfrich photometer

	Temperature ° C.	Type of spectrum	Particulars of filters		
Filter no.	...	...	S 75	S 72	S 61
Effective wave-band*, mμ	...	...	730–770	700–760	600–650
Centre of gravity of filter, mμ	...	...	750	729	619
			Effective extinction coefficient		
Calculated from data of:					
James (1938)	Room	Colour temperature 2800° K.	2·24	1·74	...
,,	,,	Equal energy	2·23	1·78	0·28
,,	,,	Mean noon sunlight	2·29	1·76	...
Collins (cit. James)	0·5	,, ,,	2·61	2·02	0·29†
,, ,,	26	,, ,,	2·79	2·14	...
Ganz (cit. James)	12	,, ,,	2·54	1·55	...
Values adopted as correction terms			2·5	1·8	0·28

* These are the limits within which the transmission exceeds one-half the maximum. For purposes of summation, transmissions exceeding one-tenth were included.

† Approximate value obtained by inspection (strictly for equal energy spectrum).

REVISION OF THE ORIGINAL DATA

A list of the numerical revisions made necessary by the change in our correction factors will be found at the end.

The revised values of the ratio μ_λ/μ_{470} for the S 61, S 72 and S 75 filters still show that very turbid waters transmitted red light as well as or better than blue. When μ_λ is graphed against μ_{470}, the relationship may be expressed surprisingly well by the linear equations given in Table II. The constant terms for the red filters are almost the same as those applied to correct for the absorption by distilled water. For the remaining filters similar terms would be quite small. The views expressed in the earlier paper as to the transmission of red light still apply up to about 680 or 700 mμ. Above this the molecules of water itself manifest strong absorption of light, and in fairly clear waters this is all important, but that part of the extinction coefficient, which was due to dissolved and suspended solids, was always least in the deep red. These factors work in opposition, and as a result the minimum extinction coefficient or maximum transmission for our more turbid waters was to be found around 600 mμ.

The ratios of the wave-length at the centre of gravity of the blue S 47 filter compared with the corresponding wave-lengths of the other filters, also given in Table II, show that that part of the extinction coefficient due to suspensoids and possibly to matter in solution is inversely proportional to the first power of the wave-length. The relationship is sufficiently close that, if only one extinction coefficient were known, that for any other wave-length could be calculated with an accuracy sufficient for most practical purposes.

TABLE II. RELATION BETWEEN EXTINCTION COEFFICIENTS AND WAVE-LENGTH

(Revision of original Table III)

Filter	Equation connecting μ and μ_{463}	Ratio of wave-length of blue filter to that of the others (at centres of gravity) $\frac{463}{\lambda}$
S 43	$\mu_{434} = 1{\cdot}11\,\mu_{463}$	1·07
S 47	$\mu_{463} = 1{\cdot}00\,\mu_{463}$	1·00
S 50	$\mu_{494} = 0{\cdot}92\,\mu_{463}$	0·94
S 53	$\mu_{530} = 0{\cdot}86\,\mu_{463}$	0·87
S 57	$\mu_{572} = 0{\cdot}82\,\mu_{463}$	0·81
S 61	$\mu_{619} = 0{\cdot}35 + 0{\cdot}73\,\mu_{463}$	0·75
S 72	$\mu_{729} = 1{\cdot}80 + 0{\cdot}68\,\mu_{463}$	0·64
S 75	$\mu_{750} = 2{\cdot}54 + 0{\cdot}64\,\mu_{463}$	0·62

The absorption index, K, defined by the equation

$$\mu_{SW} - \mu_{DW} = \frac{4\pi K}{\lambda},$$

is a constant for each water; $\mu_{SW} - \mu_{DW}$ is, in effect, a measure of absorption by suspensoids.

The dependence of scattering of light by particles in sea water upon various powers of the wave-length has been discussed by Kalle (1938).

REVISED DATA (COOPER & MILNE, 1938)

P. 510, Table I: Extinction coefficients of distilled water: Accepted values for filters S 61, S 72 and S 75 are 0·28, 1·8 and 2·5 respectively.

P. 514, l. 26: *For* "($\mu_{500} = 5{\cdot}0$; $\mu_{720} = 3{\cdot}8$)" *read* "($\mu_{500} = 5{\cdot}0$; $\mu_{720} = 5{\cdot}3$)".

P. 514, Table II: For last line of figures, read:

μ_{720} 5·9 8·2 7·5

P. 515, ll. 1 and 2 from bottom: *Read:* "the intensity of red and blue light entering the water was cut down to one-thousandth within 0·5 and 0·6 m. respectively ($\mu = 13·5$ and $16·5$)."

P. 516, Fig. 3: The plots at 610, 720 and 750 mμ should be 0·1, 1·3 and 2·0 units higher respectively. A minimum extinction coefficient occurs at about 600 mμ in most cases.

P. 517, Table III: See p. 394.

Pp. 519 and 520, Figs. 4 and 5: All curves should show less relative energy in the dark red. The statement about preferential transmission of red light does not apply above about 680 mμ.

P. 522, Table IV.

DEPTH IN METRES AT WHICH INTENSITY OF RED LIGHT IS REDUCED TO 1 % OF THAT INCIDENT ON THE SURFACE

Assuming 15 % Surface Loss; $\mu d_1 = 4·44$

Centre of gravity of filter mμ	Breakwater No. 1 Buoy	Drake's Island No. 1 Buoy	Hamoaze No. 1 Buoy	Hamoaze No. 7 Buoy	Hamoaze No. 15 Buoy	Neal Point Buoy
		February 18 1937. Spring tide. High water				
619	2·35	1·97	1·85	1·41	1·30	...
729	1·37	1·26	1·22	1·02	0·99	...
750	1·09	1·00	1·04	0·86	0·82	...
		February 16 1937. Spring tide. Low water				
619	1·93	1·95	1·10	0·78	0·53	0·35
729	1·33	1·25	0·89	0·69	0·48	0·33
750	1·04	1·07	0·76	0·60	0·46	0·35
		June 17 1937. Neap tide. High water				
619	3·86	4·27	3·06	2·48	2·54	2·48
729	1·77	1·78	1·60	1·42	1·40	1·39
750	1·38	1·40	1·27	1·18	1·19	1·15
		June 23 1937. Spring tide. Low water				
619	5·00	3·21	2·74	2·04	1·41	0·98
729	1·95	1·60	1·48	1·27	1·00	0·79
750	1·49	1·27	1·16	1·05	0·90	0·71

P. 525, Table V.

DAYLIGHT FACTORS AT BOUNDARIES OF ALGAL ZONES ON BUOYS

No correction for surface loss

	Zone I		Zone II	
	Plymouth Sound	Hamoaze No. 7 Buoy	Plymouth Sound	Hamoaze No. 7 Buoy
Depth of bottom of zone, m. ...	0·18	0·097	0·356	0·223
Daylight factor, %:				
June 17 (high water)	78·3	80·0	62·3	61·8
June 23 (low water)	76·4	77·8	59·2	56·5
February 18 (high water)	65·4	69·8	43·7	45·0
February 16 (low water)	60·9	52·4	37·7	23·3

TABLE VI. DAYLIGHT FACTORS AT BOTTOM OF TWO BUOYS (WITHIN ZONE III)

	Mallard Buoy (Plymouth Sound)			Hamoaze No. 7 Buoy		
Date and state of tide	Depth of bottom of buoy m.	Daylight factor %	Wave-length of maximum transmission mμ	Depth of bottom of buoy m.	Daylight factor %	Wave-length of maximum transmission mμ
June 17 (high water)	2·19	7·41	570	2·47	0·97	570
June 23 (low water)	2·19	5·51	570	2·47	0·35	600
February 18 (high water)	2·19	0·85	580	2·47	0·020	600
February 16 (low water)	2·19	0·34	620	2·47	0·000025	620

P. 524, Table VII. All the extinction coefficients given in the last column should be increased by 1·3.

P. 526, l. 3: *For* "0·36 and 17 m. (ratio 1 : 48)" *read* "0·33 and 17 m. (ratio 1 : 52)."

P. 526, Table in Summary: The values for red light (610–640 and 710–760 mμ) should be increased by 0·1 and 1·33 respectively.

SUMMARY

New values for the extinction coefficient of red light in distilled water require that published results for this coefficient in waters from the Tamar Estuary shall be increased by 0·1, 1·3 and 2·0 at 610, 720 and 750 mμ respectively. Earlier deductions as to the favourable transmission of red light in turbid estuarine waters apply only up to about 680 mμ. That part of the extinction coefficient due to suspensoids and colouring matter was inversely proportional to the first power of the wave-length. Other conclusions remain unaffected.

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THE PLANKTON IN THE UPPER REACHES OF THE BRISTOL CHANNEL

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(Text-figs. 1–13)

CONTENTS

Introduction

The work embodied in this report was aimed, in conjunction with shore work, at determining the causes of the poverty of the marine fauna and flora in the Cardiff area. The plankton observations were made at a station which was visited 36 times between September 6 1936 and August 25 1937, with an unavoidable absence of visits in October. In addition some results obtained in September 1937 are used.

In so far as the area can be considered as estuarine, this work serves to fill a gap in annual quantitative data for British estuaries. Meek (1923*a*) has dealt with some isolated tow-nettings from the Tyne estuary and, later, Jorgensen (1929) made a more intensive investigation in the same estuary, but did not begin observations until May, thus missing the winter and spring conditions. The Tees survey (Alexander, Southgate & Bassindale, 1935) included counts of *Eurytemora*, marine copepods and *Coscinodiscus*, but variations throughout the year are not noted. Wells (1938) investigated the plankton of the Thames estuary over a number of years. This work, also, was mainly qualitative.

Area 12 of the plankton survey, from 1902 to 1908, of the International Council (Ostenfeld, 1931) was concerned with the Bristol Channel, but the investigations were conducted well outside the present area.

THE STATION

Although it was presumed that the channel in the vicinity of Cardiff had estuarine characteristics, it was decided to restrict observations to one station, and the Cardiff Roads, shown in Fig. 1, was selected. This was largely a matter of convenience, since work was done from a hired motor boat moored at the mouth of the river Ely, and the Cardiff Roads is the nearest place where the depth at high water exceeds 5 fathoms. The position is also sheltered from the prevailing westerly winds so that surface conditions are usually suitable for working there. Since, however, the boat was moored at the mouth of the river, it was very inconvenient to approach the station at low water, and it was owing to this difficulty that the low-water observations are so scanty. This work, therefore, is mainly concerned with high-water conditions.

The Cardiff Roads are about 1¼ miles from the coast, 2 miles from the mouth of the nearest river, the Ely, over 2½ miles from the mouth of the Taff, and 4 miles from the Rhymney. From a width of 13 miles across at Barry, the Bristol Channel suddenly narrows to 8 miles across at the level of the station, and retains this width until near Newport. From Newport there is a further narrowing to the Severn river.

On the up-channel side of the station there is an entry of a large bulk of fresh water derived from the watersheds of the three rivers close to the station, the more distant rivers, the Usk, Wye, Severn, Avon, and a number of smaller rivers. This implies that the station is placed in a buffer area between the sea water of down-channel and the fresh water entering on the up-channel side. Much of the biology of the plankton is dependent on this fact and the relative effect of these bodies of water.

To the narrowing of the channel must be attributed the speed of the tidal streams. At spring tides the stream moves at a normal maximum of 4½ knots in the centre of the channel but may reach higher speeds, and, at neap tides, about 2 knots. The narrowing also affects the height of the tide, causing a spring rise for Penarth, which is probably closely comparable to the station, of 37¾ ft., and a neap rise of 27¾ ft. Tide tables are published in nautical almanacs giving the height of the tide for Penarth, and these values have been used in considering the results obtained.

Since the speed of the tidal streams decreases from the centre of the channel to the coast, no figures can be given for the speeds affecting the investigated area. If, however, we allow that the maximum speed of the tidal stream in the investigated area at neap tide is 1 knot, then the total drift of water within the tidal stream that forms a high tide at the station is 3·86 nautical miles. At spring tide, with a presumed maximum of $2\frac{1}{2}$ knots, the total drift is 9·66 nautical miles, giving a difference of approximately 6 miles. With speeds of 1 and 2 knots the difference is about 4 miles. Since observations were made at the station at various phases of the tide, the records on

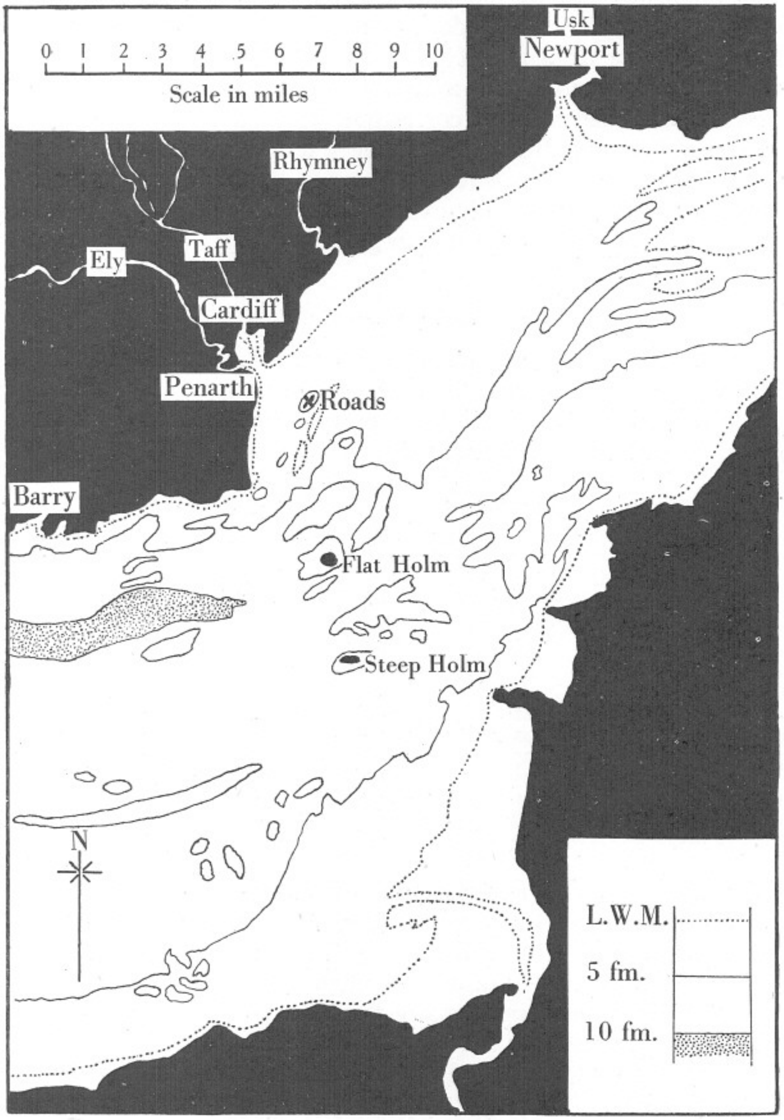

Fig. 1. The upper reaches of the Bristol Channel. The station worked is marked ×.

different occasions refer, in reality, not to one point, but to a series of points along a length of channel of the order of about 4 miles. On any occasion, the particular point investigated can be defined in relation to the points on other occasions by the height of the tide, i.e. the higher the tide the farther down-channel is the point from which water has come to form a high tide at the station. Having recognized this meaning of height of tide, it becomes clear that a factor which has a direct correlation with the height of the tide must have an increasing value from up-channel to down-channel, and a factor with an inverse correlation must have a decreasing value. These remarks should render what follows more readily explicable.

A number of soundings at the station gave an average depth of about 13 m.

The Physical and Chemical Factors

Coincident with the tow-nettings, water samples were taken at the station, transferred in pressure bottles to the laboratory, and analysed within 2 or 3 hr. of collecting. Table I gives the average values of some factors for each month for the surface water at high tide.

Table I. Average Values per Month of High-Tide Surface Water

Month	No. of samples	Salinity $^0/_{00}$	*p*H	P_2O_5 mg. per m.^{3}	Dissolved oxygen c.c.	Temp. of water ° C.
Sept.	3	27·1	8·10	69	5·51	17·6
Nov.	2	26·3	7·85	85	5·76	10·1
Dec.	2	25·8	7·90	72	6·66	7·7
Jan.	3	22·5	7·95	55	7·13	7·4
Feb.	2	22·3	7·80	54	7·47	6·4
March	4	22·9	8·00	33	7·19	5·8
April	3	25·4	7·95	44	6·70	9·0
May	4	25·2	8·00	35	6·21	13·0
June	5	26·3	8·05	33	5·80	16·0
July	5	27·3	8·05	36	5·36	17·1
Aug.	3	27·8	8·10	36	5·36	18·9

Salinity. The salinity was determined by titration with silver nitrate.

The average salinity values (Table I) show a steady decrease from September to a minimum in February, followed by a steady rise, except for a slight deviation in May, up to August. This change in salinity demonstrates the effect of the fresh water coming down from the rivers, an effect which must be cumulative and persistent over a period of time.

The minimum value of 19·8 $^0/_{00}$ was obtained on January 25, and the maximum of 28·0 $^0/_{00}$ on August 25. Fig. 2 shows the changes in value throughout the year. This curve, compared with the curve for the height of tide, shows a well-marked direct correlation between the two factors. That such a correlation exists implies that the bulk of fresh water entering the channel is slow, as well as cumulative, in effect, otherwise variations in rainfall between two successive samples would disturb the correlation.

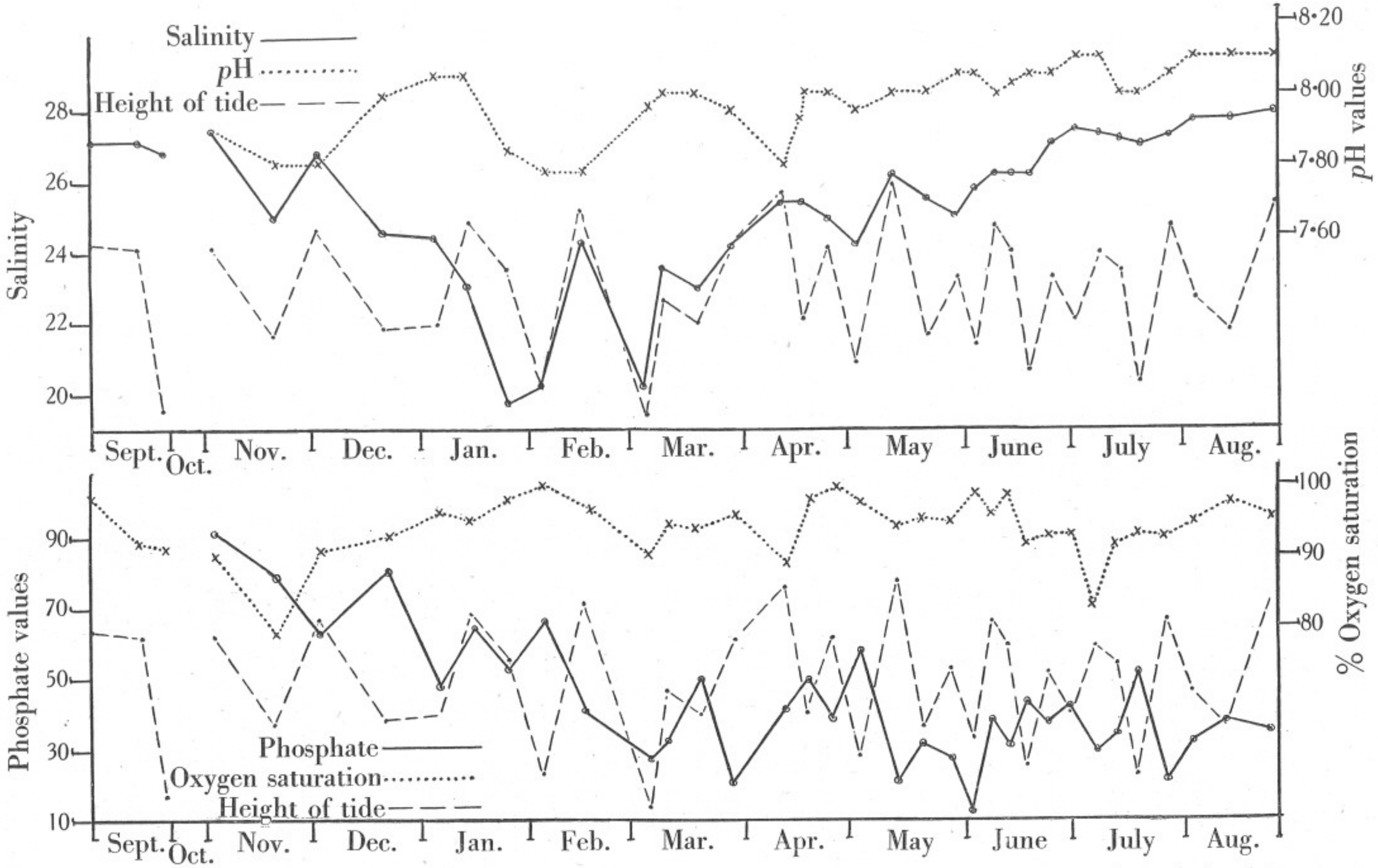

Fig. 2. The relation between the height of tide and the values of salinity, pH, percentage oxygen saturation and phosphate for samples taken at the surface at high water.

Water samples were taken on several occasions at various depths from the surface to the bottom by means of the Matthew's Fishery Water Bottle.* Except for one occasion there was no gradient in salinity. The exception was on July 27 (Table II), but this stratification was probably the result of the very heavy rain that fell over a wide area on July 15. A comparison of the weekly rainfall at Ross-on-Wye (*Meteorological Office Report*, Weekly Weather Records) with the changes in salinity suggests that the rainfall in the Wye and Severn catchment areas is effective at the station some 10–14 days after the fall.

TABLE II. SALINITIES $^0/_{00}$ AND SURFACE TEMPERATURE ON JULY 27

High water: 10.20 a.m., 10.23 p.m.; low water: 4.15 p.m.

Time	10.20 a.m.	1.5 p.m.	4.20 p.m.	6.15 p.m.	7.0 p.m.	10.10 p.m.
Surface	27·3	26·7	25·6	26·5	27·2	27·4
4 m.	27·6	26·8	25·9	..	..	28·0
8 m.	28·0	..	25·9	..	..	28·2
12 m.	28·0	..	..	..	..	28·3
Temp. ° C.	17·5	17·2	17·0	17·0	16·9	16·8

Temperature. The temperature readings are correct to 0·1° C. The determined annual range was from 5·2° C. on March 10 to 19·3° C. on August 13. Diurnal variations were small. Table II gives the variation with time and state of tide on July 27. Since this day was colder than the average for the month this range is probably greater than normal.

p*H.* The *p*H was determined by McClendon's (1917) method with cresol red as indicator.

Since it was found too inconvenient to evaluate the *p*H of the sample immediately, the delay of at least 1 hr. may have caused a change in the value. In addition to this, the salinity varied substantially from time to time, so that readings were made and corrected to an accuracy of *p*H 0·05 only.

The values ranged from *p*H 8·1, which is comparable to normal sea water, to *p*H 7·80. Table I makes it clear that the *p*H change did not follow the salinity change, although the maximum and minimum average values of both occurred in the same month. The change from time to time, as shown in Fig. 2, was erratic, and, unlike the other three factors in this figure, bears no relation to the height of the tide. It is, however, possible that this lack of correlation, over short periods of time, is due to the degree of accuracy being insufficiently fine.

Phosphates. The phosphates were estimated by Atkins' (1923) modification of Denigès' method and the values calculated to milligrams of P_2O_5 per cu. m.

Table I shows that the phosphate values were high up to February, dropped suddenly to 33 mg. in March, and remained about this level until the end of the period. Some of the phosphate that enters the channel as sewage may be precipitated as ferric phosphate. The mud at low water between the Rhymney and Newport contains 4–5 % of iron in its dry weight.

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* I am indebted to the authorities of the Plymouth Marine Laboratory for the loan of his instrument.

The determined range was from 90 mg. per cu. m. on November 3 to 12 mg. on June 3. Fig. 2 shows the changes throughout the year and further shows that these changes have an inverse correlation with the height of the tide. At the beginning of the period the inverse correlation is not good, but this is probably due to the greater intervals between successive samples during this period. From February onwards the inverse correlation is good, the biggest deviation being the lowest value of 12 mg. on June 3.

The values given in Table III for the change of phosphate value with the state of the tide on July 27 show that there was a higher concentration of phosphate at low water than at high water.

TABLE III. CHANGES OF PHOSPHATE AND OXYGEN SATURATION WITH STATE OF TIDE ON JULY 27

	High water	Low water	High water
Phosphate	22	37	22
Oxygen saturation	93	87	92

Dissolved Oxygen. The oxygen content was determined by Winkler's titration method, and the values for percentage oxygen saturation calculated from Fox's (1907) tables. The precaution advised by Alsterburg (1926) of not permitting the sample, after adding the Winkler's reagents, to stand for more than 15 min. before acidifying, was adopted. This precaution minimizes possible errors due to organic matter and ferric ions.

The determined range for high water was from 5·26 c.c. per l. on August 25 to 7·78 c.c. on February 4. The percentage saturation range was from 79% on November 20 to 100% on February 4 and April 25. This value of 79% must be regarded as exceptional, since the oxygen saturation value was over 90% in 35 out of 38 samples taken at high water. This implies that the oxygen saturation is more a function of temperature and salinity than of any other factors. In Fig. 2 the changes in percentage oxygen saturation are shown, and, though by no means well marked, there is an apparent inverse correlation with the height of tide.

Table III shows that on July 27 the oxygen saturation was less at low water than at high water, and other samples taken at low water have a lower oxygen saturation than is general for high-water samples.

Opacity. The amount of suspended matter was considerable. A Secchi's disk of 9 in. diameter became invisible at depths of little more than 0·5 m. at high water. This is approximately the same as in the Tees estuary (Alexander *et al.* 1935) at the same salinity. However, the value of 0·5 m. is a generous one, since the readings were made at slack water on calm days. As a result of the gradual deposition of silt under such conditions the water becomes clearer. With the beginning of the tidal stream the silt is again stirred up, and a swell, even at slack water, prevents deposition.

The water is more opaque at low water than at high water and, as on July 27, a pronounced change in opacity may occur about mid-water flood.

Note must be made of the substantial quantities of coal dust present, as indicated in the tow-nettings. Usually its presence was more clearly marked in the deep hauls, but it was occasionally found in the surface hauls. This depended on the state of the water.

THE BACTERIA

From February to August 1937 nine samples of sea water were taken at the station, and the bacteria investigated to a limited extent. All the samples were taken at high water. The procedure adopted was, as nearly as possible under the conditions of working, that recommended by the Ministry of Health (1936).

The examinations made were:

(*a*) Agar count at 20° C. (room temperature), Table IV.
(*b*) Agar count at 37° C.
(*c*) *Coli-aerogenes* count.

TABLE IV COLONIES DEVELOPING AT ROOM TEMPERATURE

Date	0·5 c.c.	0·5 c.c.	0·1 c.c.	0·1 c.c.	Total	Average per c.c.
Feb. 15	209	183	..	..	392	392
Mar. 6	238	208	45	..	491	446
April 11	340	290	..	..	630	630
May 13	321	..	60	69	450	643
June 3	95	..	27	18	140	200
July 7	168	175	56	52	451	376
July 19	115	112	15	13	255	213
Aug. 25	129	..	27	33	189	270
	Total number of colonies counted				2998	
	Average per c.c. determined for the period					396

Owing to the spreading over of the plates by sporing organisms, the counts of colonies developing at 37° C. (Table V) are generally minimum. A number of the incubated plates were rendered useless and were discarded. As, frequently, the whole batch of plates was affected, counts were made of the better plates as long after 24 hr. and as near to 48 hr. as possible. The counts on July 7 and August 25 were of good plates incubated for the full 48 hr., and it is seen that these give quite the highest numbers of the series.

TABLE V. COLONIES DEVELOPING AT 37° C.

Date	1 c.c.	1 c.c.	0·5 c.c.	0·5 c.c.	Total	Average per c.c.
Feb. 15	24	..	15	..	39	26
Mar. 6	84	69	..	..	153	76
Mar. 10	58	53	33	..	144	58
April 11	..	..	29	23	52	52
May 13	..	..	..	32	32	64
June 3	..	..	18	16	34	34
July 7	..	..	94	..	94	188
July 19	..	..	47	50	97	97
Aug. 25	..	..	67	76	143	143
	Total number of colonies counted				788	
	Average per c.c.					72

The *coli-aerogenes* counts (Table VI) were obtained by inoculating the sea water and dilutions of it, made by adding the sample to sterilized tap water, into tubes of MacConkey broth. The tubes were incubated for 1 or 2 days, and, from the number of tubes giving a positive reaction for acid and gas, the probable number of *coli-aerogenes* colonies in the initial sample was obtained by reference to McGrady's tables (see Ministry of Health, 1936). The numbers obtained were so high that it was considered unnecessary to distinguish between *Bact. coli* and *Bact. aerogenes*, especially as there are several intermediate types.

TABLE VI. COLONIES DEVELOPING IN MACCONKEY BROTH

Quantity of sample in each tube ..	10 c.c.	1 c.c.	0·1 c.c.	0·01 c.c.	Probable no. of colonies per 100 c.c.
No. of tubes used ..	5	5	5	5	
Date	No. of tubes giving positive reactions				
Feb. 15	5	5	4	..	1600
Mar. 6	5	5	4	3	1600
Mar. 10	..	5	3	..	900
April 11	..	5	4	1	1600
May 13	..	5	5	3	1800 +
June 3	..	2	0	..	..
July 7	..	5	3	..	900
July 19	..	5	4	1	1600
Aug. 25	..	5	5	2	1800 +

The counts given in Tables V and VI, particularly in the latter, indicate a measure of sewage pollution.

Comparative results are given by Meek (1923 *b*) for the Tyne estuary. On agar incubated at 37° C. 1000 colonies per c.c. were recorded for March, increasing to 2400 in May. *Bact. coli* remained constant at about 100 per c.c. throughout. These numbers are very much higher than those for the plankton station. Lloyd (1930), on the other hand, found lower numbers of colonies developing at room temperature in the surface water in the Clyde Sea area, even in the heavily polluted Greenock and Cumbrae Deep areas. However, in making strict comparison between the three sets of results, the influence of differences of technique on the numbers obtained has to be kept in mind. In this connexion the recent work of Dianova & Voroshilova (1935) on the salt composition of the medium and the specificity of marine bacteria is important.

THE PLANKTON

All the tow-nettings were made with a conical tow-net with a mouth 0·5 m. in diameter and silk of 100 meshes to the inch. The net was towed horizontally for 20 min. at the surface, and at times for 10 min. at a depth of 2–4 m. from the bottom. Particular attention was paid to the surface hauls which were fully analysed quantitatively, but, owing to the greater amount of detritus, the deep hauls could not be treated in such detail. The method of counting was rigorously standardized from the beginning, counts being made in a subsample on a ruled slide. The subsample consisted of 0·5 c.c. and was removed while the plankton sample was being shaken. At least two counts were made. If the difference in the two counts was more than 10% further counts were made until two counts gave such consistency; usually, however, two counts were sufficient.

Table VII gives the distribution of the tow-nettings for each month. A few of these were taken at night, but, as there was no perceptible difference between night and day hauls, they are not specifically noted.

TABLE VII. DISTRIBUTION OF TOW-NETTINGS IN EACH MONTH

	Sept.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Total
High water: Surface	3	2	2	3	2	4	3	3	5	6	3	36
Deep	2	2	2	3	1	3	2	1	.	.	.	16
Low water: Surface	.	1	1	.	.	.	.	.	.	1	.	3
Deep	.	1	1	.	.	.	.	.	.	.	.	2
Other tow-nettings	.	.	.	.	.	.	.	.	.	2	2	4
									Total examined			61

Table XII gives a list of the species identified and the average numbers in the high-water surface counts for each month, except when otherwise noted. This list is not complete, some species present in very small numbers being omitted. Only numbers of 1000 or more for diatoms, and 500 or more for copepods and larval forms, are considered significant, and other numbers are not shown except when the maximum number obtained is given as an indication of frequency and seasonal distribution.

Diatoms

Twenty-four species of diatoms (Table XII) have been identified and all these, except the estuarine species *Nitzschia sigma* W.Sm.,* are typically marine. The bulk of the diatoms collected consisted of *Biddulphia sinensis* Grev., *Rhizosolenia setigera* Brightw., *Biddulphia regia* M. Schultze and *Bacillaria paradoxa* Gmel. Next in importance came *Streptotheca thamensis* Shrubs., but even though increasing to the end of the period, it was throughout overshadowed by the numbers of the four main diatoms. Centrifuged samples indicated that *Skeletonema costatum* (Greville) and small *Coscinodiscus* species were the most frequent diatoms in the summer and not the diatoms caught by the tow-net. This is to be expected from the collecting method used, and it is to be understood that the present account of the diatoms in this area is virtually reduced to a consideration of four species only.

The average total diatom counts for each month are given in Table VIII and Fig. 3. From these it is apparent that the maximum number occurred in November, and the minimum in April. These numbers are, however, not particularly instructive. From the middle of November onwards *Biddulphia sinensis* gradually decreased in numbers and yet it made up more than 50%

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* I am indebted to Dr M. V. Lebour for confirming this identification.

of the samples until the beginning of May. This decline, therefore, obscures the changes in numbers of the other diatoms. Table VIII and Fig. 4 give the average numbers of all diatoms counted, with the exception of *B. sinensis*. These showed a minimum in December with a steady rise to the highest number in August. The steadiness of this rise is noteworthy. Except between January and February the diatom numbers are not doubled from one month to the next.

By comparing the two numbers for each month in Table VIII it can also be seen that the samples in August 1937 were very different in composition from those in September and especially November 1936. The tow-netting taken at the end of September 1937 still showed this difference. In the beginning of the period *B. sinensis* made up practically the whole of the samples, while at the end of the period it was relatively unimportant. The proportion *B. sinensis* to other diatoms changed from 7 : 2 in September and 34 : 1 in November to 1 : 26 in August.

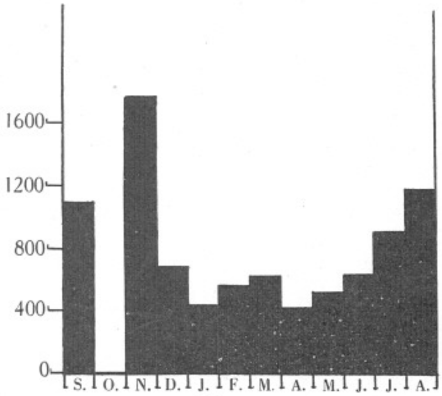

Fig. 3.

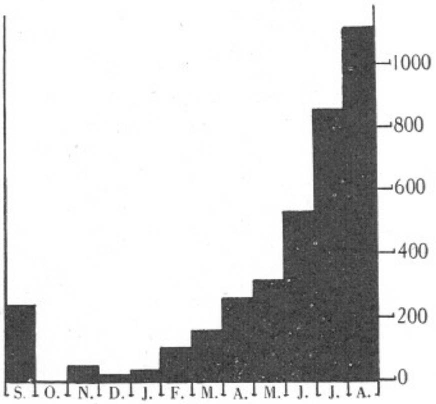

Fig. 4.

Fig. 3. The average numbers per month of all the diatoms counted in the tow-nettings. (No observations in October.)

Fig. 4. The average numbers for each month of all the diatoms except *Biddulphia sinensis* in the tow-nettings. (No observations in October.)

TABLE VIII. AVERAGE NUMBER OF THOUSANDS OF DIATOMS PER MONTH

	Sept.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Total diatoms	1080	1750	672	432	567	620	420	518	625	900	1170
Total less *Biddulphia sinensis*	240	50	22	32	107	160	260	318	533	860	1127

In Fig. 5 the changes in the values for each of the four main diatoms, expressed as percentages of the total diatoms counted, are related to the height of the tide. A series of values of under 10% for any diatom is considered insignificant.

On the basis of the information in this figure, the period of survey can conveniently be divided into four periods.

September 19–February 4. During this period *B. sinensis* made up more than 90% of the diatom samples. This made the changes in percentage of all other diatoms insignificant and, in addition, made the changes in *B. sinensis* itself insignificant so that this period is not included in Fig. 5.

February 4–April 17. This period is shown in Fig. 5. On February 4 the percentage value of *B. sinensis* was 97% and of *Bacillaria paradoxa* 1%. On February 15 there was a change to 74% and 20%. This is the first stage of a period in which the values of *Biddulphia sinensis* varied inversely, and those of *Bacillaria* varied directly, with the height of the tide.

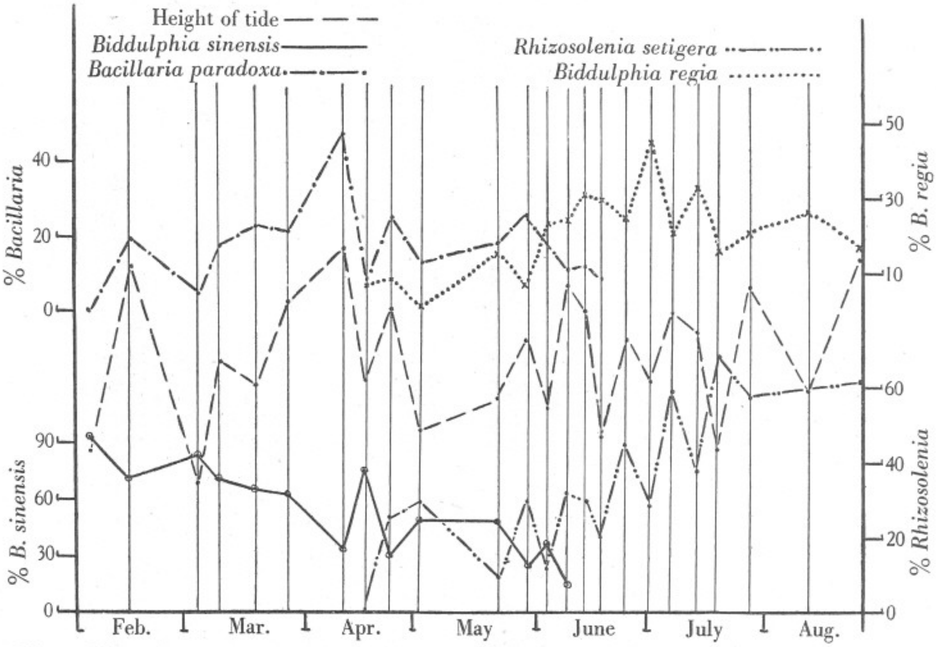

Fig. 5. The relations between the height of tide and the percentage of the four main diatoms to the total diatoms counted in the samples from February 4 to August 25.

April 17–June 18. During this period a transition occurred between the conditions in the last period and the next. *Biddulphia sinensis* and *Bacillaria* were still correlated with the height of the tide, but approached the value of 10% and by June 18 became insignificant. *Rhizosolenia setigera* suddenly jumped from under 1% on April 17 to 25% on April 25. Up to May 21 the values had no distinct relation with the height of the tide, but from that date they were directly correlated. *Biddulphia regia* was insignificant until May 21 (although Fig. 5 includes values from April 25) and from then on was inversely correlated with the height of the tide.

June 18–August 25. During this period only two diatoms were concerned. The percentage values of *Biddulphia regia* continued their inverse relation, and those of *Rhizosolenia setigera* their direct relation with the height of the tide. These correlations are well marked and do not break down until July 19. From then onwards the correlations are indefinite, the values differing little from time to time.

Within the actual total numbers of diatoms there are (Fig. 5) diatoms showing different relations with the height of the tide. Although effective in different periods *Biddulphia sinensis* and *B. regia* agree in being inversely correlated, and *Rhizosolenia* and *Bacillaria* agree in being directly correlated with the height of the tide. In Fig. 6 this distinction is recognized. Here are given the changes in the actual numbers obtained of *Biddulphia sinensis* plus *B. regia*, and the changes in the numbers of the remaining diatoms. In the first of these there is a clear inverse relation with the height of the tide and in the latter a direct relation. The correlations are by no means exact, but since we are dealing with numbers in which enormous variation may occur, owing to the quantitative method used, such a result is to be expected.

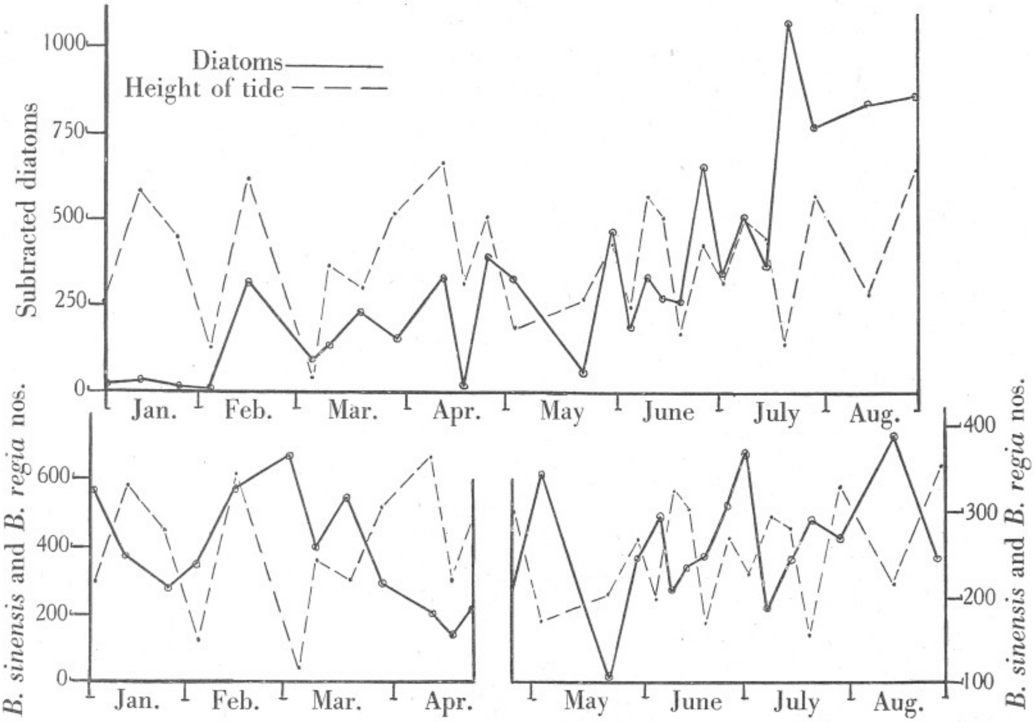

Fig. 6. The relation between the height of the tide and (1) the numbers of *Biddulphia sinensis* plus *B. regia*, (2) the numbers of the remaining diatoms, in the samples from January 5 to August 25.

There is no persistent correlation between the height of the tide and the total number of diatoms, and these correlations become apparent only when a distinction is made between the diatoms according to the information in Fig. 5.

On July 27 five tow-nettings were taken from one high water to the next. Table IX gives the percentage composition of the diatoms in each tow-netting. These values are plotted in Fig. 7 and show that the composition varied considerably between high water and low water. At high water *Rhizosolenia* was dominant and at low water *Biddulphia regia* made up the bulk of the catch. The values for *Bacillaria* and *Biddulphia sinensis* indicate also, in so far as one can accept changes within this range of values, that *Bacillaria* decreased and *Biddulphia sinensis* increased in percentage values at low tide. Care is required in considering these, since the values at high water were conditioned by *Rhizosolenia* and at low water by *Biddulphia regia*.

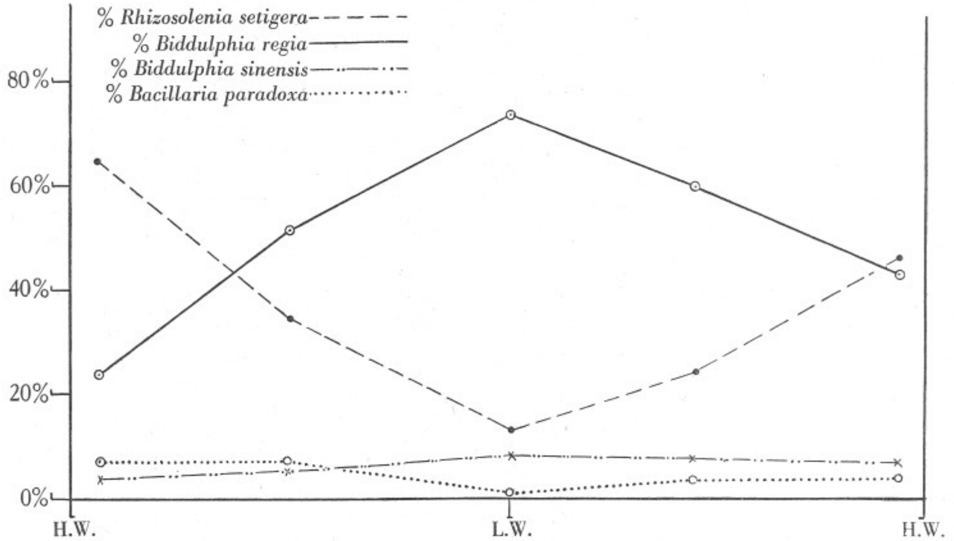

Fig. 7. The changes in the percentage composition of the diatom samples with state of tide on July 27.

TABLE IX. PERCENTAGE COMPOSITION OF DIATOMS ON JULY 27

	Rhizosolenia	*Biddulphia regia*	*Bacillaria*	*Biddulphia sinensis*
High water	65	24	7	4
Mid-water ebb	35	52	8	6
Low water	14	74	2	9
Mid-water flood	25	61	5	9
High water	46	43	5	6

The diatom numbers in each haul are relative only to one another. In order to obtain numbers which could be compared with those from other areas, water samples of 20 c.c. were centrifuged at intervals from the end of April to July and the diatom chains counted. Owing to the considerable quantities of silt there was a limit to the volume of water that could be centrifuged, and, even so, the counts obtained are minimum owing to the masking effect of the deposited silt. The counts were so low, however, that errors are unimportant. The counts varied from 72 diatom chains per 20 c.c. on June 3 to 22 on May 13. Fig. 8 shows an inverse relation between the numbers and the height of the tide. Samples in November gave counts of under 10 diatom chains per 20 c.c. and sometimes there were no diatoms present.

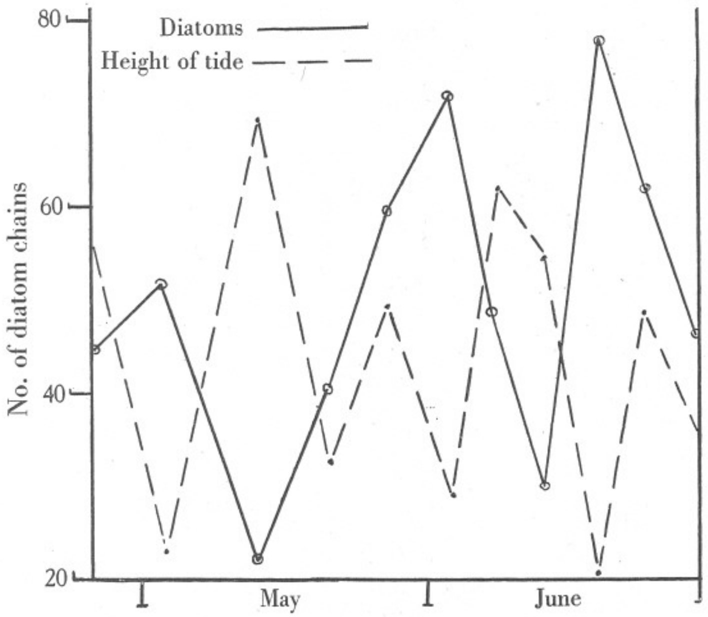

Fig. 8. The relation between the height of tide and the number of diatom chains in 20 c.c. of water from April 25 to July 1.

Copepods

Only eight species of pelagic copepods (Table XII) are recorded from the station, and, of these, five species alone were present in significant numbers. These were, in order of importance, *Eurytemora affinis* (Poppe), *Acartia bifilosa* (Giesbrecht), *Euterpina acutifrons* (Dana), and, of much lesser significance, *Oithona nana* Giesbrecht and *Centropages hamatus* (Lilljeborg). The other species were present sporadically and in small numbers. Fig. 9 presents the seasonal distribution in the numbers of the three main species.

Eurytemora affinis was the most interesting copepod obtained. Variations in numbers from time to time were sufficiently great to represent changes in actual density at the station, rather than changes due to unequal or inefficient sampling by the tow-net. I have followed Gurney (1931) in not separating the form *E. hirundoides* from *E. affinis*. As yet the copepodid stages and adults have not been analysed, and the numbers refer to the total *Eurytemora* irrespective of stage.

While a varying number of specimens were obtained outside this period, *Eurytemora* reached significant numbers, i.e. 500 or over, between November 3 and June 8. The highest average for any month occurred in April (Fig. 9) with over 28,000, and this was closely followed in May with nearly 27,000. The highest number obtained at the surface was 59,000 on May 21, and it is this number which makes the average for May high, since on May 27 a sudden decrease in numbers was obtained (Table X). A further decrease occurred on June 8. The rapidity of this decrease is striking.

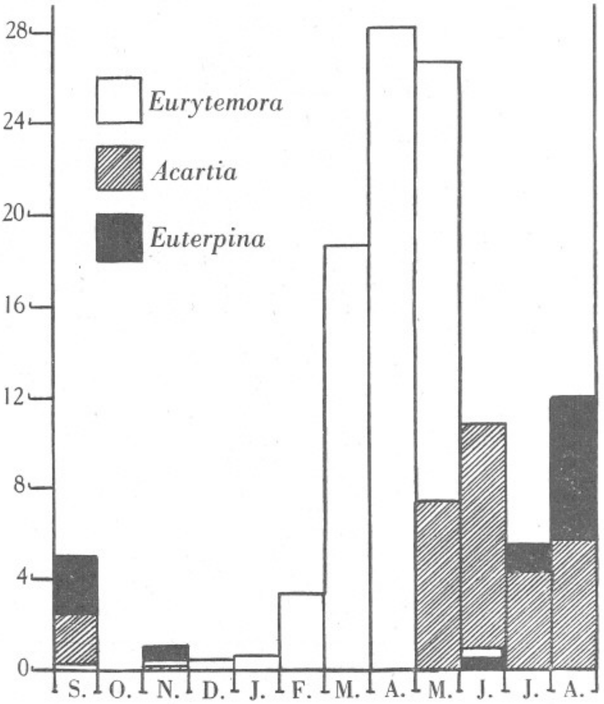

Fig. 9. The average numbers per month of the three chief copepods. (No observations in October.)

Table X. Numbers of *Eurytemora* and *Acartia*

Date ..	Apr. 25	May 3	May 21	May 27	June 3	June 8
Eurytemora	32,000	17,000	59,000	3,200	4,800	400
Acartia	..	800	20,000	1,600	25,600	13,200
Height of tide	37 ft. 4 in.	30 ft. 6 in.	32 ft. 4 in.	35 ft. 1 in.	31 ft. 8 in.	38 ft. 5 in.

In Fig. 10 the numbers of *Eurytemora* obtained at the surface are plotted against the height of the tide. Allowing for the sudden drop in numbers on May 27, there appears to be a direct correlation, with a single exception in the coincidence of the form of the curves on April 17. In this figure, also, is plotted the numbers of diatoms caught in the period, and there is, here, an inverse relation between surface *Eurytemora* and diatoms. This is to be expected, since, during this period, *Biddulphia sinensis* made up more than 50% of the diatoms collected and the diatom curve will tend to follow the *B. sinensis* curve, i.e. inverse with the height of the tide.

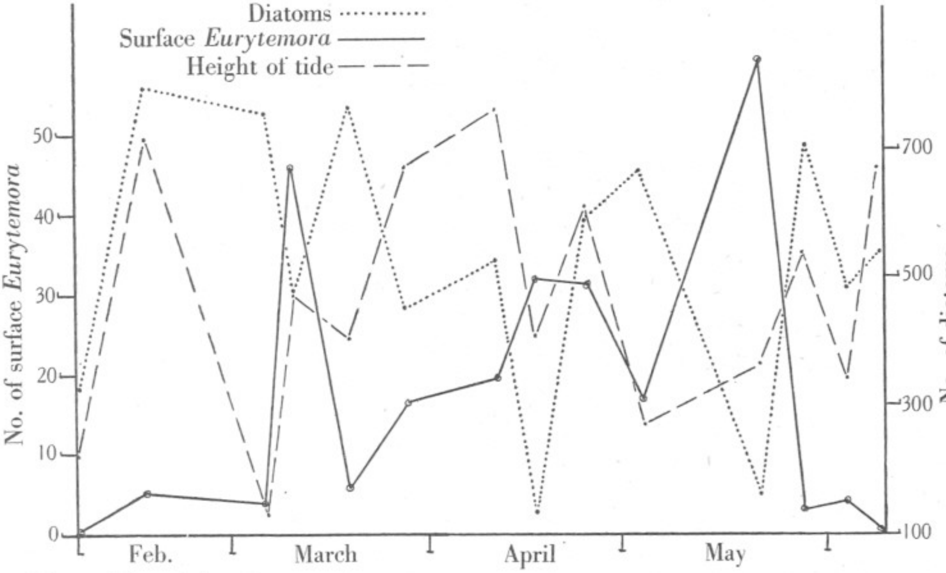

Fig. 10. The relations between the surface *Eurytemora* and (1) height of tide, (2) total diatoms, from February 4 to June 8.

Over a short period quantitative data for *Eurytemora* are available from deep hauls. The numbers obtained were higher than at the surface. On March 6, for example, over a quarter of a million were obtained in 10 min. towing. Since the duration of the bottom hauls was half that of the surface hauls the numbers are here doubled. The total numbers of *Eurytemora* obtained from the surface and the bottom, when both records are available, are plotted in Fig. 11. In contrast to Fig. 10 there is an inverse correlation with the height of the tide. The surface *Eurytemora*, expressed as percentages of the total *Eurytemora* obtained, are also plotted in this figure, and their direct correlation with the height of the tide is well marked.

Acartia bifilosa. This species was present in significant numbers up to November 20 and from May 3 to the end of the survey. Table X shows that, at about the time of the rapid decline in numbers of *Eurytemora*, there was a rapid increase in the numbers of *Acartia*, and also that, during this period, the numbers were inversely correlated with the height of the tide. On no occasion after June 8 were more than 10,000 *Acartia* obtained, so that variation in numbers may well have been due to unequal sampling.

Euterpina acutifrons agreed closely with *Acartia* in time distribution, and was significantly present up to November 27 and from May 27 to the end of the survey. In this species, the rise in numbers to the maximum of 20,000 on August 25 was gradual.

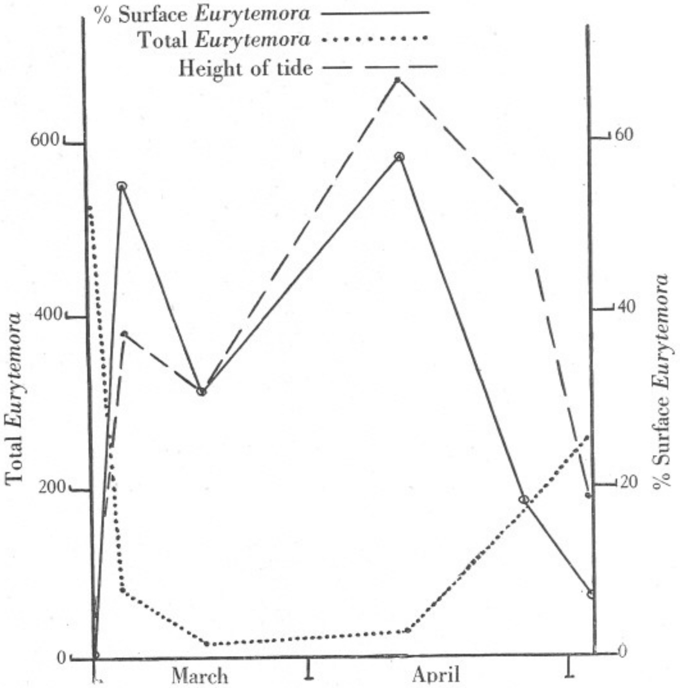

Fig. 11. The relations between the height of tide and (1) total *Eurytemora*, (2) percentage of the total *Eurytemora* at the surface.

Oithona nana was present in significant numbers from September 6 to December 21. Except for a few specimens at the end of the period of survey it did not reappear.

Centropages hamatus was present in significant numbers only in September, though a few specimens were present at the end of the survey.

Table XI gives the percentage distribution of the copepods with the state of the tide on two separate occasions. We see here that *Eurytemora* was quite unimportant at high water, but was the dominant form at low water. *Euterpina* was the most frequent copepod at high water, while *Acartia* appears to maintain an even percentage value, but it must be realized that its value at high tide is determined by *Euterpina* and at low water by *Eurytemora*. *Oithona* was present at one high tide only.

TABLE XI. PERCENTAGE DISTRIBUTION OF COPEPODS WITH STATE OF TIDE

Date	State of tide	*Acartia*	*Eurytemora*	*Euterpina*	*Oithona*
July 27 1937	High water	41	4	55	..
	Low water	40	58	2	..
Sept. 20 1937	Low water	22	58	20	..
	High water	24	..	67	9

The list of harpacticids (Table XII) is not unusual for estuaries or turbulent water. *Tisbe furcata* (Baird) was interesting in that a number of the specimens carried the suctorian *Ophryodendron trinacrium* Grüber.

Other Organisms

The remaining species and larval forms identified are of little interest. As with the diatoms and the copepods, there is a distinct poverty in the number of species. A considerable number of the species listed in Table XII cannot be considered pelagic, and are present in the tow-nettings, as commonly in other estuaries, owing to the turbulence of the water.

As regards the larger Crustacea, the numbers give little indication of their frequency since the tow-net used was too fine to catch good samples of, for example, the mysids. The largest number of mysids taken was 75 at the surface at low water. Usually there were more mysids and amphipods in the deep hauls, but there was no marked difference between day and night hauls.

Of the larval forms, cirripede nauplii were present in greatest numbers, with a maximum in April. The numbers for the polychaete larvae are shown as those of *Nerine*, *Autolytus* and general polychaete larvae. This latter group, during the summer and autumn, consisted mainly of the larvae of *Sabellaria alveolata* Linn. (Wilson, 1929) which is common along the shore and is probably present on the bottom under the plankton station.

Both *Podon polyphemoides* and ascidian tadpoles appeared suddenly on June 18 and had virtually disappeared by June 24.

DISCUSSION

It has already been explained (p. 398) that, because of the varying speeds of the tidal stream, the observations made at the station are referable to a series of points along a length of the channel, and that the points can be related to one another by the height of the tide, i.e. the highest spring tide refers to the farthest point concerned down-channel and the lowest neap tide to the nearest point down-channel, and so on. Therefore the vertical measure of the height of the tide can be used as a horizontal measure of a length of channel. This length will be referred to as the investigated length.

TABLE XII. LIST OF SPECIES AND AVERAGE MONTHLY CATCHES

	Sept.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Diatoms. Nos. refer to thousands											
cMelosira Borreri Greville	..	..	..	..	+	+	+	3*	..	..	..
cParalia sulcata (Ehr.)	+	+	+	1·4	3	5·4	7	3	1·5	+	+
Coscinodiscus Grani Gough	58	4·5	1·2	..	..	..	..	..	..	+	+
Coscinodiscus excentricus Ehr.	+	3	3	7	4	9	11	13	18	7	5
Coscinodiscus radiatus Ehr.	+	3	2	+	1	1	3	4	7	2	+
Actinoptychus undulatus (Bail.)	..	..	+	+	+	2	3	2	4·6	1	+
Actinoptychus splendens (Ehr.)	..	..	..	..	..	+	+	..	..	..	..
cSkeletonema costatum (Greville)	..	..	..	..	+	6	+	9	11	10	4·2
Rhizosolenia setigera Brightw.	14	13	3	4	4·5	4	6	130	190	500	650
cChaetoceros borealis Bailey *cChaetoceros didymis* Ehr.	1	..	..	..	..	..	..	..	4·6	18	2
Biddulphia mobiliensis Grev.	..	..	..	..	6	7·4	9	6	1·6	1	..
Biddulphia regia M. Schultze	57	16	4	1	4·4	11·5	9	30	168	230	210
Biddulphia sinensis Grev.	840	1700	650	400	460	460	160	200	92	40	43
Biddulphia favus (Ehr.)	..	..	..	..	..	+	0·6	+	+	+	..
cBellarochia malleus (Brightw.)	+	+	+	..	..	+	+	0·6*	+	+	+
Dytilium Brightwelli (West)	..	..	..	..	..	..	..	1*	+	2*	..
cStreptotheca thamensis Shrubs.	..	..	..	..	..	1	15	14	36	30	75
cAsterionella japonica Cleve & Möller	+	+	..	..	..	..	..	..	..	0·5	+
cAsterionella kariana Grun	..	..	..	+	+	..	..	+	..	..	..
cThalassiothrix nitzschioides Grun	9·4	4	1	2	0·7	1·5	1	+	+	+	+
Gyrosigma sp. Hassal	..	+	+	..	..	+	..	..	..	..	..
cBacillaria paradoxa Gmel.	28	21	10	7	85	80	140	100	70	60	76
Nitzschia sigma W. Sm.	2	3	3·7	3	1·5	3·5	1	+	2	1	1
Other phytoplankton organisms. Nos. refer to thousands											
Ceratium furca (Ehrenberg)	+	..	..	1*	..	+	0·7*	+	+	+	+
Halosphaera viridis Schmitz	..	..	..	..	+	1	1·6	2	+	..	..
Copepods. Nos. refer to hundreds											
Calanus finmarchicus (Gunnerus)	..	..	..	..	0·01*	0·01*	0·01*	..	..	..	..
Pseudocalanus elongatus Boeck	..	..	..	+	+	1*	4*	+	+	..	..
Centropages hamatus (Lillejeborg)	6·5	+	+	..	..	..	..	+	..	+	..
Temora longicornis (O. F. Müller)	..	..	+	0·02*	+	..	..	..	..	..	..
Eurytemora affinis (Poppe)	+	5	5	6	34	187	284	267	10	+	..
Acartia bifilosa (Giesbrecht)	23	+	..	..	..	..	..	75	109	53	59
Euterpina acutifrons (Dana)	50	10	+	..	..	..	..	+	5	44	120
Oithona nana Giesbrecht	6	+	11	+	+	..	..	..	..	+	..
Cladocera											
Podon polyphemoides Leuck.	..	..	..	..	..	..	..	..	5600*	..	..
Peracarids											
Gastrosaccus spinifer (Göes)	..	..	75*†	7	1	2	2	..	..	..	..
Schistomysis spiritus (Norman)	23	+	4	..	..	1	6	..	1	7	7
Schistomysis ornata (G. O. Sars)	..	..	..	2*	..	..	..	..	..	..	..
Mesopodopsis slabberi (van Ben.)	..	..	..	..	..	..	12	8	21	10	5
Neomysis integer (Leach)	..	..	..	..	..	1*	..	..	..	..	..
Diastylis rathkei (Kröyer)	1*‡	1*‡	..	..	..	..	..	..	..	..	..
Eurydice pulchra Leach	1*	..	..	..	..	..	..	..	..	..	..
Sphaeroma serratum (Fabr.)	..	..	..	1*	..	..	..	..	..	..	..
Notropis swammerdami (H. Milne-Edwards)	..	..	1*	..	..	..	..	..	..	..	..
Gammarus locusta (L.)	1*	..	..	..	..	..	..	..	..	..	..
Gammarellus homari (Fabr.)	..	..	..	12	8	3	..	..	..	..	..
Decapods											
Pandalus montagui Leach	..	3*†	..	1	..	..	..	..	..	..	..
Crangon vulgaris L.	..	1*†	..	..	..	..	..	..	..	..	..
Pasiphaë sivado (Risso)	..	..	..	..	..	..	..	..	..	2*	..
Other zooplankton species											
Foraminifera	800	+	+	..	..	..	..	..	..	..	..
Pleurobrachia pilosa (O. F. Müller)	2	1	..	+	..	..	..	..	16	17	6
Sagitta setosa J. Müller	2	..	..	..	..	..	..	+	3	1	2
Eteone sp.	..	..	..	2*§	..	1*	..	..	..	..	..
Oikopleura dioica Fol.	200	..	..	..	..	..	..	..	300	400	1000
Elvers	..	..	..	..	..	2*	..	..	..	..	..
Larval forms											
Ephyra	..	..	..	..	2*	..	..	..	..	..	..
Cyphonautes	..	+	..	..	..	..	..	..	..	..	..
Echinoplutei	10*	..	..	..	..	..	..	..	..	..	..
Post larval asteroids	10*	..	..	..	..	..	..	..	..	..	..
Larvae of *Nerine*	..	..	..	..	..	1200	900	400	+	..	..
Autolytus larvae	..	..	..	..	..	+	4	+	+	+	..
Polychaete larvae	500	500	+	500	800	3800	1600	2800	6000	7600	4000
Nauplii	..	1000	500	3600	4100	1900	1900	5000	860	1400	1600
Cirripede nauplii	2200	560	..	..	700	12400	16000	8000	10000	7300	2000
Cypris larvae	300	+	..	..	..	..	..	750	+	..	..
Cryponiscid	..	..	..	..	..	+	..	..	..	+	..
Macruran protozoea	..	..	..	..	..	2*	..	..	..	..	..
Megalopa	..	..	..	..	..	..	..	..	..	..	5*
Zoea of *Carcinus maenas*	250	..	..	..	..	..	..	..	..	+	+
Gastropod larvae	550	..	..	..	..	500	+	+	3100	4000	2600
Lamellibranch larvae	200	200	1100	950	200	950	700	300	..	..	..
Ascidian larvae	10*	..	..	..	..	..	..	..	600*	+	..
Ovum hispidum hystrix	..	..	..	..	..	..	..	..	+	700	1400

Harpacticids

Longipedia minor Scott
Canuella perplexa Scott
Ectinosoma curticorne Boeck
Zaus goodsiri Brady

Alteutha interrupta (Goodsir)
Tisbe furcata (Baird)
Tachidius littoralis Poppe
Tachidius discipes Giesbrecht
Macrocheiron fucicolum Brady

* Number in a single tow-netting only. † Low-water haul. ‡ Bottom haul.
§ Night tow-netting. + Indicates presence. *c* Numbers refer to chains of diatoms.

Since the higher the tide the farther down-channel is the point involved in investigation, the direct correlation between the salinity and height of tide (Fig. 2) means that there was a gradient of increasing salinity from up-channel to down-channel. Quite apart from this correlation, such a gradient is to be expected, and the existence of the correlation serves as a check on the distinction between observations at a single point and along a length of channel. It also shows that changes in values along the investigated length are sufficiently large to be easily observable. While normal variations in rainfall do not upset this correlation, it is possible, as Prof. Tattersall suggests, that the local influx of fresh water from the Taff and adjacent rivers causes a steeper gradient in the region of the station than farther out in the channel. If this is so the range of values at a station nearer mid-channel would not necessarily be greater than at the present station, in spite of the increase of the investigated length due to greater divergence in the speeds of the spring and neap tidal streams.

The inverse relation between the phosphate values and the height of the tide indicates a gradient of decreasing phosphate. This is further borne out in Table III which shows that phosphate values are higher at low water. Low water observations refer, of course, to a point considerably farther up-channel than the investigated length.

Table III shows, also, that the oxygen saturation was less at low water than at high water, yet Fig. 2 appears to indicate, from the inverse correlation with the height of tide, that there was a decreasing oxygen saturation from up-channel to down-channel. These results are not necessarily inconsistent. Meek (1923*b*) found that the dissolved oxygen content in the Tyne estuary is almost entirely dependent on fresh water from upstream. The fresh water coming down from the Taff, Ely and Rhymney may be effective in the same way and cause a gradient in oxygen saturation from the point of entry of the fresh water. The water that forms a state of low water at the station is only for a short period under the influence of these rivers, and, with the flood tide, moves up-channel outside their range. In this position it may enter a region where the effect of contaminating products causes a lower saturation.

There are, undoubtedly, gradients of other factors which have not been determined, particularly opacity, and possibly contaminating products as suggested by the higher phosphate and lower oxygen saturation at low water. While it is not possible to define all the gradients individually, the values for the height of the tide summate the effects of the gradients as a whole.

The water within the investigated length cannot be regarded as distinct and isolated. It becomes mixed with, and replaced by, partially or ultimately completely, up- or down-channel water. This process is, in the last place, dependent on the fresh water flowing into the estuary and channel on the up-channel side. When the up-channel water is dominant there is a drop in salinity in the investigated length. With decreasing rainfall the effect of the up-channel water is lessened, and the down-channel water mixes with, and replaces, the water in the investigated length, thus increasing the salinity. The measure of the dominance of one or other body of water is given by the average monthly salinities (Table I), and changes in values indicate that up-channel water entered the investigated length from September to February, and that down-channel water entered from February to the end of the period. Apparently this process had an annual cycle. The water that entered the investigated length from up- or down-channel brought in with it its own plankton, which mixed with the plankton already there, and gave to this mixed plankton a new environment. This process is referred to as the annual cyclical movement.

An outflow of less saline water along the surface of the channel, as in the Tees estuary (Alexander *et al.* 1935), appeared to play a negligible part and occurred only under exceptional conditions. This is easily understandable since the bulk of fresh water enters the channel far above the investigated length, and the mixing processes, induced by the tidal streams, would cause any such layer to lose its identity before arriving at the investigated length.

The correlations in Fig. 5 show how the diatoms can be related to one another within the investigated length. The percentages of *Biddulphia sinensis* and *B. regia* in the samples agree in being inversely correlated, and the percentages of *Rhizosolenia setigera* and *Bacillaria* agree in being directly correlated with the height of the tide. Two periods are distinguished. From February to May the correlations determine that *Biddulphia sinensis* was more up-channel than *Bacillaria*, and from June to August that *Biddulphia regia* was more up-channel than *Rhizosolenia setigera*.

Biddulphia sinensis → *Bacillaria*.
Biddulphia regia → *Rhizosolenia*.

Further, from the correlations in Fig. 6 it is clear that, in their respective periods, *Biddulphia sinensis* and *B. regia* were more frequent on the up-channel side, and *Rhizosolenia* and *Bacillaria* on the down-channel side.

Of the copepods only *Eurytemora* varies sufficiently along the investigated length to permit of analysis. Fig. 10 indicates, from the direct correlation, that the surface *Eurytemora* were more frequent down-channel than up-channel. This appears to be quite inconsistent with the known behaviour of *Eurytemora* in other areas (Percival, 1929). When, however, the total *Eurytemora* obtained, both from surface hauls and deep hauls, are plotted against the height of the tide (Fig. 11) an inverse correlation appears which shows that actually *Eurytemora* was a more up-channel form. The direct correlation between the height of the tide and the percentage of the total *Eurytemora* at the surface (Fig. 11) shows that the farther down-channel the more evenly were the *Eurytemora* distributed from the surface to the bottom. On the up-channel side of the investigated length the large majority of the *Eurytemora* was at the bottom. It is this variation in vertical distribution along the investigated length which explains the difference in correlations between the surface *Eurytemora*, the total *Eurytemora*, and the height of the tide.

From the plankton results the annual cyclical movement is illustrated best by the copepods. Since the changes in average monthly salinities measure the influx of up- or down-channel water, a comparison of Fig. 9 with the average salinities for the corresponding months (Table I) suggests that the copepod succession from up-channel to down-channel was

Eurytemora → *Acartia* → *Euterpina*.

This evidence is insufficient, since some factor, such as temperature, may determine the succession, but the composition values for July 27 (Table XI) do, however, substantiate the suggestion and allow the addition

Euterpina → *Oithona*.

Since *Centropages hamatus* followed *Oithona* in time, the succession can probably be further extended to

Oithona → *Centropages*.

It is of interest that, working at one place in the channel, a succession of copepods was obtained which illustrates part of the range from purely brackish water species to purely oceanic species (see Russell, 1936, quoting Ostenfeld, 1931).

Brackish water, e.g. *Eurytemora affinis*.
Littoral, and brackish water, e.g. *Acartia bifilosa*.
Neritic, low salinity, e.g. *Oithona nana*.
Neritic, e.g. *Centropages hamatus*.

The data which have been presented indicate the importance of space, and an ideal figure representing the changes in the plankton should be three dimensional, with a consideration of the changes in relation to both time and space. Apart from the difficulties of constructing such a figure, the available data are not sufficient for such a construction. In Fig. 12 is shown a strictly diagrammatic attempt, without attention to scale, to give the arrangements of the four main diatoms. *AB* represents the investigated length of channel concerned from February to the end of April, with *A* on the up-channel side, and *CD* represents the length between June 18 and the end of the period, with *C* on the up-channel side. The sections *AB* and *CD* depend on the relations shown in Fig. 5 and Fig. 6.

If the change in the diatoms *AB* to *CD* was continuous, then some such arrangement as in *BC* seems possible. It is to be understood that the sections *AB* and *CD* are the assumed arrangements at any point instant of time, and are, therefore, purely spatial arrangements. They are synthesized from a number of observations. The spatial arrangement in the interval between the conditions in *AB* and *CD* cannot be determined from available observations, and, since the hypothetical arrangement in *BC* represents a process of change, the time element cannot be neglected. It may be expected that *AD* represents the length of channel involved in the annual cyclical movement, thus making the arrangement in *BC* also spatial. If this was so, then it is clear *Bacillaria* should have been more up-channel than *Biddulphia regia*.

Against the height of the tide in Fig. 13 are plotted the number of *Biddulphia regia* to one *Bacillaria* on each occasion from June 3 to the end of the survey. The inverse relation indicates that the farther up-channel, the more *Biddulphia regia* were there compared with *Bacillaria*. Therefore, *Biddulphia regia*, even in this period, was more up-channel than *Bacillaria*. This means that the decrease of *Bacillaria* was not due to a movement up-channel out of the investigated length, but an actual decrease independent of, at least directly, the annual cyclical movement. In this it agreed with *Biddulphia sinensis*, which decreased from November onwards, and was, therefore, also independent of the annual cyclical movement. It should be noticed that, provided the idea of the annual cyclical movement is true, the decrease in *Bacillaria* was more extensive than the figures indicate. Since it was during the whole period a down-channel form, *Bacillaria* should have increased in numbers with the inflow of down-channel water, in agreement with the extension of the gradient in numbers along the investigated length (Fig. 5). That the decrease in *Bacillaria* cannot be considered as a change dependent on space does not deny the possibility that the annual cyclical movement was largely concerned in the change from *AB* to *CD*. The succession shown in the copepods would be expected to have its counterpart in the diatoms.

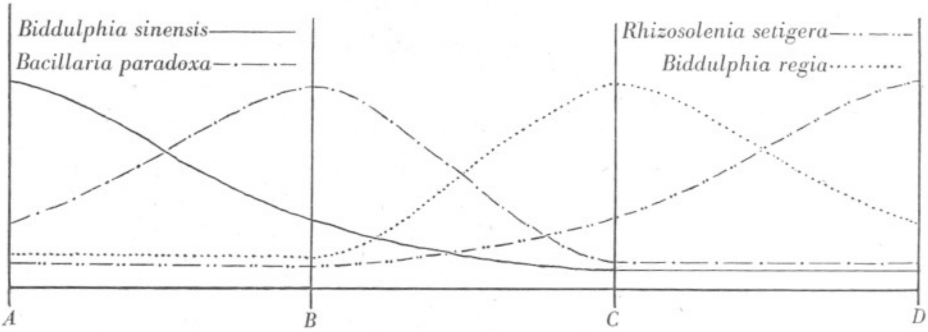

Fig. 12. Diagrammatic representation of the arrangement of the diatoms. See text.

While it is recognized that the section *BC* is hypothetical, it is considered that the sudden increase of *Rhizosolenia setigera* on April 25 (Fig. 5) does not necessarily deny the possibility of a continuous change from *AB* to *CD*. Such sudden changes in numbers of some species occurred on occasions without corresponding changes in associated forms. *Eurytemora* decreased suddenly on May 27 and *Acartia* increased on May 21 (Table X), *Podon* *polyphemoides* and ascidian larvae appeared suddenly on June 18, post-larval asteroids and ascidian larvae were found on September 26 (Table XII) and *Rhizosolenia* increased in numbers again on July 19 (Figs. 5, 6).

The consideration of the extent of phytoplankton production involves both a consideration of the actual density of the population and the variations in production throughout the year. On the first question the centrifuge counts (Fig. 8) clearly indicate a marked poverty. The maximum number of 72 chains per 20 c.c. may be compared with the frequent numbers of over 10,000 per 20 c.c. of Marshall & Orr (1927) and Steeman Nielsen (1935).

The second question is complicated by the cyclical movement, and it is necessary to neglect space and consider chiefly time. In so far as the results from samples can be accepted, it appears from Table VIII and Fig. 4 that there was no spring maximum, and that, though there was an increase in numbers from January onwards, there was no sudden rise such as usually occurs in other areas. It is, of course, clear that, because of the annual cyclical movement, the amount of production cannot be deduced from such factors as the phosphate, as was done in general terms by Hardy (1935) and in detail by Harvey (1934*b*).

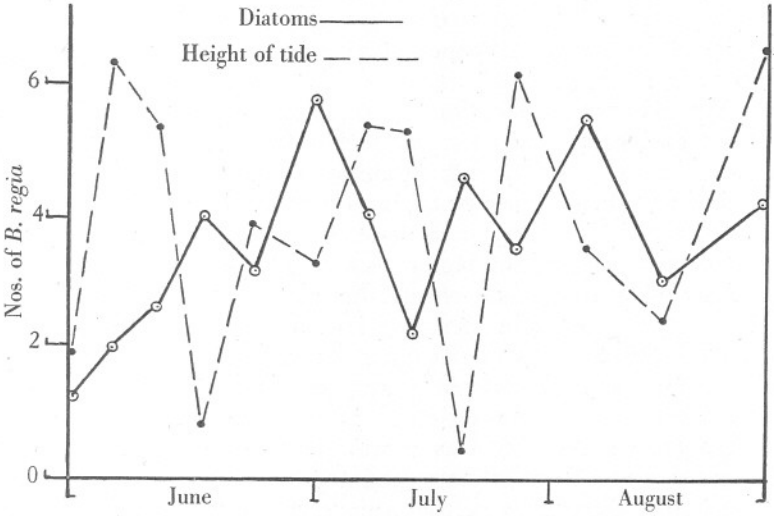

Fig. 13. The relation between the height of tide and the number of *Biddulphia regia* to one *Bacillaria paradoxa* chain in the samples from June 3 to August 25.

The major inhibiting factor on the phytoplankton production must be considered to be the instability of the water which is caused by the tidal streams. Since Gran's (1931) insistence on the importance of this factor, a considerable body of work has accumulated illustrating this. We may select as examples Steeman Nielsen (1935) and Hart (1934). The action of this factor is to remove the diatoms away from the photosynthetic zone into a deeper zone, where photosynthesis cannot take place, thus giving the diatoms less time for effective assimilation. The results of Steeman Nielsen (1935) are particularly striking. He found, for example (p. 70), that the spring increase occurred a month earlier in the stable Icelandic fjords than in the open coastal water, and production begins here only when the water is stabilized.

Owing to the shallowness of the Bristol Channel in the area concerned, instability in itself is not a sufficient explanation, since what is really effective is the ratio between the depth of the photosynthetic zone and the depth of swirling of the diatoms. Arising from the instability is the excessive quantity of silt and detritus in suspension, and it is this that makes the photosynthetic zone into a small layer and so increases the ratio. As it is the ratio which is effective, the conditions of production down-channel are not necessarily better than up-channel, in spite of the gradient of transparency, since farther down-channel the water is deeper. The gradient of decreasing phosphate must also be kept in mind.

The phytoplankton production is, therefore, largely controlled by the opacity, a factor which, in itself, has received much emphasis (Marshall & Orr, 1928; Pettersson, Höglund & Landberg, 1934), and the instability. These restrictions on production may be taken as approximately constant throughout the year. The nutritive salts, or, at least the phosphates and by implication the nitrates, were not limiting factors at any time, and, as far as the data go, light appears to be controlling within the limits imposed by the instability. An increase in the amount of light gave rise to an increase of photosynthesis, but, since the effectiveness of the light was reduced by the instability, the increase in photosynthesis did not correspond in degree with the increase of light. The absence of a limiting effect by the nutritive salts permitted the increased photosynthesis, such as it was, to proceed through the summer months and so give a form of diatom numbers shown in Fig. 4.

A contributory factor may be the salinity. Since the diatoms are typically marine, the lower the salinity the greater is the deviation of the environment from normal, but, while this may itself restrict production, the chief effect, probably, is to restrict the number of species and to debar species which might otherwise engage in a sudden increase. The suspended material may restrict certain species and this, possibly, partially accounts for the poverty in the number of species, particularly in the zooplankton. Ostenfeld (1931) maintains that the absence of several organisms in the eastern part of the English Channel and southernmost North Sea is due to this cause.

It would appear that the causes of the poverty of the fauna and flora in the plankton is due to the instability, with resulting siltiness, and the low salinity. The bacteriological examination of the water indicates sewage pollution, but, since the oxygen saturation is not appreciably lowered, it is unlikely that this pollution acts as an inhibitory factor. Sewage pollution may, in fact, favour plankton production. Välikangas (1926) has found that in the harbour of Helsingfors the greatest production of plankton takes place in the Bay of

Tölö where the greatest amount of sewage from the town of Helsingfors is poured out.

A note on the quantitative method may not be out of place. The poverty of the phytoplankton and the amount of suspended matter present considerable difficulties. Analyses of small samples of water, as in the centrifuge and sedimentation processes, would not give results of the type such as have been here presented, especially as the volume, small as the processes demand, is made smaller by the amount of silt that would be deposited. The silt introduces difficulties, also, in filtration of larger samples of water, since, even with a filter of 100 meshes to the inch, the volume of inanimate matter was usually greater, and often several times greater, than the volume of plankton, with resulting difficulties in the analysis of the samples. The use of a fine filter or phosphorbronze (Hentschel, 1936) would therefore defeat its own object, and towing with a very fine net would have a similar result. Possibly the only improvements on the method, as far as present methods go, would be the use of a meter (Harvey, 1934*a*) or a centrifugal pump (Gibbons & Fraser, 1937).

It is recognized that the method resulted in a selection of the diatoms, missing out comparative samples of, for example, *Skeletonema costatum*, and collecting the larger diatoms. However, the majority of the diatoms listed in Table XII would have been caught at least as efficiently as *Bacillaria* and *Rhizosolenia setigera*, and an accumulation of these would have given an indication of a sudden rise in numbers had there been one. Gran (1932, p. 346) maintains that "The total sum of all species could not be used to measure the productivity of an area of the sea any better than the study of the growth and decrease of the dominant species". The present discussion on phytoplankton production depends upon this.

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The field and laboratory work was done while I held a Post-graduate Studentship of the University of Wales.

SUMMARY

The plankton collected at a station in the Bristol Channel in 1936–7 is related to chemical and hydrographical factors.

On account of the varying speeds of the tidal streams, the observations

refer, not to a single point, but to a series of points along a length of the channel.

Gradients of salinity, phosphate, and a possible complicated gradient of oxygen saturation, were determined, and, from the average monthly salinities, an annual cyclical movement of water deduced.

From February to June *Biddulphia sinensis* was a more up-channel form than *Bacillaria paradoxa*, and, from June to August *Biddulphia regia* was more up-channel than *Rhizosolenia setigera*. In this latter period *Biddulphia regia* was more up-channel than *Bacillaria*.

From being concentrated at the bottom on the up-channel side *Eurytemora* became more and more evenly distributed from the surface to the bottom on the down-channel side.

Owing to the annual cyclical movement, a succession of copepod species from up-channel to down-channel entered the investigated length.

The decrease in numbers of *Biddulphia sinensis* and *Bacillaria* was, directly, independent of the annual cyclical movement, but this movement may have been effective to some extent in transforming the diatom population.

The instability of the water and the opacity, which result from the tidal streams, are the major inhibiting factors on diatom production. Low salinity and excessive siltiness are possible contributory factors.

Phosphate was not, at any time, a limiting factor, and diatom production was, apparently, controlled by light within the limits imposed by the inhibiting factors.

The bacteria indicate some sewage pollution.

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ON THE GROWTH AND FEEDING OF YOUNG HERRING IN THE CLYDE

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(Text-figs. 1–12)

CONTENTS

In an earlier paper (Marshall, Nicholls & Orr, 1937) the growth of the herring in the Clyde was followed from spawning to metamorphosis. The present paper gives the results of an attempt to continue this work from metamorphosis up to the formation of the first winter ring.

During the course of the work two distinct groups of herring were met with. Those of the first, which were obtained from July 1936 to May 1937, were caught in Kames Bay and on Fairlie Sands; the second, of which only a few catches were taken, in 1937 and 1938, were smaller and were caught offshore. As will be shown below, the second group is composed of herring which were spawned in the Clyde sea-area in spring and are therefore of the same origin as those discussed in the previous paper.

COLLECTION OF MATERIAL

Larvae which had been followed from hatching off Brown Head on the coast of Arran to metamorphosis in the mouth of Loch Fyne about the beginning of June 1935 disappeared thereafter. In spite of an intensive search of the area during the following months, both in sandy bays using a fine-meshed shore seine and in deeper water using a Poole sprat trawl, very few were found. In July 1936, however, young herring about 80 mm. long were captured in Kames Bay, a shallow sandy bay on the island of Cumbrae. From then on successful hauls were made at about weekly intervals in the same bay till the end of September when numbers became very small. Thereafter till the following May they could be caught near Hunterston Perch on Fairlie Sands on the Ayrshire coast. At this position the sand-flat shelves steeply down to a depth of several fathoms, and hauls were always made at low tide when it was possible to use the shore seine on the slope. The best hauls were taken about dusk or dawn on moonless or overcast nights. On several occasions both in Kames Bay and on Fairlie Sands the herring disappeared as soon as the moon rose.

Catches on Fairlie Sands were not obtained so regularly as in Kames Bay and it was sometimes necessary to make hauls on several nights before a catch was obtained. Sometimes an indication of herring was given by gannets diving, by the presence of seals or by hearing the fish break the surface. The catches were smaller after November 1936, and after May 1937 they were irregular. By the end of May the new brood of young herring appeared again in Kames Bay and these were followed for a few weeks to compare with the previous year's fish.

The number of herring in a haul with the fine-meshed shore seine varied very considerably. Sometimes several hauls were required to obtain sufficient fish for a sample (a few hundred), but occasionally many thousands were captured in a single haul.

Other types of gear were tried with varying degrees of success. A Saltash tuck seine with a fine-meshed bag proved unsuccessful in spite of being used immediately after the shore seine had made a successful haul. A Poole sprat trawl was uniformly unsuccessful even at times when herring were present in large numbers. A series of drift nets with mesh varying from 57 to 75 rows per yard was useful offshore, but since the shore seine provided an unselected sample the use of drift nets was discontinued.

For offshore work a sprat ring net similar to that used at Newhaven, in the Firth of Forth, was used and was successful at times when the herring could be located by means of phosphorescence in the water. Unfortunately the mesh in the bag of the net was too large (90 rows per yard) to retain all the smallest fish so that sampling for these was not accurate. Successful catches of the small offshore fish were made in July and August 1937 in Loch Striven, the Kyles of Bute and round the Cumbrae, and in October 1937 and March 1938 in Loch Striven.

To find the fluctuations of numbers with time of night and to provide material for a study of the food over a night, a series of hauls was made at 2 hr. intervals in Kames Bay on August 14 1936 (Fig. 12 A). At 5 p.m. (B.S.T.), although it was full daylight and there were a number of bathers and small boats in the bay, 132 herring were caught in one haul; at 7 p.m. the number rose to 4000–5000. The best catch (estimated at over 10,000 fish) was obtained at 9 p.m., the time of sunset. After this catches decreased rapidly; at 11 p.m. when it was quite dark, there were 450; at 1 a.m., 8; at 3 a.m., 12. At 5 a.m. when dawn was breaking there was a slight increase to 293, but by 7 a.m. there was a decrease again to 6 and hauls were discontinued.

RACE DETERMINATION

As has already been mentioned, the young herring captured in the Clyde belong to two different groups which may conveniently be described as inshore and offshore. The inshore fish were considerably larger than the offshore fish and it became necessary to find out whether two distinct races of herring were present, and if so which, if either, represented the Clyde spring-spawned fish.

In defining different races of herring, one of the most usual methods is to count the number of vertebrae in a representative sample of fish; the mean number is indicative of the race. Wood (1936) found the mean number of vertebrae in spring-spawning herring in the Clyde to vary between 57·03 and 57·21. It should be noted that Wood follows Williamson (1914) and Johansen (1919) in including the first segment of the terminal ossicle in the vertebral count. In this paper we have followed Orton (1916) and Ford (1928*a*) in excluding it. Four samples of adult Clyde herring taken in the autumn of 1937 and the spring of 1938 gave counts from 55·88 to 56·06, which shows a good agreement with Wood's figures when the necessary allowance of one vertebra is made. A statistical comparison (see Ford, 1928*a*, p. 259) showed that these four samples agreed well with one another and with Wood's samples.

Counts were also made on samples of the young herring caught and it was found that the herring of the offshore group had a vertebral count varying from 55·97 to 56·16 showing agreement with the Clyde spring-spawned fish. Those caught in Kames Bay and on Fairlie Sands (the inshore group) belonged to a different race with a lower vertebral count varying from 55·40 to 55·65.

In Fig. 1 are shown percentage frequency graphs of the number of vertebrae based on the commercial catches of Clyde fish (A), the offshore samples of young herring (B), and the inshore samples (C). The results of the individual counts are given in Table I. It will be seen that whereas curves (A) and (B) show a sharp peak, as is typical of Wood's spring spawners, (C) is of a quite distinct type. There seems little doubt therefore that the offshore fish are Clyde spring-spawned herring. Similar counts to those of the offshore fish were obtained on the 1935 brood in three successive years (Table I, C). As premetamorphosis fish they were caught in Loch Fyne in 1935 (May 30), as one year old fish in Kames Bay in 1936 (August 10), and as two year old fish in Millport Bay in 1937 (May 26), and the vertebral counts were 56·07, 56·07 and 55·97 respectively.

The curve shown by the inshore fish (C) is similar to Wood's autumn-spawning type. There is, however, little evidence of any autumn spawning in the Clyde. Tow-nettings were taken for larvae and the gonads of adult fish examined in autumn, but no indication was obtained that any spawning takes place before March. Wood (1937, p. 18) believes that the Clyde "estuary" is a nursery for adolescent herring groups until they reach sexual maturity.

It is possible therefore that the origin of these herring is to be sought outside the Clyde. Samples of adult herring were obtained from the North of Ireland fisheries in April and September 1938, and from the Isle of Man fisheries in April and July 1938. The first were spent and their vertebral count showed the typical curve for spring-spawning herring (average number of vertebrae 55·97). The second were mostly full and showed the autumn-spawning type of curve (average number of vertebrae 55·63). The Isle of Man fish were spent in April and recovering spents at the beginning of July and showed the typical autumn-spawning type of curve (average number of vertebrae 55·49 and 55·53 in April and July respectively). A statistical comparison showed that the North of Ireland fish in September and both lots of Isle of Man fish agreed

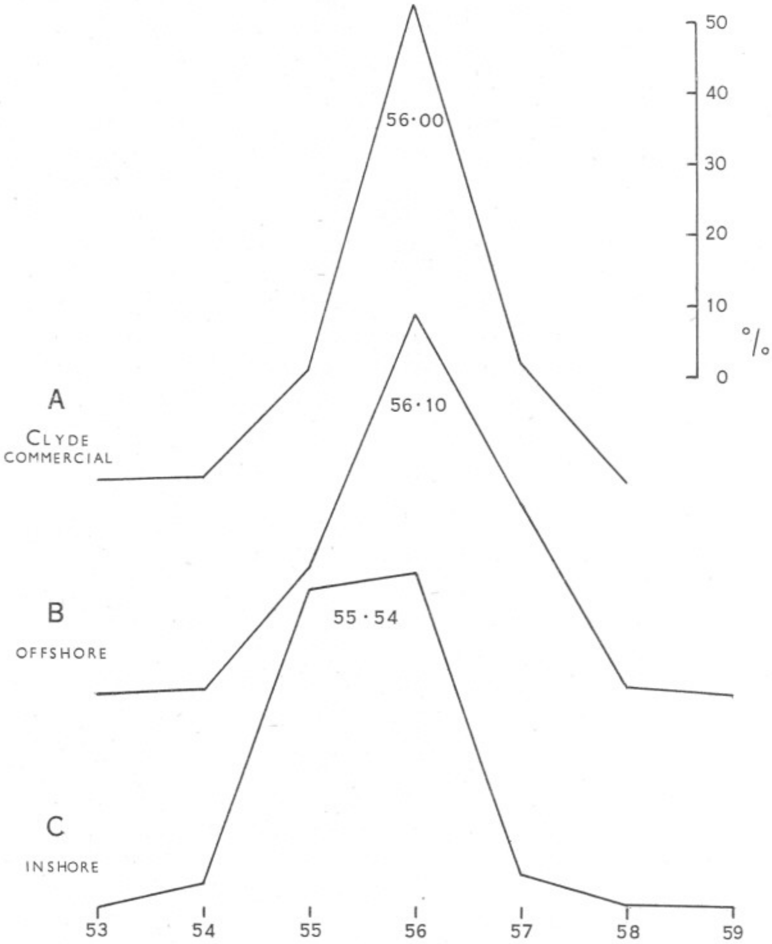

Fig. 1. Percentage frequency distribution curves of vertebral counts.

Table I

A. Vertebral Counts of Inshore Herring

Date	No. of fish	Percentage number of vertebrae									Mean	σ
		52	53	54	55	56	57	58	59	60		
13–14 & 14. vii. 36	102	..	..	2·0	47·1	44·1	6·9	..	..	..	55·56	0·654
20. vii. 36	106	..	..	0·9	41·5	52·8	4·7	..	..	..	55·61	0·595
27. vii. 36	103	..	..	7·8	44·7	42·7	2·9	1·0	..	1·0	55·49	0·850
10. viii. 36	111	..	..	2·7	51·4	41·5	4·5	..	..	..	55·48	0·630
14. viii. 36	109	..	..	4·6	37·6	52·3	5·5	..	..	..	55·59	0·670
31. viii. 36	110	..	..	6·4	40·9	49·1	3·6	..	..	..	55·50	0·674
23. ix. 36	111	..	..	4·5	48·7	42·4	4·5	..	..	..	55·47	0·658
29. ix. 36	111	..	..	4·5	37·8	52·3	5·4	..	..	..	55·59	0·667
5. x. 36	110	..	..	3·6	46·4	43·6	5·5	0·9	..	..	55·54	0·700
13. x. 36	110	..	..	1·8	37·3	55·5	4·6	0·9	..	..	55·65	0·642
28. x. 36	110	..	..	1·8	49·1	42·7	6·4	..	..	..	55·54	0·645
3. xi. 36	110	..	..	0·9	40·9	51·8	6·4	..	..	..	55·64	0·617
13. xi. 36	110	..	..	2·7	56·4	39·1	1·8	..	..	..	55·40	0·578
27. xi. 36	109	..	..	1·8	43·1	51·4	3·7	..	..	..	55·57	0·599
11. xii. 36	110	..	..	4·6	42·7	49·1	2·7	0·9	..	..	55·53	0·673
28. xii. 36	110	..	..	5·5	50·9	40·9	2·7	..	..	..	55·41	0·640
12 & 13. i. 37	110	..	..	3·6	39·1	51·8	5·5	..	..	..	55·59	0·654
9. ii. 37	110	..	..	5·5	41·8	47·3	5·5	..	..	..	55·53	0·687
15. iii. 37	110	..	..	5·5	41·8	45·5	6·4	0·9	..	..	55·55	0·737
29. iii. 37	110	..	..	4·6	35·5	55·5	4·6	..	..	..	55·60	0·653
13. iv. 37	79	..	..	1·3	51·9	40·5	6·3	..	..	..	55·52	0·638
28–29. iv. 37	110	..	..	0·9	48·2	47·3	3·6	..	..	..	55·54	0·585
13. v. 37	110	..	..	3·6	43·6	45·5	6·4	0·9	..	..	55·57	0·710
27. v. 37	109	..	..	..	46·8	49·5	3·7	..	..	..	55·57	0·567
21, 22 & 25. vi. 37	110	0·9	..	0·9	40·0	55·5	2·7	..	..	..	55·57	0·656
15. vii. 37	110	..	..	4·6	51·8	40·9	1·8	0·9	..	..	55·43	0·656
28. vii. 37	83	..	..	2·4	49·4	42·2	6·0	..	..	..	55·52	0·651

From 23. ix. 36 to 13. iv. 37 inclusive and on 13. v. 37 the herring were taken from Fairlie Sands, and on the remaining dates from Kames Bay.

B. Vertebral Counts of Offshore Herring

Date	Source	No. of fish	Percentage number of vertebrae						Mean	σ
			53	54	55	56	57	58		
8–9. vii. 37	Loch Striven	110	..	..	20·0	46·4	30·9	2·7	56·16	0·773
2. viii. 37	The Tan	110	..	1·8	16·4	50·0	30·9	0·9	56·13	0·756
5. x. 37	Loch Striven	102	..	1·0	18·6	60·7	19·6	..	55·99	0·652
21. iii. 38	Loch Striven	251	..	..	16·7	56·6	26·3	0·4	56·10	0·661

C. Vertebral Counts of 1935 Brood Fish

30. v. 35	Otter Spit	113	..	..	13·3	66·4	20·4	..	56·07	0·578
10. viii. 36	Kames Bay	106	..	..	19·8	55·7	22·7	1·9	56·07	0·707
26. v. 37	Millport Bay	110	..	..	15·5	71·8	12·7	..	55·97	0·533

D. Vertebral Counts of Commercial Samples

15. x. 37	Clyde	100	..	..	12·0	73·0	15·0	..	56·03	0·521
9. xi. 37	Clyde	108	..	..	26·9	58·4	14·8	..	55·88	0·637
10. iii. 38	Clyde (Brown Head)	110	..	0·9	11·8	70·9	16·4	..	56·03	0·566
16. iii. 38	Clyde (Ballantrae Banks)	110	..	0·9	12·7	65·5	20·9	..	56·06	0·610
8. iv. 38	Isle of Man	217	0·5	5·1	45·6	43·3	5·1	0·5	55·49	0·715
13 & 19. iv. 38	North of Ireland	183	..	..	20·8	62·8	15·3	1·1	55·97	0·637

with the inshore young herring from the Clyde; in addition they agree fairly well with summer-spawned Isle of Man herring examined by el Saby (1932).

Because of their proximity to one another, it might be expected that the two groups of young herring would be mixed in the catches. The distinction between the two groups of fish, however, is sharp and it seems improbable that any appreciable intermixture takes place. In July when both types of fish were caught about the same time, there was hardly any overlapping in the size frequency distribution curve (see Table II and Fig. 2). The vertebral counts also are distinct in the groups and, within each group, show statistical agreement. Either then the two groups of fish keep apart or the degree of intermixture is comparatively constant. This last possibility is ruled out for the following reason. Since the offshore herring, which had a high vertebral count, are considerably smaller than the inshore fish, it would be expected that if mixing were taking place, the smaller fish of any sample would have a higher average number of vertebrae than the sample as a whole. No such difference actually exists so that we may conclude that the two groups keep apart and that mixture is negligible.

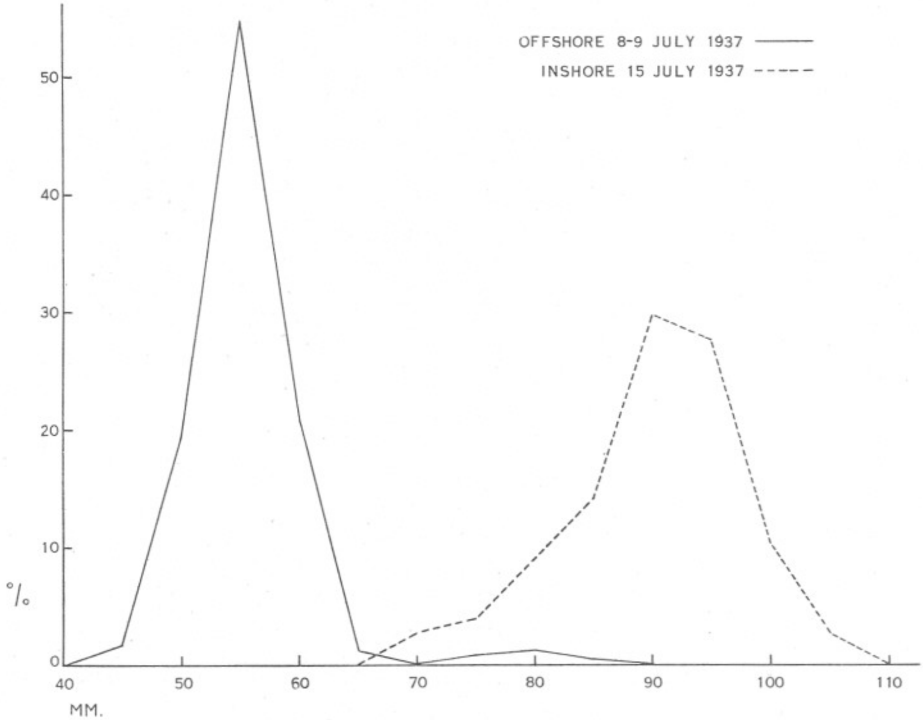

Fig. 2. Percentage frequency distribution curves of lengths for comparison of inshore and offshore young herring.

TABLE II. LENGTH MEASUREMENTS OF INSHORE HERRING

1936

mm.	13 & 14. vii.	20. vii.	27. vii.	3 & 4. viii.	10. viii.	14 & 15. viii.	19. viii.	24. viii.	31. viii.	8. ix.	15. ix.
55	..	1	2	..	..	..	..	..	..	..	..
60	2	1	1	1	..	..	..	..	..	..	..
65	10	1	..	1	..	..	..	..	..	..	..
70	17	13	2	2	..	..	2	..	..	..	..
75	30	56	6	12	3	9	2	..	..	..	..
80	90	86	21	47	8	20	9	..	..	..	4
85	124	155	94	109	15	32	4	1	1	..	18
90	26	226	294	175	28	79	7	4	..	..	38
95	1	92	155	134	62	128	1	9	..	4	44
100	..	7	44	33	130	238	3	38	2	14	73
105	..	..	3	6	181	397	5	80	14	29	97
110	..	..	..	..	67	245	3	110	24	71	122
115	..	..	..	..	7	60	..	78	53	77	75
120	..	..	..	..	..	1	1	39	85	81	23
125	..	..	..	..	..	..	..	2	83	47	5
130	..	..	..	..	..	..	..	1	16	17	3
135	..	..	..	..	..	..	..	..	..	1	1
140	..	..	..	..	..	..	..	..	..	..	..
145	..	..	..	..	..	..	..	..	..	..	..
150	..	..	..	..	..	..	..	..	..	..	..
155	..	..	..	..	..	..	..	..	..	..	..
160	..	..	..	..	..	..	..	..	..	..	..
165	..	..	..	..	..	..	..	..	..	..	..
170	..	..	..	..	..	..	..	..	..	..	..
Total	300	638	622	520	501	1209	37	362	278	341	503
Median	85·1	90·2	93·2	92·4	105·1	107·1	91·0	112·4	122·7	118·8	109·2
Average	83·4	88·4	92·5	91·7	103·4	105·4	92·6	111·6	121·1	117·8	107·2

1936

mm.	23. ix.	29. ix.	5. x.	13. x.	28. x.	3. xi.	11. xi.	13. xi.	27. xi.	11. xii.	28. xii.
55	..	..	..	..	..	..	..	..	..	..	..
60	..	..	..	..	..	..	..	..	..	..	..
65	..	..	..	..	..	..	..	..	..	..	..
70	..	..	..	..	..	..	..	..	..	..	..
75	..	..	..	..	..	..	..	..	..	..	..
80	..	..	..	..	..	..	..	..	..	..	..
85	..	..	..	..	..	..	..	..	..	..	..
90	..	..	..	..	..	..	..	..	..	..	..
95	..	..	..	..	..	..	1	..	1	..	..
100	2	..	..	..	..	..	..	..	..	..	1
105	1	1	1	..	..	..	1	..	1	..	1
110	8	6	..	1	..	1	3	..	4	..	3
115	29	38	4	7	1	2	17	2	12	3	26
120	74	64	13	45	4	11	58	13	46	16	49
125	94	98	80	142	46	55	90	56	108	65	109
130	32	65	129	218	94	62	40	91	127	120	81
135	10	16	58	169	88	70	18	65	74	53	40
140	..	..	11	19	46	35	6	38	21	36	8
145	..	..	..	1	7	13	1	20	11	21	1
150	..	..	..	..	..	2	..	..	2	5	..
155	..	..	..	..	..	..	1	..	..	2	..
160	..	..	..	..	..	..	..	..	..	..	..
165	..	..	..	..	..	..	..	..	..	..	..
170	..	..	..	..	..	..	..	..	..	..	..
Total	250	288	296	602	286	251	236	285	407	321	319
Median	125·8	126·8	131·8	132·8	134·9	134·5	127·1	133·7	131·1	133·3	128·4
Average	124·8	125·8	131·2	131·7	134·6	133·9	126·8	133·9	130·8	133·9	128·1

1937

mm.	12 & 13. i.	9. ii.	15. iii.	29. iii.	13. iv.	29. iv.	13. v.	26 & 27. v.	31. v.	21, 22 & 25. vi.	15. vii.	28 vii.
55	..	..	..	..	..	..	..	..	..	3	..	..
60	..	..	..	..	..	..	..	..	..	16	..	..
65	..	..	..	..	..	..	..	..	..	26	..	..
70	..	..	..	..	..	..	..	..	..	89	4	..
75	..	..	..	..	..	..	..	..	..	84	6	..
80	..	..	..	..	..	..	..	..	..	31	14	2
85	..	..	..	..	..	..	..	..	..	4	22	1
90	..	..	..	..	..	..	..	..	..	..	46	12
95	..	..	..	..	..	..	..	..	..	..	43	17
100	..	..	..	..	..	..	..	..	..	..	16	25
105	..	..	1	..	..	..	..	..	..	..	4	18
110	1	1	2	..	..	1	2	..	..	..	..	4
115	..	4	4	..	2	7	8	..	..	..	..	2
120	8	13	24	4	6	15	27	1	1	..	..	..
125	23	65	46	56	20	27	59	3	9	..	..	..
130	55	67	63	121	28	26	75	6	9	..	..	..
135	41	45	39	90	25	34	48	12	27	..	..	..
140	22	19	16	32	9	32	64	10	36	..	..	..
145	21	4	4	6	2	12	27	27	40	..	..	..
150	4	..	2	3	..	2	9	42	20	..	..	..
155	1	..	..	..	..	..	2	102	8	..	..	..
160	..	1	..	..	..	1	1	55	3	..	..	..
165	..	..	..	..	..	..	1	20	3	..	..	..
170	..	..	..	..	..	..	..	3	..	..	..	..
Total	176	219	201	312	92	157	323	281	156	253	155	81
Median	135·2	132·0	132·0	134·0	133·3	135·6	134·4	156·9	144·5	74·6	94·1	101·3
Average	135·7	131·8	131·2	133·8	132·6	134·3	134·8	154·7	143·7	73·8	92·1	100·5

On two occasions (August 10 1936 and May 26 and 27 1937) offshore and inshore herring were obtained in numbers in the same hauls. On each occasion, however, they belonged to different year groups, as was discovered by scale examination, and the older fish were always offshore fish. These are smaller than inshore fish of the same age and in both sets of hauls were of similar size to inshore fish of the preceding year class. This mixture then was probably due to the tendency of herring of similar size to shoal together irrespective of age (Ford, 1928*b*, p. 291). In the catches from Kames Bay and Fairlie Sands it was usual to find a few fish belonging to the previous year class. These were omitted from the samples and they were never numerous enough for reliable size measurements or vertebral counts.

A considerable number of the backbones examined (25% in the inshore fish and 19% in the offshore fish) showed complex vertebrae, i.e. vertebrae which, although the centrum shows no sign of division, have double spines dorsally, ventrally or both (Ford, 1933). These complex segments are not uncommon among herring and if they are counted as one vertebra the vertebral counts are too low. It has been found (Kändler, 1932; Ford, 1937) that if they are counted as one and a half vertebrae the normal average is obtained, and our results confirm this. The difference between the average of those with normal and those with abnormal vertebrae (when the latter are counted as one) was in inshore fish 0·49 and in offshore fish 0·46. In Table I, however, the complex vertebrae are given the value of one. Fused vertebrae, which were very rare, are given their full value.

SIZE AND GROWTH

From each catch a sample of fish, at least 250 if possible, was taken and the length measured from the tip of the snout to the tip of the longest caudal fin ray on the ventral fluke. The fish were measured within an hour or two of capture on nearly all occasions, since it was found that standing overnight or fixation in formalin caused a shrinkage amounting to several millimetres.

Inshore herring. The results of the measurements of these fish are given in Table II and Figs. 3 and 4. In Fig. 3 are shown the median lengths of the samples of inshore and offshore herring. The upper curve which is that for the inshore herring shows that there was an increase in length from the first observation in 1936 (July) till the middle of October 1936 amounting to just over 50 mm. giving an average weekly increase of about 3·5 mm. This is slightly greater than the weekly size increment in premetamorphosis Clyde spring-spawned herring. From the beginning of November 1936 until the beginning of April 1937 there was no increase in the median length, but fish caught at the end of May 1937 were considerably larger. The catches made during April were small and it is doubtful when the new year's growth actually started. On April 13 a few of the herring and on May 14 the majority showed the new winter ring at the edge of the scale. In June and July 1937 several samples of the new brood of inshore herring were obtained and their median lengths are also shown on the curve.

In Fig. 4 are shown the size frequency distribution curves of the samples grouped in fortnightly periods, omitting for simplicity the majority of the winter samples when there was no growth. The curves show the gradual increase in length up to October and the new growth in spring. The new brood of 1937 which was caught earlier than in 1936 is also shown. The smallest fish caught, nine in number, were taken on May 27 1937 and varied from 47 to 56 mm. in length. The curves show that in general the samples were homogeneous and that the size range within a sample did not increase much during the year.

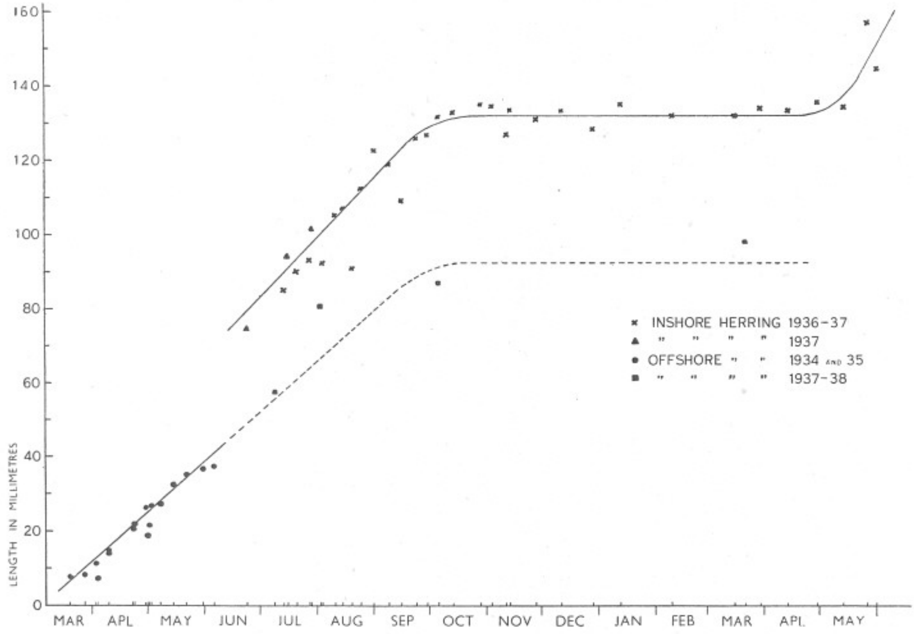

Fig. 3. Median lengths of inshore and offshore young herring, showing rate of growth.

Offshore fish. Only four catches of offshore fish in their first year were obtained, two during the growth period, one near the end of the growth period, and one near the end of the winter non-growing period. The points representing median lengths fit on an extension of the growth curve for premetamorphosis Clyde herring taken in 1935 and run parallel to but well below that for the inshore fish (Table III and Fig. 3). Thus in July 1937 the inshore fish had a median length of 94·1 mm. and the offshore fish 57·6 mm., while in March 1938, when they were forming their first winter ring, the inshore fish had a median length of about 132 mm. and the offshore about 98 mm. The size given for the offshore fish is probably on the large side since,

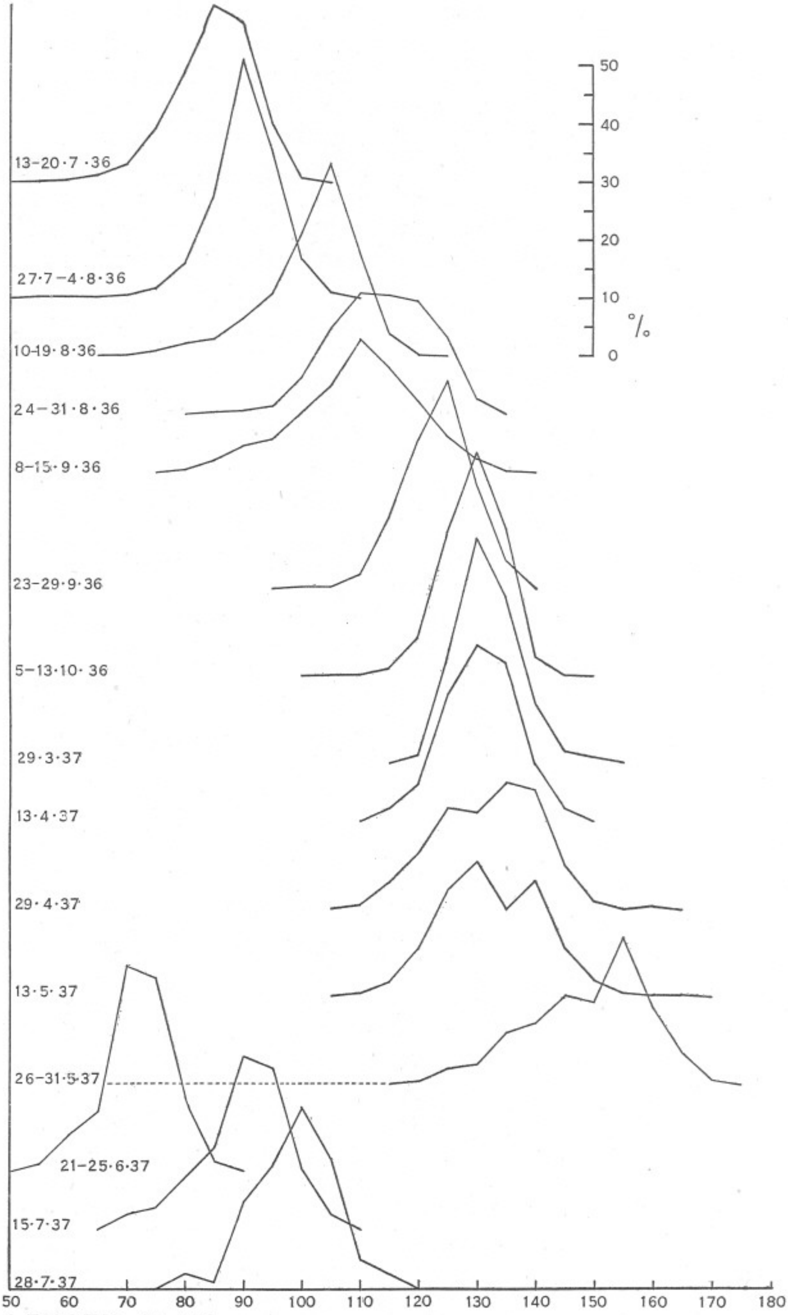

Fig. 4. Percentage frequency distribution curves for lengths of inshore herring, grouped in fortnightly periods.

as mentioned above, a proportion escaped through the meshes of the net. The 1935 brood of Clyde fish appeared again in the catches of 1936 (August 10) and 1937 (May 26 and 27); their median lengths on these dates were 131·3 and 164·4 mm. respectively.

Since the inshore and offshore herring differ considerably in length at the time the first winter ring is formed, it is possible to differentiate between the two groups at any later stage. If we assume that the rate of growth of the scale is proportional to the rate of growth of the fish (Lea, 1910), we can, by measurements on the scale, calculate the size of the fish at the end of each year's growth.

TABLE III. LENGTH MEASUREMENTS OF OFFSHORE HERRING

mm.	8, 9. vii. 37	2. viii. 37	5. x. 37	21. iii. 38
45	4	..	..	..
50	48	..	..	..
55	136	..	1	..
60	51	..	..	..
65	3	..	2	..
70	..	5	1	..
75	2	135	14	2
80	3	167	82	14
85	1	32	93	43
90	..	2	56	41
95	..	..	18	39
100	..	..	9	42
105	..	..	7	36
110	..	..	10	19
115	..	..	6	10
120	..	..	..	4
125	..	..	1	..
Total	248	341	300	250
Median	57·6	80·7	87·0	98·2
Average	57·6	80·4	88·9	98·1

Scales were taken from each fish of the measured samples of Clyde, North of Ireland and Isle of Man adult herring and the size when the first winter ring was laid down measured, using the technique described by Ford (1928*a*). The average size of the herring in the four commercial catches from the Clyde fishery at the time of formation of the first winter ring (l_1) was found to vary between 91 mm. and 110 mm. This compares with the average winter length of 93 mm. estimated for offshore herring (Fig. 3). The commercial samples from the North of Ireland fisheries gave average l_1 values of 121 mm. and 122 mm. Those from the Isle of Man fishery gave average l_1 values of 122 mm. and 120 mm., figures above those obtained from the Clyde commercial catches and the Clyde offshore fish, and nearer that for the Clyde inshore young herring (132 mm., Fig. 3). The results are not conclusive but further confirm the Clyde origin of the offshore fish and lend some support to the possibility that the inshore herring may have originated in the Isle of Man or North o Ireland grounds as was suggested by the vertebral counts.

Information on the rate of growth of herring during their first year is rather meagre. Among the earliest records are those of Meyer (1878) on spring-spawned herring in the Baltic. The rate of growth found by him for the period June 11 to November 14 (2·5 mm. per week) is lower than that found for Clyde spring-spawned herring (offshore type). On the other hand, at the end of the first year the Baltic herring reached a length of 138 mm., whereas the Clyde spring-spawned herring measured only 98 mm. The discrepancy is due to the Baltic herring growing during the winter, whereas those in the Clyde do not. Ford (1928*c*) measured the rate of growth of young herring from the Tamar and Lynher estuaries during their first year. The spawning at Plymouth in 1926–27 extended from October to March, so that the difference in age between the youngest and oldest might be several months. The median length of the Tamar fish on May 26 was 55·9 mm. and on October 7 127·1 mm., giving an average weekly increment during the growth period of 3·2 mm. which is close to that for the inshore Clyde fish (3·5 mm.). Similarly the length during the winter non-growing period was about 124 mm. in the Tamar fish as against 132 mm. for the inshore fish in the Clyde.

WEIGHT

Of the herring caught, samples at definite sizes were weighed at intervals throughout the year. The sizes chosen were at approximately 5 mm. intervals over the whole size range in each sample. The fish were usually weighed fresh after removal of adherent moisture and then dried to constant weight at 105° C. The results of the dry weight determinations are shown in Table IV and Fig. 5. It is found that the weights of the inshore herring fall into two groups, (*a*) those of the growing period from July 4 1936 to November 27 1936 and from April 29 1937 to May 27 1937, and (*b*) those of the winter period (in the intervening months) when growth had stopped. The relation between the dry weight in gm. (W) and the length in mm. (L) during the growing period is given by the equation $W = 0{\cdot}000000258L^{3{\cdot}37}$, and during the winter period by $W = 0{\cdot}0000001095L^{3{\cdot}47}$. For adult herring from the English Channel, Fraser (1931) using the wet weight determinations of Orton (1916) found $W = 0{\cdot}00000337L^{3{\cdot}149}$.

For the Clyde inshore herring, the weight of the *fresh* fish fell only slightly during the non-growing period, but the water content was relatively higher and the fat content relatively lower so that there was a fall in dry weight as is seen in Table IV and Fig. 5. When growth began again in the spring, the fat content rose, the water content fell, and the points come once more on the upper curve. Similar conditions in adult herring have been described by Lea (1911). Fig. 6 shows the relationship between the logarithms of the weights and the logarithms of the lengths for growing and non-growing periods. The points obviously lie on two straight lines, the equations for which (given above) were derived by calculation from the data.

The transition from the curve for the growing period to that for the non-growing period is shown in the weights of herring on February 9 (Table IV).

The points representing the weights of the smaller herring lie on or near the upper curve of Fig. 5, while those for the larger herring lie on or near the lower. Thus the larger herring reach winter weight conditions in advance of the smaller herring. Similarly on April 29 the weights of the smaller herring are on or near the lower curve while those of the larger herring approach the upper curve. Thus the larger herring reach summer conditions in advance of the smaller herring.

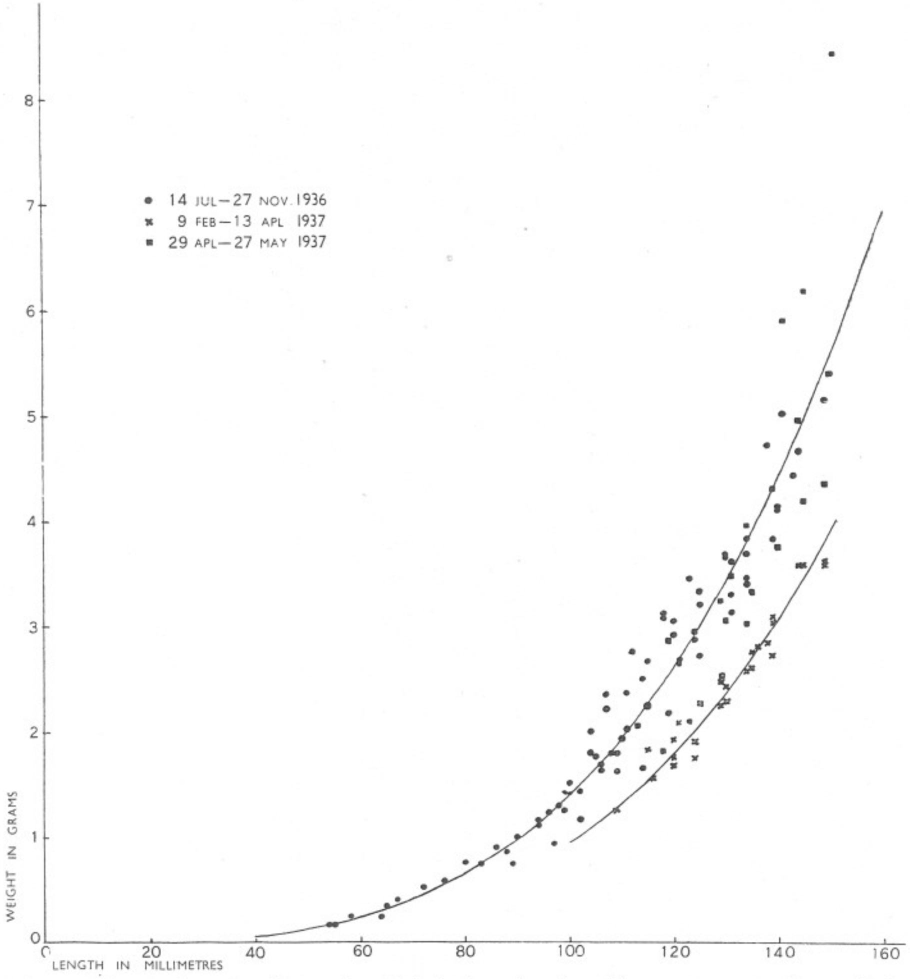

Fig. 5. The relation of weight to length in inshore herring. Upper curve, growing periods; lower curve, non-growing period.

It will be noticed that the growth period according to weight seems to continue for longer than the growth period according to length and that increase in weight begins again in spring before any increase in average length. As will be shown later, this is related to fat storage.

Only a small number of weighings of offshore (Clyde spring-spawned) herring were made and the weight-length relationship found is only slightly different from that for inshore fish (Table IV and Fig. 7). For the growing period the relationship is given by $W=0{\cdot}0000000436L^{3{\cdot}75}$ but for the winter non-growing period the values were lower and the equation is $W=0{\cdot}0000000172L^{3{\cdot}88}$. The offshore fish in the premetamorphosis stage had a weight-length relationship of $W=0{\cdot}0000020L^{4{\cdot}52}$ (Marshall, Nicholls and Orr, 1937).

TABLE IV. LENGTH, WET-WEIGHT AND DRY-WEIGHT MEASUREMENTS

Inshore fish

14. vii. 36			20. vii. 36			27. vii. 36			3 & 4. viii. 36			10. viii. 36			14. viii. 36		
mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.
67	1·95	0·41	58	1·25	0·26	54	0·74	0·18	96	5·41	1·25	94	5·13	1·17	104	8·33	2·01
72	2·48	0·53	65	1·73	0·36	55	0·74	0·17	97	4·70	0·94	96	5·65	1·24	107	10·35	2·36
76	2·79	0·59	99	6·22	1·43	64	1·07	0·25	102	5·72	1·18	99	6·14	1·43	112	10·24	2·76
80	3·46	0·77	104	7·89	1·81				102	6·54	1·44	100	6·33	1·42	118	11·76	3·08
83	3·46	0·76							108	7·72	1·80	105	7·42	1·77			
86	4·03	0·91										107	8·92	2·22			
88	4·09	0·87										109	6·88	1·63			
90	4·44	1·01										114	9·77	2·50			
94	5·14	1·12										115	10·13	2·67			
												118	11·45	3·13			

19 & 24. viii. 36			31. viii. 36			29. ix. 36			5 & 13. x. 36			27. xi. 36			9. ii. 37		
mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.
120	11·92	3·05	89	..	0·76	106	7·62	1·70	124	..	2·87	98	5·54	1·31	115	8·16	1·85
123	13·26	3·46	100	..	1·52	111	8·93	2·03	131	..	3·62	109	7·45	1·80	121	9·82	2·09
125	13·01	3·21	106	..	1·64	121	11·78	2·69	131	..	3·30	114	8·06	1·66	125	11·10	2·27
			110	..	1·94	130	14·42	3·68	134	..	3·69	119	8·46	2·18	129	11·97	2·47
			115	..	2·26	134	15·29	3·46	134	..	3·84	125	11·68	2·72	136	13·66	2·81
			120	..	2·92	138	17·17	4·73	140	..	4·11	131	12·65	3·13	139	15·07	3·09
			125	..	3·33	141	19·32	5·02	140	..	4·14	134	13·82	3·40	145	17·38	3·58
			130	..	3·66				144	..	4·67	139	15·68	3·83			
												143	17·35	4·44			
												149	20·35	5·15			

15. iii. 37			29. iii. 37			13. iv. 37			29. iv. 37			13. v. 37			26 & 27. v. 37		
mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.
109	6·40	1·26	120	9·61	1·93	116	8·16	1·56	113	9·18	2·06	111	9·56	2·37	121	..	2·65
120	8·94	1·76	124	9·68	1·92	120	8·84	1·68	118	8·50	1·82	119	11·53	2·86	129	..	3·24
124	9·97	1·91	130	12·33	2·43	124	9·39	1·75	123	10·32	2·10	124	12·42	2·95	131	..	3·47
130	11·72	2·29	135	13·13	2·60	129	11·52	2·25	129	12·19	2·53	130	13·06	3·05	134	..	3·96
135	13·82	2·75	138	14·48	2·84	134	12·83	2·57	134	14·25	3·02	135	14·57	3·32	141	..	5·90
139	14·89	3·03	149	17·76	3·58	139	14·36	2·72	140	16·67	3·75	139	17·64	4·31	145	..	6·17
144	17·68	3·58							145	18·84	4·19	144	19·54	4·96	151	..	8·42
149	18·04	3·62							149	19·86	4·35	150	22·81	5·40			

Offshore fish

10. viii. 36			26. v. 37			21. iii. 38		
mm.	gm.	gm.	mm.	gm.	gm.	mm.	gm.	gm.
119	10·93	2·76	155	..	7·26	73	1·85	0·33
122	10·87	2·64	160	..	7·40	80	2·26	0·47
124	13·06	3·49	164	..	9·51	85	2·62	0·52
125	12·42	3·09	170	..	10·24	90	3·15	0·65
129	14·73	4·16	175	..	11·76	95	4·09	0·86
131	14·95	3·80	181	..	12·31	100	4·77	0·99
133	14·66	3·70	185	..	13·17	105	5·66	1·15
139	16·89	4·28	190	..	15·66	110	6·92	1·46
140	18·34	4·81				115	7·47	1·60
144	18·57	4·78				119	9·11	1·94
145	21·36	6·25				126	10·24	2·32

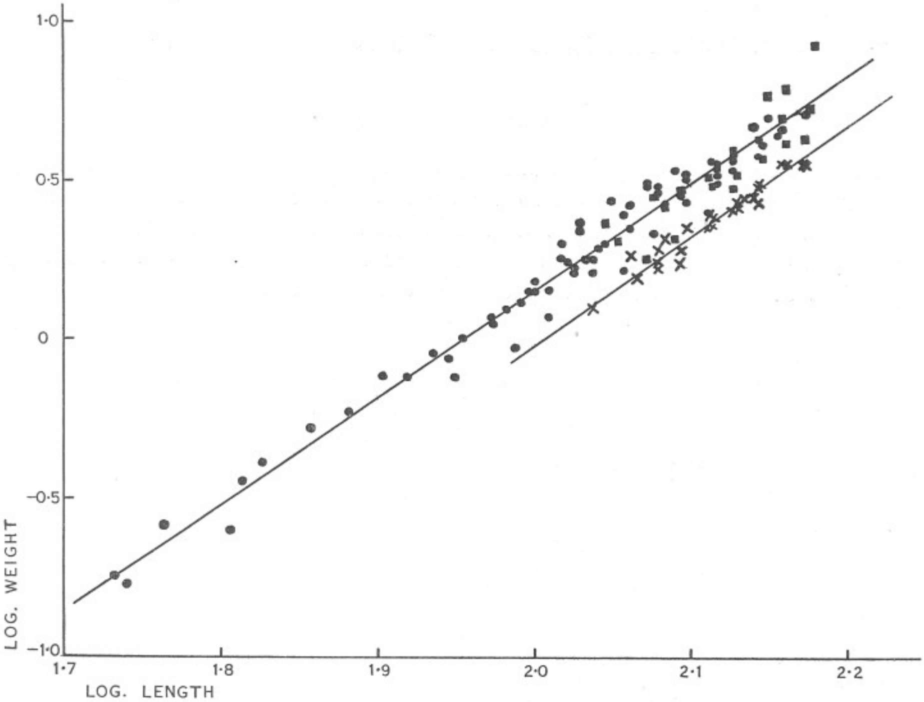

Fig. 6. Relation of logarithm of weight to logarithm of length in inshore herring. Upper curve, growing periods; lower curve, non-growing period. The points are the same as in Fig. 5.

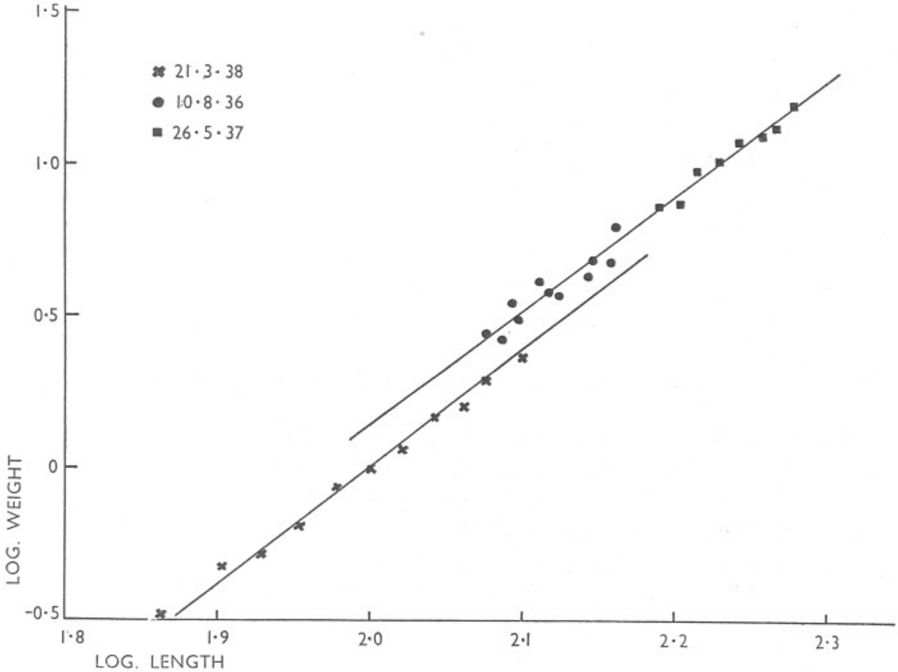

Fig. 7. The relation of logarithm of weight to logarithm of length in offshore herring. Upper curve, growing period; lower curve, non-growing period.

Chemical Composition

From the catches of inshore herring a sample of fish of about the average size for the catch was dried to constant weight at 105° C. and used for determination of protein, fat and ash. Protein was determined by the Kjeldahl method, fat by the Soxhlet ether extraction method and ash by ignition. The results are shown in Table V and Fig. 8.

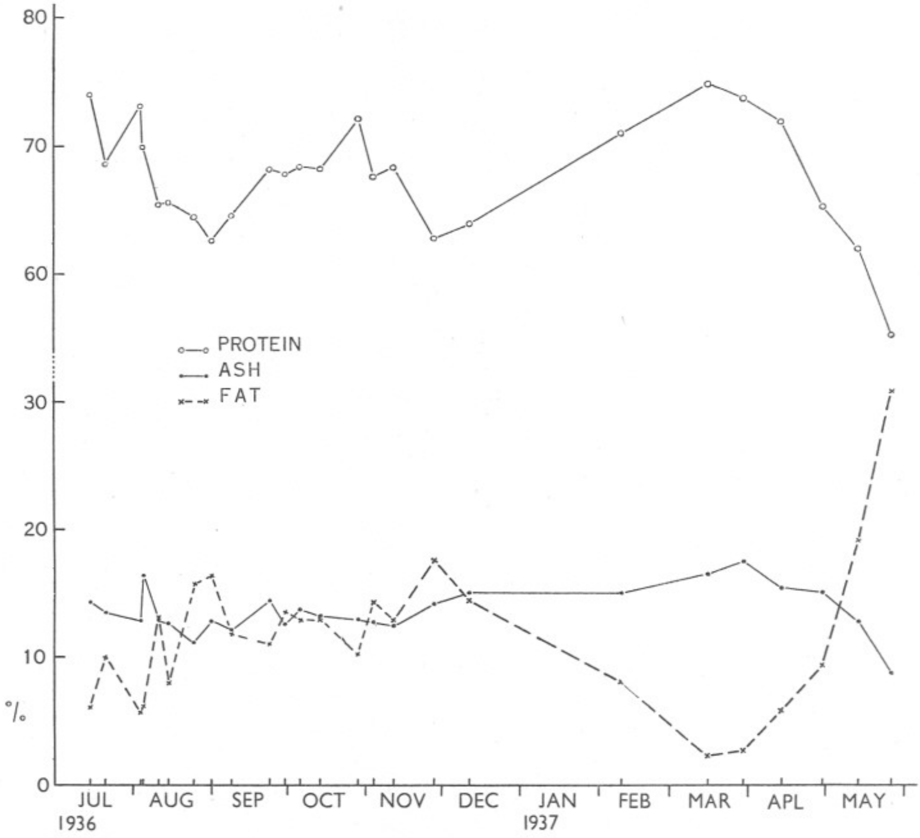

Fig. 8. Percentage chemical composition of inshore herring.

The fat content varied from 2 to 30%. During the growing period it was irregular with a tendency to increase up till the end of November when it was about 17%. This is at least 4 weeks after growth in length had ceased, which suggests that fat was being stored for utilization during the winter. Similarly although fat was increasing from the middle of March onwards, showing that the fish were recuperating, there was no increase in length until May. During the winter only a few analyses were made. By February the fat content had fallen considerably and its lowest value was reached on March 15 when it was about 2%. Thereafter there was a steady and rapid increase up to 30% at the end of May.

The minor irregularities in the fat results may be due to some extent to the size of the fish used in the sample. On August 10 1936, when the size range was considerable, analyses were made on fish of different size ranges. The results (Table V) show that the fat percentage increased with increasing size of fish.

TABLE V. CHEMICAL COMPOSITION

Date	Size mm.	Dry weight		
		Protein %	Fat %	Ash %
14. vii. 36	80–90	74·01	6·15	14·33
20. vii. 36	90–100	68·56	9·94	13·52
3. viii. 36	90–100	73·13	5·69	12·80
4. viii. 36	90–100	69·88	6·15	16·38
10. viii. 36	100–110	65·35	13·16	12·76
10. viii. 36	110–120	61·70	16·72	12·98
*10. viii. 36	120–130	60·30	21·17	12·04
*10. viii. 36	130–140	57·37	25·42	12·65
14. viii. 36	100–110	65·57	7·90	12·63
24. viii. 36	..	64·52	15·71	11·13
31. viii. 36	120–130	62·62	16·41	12·83
8. ix. 36	120–130	64·57	11·81	12·12
23. ix. 36	120–130	68·18	11·04	14·43
29. ix. 36	135–140	67·82	13·55	12·51
5. x. 36	130–140	68·37	12·89	13·70
13. x. 36	130–140	68·21	12·88	13·16
28. x. 36	130–140	72·06	10·18	12·90
3. xi. 36	130–140	67·61	14·35	12·71
11. xi. 36	120–130	68·26	12·88	12·39
27. xi. 36	130–140	62·82	17·61	14·13
11. xii. 36	130–140	63·92	14·48	14·99
9. ii. 37	130–140	70·98	8·10	15·03
15. iii. 37	130–140	74·85	2·36	16·48
29. iii. 37	130–140	73·78	2·75	17·49
13. iv. 37	130–140	71·98	5·86	15·40
29. iv. 37	130–140	65·40	9·38	15·13
13. v. 37	135–145	62·04	19·22	12·77
26. v. 37	160–170	55·32	30·90	8·82

* Offshore, 1-ring herring.

The protein content varied from 55 to 74% and its fluctuations correspond inversely to those of the fat content. As has been mentioned above, the fall in fat content in winter is accompanied by an increase in water content so that the wet weight and weight of protein change comparatively little.

In Table VI and Fig. 9 are shown the calculated weight and composition of the average inshore herring during its first year. The lengths and weights were obtained from the curves in Figs. 3 and 5 with interpolations for the change from summer to winter composition. The chemical composition was calculated from the results given in Table V.

The analyses of the chemical composition of the inshore herring show much the same type of seasonal fluctuation as has been found for herring muscle by Bruce (1924), Channon & el Saby (1932), and by Lovern & Wood (1937). The last authors found a minimum fat content in April of about 2–3 % which is the same as that of the inshore fish in March. This low value is found at the end of the winter period of poor plankton. The maximum value found for fat by Lovern & Wood (over 20 %) is lower than the maximum found in inshore fish (31 %), but agrees in that it occurred at the time when food was abundant both in the plankton (spring) and in the herring guts (Table VII and Fig. 10). Similarly for spring-spawning herring, Lovern & Wood found values generally high during the summer with a slight additional rise in the late autumn (maximum in December). The fat values for inshore fish remained high during the summer and did not fall till December or later. A close comparison of the results obtained on the young Clyde fish (inshore type) with those of other workers is not possible for two reasons; firstly, in the fish analysed in the present report the whole fish was used and not either the muscle or separate organs; secondly, the influence of the state of sexual maturity on the fat content of different tissues of adult herring is important.

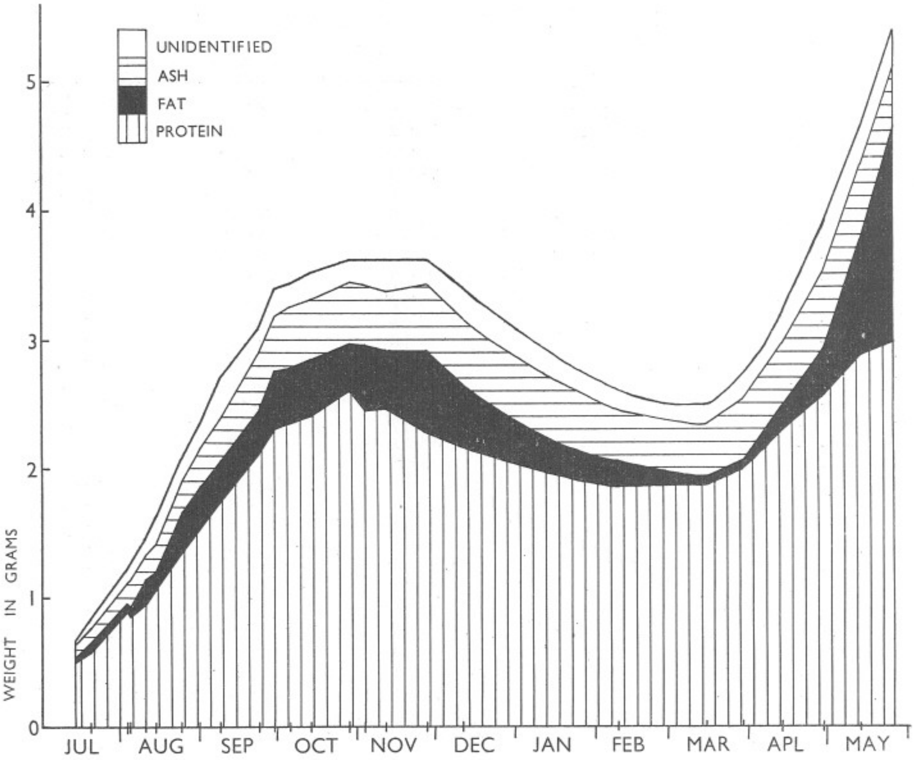

Fig. 9. Dry weight and chemical composition of the average inshore herring.

It would have been desirable to remove the gut contents before analysis, but this omission is likely to have had only a very small effect. In the most striking case where food was abundant and consisted chiefly of *Calanus*, it is possible to calculate the effect of the inclusion of the gut contents. Each fish on May 26 1937 contained on an average almost 2000 *Calanus* and since the composition of *Calanus* is known (Marshall, Nicholls & Orr, 1934) it can be calculated that the gut contents accounted for about 0·06 gm. fat, i.e. only about 1 % of the fat in the fish is represented by the *Calanus* in the gut. At other times the food in the gut is negligible from this aspect.

TABLE VI. COMPOSITION OF AVERAGE HERRING

Date	Av. length mm.	Av. wt. gm.	Protein gm.	Fat gm.	Ash gm.
14. vii. 36	80	0·67	0·50	0·04	0·10
20. vii. 36	86	0·85	0·58	0·08	0·11
3. viii. 36	96	1·22	0·89	0·07	0·16
4. viii. 36	96	1·22	0·85	0·08	0·20
10. viii. 36	101	1·46	0·95	0·19	0·19
14. viii. 36	104	1·63	1·07	0·13	0·21
24. viii. 36	112	2·07	1·34	0·32	0·23
31. viii. 36	116	2·35	1·47	0·39	0·30
8. ix. 36	121	2·70	1·74	0·32	0·33
23. ix. 36	126	3·09	2·11	0·34	0·45
29. ix. 36	129	3·39	2·30	0·46	0·42
5. x. 36	130	3·43	2·34	0·44	0·47
13. x. 36	131	3·52	2·40	0·45	0·46
28. x. 36	132	3·61	2·60	0·37	0·47
3. xi. 36	132	3·61	2·44	0·52	0·46
11. xi. 36	132	3·61	2·46	0·46	0·45
27. xi. 36	132	3·61	2·27	0·64	0·51
11. xii. 36	132	(3·38)	2·16	0·49	0·51
9. ii. 37	132	(2·60)	1·85	0·21	0·39
15. iii. 37	132	2·50	1·87	0·06	0·41
29. iii. 37	132	(2·70)	1·99	0·07	0·47
13. iv. 37	132	(3·18)	2·29	0·19	0·49
29. iv. 37	135	3·91	2·56	0·37	0·59
13. v. 37	142	4·63	2·87	0·89	0·59
26. v. 37	156	5·38	2·98	1·66	0·47

Values in brackets are interpolated.

FOOD AND ITS RELATION TO THE PLANKTON

When a catch of herring was taken, a number of fish was immediately preserved in formalin for food examination. From ten of these the guts were removed, opened, and the contents mixed. The volume of this was measured after settlement and a subsample was then examined and all recognizable organisms counted. When the fish were small or the guts nearly empty, a larger number was used, but the results are always expressed as the number of organisms per fish. They are shown in Table VII and Fig. 10. The numbers of herring used are of course too small for accurate quantitative work, but the results give a good indication of the type of food being eaten.

TABLE VII. FOOD OF INSHORE HERRING

Date	Volume c.c.	*Calanus*		Harpacticoid copepods		Other copepods		Cladocera		Ostracods and cirripedes		Cyphonautes		Molluscan larvae		Other organisms	Total no.	Notes
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
14. vii. 36	..	..	..	2452	90·4	238	9·0	2	0·1	5	0·2	..	..	1	0·1	13	2711	3 amphipods
20. vii. 36	0·5	146	15·1	74	7·6	459	47·5	246	25·4	9	0·9	2	0·2	22	2·3	11	967	..
27. vii. 36	0·4	..	..	71	13·2	213	39·4	102	18·9	8	1·5	64	11·9	70	13·0	12	540	..
3. viii. 36	0·7	4	0·2	9	0·5	988	56·8	699	40·2	13	0·7	9	0·5	14	0·8	2	1738	..
10. viii. 36	1·3	453	31·6	3	0·2	504	35·2	413	28·8	6	0·4	20	1·4	13	0·9	23	1434	12 polychaete larvae
14. viii. 36	0·5	8	1·7	146	30·2	99	20·4	54	11·2	34	7·0	117	24·2	19	3·9	7	484	..
24. viii. 36	0·6	1	0·1	9	0·5	176	9·4	6	0·3	22	1·2	1643	88·2	7	0·4	..	1864	..
31. viii. 36	1·05	1	0·3	3	1·0	207	69·0	11	3·7	4	1·3	64	21·3	1	0·3	8	300	..
8. ix. 36	0·9	1	0·1	7	0·9	592	73·6	9	1·1	5	0·6	114	14·2	15	1·9	61	804	61 polychaete larvae
15. ix. 36	0·7	..	..	2	0·1	165	5·3	11	0·4	12	0·4	2896	93·2	19	0·6	4	3109	..
*21. ix. 36	1·3	635	65·3	..	..	310	31·9	..	..	..	..	..	..	..	..	30	973	24 *Oikopleura*
23. ix. 36	0·55	..	..	3	0·7	405	94·2	6	1·4	1	0·2	9	2·1	1	0·2	5	430	..
29. ix. 36	0·55	..	..	9	2·7	317	95·5	1	0·3	..	..	..	..	..	..	5	332	..
5. x. 36	0·7	27	6·6	1	0·2	324	79·9	2	0·5	2	0·5	47	11·6	1	0·2	2	406	..
13. x. 36	1·1	435	39·6	..	..	467	42·6	18	1·6	6	0·5	3	0·3	1	0·1	168	1097	2 *Zoeae*; 3 *Megalopa*; 2 mysids; 2 *Sagitta*; 155 *Oikopleura*
28. x. 36	0·8	12	2·5	2	0·4	405	89·4	5	1·1	..	..	8	1·8	1	0·2	20	453	4 amphipods; 5 *Zoeae*
3. xi.36	0·6	46	35·7	4	3·1	73	56·6	..	..	1	0·8	1	0·8	2	1·6	2	129	..
13. xi. 36	1·8	575	74·3	..	..	194	25·1	..	..	..	..	..	..	..	..	5	774	1 euphausid; *Sagitta* present
27. xi. 36	0·45	22	38·6	2	3·5	32	56·1	..	..	..	..	..	..	..	..	1	57	..
11. xii. 36	0·2	1	3·7	..	..	24	88·9	..	..	..	..	..	..	..	..	3	27	..
28. xii. 36	0·25	1	2·6	1	2·6	35	92·2	..	..	..	..	..	..	..	..	1	38	1 *Sagitta*
13. i. 37	0·6	28	49·1	..	..	16	28·1	..	..	..	..	..	..	..	..	13	57	11 *Sagitta*; 1 amphipod
9. ii. 37	0·8	66	80·5	2	2·4	9	11·0	..	..	..	..	..	..	..	..	4	82	Remains of fish larva; *Sagitta* present
†1. iii. 37	0·6	40	57·1	1	1·4	9	12·9	..	..	..	..	..	..	..	..	18	70	4 amphipods; 10 *Cumacea*; 1 mysid; remains of fish larva and *Sagitta*
15. iii. 37	0·45	12	27·9	5	11·6	12	27·9	..	..	..	..	..	..	..	..	13	43	8 amphipods; 5 *Cumacea*; remains of *Sagitta*
29. iii. 37	0·5	1	0·8	20	15·9	11	8·7	..	..	83‡	65·9	..	..	..	..	11	126	‡All cirripede nauplii
13. iv. 37	0·8	60	10·0	6	1·0	94	15·7	..	..	426‡	71·2	..	..	1	0·2	10	598	‡215 cirripede nauplii; 211 cyprids
29. iv. 37	0·9	5	0·4	8	0·6	21	1·6	..	..	1286‡	97·4	..	..	..	..	1	1321	‡104 cirripede nauplii; 1182 cyprids
13. v. 37	1·3	317	67·4	3	0·6	124	26·4	4	0·9	14	3·0	..	..	..	..	8	470	..
†26. v. 37	3·75	1856	92·0	9	0·4	132	6·6	11	0·5	2	0·1	..	..	..	..	7	2017	..
27. v. 37	2·0	521	61·6	49	5·8	241	28·5	21	2·5	4	0·5	..	..	..	..	9	845	..
31. v. 27	0·7	26	4·4	347	58·2	113	19·0	75	12·6	3	0·5	..	..	2	0·3	30	596	12 mysids; 12 decapod larvae
11. viii. 37	0·6	1	0·2	1	0·2	627	98·8	..	..	..	..	..	..	..	..	4	634	..

* Drift nets. † Sprat ring net.

During the growing period both in summer 1936 and spring 1937, the gut contents of the inshore fish varied considerably both in volume and composition; in the winter the majority of the guts were almost empty.

For most of the year copepods formed the bulk of the food, but in spring and summer, at times when other organisms were common in the plankton, an individual catch might show these in abundance. Thus during August and September Cyphonautes (polyzoan larvae) were common and on September 15 rose to 93 % of the total numbers. In spite of their large numbers, however, they are relatively unimportant as food because of their small size. On September 15 when 2896 Cyphonautes were present the total volume of the food was only 0·7 c.c. In spring cirripede larvae, both nauplii and cyprids, were abundant and on April 29 formed 97·4 % of the food. Cirripede larvae and Cyphonautes were the most important organisms occurring sporadically, but, as may be seen in Table VII, euphausids, Cladocera, *Sagitta*, and *Oikopleura* all appeared at times. Occasionally a few *Chironomus* larvae were ingested.

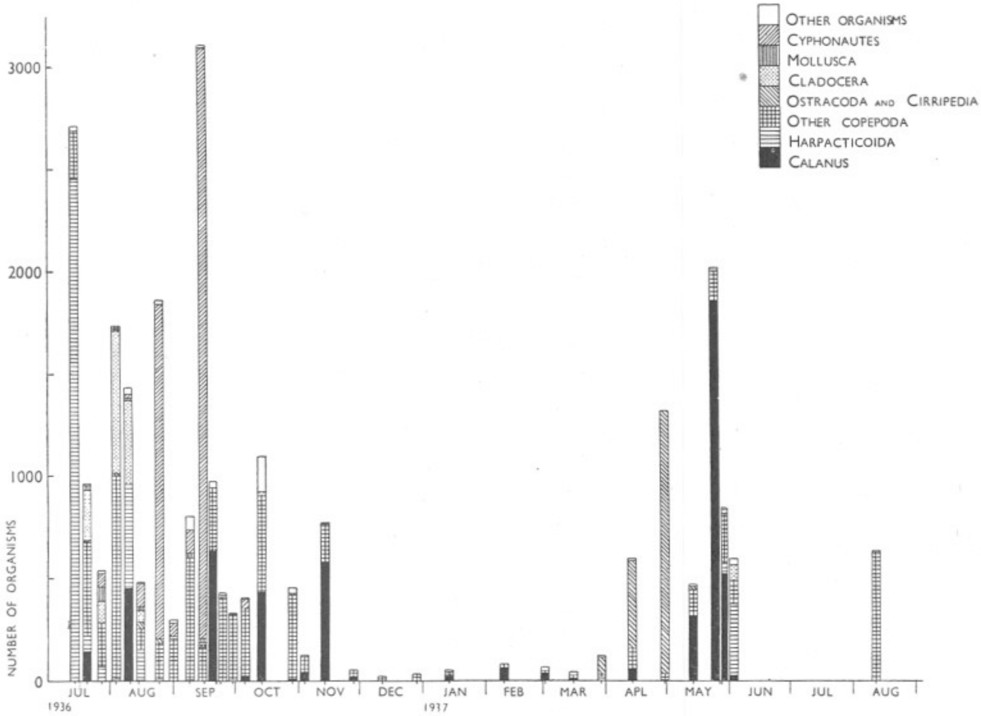

Fig. 10. Composition of the food of inshore herring.

Of the copepods *Calanus*, although not the most numerous, was certainly the most important owing to its large size. In Fig. 11 which shows the volume of the gut contents each of the peaks, with the exception of that on August 31, is due to the presence of *Calanus*. In May when the herring were increasing rapidly in weight and fat content, they were feeding mainly on *Calanus* and on May 26 there were 1856 *Calanus* per gut. The other copepods were most numerous from July to October, *Pseudocalanus*, *Microcalanus*, and *Acartia* appearing for longer than *Centropages*, *Temora*, and *Oithona*. All disappeared almost completely during the winter to reappear again in May. In July to August 1936 and in May 1937 harpacticoid copepods were common and on one occasion, July 14, each gut contained on the average 2452, forming 90% by number of the food organisms. They were mostly littoral forms, e.g. *Dactylopusia* and other thalestrids, showing that the herring were feeding inshore.

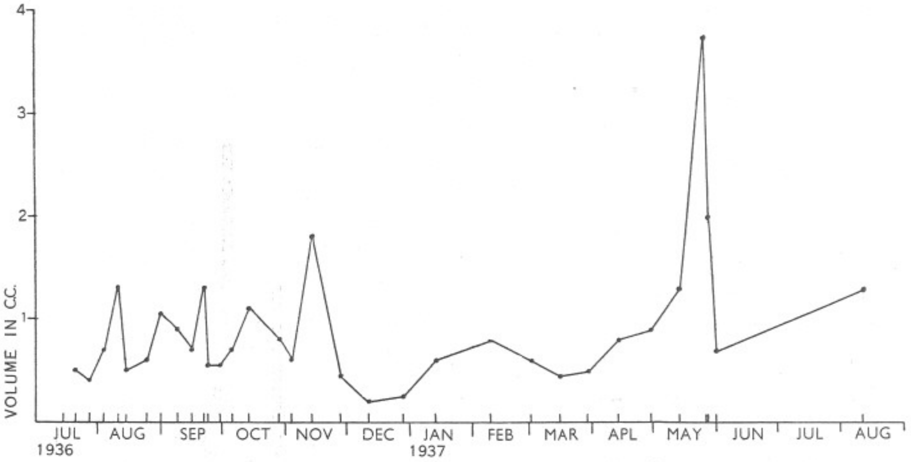

Fig. 11. Volume of the food of inshore herring.

On August 14–15 when herring were caught at 2 hr. intervals during the night, a sample of the guts was examined from each catch (Table VIII and Fig. 12). The volume of the food was never large but was highest from 7–11 p.m. and lowest from 1–5 a.m. Copepods were the most numerous organisms in the catch at 5 p.m. when there were over 1000 harpacticoids per gut. In the catches from 7–11 p.m. *Centropages*, *Temora*, Cladocera, cirripede larvae, and Cyphonautes formed the bulk of the food, as they did also in the later catches, although very much less numerous. It was hoped to find out if the fish came inshore to feed and at what time during the night they fed most, but from this point of view the results were disappointing. The harpacticoids in the first catch indicate that they had been feeding inshore, but in this, as in all catches, most of the food was in the intestine and little in the stomach which suggests that they were not feeding at the time they were caught.

A comparison of the volume of the gut contents with the growth curve for the herring and their chemical composition shows general agreement (Figs. 11, 3 and 8). When the volume of the food was high during the summer of 1936 and the spring of 1937, the herring were growing. During the winter period when food was scarce, growth had stopped and the fish were losing fat. Although growth had stopped by the middle of October, the volume of the food was still considerable and it has been shown that it was being used partly for fat storage; similarly the food showed an increase in spring some time before any increase in length began, correlated with the replacement of fat lost in winter.

Only six samples of offshore (Clyde spring-spawned) fish were examined and they showed little difference from the inshore fish in their food except that there was a reduction in the number of harpacticoid copepods.

TABLE VIII. FOOD OF HERRING DURING NIGHT OF AUGUST 14–15 1936

Time	No. of herring	Volume of food in c.c.	*Calanus* no.	Harpacticoid copepods no.	Other copepods no.	Cladocera no.	Ostracods and cirripedes no.	Cyphonautes no.	Molluscan larvae no.	Other organisms no.	Total no.
5 p.m.	132	0·5	..	1063	203	103	124	438	25	14	1970
7 p.m.	4500	0·7	43	9	144	140	53	65	45	10	509
9 p.m.	> 10000	0·7	4	3	192	114	33	30	16	12	404
11 p.m.	450	0·7	7	21	97	40	22	340	10	9	546
1 a.m.	10	0·3	..	34	29	14	20	11	14	7	129
3 a.m.	12	0·3	..	31	45	6	11	23	17	5	138
5 a.m.	293	0·25	..	1	8	1	..	9	4	..	23
7 a.m.	6	0·45	13	8	71	17	5	20	15	5	154

When a catch of herring was made, a tow-netting was taken in the same place. A 5 minute haul was made with a 40 cm. fine silk net at about 1 m. below the surface and all organisms counted after preservation in formalin. The results are given in Table IX, in which the organisms are grouped as in Table VII to assist comparison. The figures for August 14 1936 are averages based on all the hauls taken during the night of August 14–15. The object was to compare the plankton with the food in the herring guts.

As might be expected, the plankton hauls were poor during the winter period and rich during the spring and summer but, apart from this general relationship, the comparison with the contents of the herring guts is disappointing. The reason for the poor agreement between plankton hauls and gut contents is probably that on the majority of occasions the herring had been feeding offshore, whereas the tow-nettings were taken inshore where the herring were actually captured.

The most striking agreement was from March 29 to April 29 when cirripede larvae, first as nauplii and then as cyprids, were abundant both in the tow-nettings and in the food. *Oikopleura* was common in both on September 21 and October 13. Cladocera were represented seasonally in the guts according to their appearance in the plankton, as was also Cyphonautes. With animals like the last, which occur in swarms, it is largely a matter of chance whether they are richly represented in the catch or not. Although Cyphonautes was

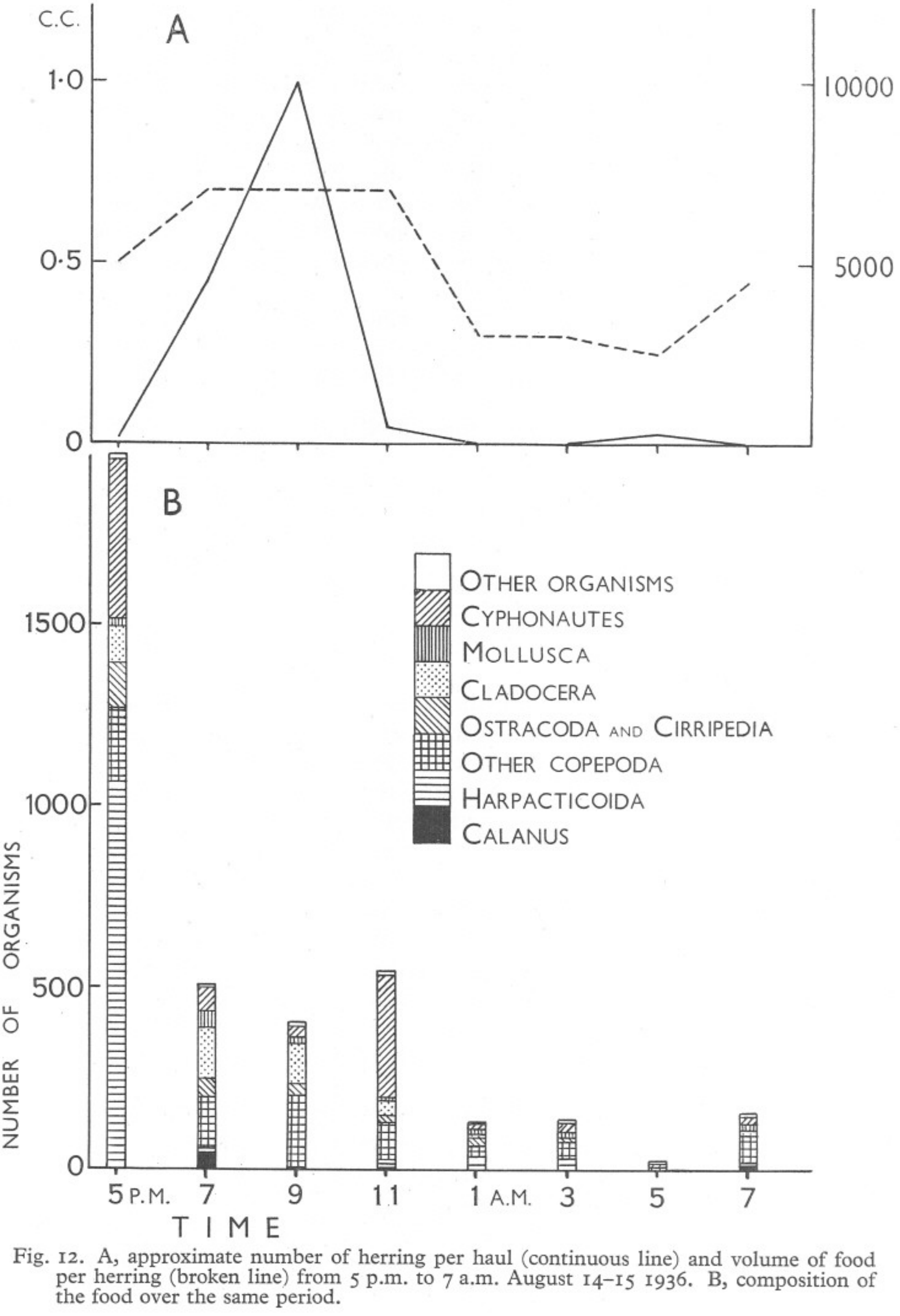

Fig. 12. A, approximate number of herring per haul (continuous line) and volume of food per herring (broken line) from 5 p.m. to 7 a.m. August 14–15 1936. B, composition of the food over the same period.

TABLE IX. PLANKTON

Date	*Calanus*		Harpacticoid copepods		Other copepods		Cladocera		Ostracods and cirripedes		Cyphonautes		Molluscan larvae		Other organisms		Total no.	Notes
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
†18. vii. 36	160	2·0	160	2·0	5400	68·5	460	5·8	110	1·4	10	0·1	540	6·9	1040	13·2	7880	670 polychaete larvae; no amphipods
28. vii. 36	..	..	180	1·7	3990	38·2	90	0·9	..	..	390	3·7	4440	42·5	1350	12·9	10440	900 echinoderm larvae; 450 polychaete larvae
4. viii. 36	..	..	280	3·5	3000	37·1	3080	38·1	80	9·9	200	2·5	730	9·0	720	8·9	8090	380 polychaete larvae
10. viii. 36	..	..	1050	17·0	4150	67·4	150	2·4	210	3·4	70	1·1	100	1·6	430	7·0	6160	290 polychaete larvae
14. viii. 36	2	0·0	163	1·7	4214	43·6	464	4·8	307	3·2	1161	12·0	3019	31·2	334	3·5	9662	..
19. viii. 36	10	0·8	320	26·2	140	11·5	..	..	30	2·5	10	0·8	400	32·8	310	25·4	1220	140 polychaete larvae
24. viii. 36	..	..	130	3·0	2985	69·7	30	0·7	145	3·4	80	1·9	545	12·7	365	8·5	4280	230 polychaete larvae
31. viii. 36	4	0·3	70	5·2	470	35·0	78	5·8	4	0·3	8	0·6	88	6·6	622	46·3	1344	550 *Oikopleura*
8. ix. 36	..	..	90	1·9	3590	77·7	40	0·9	40	0·9	150	3·2	420	9·1	290	6·3	4620	170 polychaete larvae
15. ix. 36	10	0·3	55	1·5	2735	75·3	15	0·4	10	0·3	140	3·9	125	3·4	540	14·9	3630	215 *Oikopleura*
23. ix. 36	40	0·1	60	0·1	9940	22·6	40	0·1	20	0·0	60	0·1	3740	8·5	30060	68·4	43960	28000 Rotifers; 1460 *Oikopleura*
29. ix. 36	10	0·1	440	4·8	4760	52·0	20	0·2	..	..	..	..	2590	28·3	1328	14·5	9148	420 *Cumacea*; 360 *Oikopleura*; 350 polychaete larvae
5. x. 36	..	..	580	17·3	2590	77·3	..	..	50	1·5	..	..	80	2·4	50	1·5	3350	..
14. x. 36	..	..	600	4·2	6420	44·9	270	1·9	..	..	..	..	390	2·7	6600	46·2	14280	5100 Rotifers; 600 Turbellaria; 390 *Oikopleura*; 390 polychaete larvae; no decapods, mysids nor *Sagitta*
28. x. 36	..	..	310	6·4	3420	70·5	10	0·2	10	0·2	100	2·1	450	9·3	550	11·3	4850	240 polychaete larvae; no amphipods nor decapods
3. xi. 36	..	..	60	2·1	1600	55·1	..	..	1	0·0	15	0·5	255	8·8	971	33·5	2902	610 polychaete larvae
11. xi. 36	3	0·1	45	1·2	2410	61·7	..	..	..	..	10	0·3	1015	26·1	408	10·5	3891	370 polychaete larvae; 10 *Sagitta*; no euphausids
27. xi. 36	20	0·6	65	1·9	2470	70·5	5	0·1	5	0·1	..	..	760	21·7	180	5·1	3505	90 polychaete larvae
11. xii. 36	..	..	2	0·9	194	88·9	..	..	..	..	..	..	..	..	22	10·1	218	16 *Cumacea*
28. xii. 36	6	0·4	30	2·0	1274	85·0	..	..	..	..	2	0·1	142	9·5	44	2·9	1498	34 polychaete larvae; 2 *Sagitta*
13. i. 37	5	1·6	17	5·3	122	38·2	..	..	..	..	..	..	49	15·4	126	39·5	319	93 Rotifers; 5 amphipods; no *Sagitta*
9. ii. 37	10	5·8	6	3·5	67	38·7	..	..	2	1·2	..	..	57	33·0	31	17·9	173	No fish larvae nor *Sagitta*
15. iii. 37	..	..	90	9·6	342	36·4	..	..	326*	34·7	4	0·4	94	10·0	84	9·0	940	*324 cirripede larvae; 64 polychaete larvae; amphipods, *Cumacea* and *Sagitta* present
29. iii. 37	2	0·3	10	1·5	70	10·4	..	..	501*	74·2	..	..	18	2·7	74	11·0	675	*All cirripede nauplii; 66 polychaete larvae
†13. iv. 37	6340	25·6	..	..	3640	14·7	60	0·2	12340*	49·7	..	..	60	0·2	2360	9·5	24800	*All cirripedes, mainly nauplii; 1680 *Oikopleura*
29. iv. 37	54	3·6	105	7·0	66	4·4	16	1·1	1074*	72·0	..	..	3	0·2	174	11·7	1492	*All cirripedes, mainly cyprids; 77 polychaete larvae
13. v. 37	202	4·8	82	2·0	1066	25·5	1334	31·9	712	17·0	4	0·1	332	7·9	456	10·9	4188	252 euphausid larvae
27. v. 37	6	0·4	312	19·8	548	34·8	290	18·4	30	1·9	..	..	353*	22·4	34	2·2	1573	*Mainly *Littorina* eggs

† Offshore hauls.

not obtained on the dates they occurred in the herring guts, additional tow-nettings taken in Kames Bay about the same time showed that they were abundant. Among the herring also not all the guts contained Cyphonautes, but in four out of ten the intestine was packed with them.

The most marked disagreement is in the numbers of *Calanus*, which was frequently common in the guts and quite absent from the plankton hauls. This suggests that the herring had been feeding offshore before they were caught although the presence of typical littoral plankton, e.g. harpacticoids, shows that they fed inshore as well. On only one occasion was *Calanus* abundant in the tow-nettings and that was when the haul was taken further off shore than usual.

Much has been written on the food of the herring, but it has been mainly concerned with adult fish. In general it is agreed that herring on the west coast of Scotland depend more upon copepods and euphausids than do those on the east coast (Scott, 1907) which have a larger proportion of young fish (mainly *Ammodytes*) in their diet (Hardy, 1924; Savage, 1937). The food of young herring about 50–150 mm. in length is dealt with in papers by Hardy (1924), Jespersen (1928), Wailes (1936), and Battle *et al.* (1936).

In general our results are in good agreement with theirs. Copepods form the chief part of the diet and other organisms appear seasonally. Hardy, (1924) dealing with whitebait herring in the Thames estuary, found copepods, chiefly *Pseudocalanus*, *Temora*, and *Eurytemora* common, harpacticoids numerous at times, and cyprid larvae of cirripedes important in spring and autumn. Ogilvie (1934) mentions that harpacticoids, though rare in adult herring, are frequent in young herring. Jespersen's (1928) young herring of 6–14 cm. were caught only in the winter half-year but they were then feeding voraciously, mainly on copepods (*Temora*, *Pseudocalanus* and *Paracalanus*). He found that non-crustaceous food was more important for young than for adult fish. Cyphonautes, however, was rarely taken although common in the plankton from February to March, which contrasts with our results. Another contrast is that his herring (adults in this case) appeared to select cirripede cyprids even when the nauplii were more abundant, whereas ours took both in large quantities according to their abundance in the plankton.

Only a few records are available of the fluctuations in the gut contents over the day. Jespersen (1928) compared the quantity of food and the numbers of copepods per fish in samples of young herring from the west coast of Denmark and found that feeding decreased during the night, i.e. from 10 p.m. till 10 a.m. Lissner (1925) dealing with adult herring in the North Sea concluded that they are attracted by a weak light, begin to feed in the afternoon and stop towards morning. Mužinić (1931) found that feeding was at its maximum from about 5 p.m. to 9 p.m. and then slackened off till 4 or 5 a.m. when there was a slight increase. This compares well with the feeding of the inshore herring from the Clyde (Fig. 12 A). Battle *et al.* (1936) state that young herring require a good light to feed by and that although moonlight is bright enough, starlight is not. For this reason the plankton present in the surface waters is most important for them.

We wish to thank the staff of the laboratory for their co-operation in the work. We are indebted to the Fishery Board for Scotland for the loan of a shore seine and for the herring reports obtained through their Fishery Officers in the Clyde; also to the Fisheries Laboratory, Lowestoft, for the loan of a shore seine and drift nets.

SUMMARY

Two groups of young herring are found in the Clyde. One, the offshore group, has been identified as Clyde spring-spawned herring; the other, the inshore type, is of unknown origin. The former were caught only occasionally, the latter at fairly regular intervals during their first year.

The two groups are distinguished by differences in size and identified as belonging to different races by vertebral counts. There was no intermixture.

The offshore herring which metamorphosed at the end of May when 40–50 mm. long, reached a length of 90–100 mm. in winter. The inshore fish which were about 50 mm. long in May reached a length of about 130 mm. in winter.

Equations are given showing the relation between length and weight for inshore and offshore herring during the growing and non-growing periods.

Determinations were made of water, fat, protein and ash content of inshore herring. The fat content rises during the summer and falls in winter. It varies inversely with the water content. The fat content continues to increase for some weeks after growth in length has stopped in winter and rises also in spring some time before increase in length begins again.

The food was examined throughout the year and compared with plankton hauls. It consisted mainly of copepods although other organisms were common at times when abundant in the plankton.

A series of hauls made over a night showed that herring were most abundant inshore at dusk and dawn and contained most food from 7–11 p.m.

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THE SWIMMING AND BURROWING HABITS OF SOME SPECIES OF THE AMPHIPOD GENUS *BATHYPOREIA*

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(Text-figs. 1–2)

CONTENTS

Introduction

The amphipod family Haustoriidae is represented in the coastal waters of Great Britain by three genera, *Haustorius*, *Urothoë* and *Bathyporeia*, all three of which are characterized by the habit of burrowing into the soil of the sea floor. Dennell (1933) deals at some length with the swimming and burrowing habits of *Haustorius arenarius* in which he shows that (p. 387) "the animal swims on its back by the beating of the powerful pleopods... burrowing is a modification of the swimming movements, and is dependent on the expulsive action of the swimming current". Crawford (1937), referring to the burrowing Amphipoda of certain soils of the sea bottom near Plymouth, states (p. 638), "The local Haustoriidae burrow almost entirely by ejecting sand grains in a powerful swimming current", and in giving some details of the burrowing of *Urothoë brevicornis* and *U. grimaldii* var. *poseidonis* states that it is very similar to that of *Haustorius*. Schellenberg (1929), in dealing with the burrowing habits of some amphipods, gives an outline description of the process in *Bathyporeia robertsoni* Bate (probably *B. sarsi* Watkin, 1938). The author in this paper shows that the swimming and burrowing habits of four species of *Bathyporeia*, namely, *guilliamsoniana* (Bate), *elegans* Watkin, *pelagica* (Bate) and *pilosa* Lindström, are alike and similar to that of *B. robertsoni* as described by Schellenberg, but the process is described in much further detail. The burrowing habit differs considerably from that described by Dennell in *Haustorius*, in that it is dependent upon the sweeping action of the second gnathopods and first and second peraeopods aided by the first and second uropods; the swimming current takes no part in the process. The method of burrowing is more similar to that of *Ampelisca* which Crawford

(1937) states (p. 638) "is not dependent on a flow of water set up by the pleopods but is performed by the scraping and hauling of the gnathopods, aided by the urosome as soon as it gets a purchase".

SWIMMING HABIT

Movement has been observed in the four species *guilliamsoniana* (Bate), *elegans* Watkin, *pelagica* (Bate) and *pilosa* Lindström. No differences between the movement of the four species were observed. There are two types of movement, swimming and burrowing, which may follow each other in rapid succession. Swimming movements are effected by the pleopods. During this type of movement the body is held rigid and fully extended, the flagella of the antennules and antennae are laid back alongside the body, the pleon is fully extended which brings the first and second uropods close up beneath it, the third uropods depressed and in line with the pleon and converged in the median line (Fig. 2*a*). Thus a definite "stream-lining" effect is obtained.

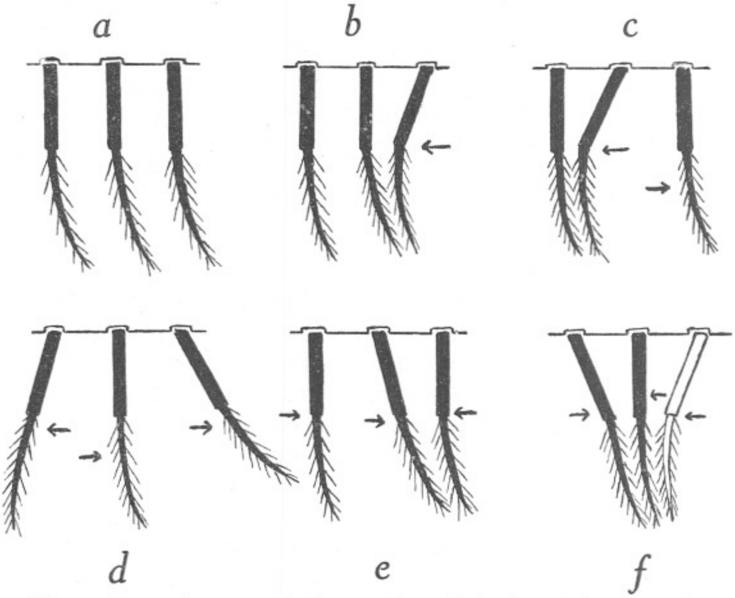

Fig. 1. Successive stages in the metachronal rhythm of the pleopods.

Pleopod movement is of the metachronal type. At rest the three pairs of pleopods are held hanging vertical to the pleon, and each pair is capable of a forward and backward movement from the rest position. Fig. 1*a* shows diagrammatically the rest position. Swimming commences with a forward movement of the third pair (*b*). The second pair now begin their forward movement and the third pair begin their backward movement and are now in the vertical position thus an inter-limb space is created between the second and third pairs and water flows in to fill this space (*c*). The first pair now commence their forward movement, the second pair have moved back to the vertical position, whilst the third pair are at the end of their backward stroke (*d*). Thus an inter-limb space is created between the first and second pairs and water flows in to fill this space. The first pair now begin the backward movement, the second pair have completed the backward stroke and the third pair are recovering to the rest position (*e*). Thus water is forced out between the second and third pairs. The completion of the beat is shown in (*f*) in which the first pair are at the end of their backward stroke, the second pair recovered to the rest position, whilst the third pair are moving forward for the next beat. Thus water is forced out from between the first and second pairs. Successive beats follow each other rapidly and the animal is driven forward through the water.

The rami of the pleopods are fringed with long feathered bristles (Fig. 2*g*). The bristles of the proximal end of the outer ramus are shorter and point outwards, those of the proximal end of the inner ramus are modified to serve as coupling hooks. The latter bristles have bifid tips with spear-shaped ends (g_2) and between the bifid tip the corresponding bristles of the opposite pleopod fit. The interlocking bristles can move freely within each other but cannot be withdrawn, due to the spear-shaped tips. The pleopods of a pair are further linked together by coupling hooks on the distal end of the basal joint (g_1). There are normally two coupling hooks on each basal joint, each with three recurved hooks into which the corresponding hooks of the opposite pleopod fit.

As a result of swimming movements currents of water are drawn in between the appendages and thus over the gills. The joints of some of the appendages are expanded and modified to form a ventral groove in which the gills are protected and in which in the female the brood pouch is formed. Anteriorly this groove is formed by the large coxal plates of the second peraeopods, the coxal plates of the second gnathopod and the first peraeopod lie alongside the mouthparts and are protective to them. Thus the ventral groove begins in the region of the second peraeon segment. The coxal plates of the third, fourth and fifth peraeopods are small and take no part in the formation of the ventral groove; their reduction is compensated by the expansion of some of the joints of these appendages. Peraeopod 3 is a doubly geniculate appendage, the basis is expanded and lies normally beneath its segment, the ischium is small and the large expanded merus is held at an angle of less than 90° to the basis and thus comes to lie partly at the side and partly posterior to it. The expanded basis of peraeopod 4 continues the side of the groove and it is completed by the basis of peraeopod 5 which is expanded on its posterior margin only. Thus the ventral groove is a feature of the peraeon only.

The first epimeral plate of the pleon is small; between it and the basis of peraeopod 5 is a space through which the water from the ventral groove passes out. The second and third epimeral plates are well developed, the

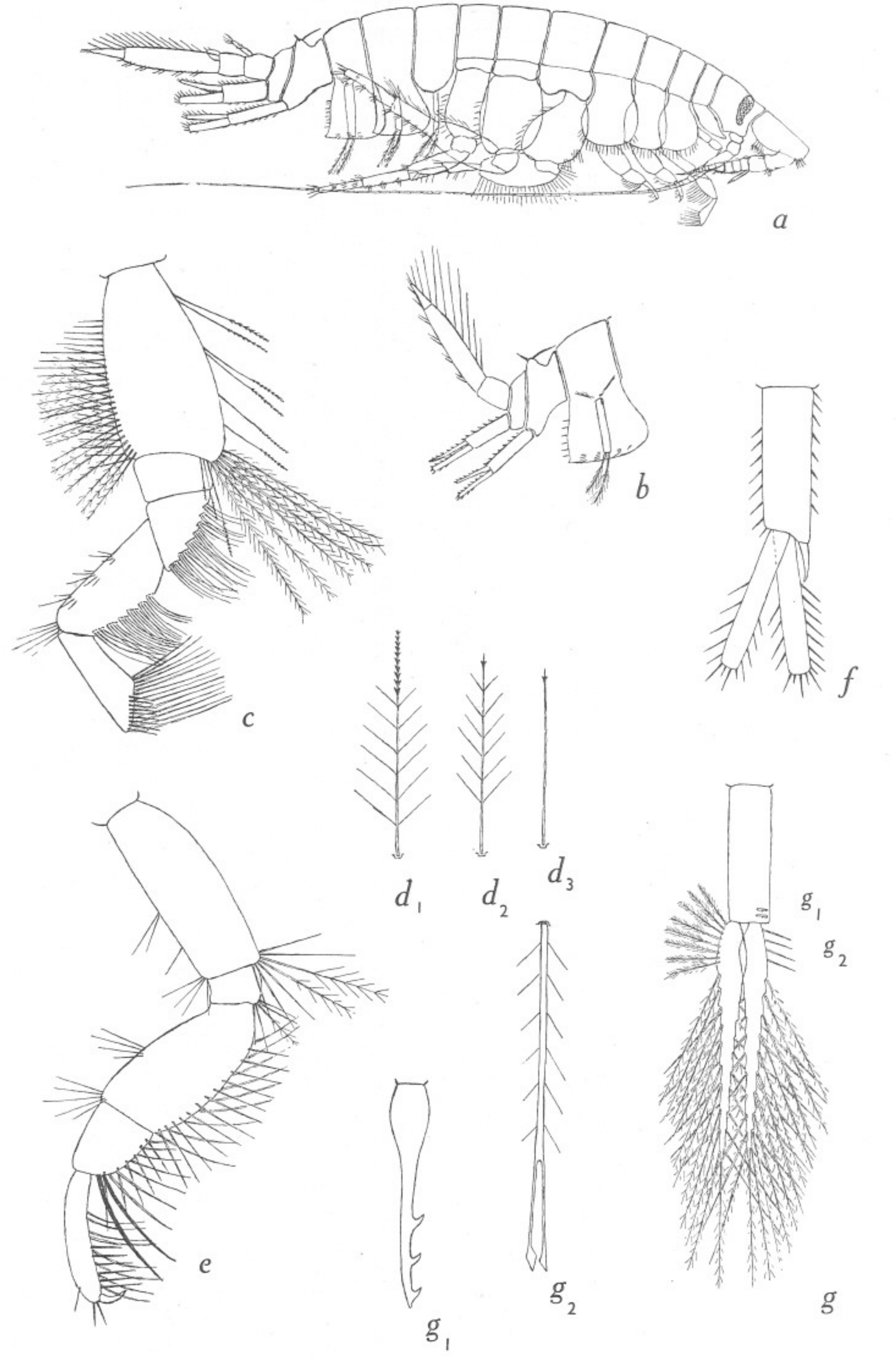

Fig. 2. Drawings based on *Bathyporeia guilliamsoniana* (Bate): *a*, animal in the swimming position; *b*, urosome in the burrowing position; *c*, second gnathopod; d_1, d_2, d_3, armature of bristles on anterior border of merus and carpus of the second gnathopod; *e*, first peraeopod; *f*, first uropod; *g*, pleopod; g_1, coupling hook on basal joint; g_2, modified feathered bristle from base of inner margin of inner ramus.

second is convex anteriorly and concave posteriorly, the third typically rounded at both ends. Between the epimeral plates the pleopods work in unison to draw water through the ventral groove, and the urosome bends forward to push sand particles backwards beyond the animal.

Two main water currents are drawn into the ventral groove. The main current enters anteriorly between the second gnathopods. This current passes in well beneath the mouthparts; there is no evidence that any part of it is deflected on to the mouthparts and thus no evidence of feeding on small particles held in suspension in the water current. The entrance to the ventral groove is guarded by a series of long heavily feathered bristles which arise from the distal end of the basis of the second gnathopod; they form an effective filter for the larger particles suspended in the water current. A subsidiary water current is drawn in between the anteriorly directed second peraeopod and the backwardly directed third peraeopod. This opening is guarded by an extensive development of feathered bristles along the anterior margin of the basis, ischium and merus of peraeopod 3, and the water current is thus filtered of the larger particles in suspension. The feathered bristles are directed inwards, and although those of the opposite sides do not meet they form an effective filter for practically all the water that enters at this point.

BURROWING HABIT

The second type of movement is that of burrowing into the sand which is the natural home of the species in this genus. Evidence is now available to show that the various species feed by cleaning sand particles and that swimming is a diversion probably connected mainly with reproduction when the tide covers them. They thus resemble in their habits certain cumaceans (Foxon, 1936) which live in the same type of habitat, and in some localities, e.g. Kames Bay, Millport, may be found in the same samples.

It has been shown that in the swimming movement the body is held rigid and fully extended with the pleon in line with the rest of the body, the first and second uropods laid back beneath the urosome, the third uropods extended and in line with the pleon and with their right and left rami converged to the middle line. The rami can be easily diverged from each other and it is suggested, although proof is difficult to obtain, that they may act as steering organs. The swimming movement eventually carries the animal head first into the sand when swimming ceases and burrowing begins. Penetration of the sand is assisted by the form of the antennule. The basal joint is large and extends forward in direct line with the head, with its proximal end broad and narrowing to the distal end, which thus is spear-shaped when viewed from above and acts as a ploughshare to penetrate the sand. The distal end of this basal joint carries a group of spines which point outwards in the direction in which the animal enters the sand. The second joint arises from the underside of the basal joint, and the whole limb beyond the basal joint can be laid back alongside the body. Similarly, the antenna can be laid back alongside the body, so that the anterior end of the body is triangular when viewed from the side and above, making rapid penetration of the sand an easier matter. The force of the swimming movement is sufficient to carry the head end into the sand and thus to bring the sand within reach of the burrowing appendages, which are the second gnathopods and first and second peraeopods. These three pairs of appendages are so arranged that the second gnathopods lie parallel with each other in the median plane; the first peraeopods diverge somewhat and come to lie outside and somewhat posterior to the second gnathopods; the second peraeopods also diverge and come to lie outside and somewhat posterior to the first peraeopods. The first gnathopods are small and are used as feeding appendages and are intimately associated with the mouthparts; thus the area beneath the head is free for the action of the burrowing limbs, which are flexed at the ischium and can be projected forward and drawn back with rapidity. The second gnathopods can extend forward to beneath the antennules; they sweep the sand particles backwards to within reach of the first and second peraeopods, which sweep the sand backwards towards the posterior end of the animal where it comes within reach of the first and second uropods. The three anterior burrowing limbs do not work in unison, the second gnathopod has a more rapid action than the peraeopods, all three, however, take a deep backward sweep before being drawn upwards and pushed forward for the next sweep.

Associated with the second gnathopods and first and second peraeopods in the burrowing movement are the first and second uropods. They, by means of flexion and extension of the pleon, sweep sand particles backwards beyond the animal. The pleon is flexed between the third and fourth segment. As a result of the flexion of the pleon the first and second uropods are carried forward, but the more delicate third uropods are raised into a vertical plane and are thus removed from the sphere of action of the first and second uropods (Fig. 1 *b*). The outer rami only of the third uropods are well developed and their inner margin is fringed with an edge row of long feathered bristles. The rami can be diverged from each other, and when held in the vertical position on the flexion of the pleon they are always widely separated. They thus act as a brake on the rapidity of the forward movement.

It is instructive to observe the animal attempting to burrow in very shallow sand in a dish, thus imitating and prolonging the first movements in the burrowing process. The abdomen is held in extension and sand particles are shot backwards beyond the animal from the second gnathopods and first and second peraeopods, being caught in the water issuing in the swimming movement from the ventral groove. This movement continues until full swimming in the water recommences. When the animal enters sand sufficiently deep to cover the body the first sweeps of the anterior burrowing appendages cause the sand particles to be flung back in the same way, but as the weight of sand to be moved becomes greater the rate of movement is reduced, the pleon is flexed, the first and second uropods come into service and the third uropods are raised, further reducing the speed of forward movement which now becomes a crawl through the sand. When the animal is placed on dry sand no attempt is made to burrow, but when the sand is damped but not covered with water penetration can be effected. On substituting mud for the sand no attempt is made to burrow, the animal swims round spasmodically and mud particles accumulate on the appendages.

The first and second uropods are robust appendages with the two rami of about equal length with no linking apparatus between the basal joints of a pair. The rami usually converge, but in the backward sweep, i.e. pleon extension, they diverge and thus cover a greater area, the arrangement of spines on their margins closing the gap between them. On the flexion of the pleon they converge and can thus be drawn back freely. The first uropods are longer than the second pair and work to the outside of them, thus sweeping sand away from underneath the whole width of the pleon.

The function of the third, fourth and fifth peraeopods in the burrowing process is somewhat doubtful, although some of their functions seem clearly indicated. It has already been shown that the basis of the three limbs and the merus of peraeopod 3 take part in the formation of the ventral groove. The merus of peraeopod 3 is fringed on its outer face near the anterior border with a row of spines which project outwards at right angles to the face of the joint. Their function is to prevent sand particles falling from above on to the edge row of feathered bristles of the merus which filters the water current entering at this point. The carpus and propodus of peraeopod 3 are reduced, geniculate to the merus and held alongside the body outside the expanded basis of peraeopod 4. The outer edge of the propodus carries a row of spines held at right angles to the outer face of the joint, and thus they also prevent sand particles from falling from above. The basis of peraeopod 4 is held normally beneath its segment, but from the ischium the limb is flexed to be held alongside the body outside peraeopod 5 and pointing dorsally and posteriorly, and diverging outwards. It is capable of a slight up-and-down and back-and-fore movement. Its main function seems to be the prevention of sand particles from falling from above; the inner face of the joint carries a group of spines and bristles which extend across the space between it and the body. It may assist burrowing by a forward push against the sand particles. Peraeopod 5 is also flexed at the ischium and is held pointing downwards and backwards. In its armature it carries a less number of bristles but a greater number of spines. This appendage from its position cannot prevent sand particles from falling from above; it may prevent sand particles falling inwards from the sides or possibly help to push the animal forward through the sand.

A detailed description of the second gnathopod, first and second peraeopods and first and second uropods will assist an understanding of the way in which these appendages are modified to serve the burrowing process.

Second gnathopod (Fig. 2*c*). The basis is rectangular in outline; the anterior edge carries a row of simple bristles, the outer face a row of heavily feathered bristles, the inner face a row of serrated bristles, the posterior edge a few long serrated bristles. The distal edge of this joint carries a row of long feathered bristles, which together with those of the opposite limb form an effective filter to prevent large particles entering the ventral groove along with the main water current which enters through them. The ischium is small and square with a tuft of feathered bristles and simple bristles on its distal posterior edge; the feathered bristles assist in filtering the water current. The merus, carpus and propodus project forwards. The merus is triangular with up to seven groups of bristles on its posterior margin, each group is horseshoe-shaped, the end bristles of each group are simple bristles (d_3), the centre ones serrated feathered bristles (d_1) with feathered bristles in between (d_2). The carpus has a curved posterior edge which carries up to seven groups of bristles as on the merus. The anterior edge of the carpus carries about four groups of simple bristles. The propodus is spatulate with the ventral surface deeply grooved; the edges of the groove carry a row of well-developed spines which are fundamentally arranged on the same horseshoe-shaped pattern as on the merus and carpus, but the rows are broken by the hollow ventral surface. On the outer edge at the distal end is a row of about twelve stout spines. The bristles on the merus, carpus and propodus are curved backwards to assist in the process of sweeping the sand particles. The dactylus is absent.

The *first peraeopod* (Fig. 2*e*) is stoutly built with the basis rectangular with a few serrated bristles and ordinary bristles on the anterior margin near the base and with a few feathered bristles and ordinary bristles near the apex. The feathered bristles assist in filtering the water current entering the ventral groove. The ischium is small and square. The merus and carpus are broad with an inner face row and outer face row of simple bristles on the anterior margin. The distal end of the anterior margin of the carpus bears a pair of much stronger spines with two other spines not so well developed. These strong spines are somewhat hooked and are the main sweeping structures on this appendage. The merus and carpus are somewhat geniculate to the ischium. The propodus is smaller, not swollen, with the anterior edge carrying a row of spines and the outer and inner faces with a row of bristles. The dactylus is claw-like. The inner and outer face rows of bristles on the merus, carpus and propodus extend laterally to aid the sweeping movement. The absence of heavily feathered bristles show this appendage to be mainly sweeping in function and not assisting to any extent in filtering the water current entering the ventral groove.

The *second peraeopod* is built on the same plan as the first with the same armature; the only comment necessary is that the spines of the edge row on the propodus are stronger.

The *first* and *second uropods* (Fig. 2*f*) are stoutly built with the basal joint carrying a row of small spines on outer and inner edges. The distal end of the outer edge carries a stout spine. The inner and outer rami are of equal length and the inner arises immediately dorsal to the outer. The inner and outer rami carry a row of stout spines along their inner and outer edges and around the apex. The ventral surface carries a few scattered bristles.

This work was in part carried out at the Laboratory of the Scottish Marine Biological Association, Millport. My thanks are due to the Director, Mr Richard Elmhirst, for the facilities afforded me there.

SUMMARY

The swimming and burrowing habits of four species of the genus *Bathyporeia* have been examined and shown to be alike, and similar to *B. robertsoni* as described by Schellenberg.

Swimming is effected by the pleopods working in a metachronal rhythm, drawing water along a ventral groove formed by the coxal plates of the second peraeopods and the expanded bases of peraeopods 3, 4 and 5.

The main water current enters anteriorly between the second gnathopods and is filtered by a series of feathered bristles borne on the basis of this appendage; a subsidiary current enters between the second and third peraeopods.

Burrowing is effected mainly by the sweeping action of the second gnathopods and first two pairs of peraeopods, aided by the first and second uropods as soon as they can obtain a purchase on the sand. The adaptation of these three pairs of appendages to burrowing is explained.

The method of burrowing is different from that described by Dennell for *Haustorius* and appears to be more nearly related to that of *Ampelisca*.

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THE PELAGIC PHASE IN THE LIFE HISTORY OF THE AMPHIPOD GENUS *BATHYPOREIA*

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(Text-figs. 1–2)

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INTRODUCTION

During the year 1936 Mr Richard Elmhirst, Director of the Scottish Marine Biological Station at Millport, carried out a series of tow-nettings across the waters of Kames Bay at varying times after nightfall, mainly between the hours of 10 p.m. and midnight, to ascertain the nature and extent of the faunal immigration into the bay from outside waters, and of the nocturnal and vertical movement of the fauna which normally lies buried in the sand during the day. The net employed was an ordinary coarse tow-net supplemented on occasions with a finer net; however, the latter net captured but few amphipods and its results will be neglected in the present paper. The method employed was to walk into the water at the edge of the incoming or outgoing tide to a depth of about 2 ft. and to walk across the bay following the edge of the tide trailing the net about 10 ft. posteriorly and at arms' length into the tidal flow, thus avoiding as far as possible disturbances set up in the water by the walking movement. Each sample consisted of a tow of approximately 100 yards. Thus the position of each sample is determined by the time it was taken in relation to the day of the lunar month, and samples were obtained at all levels from high-water mark to low-water mark. I am indebted to Mr Elmhirst for the opportunity of examining the *Bathyporeia* taken in these samples and for his critical comments during this investigation.

THE *BATHYPOREIA* FAUNA OF THE SAND OF KAMES BAY*

An appreciation of the nocturnal vertical movement of these amphipods into the water of the tidal flow necessitates an analysis of their abundance in the intertidal sand. During the latter half of March 1937 a series of three traverses were sampled from the high-water mark of spring tides to the low-water mark of spring tides. Each traverse was sampled at intervals of approximately 5 yards. Each sample consisted of the fauna obtained by sieving the sand from a surface area of one square foot, to a depth of 1 in., through a brass wire sieve of 30 meshes to the linear inch. Experience on Kames Bay and elsewhere had shown that these amphipods do not bury themselves deeply into the sand and that the total population, apart from the newly hatched forms, which can pass through the meshes of the sieve, may be captured by this means.

The first traverse was taken approximately along the centre line of the bay and the second and third respectively at a distance of 20 yards to the left and right of the first. The result of the analysis of the samples is given in Table I and is represented diagrammatically against the background of the bay in Fig. 1.

Four species of the genus *Bathyporeia* occur in this area (Watkin, 1938), *B. pilosa* Lindström, *B. pelagica* Bate, *B. elegans* Watkin, and *B. guilliamsoniana* Bate, and they show a definite zonation from high-tide mark to low-tide mark. The upper 30 yards of the 200 yard traverse show a complete absence of *Bathyporeia*. The next 45 yards is occupied by *B. pilosa*, which forms a definite belt across the bay sharply demarcated at its upper and lower limits, particularly the latter. The concentration of individuals in the centre of this belt is particularly heavy; as many as 1205 individuals per square foot are recorded, with an average number per sample of almost 300. The sharpness of the lower edge of the belt is particularly noteworthy; thus sample 12 in traverse three gave 1205 individuals whilst 5 yards nearer low-water mark the number drops to 42 and the next sample records the lower limit of the belt. There follows an area of 35 yards, samples 16–22, barren of *Bathyporeia*, with *B. pelagica* appearing in sample 23 and forming a second well-defined belt across the bay 40 yards in width, disappearing in sample 31. The concentration of *B. pelagica* is very considerably less than that of *B. pilosa*, averaging less than 20 per square foot. The third zone is formed by *B. elegans* which overlaps to some extent the *B. pelagica* zone and continues to the

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* Kames Bay, Great Cumbrae, is a comparatively small bay bounded on the north-land side by the Marine Promenade of Millport and on the east and west side by sandstone rocks which wear down to a fine reddish sand unmixed with clay which forms the soil of the bay. On the south side the bay is open to the waters of the Firth of Clyde, the island of Little Cumbrae and the two promontories which form the southern points of the island of Great Cumbrae afford some protection. At low tide a stretch of sand roughly 280 yards from east to west and 180 yards from north to south is exposed.

TABLE I. NUMBER OF INDIVIDUALS OF *BATHYPOREIA* PER SQUARE FOOT IN TRAVERSES I, II AND III

Sample numbers in each traverse are at corresponding tide levels
A, *B. pilosa*; B, *B. pelagica*; C, *B. elegans*; D, *B. guilliamsoniana*

Sample no.	Traverse I				Traverse II			
	A	B	C	D	A	B	C	D
1–6	None				None			
7	..	..	..	..	8	..	..	..
8	6	..	..	..	110	..	..	..
9	428	..	..	..	106	..	..	..
10	166	..	..	..	361	..	..	..
11	708	..	..	..	507	..	..	..
12	39	..	..	..	923	..	..	..
13	8	..	..	..	6	..	..	..
14	7	..	..	..	5	..	..	..
15	4	..	..	..	..	..	..	..
16–22	None				None			
23	..	..	..	..	..	2	..	..
24	..	1	..	..	..	18	..	..
25	..	8	..	..	..	31	..	..
26	..	9	..	..	..	126	..	..
27	..	7	..	..	..	37	..	..
28	..	1	..	..	..	5	..	..
29	..	3	..	..	..	3	..	..
30	..	7	11	1	..	..	10	1
31	..	1	4	..	..	..	18	..
32	..	..	16	..	..	..	41	5
33	..	..	26	5	..	..	35	..
34	..	..	37	5	..	..	39	1
35	..	..	47	5	..	..	29	4
36	..	..	37	4	..	..	..	..

Sample no.	Traverse III				Average for three traverses			
	A	B	C	D	A	B	C	D
1–6	None				None			
7	95	..	..	..	34	..	..	..
8	357	..	..	..	158	..	..	..
9	483	..	..	..	339	..	..	..
10	860	..	..	..	462	..	..	..
11	788	..	..	..	668	..	..	..
12	1205	..	..	..	722	..	..	..
13	42	..	..	..	19	..	..	..
14	..	..	..	..	4	..	..	..
15	..	..	..	..	1	..	..	..
16–22	None				None			
23	..	4	..	..	..	2	..	..
24	..	6	..	..	..	8	..	..
25	..	17	2	..	..	19	1	..
26	..	47	..	..	..	61	..	..
27	..	99	2	..	..	48	1	..
28	..	29	6	..	..	12	2	..
29	..	21	12	1	..	9	4	..
30	..	10	21	7	..	6	14	3
31	..	1	14	..	..	1	12	..
32	..	..	22	..	..	..	26	2
33	..	..	39	5	..	..	33	3
34	..	..	38	10	..	..	38	5
35	..	..	27	1	..	..	34	3
36	..	..	..	..	..	..	12	1

low-water mark of spring tides; sampling beyond low-water mark shows its presence to some depth. Its heaviest concentration is near low-water mark and its abundance is somewhat less than that of *B. pelagica*. The fourth species, *B. guilliamsoniana*, occurs but spasmodically amongst the *B. elegans*, but it becomes abundant and dominant beyond low-water mark.

All the individuals of *pelagica*, *elegans* and *guilliamsoniana*, and a sample of *pilosa*, from the first traverse, were examined to ascertain the proportion of males to females, and of adult forms to immature forms. Adult maleness was determined by examination of the antenna, adult femaleness by either the presence of eggs in the brood pouch, by setose oostegites, or by the antennae. Individuals are listed as immature if the secondary sexual characters have begun their development, young if the secondary sexual characters are not shown. The result of this analysis is shown in Table II.

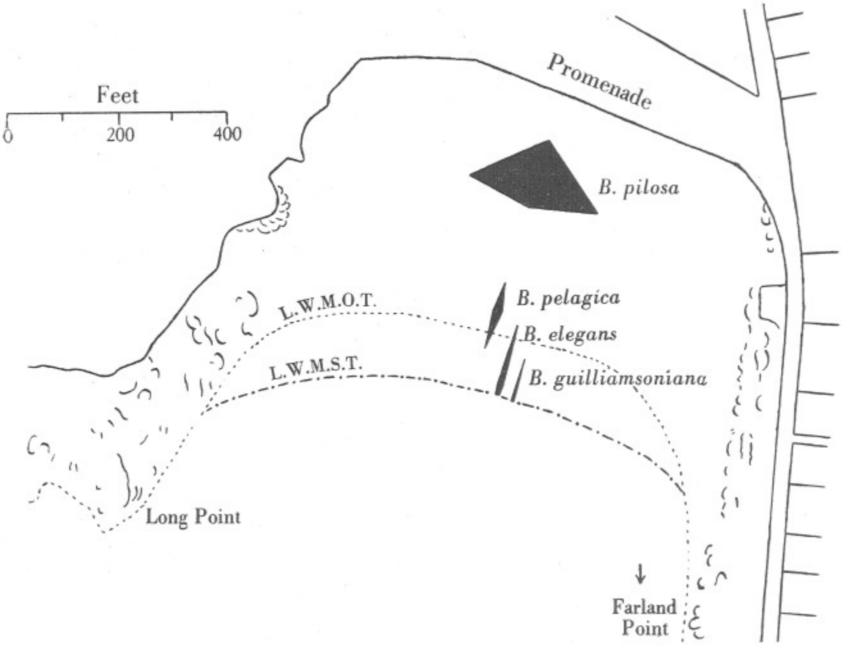

Fig. 1. Zonation of *Bathyporeia* in Kames Bay. Horizontal scale = 1000 individuals to 1 in.

Thus, neglecting the differences between the species, the adult male population represents about 15%, the adult female population about 26%, giving a ratio of adults to immature and young forms of 41 to 59. It must be borne in mind that the sampling took place in March, when it may be expected that the proportion of adult forms would be at its peak after the almost complete winter cessation of reproduction and before many, if any, of the new season's population are released from the brood pouch. The above figures show a small percentage only of young forms; this percentage is probably too low since some young forms, if present, may have escaped through the meshes of the sieve. However, from the point of view of subsequent discussion the main points to be noted are the small percentage of adult males and the much larger percentage of adult females.

TABLE II. ANALYSIS OF THE SEX AND AGE OF THE SPECIES

	Adult ♂	Adult ♀	Immature ♂	Immature ♀	Young forms	Total
B. pilosa	175	260	264	176	14	889
B. pelagica	5	32	66	63	56	222
B. elegans	13	36	13	43	67	172
B. guilliamsoniana	2	3	3	1	2	11
Total	195	331	346	283	139	1294

ANALYSIS OF THE TOW-NET SAMPLES

Table III gives the date, time of day when the sample was taken, age of the moon in days, time of high water and species content of forty-six samples taken during the year 1936. One sample only, that of September 30, did not contain any *Bathyporeia*. Thus it may be assumed that some vertical migration into the tidal waters takes place on almost every night of the year. Other samples taken at various times in previous years confirm this view. However, the number per sample is very variable. Of the forty-five samples containing these amphipods twenty gave less than ten individuals, seventeen samples from ten to fifty individuals, four samples from fifty to 100 individuals, four samples from 100 to 200 individuals and one only over 300 individuals. In view of the high concentration in the sand larger numbers might have been expected in the samples.

The total numbers for each species show a direct relationship to the numbers in the sand with *pilosa* as the dominant form, with *pelagica* and *elegans* in considerably less numbers and with an occasional *guilliamsoniana*. The individual samples show that, in the main, each species retains its purity when migrating into the tidal waters. Thus thirty-two of the forty-five samples show one species only, eight a mixture of two species, four a mixture of three species, and one only with the four species. It may be expected that *pilosa*, since it occupies a definite zone on the beach away from the other species, would retain its purity. This is so, in that twenty-two of the samples contain this species only and where it does occur in a mixed species sample its numbers are few. On the other hand, the *pelagica* and *elegans* belts overlap to some extent and some admixture of the two species in the tidal waters may be expected. However, here again the samples containing these species are remarkably pure, and where a mixture of more than one species occurs the sample is dominated by one of the species.

It is noteworthy that the species retain their zonation so closely when swimming in the tidal waters, which suggests that the time they remain in the water is short. Possibly they make several short excursions from the sand, which may account to some extent for their scarcity, compared with the numbers in the sand and also their ability to remain within the orbit of their belt in the sand. Their behaviour in a dish in the laboratory, although under

TABLE III. NUMBER OF INDIVIDUALS OF EACH SPECIES PER SAMPLE

A, *B. pilosa*; B, *B. pelagica*; C, *B. elegans*; D, *B. guilliamsoniana*

Date	Age of moon in days	G.M.T.	Time of H.W. p.m. G.M.T.	A	B	C	D	Total
7. i. 36	14	10.00 p.m.	11·44	23	..	..	..	23
15. i. 36	22	7.30 p.m.	4.16	..	8	..	..	8
21. i. 36	28	10.30 p.m.	10.32	1	..	..	..	1
12. ii. 36	20	8.30 p.m.	3·7	..	..	1	..	1
13. ii. 36	21	7.00 p.m.	3·37	..	4	..	..	4
4. iii. 36	12	11.00 p.m.	10.38	21	..	..	..	21
19. iii. 36	27	9.00 p.m.	9.34	152	..	..	..	152
24. iii. 36	2	4.30 p.m.	1·0	56	..	1	..	57
29. iv. 36	9	10.15 p.m.	6.53	..	16	2	..	18
30. iv. 36	10	10.15 p.m.	8.10	11	..	..	1	12
1. v. 36	11	10.15 p.m.	9.13	3	..	..	..	3
2. v. 36	12	10.15 p.m.	10.2	32	..	..	..	32
3. v. 36	13	10.15 p.m.	10.44	69	..	..	..	69
9. v. 36	19	10.15 p.m.	1.55	2	6	8	1	17
14. v. 36	24	10.15 p.m.	5.55	145	..	..	..	145
15. v. 36	25	10.15 p.m.	7.9	1	37	2	..	40
16. v. 36	26	10.15 p.m.	8.17	4	8	..	1	13
17. v. 36	27	10.15 p.m.	9.19	5	5	..	..	10
21. v. 36	2	10.15 p.m.	0.22	24	..	..	..	24
1. vi. 36	13	10.15 p.m.	10.0	37	..	..	..	37
10. vi. 36	22	11.00 p.m.	3.49	5	3	1	..	9
12. vi. 36	24	10.30 p.m.	5.32	..	..	1	..	1
16. vi. 36	28	10.30 p.m.	9.52	37	..	..	..	37
23. vi. 36	5	1.00 a.m.	3.12	143	1	..	..	144
26. vi. 36	8	11.00 p.m.	5.12	..	1	5	..	6
27. vi. 36	9	1.00 a.m.	5.58	..	..	7	..	7
2. vii. 36	14	11.00 p.m.	10.53	14	..	..	..	14
14. vii. 36	26	11.00 p.m.	8.23	..	11	..	..	11
21. vii. 36	4	11.00 p.m.	2.9	..	8	..	..	8
31. vii. 36	14	11.00 p.m.	10.30	370	..	..	..	370
12. viii. 36	26	11.00 p.m.	8.11	2	71	..	..	73
21. viii. 36	5	11.00 p.m.	2.41	..	2	..	..	2
31. viii. 36	15	11.00 p.m.	11.35	1	..	..	..	1
8. ix. 36	23	9.45 p.m.	5.11	..	4	81	1	86
15. ix. 36	1	10.00 p.m.	0.6	32	..	..	..	32
24. ix. 36	10	10.00 p.m.	5.28	1	12	..	..	13
30. ix. 36	16	10.00 p.m.	11.50	..	..	..	..	..
5. x. 36	21	10.30 p.m.	3.9	..	..	4	..	4
20. x. 36	6	10.30 p.m.	2.41	..	3	..	..	3
28. x. 36	14	10.30 p.m.	10.38	1	..	..	..	1
7. xi. 36	24	8.30 p.m.	7.0	2	..	..	..	2
12. xi. 36	29	10.30 p.m.	11.24	1	..	..	..	1
25. xi. 36	12	8.30 p.m.	9.14	3	..	..	..	3
11. xii. 36	28	11.30 p.m.	10.58	6	..	..	..	6
14. xii. 36	2	8.45 p.m.	0.25	26	..	..	..	26
22. xii. 36	10	7.30 p.m.	6.19	4	..	..	..	4
			Total	1234	200	113	4	1551

totally different environmental conditions, confirms this, for an individual will emerge from the sand, swim around the dish for a short time and return to the sand. It cannot be imagined that they perform extensive migrations during the intertidal period and yet return to their particular zone area with the retreating tide. This zonation is further shown when the time of sampling is related to the time of high water. The high-tide samples are composed of *pilosa*, the mid-tide samples of *pelagica*, and the low-tide samples of *elegans*. When the samples are related to whether they were taken on a falling tide or a rising tide little of value emerges. In general the average number per sample is greater when taken on a falling tide. This point requires further investigation.

An analysis of the individuals of each species into their sexual age, for the population obtained by the tow-net, is given in Table IV.

The immediate noticeable feature is the preponderance of adults over immature forms as compared with the numbers shown in Table II for the sand population. The total figures for all species combined give an adult population of over 57% as compared with 40%. Of the 57% of adults, 42% are adult males and 15% adult females. Thus the dominance of adult males in the tow-net samples is a marked one.

Considering the species individually, of 1234 individuals of *pilosa* taken in the tow-net samples 57% are adults (40% males and 17% females), 31% are immature and 12% young forms. This dominance of adults is actually much more marked than the actual figures suggest since of the 148 young forms, 123 were obtained in two samples, those of June 23 and July 31. A study of the individual samples shows a considerable variation. An occasional sample like that of March 24 is composed almost entirely of adult males; no sample shows a dominance of adult females. Other samples are composed almost entirely of a mixture of adult males and females, such as that of May 14, whilst others show a high percentage of immature and young forms.

A similar variable population occurs in *pelagica* with 70% adult (60% male and 10% female), with 18% of immature forms and 12% of young forms. As in *pilosa* some samples are almost completely composed of adult males, others with a mixed population of adult males and females, and others with a considerable percentage of young forms.

The figures for *elegans* show the same general outline as in the above two species with a marked dominance of adult males. Only four individuals of *guilliamsoniana* were taken and of these three were adult males.

TABLE IV. NUMBER OF INDIVIDUALS IN EACH SAMPLE AT EACH MATURITY STAGE

A = adult males, B = adult females, C = immature males with secondary sexual characters developing, D = immature females with secondary sexual characters developing, E = young males and females with no secondary sexual characters showing.

Date	A	B	C	D	E	Total
			B. pilosa			
7. i. 36	4	..	12	3	4	23
21. i. 36	..	..	..	1	..	1
4. iii. 36	13	6	2	..	..	21
19. iii. 36	76	21	34	19	2	152
24. iii. 36	52	1	2	1	..	56
30. iv. 36	9	..	2	..	..	11
1. v. 36	2	..	..	1	..	3
2. v. 36	22	7	1	2	..	32
3. v. 36	59	6	2	2	..	69
9. v. 36	2	..	..	..	..	2
14. v. 36	98	37	10	..	..	145
15. v. 36	1	..	..	..	..	1
16. v. 36	3	1	..	..	..	4
17. v. 36	2	..	3	..	..	5
21. v. 36	14	8	..	..	2	24
1. vi. 36	27	3	2	..	5	37
10. vi. 36	3	..	1	..	1	5
16. vi. 36	2	12	6	9	8	37
23. vi. 36	64	12	13	3	51	143
2. vii. 36	5	6	2	1	..	14
31. vii. 36	16	69	81	131	73	370
12. viii. 36	2	..	..	..	..	2
31. viii. 36	1	..	..	..	..	1
15. ix. 36	12	7	4	7	2	32
24. ix. 36	..	..	1	..	..	1
28. x. 36	..	..	..	1	..	1
7. xi. 36	1	..	1	..	..	2
12. xi. 36	..	..	..	1	..	1
25. xi. 36	1	..	1	1	..	3
11. xii. 36	1	1	4	..	..	6
14. xii. 36	3	6	11	6	..	26
22. xii. 36	..	..	3	1	..	4
Total	495	203	198	190	148	1234
			B. pelagica			
15. i. 36	2	1	3	2	..	8
13. ii. 36	2	..	..	1	1	4
29. iv. 36	13	3	..	..	..	16
9. v. 36	4	..	..	2	..	6
15. v. 36	35	1	1	..	..	37
16. v. 36	4	1	1	2	..	8
17. v. 36	3	2	..	..	..	5
10. vi. 36	2	1	..	..	..	3
23. vi. 36	1	..	..	..	..	1
26. vi. 36	1	..	..	..	..	1
14. vii. 36	5	..	3	3	..	11
21. vii. 36	5	2	1	..	..	8
12. viii. 36	31	4	6	7	23	71
21. viii. 36	1	..	..	1	..	2
8. ix. 36	2	..	1	1	..	4
24. ix. 36	8	3	1	..	..	12
20. x. 36	1	2	..	..	..	3
Total	120	20	17	19	24	200

TABLE IV (CONTINUED).

Date	A	B	C	D	E	Total
			B. elegans			
12. ii. 36	1	..	..	..	..	1
24. iii. 36	..	..	1	..	..	1
29. iv. 36	2	..	..	..	..	2
9. v. 36	8	..	..	..	..	8
15. v. 36	2	..	..	..	..	2
10. vi. 36	1	..	..	..	..	1
12. vi. 36	1	..	..	..	..	1
26. vi. 36	1	..	1	..	3	5
27. vi. 36	3	..	..	..	4	7
8. ix. 36	9	18	27	18	9	81
5. x. 36	2	..	1	1	..	4
Total	30	18	30	19	16	113
			B. guilliamsoniana			
30. iv. 36	1	..	..	..	..	1
9. v. 36	1	..	..	..	..	1
16. v. 36	1	..	..	..	..	1
8. ix. 36	..	..	..	1	..	1
Total	3	..	..	1	..	4

RELATIONSHIP TO LUNAR MONTH

The age of the moon in days (with day 1 as the first day of the new moon) is given in the second column in Table III. If Table IV be rearranged according to the day of the lunar month and all species be considered together irrespective of the season of the year, an indication of the relationship of the occurrence of *Bathyporeia* with the lunar month can be obtained. These results are represented graphically in Fig. 2.

The result is somewhat uneven, but in general it may be concluded that the number of individuals appearing in the tidal waters increases to two maxima which correspond to the periods immediately preceding full moon and preceding and partly overlapping the appearance of the new moon. The period immediately following the full moon is marked by a distinct scarcity of individuals, although this period is rather inadequately covered by the sampling. A second period of scarcity occurs at the end of the first quarter and beginning of the second quarter. A more detailed study shows that there is no difference in the nature of the population at the two maxima; both are represented by the same proportion of males to females and of adults to young forms. In the figure all four species are grouped together, an attempt to show variability in the behaviour of *pilosa*, *elegans* and *pelagica* gave negative results and detailed figures have consequently been omitted.

DISCUSSION

The vertical movement of amphipods whose habitat is normally in the soil of the sea floor is a phenomenon which has been recorded by many authors. It is, however, necessary to distinguish between species whose vertical movements are mainly confined to the hours of daylight and those in which such movement occurs only during the hours of darkness. It is with the latter only that this paper is mainly concerned, and reference to three main papers may be made, Tattersall (1913*a*), Elmhirst (1932), and Fage (1933). Tattersall in his survey of the amphipods of Clare Island and the adjoining district states that "tow-netting in the harbours and shallow waters...at the surface at night has yielded excellent results", and of eighty-four species of gammarid amphipods recorded eleven are given as occurring in plankton samples taken during the night and twelve as taken in plankton samples during the daytime. Only one species, *Guernea coalita* (Norman), is recorded as occurring in both the day and night hauls. He gives no details of the sex or age of the specimens captured, but informs me that they were predominantly adult males. Tattersall in another paper (1913*b*) shows that many mysids and cumaceans become abundant in the surface waters at night time. Elmhirst records the night tidal migrants of Kames Bay and shows that *Gammarus locusta*, *Bathyporeia* sp., *Pontocrates* sp., *Megalopurus agilis*, *Periculodes longimanus* and *Paratylus swammerdami* are common forms in the night population of the waters of this bay, together with the mysid *Schistomysis spiritus* and the cumacean *Pseudocuma cercaria*. Some details of the age and sex are given. Fage in an

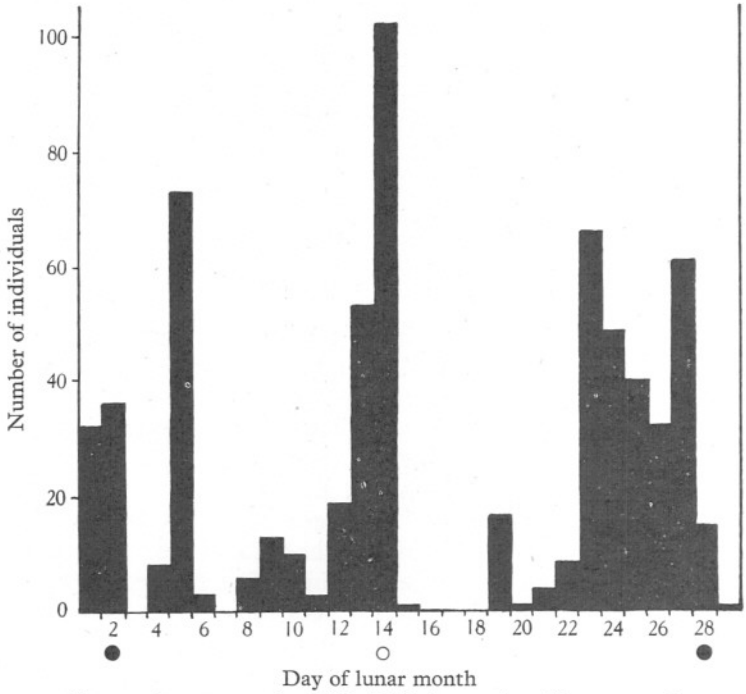

Fig. 2. Average number of individuals per day of lunar month.

extensive series of samples taken at Banyuls-sur-Mer and at Concarneau at night time, "autour d'un foyer lumineux", records thirty-four species of gammarid amphipods together with several mysids and cumaceans. Comparing the lists of amphipod species as given by Tattersall, Elmhirst and Fage it is interesting to note that seven species are common to the lists of Tattersall and Fage, namely, *Metaphoxus pectinatus* and *fultoni*, *Synchelidium haplocheles*, *Perioculodes longimanus*, *Apherusa bispinosa*, *Dexamine spinosa* and *Guernea coalita*; five common to the lists of Elmhirst and Fage, namely, *Gammarus locusta*, *Bathyporeia* sp., *Pontocrates* sp., *Perioculodes longimanus* and *Paratylus swammerdami* (*Nototropis schwammerdami*); and but one only common to all three lists, namely, *Perioculodes longimanus*. Patience (1909) records *Metaphoxus pectinatus* and *fultoni* as occurring at night time in the waters of the Firth of Clyde, thus making three species common to all three areas. Several authors refer to the vertical migration of *Apherusa bispinosa*, *Dexamine spinosa* and *Guernea coalita* (see Fage, pp. 211, 218 and 233 respectively). Russell (1925) shows that samples, taken at various depths up to 50 m. in the waters of the English Channel on a moonlight night in July, contain various benthic amphipods belonging to the genera *Monoculodes*, *Bathyporeia* and *Ampelisca*. A species of the genus *Urothöe* was exceptional in being present in the surface waters at 9 p.m. He further shows (1927) that in a series of samples taken during the day truly benthic amphipods are absent except for species of the genus *Apherusa*, mainly *A. ovalipes* and *A. clevei*. Fish (1925) records, particularly, four species of amphipods in tow-nettings taken at Wood's Hole, *Batea secunda*, *Stenothöe cypris*, *Monoculodes edwardsi* and *Calliopus laeviusculus*, the two former breeding during the summer, the two latter during the winter, but does not distinguish between day and night migrants. He is doubtful of the extent to which nocturnal vertical migration affects the night population of amphipods in the surface waters. It is interesting to note, however, that of the twenty-six species of benthic gammarid amphipods listed, seventeen belong to families which also show night migrants in the lists of Tattersall and Fage.

Thus it may be stated that it is a feature of certain species of benthic amphipoda, particularly some of those belonging to the families Haustoriidae, Phoxocephalidae, Oedicerotidae, Calliopiidae, Atylidae and Dexaminidae, to perform nocturnal vertical movements. How far such movements are a normal feature of all the species in these families and whether such movement is spasmodic and of rare occurrence in other families, such as certain species in the families Lysianassidae, Ampeliscidae and Gammaridae, must remain a matter of interesting speculation pending much further detailed investigation.

The nocturnal vertical movement of pelagic amphipods, particularly the Hyperiidae, is well known from the work of Hardy & Gunther (1935) and also Barnard (1932) on the plankton samples of the *Discovery* Expedition, and the nocturnal vertical movement of macroplankton organisms in general from the work of Russell at Plymouth. It is unnecessary to review this literature in this paper since it is probable that such movement of planktonic organisms is of a fundamentally different nature.

A discussion of the factors which control this nocturnal vertical migration must remain largely speculative pending further detailed investigation. That the movement is not a feeding one seems fairly definite, since observations on *Bathyporeia*, as yet unpublished, show that they feed when lying buried in the sand by projecting sand particles on to the mouthparts and removing the particles of food that cling to the particles. Foxon (1936) also shows that the cumacean *Pseudocuma longicorne*, which lives in the same habitat, feeds in essentially the same way; in fact it is probable that all benthic littoral amphipods, mysids and cumaceans feed whilst associated with their sea-floor habitat. The association of the movement with the reproductive cycle is suggested by the concentration of adults as compared with young forms. The evidence given above for *Bathyporeia*, that given by Elmhirst (1932) for other amphipods in Kames Bay, and many of the species referred to by Fage (1933), shows that adults are predominant. The results given by Fage, however, mostly show a much higher percentage of adult males compared with my figures for *Bathyporeia*. This is probably due to the effect of the lantern in concentrating the adult males with their large eyes and in having a less effect on the adult females with their smaller eyes. Fage (1933), Foxon (1936) and Tattersall (1938) show that similar concentrations of adults as compared with young forms occur also in some mysids and cumaceans. Thus an explanation of this movement may, in part, be found to be associated with the breeding cycle. Foxon's suggestions that "it is unlikely that the formation of pairs would take place in the sand", and that the "function of the swarm is to enable the males and females to locate each other", will apply equally well to *Bathyporeia* and to the mysids, but it does not explain the presence of numerous immature forms in occasional samples. Tattersall suggests that the vertical movement of immature mysids is probably connected with environmental factors such as temperature, again a suggestion that will apply equally well to *Bathyporeia*. Fage shows that in certain species such as *Gammarus locusta* immature forms only are obtained. Thus it is probable that different factors may control the movements of adults and young immature forms. Foxon further suggests in connexion with *Pseudocuma longicorne* "that where swarms consisting entirely of males are formed, these are forming a swarm into which the females can enter and so find a mate", a suggestion which implies that the males occupy a higher level in the water than the females. This paper supplies no evidence that the males of *Bathyporeia* occupy a higher level in the water than the females, since the method of capture at the water's edge of the tidal flow does not permit of sampling at various depths, in fact all samples show a mixed population. From further data collected by Mr Elmhirst during 1932 and again during 1936, when samples were obtained at the surface and at a depth of 2 fathoms, it may be concluded that the *Bathyporeia* population is always a mixed one and no swarms consisting entirely of males occur. Fage, however, shows that in four species of the genus *Urothöe* no female or immature specimen was captured within or without the orbit of the light employed. Thus in certain species a factor which affects adult males only may have to be considered.

Foxon further suggests that "the females come to the surface when eggs are about to hatch so that the young may have the same opportunity of dispersal". Tattersall has made the same suggestion in regard to certain mysids. This suggestion does not account for the presence of adult males and implies that some females with well-developed embryos in the brood pouch would occur. No female with young in the brood pouch was taken throughout this series of samples, the numbers with eggs were relatively few, and the presence of small newly hatched forms in the samples which cannot have escaped through the meshes of the net is not indicated. However, from observations made by Mr A. K. Vaughan of the University College of Wales (as yet unpublished) on the life cycle of *Bathyporeia* on the Cardiganshire coast, it is known that the release of the young forms from the brood pouch takes place at the periods of new and full moon. Whether this fact bears a relationship to the increase in numbers in the tidal waters in the periods immediately preceding new and full moon must remain at the moment problematical.

The above discussion attempts to find factors controlling the nocturnal vertical movement of *Bathyporeia* almost entirely in relation to the breeding habits of the species; other factors, however, such as purely physical ones, should be considered. Wheeler (1937), in a discussion concerning the vertical movement of prawns off Bermuda, reaches the tentative conclusion that, of the factors controlling periodicity, light is the most likely one as inhibiting their activity. The evidence on *Bathyporeia* in this paper shows that periodicity in relation to the lunar cycle is a feature of the vertical movement of the species in this genus. That light is not a major controlling factor in the genus is shown by the fact that the numbers in the tidal waters increase in the periods immediately preceding new and full moon, but that it is a factor seems definitely indicated in that they do not occur in the tidal waters during the day time.

A reference to Table III shows that individuals were taken in all the months of the year with larger numbers in the samples from March to September than in the autumn and winter months. The largest samples were obtained in July, March, May and June in that order, so that temperature cannot be regarded as an important factor apart from the effect which it may have on the breeding cycle.

There remains the possible influence of the tides, which may be regarded as an important factor in an intertidal area. That this factor is of importance is shown by the increase in numbers in the two periods which correspond to the highest rises of the tide in each lunar month. It may be noted that the life cycle of *Bathyporeia* is such that each batch of eggs takes about 15 days to develop, hatch and be released from the brood pouch. In *B. pilosa* broods are produced all the year round, but with larger numbers breeding during the spring and summer months, with an occasional extensive brood during these months, which may account for the larger numbers in the samples at definite periods. It may be noted that *B. pilosa* appears in the samples all the year through. With *B. pelagica* breeding is confined to the spring and summer months, and it appears in the tidal waters apart from an occasional specimen during these months only. The results obtained by Fage (1933) and Fish (1925) show that certain species appear in the plankton for periods of time only, some are summer migrants, others winter migrants. Thus a knowledge of the period and length of the breeding cycle for each species is necessary. Clark (1925) has shown an interesting connexion between the tides and the breeding of the fish *Leuresthes tenuis*.

It appears that the nocturnal vertical movement of *Bathyporeia* into the waters of the tidal flow is determined, in part, by the influence of the tides which has some relation to the double breeding cycle in each month.

SUMMARY

The zonation of four species of the genus *Bathyporeia*, from high-water mark to low-water mark, in Kames Bay, Millport is given. *B. pilosa* occurs above the high-water mark of neap tides, *B. pelagica* is a mid-tidal form and *B. elegans* a low-water form. The latter, together with *B. guilliamsoniana*, extend beyond low-water mark. Of the population, as it occurs in the sand, 15% are adult males, 26% adult females, giving a proportion of adult to immature forms of 41 : 59.

The analysis of forty-five tow-net samples taken across the bay at night shows that the same zonation of the species is maintained; the proportion of adults to immature forms in these samples, however, is as 57 : 43, and of the 57% of adults, 42% are adult males, 15% adult females.

When the samples are related to the day of the lunar month it is shown that the numbers increase in the tidal waters in the periods immediately preceding the full moon and preceding and partly overlapping the new moon.

A nocturnal vertical movement appears to be a marked feature of a few benthic amphipod families, but the factors which cause this migration are problematical and require much further investigation.

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THE PHYSIOLOGY OF MATURATION AND FERTILIZATION IN *POMATOCEROS TRIQUETER* (L.) I. THE NATURE OF THE MATERIAL

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(Text-figs. 1–5)

While investigating the physiology of maturation and fertilization in the marine worm, *Pomatoceros triqueter*, it soon became evident that there was great variability in the reactions of the material, not only among the egg batches from different worms but even in the eggs liberated from one worm. For accurate interpretation of the results it was important that some knowledge of this variability should be obtained. This paper is therefore devoted to the individual and seasonal variations shown by the material.

MATERIAL

The animals were collected from the north side of Cullercoats Bay. The principal sources were the "Gut" and the hollow close to the north breakwater.

During the course of the work over 3000 worms were examined, the sex ratio being about five females to one male. The distribution of the sexes appeared to be haphazard, but the material was selected to give large numbers of eggs, large specimens forming the greater number of those examined. A statistical study of different size classes might show a reversal of sex in the animal or a size difference between the sexes.

METHODS

The material for the studies on seasonal variation, carried out between September 1934 and September 1935, was collected the day prior to or on the morning of the experiment. For other work, apart from certain special cases to be referred to later, the material was never kept in the aquarium for more than three days before use.

To collect the germinal products the stone on which the animal had formed its tube was first washed with tap water, the tube chipped away, and the worm removed. If a female, it was quickly rinsed in tap water, then in Berkefeld filtered sea water, and finally placed in the dish of sea water or experimental solution in which the eggs were required. Since liberation proceeds very rapidly once the tube is broken, the above steps must be carried out quickly.

Practically complete liberation occurs within 10 min. of opening the tube. Uniform crystallizing dishes about 5 cm. in diameter and in depth were used for the experiments. No special precautions were taken to control the temperature, but records were kept throughout each experiment. The *p*H of the experimental solution was determined colorimetrically before and after each experiment.

Previous workers have usually paid little attention to the accuracy of results obtained from egg counts, although Fuchs (1915) working on *Ciona* drew attention to the possible errors associated with egg counts and the desirability of making check counts. The chief source of error in the present work was the difficulty of obtaining a uniform mixture of eggs for distribution to the various solutions. The best method was to shake the eggs thoroughly in 25 c.c. of solution and then by a rotary motion collect them in the centre of the dish. A fine pipette was then used to transfer the eggs. In filling the pipette eggs were collected from different regions of the egg mass and approximately the same number of eggs was present in each transfer. Counts were made in the dish, not by transfer to a slide which in itself introduces a source of error. Unfertilized eggs were fixed by the addition of formalin after a lapse of $4\frac{1}{2}$ hr., for then practically all eggs capable of maturing had done so. Results had sometimes to be based on counts of only 300–400 eggs. The degree of accuracy of the method as tested by repeated countings was $\pm 3\%$ for mature eggs and fertilization stages and $\pm 10\%$ for immature oocytes.

Males were transferred immediately to about 5 c.c. of sea water instead of being washed in tap water. After liberation of the sperm the worm was removed and the mixture shaken. When more than five batches of eggs had to be fertilized the method advised by Fuchs for making a sperm suspension proved more satisfactory; that is, to dilute the original suspension and ensure thorough mixing by pouring the liquid from one vessel to another about twenty times. Fertilizations were made by adding one or more drops of sperm suspension and fertilized batches were fixed 5 hr. after the addition of sperm.

The Form of the Egg

When shed from the worm the eggs are primary oocytes (Fig. 1) and they are usually light red in colour. Each egg possesses a large, clear germinal vesicle, the diameter of which is half that of the egg. A large asymmetrically placed nucleolus is present in each nucleus. The cytoplasm is finely granular, with the granules evenly distributed. The egg is surrounded by a vitelline membrane 2μ in thickness, the surface of which is covered by a system of fine furrows or indentations. By cytolysing eggs in distilled water the membrane was shown to be very tough. When liberated the eggs are usually irregular or flattened. The mean diameter of flattened eggs, based on measurements of 50–100 eggs, varies with the batch. In the majority it is $65\text{–}70\mu$, but eggs giving a mean diameter of over 80μ have been recorded.

A varying percentage of the eggs liberated, depending on the ripeness of the batch, mature. This change is characterized by the breakdown of the germinal vesicle, the egg becoming purple-red in colour and approximately spherical. The mean diameter of such eggs was 63–68 μ. A comparison of the measurements of flattened primary oocytes and mature eggs indicates the marked variation in the form of the primary oocyte (Fig. 2). The change from primary oocyte to mature egg took place mainly in the 4 hr. following liberation; longer intervals in sea water without fertilization resulted in cytolysis. The common cytolytic form superficially resembled the blastula stage of a fertilized egg. The earliest time for the appearance of these "pseudoblastulae" was 6 hr. after liberation, but their formation depended on the ripeness of the material. Ripe material, that is a batch of eggs giving a high percentage of mature eggs, would show complete cytolysis 24 hr. after liberation. In an unripe batch this was delayed, some fertilizable primary oocytes remaining 80 hr. after liberation.

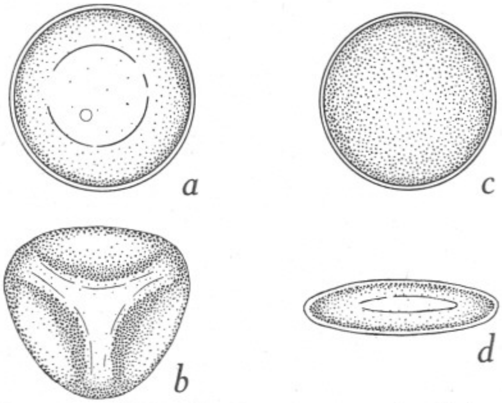

Fig. 1. *a*, flattened primary oocyte viewed from above, showing the germinal vesicle with excentric nucleolus; *b*, misshapen primary oocyte, this is the usual appearance of eggs after liberation; *c*, an egg which has matured; *d*, flattened primary oocyte viewed from the side, this corresponds to the form described by Hörstadius.

These observations do not agree with the description of the material given by Hörstadius (1923). He considered that the primary oocytes were flattened and that, after breakdown of the germinal vesicle, the long diameter decreased and the breadth increased but the egg did not become spherical. The flattened forms are, however, not of one shape. Only extremely flattened eggs agree with Hörstadius's description of the primary oocyte. In no case did the mature egg resemble the stage described by Hörstadius. In the first place, it tended to become approximately spherical. Secondly, from both the figure and the description it should have shown a prominent perivitelline space; this could not be observed. By treating mature eggs and even fertilized eggs with hypertonic sea water the withdrawal of the egg protoplasm from the vitelline membrane could be watched, giving clear evidence that no perivitelline space existed before or after fertilization.

Hörstadius also stated that the eggs underwent a reduction in volume on reaching maturity. In view of the large variation in shape, such a conclusion cannot be arrived at from measurements of the long and short diameters of primary oocytes and mature ova.

Under the conditions of liberation which existed in these experiments the eggs were accompanied by a varying number of immature oocytes. The smallest of these are colourless, but as size increases the colour approximates to the light red of the primary oocyte. They can, as a rule, be distinguished from primary oocytes by a zone free from granules beneath the vitelline membrane.

The rounding off of eggs has been attributed to a change in permeability. In both *Asterias glacialis* (Dalcq, 1923, 1924) and *Sabellaria vulgaris* (Waterman, 1936), where such an explanation has been advanced, rounding off occurs immediately on contact with sea water and the germinal vesicles remain intact. In *Pomatoceros* conditions appear to be different. Mere liberation into sea water is not enough to produce rounding off; some eggs even after 24 hr. in sea water may still be misshapen. Here, rounding off is associated with the breakdown of the germinal vesicle. At first it appeared as though certain eggs with complete germinal vesicles also underwent the change, but, since such eggs were darker in colour than the unchanged primary oocytes, it is probable that the nuclear membranes whilst visible were not intact.

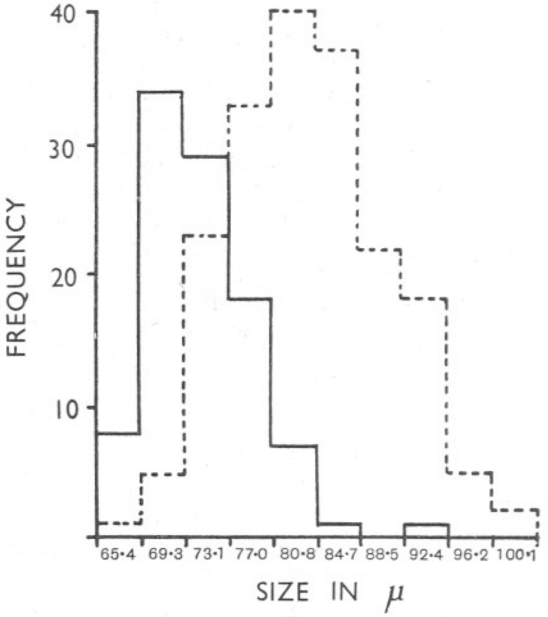

Fig. 2. A comparison of the variations in diameter of primary oocytes with eggs of the same batch which have matured. Mature eggs ———, primary oocytes – – – – – – – –. A marked reduction in diameter is associated with this change and is due to the eggs rounding off. Primary oocytes $82{\cdot}2\,\mu \pm 6{\cdot}8$. Mature eggs $72{\cdot}8\,\mu \pm 4{\cdot}7$.

In animals such as *Pomatoceros*, where it is not an immediate phenomenon following liberation into sea water, rounding off can be explained without assuming a change in permeability. Hobson (1932) indicated that the vitelline membrane had elastic properties. If such a membrane were distorted owing to the combined effects of the eggs being packed tightly in the body cavity and being forced out of a slit-like aperture on liberation, it would tend to return to its normal form unless some opposing force were exerted on it. Such resistance would exist if the egg protoplasm was in a very viscous condition. If, on maturing, the viscosity decreased or movements took place in the protoplasm, the elastic properties would result in the egg becoming spherical. Evidence from other species suggests that such changes might occur. Harris (1935) has recorded marked streaming movements and a large drop in viscosity following the breakdown of the germinal vesicle in eggs of *Sabellaria alveolata*. In *Nereis* (Hoadley, 1934) the internal changes associated with the disappearance of the germinal vesicle are so violent that distortions occur in the shape of the eggs.

The Normal Course of Maturation and Fertilization

Maturation is not completed before fertilization. The egg can progress as far as the breaking down of the nucleus, but it must await insemination before the polar bodies are liberated. It is therefore like the majority of annelids, molluscs and echinoderms other than certain echinids.

In ripe eggs, the spermatozoon enters within 5 min. of adding the sperm, and, if it has not already done so, the germinal vesicle breaks down and the egg becomes spherical. Normally no membrane is raised on fertilization, although in exceptional cases a perivitelline space may appear at one or more points. No change was noticed in the attachment of the membrane to the egg surface until the polar bodies were liberated. Then the membrane was stretched in the region where the polar bodies lay and its internal and external limits could be clearly seen. There is nothing to suggest that the membrane is in any way different from the vitelline membrane of the unfertilized egg. An observation which pointed to the polar bodies being attached to the membrane was made when treating fertilized eggs with hypertonic sea water. The egg protoplasm withdrew from the membrane, but the polar bodies remained attached to both membrane and egg surface, being drawn out between them.

The minimum time for the disappearance of the nuclear membrane and the appearance of uniformity of colour in the egg was 6 min. at 16° C. The two polar bodies were liberated at about 30 and 50 min. respectively. There was no reconstitution of the pronucleus and the eggs reached the first cleavage after an interval of about 100 min. These figures are only approximations, for a considerable amount of variation occurs.

Variation in the Material

Variations in the physiological condition of the eggs liberated from different worms were often recorded. There was a wide range of variation in the percentage of unfertilized eggs which matured. In fertilized eggs there might be present at the same time two- to eight-cell cleavage stages. In addition there might be a number of eggs into which sperm had penetrated, but which had not undergone division. It was an exception to find a batch of eggs which proceeded through the various cleavage stages as uniformly as is found in the majority of echinoderms. Evidence of variability was also brought to light in the experimental studies.

Variation in the Degree of Maturity

It was thought that the number of unfertilized eggs which matured would be a measure of the ripeness of the material. An attempt was made, therefore, to see whether the degree of maturity was directly related to fertilizability, as measured by the proportion of eggs undergoing cleavage. The procedure was to fertilize ten batches of eggs with sperm from the same male, an unfertilized control series being kept. These experiments failed to show that such a relationship existed.

Variation in Individual Egg Batches

The egg batch of one worm could be divided up by allowing the worm to remain in one dish only for a short time, after which it was transferred to another, and so on through a series of dishes. In general, it was found that the number of mature eggs was highest in the batches liberated last (Fig. 3). With the gradual rise in the number of mature eggs there was a sharp drop in the total number of eggs and immature oocytes (Table I).

TABLE I. TYPICAL SERIES OF RESULTS OBTAINED BY THE TRANSFER METHOD

The time of transfer given in column 1 is the time of removal of the worm from one dish to the next in the series.

Time of transfer	No. of eggs liberated	No. of immature oocytes	No. of eggs lib./sec.	Immature oocytes lib./sec.
15 sec.	8800	340	587	23
30 ,,	2400	440	160	29
40 ,,	780	180	78	18
1 min.	740	60	37	3
2 ,,	740	30	12	0·5
3 ,,	780	120	13	2
4 ,,	800	90	13	1
8 ,,	1160	100	5	0·4
16 ,,	1650	150	4	..
16+ min.	1150	200	..	..

Such transfer series, by providing fractions more uniform in themselves with respect to maturity than a complete egg batch, allowed further tests to be made regarding a relationship between maturity and subsequent cleavage. These experiments showed that the increase in maturity common in transfer experiments could sometimes be associated with increased cleavage (Table II).

Whilst the difference in the degree of cleavage between the units of a transfer series might be negligible, it should be noted that on no occasion did a unit with few mature eggs give a cleavage value significantly higher than that given by a unit containing a greater proportion of mature eggs. In the experiments where whole batches were used it was quite common to find that batches with few mature eggs gave better fertilizations than others apparently more mature. It is evident from the difference in behaviour between units in a transfer series and complete egg batches, that it would be possible for fundamental physiological differences to be veiled by utilizing data gained only from experiments using the whole egg batch as a unit.

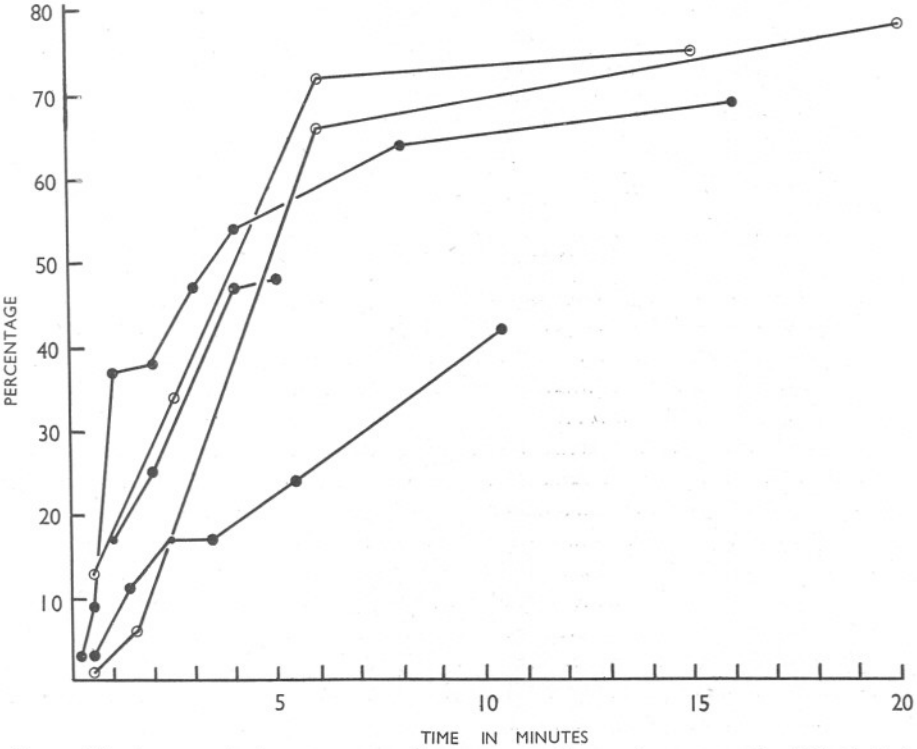

Fig. 3. The increase in the percentage of mature eggs shown when an egg batch is divided up into a number of units by the transfer method. The percentage of mature eggs in a unit is recorded against the time at which the worm was transferred from that unit.

The mechanism whereby unripe material is liberated first has not yet been elucidated. It may be dependent on the relative positions of the unripe germ cells and the segmental openings. Certainly this liberation of unripe and then ripe eggs does not result from a linear distribution in the body cavity according to ripeness. For in a series of nineteen experiments in which worms were cut in half, nine showed the anterior region to contain the higher percentage of ripe eggs and four the hinder region.

TABLE II. TRANSFER EXPERIMENTS SHOWING THE RELATIONSHIP BETWEEN MATURITY AND CLEAVAGE

The percentage of mature eggs in the unfertilized sample and the percentage cleavage after fertilization are given for each transfer unit.

	Transfer units					
Mature eggs	0	9	29	..	..	..
Cleavage	25	32	68	..	..	..
Mature eggs	1	2	1	24	..	..
Cleavage	8	9	14	24	..	..
Mature eggs	4	6	9	9	14	20
Cleavage	49	47	52	51	55	58

NATURAL SPAWNING

In view of the heterogeneity of the eggs liberated when the tube is opened, attention was turned to the possibility of collecting naturally spawned eggs. The aim was to see whether, in natural liberation, the eggs are more homogenous and whether the immature oocytes, which in artificial liberations are discharged first, are conserved.

Stones with one or more worms were kept in separate finger bowls, partially immersed in an aquarium tank and aerated. They were examined daily for signs of spawnings and, since none occurred, were opened after being two months in the aquarium. They contained high percentages of cytolysed eggs and yielded poor fertilizations. In one sample 50% were cytolysed and only 10% of the remainder were capable of fertilization; these, however, gave normal larvae. A characteristic of many of the eggs was the occurrence of vacuoles in the cytoplasm; as many as five were noted in some of the eggs. This condition was also shown by worms which had been kept in the aquarium tanks for the same period. The presence of large numbers of cytolysed eggs was common during the first three months of these artificial conditions. After that, some of the worms showed complete absence of germinal products, others showed a few normal primary oocytes and were quite free from cytolytic forms. After five to ten months a very significant change had occurred. Compared with the fresh material from the shore the egg content was about ten times as great; egg batches of 10,000–40,000 eggs being the rule. This tank material was quite normal and made possible experiments of a size which had hitherto been impracticable. Since all the worms kept in these artificial conditions contained a large store of eggs or sperm and since no natural spawnings were observed, the state must have arisen from continual production with non-liberation.

SEASONAL VARIATION

Very early in the work it was found that the possibility of obtaining a good batch of eggs, from the point of view of numbers and fertilizability, varied during the month. Daily records of maturity and cleavage were kept. In view of the fact that the results did not reveal any definite periodicity the observations were extended to include the percentage of immature oocytes, the number of eggs, the relative proportions of the different cleavage stages, and the relative numbers of ripe and unripe females.

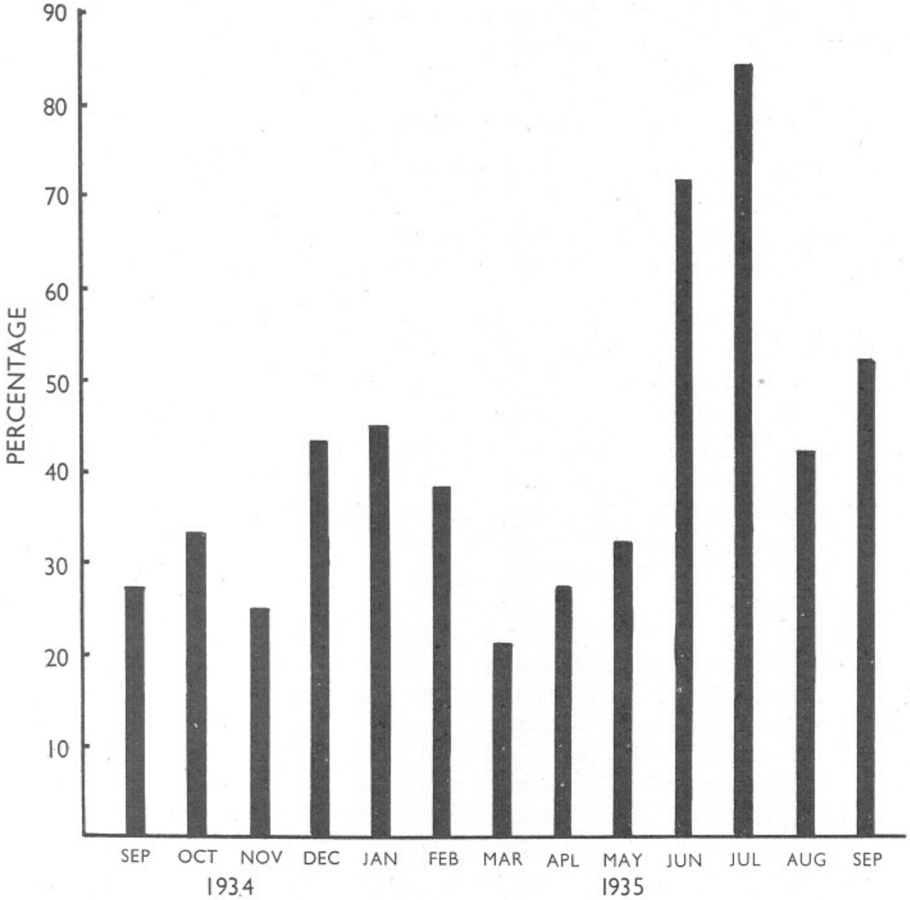

Fig. 4. The variation in cleavage throughout the year, as shown by the percentage of samples giving a cleavage value of or greater than 80 %.

No definite lunar periodicity was found. The immature oocytes, which were only recorded over a period of three months, showed no signs of a periodicity.

As regards mature eggs and cleavage there was a slight tendency for the highest values to occur about the time of the full moon, but this was not definite. The cleavage results showed a period lying between the full moons of June and July of consistently high values. This June–July deviation cannot be attributed to the existing laboratory or sea temperatures for similar temperature variations occurred at other times. The general trend of the observations suggests it to be a period of special breeding activity, although the possibility of fertilization exists throughout the year (Fig. 4).

VARIATION IN MATURITY

Whilst the data referred to above failed to demonstrate a lunar periodicity, they served a more positive purpose in that the frequency distributions of percentages of immature oocytes and of mature eggs indicated the nature of the change undergone in the transformation of an immature oocyte into a mature egg.

In the maturity distribution (Fig. 5) it will be seen that the major class consists of egg batches containing 0–10 % mature eggs. The distribution of immature oocytes (Fig. 5) is that of a skewed normal distribution. The skew is probably the result of the technique, for it was essential to obtain enough eggs for fertilized and unfertilized samples. When the number of immature oocytes was large, in particular over 90 %, the material was rarely used. The difference between the two distributions, especially when it is remembered that the mature egg is only the later stage of the immature oocyte, is remarkable. There are two possible explanations for the peculiar form of the maturity distribution.

1. That eggs are liberated as they ripen, the actual time of liberation depending on certain variable conditions. In such circumstances most females opened would show a majority of unripe eggs and the immature oocytes would be fairly constant in number.

2. That complete liberation occurs just prior to maturity, the form of the frequency distribution of maturity arising from the nature of the reactions producing maturity. If the development of maturity were dependent on a series of chemical or physical changes whose rate after initiation gradually increased, then the interval passed by a batch in the 0–10 stage would be longer than that passed in the more mature conditions. Very mature batches would, therefore, be rarely found.

The second hypothesis appears to be the more probable. The frequency distribution of immature oocytes is in keeping with it and militates against the first hypothesis. Also, the total number of eggs liberated show marked fluctuations not in keeping with the idea of gradual liberation.

VARIATION IN MATURITY THROUGHOUT THE YEAR

Table III shows the monthly variations in maturity. There was a gradual decrease in the number of higher maturity groups present as the summer months approached, culminating in July where the 0–10 class predominated. In August the higher classes again increased.

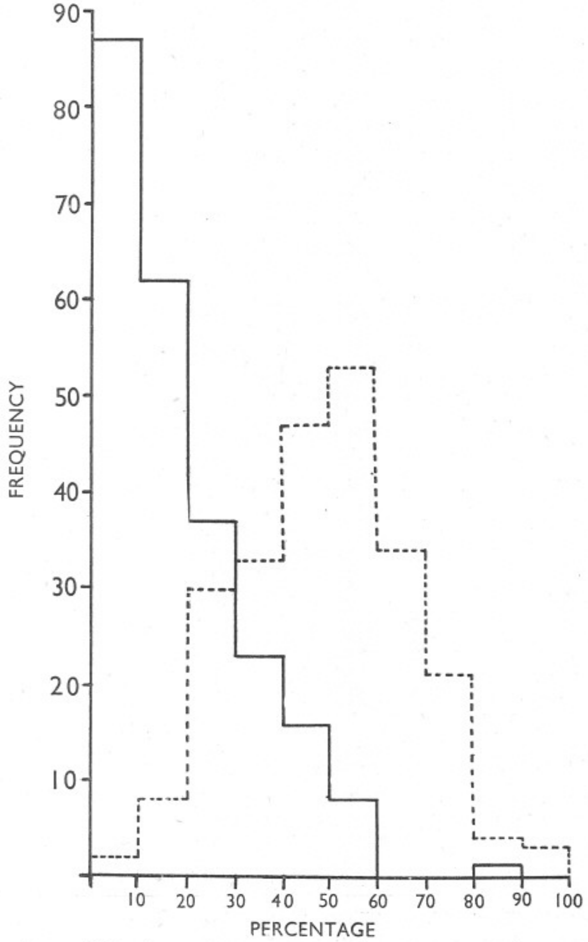

Fig. 5. A comparison of the immature oocytes and mature egg values given by the same material. Mature eggs ———, immature oocytes - - - - - - - -. Data collected June 21 to September 17 1935.

It is improbable that this monthly variation is wholly a result of the individual experiments being carried out at existing laboratory temperatures, which varied throughout the year. This conclusion is supported on the following grounds. Hörstadius has shown that up to 16° C. the percentage of mature eggs increases with rise in temperature. Above that temperature there was a decrease and at 22° C. 81 % of the eggs were deformed. If the present results arose principally from the effect of temperature at the time of the individual experiments, then the data when classified on a temperature basis should show the higher maturity values increasing with rise in temperature up to 16° C. and then decreasing. As will be seen by reference to Table IV, where the data have been reclassified, this is not so. Also, experiments to see the effect of allowing parts of the same egg batch to mature at different temperatures have shown that summer material can develop, without cytolysing, at higher temperatures than winter material (the degree of resistance of eggs to warmth depends on a number of factors and is at present being investigated). It seems reasonable to conclude, therefore, that whilst the temperature at which the experiment is conducted will undoubtedly influence the degree of maturity, it is not the principal factor concerned in producing the variation already outlined.

TABLE III. MATURITY DETERMINATIONS

This table gives the results of all maturity determinations made under standard conditions. The individual maturity percentages are classified in groups differing by 10 %, the number of results in each group being expressed as a percentage of the total number of determinations made during the month.

	Percentage maturity groups										Total
Month	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	no. of results
Dec. 1934	22	15	30	11	15	4	..	..	4	..	27
Jan. 1935	19	21	17	15	15	6	4	2	..	..	52
Feb.	29	33	19	14	..	..	..	5	..	..	21
Mar.	24	51	18	3	3	..	..	..	..	..	33
Apr.	25	33	21	4	8	4	4	..	..	..	24
May	38	28	24	7	..	3	..	..	..	..	29
June	33	28	20	9	2	4	2	..	..	2	46
July	52	19	15	2	7	4	..	..	..	..	80
Aug.	34	24	19	13	9	1	..	..	..	..	79
Sept. 1–17	24	35	12	16	4	8	..	..	..	..	49

TABLE IV. MATURITY VALUES CLASSIFIED ON A TEMPERATURE BASIS

The results are classified according to the mean temperatures of the experiments. The number of results in each percentage maturity group has been expressed as a percentage of the total number of results in that particular temperature group.

	Percentage maturity groups										Total
Temperature ° C.	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	no. of results
9·9–10·8	32	48	4	4	4	..	4	4	..	..	25
11·0–12·9	24	24	22	14	7	4	2	1	..	..	90
13·0–14·9	25	24	32	7	5	7	..	..	1	..	76
15·2–16·8	27	31	17	13	6	3	1	..	..	..	96
17·3–17·8	45	26	11	6	10	..	..	..	..	2	62
19·0–21·0	43	23	14	7	9	4	..	..	..	..	91

On p. 492 a hypothesis has been advanced to explain the form of the maturity frequency distribution. The variation in the monthly classes would signify an acceleration in the speed of the reactions concerned. Such an increase in the rate of production of ripe eggs would explain the gradual disappearance of the higher maturity values during the summer months.

DISCUSSION

The description of the eggs of *Pomatoceros* given here differs in certain respects from that of Hörstadius. Whilst the explanation might be that the earlier observations were based on a relatively small sample of eggs, the possibility of fundamental differences between the material at Cullercoats and at Kristineberg must be borne in mind.

From the viewpoint of cell permeability shape changes in eggs are of special importance, for it has been usual to treat the egg as spherical when calculating volume change. If the changes recorded here and by Hörstadius were of general occurrence then much of the work done on egg permeability would be vitiated.

The danger of static and dynamic variability leading to confusion in biological work, especially in cytological experiments, has been emphasized (Gray, 1931). Yet quantitative examinations of variability in populations of germ cells are few, although innumerable qualitative references occur in the literature of experimental cytology. A series of investigations by Goldforb has done much to record variability in the sea urchins, *Toxopneustes*, *Hipponoe* and *Arbacia*. The characters studied (Goldfarb, 1917) were size, shape, number of eggs with or without jelly layer, rate of membrane formation, and number and rate of cleaving eggs. These characters were held to be correlated and by knowing certain of them the behaviour of a batch of eggs could be forecast. Goldforb repeatedly asserts that exact forecasts of the physiological condition of eggs could be made, but his results do not always support this view. When studying the variation in agglutinating power of egg waters (Goldforb, 1929), the results given consideration are not the agglutinating times of sperm based on individual samples of egg water, but averages of the individual results. Fundamentally, it is equivalent to using mixed batches of eggs. The individual results (see tables II*a* and II*b*, Goldforb, 1929) used to give the averages often show marked differences. In one an average of 115% is obtained by averaging 300, 50, 112 and 0% (these figures represent percentage increase in agglutinating time after the egg batches have been kept for a common time). These average results are held to show "...progressive and marked increase in agglutination time with ageing of eggs". Such results indicate that whilst a trend towards "poor" and "good" might be obtained, individual batches could not be classified as such with any accuracy prior to carrying out the experiment.

Grave (1928) working on *Cumingia tellinoides* and Grave & Oliphant (1930) on *Hydroides hexagonis* record variations in the physiological condition of the germinal products, but no attempt at such a definite analysis as that of Goldforb is made.

Dalcq (1928) showed that in *Barnea candida* an increase in maturity could be produced by increasing the concentration of the potassium or calcium ion in the basic physiological solution. The relationship was in the form of a sigmoid curve. The explanation advanced was that the eggs leaving the ovary were not in a uniformly ripened series, but contained a major class which responded to a certain concentration.

Both Goldforb and Grave & Oliphant consider the poorest lot of eggs to be those giving the most rapid disintegration. This criterion is open to question. Grave & Oliphant use as their standard for disintegration two types of cytolysis. One of these, from their descriptions, bears a striking resemblance to the "pseudoblastulae" cytolysis of *Pomatoceros*. Now, in *Pomatoceros*, eggs which mature are the first to give rise to pseudoblastulae. A batch of eggs maturing rapidly after liberation will give rise to the cytolytic form before a batch of primary oocytes, although they need not be in an inferior physiological condition.

None of the work quoted offers any means whereby the effects of variation can be cut out or rendered more manageable. Fry (1936) describes a method for obtaining egg batches of common cleavage times in *Arbacia*. It amounts to carrying out tests on several batches of eggs and finding those with the same cleavage time. Such batches are then mixed and used for experiments. The problem is not so simple in *Pomatoceros*, for in this species it is clearly evident that the degree of variability among batches, especially where comparison is to be made among fertilized batches, is such as to prevent adequate knowledge of the processes leading to maturation being obtained from experiments on the whole egg batch. This variability is present in other marine worms also, for Grave & Oliphant record that for *Hydroides* "The variation in longevity within a single lot of eggs is greater than in the case of other species studied to date". Fortunately in *Pomatoceros* it is possible to divide up the egg content of a female into more uniform parts. The transfer series, as well as reducing the unwieldly extent of static variability, offers a means of studying eggs of different growth periods, which at least are uniform in that they have developed within a common environment. The use of this method can undoubtedly be advanced to the study of other worms. One drawback which exists in its application is the small size of the egg batch.

The problem of natural spawning is important, for it is in terms of the eggs so liberated that the results should be interpreted. In animals like *Pomatoceros* where primary oocytes can be successfully fertilized, it is not essential that naturally spawned eggs should be on the point of maturing. In view of the fact that the worm is capable of giving fertilizable eggs at all times of the year and that no knowledge of communal spawning exists, it is possible that liberation occurs before complete ripeness of the batch. In those circumstances the fertilizable life of the egg would be longer and the chance of fertilization greater than if it were liberated on the point of maturity.

The greater part of this work was carried out at the Dove Marine Laboratory, Cullercoats, during the tenure of a post-graduate studentship awarded by Armstrong (now King's) College, Newcastle-on-Tyne. I wish to acknowledge assistance given by the staff of that laboratory, especially Prof. A. D. Hobson under whose direction the investigation was made.

SUMMARY

The description of the material used in the present investigation does not conform with that of Hörstadius. Attention is drawn to this lack of agreement.

A hypothesis based on viscosity changes within the egg has been advanced to explain the rounding off of eggs in sea water.

By means of what is called the transfer series the egg batch has been divided into a number of parts; the last liberated eggs being the ripest. The occurrence of large numbers of egg batches giving low maturity values has been attributed to maturity resulting from a series of changes whose rate after initiation gradually increases.

Attention is drawn to seasonal changes in the reactions of the germinal products. No definite lunar periodicity is shown in the ripening of the eggs.

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SUBSTANCES CONTROLLING THE GROWTH OF A DIATOM

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The centric diatom *Ditylum brightwelli* Ehrb. is found in the English Channel during the spring and autumn; in common with many other species it "dies out" during the summer months.

Its life history has been investigated by Gross (1937*a*) who used for this purpose cultures in sterilized sea water enriched with nitrates, phosphates and soil extract, a culture medium known as "Erdschreiber" (Gross, 1937*b*). The succession of events in its life history may be epitomized as follows. The cells make vegetative divisions for a period of several months, the successive generations of daughter cells becoming narrower. Under some conditions, such as crowding, low temperature and low light-intensity, the contents of a cell may retract into a sphere (resting spore) within the skeleton, and, on change of conditions, this resting spore will grow to refill the parental skeleton. This can happen within a few hours if conditions become suitable soon after the formation of the resting spore. Normal vegetative growth then continues. After many divisions have taken place, with consequent reduction to a certain diameter, the cells become capable of forming auxospores; this takes place under certain conditions of environment, and, if it does not take place, vegetative division continues. This may continue, no auxospores being formed, until the diameter of the cells falls to 10–13μ, when they die. On the other hand, if auxospores are at any time formed, then either one of two things happens. If the environment is unsuitable the auxospores do not develop but perish; if the environmental conditions are suitable the auxospores develop into broad cells with diameters up to 100μ, several times broader than the parent of the auxospores. These broad cells after several months of vegetative division, and reduction in size, again become capable of forming auxospores.

Gross found that formation of auxospores was, *inter alia*, brought about by transferring the cells from "Erdschreiber" into natural sea water, but that in sea water the conditions were unsuitable for their further development and they perished.

I am indebted to Dr Fabius Gross for a subculture of this diatom in "Erdschreiber". During the late summer and early autumn of 1937, I found that when it was transferred to natural sea water enriched with nitrates, phosphates, silicates and iron, most of the cells formed auxospores which did not develop and the remaining cells did not make vegetative divisions. The inability of auxospores to develop in natural sea water collected in the summer was not solely due to lack of phosphates, nitrates, silicates and iron. If a small quantity of soil extract was added to the enriched natural sea water, an inoculum of actively dividing diatoms would continue to grow vigorously by vegetative division. This happened repeatedly. It was found that the soil extract could be replaced by an extract of *Fucus* or of *Ulva* which had been allowed to undergo bacterial breakdown and had been separated from the bacteria and colloids by dialysis.

Sea water collected in late October 1937 behaved quite differently from sea water collected during the summer. When this was sterilized and enriched to the same extent with phosphates, nitrates and iron, the diatoms would grow in it without any further addition of soil or algal extract. This was found to happen with samples of water collected from time to time throughout the winter of 1937 and spring 1938.

Meanwhile a second strain of this species was grown from a single cell isolated from the sea in April 1938. It made considerable growth in water collected during the spring and during the previous winter, enriched with nitrates, phosphates and iron. In water collected during July 1938, similarly enriched, both strains formed auxospores which did not develop.

The inference drawn from these observations on growth in natural sea water is that these two particular strains of *Ditylum brightwelli* require for continued growth, not only a supply of available nitrogen, phosphate and iron, but, in addition, some other accessory substance or substances, whose concentration in offshore sea water was less than the necessary limit during the summer of 1937 and 1938. In the autumn of 1937 either the accessories were re-formed or a body of water containing the accessories had moved into the area. This "*fertile water*" either lost its fertility or was gradually replaced during the early summer of 1938. Samples of offshore water collected during the autumn and winter of 1938 behaved in the same way as water collected during the summer—there was no return of "fertile water".

Accessory substances are not necessary for the growth of all species of marine diatoms. Indeed, the pennate diatom *Nitzschia closterium* forma *minutissima* has been found capable of continued growth in synthetic media free from organic matter (Peach & Drummond, 1924). Originally isolated by Allen & Nelson (1910) it has had widespread use in many laboratories, and is remarkable for the ease with which it may be kept in culture in natural sea water enriched with nitrate, phosphate and iron. However, it has been repeatedly observed that if soil extract or an algal extract is added to the enriched sea water, the rate of growth is markedly increased. This happens even if the water is heavily loaded with both iron and silicate in addition to nitrate and phosphate (Harvey, 1933). With this *Nitzschia*, the accessory substances do not act as nutrients *necessary* for growth, as they do for the two strains of *Ditylum*, but they act as a growth stimulant.

Throughout this investigation, the natural and artificial sea waters used in the experiments were enriched with 30 mg. nitrate N, 3 mg. phosphate P and *ca.* 0·1 mg. Fe as ferric ammonium citrate per litre. The quantities of nitrogen and phosphorus added are in similar proportion to each other as they are in diatoms, are sufficient for the growth of several thousand *Ditylum* cells per c.c., and are in the region of optimum concentration. The amount of iron added was considered to be in gross excess of requirements, provided it remained available (Harvey, 1937).

It was thought that silicate occurring in the various extracts which were added in the experiments might have affected growth, but experiments did not show this to be so, sufficient being derived from the glass vessels. Later, when a means of producing very luxuriant growth had been discovered, it was found necessary to add silica if more than a few thousand cells per c.c. were to develop in the culture medium.

The method used in carrying out the biological tests was to transfer *Ditylum* cells from an *actively dividing* culture to the medium used for the control, collect them by centrifuging or allowing to sediment, again suspend them in the medium and sediment, then suspend them once more in the medium and use equal volumes of this to inoculate equal volumes of the control and experimental media. The cells were then kept, either at room temperature or at 13° C., where they received nearly equal illumination, but no direct sunlight. When relatively small differences in growth rate or final "crop" were to be observed, the glass vessels were kept on a slowly rotating table, so that they averaged equal illumination, and in order that the "lens effect" in the cylindrical vessels should be eliminated.

Growth in Artificial Sea Water

Allen (1914) found that the diatom *Thalassiosira gravida* would make no growth in an artificial sea water made with glass-distilled water and pure chemicals, providing chloride, sulphate, sodium, potassium, magnesium and calcium in the proportions in which they occur in sea water, with the hydrogen-ion concentration adjusted with bicarbonate and enriched with phosphate, nitrate and iron. The further addition of bromide and iodide was without effect, but, if a very small proportion of natural sea water or of a boiling water extract of *Ulva* was added, then a rich growth of this diatom took place.

From a long series of experiments he concluded that natural sea water contained a substance or substances of organic nature which was necessary for the growth of this diatom.

Peach & Drummond (1924) investigated the growth of *Nitzschia closterium* forma *minutissima* in similar artificial sea water and found that this species would grow without any addition of accessory substances. This species appears unique among marine diatoms for the adverse conditions, fatal to other species, which it will withstand. Allen (private communication) repeated Peach & Drummond's experiments with similar result, but observed that better (more rapid) growth was brought about by the addition of a small proportion of natural sea water or of *Ulva* extract. It has been repeatedly observed by the writer that the addition of such extracts hastens the growth of *Nitzschia*, even in sea water heavily enriched with inorganic nutrients.

A similar "artificial sea water" was made and enriched with nitrate, phosphate and iron. Neither strain of *Ditylum* would grow in it. With the addition of 5 % of natural winter sea water a rather poor growth resulted, while with the addition of soil or of algal extracts a considerable growth took place. The very small additions, which had been found by Allen to suffice for the growth of *Thalassiosira*,* were insufficient for the growth of *Ditylum*.

It is thought that the *Nitzschia* species can itself make the organic accessories which it needs for continued growth, that is to say that the accessories are of the nature of phytohormones. In support of this hypothesis are the observations that addition of "accessories" to the water causes more rapid growth, and an observation that it grows more rapidly in water in which the species has been growing previously (Harvey, 1933).

Experiments on the growth of *Ditylum* soon showed that the active principles which are present in soil extract and in algal extracts could be separated into two groups. The one, which will be termed group "A" accessories, was readily adsorbed on carbon and was insoluble in aqueous butyl alcohol. The other, group "N" accessories, was not adsorbed on carbon and was soluble in aqueous butyl alcohol.

The addition to "artificial sea water" of "A" alone, or of cystine which has been found capable of replacing it (p. 504), would not allow a vigorous growth of *Ditylum*. There was some other factor "N" in soil and algal extracts and in natural sea water which was not adsorbed on carbon and which was necessary in addition to "A" for a vigorous growth and considerable crop of *Ditylum*.

The action of the group "N" accessories is complementary to the action of group "A". The increase in growth of *Ditylum* brought about by the addition of "A" and "N" together exceeds the sum of the increases brought about by the addition of each singly; the effect of "A" is enhanced by the presence of "N" and vice versa.

The following experiments are typical.

Ditylum cells were washed in "artificial sea water", and equal volumes of media were inoculated with equal volumes of the *Ditylum* suspension on February 14. On February 28 counts were made with the following result:

Nature of medium	Cells per c.c.
Artificial sea water, enriched N, P and Fe	180
,, ,, + *Fucus* extract*	*circa* 2200
,, ,, + carbon adsorbed fraction†	678
,, ,, + non-adsorbed fraction†	517
,, ,, + carbon adsorbed† and non-adsorbed fractions†	1818

The *Fucus* extract had been broken down by bacteria and dialysed. The quantity * added per litre of artificial sea water was derived from 14 g. of *Fucus*. After fractionating by adsorption on carbon, the quantity of either fraction † added per litre of water was derived from 20 g. of *Fucus*.

The increase of cells per c.c. was

498 due to the addition of the adsorbed fraction,
1301 due to the addition of the adsorbed fraction in the presence of the non-adsorbed fraction.

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* The extract from 0·5 g. of *Ulva* added to 1 l. of "artificial sea water" was sufficient for the growth of *Thalassiosira*.

In another experiment, carried out in the same manner, the following counts after 8 days' growth (March 15–23) were made:

Nature of medium	Cells per c.c.
Artificial sea water enriched N, P and Fe	70
,, carbon adsorbed fraction from *Fucus* extract	145
,, non-adsorbed fraction from *Fucus* extract	225
,, non-adsorbed + adsorbed fraction	780
,, ,, + adsorbed fraction from "winter" sea water	590
,, ,, + cystine (20 mg./l.)	1600
,, Cystine (20 mg./l.)	330

The increase in number of cells per c.c. was

260 due to the addition of cystine,

1375 due to the addition of cystine in the presence of the non-adsorbed fraction,

75 due to the addition of the adsorbed fraction,

555 due to the addition of the adsorbed fraction in the presence of the non-adsorbed fraction.

The complementary effect of the two fractions was even more marked in several other experiments. Thus in one experiment the increase in number of cells per c.c. was

109 due to addition of cystine alone,

1636 due to addition of cystine alone in presence of non-adsorbed fraction from *Fucus*.

ON THE NATURE OF "A" ACCESSORIES

Soil extract has been extensively used as a source of "growth stimulant" or "accessory nutrients" in rearing various marine larvae, Protozoa and algae *in vitro*. It appears that its effect is not limited to very simple organisms. Pringsheim, who has reviewed the literature (1936), has found from his own experiments on flagellates that the active principle was adsorbed on carbon and was insoluble in ether and in alcohol.

From experiments on the growth of *Ditylum* in enriched sea water collected during the summer of 1937 it was found that an active principle was adsorbed on carbon from either neutral or acid soil and algal extracts. It could be eluted from the carbon by a solution of ammonia or by boiling with acid alcohol and subsequent treatment with alkali. The addition of this eluted preparation to enriched "summer" sea water rendered it capable of growing a considerable crop of *Ditylum*.

The most convenient method was to boil soil extract, or algal extracts which had undergone bacterial decomposition, or natural sea water, with "decolorizing carbon" which had previously been heated to a dull red and washed with hot hydrochloric acid. The carbon, after washing with water, was then eluted at least twice with a hot $2\frac{1}{2}$ % solution of ammonia in 60 % acetone. The solution was evaporated, dissolved in dilute sodium bicarbonate, dried to get rid of ammonium, and then neutralized.

It was also found that on heating under a reflux with acid alcohol, the active "A" principle passed into solution, presumably as an ester or internal anhydride. On drying and heating with sodium bicarbonate to saponify, active preparations were obtained. The active principle, as Pringsheim had found, was not dissolved in boiling alcohol or ether from either acid, alkaline or neutral residues.

The "ester" after freeing from acid alcohol was sometimes insoluble and sometimes partly soluble in ether. Ether solutions, which were active after saponification, were obtained in some instances, but not always.

It is concluded from these experiments that part at least of the "A" activity is due to an organic acid. The difficulty of obtaining an ether solution of its "ester" suggests that it has either basic properties or many hydroxyl groups.

The concentration of the active principle from extracts of algae presented difficulties, mostly owing to its being readily adsorbed on the impurities when they were precipitated. Continuous extraction with warm aqueous butyl alcohol proved useful, as this removed many of the amino acids and left the "A". To assess the losses which occurred during each step of a process was also impracticable during the earlier steps, since the addition of a crude extract to sea water led to a considerable development of bacteria which sometimes, but not always, interfered materially with the growth of the diatoms.

The proliferation of bacteria in a diatom culture is usual when the diatoms start to die and their soluble organic constituents diffuse into the water, providing food for the bacteria. It has been frequently considered that the growth of bacteria kills the diatoms, and this undoubtedly happens in some cases. In other cases, the growth of bacteria appears to be the effect rather than the cause of the diatoms' death. In many instances it has been noticed that the addition of an algal extract has led to the culture becoming cloudy with bacteria, but, in spite of this, the addition has brought about increased growth of the diatom.

If the addition of a preparation led to little or no increased growth there was always the possibility that, although active, its effect had been offset by the growth of bacteria. Moreover, a falling off in activity has been noticed in many of the preparations, some of which became entirely inactive in the course of a few weeks. The growth of bacteria appears to have little or no destructive effect on active preparations, but the growth of moulds destroys the active principle.

With regard to the quantities of these algal extracts which were necessary to show a well-marked effect on the growth of *Ditylum*, it was found that the extract from 5 g. of *Fucus*, made by boiling with *N*/10 HCl, drying, washing with alcohol and ether and neutralizing, was about the minimum necessary for addition to 1 l. of sterilized "summer" sea water.

Several organic acids, or chemical groups which occur in these acids, are known, which affect the growth of plants and Protozoa, in some cases being *necessary* for the growth of a particular species in artificial media. Hammett (1930) has found that a number of compounds containing divalent sulphur affect the growth of plants and *Paramoecium*; of these substances cystine is typical.

The effect of adding *l*-cystine to water collected during the summer of 1937 was tried and found to give positive results. It brought about a moderate growth provided it was added in relatively large quantity, 10 mg./l. or more. At concentrations of 1 mg./l. or less it had only a slight effect. Later experiments with natural sea waters suggested that 0·5 mg./l., or possibly rather less, may materially increase the growth of this diatom, provided iron, manganese, silica and naturally occurring organic compounds were present in sufficiency.

It is thought that "A" occurring naturally in sea water is unlikely to be cystine only, although this occurs abundantly in marine algae (Mazur & Clark, 1938), and is readily adsorbed on carbon under the conditions employed in this investigation. In the first place the total organic matter in sea water probably does not exceed 5 mg./l. (Keys, Christiensen & Krogh, 1935), and in the second place an active preparation has been obtained by adsorption on carbon from an algal extract which had been deaminated.

Cystine, however, has proved most valuable throughout these experiments as a *substitute for group A*, and, incidentally, for keeping stock cultures healthy and actively dividing.

Glutathione which contains the same $—S.CH_2.CH(NH_2)COOH$ group and methionine $CH_3.S.CH_2.CH(NH_2)COOH$ were found to act in the same way as cystine.

It is known that the addition of aneurin (vitamin B_1) is effective in promoting the growth of various moulds, bacteria and the embryos of higher plants in synthetic media, and that in some cases its immediate degradation products are equally effective.

The effect of adding aneurin chloride in place of "A" accessories was then tried. It was usually found to be active in concentrations down to about 0·25 mg./l.

Growth of *Ditylum* after 10 days (May 13–24) in artificial sea water containing nitrates, phosphates, iron, "additional salts" and N_R (see p. 507):

	Cells per c.c.
With no further addition	526
With 1·25 mg./l. aneurin chloride	1170
With 0·25 ,, ,,	1000
With 0·05 ,, ,,	700

Again, the concentration of aneurin is much higher than could be expected to have occurred in sea water during the winter of 1938, and, moreover, in several instances it has been found to have no effect.

Another pure substance, the supply of which affects the growths of higher plants, yeasts and moulds, is biotin, the bios II of yeast, a basic sulphur containing acid, isolated from the yolk of eggs by Kögl & Tonnis (1936) after a long series of fractionations which included adsorption on carbon and "esterification". Starting from an extract of egg yolks and following the earlier stages of fractionation (precipitation of impurities with 50 % acetone, 85 % alcohol, normal and basic lead acetate, followed by adsorption on carbon), a crude preparation was made of which the biotin content was calculated from Kögl & Tonnis's data. This was found to be active, replacing "A", in experiments with *Ditylum* in artificial sea water. Provided that its activity was due entirely to its biotin content and not to impurities, the concentration necessary was of the order of 0·005–0·01 mg. biotin per litre.

This is more nearly approaching a concentration which might conceivably occur in the sea; moreover, the active principle in algal extracts behaves like biotin in so far as its properties have been observed.

It has been found by Kögl & Haagen-Smit (1936) that aneurin and biotin together had more effect on the growth of pea embryos in synthetic media than the effect of each singly. Kögl & Fries (1937) have found that some moulds require aneurin while others need biotin also for growth in synthetic media. With this in view, the effect of a mixture of 0·002 mg. aneurin and 0·0005 mg. "biotin" per litre was tried in place of "A", but found inactive towards the growth of *Ditylum* at these dilutions.

These experiments show that "A" activity is not limited to a single compound, and lend support to the idea that "A" occurring naturally in sea water may be a group of substances. It is perhaps significant that both aneurin, biotin, cystine and the related compounds glutathione and methionene all contain divalent sulphur.

ON "N" ACCESSORIES OCCURRING IN ALGAL EXTRACTS

The crude preparations of "N", left after adsorbing the "A" content on carbon from algal or soil extracts, would naturally contain traces of various elements which may be beneficent or necessary for the growth of *Ditylum* and may be absent in this artificial sea water. Boron, which is present to the extent of some 5 mg./l., is an instance, none having been included in the artificial sea water.

It was found that the addition of borate did cause improved growth of *Ditylum*, and the further addition, to the extent of 0·025 mg./l., of copper, manganous, arsenic as arsenate, cobalt and zinc salts caused an even slightly better growth. It is seen from the following experiment that these "additional salts" did not take the place of "N".

Equal quantities of *Ditylum* were used to inseminate equal volumes of artificial sea water enriched with nitrates, phosphates and iron. After 7 days' growth (March 25–April 1) the following populations were found:

	Cells per c.c.
In artificial sea water	46
,, ,, + cystine 20 mg./l.	160
,, ,, + cystine + "additional salts"	315
,, ,, + cystine + non-adsorbed fraction of *Fucus* extract	1190

The further addition of iodate, iodide and bromide was without apparent effect.

Experiments with non-adsorbed fractions of algal extracts showed that the active "N" principle was insoluble in ethyl and butyl alcohol, in acetone and in ether, but soluble in warm aqueous butyl alcohol. It was destroyed by ashing and by boiling with acid alcohol, suggesting that it is an organic compound.

It was found that treatment with normal aqueous hydrochloric acid at boiling-point, with subsequent evaporation and neutralization, made the fraction more active. It is concluded that part of the "N" occurred in combination in the algal extract and was set free by this treatment. Similar treatment with sodium bicarbonate had little effect on the activity. The extent to which such activation is brought about by evaporating with normal hydrochloric acid is shown in the following experiment:

Equal quantities of *Ditylum* were added to equal volumes of artificial sea water enriched with nitrate, phosphate, iron, "additional elements" and cystine. After 7 and 14 days' growth the number of cells were counted (April 12–26):

	After 7 days	After 14 days
No addition	86	95
+non-adsorbed fractions of *Fucus* extract	230	1875
+same treated hydrochloric acid	267	4060

Fractionation of *Fucus* extracts after the "A" had been adsorbed on carbon was attempted. It was found that the active "N" principle was not precipitated by normal lead acetate, but was partly precipitated by basic lead acetate, from which it could be regenerated by the action of sulphuretted hydrogen.

The fraction so obtained, which will be termed "N$_R$" when added to artificial sea water together with "A" or cystine or aneurin chloride or crude "biotin", allowed a vigorous growth of *Ditylum* to take place with the production of some thousand cells per c.c.

Several preparations of "N$_R$" have been made from algal extracts which were first treated with normal lead acetate, filtered, and then made alkaline with ammonia; the washed precipitate was suspended in water made acid with HCl, treated with sulphuretted hydrogen for 24 hr. or longer, boiled and filtered clear from lead sulphide. After boiling with carbon the solution was evaporated, dissolved in dilute sodium bicarbonate and again dried to get rid of ammonium. After neutralization, these preparations showed an activity complementary to accessory "A" or cystine.

The growth which occurred in five illustrative experiments is shown in the following table. The experiments were made in the manner already described:

Nature of medium	Cells per c.c.
Exp. (April 12–19).	After 7 days
Artificial sea water	27
,, ,, +adsorbed fraction from *Ulva*	25
,, ,, +non-adsorbed fraction from *Fucus*	73
,, ,, +adsorbed+non-adsorbed fractions	206
,, ,, +N$_R$	34
,, ,, +N$_R$+adsorbed fraction+"additional salts"	150

Exp. (April 25–May 5).	After 7 days	After 10 days
Artificial sea water containing "additional salts"	29	*circa* 29
,, ,, +N$_R$	42	74
,, ,, +aneurin chloride 0·25 mg./l.	29	*circa* 29
,, ,, +N$_R$+aneurin chloride	250	320
,, ,, +cystine 20 mg./l.	119	156
,, ,, +cystine+N$_R$	590	*circa* 590
*Natural sea water (sample *a*) collected April 1938	461	860
* ,, ,, (sample *b*) collected January 1938	220	350

* Enriched with nitrate, phosphate and iron to the same degree as the artificial sea water.

Nature of medium	Cells per c.c.
Exp. (May 13–23).	After 10 days
Artificial sea water containing "additional salts"	184
,, ,, $+N_R$	254
,, ,, +cystine 20 mg./l.	456
,, ,, $+N_R+$cystine	1120
Exp. (March 29–April 4).	
Artificial sea water	61
,, ,, $+N_R$	82
,, ,, +cystine 20 mg./l.	170
,, ,, +cystine$+N_R$	645
,, ,, +non-adsorbed fraction from *Fucus*	84
,, ,, +non-adsorbed fraction+cystine	1720
Exp. (April 30–May 9).	After 6 days
Artificial sea water with "additional salts" and with N_R	42
,, ,, +cystine 10 mg./l.	372
,, ,, +biotin 0·01 mg./l. containing "impurities"	277
,, ,, +biotin 0·001 mg./l.	122
* Natural sea water collected January 1938	561

* Enriched with nitrate, phosphate and iron to the same degree as the artificial sea water.

It was particularly noticeable that, in order to obtain a good growth of *Ditylum* in artificial sea water, preparations of "A" and "N" derived from relatively large quantities of algae were required. It is assumed that losses in preparation were great. The additions of an extract of *Fucus*, which had undergone bacterial breakdown and had not been subjected to further fractionation, allowed a considerable growth of *Ditylum* when added in much smaller quantity.

It was observed that preparations of "A" and "N" were liable to lose activity on keeping. Sometimes they lost their activity within a few weeks; in one instance this happened with a preparation which had been kept with its activity apparently unimpaired for several months previously.

The growth of *Ditylum* in artificial sea water with added accessories was rarely so good as in a natural, freshly collected "1937–8 winter" sea water. No general conclusion can be drawn, but it does seem likely that the "A" and "N" in "fertile" sea water are both groups of substances, and that the preparations used in these experiments did not contain all the accessories needed for optimum growth, although they sufficed for a moderate growth owing perhaps to their having been added in large quantity.

These experiments with artificial sea water have indicated that "A" accessories—replaceable by the $\text{—S—CH}_2\text{CH(NH)}_2\text{COOH}$ group—are necessary for the growth of *Ditylum*. They even suggest that, for this species and for the media employed, "A" accessories act as a limiting factor in the sense used by Blackman. An alternative view, perhaps better justified by subsequent experiments is that there are a number of accessory or growth promoting substances, including the potent $\text{S—CH}_2\text{—CH(NH)}_2\text{COOH}$ group, an increase in any one of which *tends* to bring about increased growth, and will do so if other, interacting, substances are present.

CONCERNING THE "PHYSIOLOGICAL STATE" OF THE INOCULUM

When inoculating a series of samples of media to test their relative capacity to support a growth of *Ditylum*, actively dividing cells from a stock culture were always used. Excellent agreement between their growth in duplicate samples was obtained. On repeating the experiments, using actively dividing cells, usually from a subculture of the stock previously used, similar results were usually obtained. In the repeat experiments conditions for growth were not *identical* with the conditions in the previous experiment. In particular, the illumination differed during the course of the two experiments. Occasionally it happened that the repeat experiments did not show the beneficial effects of an added accessory which were shown in the previous experiment; this happened several times when using vitamin B_1 in place of "A".

It was apparent that the response of the inoculum to the conditions of the experiment—light, quantities and nature of the "A" and "N" accessories added, temperature, etc.—depended to some extent on the "physiological state" of the *Ditylum* cells in the inoculum. For instance, it was noticed that insemination with very rapidly dividing cells, such as occurred in a stock culture during bright weather at 13°, tended to give a greater crop than an insemination with less rapidly dividing cells, as from a stock culture at 8°, or one at 13° in which a considerable growth had already taken place, the growth rate had started to slow down and the cells had become darker in colour than during the earlier and more rapidly growing phase.

The optimum conditions of light, for growth of a diatom, appear to depend to a large extent upon the conditions under which the diatom has lived previously, that is upon its "physiological state". This is illustrated in the following experiment:

A culture of *Biddulphia mobiliensis* was divided into two parts. One was then grown in a north window in relatively dim December light. The other portion was grown near an electric bulb immersed in a bowl of running water; it received continuous light of some 18,000 lux. The illumination was measured with a photometer which Dr W. R. G. Atkins had very kindly rated for me in diffuse daylight of varying intensities against a similar photometer which had been standardized in artificial mean noon sunlight. At the end of a week, subcultures of each of these cultures were made in sea water enriched to the same extent. Each subculture was divided into ten glass vessels. They were immersed in two water-baths, one kept at 13° C., the other at 18° C., at different distances from an electric bulb which was immersed in each bath. The light which each vessel of culture received was measured. After 72 hr. continuous light, a sample from each vessel was taken out, poisoned, and the diatoms per c.c. counted. From these counts the percentage increase in number of cells which had taken place during the 72 hr. was calculated:

	Temp. ° C.	Percentage increase in 72 hr. at				
		28,000	18,000	8000	4100	1400 lux
Cells grown previously in dim light	18°	7	68	66	106	87 %
	13°	24	16	14	8	2 %
Cells grown previously in continuous light at 18,000 lux	18°	171	236	190	123	98 %
	13°	31	70	105	67	36 %

This experiment is interpreted as showing that the optimum light intensity varies both with the "physiological state" of the diatom and with the temperature, and, furthermore, that the effect of temperature on growth rate varies with both the light intensity and the physiological state of the diatom.

In view of this experiment it is not surprising that similar results were not invariably obtained in experiments carried out at different illuminations and with inseminations of *Ditylum* which had lived under different conditions. In these experiments the nature and quantity of the added substances were controlled, but the other two variables—"physiological state" of the inoculum and light intensity—were not controlled. Moreover, it is possible that differences in the "physiological state" of the diatom are bound up with differences in its capacity to synthesize factors which it requires for vigorous growth, or to utilize partly preformed substances in the external medium for the final production of its requirements. Thus it has been found for some species of bacteria, that they lose their power of growing in synthetic media if they have been cultured persistently in a medium containing a preformed "factor".

Some observations made in particularly dull weather during the winter of 1938 suggest that, for slow growth, the requirements of *Ditylum* are different from its requirements for rapid growth during bright weather. It was noticed that the response to addition of organic compounds having "N" activity was less than during bright weather.

GROWTH IN NATURAL SEA WATER

When actively growing cells of *Ditylum* were transferred to natural sea water, collected several miles offshore at intervals during the summer of 1937 and enriched with nitrate phosphate and iron citrate, they ceased making vegetative divisions and many of the cells formed auxospores which did not develop. In water collected in late October 1937, enriched in the same way, the cells continued vegetative growth, and this ability of the water to support vegetative growth was found in samples collected at intervals during the winter and following spring, mostly at a distance of 20 miles offshore. By June 1938 there was a falling off in the growth which the water would support after enrichment.

Water collected from the surface on July 12, filtered and enriched, allowed only a few divisions to take place. Water collected on July 24 from 5, 15 and 50 m. at a position 20 miles offshore would allow no more than some two divisions to take place before either auxospores developed or the cells died.

On July 28 water samples collected on July 12 and 24 were filtered, sterilized and enriched with N, P and Fe in identical manner, they were inseminated with 21 cells of *Ditylum* (strain T) per c.c. On August 2 counts were made:

	Vegetative cells	Auxospores
Surface water of July 12	268	55 per c.c.
From 15 m. July 24	4	65
,, 5 ,, ,,	Similar to 15 m.	
,, 50 ,, ,,	Similar to 15 m.	

On July 29 a similar experiment was made using strain G *Ditylum* of which 19 cells per c.c. were added. On August 3 counts were made:

	Vegetative cells	Auxospores
Surface water of July 12	106	38 per c.c.
From 15 m. July 24	10	30

These waters collected in July 1938 behaved towards *Ditylum* in the same way as waters collected at intervals during the summer of the previous year.

The July waters responded to the addition of cystine alone, allowing a healthy growth and moderate crop of *Ditylum*, with the exception of water from 15 m. collected on July 24. This sample only gave a poor growth with the addition of cystine, but with the further addition of an *Ulva* preparation containing "N" it behaved as the others did with the addition of cystine only, giving a healthy and vigorous growth.

In the course of experiments on the growth of *Thalassiosira gravida* in artificial sea water Allen (1914) had observed that the addition of a small percentage of sea water from the aquarium tanks was more effective in promoting a vigorous growth of the diatom than similar additions of water collected from the sea. "The tanks of the Plymouth Aquarium are worked on a closed system of circulation, the same sea water being circulated over and over again, so that the principal difference between the water taken from them and that obtained from outside consists in the greater abundance in the tank water of organic compounds, which result from the metabolism of living organisms." He further observed that "on the whole, samples of water taken from Plymouth Sound give better growths than are obtained from samples from the English Channel in the neighbourhood of the Eddystone". The water in Plymouth Sound contains more organic matter in solution than water from the open sea. It receives a continuous discharge of sewage in addition to land drainage.

On August 12 samples of water were collected from

(*a*) The entrance to Plymouth Sound, off Penlee Point, at half ebb from near the surface.

(*b*) A position 6 miles offshore from near the surface.

(*c*) A position 20 miles offshore from near the surface.

(*d*) A position 20 miles offshore from 5 m. depth.

(*e*) A position 20 miles offshore from 50 m. depth.

Each sample was filtered, heated, and enriched with nitrate, phosphate and iron on August 17 in identical manner and inseminated with the same quantity of *Ditylum* cells washed with and suspended in artificial sea water. The cells were of various sizes, some narrow and long, others broad and short which had developed from auxospores about a week previously; the initial population of added cells was 28 per c.c. Two days later auxospores had developed in the waters from 5 and 50 m. depths.

On August 22 counts of the living cells were made:

	Cells per c.c., including auxospores
In water from surface, 20 miles offshore	284, many auxospores
,, ,, 5 m., 20 miles offshore	53, nearly all auxospores
,, ,, 50 m., 20 miles offshore	138, nearly all auxospores
,, ,, surface, 6 miles offshore	238, many auxospores
,, ,, surface, off Penlee Point	1800, no auxospores

As part of this experiment the same series was also grown, with the addition of 10 mg./l. of cystine. The same quantities of the same *Ditylum* were used to inseminate the waters. The same light conditions and temperature prevailed, the cultures being kept on a revolving table. On August 22 counts were made:

	Cells per c.c. including auxospores
In water from surface, 20 miles offshore + cystine	2400, no auxospores
,, ,, 5 m., 20 miles offshore + cystine	437, some auxospores
,, ,, 50 m., 20 miles offshore + cystine	284, nearly all auxospores
,, ,, Surface, 6 miles offshore + cystine	*circa* 2400, no auxospores
,, ,, Surface, off Penlee Point + cystine	2500, no auxospores

Similar results were obtained in further experiments. With the onset of winter of 1938 there was no change in the inability of offshore water to support a vegetative growth of *Ditylum*. Waters collected on November 4 and 29 1938 and January 2 1939 proved singularly infertile after enrichment with nitrate, phosphate and iron.

Meanwhile a series of experiments had shown that lack of manganese in natural sea waters could render them infertile for *Ditylum*. The addition of 20 mg. Mn per cubic metre to the November 4 and 29 waters in both cases made them capable of supporting a considerable, yet limited, growth of *Ditylum*, some 2000 cells per c.c. being produced.

Offshore sea water collected on November 29 1938 and on January 2 1939 were filtered, heated to 90° C. and enriched with N, P and Fe in identical manner on January 6, when both were inseminated with 26 cells of *Ditylum* per c.c. Additions of cystine and manganese were made and the cultures kept at 13° C. in glass vessels resting on a mirror below a skylight in order to increase illumination. Counts were made at intervals with the following result:

	Cells per c.c. after				
	3 days	5 days	9 days	13 days	22 days
January 2, water:					
No further addition	40	38*a*	*d*	..	..
No further addition	37	26*a*	*d*	..	..
+ 5 mg./l. cystine	62	66	*d*	..	..
+ 2 mg./m.^{3} manganese	59	85	223*a*	428*a*	..
+ 10 mg./m.^{3} manganese	62	100	540	1110	2420
November 29, water:					
No further addition	43	40*a*	*d*	..	..
No further addition	33	33*a*	*d*	..	..
+ 5 mg./l. cystine	40	35	*d*	..	..
+ 10 mg./m.^{3} manganese	72	129	613	1560	1950
+ 10 mg./m.^{3} manganese, 0·25 mg./l. cystine	86	156	860	2130	2150
+ 10 mg./m.^{3} manganese, 1 mg./l. cystine	79	180	920	2040	2540

a denotes presence of auxospores. *d* denotes disintegrating cells.

With the freshly collected January water, adding a considerable dose of cystine prevented auxospore formation but had little effect on growth. Adding manganese, on the other hand, had a marked effect on growth.

With the water collected on November 29, cystine alone had no effect on growth but prevented auxospore formation. In the presence of an ample sufficiency of manganese, the effect of added cystine became apparent even at a concentration of 0·25 mg./l.

The water collected on January 2 only made a poor growth with the addition of 2 mg. Mn/m.^{3}, so an experiment was made to find out whether the effect of small additions of manganese was considerably augmented by small additions of cystine. It showed that the addition of 0·5 mg. Mn/m.^{3} had little effect and the addition to this of 0·5 mg. cystine per litre brought about only a slight further increase. This small quantity of cystine caused only a small increase to the growth brought about by the addition of 2 mg. Mn/m.^{3} when added in conjunction with it.

EFFECT OF STORAGE ON NATURAL SEA WATER

A quantity of offshore sea water collected in January 1938 was filtered and, after enrichment with nitrates, phosphates and iron, found able to support a healthy growth of *Ditylum* yielding a moderate crop. Unfortunately no counts were made of the final crops these "fertile 1937–8 winter waters" would yield—it is assessed as some 2000 cells of mixed diameters per c.c.

After being stored in a glass vessel for several months, it was noticed that this water would no longer support a good growth. By the end of July it would only allow a few cell divisions to take place. The growth was not greatly increased by the addition of cystine, nor was it increased by keeping the culture cool and in a dim light approaching more closely the physical conditions earlier in the year.

The effect of adding an extract of *Ulva*, from which the "A" active substances had been adsorbed on carbon, was tried. The addition of this alone did not lead to a vigorous growth such as occurred when both this and cystine were added together.

A carboy of open sea water had been collected in December 1937 and stored, unfiltered. When this was examined in September, after nine months' storage, it was found to behave in the same way as the water stored after filtration in a flask. After enrichment with nitrates, phosphates, and iron and insemination with the diatoms, these made at most one or two vegetative divisions before forming auxospores, which did not develop. The addition of cystine alone brought about a very slight increase in vegetative growth before auxospore formation set in. The addition of yeast extract which had been freed from "A" by continuous extraction with butyl alcohol had almost no effect. The addition of both cystine and extract caused a healthy and vigorous vegetative growth—approximately 1600 cells per c.c. being produced.

ON THE NATURE OF ORGANIC "N" ACCESSORIES

Some information concerning the nature of "A" accessories had been obtained from the experiments with artificial sea water, but virtually no information concerning the "N" group except that an organic substance or substances were probably a necessary part. The next step was clearly to investigate the "N" substances lacking in stored sea water. As a preliminary a number of physiologically active compounds, including heteroauxin, vitamins B_2 and C, β-alanine and nicotinic acid, were tried out for "N" activity without result. Having arrived at this impasse, the following line of reasoning provided the first clue:

(i) A very active preparation of "N" had been obtained by continuous extraction of a yeast extract with (aqueous) butyl alcohol, a process which carries over many of the amino acids.

(ii) It had been noticed that a heavy dosage with cystine of artificial sea water would allow a certain but limited amount of growth by the diatom. This suggested that the $—CH_2—CH(NH_2)COOH$ grouping in cystine itself had a small measure of "N" activity. With this possibility in view the effect of *dl*-α-amino-propionic acid (α-alanine) was tried as a substitute for "N".

When added in concentrations between 1 and 10 mg./l. to stored sea water enriched with nitrates, phosphates, iron, cystine and in some cases manganese, it brought about an increased growth of the diatom in twelve experiments, and no increase in one experiment. Moreover, the effect was complementary to that of cystine in the two following experiments:

Sea water collected January, filtered and stored, enriched with N, P, Fe and inseminated with *Ditylum* September 2 1938. Counts made September 8:

	Cells per c.c.
No further addition	211
10 mg./l. cystine	616
10 mg./l. *dl*-α-alanine	459
10 mg./l. cystine + 10 mg./l. α-alanine	1800

Similar procedure; inseminated September 5, counts made September 10:

	Cells per c.c.
No further addition	135
5 mg./l. cystine	510
5 mg./l. *dl*-α-alanine	266
5 mg./l. *dl*-α-alanine + 5 mg./l. cystine	1450
1 mg./l. *dl*-α-alanine + 5 mg./l. cystine	1250

It is noteworthy that the addition of α-alanine alone had little effect. Subsequent experiments suggested the possibility that the activity of α-alanine and other organic substances might conceivably be due to traces of impurity, such as manganese, but the relative inactivity of these organic substances when added alone without cystine is opposed to this possibility.

Experiments with this and other substances to find whether their addition caused growth in stored sea water enriched with cystine are summarized in the following table, and show that a number of related amino acids do not share the activity of α-alanine.

The effect of adding lactate, in which the amino group occurring in α alanine is replaced by a hydroxyl group, was tried and this was found to be active. Its activity is shared by a number of hydroxy compounds as shown in the table. It may be significant that these hydroxy compounds share the property of forming complexes with iron and with manganese, rendering both metals less readily precipitated in alkaline media. Thus gluconic acid forms complexes with the salts of both metals which are not precipitated in *N*/2 sodium hydroxide.

	Increased growth	No increased growth
dl-$CH_3CH(NH_2)COOH$ *dl*-α-alanine	In 12 exp.	In 1 exp.
d-$CH_3CH(NH_2)COOH$ *d*-α-alanine	1 ,,	3 ,,
$CH_2(NH_2)CH_2COOH$ β-alanine	0 ,,	2 ,,
CH_3CH_2COOH propionic acid	0 ,,	2 ,,
dl-$CH_3CH(OH)COOH$ *dl*-lactic acid	2 ,,	0 ,,
$CH_2(OH)COOH$ glycollic acid	0 ,,	2 ,,
$CH_2(NH_2)COOH$ glycine	Slight in 2 exp.	1 ,,
$CH_3COCOOH$ pyruvic acid	1 exp.	1 ,,
l-$(CH_3)_2.CH.CH_2.CH(NH_2)COOH$ *l*-leucine	Slight in 1 exp.	1 ,,
d-$(CH_3)_2.CH.CH_2.CH(NH_2)COOH$ *d*-leucine	0 exp.	1 ,,
$C_8H_6NCH_2CH(NH_2)COOH$ *l*-tryptophane	0 ,,	3 ,,
dl-proline	0 ,,	2 ,,
$C_5H_6(OH)_5COOH$ gluconic acid	5 ,,	3 ,,
d-$C_5H_6(OH)_5CHO$ dextrose	9 ,,	0 ,,
l-$C_5H_6(OH)_5CHO$ laevulose	1 ,,	Doubtful in 1 exp.
$C_6H_6(OH)_6$ inosite	Slight in 3 exp.	1 exp.

In the majority of experiments the quantity added was 2 mg./l., and in many of the experiments the increased growth took place in the presence of added manganese.

It was noticed that the effect of α-alanine appeared less marked as the days became shorter and the diatoms were growing less rapidly. The experiments made in early September with water collected during the previous January were repeated during a period of dull weather starting on November 4 when the samples were inseminated with *Ditylum*. Counts were made on November 7, 9 and 12, when many cells had disintegrated.

	Cells per c.c.		
	Nov. 7	Nov. 9	Nov. 12
January water enriched N, P, Fe	94	66	38
Ditto + 2 mg./l. α-alanine	103	78	39
Ditto + 5 mg./l. cystine	207	420	200
Ditto + 5 mg./l. cystine + 2 mg./l. α-alanine	303	545	460
Ditto + 5 mg./l. + 2 mg./l. dextrose	1040	..	1300

This experiment shows the greater "N" activity of dextrose and bears out the general observation that the effect of α-alanine was less during slow growth in dull weather.

EFFECT OF MANGANESE

In these experiments with organic compounds possessing N activity the final growth or crop was not a large one. It was clear that further growth was held back through lack of some further accessory nutrient.

During the course of the earlier work it had been found that growth in artificial sea water was increased by adding 25 mg./$m.^3$ of Cu, Mn, Co, Zn and As as salts. The effect of this same mixture of "additional salts" was tried on growth in stored sea water enriched with N, P, Fe, cystine and α-alanine. It brought about a very markedly increased crop. The following experiment shows that the effect is due, for the most part, to the manganese.

Stored sea water enriched with N, P, Fe, 5 mg./l. cystine and 1 mg./l. α-alanine was inseminated with *Ditylum* in October 5. On October 11 the following observations were made:

In the water as above with no further addition	Almost all auxospores
With 5 mg./$m.^3$ Cu, Co and Zn	Slight increase about 50 % auxospores
,, ,, Cu, Zn	
,, ,, Mn, Cu, Co, Zn, As	Heavy growth, no auxospores
,, ,, Mn, Co, Zn	
,, ,, Mn, Cu, Zn	
,, ,, Mn, Cu, Co	
,, ,, Mn, Cu, Co, Zn	
,, ,, Mn, Cu	

It appeared from these and subsequent experiments that a very small supply of *manganese promotes continued growth* of this *Ditylum*.

The amount of manganese in samples of ocean and inshore waters from various depths has been estimated by Thompson and Wilson (1935), who found quantities varying between 1 and 10 mg./$m.^3$

Continued experiments with stored sea water showed that after enrichment with N, P and Fe, the addition of 10 to 20 mg./$m.^3$ of manganese would allow a moderate, and sometimes good, growth of *Ditylum*. Furthermore, offshore water collected on November 4 1938 which after filtering, sterilizing and enrichment with N, P and Fe, allowed no growth of the *Ditylum*, behaving like stored water, on adding 10 mg./$m.^3$ of manganese permitted a good growth to take place. Waters collected on November 28 1938, and January 2 1939 behaved in the same manner.

When cystine and one of the "N"-active organic compounds, as gluconic acid or dextrose, was added to the enriched stored sea water in addition to manganese, still further increased crops were obtained. A number of experiments were made, with such additions and it became apparent that growth was finally brought to a standstill through lack of another necessary substance. The water, amply enriched with N and P and, as far as one could judge, with Fe, Mn and organic compounds, having A and N activity, would not allow more than some five or six thousand cells per c.c. to develop. When such numbers were approached the cells became misshapen and had the general appearance of diatoms lacking silica.

A dilute solution of commercial waterglass was neutralized with hydrochloric acid to give a sol containing *ca.* 0·1 % of SiO_2. On adding this to provide some 20 mg. SiO_2/l. to stored sea water enriched with N, P, Fe, Mn, cystine and gluconic acid, a growth exceeding 20,000 cells per c.c. was obtained, turning the medium the colour of pale sherry. It was found that without the cystine and gluconic acid a very considerable growth would take place provided manganese and silica were added. It appeared either that the commercial water glass contained (? organic) accessory substances or that the stored sea water contained sufficient to allow a very heavy growth in the presence of ample SiO_2 and Mn. Neutral silica sols were therefore made from the same sample of waterglass after it had been dried and heated to bright redness in a platinum crucible and also from silicon tetrachloride. The growth induced by adding these was less rapid than the growth induced by adding the sol made from (unheated) waterglass, but the final growth in all three cases was similar. A reagent blank containing the sodium hydroxide and hydrochloric acid used in making the sol from silicon tetrachloride was included in the experiment.

Stored sea water enriched with N, P, Fe and 0·02 mg. Mn/l., inseminated November 4. Counts made November 14 and 21.

	Nov. 14 cells per c.c.	Nov. 21
No further addition	1210	*circa* 2700
With reagent blank	1240	,, ,,
With 20 mg. SiO_2/l.		
ex commercial waterglass	3440	*circa* 15,000
ex commercial waterglass (heated)	1860	,, ,,
ex $SiCl_4$	1860	,, ,,

Thus it appears that commercial waterglass contains some substance other than silica which increases the diatoms growth rate, and that this substance is destroyed or rendered inactive by heating to redness.

This and a number of subsequent experiments all showed that the stored natural sea water would support a growth when manganese and silica were added, without any addition of organic accessories other than the citrate added with the iron. The further addition of reasonably small quantities of cystine, 1–2 mg./l., led to heavier crops.

Experiments were made to find the quantity of manganese required to allow a continued and vigorous growth of *Ditylum* in stored sea water in the presence of other accessories added in ample quantity, i.e. silica, cystine, iron and gluconic acid. These indicate that 1–2 mg./$m.^3$ of manganese is sufficient, while greater concentrations cause more rapid growth, but not necessarily greater crops.

Exp. (Dec. 2–9).

	Cells per c.c.	
	Dec. 6	Dec. 9
No manganese added	75	*circa* 75
2 mg./m.^{3} manganese added	1900	*circa* 7000
5 mg./m.^{3} manganese added	2740	7000
10 mg./m.^{3} manganese added	3500	7000

Exp. Dec 14–29.

	Cells per c.c. Dec. 29
No manganese added	175
0·5 mg./m.^{3}	1400
1·0 mg./m.^{3}	2000

THE EFFECT OF OTHER INORGANIC SALTS

The experiments with stored sea water had amply shown that it would support a luxuriant growth resulting in an exceptionally heavy crop of the diatom if there were added nitrate, phosphate, silicate, iron as a complex with a polyhydroxy organic compound, manganese and the $-S-CH_2CH(NH_2)COOH$ group. Later, in 1939, it was found that such additions would no longer allow such heavy crops, due, apparently, to the long periods of very dull weather or to a further change in the stored water. Alanine and dextrose when added in addition had little effect in promoting greater crops, although both these substances had a marked effect when added to the same water enriched in the same way, but without added silicate during the previous September and November. Meanwhile, experiments made with artificial sea water had suggested that other elements occurring in minute traces in the water may play some part in the diatoms' metabolism. With this possibility in view the effect of adding such "trace elements" to the stored sea water in addition to iron, manganese and cystine was tried. A preparation containing a representative mixture of such elements likely to play a part in plant metabolism was made by boiling coal ash with hydrochloric acid, evaporating, redissolving and neutralizing to *p*H 7. In three experiments this was found to have a marked effect in increasing the diatoms' growth.

I have great pleasure in acknowledging the many helpful suggestions I have received during the course of this investigation, particularly those from Dr L. H. N. Cooper, Dr W. R. G. Atkins, F.R.S. and Dr E. J. Allen, F.R.S.

SUMMARY

The diatom *Ditylum brightwelli* requires, for vigorous growth in artificial sea water, two organic substances, or groups of substances, in addition to inorganic salts.

The substances, or groups, act in a manner complementary to each other.

The effect of adding either is greatly increased by the presence of the other.

One accessory substance, or group, has been obtained in impure state from natural sea water and from extracts of algae by adsorption on carbon and elution. It has properties of an inorganic acid or internal anhydride. Several organic compounds containing sulphur possess similar activity, including compounds containing the $—S—CH_2—CH(NH_2)COOH$ group.

The other substance or group has been obtained in impure state from extracts of algae and of yeast. A number of organic compounds have been found to possess similar activity.

The diatom required manganese, a concentration of one part per thousand million being sufficient for vigorous growth.

The diatom made good growth when transferred to natural sea water, enriched with nitrate, phosphate and iron, collected from offshore at intervals between October 1937 and June 1938.

The diatom ceased growth and formed auxospores when transferred to natural sea water, enriched in the same manner, collected from offshore during the summer of 1937 and during the period between July 1938 and January 1939.

These natural sea waters could be rendered fertile in some cases by adding a compound containing the $—S—CH_2CH(NH_2)COOH$ group and in some cases by adding 1–2 mg. $Mn/m.^3$

Water collected during the winter of 1938 became infertile after nine months storage, due to loss of available manganese.

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THE BIOLOGY OF *CHTHAMALUS STELLATUS* (POLI)

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(Text-figs. 1–4)

INTRODUCTION

Chthamalus stellatus (Poli) is a littoral barnacle, very similar both in habitat and in external appearance to another shore barnacle, *Balanus balanoides*. Actually with practice the two species can nearly always be distinguished *in situ* on the rocks (see Moore, 1936, p. 704). Although these two species appear to occupy similar ecological niches on the shore, their geographical distributions are different, since *Chthamalus* replaces *Balanus balanoides* progressively towards the south and west of the British Isles, and eastwards in the English Channel. In this paper an attempt is made to bring together what is known of the biology of *Chthamalus stellatus*, and to make a comparison between this species and *Balanus balanoides*.

MATERIAL AND METHODS

The observations reported in this paper are of very scattered origin, and concern the geographical distribution, relationships with other organisms, and growth rate of the barnacle *Chthamalus stellatus*.

For the investigation of the geographical distribution of *Chthamalus*, records of its presence or absence around the coasts of the British Isles were made by both authors, and also by a number of people who kindly sent material for examination. Except when observers were familiar with the differences between *Chthamalus* and *Balanus balanoides*, such material was always examined personally by one of the authors, and no records of absence were accepted from doubtful sources. The results of this collection of data are described and discussed on pp. 527–31, and summarized in Table I.

Notes on the general relationships of *Chthamalus*, and counts of its density, were made both on the Dorset coast and in the neighbourhood of Loch Sween by J. A. Kitching. Corresponding observations were made in Devon and Cornwall and in the north and west of Scotland by H. B. Moore, with the assistance, in Scotland, of Mr G. M. Spooner. The methods of counting and

TABLE I

Locality no. (see map)	Name of locality	Abundance of *Chthamalus*	Observer, or reference to previous record	Date of field observations
1	Nybster, Caithness	Only 2 found in 15 min. search; *Chthamalus* confined to extreme top of barnacle zone	H. B. Moore	28. vi. 36
2	Dunnet Hd., Caithness	Only 6 seen in 10 min. search; *Chthamalus* confined to top 20 cm. of barnacle zone	H. B. Moore	27. vi. 36
3	Skullomie, Sutherland	Up to 120 per m.^{2}; confined to top 50 cm. of barnacle zone	H. B. Moore	27. vi. 36
4	Geodha Chobhair, Sutherland	Up to 2200 per m.^{2}; confined to top 140 cm. (*ca.*) of barnacle zone	H. B. Moore	24. vi. 36
5	Bay of Stoer, Sutherland	Up to 3800 per m.^{2}	H. B. Moore	22. vi. 36
6	Seana Chamas, Ross	Present	H. B. Moore	20. vi. 36
7	Sgòir Beag, Vatternish, Skye	Abundant; occupying 50–100 cm. at top of barnacle zone	H. B. Moore	15. vi. 36
8	Scurrival Point, Barra, Outer Hebrides	Present	*Mrs N. McMillan	13. vii. 37
9	Breivig Bay, Barra, Outer Hebrides	Present	*Mrs N. McMillan	6. vii. 37
10	Òb Allt an Daraich, Skye	Up to 2400 per m.^{2}	H. B. Moore	11. vi. 36
11	Port na Cullaidh, Elgol, Skye	Up to 3200 per m.^{2}	H. B. Moore	18. vi. 36
12	Ardmore Pt., Mull	Plentiful	Kitching (1935)	5. viii. 34
13	Staffa	Plentiful	Kitching (1935)	14. viii. 34
14	Fionphort, Mull	Scarce	Kitching (1935)	6. viii. 34
15	Sound of Mull, near Loch Aline	Scarce	Kitching (1935)	2. viii. 34
16	Loch Buie, Mull	Plentiful	Kitching (1935)	7. viii. 34
17	South end of Kerrera, Firth of Lorne	Scarce	Kitching (1935)	1. viii. 34
18	Ardnoe Pt., near Crinan	Rather sparse	Kitching (1935)	24. vi. 34
19	Mouth of West Loch Tarbert, Jura	Rather sparse	J. A. Kitching	26. viii. 36
20	Farland Pt., Cumbrae	Up to 480 per m.^{2}	H. B. Moore	5. vii. 35
21	Whiting Bay, Arran	A very few present	H. B. Moore	10. vii. 35
22	Macrihanish, Kintyre	Scarce	Kitching (1935)	27. viii. 34
23	Mull of Kintyre	Absent	Kitching (1935)	27. viii. 34
24	Port Stewart, Co. Londonderry	Present	*Mrs N. McMillan	26. ix. 36
25	Saltpan Rock, 1–2 miles south of Moville, Co. Donegal	Present	*Mrs N. McMillan	28. iii. 37
26	Malin Hd., Co. Donegal	Present	*Mrs N. McMillan	29. iii. 37
27	Horn Hd., Co. Donegal	Present	†Dr J. W. S. Pringle	—. viii. 36
28	Bundoran, Co. Donegal	Present	*Dr R. Lloyd Praeger	8. v. 37
29	Achill I. (west corner), Co. Mayo	Abundant; no *Balanus balanoides* present	Mr D. A. Webb	—. vii. 37
30	Achillbeg I., Co. Mayo	Abundant; about equal in numbers with *B. balanoides*	Mr D. A. Webb	—. vii. 37
31	Dukes, Dingle Bay, Co. Kerry	Present	Dr W. R. G. Atkins	—. ix. 36
32	Rossbehy, Dingle Bay, Co. Kerry	Fairly plentiful	J. A. Kitching	21. vii. 38
33	Castlecove, Co. Kerry	Abundant	*Dr S. Kemp	24. viii. 36
34	Dunmanus Bay, Co. Cork	Abundant	Miss E. M. Moore	—. viii. 36
35	Barley Cove, Co. Cork	Abundant	Miss E. M. Moore	—. viii. 36
36	Middle Calf I., Schull, Co. Cork	Present	*Miss E. M. Moore	24. viii. 36
37	Castle I., Schull, Co. Cork	Present	*Miss E. M. Moore	24. viii. 36
38	Lough Ine, Co. Cork	Present	*Miss E. M. Moore	—. viii. 36
39	Oysterhaven, Co. Cork	Abundant	H. B. Moore	10. vii. 37
40	Fountainstown, Co. Cork	Abundant	H. B. Moore	15. vii. 37
41	Ardmore, Co. Waterford	Abundant	H. B. Moore	8. vii. 37
42	Stradbally, Co. Waterford	Abundant	H. B. Moore	8. vii. 37
43	Greenore Pt., Co. Wexford	Abundant	J. A. Kitching	1. vii. 38
44	Wicklow, Co. Wicklow	Only 2 seen in about 10 m. of shore	H. B. Moore	6. vii. 37
45	Bray Hd., Co. Wicklow	Present; less than 1 per m.^{2}	H. B. Moore	6. vii. 37
46	Ireland's Eye, Co. Dublin	Sparse; only on most wave-beaten rocks	J. A. Kitching	25. vii. 38
47	Greenisland, Co. Antrim	Absent	Mrs N. McMillan	—. viii. 36
48	White Hd., Co. Antrim	Absent	Mrs N. McMillan	—. xii. 36
49	Island Magee, Co. Antrim	Absent	Mrs N. McMillan	29. ix. 36
50	Kirk Michael, I. of Man	Absent	H. B. Moore	—. —. 32
51	‡Port Erin, I. of Man	Absent	H. B. Moore	—. —. 34
52	Langness, I. of Man	Absent	H. B. Moore	—. —. 34
53	Leasowe Embankment, Wirral, Cheshire	Absent	Mrs N. McMillan	18. vi. 37
54	Penrhyn Bay, Llandudno	Absent	Mr D. P. Wilson	3. vii. 35
55	Penmon Pt., Anglesey	Absent	Mr D. P. Wilson	—. ix. 36
56	Bay near Rhosneigr, Anglesey	Absent	Mr D. P. Wilson	—. vii. 37
57	Rhosneigr, Anglesey	Present but very rare	*Mr D. P. Wilson	—. vii. 37
58	Porth Oer, Carnarvon	Present	*Mrs N. McMillan	16. v. 37
59	Bardsey I.	Present	Kidd, Pyefinch & Butler (1935)	—. —. 33

* Specimens examined by H. B. Moore.

† Specimens examined by J. A. Kitching.

‡ An early record by Marrat (1886) is of very doubtful value.

Locality no. (see map)	Name of locality	Abundance of *Chthamalus*	Observer, or reference to previous record	Date of field observations
60	Black Rock, near Criccieth, Carnarvon	Present	*Mrs N. McMillan	16. v. 37
61	Aberystwyth	Present	Walton (1915)	—. —. 15
62	Llangranog, South Cardigan	Present	†Mrs N. McMillan	18. iv. 38
63	Nash Pt., Glamorgan	Plentiful	†Mr G. E. H. Foxon	—. v. 38
64	Clevedon, Somerset	Absent	J. A. Kitching	11. iv. 38
65	Blue Anchor Bay, Somerset	Absent	H. B. Moore	10. xi. 35
66	Lee Bay, North Devon	Abundant	H. B. Moore	31. x. 35
67	Hartland Pt.	Abundant	H. B. Moore	5. viii. 35
68	Millook Haven, North Cornwall	Abundant	H. B. Moore	2. ix. 34
69	Treyarnon	Abundant; up to 70,000 per m.^{2}	H. B. Moore	26. vii. 36
70	Holywell Bay	Abundant	H. B. Moore	26. viii. 36
71	St Ives	Abundant; commoner than *B. balanoides*	H. B. Moore	24. vii. 35
72	Cape Cornwall	Abundant; up to 47,000 per m.^{2}; in very wave-exposed places no *B. balanoides* present	H. B. Moore	6. viii. 36
73	Tresco, Scilly Is.	Abundant	*Mr G. M. Spooner	22. iv. 36
74	Porth Mellin (Mullion Cove)	Abundant	H. B. Moore	28. xii. 35
75	Charlestown, South Cornwall	Abundant	H. B. Moore	28. x. 34
76	Gara Pt., Yealm	Abundant	H. B. Moore	6. iv. 35
77	Black Cove	Abundant	H. B. Moore	21. iv. 35
78	Start Pt.	Abundant	H. B. Moore	21. iv. 35
79	Hope's Nase	Abundant	H. B. Moore	22. iv. 35
80	Dawlish Warren	Abundant	H. B. Moore	31. i. 37
81	Lyme Regis	Fairly abundant	H. B. Moore	21. x. 35
82	Redcliff Pt., Weymouth	Present in a very narrow zone	H. B. Moore	11. i. 36
83	Durlston Pt.	Present	J. A. Kitching	29. vii. 36
84	Old Harry Rocks, near Swanage	Absent	J. A. Kitching	30. vii. 36
85	Freshwater Bay, I. of Wight	Absent	Fischer-Piette (1936)	13. iii. 34
86	St Catherine's Pt., I. of Wight	Absent	Fischer-Piette (1936)	27. ix. 32
87	Culver Cliff, I. of Wight	Absent	Fischer-Piette (1936)	12. iii. 34
88	Newhaven	Absent	Fischer-Piette (1936)	13. v. 33
89	Hythe	Absent	Mr P. R. Crimp	—. ix. 37
90	Dover	Absent	J. A. Kitching	—. iv. 34
91	Flamborough Hd.	Absent	Mr J. H. Fraser	—. x. 36
92	Robin Hood's Bay	Absent	Mr R. Bassindale	—. —. 36
93	Tees Mouth	Absent	Mr R. Bassindale	—. —. 36
94	Bass Rock, Firth of Forth	Absent	H. B. Moore	4. vii. 36
95	Isle of May, Firth of Forth	Absent	J. A. Kitching	8. v. 37
96	St Andrews	Absent	Miss K. M. G. Flemming	—. ii. 38
97	Aberdeen	Absent	Mr A. Milne	—.—. 36
98	Helmsdale, Sutherland	Absent	H. B. Moore	28. vi. 36
99	Bruneval, Normandy	Absent	Fischer-Piette (1932)	6. ii. 31
100	Cap de la Hève	Absent	Fischer-Piette (1932)	30. vi. 27
101	Arromanches	Absent	Fischer-Piette (1932)	7. ii. 32
102	Grandcamp-les-Bains	Absent	Fischer-Piette (1932)	28. vii. 30
103	Pointe de Barfleur	Absent	Fischer-Piette (1932)	31. vii. 27
104	Cap Levi	Absent	Fischer-Piette (1932)	18. vii. 31
105	Cap de la Hague	Absent	Fischer-Piette (1932)	31. vii. 27
106	Flamanville	Absent	Fischer-Piette (1932)	30. iv. 30
107	Carteret	Up to 100 per m.^{2}	Fischer-Piette (1932)	30. iv. 30
108	Ile Lihou, Guernsey	Very few; about 1 per m.^{2}	Fischer-Piette (1932)	30. v. 31
109	La Corbière, Jersey	A very few	Fischer-Piette (1932)	20. iv. 30
110	Saint Germain-sur-Ay	A few	Fischer-Piette (1932)	28. iv. 30
111	Granville	Scarce	Fischer-Piette (1932)	4. ix. 26
112	Cancale, pointe du Grouin	Abundant in upper part of shore	Fischer-Piette (1932)	29. vi. 30
113	Cap Fréhel	Abundant	Fischer-Piette (1932)	12. vii. 32
114	Pointe de Pordic	Abundant	Fischer-Piette (1932)	23. ii. 32
115	Bréhat	Abundant	Fischer-Piette (1932)	21. iv. 32
116	Ploumanach	Abundant in places	Fischer-Piette (1932)	27. ix. 31
117	Ile d'Ouessant	Abundant	Fischer-Piette (1936)	11–15. ii. 33

* Specimens examined by H. B. Moore. † Specimens examined by J. A. Kitching.

of determining the tissue weights of the *Chthamalus* were those already used by Moore (1935, p. 264; 1936, p. 703) in similar work on *Balanus balanoides*.

Finally, observations on the growth rate were made by H. B. Moore on a rock at Drake's Island, Plymouth Sound. For this purpose patches of *Chthamalus* at three different levels were photographed for future recognition, and the individuals were measured *in situ* at intervals. These results are shown graphically in Fig. 4, and discussed on pp. 536–8.

LARVAL HISTORY AND SETTLEMENT OF SPAT

Developing larvae were found by Bassindale (1936) in the mantle cavity of *Chthamalus* at Plymouth from July to September. In cultures these larvae reached the sixth naupliar stage in 2–3 weeks, and they would presumably have reached the cypris stage soon after had they lived. Settlement of spat took place at Plymouth during the late autumn and winter. Hatton & Fischer-Piette (1932) have given the first dates of settlement at St Malo in 1931 and 1932 as September 14 and 24 respectively, and according to Hatton (1938) settlement continues for 5 months.

The naupliar stages have been illustrated by Bassindale (1936). The larva is very much smaller than that of *Balanus balanoides*. The sixth stage nauplius has a length of only 0·49 mm., as compared with the 1·15 mm. attained by *Balanus balanoides* at this stage. Also the newly settled young of *Chthamalus* is much smaller than that of *Balanus balanoides*.

The following figures have been given by Hatton (1938), and partly by Hatton & Fischer-Piette (1932), for the population density of *Chthamalus* spat (per 1/100 sq. m.) from counts made in January or February, when settlement was complete:

	High wave exposure		Slight wave exposure	
Level	1931	1932	1931	1932
Just above high water of neap tides	21	21	48	60
Just below high water of neap tides	270	205	39	42
Mid-tide level	1830	1610	8	19
Low water of neap tides	8	5	0	0

They show that the density of settlement was greatest at the highest wave exposure, and suggest also a shift of the optimal level for settlement of the spat from about mid-tide level at the higher wave exposure to high water of neap tides at the more sheltered locality. There was no settlement in a position of extreme shelter (Hatton & Fischer-Piette, 1932).

ZONATION, AND INTERRELATIONS WITH *BALANUS BALANOIDES*

Lower limit of vertical distribution

For *Chthamalus*, as for *Balanus balanoides*, the lower limit of distribution on the shore is very variable and difficult to determine. However, it may be stated that *Chthamalus* rarely if ever occurs below low water of spring tides. It is hard to see why it should not do so in a favourable situation, since Fischer (1928) has shown that it can survive a continuous immersion of 12 months under natural conditions. It is not possible to say whether it is excluded by some physical factor, such as excessive immersion (see below), or by competition with sublittoral organisms such as coralline algae or other rock-encrusting forms. The adverse factor, whatever it may be, might only operate at some particular stage in the life history of the *Chthamalus*, e.g. on newly attached larvae. It is quite possibly the same factor which prevents *Balanus balanoides* from extending below low water of spring tides.

Actually the lower limit of *Chthamalus* is in many districts far above low water of spring tides, and it may be above high water of neap tides (Fig. 1). No doubt the same factors which locally restrict *Balanus balanoides* also operate against *Chthamalus*—as for instance the rubbing action of the fronds of the larger algae (see Kitching, 1935, p. 363), and competition for space with other sessile organisms such as *Corallina officinalis* (see Moore, 1935, p. 299, for competition of *Balanus balanoides* and *Spirorbis borealis*).

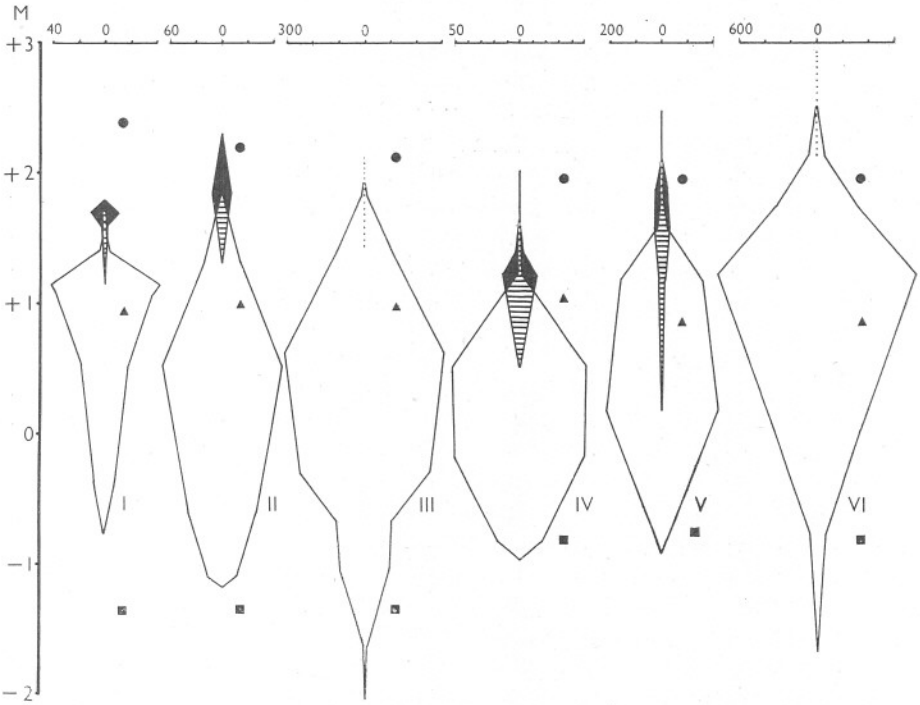

Fig. 1. Interrelation of *Chthamalus stellatus* (black) and *Balanus balanoides* (white) at a series of localities in Scotland. Quantities are measured in grammes (dry weight) of tissue per square metre. Levels (in metres) are referred to local mid-tide level. The approximate heights of other local levels are indicated as follows: mean low-water neap tides ■; mean high-water neap tides ▲; mean high-water spring tides ●. I, Òb Allt an Daraich, Skye; II, Port na Cullaidh, Elgol, Skye; III, Seana Chamas, Ross; IV, Bay of Stoer, Sutherland; V, Geodha Chobhair, Sutherland; VI, Skullomie, Sutherland.

The extent of the competition between *Chthamalus* and *Balanus balanoides*, both for space and for food, remains unknown. The food of each species requires investigation. But in localities where both species flourish there must certainly be competition for space. Denudation of the rock takes place largely during winter as a result of storms, and since *B. balanoides* spat settle in early summer, whereas *Chthamalus* spat do not settle until autumn or winter, the *Balanus* will have time to become well established before the *Chthamalus* appear. In the absence of other disturbing factors this would favour a predominance of *Balanus*, and it probably does actually do so in regions where both species are common, and especially in the lower part of the shore. This effect may quite probably determine the lower limit of *Chthamalus* in these localities.

However, some important factor, other than competition with *Balanus balanoides*, must be operative. This is shown by the fact that in certain localities the lower limit of *Chthamalus* is above the upper limit of *Balanus balanoides*. For instance, as shown in Fig. 1, at Òb Allt an Daraich, in Skye, there was a zone a few centimetres wide of fairly abundant *Chthamalus*, below which there was an almost bare zone several centimetres wide with hardly any barnacles of either species; and below this again *Balanus balanoides* was abundant. Thus the two species intermingled only to a very slight extent. Fischer also (1928) has written (translation) that "at the Point of Granville, at the margin of their geographical distribution, the *Chthamali* are practically restricted to levels which are above the limit of *B. balanoides*...". In these localities it must be presumed that the lower limit of *Chthamalus* is determined by some harmful effect of excessive immersion in sea water (see below, p. 534).

In addition, the lower limit of *Chthamalus* varies with geographical situation. For instance, at the western extremity of the English Channel the vertical distribution embraces the whole littoral region, but as *Chthamalus* approaches the limit of its geographical distribution eastwards in the English Channel, so its lower limit rises (Fischer-Piette, 1936). For geographical reasons it is not possible to demonstrate this effect so clearly up the west coast of Britain as the northern limit of distribution of *Chthamalus* is approached; but the data illustrated in Fig. 1 are sufficient to show that a similar effect exists. In the west of Scotland (e.g. Skye) *Chthamalus* is probably approaching its limit of tolerance of low temperature (see p. 530); whereas in the middle region of the Channel it is near the limit of adequate penetration eastwards of Atlantic water (see p. 528). It is of course possible that either of these factors might exert a stronger influence nearer low water, thereby restricting the *Chthamalus* to the higher levels where such influence was less. This may well be true at the Point of Granville. But at Skye by far the lowest temperature will be experienced during the period when the barnacles are left exposed to the air (in winter), and not when they are covered with water. Therefore at Skye it might be expected that if temperature were the only factor concerned the *Chthamalus* would be restricted to the lower levels, where they would be exposed for the shortest periods to the cold air. It therefore became necessary to postulate that the lower limit of *Chthamalus* is determined by a harmful effect of excessive immersion, and that *Chthamalus* is more sensitive to this influence in localities which are unfavourable in some other respect also. Such modifications of sensitivity will be discussed later (p. 534). It should be realized that it is not possible to distinguish between a harmful effect of immersion and a beneficial effect of emersion. For convenience we have followed the former interpretation. This factor appears to exert a strong influence on *Balanus balanoides* also (Moore, 1935, p. 288).

Upper limit of vertical distribution

The upper limit of *B. balanoides*, when not elevated by splash nor (possibly) depressed by *Chthamalus*, is almost exactly at high water of mean neap tides (Fischer-Piette, 1929; Moore, 1935). That of *Chthamalus* is regularly higher, but information from localities undisturbed by splash is scanty. In Loch Sween the upper limit is near high water of spring tides (Kitching, 1935).

In most of the districts where both barnacles occur their zones overlap, and it is questionable whether or not the upper limit of *Balanus balanoides* may be depressed by the competition of *Chthamalus*. Actually there is no evidence of such an effect. From a comparison of the vertical distribution of the two species in regions where *Chthamalus* is relatively scarce (Fig. 1, p. 525 of this paper) and in regions where it is as abundant as *Balanus* (Moore, 1936, Fig. 4, p. 705), it appears that the effect of the occurrence of *Chthamalus* within the *Balanus* zone is to decrease the abundance of *Balanus*, but not to exclude *Balanus* completely. The nature of the competition between the two species is unknown. If it were merely competition for available space on which to settle, the *Balanus* would be the more likely to preponderate, as already suggested. It might, on the other hand, be a case of competition for the available food. Furthermore, at Plymouth *B. balanoides* is approaching a region which is apparently unfavourable to it. This is indicated by the fact that its progressive decrease in numbers westwards in the English Channel is accompanied, towards the extreme limit of its geographical distribution, by a failure of those individuals which survive to spawn. This has been found elsewhere (Moore, 1935, p. 290) to be an indication of unfavourable conditions. This might be expected to result in a decreased range of tolerance of *B. balanoides* towards certain other environmental factors. On the other hand, *Chthamalus* encounters a progressively more congenial environment westwards down the English Channel (see p. 526), and might therefore show an accompanying extension in range of tolerance for such factors. If then, owing to overcrowding, the two species are in conflict for a limited amount of food, the one with the greater tolerance for adverse conditions may be expected to survive, and to predominate over the less tolerant.

RELATION TO ATLANTIC WATER

It has long been known that certain planktonic animals are characteristic of the enclosed waters of the North Sea, English Channel, and Irish Sea, whereas quite different ones characterize the open waters of the Atlantic and the western coasts of Ireland and Scotland washed by them. Russell (1935, 1936) has shown how some of these, and in particular *Sagitta elegans*, may be used as indicators of the movements of Atlantic water in and out of the mouth of the English Channel. An examination of the geographical distribution of *Chthamalus*, as shown in Fig. 2, makes it apparent that this species also is limited to those shores which are bathed by Atlantic water, being absent from the North Sea, upper Irish Sea, upper Bristol Channel, and eastern half of the English Channel. The only other instance, of which we are aware, of a non-planktonic organism for which a connexion has been suggested between geographical distribution and the presence of Atlantic water is *Echinus esculentus*. The occurrence of this species between tidemarks is, according to Reid (1935), limited to coasts bathed by water of Atlantic characters; although *E. esculentus* is more widely distributed at deeper levels. A comparison of the distributions of *Sagitta elegans*, intertidal *Echinus esculentus*, and *Chthamalus stellatus* reveals the following differences:

(1) In the English Channel *Chthamalus* extends plentifully up to Plymouth, and in small numbers up to Swanage; the eastern limit of *Sagitta elegans* fluctuates from the Scilly Isles eastwards; and intertidal *Echinus* only reach the Scilly Isles and the extreme tip of Cornwall.

(2) In the Irish Sea from its southern entrance *Chthamalus* penetrates farther northwards than does intertidal *Echinus*; *Sagitta elegans* occurs throughout the Irish Sea.

(3) In the Irish Sea from its northern entrance *S. elegans* and intertidal *Echinus* extend southwards to the Isle of Man, whereas *Chthamalus* does not come south of the Mull of Kintyre, as far as we know.

(4) In the North Sea, in its northern entrance, *Sagitta elegans* and intertidal *Echinus* penetrate a considerable distance southwards, whereas *Chthamalus* stops short at Dunnet Head (at the north-east corner of Scotland).

The explanation of these differences probably lies in the fact that the geographical ranges of *Sagitta elegans* and of *Echinus esculentus* extend northwards far beyond the British Isles; whereas *Chthamalus* reaches its northern limit in the north of Scotland, where it is probably limited by temperature. The unfavourable temperature conditions in the northern Irish Sea and in the north of Scotland probably render *Chthamalus* more sensitive to decrease in proportion of Atlantic water than it is in the warmer southern Irish Sea and English Channel; whereas *Sagitta elegans* and *Echinus*, not being on the edge of their geographical range, are not so affected. It must also be realized that *Sagitta elegans*, being planktonic, has a distribution that fluctuates considerably from time to time.

At present there is little or no clue as to what is the character which renders Atlantic water more favourable to *Chthamalus* than the water of an enclosed sea. It cannot be temperature, since this must be considerably higher on the Isle of Man, where *Chthamalus* is not found, than in the north of Scotland, where *Chthamalus* does occur. Similarly it cannot be salinity, since the

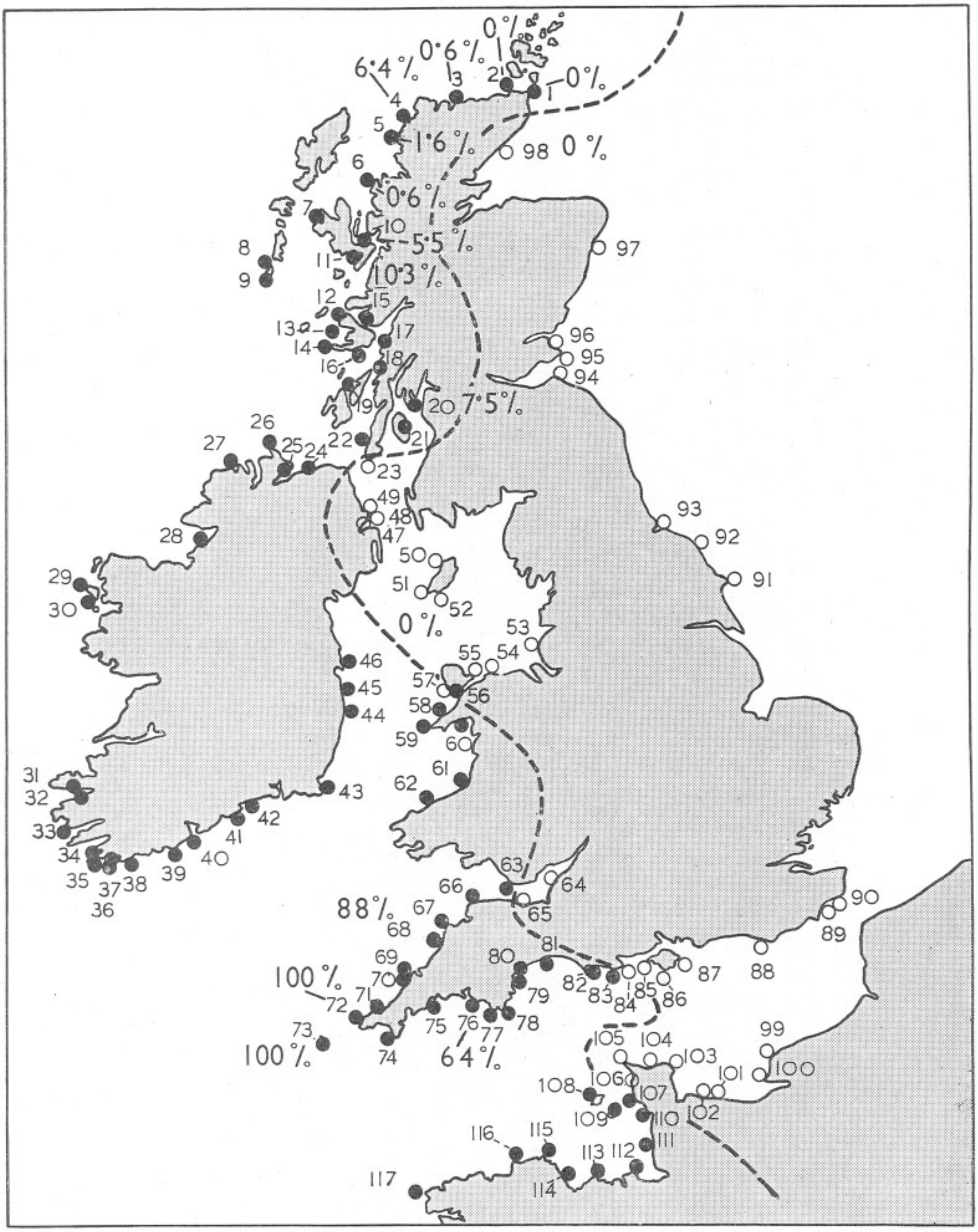

Fig. 2. The geographical distribution of *Chthamalus stellatus*. ○ indicates absent; ● indicates present. Numbers of stations (see Table I) are given in smaller type; the numbers in larger type represent the percentage by weight of *Chthamalus* in the total population of barnacles (*Chthamalus* and *Balanus balanoides*) between tide marks.

difference in salinity between the waters around Plymouth and those near Swanage is quite negligible in comparison with the range of salinity which *Chthamalus* tolerates in the estuary of the river Tamar at Plymouth. Perhaps it is the same unknown factor which Fischer-Piette (1936) has postulated to explain the preference of certain littoral species for regions which jut out into the neighbourhood of deep water, and of others for embayed regions.

EFFECTS OF TEMPERATURE, SALINITY, HYDROGEN ION CONCENTRATION, AND CURRENTS; PENETRATION UP ESTUARIES

Very little is known about the tolerance of *Chthamalus* for temperature. Moore (1935) has recorded a temperature of 36·3° C. within the mantle cavity of one *in situ* on the rocks during a low-tide period, and an even higher temperature could almost certainly be attained on a really hot day. No doubt a time factor would enter into any question as to the highest temperature consistent with survival. As regards the influence of low temperature, *Chthamalus* reaches its northern limit in the north of Scotland, where the February sea-surface temperatures, as given in Deutschen Seewarte Hamburg (1927), are about 6° C., although no doubt considerably lower temperatures occur on the shore itself. *Balanus balanoides*, on the other hand, extends northwards to the region where the shore is frozen in winter, and appears to be limited more by the mechanical action of the ice than by the low temperature.

The figures given for the salinity range tolerated by *Chthamalus* are of doubtful value for two reasons. A barnacle can close its aperture and so protect itself from a temporary lowering of the salinity of the surrounding water. And, in addition, minimal figures for salinity usually refer to the time of low water in the particular part of the estuary in question, whereas *Chthamalus* is usually restricted to a zone near the level of high water, and so is only covered by water of very much higher salinity. No figures are available for the minimal salinity which will allow either survival or feeding.

For the range of hydrogen ion concentration which *Chthamalus* can tolerate there are again no adequate figures. Fischer (1928) has recorded that *Chthamalus* can endure *p*H 9·0.

Wave action is favourable to *Chthamalus*, just as it is to *Balanus balanoides*, but water movement produced by a current can take the place of wave-produced movement. According to Fischer (1928) water movement produced in one way or the other is essential for its survival, and certainly we have found that *Chthamalus* does not survive nearly so well as *Balanus balanoides* in a tank supplied with running water and a tidal rise and fall but without wave action or a strong current. However, in Loch Sween, where conditions are for some unknown reason particularly favourable (Kitching, 1935), *Chthamalus* lives in extremely sheltered places where the current is slight. *Chthamalus* can also survive very muddy water, as for instance in the Tamar estuary.

The strong modifying influence which variation in one factor may have on the degree of tolerance of a given species for another factor is well illustrated by the relative penetration of *Chthamalus* and *Balanus balanoides* into different estuaries. In the lochs on the west coast of Scotland, where *Chthamalus* is near its northern limit, and may therefore be considered as living under generally less favourable conditions than *Balanus balanoides*, the latter penetrates usually to the extreme head of the lochs, if the shores are not too weed-grown, whereas *Chthamalus* usually stops short much nearer to the open sea. It may be either shelter from wave action or low salinity for which the tolerance of *Chthamalus* is here reduced. Farther south, in the Plymouth region, where *Chthamalus* and *Balanus balanoides* occur in more or less equal numbers, both species penetrate the estuaries of the Tamar and Avon to the same extent. Still farther south again in the neighbourhood of Roscoff, *B. balanoides*, which is here at the southern margin of its geographical range, is confined to wave-beaten rocks on the open shore, whereas *Chthamalus*, which is nearer to the centre of its range, extends far up the estuaries into the region occupied by the brackish water alga *Fucus ceranoides*.

NATURE OF SUBSTRATUM

The distribution of *Chthamalus* along the coast of the "Isle" of Purbeck (Dorset) was studied in considerable detail, since here *Chthamalus* reaches the eastern limit of its distribution in the English Channel. However, this investigation was complicated by changes in the lithology. The chief rock formations contributing to the shore of the Isle of Purbeck are the Kimmeridge clay, Portland stone, Purbeck stone, Wealden sands, lower greensand, and chalk (Strahan, 1898). Of these only the harder rocks form substrata suitable for barnacles. The Portland stone (a hard limestone), Purbeck stone (also limestone), and chalk reach the shore at various positions along the coast, and *Chthamalus* was found to show a marked preference for Portland stone (Table II). On chalk it was only recorded on the sheltered sides of boulders or on flints. *Balanus balanoides* also was usually absent from chalk, especially when this was covered with green algal slime, but was plentiful on the clean chalk of the Old Harry Rocks. In other localities the abundance (or otherwise) of *B. balanoides* corresponded fairly closely with that of *Chthamalus*. The most likely alternative explanations of the undesirability of chalk are either (more probably) that it affords insecure holding (either for the larvae or for the adults) because of its smooth or even slimy surface, or that being porous it allows the barnacles to be desiccated from below in dry weather.

At two stations on the Purbeck coast both *Chthamalus* and *Balanus balanoides* were present in sufficient numbers for a detailed examination to be made of their vertical distribution. On the eastern promontory of Lulworth Cove the levels of maximal population density of the two species are separated by a vertical distance of about $1\frac{1}{2}$ m., and in the region of overlap of the two zones both species are very sparse. This seemed to indicate that the lower limit of *Chthamalus* was here in no way influenced by the competition of *Balanus balanoides*, but was on the other hand determined by "excessive immersion", just as is already believed to apply in northern Scotland. However, such a conclusion, though possibly correct, does not necessarily follow in this instance. The rock surface at Lulworth is much rougher near the level of high water of spring tides than it is lower down, and *Chthamalus* here shows a marked preference for rough patches of rock. It is therefore possible that towards lower levels sites suitable for *Chthamalus* become progressively fewer.

TABLE II

Locality*	Rock	Nature of surface	*Chthamalus*	*Balanus balanoides*
Swyre Head†	Upper chalk	Smooth, rather slimy chalk	On flints only, sparse	Absent
Durdle Door (west side)	Purbeck stone	Generally smooth	In cracks only	In cracks only
Lulworth Cove (west side)	Portland stone	Variable	Chiefly in crevices	Chiefly in crevices
Lulworth Cove (east side)	Portland stone	Variable	Plentiful in hollows, scarce on open rock	Plentiful
Arish Mell Gap (west side)	Upper chalk	Chalk boulders	Sparse; usually only on landward side of boulders	Sparse; usually only on landward side of boulders
Arish Mell Gap (east side)	Upper chalk	Nearly vertical; smooth, green and slimy	Only one specimen found	Absent
Tilly Whim	Portland stone	Variable, chert bands	Plentiful in hollows and on chert bands	Plentiful
Old Harry Rocks	Upper chalk	Smooth	Absent	Plentiful

* Localities are given in geographical order, from west to east.

† This headland is about three-quarters of a mile west of Durdle Door, and must not be confused with another "Swyre Head" farther east.

The influence of lithology and of texture of rock surface was more strongly marked on the cliffs below the Tilly Whim caves, near Swanage. Here the bedding planes are horizontal and there is therefore a vertical succession of various bands of limestone and chert, each weathered at its surface to a characteristic texture. As will be seen from Fig. 3 and Table III, the vertical distribution of *Chthamalus* does not conform to the usual arrangement of a belt with the maximal population density near the middle, and regions of less density above and below. There are here three levels of high population density, namely, bands A, E, and G, of which E and G are especially thickly populated. E and G are hard cherty bands, with coarse-textured surface weathered in an angular way; and the surface of A is also rather coarse. D, which also has a considerable population of *Chthamalus*, has coarse cherty nodules in it. On the other hand, the bands with relatively smooth surfaces, B, C, and F, have small populations of *Chthamalus*. It is noticeable also that

Chthamalus is specially abundant in certain small upward-facing pockets in the surface of band A, although the reason for this remains doubtful.

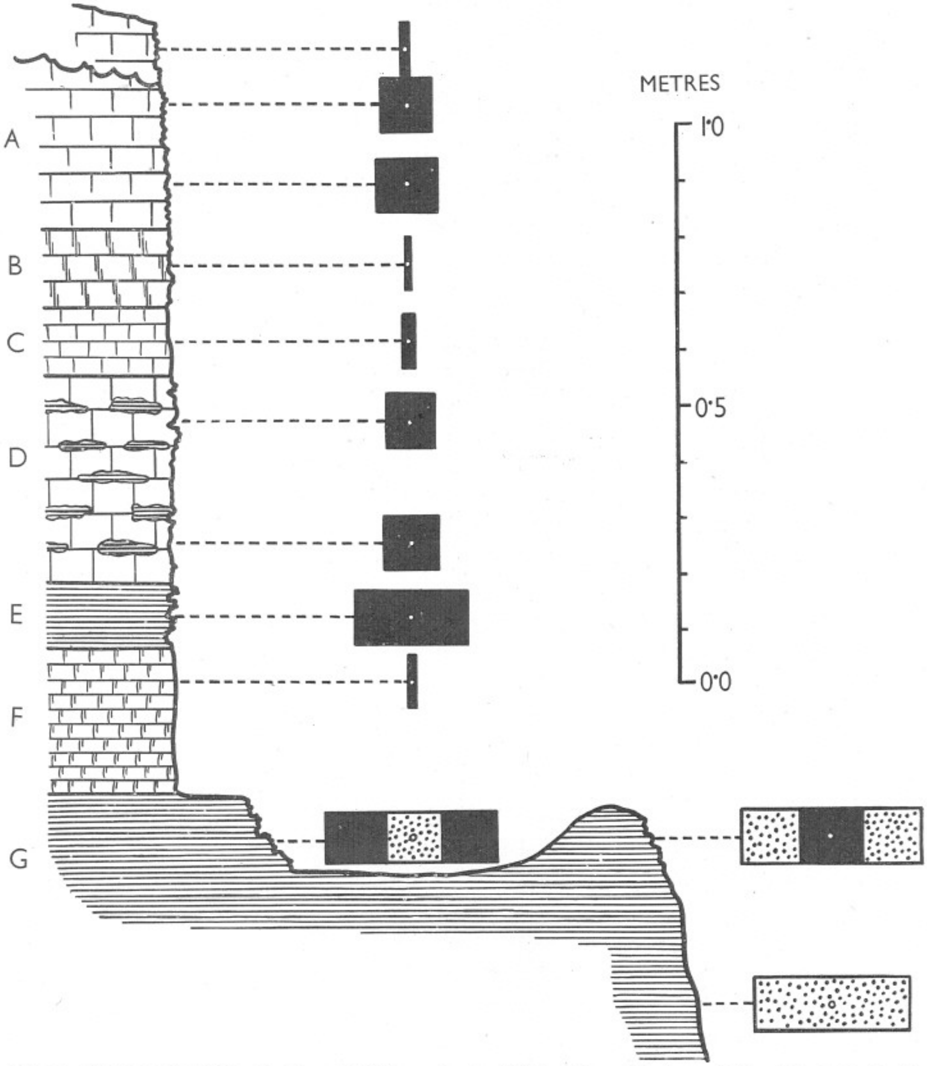

Fig. 3. The vertical distribution of *Chthamalus* (solid black) and *Balanus balanoides* (stippled) in relation to lithology at Tilly Whim, near Swanage, Dorset. For explanation of lettering see Table III. Brick pattern indicates limestone and horizontal shading indicates chert. The texture of the rock surface is indicated diagrammatically.

It appears from these observations that *Chthamalus* prefers a hard rough limestone to a soft smooth one, but it can exist on the latter in positions sheltered from violent wave action. Hatton (1938), in an experiment on

Balanus balanoides, found that the larvae failed to settle on polished marble except in depressions or scratches in the surface. He suggested that they were dragged away by water currents. On the other hand, it has also been shown (Kitching, 1935) that an excessively coriaceous surface is unfavourable to *Chthamalus*. It is also clear that on limestone shores, where the texture of the rock surface is liable to show progressive changes from high water of spring tides downwards, it is necessary to interpret with great caution observations of the vertical distribution of littoral organisms.

TABLE III

			Mean no. of barnacles per m.^{2}	
Band	Nature of rock	Nature of surface	*Chthamalus*	*Balanus balanoides*
A (top)	Limestone	Coriaceous	125	0
A (upper half)	Limestone	Coriaceous	620	0
A (lower half)	Limestone	Coriaceous	750	0
B	Limestone	Less coriaceous	80	0
C	Limestone	Smooth, greenish	180	0
D (upper part)	Limestone and chert	Uneven	600	0
D (lower part)	Limestone and chert	Uneven	670	0
E	Chert	Angular and creviced	1370	0
F	Limestone	Rather smooth, greenish	110	0
G	Chert	Hard, angular	2075	650
G (same level at outer edge of shelf)	Chert	Hard, smoother	730	2170
G (lower down, on outer side of shelf)	Chert	Hard, smoother	0	1875

N.B. The above figures can only be regarded as very rough. Each mean has been compiled from between three and ten counts. Each count was made on a separate 10 × 10 cm. square of rock surface, selected as typical of the band in question.

MODIFICATIONS OF THE RANGE OF TOLERANCE TOWARDS ENVIRONMENTAL FACTORS

In a number of instances, in attempting to explain the distribution of *Chthamalus*, we have suggested that its ability to endure conditions which are unfavourable in respect of one environmental factor may be modified by the action of another factor. For instance, its resistance to excessive immersion is thought to be reduced by deficiency of Atlantic water in the English Channel and probably by low temperature in the north and west of Scotland (p. 526). In addition, its tolerance of sheltered water and its power of penetration up estuaries are similarly reduced in both these regions. On the other hand, in the northern part of the Sound of Jura (west coast of Scotland), and in the lochs opening into it, *Chthamalus* is markedly more tolerant of environmental factors than it is on the neighbouring parts of the coast. In this quite restricted favourable area *Chthamalus* penetrates far into extremely sheltered arms of the sea lochs, and is very much more abundant than in wave-exposed, and therefore presumably favourable, situations on the western (Atlantic) coast of Jura. This curiously local vigour of *Chthamalus* is difficult to explain, but is possibly connected with the abnormal tidal conditions in this area, which are such that the *Chthamalus* zone remains uncovered but subject to splashing for prolonged periods. Whatever may be the explanation, *Chthamalus* appears in this area to be unusually tolerant of various environmental factors. Some beneficial influence appears to ameliorate locally an environment which in some other respect (probably temperature) is none too satisfactory.

It might in certain cases be argued that there is no proof that one factor may modify the tolerance of *Chthamalus* towards another factor. For instance, as already suggested, unsuitable water might prove especially adverse in the lower part of the shore, and so raise the lower limit of *Chthamalus*. It is, however, difficult to frame plausibly any comprehensive explanation of the distribution of *Chthamalus* in terms of the direct action of environmental factors upon an organism with unvarying limits of tolerance. Why, for instance, should *Chthamalus* be able to withstand in Loch Sween or in the Tamar conditions towards which, on the north-west coast of Scotland or in the English Channel, it cannot make any approach?

Although the idea of modified tolerance seems obvious enough, we have not been able to find many comparable examples. The tolerance of *Procerodes* (=*Gunda*) *ulvae* for low salinity is dependent on the presence of sufficient calcium in the external medium (Pantin, 1931), but this is probably a direct effect of the calcium ion on the permeability of cell membranes. Although it is not possible to decide definitely until the interplay of all the various factors involved has been analysed, it seems that in *Chthamalus* the modification of tolerance takes effect by a change in the general vigour of the organism, rather than by the direct action of one factor on some particular part of the organism which is especially susceptible to the influence of another factor.

Perhaps a more satisfactory parallel example is provided by the effect of competition with one another on the temperature range of fresh-water planarians. Beauchamp & Ullyott (1932), working in parts of eastern and southern Europe, investigated the distribution of *Planaria montenegrina* and *P. gonocephala* in swiftly flowing streams in which the temperature of the water increased progressively from the source downwards. In streams in which both species occurred, *P. montenegrina* occupied an upper section extending from the source down to where the temperature reached 13–14° C., and *P. gonocephala* ranged from this latter level downwards. There was scarcely any overlapping of the two species. But in certain streams, from which *P. montenegrina* was absent, *P. gonocephala* extended up to sources as cold as those normally occupied exclusively by *P. montenegrina* (e.g. 8·5° C.).

And in certain streams, from which *P. gonocephala* was absent, *P. montenegrina* extended down to parts of the stream which would, in the presence of the other species, have been too hot (16–17° C.). Thus competition seems to influence the range of temperature tolerated by these planarians.

In addition, Broekhuysen (1936) has recorded a decrease in tolerance of low salinity in *Carcinus maenas* (the shore crab) in winter, as compared with summer when the temperature is higher.

In general, therefore, it seems permissible to postulate that departure from the optimal condition for one environmental factor may reduce the tolerance range of a species for a second factor. It seems possible that this effect will be found to play an important part in the distribution of organisms in an environment such as the shore, where conditions are so exceptionally varied.

GROWTH RATE

Hatton (1938), in a supplement to previous work of Hatton & Fischer-Piette (1932), has given figures relating to the growth of newly settled *Chthamalus* at different levels, during a period of 18 months. In a wave-exposed locality the lengths (mm.) attained were:

	1930–1 spat		1931–2 spat	
	After 9 months	After 18 months	After 9 months	After 18 months
Slightly above high water of neap tides	2·6	5	2	4·1
Slightly below high water of neap tides	3·6	4·1	2·5	3·6
Mid-tide level	4·1	4·2	2·7	3·4

Thus in the first year growth was greatest at the lowest level, but in the second year at the highest. However, in a more sheltered locality growth was greatest at the highest level in both years. Seasonal variation in growth rate was slight.

An attempt was made to estimate the growth rate of *Chthamalus* at Drake's Island, Plymouth, by means of periodic measurements of a number of barnacles belonging to several year groups at three different levels on the rocks, the individual barnacles being recognized from a photograph. Unfortunately, the extreme irregularity of their outline, and the smallness of the growth observed, introduced too great an error to permit any deductions on seasonal variation in growth rate to be made, so that only the total growth for the period is used here. Hatton (1938), however, found little variation in growth rate throughout the year. The three levels selected represent the upper, middle and lower regions occupied at Plymouth by *Chthamalus*, and it is unfortunate that there is so little difference in level between them. It would be better if the experiment could be repeated somewhere where *Chthamalus* occupies a wider zone on the shore. The results obtained during 10 months' growth are shown in Fig. 4. All sizes of barnacle show definitely greater growth rates at lower levels. The newly settled 1936 spat, when measured in December 1936, averaged $\left(\frac{\text{length} + \text{breadth}}{2}\right)$ 1·61, 2·22 and

2·19 mm. respectively at the upper, middle and lower station, and in October 1937 these spat had grown to 3·07, 3·99 and 4·18 mm. respectively. On the same date the 1937 spat averaged 0·65, 0·79 and 1·15 mm. From the measurements made on Drake's Island the following averages were derived for the size $\left(\frac{\text{length} + \text{breadth}}{2}\right)$ of *Chthamalus* of various ages at the three levels

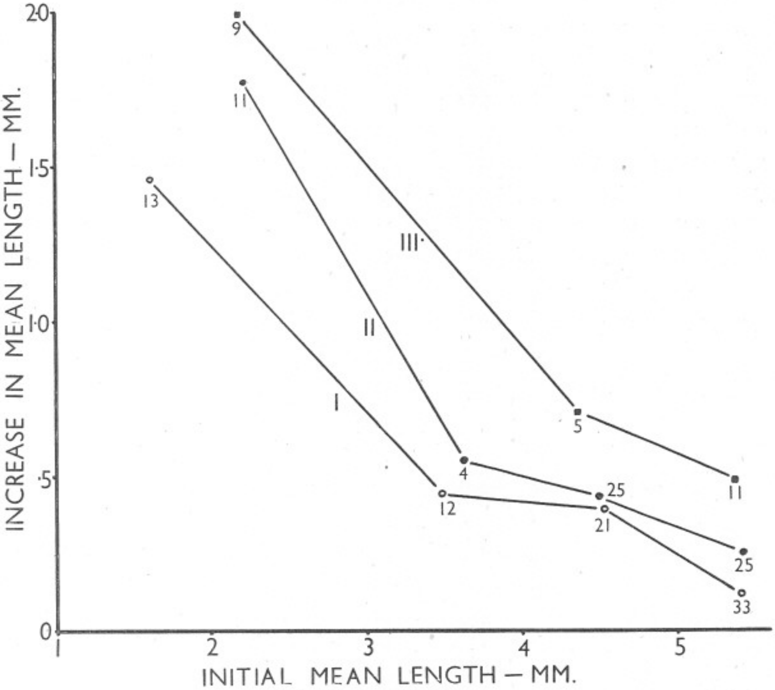

Fig. 4. Increase in mean length $\left(\frac{\text{length} + \text{breadth}}{2}\right)$ of *Chthamalus stellatus* at three levels at Drake's Island, Plymouth, between December 11, 1936 and October 20, 1937. The figures alongside the curve indicate numbers of specimens. Levels: I, +1·36 m. (O.D.); II, +0·81 m. (O.D.); III, +0·41 m. (O.D.).

in question, but it must be emphasized that these values are no more than very rough approximations:

	Number of months since settlement of spat			
Level*	12	24	36	48
+1·36 m.	2·0	3·7	4·2	4·6
+0·81 m.	3·8	4·5	5·0	5·4
+0·41 m.	3·9	5·0	5·6	6·0

* Levels above ordnance datum.

Whereas at Plymouth the rate of growth remained greatest at the lowest level, at St Malo there appears to have been some unfavourable influence at the lower levels which operated progressively more severely as the *Chthamalus* grew older, and which was also rendered more effective by lack of wave action. Further observations are desirable from localities near the centre and at the edge of the geographical range of *Chthamalus*.

MORTALITY RATE

Figures for the mortality rate of barnacles are somewhat scarce. In the sublittoral region, although in some places the barnacles may persist for long periods, in general it is probable that they appear as early colonists of areas denuded by winter storms and other means, but that they are soon smothered by later arrivals such as ascidians, sponges, and algae (Kitching, 1937). The mortality rate of *Balanus balanoides* found by Moore (1934) for a small number of individuals of over 4 months old was 35 % per annum in the lowest levels and 3 % in the uppermost. Hatton (1938) has also found a higher mortality rate for *B. balanoides* at the lower levels. The figures for *Chthamalus* on Drake's Island were as follows:

Level	No. of individuals	Mortality rate % per annum
+1·36 m. O.D.	71	3·3
+0·81 m.	70	16·6
+0·41 m.	30	46·5

Hatton (1938) found a mortality rate near St Malo of 24–25 % per 20 months around the level of high water of neap tides, and of 96 % at about mid-tide level, in a wave-beaten situation. In a sheltered situation the rate was higher.

It is noteworthy that most of the deaths at Plymouth occurred during a period of severe winter gales when the shore showed signs of considerable disturbance, and numbers of *Patella* were found with their shells fractured, apparently by blows from boulders. Probably therefore most of the *Chthamalus* were killed by stones pounded against the rocks by waves.

ENEMIES

The enemies of *Chthamalus* are much the same as those of *Balanus balanoides*, with the difference that where *Chthamalus* occupies a higher zone on the shore it is less liable to be attacked by *Purpura lapillus*. *Purpura* is undoubtedly their chief enemy. In addition no doubt many are killed or fractured by wave-borne stones and then eaten by small carnivores such as crabs and certain amphipods. Some are displaced by *Patella* in its wanderings (Hatton, 1938), and at the lower levels some are smothered by the overgrowth of algae and *Mytilus*. A certain number are probably killed and eaten by crabs and by shore fish such as wrasse. Herring gulls have been found to eat barnacles on occasions, but not in large quantities. Finally, an enemy which may play at the lower levels almost as serious a part as does *Purpura* is the polychaete, *Eulalia viridis*. As we are not aware of any previous record of the attacks made by *Eulalia* on barnacles, a note is given below of observations made by one of us (H. B. M.) at Drake's Island.

On a number of occasions when the shore was visited as the tide was receding, *E. viridis* was observed feeding in considerable numbers on barnacles on those rocks which were still wet, and on which the sun was not shining. After from half an hour to an hour of emersion the *Eulalia* had usually all disappeared. In June 1937, in an area of about 6 sq. m., about twenty *Eulalia* and a single specimen of a species of nemertine were seen feeding on *Balanus balanoides*. The movements of one *Eulalia* were watched in detail. First it moved for some distance across the barnacles. Then it stopped and explored the orifice of one barnacle for a period of a minute or more, during which it several times moved its head away a centimetre or so from the barnacle. It next applied its mouth to the opercular plates of the barnacle, the proboscis not being everted, and after a few seconds one of the plates burst outwards with an audible click. The *Eulalia* then arched its front end off the ground and everted its proboscis downwards into the aperture of the barnacle. For about 5 min. peristaltic movements were visible in the proboscis, and once or twice this was withdrawn for an instant from the barnacle. At the end of this time the worm left the barnacle, and moved off in search of another; and on examination the shell of the barnacle was found to have been completely emptied.

Acknowledgements

We gratefully acknowledge the assistance of all those who helped in making the observations recorded in this paper and in obtaining material from the various localities recorded in Table I. We wish to thank in particular the staff of the Plymouth laboratory and the crew of the M.B. *Gammarus*. We are indebted to Professor C. M. Yonge for his criticism of the manuscript.

Summary

Chthamalus stellatus is a littoral barnacle very similar in habitat and general ecological relations to another littoral barnacle, *Balanus balanoides*. In those localities where both species flourish they compete considerably for space, and also possibly for food. The fact that *B. balanoides* settles sooner after the winter storms than does *Chthamalus* probably favours the former.

Chthamalus is characteristically a southern species and *Balanus balanoides* a northern one, but the north and south ranges of the two species overlap in the British Isles and in France. The determining factor is presumably temperature.

Chthamalus is an Atlantic species, and *Balanus balanoides* is more characteristically a North Sea species. The essential factor in Atlantic water remains unknown, although in respect of a need for Atlantic water *Chthamalus* resembles *Sagitta elegans* and intertidal *Echinus esculentus*.

Except in especially favourable districts (see below), *Chthamalus* is usually

restricted to a zone in the upper part of the shore, whereas *Balanus balanoides* occupies a zone mainly below the *Chthamalus* zone. In certain places where the two zones do not overlap it is clear that the lower limit of *Chthamalus* is determined, not by the competition of *Balanus balanoides*, but by some adverse effect of excessive immersion.

Towards its northern limit of geographical distribution and towards regions where Atlantic water is deficient, *Chthamalus* extends less far down on the shore and less far into sheltered waters and estuaries. It seems that its tolerance of excessive immersion and of certain conditions characteristic of bays and estuaries becomes reduced in an environment unfavourable in respect of Atlantic water or temperature.

On Drake's Island, Plymouth, the growth rate and rate of mortality of *Chthamalus* were found to be greater at the lower levels.

The chief enemy of *Chthamalus*, as of *Balanus balanoides*, is *Purpura* (=*Nucella*) *lapillus*. The polychaete *Eulalia viridis* was observed feeding on *Balanus balanoides*. Many barnacles are probably killed by stones pounded against them by the waves.

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ON A NEW SPECIES OF *GAMMARUS* (*G. TIGRINUS*) FROM DROITWICH DISTRICT

By E. W. Sexton, F.L.S.

With an Appendix by L. H. N. Cooper, D.Sc., F.I.C.

From the Plymouth Laboratory

(Plates IV–VI)

The new species of *Gammarus* here described was first noted in 1931 by Prof. H. Munro Fox, to whom I am indebted for the opportunity of examining it. He found it in abundance in the Droitwich district, living in the brackish waters of the River Salwarpe near the town, in the Canal, and in Wyken Slough near Coventry.

Droitwich is an inland town in Worcestershire, situated on the Salwarpe, a tributary of the River Severn. It is famous for its natural alkaline brine springs, and it is these which make the waters of the district saline.

In August 1938 a quantity of fresh material was collected by Mr G. M. Spooner and Miss M. Mare, who visited several localities in Worcestershire, Warwickshire, and north Gloucestershire. The species was found in numbers at Wyken Slough and at Droitwich (River Salwarpe), and in small numbers in the River Avon at Tewkesbury. The two former localities were already known to Prof. Munro Fox as haunts of *Gammarus tigrinus*. Tewkesbury was visited because of the occurrence of some unknown amphipods in a collection made by Mr G. I. Crawford in 1935. In the light of the fresh material obtained, these specimens are now recognized as the young of *G. tigrinus*. No new localities were discovered. Those visited are listed in the following table, which also gives the occurrence of the only other species of *Gammarus* collected, namely, *G. pulex*.

Locality	*G. tigrinus*	*G. pulex*
River Chelt, Gloucester-Tewkesbury road	o	Abundant
River Avon, Tewkesbury	Sparingly	Scarce
River Arrow, Alcester	o	Scarce
Stratford Canal, north-west of Stratford-on-Avon	o	o
Wyken Slough, north-east of Coventry	Abundant in the lake and connecting streams	o
Oxford Canal, near the above	o	Scarce
River Blythe, Patrick Br.	o	o
Earlswood Reservoir, Canal on north-east side	o	o
Earlswood Reservoir, outflowing stream	o	Numerous
Droitwich, River Salwarpe at exit from town	Abundant	o

(o = species looked for and not found.)

Samples of the water at Wyken Slough, at Droitwich, and at Tewkesbury were taken, and analyses made by Dr L. H. N. Cooper (see Appendix, p. 549). The two former have a high mineral content of peculiar composition, and the latter was found to contain a higher quantity of dissolved matter than is usual in rivers of comparable size.

Mr Spooner supplies the following data on the habitats in which he collected *G. tigrinus*. At Tewkesbury the species was found patchily, at the edge of the river among leaves, roots, and debris around the base of *Glycera aquatica** plants. Here corixids were abundant and *Asellus* common. The few specimens of *Gammarus pulex* obtained were apart from the patches of *G. tigrinus*. Wyken Slough is a shallow lake of about 5 acres extent lying in a hollow on the course of an upper branch of the River Sowe, a tributary of the Avon. It marks a point where Carboniferous, Permian, and Upper Trias strata abut. The bottom consists of black mud, but there is no lack of vegetation round the edges, and the fauna is rich. *G. tigrinus*, *Potamopyrgus jenkinsi*, and a corixid were among the dominant species. The former was sieved in quantity from growths of *Myriophyllum spicatum** and roots of *Oenanthe*: it occurred all round the lake and in both the inflowing and outflowing streams. The River Salwarpe, at the point where the collection was made (on the west side of Droitwich), is a shallow and fairly fast-flowing stream, with a stony bottom deficient in vegetation. The *Gammarus* were collected in numbers from the undersides of stones and bricks lying in the stream. *Potamopyrgus jenkinsi* was present in great abundance—in one place accumulated in such numbers as to form a bottom deposit, in which many *Gammarus tigrinus* were sheltering.

The *Gammarus* collected in the above localities in August were brought back to Plymouth alive and kept under observation in the laboratory. Further consignments of living material were sent by Prof. Munro Fox in November and December 1938.

All the *Gammarus* species which have been studied so far at Plymouth show great adaptability to changes of salinity, provided only that the changes are made very gradually and extended over a considerable period of time. A sudden transference from one medium to the other causes instant death. By working in this way, and with the young rather than with the adult, I have been able to bring, amongst others, the fully marine *G. locusta* to live and breed in fresh water, and, in the reverse direction, *G. pulex* from fresh water to sea water, with no ill effects either during the transition period or afterwards.

The new species has shown an even greater adaptability than the others. Owing to a shortage of the Droitwich water many different mixtures were tried to find the salinity best suited to the animals' needs. This was found to be the "standardized" water used in the *G. chevreuxi* work, namely, one part sea water to four of fresh water from the Plymouth supply, which gives a mixture of about 7‰ salinity. The adults, it is true, died off rather quickly in it, but the young from their matings survived and are still alive and healthy,

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* Miss M. Mare det.

some in pure Droitwich river water, some in pure standardized, and others in a mixture of half and half. They are not yet mature (February), but that no doubt is due to winter conditions retarding development.

Gammarus tigrinus n.sp.

Unless otherwise stated, the following description refers to the fully adult male, and the sizes and proportions given apply only to specimens which have attained the maximum growth.

Colour. The specific name, *tigrinus*, refers to the remarkable colour pattern in the living animal, stripes and bars of dark pigment on a light background.

The body colour is a delicate pale green, yellowish green in the male, bluer in the female. The stripes, of deep indigo blue, run transversely across each segment, and extend to halfway down the sides. In addition to the dark stripe or stripes, each segment is banded posteriorly with a clear yellow, so that the effect, seen dorsally, is of alternating bars of pale green, deep blue almost black, and bright gold. From the lateral view the effect is even more striking. Just below where the stripes end, halfway down the sides, two longitudinal bands of colour run along the whole length of the body. The lower one is darkly pigmented and follows the line of the intestine; the upper one is brilliant red. A colour pattern of this type has not been noted, as far as is known, in any other species of *Gammarus*, and would appear to be peculiar to *G. tigrinus*. In some specimens the red shades off on either side, through orange to pale pink, in others it remains as a sharply defined red band. The usual clusters of "oil globules" in the first three pleon segments are also bright red.

The figure of the whole animal (Pl. IV) was taken from a freshly captured specimen, while the colouring was still vivid. In captivity, a steady loss of colour takes place, particularly in the red; after death, and in preservatives, all traces of it vanish.

Size. Compared with others of the genus, *G. tigrinus* is a fairly large species, the males looking larger than they actually are, by reason of the masses of fine hairs on the appendages. The full-grown male averages from 10·5 to 12·5 mm., the female from 8·5 to 10 mm. These measurements are taken, not, as is frequently done, along the dorsal curve, but with the animal straightened out on a micrometer scale, from the tip of the rostrum to the insertion of the telson.

Sex. The difference between the sexes is much more noticeable than is usual in the genus. All the appendages of the male are larger in proportion, the gnathopod hands, for example, being twice the length of those of the female. The male carries masses of the long fine curved sensory hairs peculiar to this species on its second antenna, first, second, fourth and fifth peraeopods, while the female has none of these, and fewer spines and setae than the male. Breeding appears to continue throughout the year.

The gonads and eggs of the female are dark green in colour. The eggs are comparatively large and numerous; the highest number of young, so far, hatched in one brood in laboratory conditions was 50. There are four pairs of incubatory lamellae, or brood-plates, attached to peraeon segments 2–5, of very unusual proportions, two large and two small. The first is the largest and carries the greatest number of fringing hairs, forty-nine in the specimen figured (Pl. V, fig. 11). The second is almost the same size and shape, with 35 hairs; the third is suddenly very small, narrow, strap shaped, with 22 hairs; whilst the fourth, with 14 hairs, is even smaller and narrower. In another younger female examined, the hairs numbered respectively, 31, 26, 13 and 10.

Owing to its curious conformation the pouch looks only about half the ordinary size of the *Gammarus* pouch, and the eggs when deposited appear to lie farther forward under the peraeon than is usual.

The *body* is slender, with the back evenly rounded. The *cuticle* is microscopically spinulose all over (Pl. VI, fig. 27). The spinules are small and not so produced on the anterior part of the body, giving it a surface like a rough file or rasp, but on the pleon, particularly on the dorsal region, they are much longer, very dense and sharp, and look, under a high power, like a thick prickly fur. Scattered over the body and appendages, and inset either singly or in groups, are numerous sensory processes, in little smooth indentations (see figure).

The *gills* are large, oval in shape, and are carried on peraeon segments 2–7. Two are figured (Pl. V, figs. 7, 12).

Side-plates 1–4 are deeper than the corresponding segments, all with an unusually large number of setae inset on the margins, and fine delicate semi-transparent setae on the under surfaces. The fourth side-plate (which is used as a distinguishing specific character) is longer than it is broad, with the posterior expanded portion rounding off into the inferior margin, and inset with five long setae (Pl. V, fig. 13). The sixth and seventh side-plates are both furnished with fine long setae anteriorly, the seventh being noticeable for the number of serrations and setae behind (see Pl. VI, fig. 17).

Pleon segment 1 (Pl. VI, fig. 18) with 12–14 long delicate setae inset in a row around the anterior angle of the epimeron, is deeply notched behind for the insertion of a seta. In pleon segments 2 and 3 (Pl. VI, figs. 19, 22) the epimeral plates are produced acutely backwards, the second with a number of stout spines and setae on the anterior half, and the third with a row of spines and setae on the inferior margin. The *pleopods* (Pl. VI, fig. 18) are long and slender; the rami are subequal and about twice the length of the peduncle, each with a large basal joint, and about 20 small joints furnished with two long plumose setae apiece. The peduncle of pleopod 3 has a large radiating fan of hairs at the anterior distal angle. One of the cleft spines of the basal joint, inner ramus, is figured (Pl. VI, fig. 20). Pl. VI, fig. 21 shows the unusually elaborate form of the coupling spines in this species.

The *head* is about half as long again as peraeon segment 1, measured along the dorsal line. The rostrum is not produced; the lateral lobes are vertically truncate, the upper and lower angles rounded: there is a rather deep sinus below.

The *eyes* are large and reniform; the ommatidia were about 90–100 in number in the specimen examined; the retinal pigment is black, with the "accessory pigment" forming a white reticulation.

Antenna 1 (Pl. V, fig. 1) is much shorter than antenna 2, being only about three-quarters its length. The first joint of the peduncle is large and broad, and almost as long as the second and third taken together. It carries on its upper surface the "sensory groove" (first noted in *Gammarus chevreuxi*, *Journ. Mar. Biol. Assoc.*, vol. XIII, 1924, p. 386), containing, in the full-grown animal, seven very finely plumose sensory hairs, graduated in size, the longest at the distal end of the groove, each mobile in a little socket. The *primary flagellum* appears to be very fragile; in many specimens it was found broken or regenerating, rarely complete. The longest counted, for the male, consisted of 26–27 joints, with an *accessory flagellum* of 6 joints, the terminal joints in both, minute; for a female of 10 mm. length, the primary numbered 26, and the accessory 6, younger females had 16–23 joints in the one, 4–5 in the other. The *flagellum* is almost glabrous, the setae few and short. Each joint, except the formative proximal and the terminal, carries a small stalked calceolus inset on the inner surface (Pl. V, fig. 2).

Antenna 2 of the male (Pl. V, fig. 3), is noteworthy for the length of the fourth and fifth peduncle joints, the latter being the longer. The fourth carries on either side about 9, the fifth about 11 groups of long flexible setae set fanwise on the outer surface; in addition the fourth joint is furnished with several stout spines on its upper surface, 3 on the outside, in the specimen figured, and 1 on the inner side. The *flagellum* has about 14 joints, each with a group of the long setae on the upper and lower margins. The inner surface of the fourth and fifth joints of the peduncle, and 9—10 joints of the flagellum is clothed with masses of the delicate lightly curled hairs peculiar to this species, set in clusters.

In the female (Pl. V, fig. 4) these hairs are entirely lacking. The flagellum in the 10 mm. specimen referred to above numbered 14 joints.

The *gnathopods* show a great disparity in size and armature between the sexes.

Gnathopod 1: in the male (Pl. V, fig. 5) the hand is oblong oval and the palm crenulate, oblique, with a large truncated median spine. At the palmar angle, the long claw shuts down between the "angle-groups" (Pl. V, fig. 6), with one stout and one short curved spine inset together above the tip on each side, and one short curved spine on the upper surface at the tip, with two underneath.

Gnathopod 2: in the male (Pl. V, fig. 7). The hand is broader in proportion to its length than in gnathopod 1; and the palm is sinuous and crenulate, more nearly transverse. It has the truncated median spine on the margin, and, at the palmar angle, a graduated row of three stout spines on the outer side, and two groups of short curved ones underneath (Pl. V, fig. 8).

None of the characteristic sensory curled hairs are developed on the gnathopods.

In the female there is very little difference in the two gnathopod hands (Pl. V, figs. 9, 10). The palm is more oblique in gnathopod 1. They are only half the length of the hands of the male. Gnathopod 2 carries the first and largest of the brood-plates (Pl. V, fig. 11).

The peraeopods. The most noticeable point about the peraeopods, and one which distinguishes this species from the others of the genus, is the sensory armature. Generally speaking, the sensory hairs peculiar to a species which develop in the male at sexual maturity occur only on the first pair of the peraeopods. In *G. tigrinus* the second, fourth, and fifth as well as the first are all furnished with masses of the curled hairs on the under-surface of joints 4–6. The peraeopods carry also dense clusters of strong flexible setae set fanwise on the margins, and groups of various sized sensory spines, including the slender setiform kind developed on these appendages in *Gammarus*. In addition, a new and hitherto undescribed type of spine, is found on the anterior margins of a few of the joints. This is the "stiletto spine" (Pl. VI, fig. 15) shaped like a stiletto, with a strong flat shaft, broad at the base and tapering off to a pointed end.

The fourth is the longest of the peraeopods. The anterior margins of the basal joints of the three hinder peraeopods (Pl. VI, figs. 14, 16, 17) are inset with stout spines; the margin of the posterior expansion in each has a row of setae of varying lengths; the under surfaces in all three carry numbers of the delicate hyaline setae.

In peraeopod 3, the hind-lobe is free; in 4 and 5 the expansion narrows gradually to end in a group of spines and setae at the distal angle.

The peraeopods of the female resemble those of the male, though smaller in all their parts. They have the same groups of long setae and sensory spines, fewer in number, but none of the curled hairs. The stiletto spines are also present, very poorly developed as compared with the male.

The *uropods*. Uropod 1 (Pl. VI, fig. 23) is the longest of the three, a third as long again as the second (Pl. VI, fig. 24). In the third, outer ramus 2-jointed; inner ramus about two-thirds the length of the outer. Both carry spines, clusters of setae, and long plumose setae of two kinds, those bordering the margins being straight with firm shafts. The second kind are few in number, very fine and flexible, curving over at the tips.

The *telson* (Pl. VI, fig. 26) is cleft to the base. There is a lateral group of one spine and setae on each side, and a group of two spines and setae on the apices.

To summarize briefly, the principal distinguishing characters of the new species, *Gammarus tigrinus*, are to be found:

1. *In the antennae*: antenna 1 shorter than antenna 2; accessory flagellum 5–6 jointed; fourth and fifth joints of peduncle of antenna 2 in the male exceptionally

long, the fifth the longer, flagellum about equal in length to these 2 joints together.

2. *In the gnathopods:* the difference in size of the hands between the adult male and female.

3. *In the peraeopods:* the shape of the fourth side-plate (Pl. V, fig. 13).

4. *In the uropods:* the proportions of the rami in the third, the inner ramus two-thirds the length of the outer.

5. *In epidermal structures:* in particular, in the long curled sensory hairs peculiar to the male of this species, and the positions in which they occur.

APPENDIX

By L. H. N. Cooper

Qualitative examination of waters from the Midlands of England, in which Prof. Munro Fox had found the new species, *Gammarus tigrinus*, described above by Mrs Sexton, showed them to be of unusual composition suggesting the need for a quantitative analysis.

METHODS OF ANALYSIS

Standard methods of analysis were used. Sodium was not determined directly but was calculated as the equivalent of alkali required to satisfy the acid radicals not already taken up by calcium and magnesium. Potassium was not present in considerable amount.

The salts composing "total solids" dried at 180° C. should be anhydrous except for magnesium sulphate which is likely to contain one molecule of water of crystallization. This has been computed for Table I. At 180° C. also, bicarbonate must have been converted to carbonate and allowance has been made for this in calculating the composition of total solids.

The positions of the waters sampled in August 1938 have been described by Mrs Sexton. Those from the Droitwich river (River Salwarpe) and from Wyken Slough, which resemble neither diluted sea water nor the hard waters found in limestone districts, must have their origin in the salt springs of the district. The chloride, although not proportionately as high as in sea water, is yet remarkable for an inland stream. Calcium and magnesium are not very much greater than they would be in diluted sea water of the same chlorinity. Bicarbonate and sulphate are high and are present in part as sodium salts, the origin of which is not obvious; unless it be by base exchange between dissolved sodium chloride and limestone rocks. *p*H determined after the samples had stood 18 days was as high as 9·0 in the water from Wyken Slough and 8·8 in that from the Droitwich river. In a sample taken from the Droitwich river on December 8 1938, after a period of heavy rainfall, *p*H had fallen to 7·9 but otherwise the water resembled the August sample somewhat diluted. It is evident that these waters provide an unusual ecological environment and deserve a more systematic investigation than has here been possible. Excess base, *p*H and chloride are expecially worthy of further study.

The water from the Avon at Tewkesbury, in which a few specimens of *G. tigrinus* were found, although essentially a normal hard water contains sufficient sodium chloride to suggest some admixture of saline water of the Droitwich type. Nearly 10% of the "total solids" remained unaccounted for. Part may be attributed to organic matter in which the water was rich. Since the Avon water was not the main subject of study, the cause of the deficiency was not further sought.

TABLE I.

COMPOSITION IN MILLI-EQUIVALENTS PER LITRE

		Droitwich River		Wyken Slough	River Avon Tewkesbury
	Droitwich Spa*	Aug. 1938	Dec. 1938	Aug. 1938	Aug. 1938
Na^+ (calc.)	5247·4	36·46	—	29·40	2·37
Ca^{++}	68·3	10·22	—	2·22	5·88
Mg^{++}	10·9	6·66	—	3·99	3·92
Total cations	5326·6	53·34	—	35·61	12·17
Cl'	5232·7	41·63	28·89	18·64	2·10
SO_4''	93·5	8·10	—	7·55	4·35
HCO_3'	0·4	3·32	3·02	8·79	5·72
CO_3''	—	0·29	0·00	0·63	0·00
Total anions	5326·6	53·34	—	35·61	12·17
*p*H	—	8·8	7·9	9·0	—

PERCENTAGE COMPOSITION OF TOTAL SOLIDS DRIED AT 180° C.

	Droitwich River		Wyken Slough	River Avon Tewkesbury
	Aug. 1938	Dec. 1938	Aug. 1938	Aug. 1938
Na (calc.)	26·2	—	32·4	7·0
Ca	6·4	—	2·1	15·0
Mg	2·5	—	2·3	6·1
Cl	46·0	47·1	31·0	9·5
SO_4	12·1	—	17·0	26·6
CO_3 (including HCO_3)	3·6	8·46	14·1	21·9
H_2O (in $MgSO_4.H_2O$)	1·9	—	1·7	4·5
	98·7	—	100·6	90·6
Total solids at 180° C. (g./l.)	3·202	2·174	2·135	0·785

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* Analysis by H. B. Salt, *The Natural Brine Baths of Droitwich Spa and the Surroundings*. Whately, Droitwich. (No date).

EXPLANATION OF PLATES

Plate IV

Gammarus tigrinus n.sp. × 12. The figure was drawn from a freshly captured male, from Droitwich river, November 1938. It shows the colour pattern in life. The pale green colour of the body is represented here by light grey; the bright yellow bands on the posterior margins of the body-segments by clear spaces; and the deep indigo blue of the stripes, bars and patches by the dark tint. The red is as it appears in the living animal. Most of the sensory hairs on the appendages have been omitted, for the sake of clearness of outline.

Plate V

The figures are drawn from one specimen, a male, left side. × 28, unless otherwise stated

Fig. 1. Antenna 1.
Fig. 2. Calceolus from under surface of primary flagellum, antenna 1. × 200.
Fig. 3. Antenna 2.
Fig. 4. Antenna 2, ♀. Left side.
Fig. 5. Gnathopod 1.
Fig. 6. Palmar angle, under-surface, left hand, gnathopod 1, from specimen figured, showing the groups of spines between which the claw closes down. × 57.
Fig. 7. Gnathopod 2, with gill outlined.
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Fig. 9. Hand of gnathopod 1. ♀.
Fig. 10. Hand of gnathopod 2, ♀, same specimen; gill not shown, brood-plate figured separately.
Fig. 11. Incubatory lamella, or brood-plate from gnathopod 2.
*Fig. 12. Peraeopod 1, with gill attached.
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Plate VI

*Fig. 14. Peraeopod 3.
Fig. 15. Stiletto spine from second joint, peraeopod 3. × 200.
*Fig. 16. Peraeopod 4.
*Fig. 17. Peraeopod 5.
Fig. 18. Pleon-segment 1 with pleopod.
Fig. 19. Pleon-segment 2, epimeron.
Fig. 20. Cleft spine, from basal joint of inner ramus, pleopod 3. × 200.
Fig. 21. Coupling-spines from the peduncle of pleopod 3. × 200.
Fig. 22. Pleon segment 3, epimeron.
Fig. 23. First uropod.
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Fig. 25. Third uropod. (The proportions of the rami are unusual, the inner ramus being shorter than normal, probably regenerating.)
Fig. 26. Telson.
Fig. 27. Cuticle, showing the spinules covering the body, and the inset sensory processes, from the dorsum of pleon segment 4. × 200.

* In these figures it has not been possible to represent the full number of the sensory hairs.

ON A NEW SPECIES OF *GAMMARUS* (*G. TIGRINUS*) FROM DROITWICH DISTRICT

By E. W. Sexton, F.L.S.

With an Appendix by L. H. N. Cooper, D.Sc., F.I.C.

From the Plymouth Laboratory

(Plates IV–VI)

The new species of *Gammarus* here described was first noted in 1931 by Prof. H. Munro Fox, to whom I am indebted for the opportunity of examining it. He found it in abundance in the Droitwich district, living in the brackish waters of the River Salwarpe near the town, in the Canal, and in Wyken Slough near Coventry.

Droitwich is an inland town in Worcestershire, situated on the Salwarpe, a tributary of the River Severn. It is famous for its natural alkaline brine springs, and it is these which make the waters of the district saline.

In August 1938 a quantity of fresh material was collected by Mr G. M. Spooner and Miss M. Mare, who visited several localities in Worcestershire, Warwickshire, and north Gloucestershire. The species was found in numbers at Wyken Slough and at Droitwich (River Salwarpe), and in small numbers in the River Avon at Tewkesbury. The two former localities were already known to Prof. Munro Fox as haunts of *Gammarus tigrinus*. Tewkesbury was visited because of the occurrence of some unknown amphipods in a collection made by Mr G. I. Crawford in 1935. In the light of the fresh material obtained, these specimens are now recognized as the young of *G. tigrinus*. No new localities were discovered. Those visited are listed in the following table, which also gives the occurrence of the only other species of *Gammarus* collected, namely, *G. pulex*.

Locality	*G. tigrinus*	*G. pulex*
River Chelt, Gloucester-Tewkesbury road	o	Abundant
River Avon, Tewkesbury	Sparingly	Scarce
River Arrow, Alcester	o	Scarce
Stratford Canal, north-west of Stratford-on-Avon	o	o
Wyken Slough, north-east of Coventry	Abundant in the lake and connecting streams	o
Oxford Canal, near the above	o	Scarce
River Blythe, Patrick Br.	o	o
Earlswood Reservoir, Canal on north-east side	o	o
Earlswood Reservoir, outflowing stream	o	Numerous
Droitwich, River Salwarpe at exit from town	Abundant	o

(o = species looked for and not found.)

Samples of the water at Wyken Slough, at Droitwich, and at Tewkesbury were taken, and analyses made by Dr L. H. N. Cooper (see Appendix, p. 549). The two former have a high mineral content of peculiar composition, and the latter was found to contain a higher quantity of dissolved matter than is usual in rivers of comparable size.

Mr Spooner supplies the following data on the habitats in which he collected *G. tigrinus*. At Tewkesbury the species was found patchily, at the edge of the river among leaves, roots, and debris around the base of *Glycera aquatica** plants. Here corixids were abundant and *Asellus* common. The few specimens of *Gammarus pulex* obtained were apart from the patches of *G. tigrinus*. Wyken Slough is a shallow lake of about 5 acres extent lying in a hollow on the course of an upper branch of the River Sowe, a tributary of the Avon. It marks a point where Carboniferous, Permian, and Upper Trias strata abut. The bottom consists of black mud, but there is no lack of vegetation round the edges, and the fauna is rich. *G. tigrinus*, *Potamopyrgus jenkinsi*, and a corixid were among the dominant species. The former was sieved in quantity from growths of *Myriophyllum spicatum** and roots of *Oenanthe*: it occurred all round the lake and in both the inflowing and outflowing streams. The River Salwarpe, at the point where the collection was made (on the west side of Droitwich), is a shallow and fairly fast-flowing stream, with a stony bottom deficient in vegetation. The *Gammarus* were collected in numbers from the undersides of stones and bricks lying in the stream. *Potamopyrgus jenkinsi* was present in great abundance—in one place accumulated in such numbers as to form a bottom deposit, in which many *Gammarus tigrinus* were sheltering.

The *Gammarus* collected in the above localities in August were brought back to Plymouth alive and kept under observation in the laboratory. Further consignments of living material were sent by Prof. Munro Fox in November and December 1938.

All the *Gammarus* species which have been studied so far at Plymouth show great adaptability to changes of salinity, provided only that the changes are made very gradually and extended over a considerable period of time. A sudden transference from one medium to the other causes instant death. By working in this way, and with the young rather than with the adult, I have been able to bring, amongst others, the fully marine *G. locusta* to live and breed in fresh water, and, in the reverse direction, *G. pulex* from fresh water to sea water, with no ill effects either during the transition period or afterwards.

The new species has shown an even greater adaptability than the others. Owing to a shortage of the Droitwich water many different mixtures were tried to find the salinity best suited to the animals' needs. This was found to be the "standardized" water used in the *G. chevreuxi* work, namely, one part sea water to four of fresh water from the Plymouth supply, which gives a mixture of about 7‰ salinity. The adults, it is true, died off rather quickly in it, but the young from their matings survived and are still alive and healthy,

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* Miss M. Mare det.

some in pure Droitwich river water, some in pure standardized, and others in a mixture of half and half. They are not yet mature (February), but that no doubt is due to winter conditions retarding development.

Gammarus tigrinus n.sp.

Unless otherwise stated, the following description refers to the fully adult male, and the sizes and proportions given apply only to specimens which have attained the maximum growth.

Colour. The specific name, *tigrinus*, refers to the remarkable colour pattern in the living animal, stripes and bars of dark pigment on a light background.

The body colour is a delicate pale green, yellowish green in the male, bluer in the female. The stripes, of deep indigo blue, run transversely across each segment, and extend to halfway down the sides. In addition to the dark stripe or stripes, each segment is banded posteriorly with a clear yellow, so that the effect, seen dorsally, is of alternating bars of pale green, deep blue almost black, and bright gold. From the lateral view the effect is even more striking. Just below where the stripes end, halfway down the sides, two longitudinal bands of colour run along the whole length of the body. The lower one is darkly pigmented and follows the line of the intestine; the upper one is brilliant red. A colour pattern of this type has not been noted, as far as is known, in any other species of *Gammarus*, and would appear to be peculiar to *G. tigrinus*. In some specimens the red shades off on either side, through orange to pale pink, in others it remains as a sharply defined red band. The usual clusters of "oil globules" in the first three pleon segments are also bright red.

The figure of the whole animal (Pl. IV) was taken from a freshly captured specimen, while the colouring was still vivid. In captivity, a steady loss of colour takes place, particularly in the red; after death, and in preservatives, all traces of it vanish.

Size. Compared with others of the genus, *G. tigrinus* is a fairly large species, the males looking larger than they actually are, by reason of the masses of fine hairs on the appendages. The full-grown male averages from 10·5 to 12·5 mm., the female from 8·5 to 10 mm. These measurements are taken, not, as is frequently done, along the dorsal curve, but with the animal straightened out on a micrometer scale, from the tip of the rostrum to the insertion of the telson.

Sex. The difference between the sexes is much more noticeable than is usual in the genus. All the appendages of the male are larger in proportion, the gnathopod hands, for example, being twice the length of those of the female. The male carries masses of the long fine curved sensory hairs peculiar to this species on its second antenna, first, second, fourth and fifth peraeopods, while the female has none of these, and fewer spines and setae than the male. Breeding appears to continue throughout the year.

The gonads and eggs of the female are dark green in colour. The eggs are comparatively large and numerous; the highest number of young, so far, hatched in one brood in laboratory conditions was 50. There are four pairs of incubatory lamellae, or brood-plates, attached to peraeon segments 2–5, of very unusual proportions, two large and two small. The first is the largest and carries the greatest number of fringing hairs, forty-nine in the specimen figured (Pl. V, fig. 11). The second is almost the same size and shape, with 35 hairs; the third is suddenly very small, narrow, strap shaped, with 22 hairs; whilst the fourth, with 14 hairs, is even smaller and narrower. In another younger female examined, the hairs numbered respectively, 31, 26, 13 and 10.

Owing to its curious conformation the pouch looks only about half the ordinary size of the *Gammarus* pouch, and the eggs when deposited appear to lie farther forward under the peraeon than is usual.

The *body* is slender, with the back evenly rounded. The *cuticle* is microscopically spinulose all over (Pl. VI, fig. 27). The spinules are small and not so produced on the anterior part of the body, giving it a surface like a rough file or rasp, but on the pleon, particularly on the dorsal region, they are much longer, very dense and sharp, and look, under a high power, like a thick prickly fur. Scattered over the body and appendages, and inset either singly or in groups, are numerous sensory processes, in little smooth indentations (see figure).

The *gills* are large, oval in shape, and are carried on peraeon segments 2–7. Two are figured (Pl. V, figs. 7, 12).

Side-plates 1–4 are deeper than the corresponding segments, all with an unusually large number of setae inset on the margins, and fine delicate semi-transparent setae on the under surfaces. The fourth side-plate (which is used as a distinguishing specific character) is longer than it is broad, with the posterior expanded portion rounding off into the inferior margin, and inset with five long setae (Pl. V, fig. 13). The sixth and seventh side-plates are both furnished with fine long setae anteriorly, the seventh being noticeable for the number of serrations and setae behind (see Pl. VI, fig. 17).

Pleon segment 1 (Pl. VI, fig. 18) with 12–14 long delicate setae inset in a row around the anterior angle of the epimeron, is deeply notched behind for the insertion of a seta. In pleon segments 2 and 3 (Pl. VI, figs. 19, 22) the epimeral plates are produced acutely backwards, the second with a number of stout spines and setae on the anterior half, and the third with a row of spines and setae on the inferior margin. The *pleopods* (Pl. VI, fig. 18) are long and slender; the rami are subequal and about twice the length of the peduncle, each with a large basal joint, and about 20 small joints furnished with two long plumose setae apiece. The peduncle of pleopod 3 has a large radiating fan of hairs at the anterior distal angle. One of the cleft spines of the basal joint, inner ramus, is figured (Pl. VI, fig. 20). Pl. VI, fig. 21 shows the unusually elaborate form of the coupling spines in this species.

The *head* is about half as long again as peraeon segment 1, measured along the dorsal line. The rostrum is not produced; the lateral lobes are vertically truncate, the upper and lower angles rounded: there is a rather deep sinus below.

The *eyes* are large and reniform; the ommatidia were about 90–100 in number in the specimen examined; the retinal pigment is black, with the "accessory pigment" forming a white reticulation.

Antenna 1 (Pl. V, fig. 1) is much shorter than antenna 2, being only about three-quarters its length. The first joint of the peduncle is large and broad, and almost as long as the second and third taken together. It carries on its upper surface the "sensory groove" (first noted in *Gammarus chevreuxi*, *Journ. Mar. Biol. Assoc.*, vol. XIII, 1924, p. 386), containing, in the full-grown animal, seven very finely plumose sensory hairs, graduated in size, the longest at the distal end of the groove, each mobile in a little socket. The *primary flagellum* appears to be very fragile; in many specimens it was found broken or regenerating, rarely complete. The longest counted, for the male, consisted of 26–27 joints, with an *accessory flagellum* of 6 joints, the terminal joints in both, minute; for a female of 10 mm. length, the primary numbered 26, and the accessory 6, younger females had 16–23 joints in the one, 4–5 in the other. The *flagellum* is almost glabrous, the setae few and short. Each joint, except the formative proximal and the terminal, carries a small stalked calceolus inset on the inner surface (Pl. V, fig. 2).

Antenna 2 of the male (Pl. V, fig. 3), is noteworthy for the length of the fourth and fifth peduncle joints, the latter being the longer. The fourth carries on either side about 9, the fifth about 11 groups of long flexible setae set fanwise on the outer surface; in addition the fourth joint is furnished with several stout spines on its upper surface, 3 on the outside, in the specimen figured, and 1 on the inner side. The *flagellum* has about 14 joints, each with a group of the long setae on the upper and lower margins. The inner surface of the fourth and fifth joints of the peduncle, and 9—10 joints of the flagellum is clothed with masses of the delicate lightly curled hairs peculiar to this species, set in clusters.

In the female (Pl. V, fig. 4) these hairs are entirely lacking. The flagellum in the 10 mm. specimen referred to above numbered 14 joints.

The *gnathopods* show a great disparity in size and armature between the sexes.

Gnathopod 1: in the male (Pl. V, fig. 5) the hand is oblong oval and the palm crenulate, oblique, with a large truncated median spine. At the palmar angle, the long claw shuts down between the "angle-groups" (Pl. V, fig. 6), with one stout and one short curved spine inset together above the tip on each side, and one short curved spine on the upper surface at the tip, with two underneath.

Gnathopod 2: in the male (Pl. V, fig. 7). The hand is broader in proportion to its length than in gnathopod 1; and the palm is sinuous and crenulate, more nearly transverse. It has the truncated median spine on the margin, and, at the palmar angle, a graduated row of three stout spines on the outer side, and two groups of short curved ones underneath (Pl. V, fig. 8).

None of the characteristic sensory curled hairs are developed on the gnathopods.

In the female there is very little difference in the two gnathopod hands (Pl. V, figs. 9, 10). The palm is more oblique in gnathopod 1. They are only half the length of the hands of the male. Gnathopod 2 carries the first and largest of the brood-plates (Pl. V, fig. 11).

The peraeopods. The most noticeable point about the peraeopods, and one which distinguishes this species from the others of the genus, is the sensory armature. Generally speaking, the sensory hairs peculiar to a species which develop in the male at sexual maturity occur only on the first pair of the peraeopods. In *G. tigrinus* the second, fourth, and fifth as well as the first are all furnished with masses of the curled hairs on the under-surface of joints 4–6. The peraeopods carry also dense clusters of strong flexible setae set fanwise on the margins, and groups of various sized sensory spines, including the slender setiform kind developed on these appendages in *Gammarus*. In addition, a new and hitherto undescribed type of spine, is found on the anterior margins of a few of the joints. This is the "stiletto spine" (Pl. VI, fig. 15) shaped like a stiletto, with a strong flat shaft, broad at the base and tapering off to a pointed end.

The fourth is the longest of the peraeopods. The anterior margins of the basal joints of the three hinder peraeopods (Pl. VI, figs. 14, 16, 17) are inset with stout spines; the margin of the posterior expansion in each has a row of setae of varying lengths; the under surfaces in all three carry numbers of the delicate hyaline setae.

In peraeopod 3, the hind-lobe is free; in 4 and 5 the expansion narrows gradually to end in a group of spines and setae at the distal angle.

The peraeopods of the female resemble those of the male, though smaller in all their parts. They have the same groups of long setae and sensory spines, fewer in number, but none of the curled hairs. The stiletto spines are also present, very poorly developed as compared with the male.

The *uropods*. Uropod 1 (Pl. VI, fig. 23) is the longest of the three, a third as long again as the second (Pl. VI, fig. 24). In the third, outer ramus 2-jointed; inner ramus about two-thirds the length of the outer. Both carry spines, clusters of setae, and long plumose setae of two kinds, those bordering the margins being straight with firm shafts. The second kind are few in number, very fine and flexible, curving over at the tips.

The *telson* (Pl. VI, fig. 26) is cleft to the base. There is a lateral group of one spine and setae on each side, and a group of two spines and setae on the apices.

To summarize briefly, the principal distinguishing characters of the new species, *Gammarus tigrinus*, are to be found:

1. *In the antennae*: antenna 1 shorter than antenna 2; accessory flagellum 5–6 jointed; fourth and fifth joints of peduncle of antenna 2 in the male exceptionally

long, the fifth the longer, flagellum about equal in length to these 2 joints together.

2. *In the gnathopods:* the difference in size of the hands between the adult male and female.
3. *In the peraeopods:* the shape of the fourth side-plate (Pl. V, fig. 13).
4. *In the uropods:* the proportions of the rami in the third, the inner ramus two-thirds the length of the outer.
5. *In epidermal structures:* in particular, in the long curled sensory hairs peculiar to the male of this species, and the positions in which they occur.

APPENDIX

By L. H. N. Cooper

Qualitative examination of waters from the Midlands of England, in which Prof. Munro Fox had found the new species, *Gammarus tigrinus*, described above by Mrs Sexton, showed them to be of unusual composition suggesting the need for a quantitative analysis.

METHODS OF ANALYSIS

Standard methods of analysis were used. Sodium was not determined directly but was calculated as the equivalent of alkali required to satisfy the acid radicals not already taken up by calcium and magnesium. Potassium was not present in considerable amount.

The salts composing "total solids" dried at 180° C. should be anhydrous except for magnesium sulphate which is likely to contain one molecule of water of crystallization. This has been computed for Table I. At 180° C. also, bicarbonate must have been converted to carbonate and allowance has been made for this in calculating the composition of total solids.

The positions of the waters sampled in August 1938 have been described by Mrs Sexton. Those from the Droitwich river (River Salwarpe) and from Wyken Slough, which resemble neither diluted sea water nor the hard waters found in limestone districts, must have their origin in the salt springs of the district. The chloride, although not proportionately as high as in sea water, is yet remarkable for an inland stream. Calcium and magnesium are not very much greater than they would be in diluted sea water of the same chlorinity. Bicarbonate and sulphate are high and are present in part as sodium salts, the origin of which is not obvious; unless it be by base exchange between dissolved sodium chloride and limestone rocks. *p*H determined after the samples had stood 18 days was as high as 9·0 in the water from Wyken Slough and 8·8 in that from the Droitwich river. In a sample taken from the Droitwich river on December 8 1938, after a period of heavy rainfall, *p*H had fallen to 7·9 but otherwise the water resembled the August sample somewhat diluted. It is evident that these waters provide an unusual ecological environment and deserve a more systematic investigation than has here been possible. Excess base, *p*H and chloride are expecially worthy of further study.

The water from the Avon at Tewkesbury, in which a few specimens of *G. tigrinus* were found, although essentially a normal hard water contains sufficient sodium chloride to suggest some admixture of saline water of the Droitwich type. Nearly 10% of the "total solids" remained unaccounted for. Part may be attributed to organic matter in which the water was rich. Since the Avon water was not the main subject of study, the cause of the deficiency was not further sought.

TABLE I.

COMPOSITION IN MILLI-EQUIVALENTS PER LITRE

		Droitwich River		Wyken Slough	River Avon Tewkesbury
	Droitwich Spa*	Aug. 1938	Dec. 1938	Aug. 1938	Aug. 1938
Na^+ (calc.)	5247·4	36·46	—	29·40	2·37
Ca^{++}	68·3	10·22	—	2·22	5·88
Mg^{++}	10·9	6·66	—	3·99	3·92
Total cations	5326·6	53·34	—	35·61	12·17
Cl'	5232·7	41·63	28·89	18·64	2·10
SO_4''	93·5	8·10	—	7·55	4·35
HCO_3'	0·4	3·32	3·02	8·79	5·72
CO_3''	—	0·29	0·00	0·63	0·00
Total anions	5326·6	53·34	—	35·61	12·17
*p*H	—	8·8	7·9	9·0	—

PERCENTAGE COMPOSITION OF TOTAL SOLIDS DRIED AT 180° C.

	Droitwich River		Wyken Slough	River Avon Tewkesbury
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Cl	46·0	47·1	31·0	9·5
SO_4	12·1	—	17·0	26·6
CO_3 (including HCO_3)	3·6	8·46	14·1	21·9
H_2O (in $MgSO_4.H_2O$)	1·9	—	1·7	4·5
	98·7	—	100·6	90·6
Total solids at 180° C. (g./l.)	3·202	2·174	2·135	0·785

* Analysis by H. B. Salt, *The Natural Brine Baths of Droitwich Spa and the Surroundings*. Whately, Droitwich. (No date).

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PLATE VI

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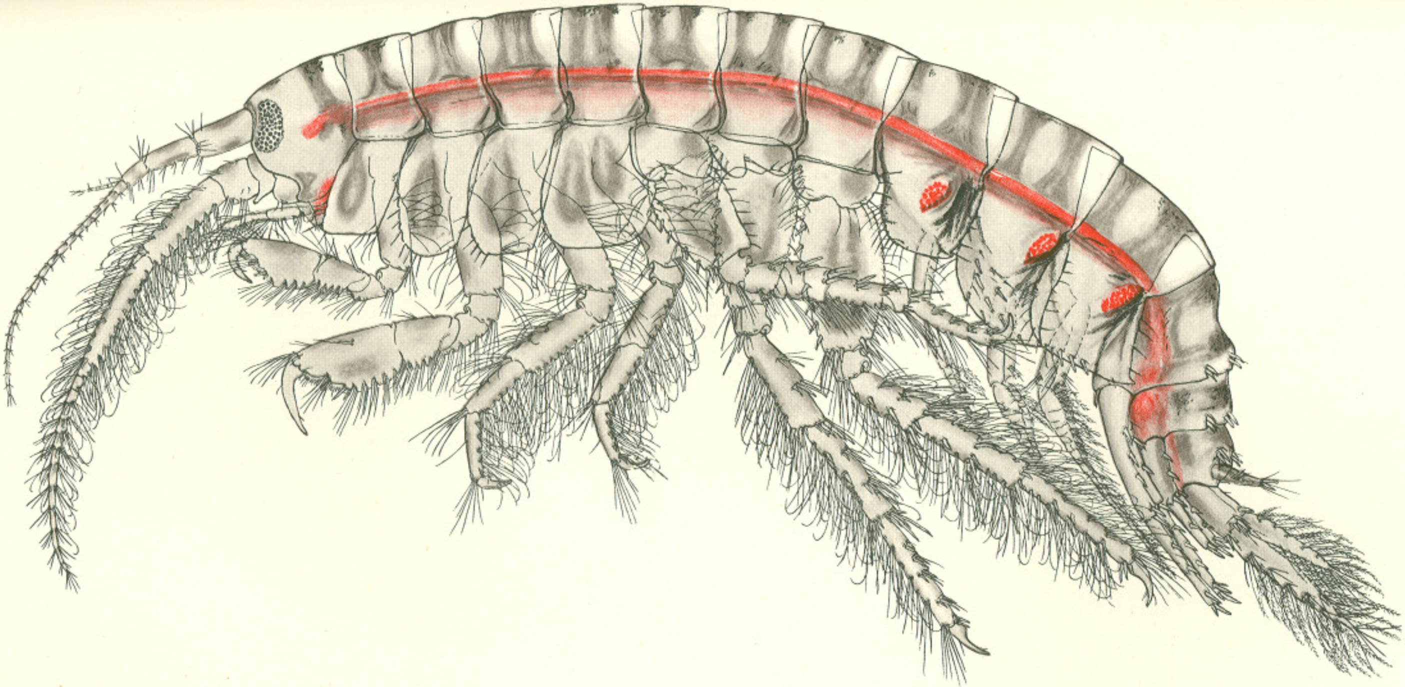

E.W.S. del.

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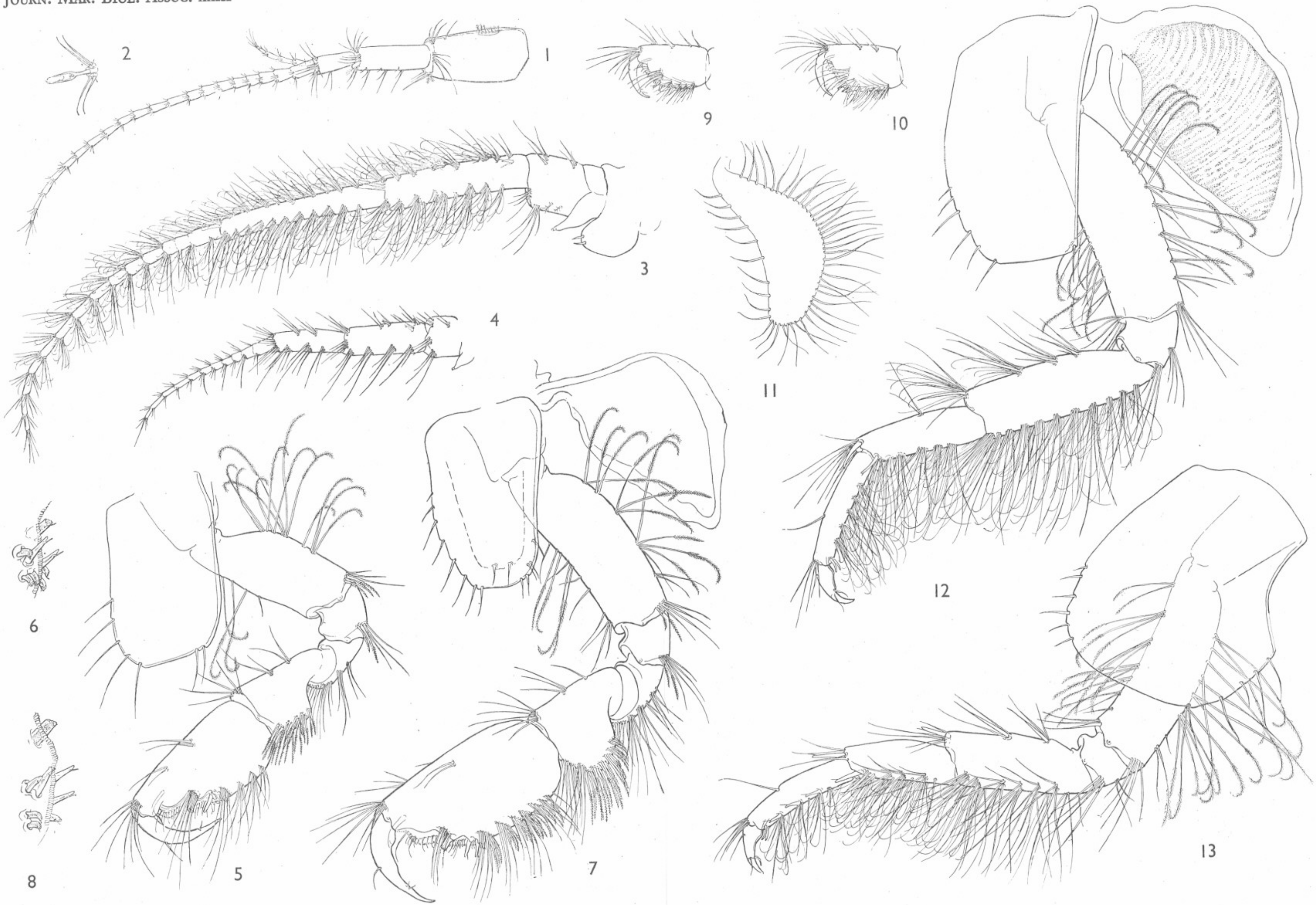
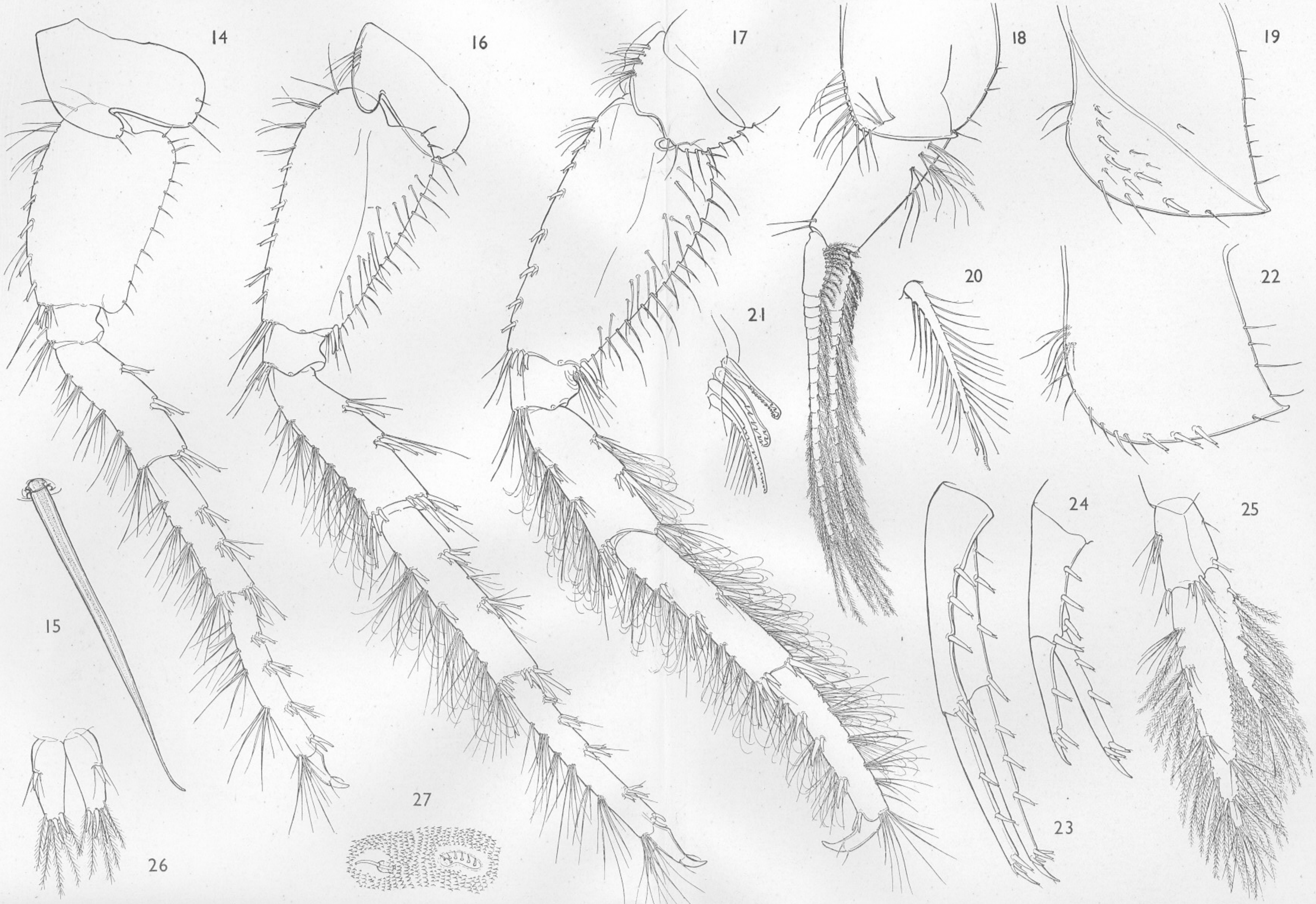
GAMMARUS TIGRINUS n. sp.

PLATE IV.

PLATE V

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STUDIES IN THE MARKET SPONGES
I. GROWTH FROM THE PLANTED CUTTING

By L. R. Crawshay, M.A.

Lately Research Officer, Sponge Fishery Investigations

(Text-fig. 1)

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HISTORICAL

EARLY KNOWLEDGE

Reconstructive growth in sponges, in its broad sense at least, was probably well known to the early Greeks. A passage is quoted by Oscar Schmidt (1862) from a translated text of Aristotle's *Historia*, in which, referring especially to his second sponge, πυκνός, the statement occurs: "Si autem abrumpantur, ex eo quod relictum est, renascuntur et complentur." This is omitted in D'Arcy Thompson's translation (1910), though in another place (548*b*, 15) a revised passage appears in nearly similar words: "but, if the sponge be broken off, it grows again from the remaining stump and the place is soon as well covered as before". It is, in any case, scarcely credible that a fact so generally known to sponge fishermen of the present day as the repair and renewed growth of a sponge from its torn attached remnant was not similarly known to fishermen of the distant past.

F. CAVOLINI, 1785, 1790

It remained for Cavolini (1785, 1853), in the years 1785 and 1790, first to throw important light on this subject, through a number of experiments made by him with living sponges. In these experiments, which he carried out in certain sea caves at Naples, Cavolini was not concerned with any general economic question, but primarily with confirming his belief in the animal nature of sponges, as against the assertion of a recent observer that they were vegetables. The question was especially concerned with contraction of sponges when touched in the sea, as observed by the ancients, apparently here referring to Aristotle (1910, 548*b*, 10), and as confirmed by statements of the divers. Of the three sponges named, as chiefly used by him, the "Spugna officinale del Linneo" may possibly have been a form of the *S. mollissima* of O. Schmidt, though this is doubtful; the "Spugna carnose" was certainly a Hircinian; of the third, the "Spugna detta alcionio foraminoso dall' Imperato", the identity is obscure.

Failure of preliminary rough tests with an iron hook, on sponges growing on the sea bottom, to show the distinct body contraction which he expected, led to his hooking them from the bottom, and redepositing them in the sea for observation. The receptacles which he used for this purpose were of red pottery; not, as stated by Marenzeller (1878), ordinary vessels, but small bells (campanelle) made of this substance, used by boys in their play and apparently similar in form to sheep bells. Inside these, suitably small sponges of 2–3 in. in diameter were placed, pierced and threaded through with a piece of cord, the ends of which were secured through the two holes at the inner end of the bell, where the clapper (battoccio) is normally fastened. The bells with their contents were then lowered into the sea and left for different periods—at first for 12 days—after which, when again raised, it was found that besides healing of the torn surfaces the sponges had made spreading attachment to the face of the bells, and in one case the author states that two were so closely grown together that he could not separate them.

Of the later observations, extending from May to August 1790, the ultimately published account (Cavolini, 1853) is rather fragmentary and lacking in continuity, being mainly, as it seems, a collection of incomplete records made by Cavolini covering this period. In these experiments, sponges were also fastened externally to the bells, some being much incised, or completely cut asunder into as many as four pieces, though whether any were planted as separate fragments is not clearly recorded. The purpose of this cutting was, in fact, further to observe the process of repair, which had previously been noted in the healing of injuries caused by the hooks, and especially injury of the torn base of the sponge removed from its natural attachment; full evidence of this repair appearing in his results. A statement in the earlier memoir (Cavolini, 1785), of the author's confirmation of the assertion by the ancients that the sponge uprooted from the rock repairs itself from the root that remains, is apparently a direct reference to the passage attributed to Aristotle by Schmidt (p. 553).

It should here be mentioned that a remark by Vosmaer (1887, p. 29), where alluding to Cavolini having maintained the animal nature of many sponges, "obwohl er meint, dass die von früheren Autoren ausgesprochene Ansicht in Betreff der Contractilität der Schwämme eine irrige sei", is incorrect. As in the care of his material, Cavolini's statements are often marked by extreme caution. But that his observations confirmed the presence of such contractility is made clear by him from an early point in his experiments; although the process is so slight and gradual that, like the movements of the hands of a clock, bodily contraction is not easy to discern. Similarly later: "Risulta da ciò che la spugna sia degli animali lo più semplice: giacchè il suo muscolare è come una cellulare diradata, come una gelatina; che anzi questa gelatina viene inceppata tra quello ramosissimo semicorneo scheletro: i suoi moti provenienti dalla sensazione, sono debolissimi, e siccome più diradato è questo muscolare, cosi il sentimento minora, come accade nel citato alcionio d' Imperato" (Cavolini, 1785, p. 271).

O. SCHMIDT, 1862–4

About or shortly before the year 1862, Oscar Schmidt first drew attention to the economic possibilities of sponge culture offered by the planting of cuttings in an article contributed by him to the *Wiener Zeitung*; as a result he was invited soon afterwards by the Austrian Government to undertake experiments in this direction with the assurance of all necessary assistance. Schmidt (1862, p. 22) had stated that it was "known" that if an entirely fresh sponge was cut into suitable small pieces, and if these were replaced in the sea, they would grow and develop to complete individuals; later alluding (Schmidt, 1878, p. 534) to the experience of naturalists in this connexion, and especially of Lieberkühn. If he was aware of the work of Cavolini, he makes no direct allusion to it. Marenzeller (1878), in his later review of the experiments of Schmidt and Buccich, seems to infer that he was not aware of it, alluding only incidentally to Cavolini's observations in a footnote, and remarking that these had later fallen into oblivion. Had it been otherwise it would seem strange that Schmidt did not at the outset perceive in the simple device of Cavolini, or more particularly in his use of pottery, a more promising medium of attachment than that of wood, which was destined greatly to increase the difficulties encountered by him and by Buccich.

Schmidt's (1864) preliminary experiments were carried out in the spring of 1863, at Zlarin, and in the Bay of Socolizza of Lesina Island, in the Adriatic. The selected sponges were small, namely, of 2–2½ in. in diameter, these being cut into from four to seven pieces. The fragments were then fastened by small wooden pegs to the bottom of wooden cases bored, in the sides and cover, with numerous holes of ¾–1 in. in diameter, and so that on a surface of 2 sq. ft.

from twelve to twenty such pieces were placed. Three such containers, with their cuttings, were then loaded with stones and sunk in the sea in a depth of 8–10 ft. Examination of the contents after 4 weeks showed that while some of the cuttings had suffered harm from deposited mud, most of them appeared to be in healthy condition. Later, however, at 3 months from planting conditions were found to be far otherwise, comparatively few of the cuttings surviving. Losses were largely attributed to accumulating mud, though at the same time much harm had been caused by interference with the experiments, and removal of the material by fishermen.

Prior to this, Schmidt had observed that certain details of method needed correction and improvement. A substitute, for example, was required for the closed cases which were detrimental. Change also was needed in the method of attachment of the cuttings to which injury had occurred in the region of the wooden pins. On the whole he was confident that the main project in view was economically practicable. He did not perceive that in cuttings of so small a size a great additional strain was placed on the material in any conditions not wholly favourable to healthy growth.

G. Buccich, 1864–72

In May of the following year, 1864, it seems that the charge of the work was passed over wholly to Gregor Buccich, a telegraph operator at Lesina, who received for some time an appropriation from the Ministry of Agriculture in Vienna, and continued the experiments for some 6 years or more, the station being finally closed in 1872. Details of the work and its results are described by Marenzeller (1878), who had access to all official reports, including especially that of Buccich on the whole subject, which forms the basis of Marenzeller's account.

From the latter it appears that the work was seriously obstructed throughout by continued disturbance, both accidentally and often intentionally, on the part of the local fishermen, and through a generally hostile attitude towards such innovations; and, in spite of the repeated protests made by Buccich, its continuance became, through this cause, impossible. In a brief allusion to the circumstances by Schmidt himself (1878, p. 535), shortly before the publication of Marenzeller's report, ultimate failure of the experiments was attributed to this cause, and secondarily to the destruction by *Teredo* of the woodwork employed. Apart from these facts he expressed no doubt concerning the satisfactory progress of the material itself, nor did he change his views regarding the economic possibilities of the general project.

Buccich's methods seem to have varied comparatively little from those previously used by Schmidt. Perforated wooden containers, in one form or another, continued to be used, the cuttings being fastened by wooden pins, which were ultimately secured in lengths of bamboo fixed in the containers. The latter were then ballasted with stones, and sunk to a depth of 5–7 m.

Alternatively, fastening of the cuttings directly to flagstones was tried, but later abandoned through harmful results, attributed to accumulations of bottom deposit. Here also, as elsewhere, allusion is made to the supposed harmful effects of direct light on the cuttings. This has at no time been borne out within my personal experience, even in the full tropical sunlight of the West Indies, or in a small number of cuttings, planted in about 5 fathoms, and observed for a few weeks, during a stay in Cyprus in 1929.

Sponges collected preparatory to planting were fastened inside a sort of fish carrier; and were later cut, with the minimum of delay, to a size stated as about 26 mm. square. They were thus presumably about 1 cu. in. in volume, and nearly the same size as in Schmidt's first experiments. It will later be shown that a cubic inch would represent about one-tenth part only of the approximate volume of the standard-sized cutting latterly adopted in the West Indian planting.

Details of growth and general progress, obtainable from the report, are very limited, and especially, as stated by Marenzeller, owing to the disturbance of any continued observations. If the statement of Buccich, that in the first year a cutting grew to two or three times its original size, had reference to volume, this would represent a fair to good rate of progress. That he should have found, as stated, less growth in the second and third years than in the first and fourth years, is well conceivable only if in the middle period a comparatively reduced food supply occurred, as in some years may happen, and perhaps through exceptional shortage in rainfall.

The estimate by Buccich, of 7 years as the approximate period required for a cutting to reach marketable size, does not necessarily indicate a slow rate of growth, but is consistent with the very small size of the cuttings as planted. From Schmidt's own more definite statements concerning the size of sponges used, and the number of cuttings made from them, the average cutting volume appears as about 1·1 cu. in. Regarding this as equivalent to a sphere of 1·28 in. in diameter, and, as against the "two or three times" increase in the first year, allowing an average annual volume growth factor of 2·0 for the whole period, in 7 years an average diameter of about 6·5 in. would be attained—or, in the more probable depressed form, a horizontal diameter exceeding this—which might reasonably be regarded as of "marketable" size. Had the cuttings been even six times the estimated volume, which again would be much below the optimum, they would have been capable, with the same average annual factor of 2·0, of reaching the same size of 6·5 in. in average diameter in about 4·4 years.

The division of sponges into very small cuttings (prompted mainly, no doubt, by the mistaken idea of economy) has been an almost invariable tendency at the outset of planting and has certainly been one of the chief factors which have retarded progress in sponge culture. There seems, however, in the present experiments, to have been much lack of discretion in the handling of the sponges between collection and planting. Reference is made to the length of time during which sponges may with safety be left out of water, or on dry land, in winter and in summer, the time sometimes extending to hours. If, as too probably, this means that such unnecessary exposure of the sponges was often allowed to occur, there can be no doubt that much of the material must have suffered harm. Eighty years previously, Cavolini had followed the extreme and safer course of not allowing his sponges to pass through the air at all.

EARLY PLANTING IN FLORIDA, 1879–98

After the experiments at Lesina, certain attempts at economic sponge culture were made on the coast of Florida, and the results are referred to by Rathbun (1887) and more especially by Moore (1908). These were, first a small experiment in about 1879 by Fogarty, a buyer in Key West, with cuttings attached to stakes and wires; which was not carried to a conclusion. Later, and for some years following, Monroe experimented at Biscayne Bay with cuttings fastened to staves, stakes, etc., but without any definite success. In a more extensive experiment, about 1897–8 by Dr Harris at Sugar Loaf Key, with some thousands of cuttings attached to galvanized wire, corrosion and breaking of the wire led to detachment and loss of the sponges with high mortality. None of these experiments yielded any serviceable data.

H. F. MOORE, 1901–5

Dr H. F. Moore was commissioned by the U.S. Bureau of Fisheries in 1901 to carry out experiments in different methods of improving the Florida fishery, and his results concerning sponge culture, extending over about 4 years, are fully described in his report (1908). Here it is only necessary to mention certain points in his work, which resulted in a very great advance in our knowledge of the subject. In methods chiefly needing attention for successful planting, a question of primary importance was the selection of a suitable medium of attachment for the planted cuttings. Ultimately, after many trials of different substances and in different forms, Moore was led to adopt the concrete disc, which, except for reduction of the original size, about 10 in. in diameter, and perhaps some modification in form, is unlikely to be surpassed for practical purposes.

For attachment to the disc, except when a short wire spindle vertically piercing the cutting was used, a piece of small-gauge aluminium wire was passed through the cutting and through a hole in the disc on either side of it, the ends being twisted together below. In this manner the cutting was secured in position until able to form its own attachment to the disc. The size of the cuttings planted was at first small; subsequently, however, it was increased to about $1\frac{1}{2}$ by $2\frac{1}{2}$ by 3 in., or with a volume stated to be about 8–10 cu. in., the latter being roughly the same as that adopted for cuttings in the West Indian experiments.

Rate of growth in the planted cuttings was found to vary appreciably in different localities. The exact details are somewhat difficult to interpret, owing to the fact that Moore expresses growth mainly in terms of an annual increase of diameter, quoted as a single quantity only. If, as is to be presumed, this was an average of three dimensions, any such increase in a massive sponge must necessarily depend much on the volume of the latter, and itself increase annually with regular growth. In two grounds selected for comparison, Sugar Loaf Sound and Anclote Key, this annual diameter increase was found to be about 0·8 in. in the former, and about 1 in. in the latter. It was accordingly deduced that growth was slower in the one locality than in the other, and Moore was led to infer that at Sugar Loaf Sound the observations indicated the presence of a dwarfed stock of slower growth. He thought that this dwarfing resulted from the intensive fishing in the shallow and more accessible waters of the Sound and from the constant removal of the larger sponges. It is conceivable that in the course of time such a result might ultimately come to pass; but it is difficult to believe that it could happen in the comparatively short period of some 50 years, since the Florida fishery first attracted commercial interest, i.e. about 1852 (Rathbun, 1887, p. 830).

The observed growth rates are, however, shown graphically in a series of curves for each of the two grounds in question, indicating the volume increase recorded periodically for different series of cuttings. Examination of these curves does not clearly bear out the conclusion that there is an appreciable difference in growth rate on the two grounds. While for Sugar Loaf Sound a factor as low as 1·76 for increase in volume occurs for one short-period series covering 12 months, the average annual factor for the three series together amounts to 1·99. As against this, the corresponding three series for Anclote Key, though more nearly uniform with factors between 2·03 and 2·16, show an average of 2·11. Also, as between the two long-period series, covering 2·9 years for Sugar Loaf Sound, and 4·0 years for Anklote Key, the difference in average for the whole period is again comparatively slight, the figures being 1·99 (the same as the average for this ground) and 2·16, respectively. It may here be mentioned that in the light of the West Indian observations an annual growth factor of 2·0 is generally to be regarded as a fair rate, though not a high one; if maintained from year to year it gives no cause to doubt the generally healthy progress of the growing material.

Moore concluded that a greater aggregate volume is ultimately attained by a greater number of cuttings: or as definitely suggested, by fifty as against twenty cuttings made from the same sponge. It is possible that this view was reached owing to the inadequate data available during the limited period of his observations; and it may also be due to incompleteness of results, through interference with the experiments, of which he complains, and other causes of material loss. Within my personal experience there is no evidence to support this view, for, assuming uniformly favourable conditions for growth, the rate of volume increase, though varying between different individual sponges, is approximately the same for a large cutting as for a small one, made from the same sponge. Apart from positively adverse conditions decrease in the growth factor only appears with approach in size to the maximum volume which the feeding capacity of the sponge can sustain, and this again varies much between different individuals.

GROWTH FROM THE CUTTING

Localities and Methods, 1921–34

This section of the paper, concerning the general subject of growth from the cutting, is based on observations and experience acquired during the course of the Sponge Fishery Investigations, which were carried out on behalf of the Colonial Office and extended over several years. The data were largely obtained on the coastal grounds of the Island of Abaco, in the northern part of the Bahamas, between the years 1921 and 1928; but included also are the results of numerous planting experiments carried out in the Lagoon of Turneffe, British Honduras, between 1926 and 1934.

In the Bahamas, sponges collected from different and often distant grounds were carried in boats fitted with flooded wells kept in communication with the sea, and thus conveyed to the experimental depot ground, closely adjacent to the Laboratory, where they were deposited, either for observation as complete sponges, or for subsequent division into cuttings. In British Honduras similar methods were followed, but as the station was located on a reef it had not the facilities of an adjacent depot; observation of the material was made by periodic visits to the different planting centres, extending over a large area within the lagoon, from within which all sponges were obtained.

For purposes of basal support, concrete discs were used throughout from an early date. The size of these ranged from $3\frac{1}{2}$ to 10 in. in diameter; the latter was used infrequently and for special purposes only, the disc chiefly used during the later part of the work being $4\frac{1}{2}$ in. in diameter at the upper surface. Except in certain grouping experiments, all discs were numbered by means of a series of short cuts made with a hacksaw, proceeding from a zero point on the upper surface and arranged on a decimal system around the different faces and edges of the disc. These marks were thus indelible; they could be extended to high numbers and with their help the progress of the cutting could be recorded over an indefinite period.

Reconstruction, Basal Growth and Rounding

Assuming the presence of favourable conditions of location and the important essential of careful handling of the material, fragmentation, carried out by the clean cutting of a market sponge, is followed in a very short period, commencing almost at once, by a lengthy process of general reconstruction, as required to equip the cutting for independent existence and self-maintenance. If all things are favourable the loss in volume directly due to cutting is, or should be, practically negligible.

It may be noted here that a normal and healthy sponge has certain requirements in which a cutting is at first partially or wholly deficient. These include the complete investment of the whole outer surface with a covering pellicle, accompanied in parts, where much exposed to the sunlight, by a protecting zone of densely pigmented cells, and normally—though not strictly essential—some basis of attachment. In addition the canal system contained within the severed fragment must be modified to suit the sponge to a separate existence.

Though a cutting may be orientated in any position, and, as mentioned by Moore, successful growth may be effected by a fragment from the interior of the sponge without any part of the dermal surface, absence of the latter is liable at least to retard progress in early growth, if not to give occasion to higher mortality. On this point the results of the experiments have led to the conclusion that the optimum conditions in planting are provided by retaining in the fragment a portion of the original dermal surface, and securing the latter downwards, against the concrete planting disc, or other base of attachment. It follows therefore that, in this position, the change involves considerable readjustment of the canal system, for the surviving remnant of the pore-bearing surface has now to be closed off to form the attaching base of the new sponge.

In the newly cut fragment (Fig. 1) it is questionable whether any one or other of these processes has a prior call. All appear to proceed simultaneously. Healing—the formation of a delicate covering on the cut surfaces—is a matter of a few hours only. Concurrently with this, closure of the larger surface apertures—no longer to function as previously, or only very partially so, in the new canal system—is more gradual, and in accordance with their size may occupy a day or two for purposes of preliminary completion. In this the process appears to be much more a matter of upgrowth and closing from within and below than of covering in at the extreme surface. The importance of this is evident in cases of local subsurface injury, where, if the injured part becomes enclosed within the body of the sponge, need may arise for the complete walling off of an interior damaged and macerating region. Such a space, especially if large, is liable not to be reoccupied, but to remain a closed cavity containing at length only the remnants of the dead skeleton. Otherwise, if close to the surface, the injured region will in due course be sloughed off, and the space will probably be refilled ultimately by new growth from below.

Simultaneously with the new dermal covering a varying number of quite small apertures are left unclosed to form the new oscula.

The number of these, though variable and differing with the characters of the sponge, is usually very small; and in small cuttings at least there may at first be no more than one. The position is also very variable, and probably determined by that point of the new surface where a section of the existing canal system provides the most complete and effective means of communication with the remainder in the adjustment of the new system of circulation. Commonly, around each of these early oscula, a delicate tubular outgrowth is soon extended, and very shortly a strongly working circulation is restored.

Concurrently with this change, pigmentation of the newly cut surfaces proceeds by gradual stages; and if light conditions are strong, becomes appreciably visible to the eye within a few hours. Complete "blackening" of the surface, which is a matter of degree in the massing of increasing numbers of deep brown pigmented cells at and below it, is more gradual, and may occupy a week or two, depending on the intensity of the sunlight. Where screened from the sun, as in certain lower parts of the subsequently growing sponge, pigmentation is much reduced or wholly absent.

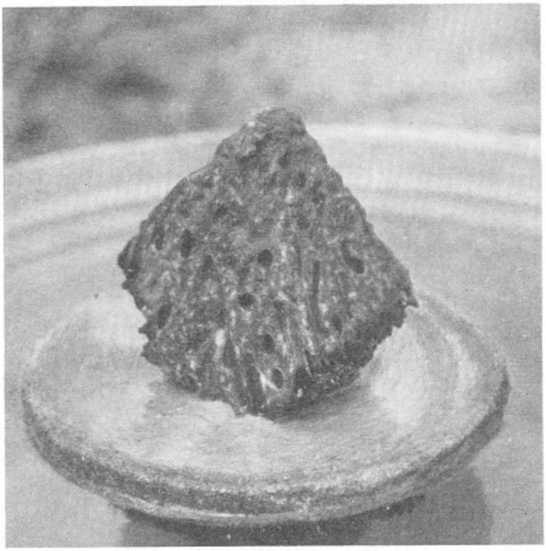

Fig. 1. Newly made cutting from a Wool sponge. The disc is 7 in. in diameter.

Progress in growth and change is necessarily very variable, depending on food supply and other conditions. Where a fixed base of attachment is present, as provided by the concrete disc to which the cutting is firmly secured (see Fig. 1), downward growth from the lower region of the cutting is a prominent feature from an early period; and continues until complete attachment to the disc is effected by the sponge itself. This process is gradual, and may occupy a period ranging from a few weeks to a few months, depending much on circumstances of location. If the position is disturbed, or the cutting insecurely fastened at the outset, attachment may never occur, the base then becoming merely rounded off and strengthened against friction by additional fibres. It should here be explained that it has been customary of late years for certain planters to follow the method adopted by Moore of fastening the cutting by a length of aluminium wire passed through it, and through a hole in the disc. The method adopted in all planting experiments here described has, however, been to secure the cutting firmly by means of a pin, passing through both cutting and disc. This pin is made from the thoroughly dried stalk of palmetto, with a small projecting head at the upper end to prevent lifting of the cutting.

When the basal attachment is completed it is usually followed by a more or less pronounced extension of the base as a thin marginal plate, and it is probable that this process has as much importance for feeding purposes in that region as for general attachment.* After a time the basal marginal extension not infrequently reaches the edge of the planting disc; it will then often pass over the edge, and continue down the side until, with increasing growth, the whole of the disc except the lower surface may become enclosed within the base of the sponge.

As previously stated, the rule adopted almost invariably, from an early period of the work, has been to plant the cutting with its portion of the dermal surface downwards and tangentially against the disc. The exact details of the fate of the original dermal covering of this part of the cutting have not been followed. It seems that, in a healthy sponge, it must be absorbed. In the ultimate basal growth there remains no visible trace of it as distinct from any other part of the original cut fragment, but in section it is usually distinguishable by its darker colour from the new growth around it. The ultimate investment of the disc, or other basis, by the cutting is so complete that in the grown sponge every detail of irregularity in the surface of the object is usually distinctly impressed on the base of the sponge and on the surface of its skeletal network after maceration.

On this subject of basal growth—perhaps more properly, in its origin at least, to be regarded as cell migration—some digression has been made from that of the general growth. Assuming that roundness is a character of the original sponge, a primary object in growth is to restore rotundity of form to a cutting which is at first roughly pyramidal (Fig. 1). This restoration takes place through a gradually increasing convexity of the separate cut faces as a first step, with, later, complete obliteration of the original lines of the cutting. Progress in this direction will vary considerably with location: on grounds where food supply is abundant, and growth rapid, rotundity may be acquired in about 3 or 4 months; on other grounds it may take twice the time, or longer.

Roundness of form, however, in any strict sense of symmetry or regularity, is very far from being an invariable rule among the market sponges, or in any one species of them; and, though the question of form variability cannot

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* It should perhaps be explained that this basal outgrowth is here regarded simply as an outward extension of the sponge body, and as functioning in continuity with it in all respects. Though not examined in detail, the conditions are apparently similar to those of corresponding basal outgrowths formed by certain Plymouth sponges, while kept under observation in the laboratory, as, for example, in *Esperella*. In these the canal system is continuous, with flagellated chambers extending almost to the extreme limits of the investment.

conveniently be discussed here, it may be stated that where any marked irregularity of growth occurs in a sponge the features constituting such irregularity will usually be shown in the cutting made from it at an early stage of growth, and independently of roundness as a premier character.

Food Supply

Rate of progress of a cutting must of necessity depend largely on food supply, the exact nature of which is uncertain. It may, however, be said that, speaking generally, most favourable conditions for growth are usually found in or near to positions subject to regular tidal interchange, combined with the presence of waste organic products carried down from land areas, or otherwise leading to deposits of partially organic mud. It is not improbable that part of the food is derived through ingestion of the finer particles of waste organic matter itself; but the fact that some of the most favourable situations are far removed from land, and chiefly characterized by heavy deposits of extremely fine calcareous mud—produced, as ascertained by Drew (1914), by denitrifying bacteria—suggests that certain bacteria form in themselves the chief source of supply.

Resting in, and largely covered by this mud, commonly at many miles from land, the sponges thrive and grow rapidly, attaining to the largest sizes reached in the Bahamas, and they often retain remarkable regularity and massiveness of form with their increasing growth. The intensity of the bacteria associated with this mud, chiefly *B. calcis*, is well shown in the fact that, at 3 miles out from the shore from the western end of the Southern Bight of Andros, Drew (1914, p. 42) estimated that there were 35,000 bacteria in each c.c. of surface water and 160,000,000 per c.c. in surface samples of the mud. He notes that the last figure may be underestimated owing to settlement of bacteria during the preparation of his cultures.

Limitation and Increase of Growth

In the absence of directly unhealthy factors life may continue in the cutting, even for some years, under conditions of food-shortage; but growth is much retarded or absent, and there may be an actual decrease in the living volume. In such conditions the existing resources of the individual appear to be primarily employed for purposes of attachment, possibly including the quest for food supply through the medium of the basal extension (p. 563), and secondarily for gradual reconstruction of the natural form. In conditions of bare maintenance this may involve some withdrawal from parts of the outer and upper surface areas, ridges and other prominences especially being vacated, and the retraction at times extending over parts of the cut surface to a depth of perhaps ½ cm. or more. Here, the new surface seals itself over, and the vacated skeleton endings shortly die and, after a month or more, slough off, leaving the new dermal surface wholly bare. In extreme cases of serious partial injury, such as possibly occur through temporary conditions of pollution, so long as it does not extend to the entire cutting, an unharmed portion may still effect recovery by walling itself off from the remainder, and in due course forming a completely rounded and separate small unit, perhaps not exceeding about 2–3 cm. in diameter.

In the normal course progress towards completion of the natural form of the original stock will continue, as stated, through rounding of the surface, with gradual obliteration of the original lines of the cutting, and (if rotundity is a character of the sponge) until complete rotundity is reached. The positions of the dominant oscula will, in the meanwhile, be adjusted to the form, and be mainly confined to the upper surface, though in some sponges these may be partly lateral. The attainment of this stage may be slow, extending over a year or more, or, on the other hand, it may occupy only a few months.

Examples in earlier experiments of completely formed small sponges of no more than 6 or 7 cm. in diameter after some 3 to 4 years from planting, appeared at first to support a prior view that growth and the life period are limited by the age of the stock from which the cutting is made. Subsequent observations, however, have not borne out this view; and, as far as is yet known, the possibilities of growth are unlimited by the age factor alone. With the passage of years a sponge grows to exceed its optimum volume in relation to its individual feeding capacity, and the available food supply around it. As with increase in volume the food shortage becomes relatively greater, maintenance of the individual as a complete whole becomes more difficult, and repair of local injuries becomes less complete. Parasitism and overgrowth also cause increasing interference with ingoing currents and excretion; and while modification of form, in adaptation to need, or often partial withdrawal from outlying parts of the skeleton, may prolong life for several years, ultimate decline and death seems rather attributable to uncontrolled growth than directly to loss of cell vitality with age. This view is in general agreement with the conclusions of Bidder (1932) regarding growth and death in sponges of this group.*

Where conditions are favourable to steady growth, increase in the cutting may range from doubling to trebling its volume, or occasionally more, in the year. Speaking generally, though different species vary in this respect, an annual growth factor of less than 2 is rather low, and suggestive of some deficiency, not necessarily harmful, in the local conditions. A factor of 2 may be regarded as fair, or a fair average, while 2·5 indicates a good rate of growth, and from the point of view of the planter is perhaps the most reliable. Increase showing an annual factor of 3 or more is high, and usually concurrent with the presence of exceptional accumulations of waste matter, which may at any time lead to disturbance of healthy conditions and harmful results to the

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* See also p. 572.

planted material. Conditions such as these may be quite favourable to sponges of investing or creeping habit, as among certain Halichondriae and others; they are, however, disadvantageous or even dangerous to the large and massive market species, easily leading to congestion of the canal system and interference with excretion, with possibly fatal results. If congestion is not extensive and amounts to no more than mechanical stoppage of the canals, it may be remedied by the sponge itself, through isolation and abandonment of the affected region which will later disintegrate and fall away. Two or three of the growing sponges from a series of cuttings in British Honduras were found to be partially dead 3 years after planting, and examination of the material showed that a large part of the incurrent canals, to a depth of 2–3 cm. below the surface, were closely choked with fine mud. It was accordingly decided to cut and replant most of the remaining members of the series, and, with few exceptions, growth in the new cuttings proceeded normally. It is to be observed that the usual method of planting, with the old dermal surface downwards, served in this case to promote recovery.

GROWTH RATE

For observations of growth rate, it is first to be considered that, with certain exceptions, the general form of most market sponges having any definite approach to symmetry, though occasionally spherical, more commonly approximates to that of a depressed spheroid. Hence, while making any necessary correction required in cases of irregularity, estimates of volume are best obtained by the formula

$$V=\tfrac{4}{3}\pi ab^2,$$

where b is half the mean of two horizontal diameters at right angles, and a is half the height. From the volume thus obtained of a sponge before planting, the fractional average for each cutting at the time of planting is known; and while the size of the cut fragments must necessarily vary, and at times considerably so, for estimates of later growth the selection of about five or six of a planted series will usually suffice to give very nearly the true average rate of progress for the whole.

If V = the initial volume at the time of planting, x = the volume reached at a future date, y = the annual factor of increase, then the ratio of increase x/V for 1 year $=y$; for 2 years $=y^2$; and for n years $=y^n$; and generally, for all values, $y^n=x/V$; or, $y=\sqrt[n]{x/V}$; $\log y=\dfrac{\log x/V}{n}$.

Frequently a value other than y is required, as for example the period at which, with V known and y approximately known or assumed, a certain volume x will be attained; or, in the inverse order, with a present volume regarded as x known and y assumed, the approximate age of the sponge is required. As with the growth factor y, n almost invariably involves a fraction, its exact or approximate value being obtainable through transposition of the last formula.

Estimates of progress in terms of the diameter were not ordinarily used for purposes of the experiments. If so required, and taking here, in each case, the average of three dimensions, if d = the original average diameter of a sponge, D = its increased average diameter, p = the annual factor of increase in the average diameter, then similarly, as in the preceding formula for volumes: $p^n = D/d$; or $p = \sqrt[n]{D/d}$, for any value concerned.

And since p is equivalent to the cube root of the volume factor y; if, for example, the latter is 2·0, and sustained for the period, the average diameter of a sponge will thus be doubled in 3 years. Manifestly this cannot continue for very many years; and herein a striking aspect of sponge growth appears, in the capacity at once to resume such rate of increase as here considered, or a higher one, in a fragment severed from a sponge in which any volume increase may have long since ceased.

Actually, the growth rate of a sponge in a given location has been found to vary appreciably over different periods, and short-period observations may at times be misleading. In an early Abaco experiment (E. 6, Velvet), for example, data for the first 8 months were unobtainable through omission to measure the original sponge. For subsequent periods the following estimates of volume increase were obtained for the average of eight cuttings:

Feb. to Jan.	10·9 months	Equivalent annual factor	2·24
Jan. to Dec.	11·7 months	,, ,,	2·30
Dec. to Aug.	7·5 months	,, ,,	2·67
Total period	$2\frac{1}{2}$ years	Average annual factor	2·36

For a single cutting, the largest of the series, the increase through the total period showed a nearly similar average annual factor of 2·34. In this, as in the preceding case, the average rate of increase is calculated direct between the original volume and that of the ultimate growth.

In the foregoing example, as was the custom at the period, the cuttings were very small, and though of unknown volume, certainly did not exceed 2·5 cu. in.

The following example may be given of the high growth rate often observed at Turneffe, British Honduras. Fifteen cuttings of a Wool sponge showed after $9\frac{1}{2}$ months an average increase equivalent to an annual factor of 2·94. This rate, however, was not maintained: one cutting, No. 5585, during the succeeding 19 months, May to December, gave an annual factor of 2·14, while in another, No. 5590, for $20\frac{1}{2}$ months, May to January, it was 2·08. No. 5590 was later replanted as eighteen cuttings on a different and distant ground and three of these cuttings, which alone were measured, gave after $18\frac{1}{2}$ months an average annual growth factor of 3·13. If now the average ultimate volume of these three cuttings—nearly 69 cu. in.— be compared with a similar eighteenth fractional part of the original average cutting at the first subdivision, i.e. 1·02 cu. in., it is seen that the average annual factor for the whole period, 4·04 years, from July 1926 to August 1930, is 2·84, or not far short of that found for the whole series at $9\frac{1}{2}$ months. The two grounds are not quite similar, but the example illustrates the potentially high growth factor of the sponge in question, at the commencement of life as a cutting.

Variability in growth rate among different cuttings of the same sponge is necessarily more pronounced where the general growth factor is high. Though probably due to different causes, there is little doubt that it is largely attributable to the different regions of the sponge from which the cuttings were made, and especially as between the base and the upper and distal parts. In the cutting of a sponge, with comparatively rare exceptions, the general aim has been to replant the whole of the sponge, and in so doing to ensure as far as possible uniformity of size in the cuttings. The method employed is first to divide the sponge vertically into nearly equal complete segments, as many as its size allows; then to subdivide each segment transversely or obliquely to the original "polar" axis of the sponge. In the result it follows that certain cuttings are wholly basal, as contrasted with others from the extreme upper region; and it seems that the stronger growth occurs from the former. Though definite data are very incomplete, this view is supported by certain records of distinctly stronger growth in known basal cuttings. Further, in the few cases observed, it is in the lower region of a young sponge that the reproductive bodies first appear, presumably marking the zone of maximum vitality.

In conditions of food shortage growth may be much retarded or even suspended for a long period, but will again be resumed with change to a more favourable location. In an extreme case, in one of the Abaco experiments, a series of cuttings from a Wool sponge, planted in what proved to be an exceptionally unfavourable position, effected complete reconstruction as well-formed little sponges; but up to 30 months from planting no growth was made, and even a distinct reduction in volume had occurred. Survivors were then moved to another position some 150 yards away, in which after 7 months, when the material had to be removed, a pronounced change with renewed growth was observed, showing approximately the equivalent of an annual factor of 1·7 (though exact results were uncertain through incomplete data). In the second position, where many sponges were placed, the growth rate was usually low, rarely reaching an annual factor of 2·0.

Size of Cuttings

The number of cuttings that can be made economically from a sponge, and the size of the individual cutting, are questions of some importance. In similarly favourable conditions, though cuttings may vary to some extent between one another, small cuttings from the same sponge should make approximately the same rate of progress, in proportion to their volume, as large ones. But it is clearly advisable to observe some uniform standard of cutting volume, whether at all times to be closely adhered to or not, to be retained as an approximate basis of reference and comparison, for records of general progress.

During the latter part of the work such a standard was adopted in the form of a spheroid of 3 by 3 in. in horizontal diameter by 2 in. in height, showing an average diameter of 2·67 in. As sufficing for practical purposes, this was then treated as the diameter of a sphere, and was the unit employed in the preparation of a scale for general reference. The following table is given in illustration: the first column showing the number of cuttings to be made from a sponge of the diameter given in the second column.

Cuttings	Sponge av. diam. in.	Cuttings	Sponge av. diam. in.
1	2·7	35	8·7
2	3·4	40	9·1
3	3·8	45	9·5
4	4·2	50	9·8
5	4·6	55	10·1
6	4·8	60	10·4
7	5·1	65	10·7
8	5·3	70	11·0
9	5·5	75	11·3
10	5·7	80	11·5
15	6·6	85	11·7
20	7·2	90	12·0
25	7·8	95	12·2
30	8·3	100	12·4

In certain of the larger sponges, in which the depression of the spheroid may be considerable, an appreciable difference will occur between its average diameter and that of the sphere of equivalent volume. But, since the diameter of the sphere is the smaller, the result, if uncorrected, will only lead to a slight increase in the size of the cuttings above the standard, and this, on the whole, is preferable in the larger sponge.

It is difficult to cut a sponge at once into more than about sixty fragments without much distortion of the ideal and compact form, and this is liable to place undue strain on the material in its reconstructive growth. It is thus advisable first to cut sponges of large size into three or four large fragments, fastening them together with a piece of line; then later, after an interval of a week or two, when the cut surfaces have healed over, to carry out the complete subdivision. This will give greater scope for orientation of the cuttings, and they will be of a better shape, without having one of the newly cut surfaces resting beneath, which is not desirable.

As an example of steady growth, with exceptional uniformity of progress among the several cuttings and a rather high rate of increase over about 2½ years, through the first stage of cutting, mention may here be made of a Wool sponge planted at Turneffe in October 1927. A special point of interest is the exceptional regularity of the sponge itself, with almost perfect symmetry of form, and with its rather numerous cloacae regularly disposed over the upper surface. This sponge, H. 281, had an average diameter of 9·5 in., and was divided into forty-three cuttings. The cuttings were therefore nearly to the standard scale (which would give 45), though made, as it happened, before any standard unit was adopted. In March 1930, one only of the series was missing. The whole of the remainder had made nearly similar and regular growth, though slightly varying in size, and, for a fairly representative series of ten measured examples, the average annual factor, for 29 months, was found to be 2·67. There can be no doubt that the exceptional symmetry of form of the original sponge was largely accountable for the pronounced uniformity of growth and progress appearing in the whole planted series.

Cuttings from Large Sponges

As bearing on the question of continued growth in cuttings made from a large sponge, the following example is deserving of record. At Turneffe there is a large-growing type of Velvet, of not infrequent occurrence, though regrettably now more scarce than in the past through lack of timely protection of the fishery. An example of this type, H. 38, was obtained in July 1926, having an average diameter of 16·7 in. During removal of the sponge to the planting ground a fragment of it, estimated at rather more than one-third, was carried away by the tide and lost. The remainder, estimated as representing a sponge of about 13·9 in. in average diameter, and equivalent to a standard cutting estimate of about 140 cuttings, was planted as a first step as nine large fragments only, variable in size, but on the average about fifteen times the cutting standard in volume.

Growth in these fragments through the first period of 9½ months could not accurately be known, through uncertainty as to the exact proportion of the sponge which they represented. Later, on the same ground, one of them, No. 6037, showed over 19 months, from May 1927 to December 1928, an annual factor of 2·1. In January 1929, another, No. 6040, having an average diameter of 12·25 in., was moved to another ground, where it was divided into four nearly equal quarters, each quarter being planted in a different position as either fifteen or sixteen cuttings. The cuttings were therefore large in size, as against a standard equivalent of 97 for the whole sponge (table, p. 569). Here, in August 1930, about 18 months later, four measured cuttings from each of two of these quarter-series—the other two not being measured—showed through this period an annual growth factor of 2·6 in one and 2·8 in the other; that is to say, at a date 49 months after the first subdivision of the original sponge, which was probably not less than 15 years old when obtained.

Breeding Process in Cuttings

In the absence of any seriously adverse conditions, no interruption or dislocation of the breeding process, as it would normally take place in the original sponge, should occur in the cutting. The only observed example of such interruption occurred among survivors of some 200 cuttings, planted on the Abaco grounds, which suffered great injury from a serious storm in October 1926. The water was driven from the ground where they were planted, and the cuttings were exposed for some hours to the rain. In this case surviving examples of known breeding material either produced no embryos in the following breeding season, or only very few, and none of these, as far as observed, reached complete larval development. In the ordinary course a fragment cut and planted from breeding stock during the breeding season will continue without check and complete the extrusion of its larvae, as in the original sponge; or, if out of the breeding season, it will proceed with the formation of its proportion of the larval brood next to follow, and will liberate them at the proper season. In Fig. 1 the developing embryos appear as whitish patches distributed over the cut surface.

Duration of Life

In other respects, a sponge grown from a cutting becomes a completely independent organism, and may be recut in due course, and repeatedly, for a long period, if not indefinitely. Allusion has already been made (p. 565) to the causes which contribute to the ultimate death of a sponge of natural growth. Under the very different conditions of repeated subdivision the question of age must still be taken into consideration. On this point it can only be stated that if allowance is made for the probable age of the sponges when cuttings were first made, the total age of the material covered by the present records would not exceed 25 years. This, though likely to be much past the period of increasing growth under natural conditions, is probably still far short of the maximum life limit in similar circumstances.

Regarding this question from another aspect, there can be no doubt that in large sponges which have reached their higher limit of growth, the continued production of larvae amounting in some cases to hundreds of thousands annually must contribute to much impoverishment of the parent. So marked is this wastage in the general body tissues, that at the close of the breeding season it is usually distinctly visible to the eye, in the pitted and impoverished appearance of a section through the living sponge.

With the artificial subdivision of any such sponge, its wider distribution, and the proportionately larger food supply made available to the smaller body, a new factor is introduced, enabling the previously arrested body growth to be resumed. Yet in such conditions the larval production of the fragment is not comparable with that of a young sponge of equal or greater volume, which for some 2 years or so from early maturity is very limited. From the time of planting, the larval production of the fragment is approximately that of its corresponding volume in the original stock and similarly to increase with its own growth.

Whether ultimately this may lead to the death of the parent or not is a question which cannot be solved from the limited records available. In the view of Dr Bidder, whose conclusions on bath sponges are based on past official reports of mine concerning resumption of growth in fragments severed from a fully grown sponge, there is no clear reason to believe that, apart from secondary causes previously referred to (p. 565), any such limitation of life and growth truly and "naturally" exists. With the repeated subdivision of the body of the sponge, in the planting of cuttings, the difficulty of continued self-maintenance beyond the maximum of individual growth is removed, and life should continue indefinitely; nor even should ultimate exhaustion of the parent occur through recurrence of the reproductive process.*

Observations covering about 2000 different sponges, and some 60,000 cuttings derived from them, yield no evidence definitely inconsistent with such a view; that is to say, no distinct example of "senescence" has been observed. From another aspect—and here disregarding partial mortality in the material through external causes, always liable to occur—the total extent of volume increase represented in repeated series of cuttings from a single sponge has, in many experiments, been very far in excess of anything conceivable under conditions of independent growth in that sponge. In the Velvet H. 38, for example (p. 570), an average annual factor of volume increase of 2·3 would in only 8 years lead to the equivalent of a sponge of about 12·8 ft. in average diameter. This would be rather more than four times the average diameter of a very large sponge of the same type, and from the same locality, which I saw several years ago in the warehouse of Messrs Cresswell in London, and probably the largest market sponge on record.

Within the range of the observations, and concerning sponges of a total estimated age not exceeding about 25 years, the recorded evidence seems thus far to support the view that life and growth in sponges of this group has no necessary limit.

I have to express my thanks to the Council of the Marine Biological Association for allowing me the facilities of the Plymouth Laboratory for the preparation of this paper; and to Dr G. P. Bidder for much valuable assistance and advice.

SUMMARY

An account of the chief earlier experiments, from those of Cavolini in 1785 to the close of Moore's experiments in Florida in 1905, is given and the general subject of reconstruction and growth in the sponge cutting is described in detail.

Under favourable conditions loss of volume in the initial stages, following cutting, should be practically negligible, the early steps in repair and recon-

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* Cf. Bidder (1932). Par. 1: "water voles, female plaice, sea anemones, and the bath sponge do not show senescence". Par. 3: "Pseudo-senescence and pseudo-specific size are often mere consequences of the decrease in ratio of surface to volume as size increases....with increase in size the relative diminution in surface requires higher velocity to feed the sponge, lengths of all channels are increased, and consequent friction becomes more than the flagella can overcome."

struction proceeding without delay after severance of the fragment from the original sponge. As chief among these, the dermal covering required for newly cut surfaces, the closing of most of the exposed apertures of the old canal system, and the retention of one or more of these for future oscula, are accomplished rapidly and, for preliminary purposes, are completed within a very few days.

Pigmentation of new surfaces exposed to the light, though very soon apparent, may require a few weeks for completion. Basal attachment (assuming contact with a fixed basal support) is also soon initiated, but may likewise occupy a few weeks, or several weeks, for purposes of complete natural support. Rotundity—or other prevailing character of form exhibited by the original stock—may be reached in a few months, or in several months, the time varying much with the conditions.

Size of cutting is, for purposes of growth alone, unimportant; the same, or approximately the same, rate of increase occurring in the same sponge under similar conditions, in large fragments or (within limits) in small ones. But, for practical convenience, an optimum size is recommended, equivalent to a sphere of 2·7 in. in average diameter, or a volume of about 10 cu. in.

Shortage of food which probably consists chiefly of bacteria may lead to complete suspension of growth, or even to reduction in volume. But in such cases, even after 2½ years, growth is normally resumed with change to more favourable conditions. Rate of growth amounts, under fair conditions, to an annual factor of volume increase of about 2·0. Under more favourable conditions a factor of 2·5 is frequent, and 3·0, or a higher rate, may be reached, though less likely to be maintained.

Breeding is in no way interrupted in the cutting, but in all respects proceeds normally as in the sponge from which the cutting was derived.

Recutting of the material at any suitable stage of its growth from planting—and so continuing at intervals, through successive series of planted cuttings grown from the same material—is attended with the same results as in the first planted series, except in so far as certain growth variation may occur with change of surroundings.

The maximum growth attainable is limited by that volume—coupled with its form—capable of self-maintenance as an independent unit by the feeding mechanism of the individual sponge. Beyond this point, death may occur through causes associated with that limitation. Whether, under conditions of repeated cutting and redistribution of the stock, life and growth can be prolonged indefinitely or not, is at present uncertain. Within the extent of the numerous recorded observations, there is no evidence inconsistent with this possibility.

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The Association was founded in 1884 at a meeting held in the rooms of the Royal Society of London with Professor T. H. Huxley in the chair. Amongst distinguished scientific men present on that occasion were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester, who 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 £16,000.

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*.

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All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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