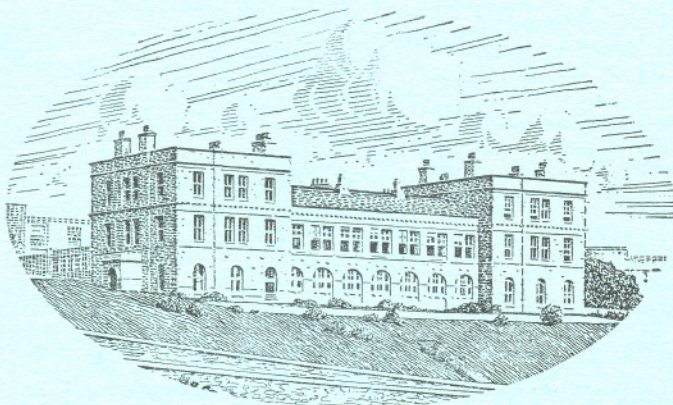


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## THE BIOLOGY OF *LIMACINA RETROVERSA*

By J. E. Morton

Department of Zoology, Queen Mary College, University of London

(Text-figs. 1-5)

The Plymouth Fauna List contains records of two genera of pteropods, *Limacina* and *Clione*. Of the first, *Limacina retroversa* (Flem.) is by far the better-known species, apparently occurring regularly at Plymouth in large numbers in townnettings from outside, and sometimes inside, the Sound. It breeds at Plymouth from June to August, and Lebour (1932) has given a detailed account of its breeding and larval stages and has discussed its role in the plankton. The second species of *Limacina* at Plymouth is *lesueurii* (d'Orbigny), which has been observed from time to time since 1906, when it was very common. The last record in the Fauna is off the Breakwater in 1920. Of the gymnosomatous pteropods, *Clione limacina* Phipps is the only species regularly occurring. Lebour (1931) has described the life history of this form, and mentions February to August as its months of greatest abundance. Its breeding season is June to August. Another gymnosome, tentatively referred to as *Clionina longicauda*, is reported by Russell (1936), and from the specimen department at Plymouth Laboratory the writer obtained some preserved specimens, not easily identifiable, of a *Pneumodermopsis* taken locally; its species is perhaps *ciliata*, recorded by Massy (1917) from the Irish Coast.

In spite of its abundance and wide distribution, *Limacina* has not been carefully studied in the living condition. In Meisenheimer's long account (1905) of pteropod morphology, the functional aspect is rather understressed. Nor did Yonge (1926), in his account of feeding mechanisms in thecosomatous pteropods, deal with *Limacina*. A knowledge of this genus would, however, undoubtedly shed much light on the study of the larger and more specialized thecosomes. Hsiao (1939) has already worked on the sexual succession and life history of populations of *L. retroversa*; the present writer has completed an account of the genital system and reproduction of *L. bulimoides* from the South Atlantic. During the summer of 1952, when abundant and active material of *L. retroversa* was available at Plymouth, an opportunity was taken to examine its movements, mode of feeding, and the structure and action of the digestive system. From similar living material of *Clione limacina*, it is later intended to give an account of the structure and reproduction of this pteropod.

I am indebted to the Director and Staff of the Plymouth Laboratory of the Marine Biological Association for their kindness during my tenure of the



University of London table; and I have especially to thank Dr D. Atkins for first obtaining samples of living material for me, and for drawing my attention to its times of abundance at International Hydrographic Station E I.

#### MOVEMENTS

*Limacina* swims with the aperture of the shell upwards and the surface of the foot widely exposed above. Its movements in a laboratory vessel consist of repeated upward migrations to the surface, describing a broadly spiral course, followed by a more rapid and direct descent during which the wings are out of action. In upward swimming the rhythmically flapping wings constitute a tractellum producing an eddy behind which the animal moves. The effective stroke appears to come about by the contraction of the sheet of muscle fibres running through the base of the ventral side of each wing, underlying the ciliated field, to be referred to below. At the resting stage, the dorsal muscle sheet of the wings would appear to contract and the thicker bases are reflected backwards against the shell to show their under-surfaces which are also ciliated. The expanded tips of the wings are transparent and non-muscular. They are held freely relaxed until a contraction of the dorsal muscle flicks the bases forward again. During descent the wings offer no resistance to the water and are held erect and close together above the animal, forming—when viewed from above—two planes at an oblique angle. The weight of the compact shell and body quickly carried the animal downwards. Fig. 1 illustrates the successive positions of the wings during swimming. From references in the literature to other Thecosomata (see, for example, Stubbings, 1937), it seems likely that the principal movements of these pteropods are in a vertical direction, with the surface of the foot widely exposed for feeding. In *L. bulimoides* there is evidence that this species performs regular diurnal depth migrations (Morton, 1954).

#### FOOD COLLECTING

*Limacina* is entirely a ciliary feeder. It apparently lives chiefly upon dinoflagellates and suitably shaped diatoms. When the Thecosomata became pelagic it is probable they had already lost the ctenidium which was used to produce food-collecting currents in ciliary-feeding prosobranchs. They rely to a much greater extent on the ciliated surfaces of the foot. Yonge (1926), in his account of feeding in the Cavoliniidae and Cymbuliidae, maintains that feeding is carried out entirely by the lobes of the foot, and the cilia at the bases of the wings. As we shall later discuss, it is clear that these families are a good deal more advanced in structure than the spiral-shelled Limacinidae, where it would appear that, in spite of the loss of the ctenidium, the mantle cavity is still the most important feature of the feeding apparatus.

We may first look at some external structures of *L. retroversa* (Fig. 2) of which the most prominent are the foot and the wings. The foot consists of



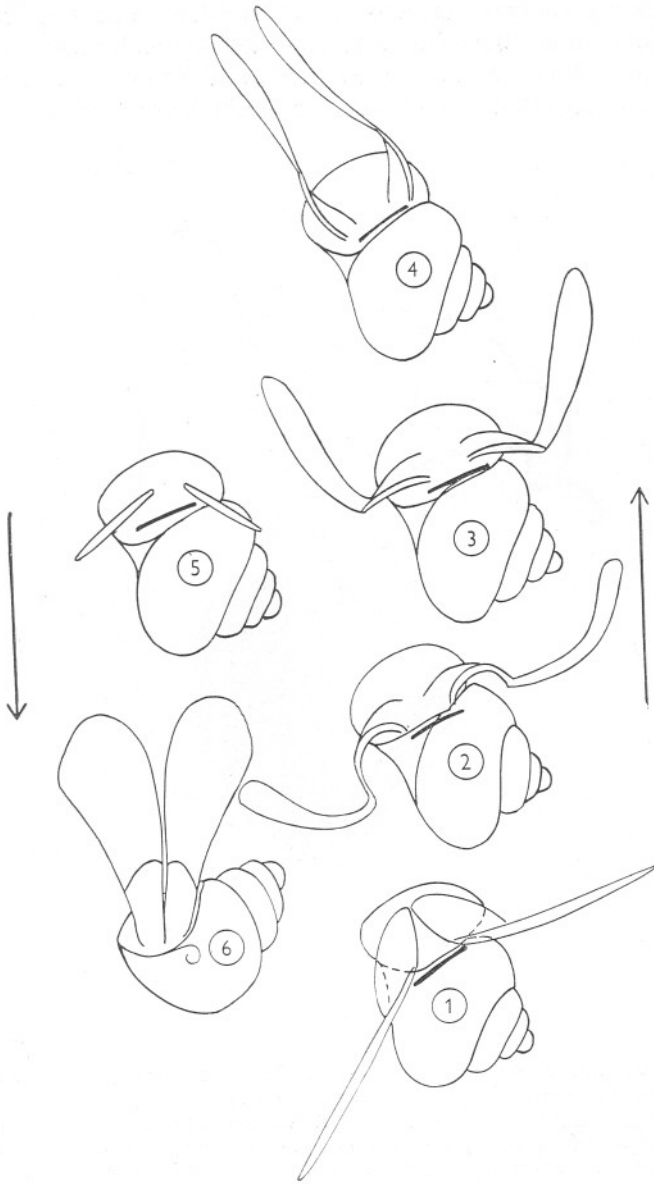


Fig. 1. *L. retroversa*. Outline drawings showing successive positions of the wings in swimming upwards (1-4), and in descending (5). 6 shows the wings held motionless and vertical, in a different view from 5.



a flat median lobe (M L) flanked by two narrower lateral lobes (LAT L). The median lobe is broadly triangular or heart-shaped, widest behind and carrying upon its back surface the transparent, chitinous operculum, golden yellow in colour. This is almost always retained in limacinids though, as Lebour (1932) points out, it may often drop off in older specimens of *L. retroversa*. The flat

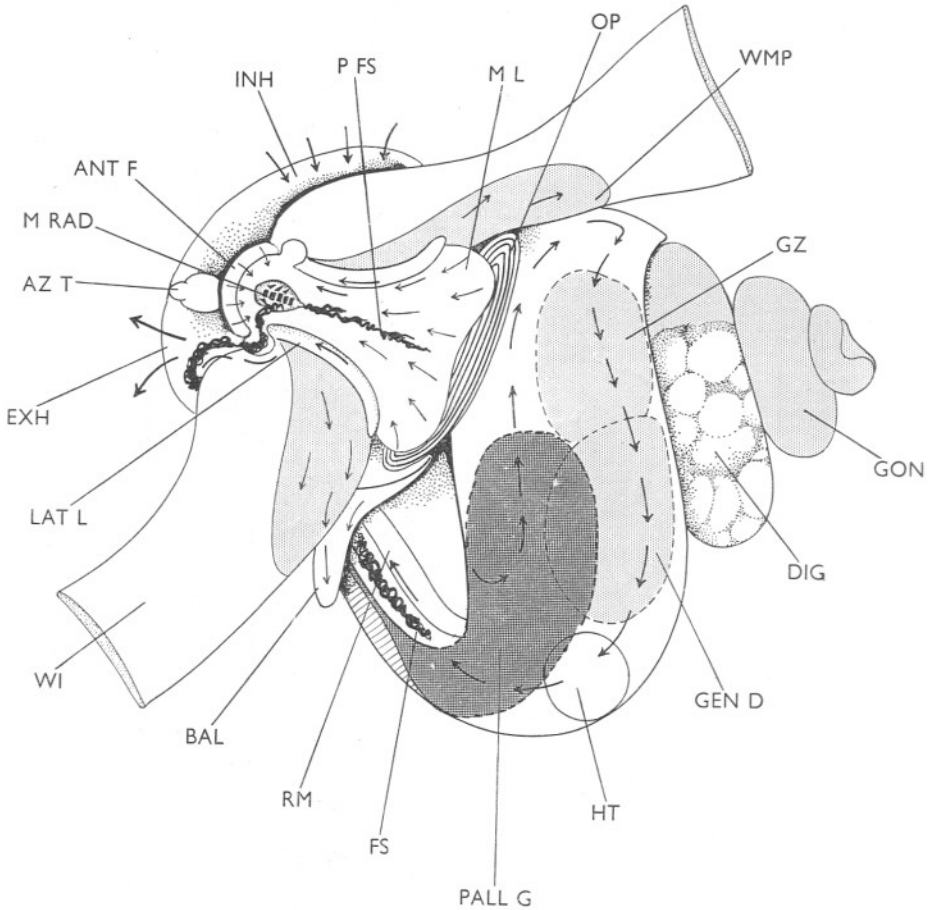


Fig. 2. *L. retroversa*, ventral view. Diagram showing the ciliary currents employed in food collecting. The surface of the foot and the ciliated portions of the wings are fully expanded, and the distal parts of the wings are omitted. The contents of the pallial cavity are shown as if transparent, the pallial mucus gland, the genital ducts and the stomach being represented by stippled areas. ANT F, anterior horseshoe-shaped fold; AZ T, azygous tentacle; BAL, balancer; DIG, digestive gland; EXH, exhalant side of pallial cavity; FS, food string within the pallial cavity; GON, gonad; GEN D, genital duct; GZ, gizzard; HT, position of the heart; INH, inhalant side of the pallial cavity; LAT L, lateral lobe of the foot; M L, median lobe of the foot; M RAD, mouth, with the radula; OP, operculum; P FS, food strings formed on the median lobe of the foot; PALL G, pallial mucous gland; RM, rectum; WI, wing; WMP, ciliated 'Wimperfeld' at base of wing.

surface of the median lobe represents the sole and is finely and uniformly ciliated, and covered with unicellular mucous glands. In front it narrows rapidly, and channels forward towards the mouth between the two lateral lobes which form straight or crescentic folds, bounding the sole on either side from the bases of the wings. These folds are dark brown or blackish in colour, and beneath them and along their edges are tracts of dense forward-beating cilia. These lead into a shallow funnel-shaped depression, enclosing the mouth, and bounded in front by a small, horseshoe-shaped fold (ANT F), lighter brown in colour. Outside the lateral lobes of the foot spring the paired swimming wings, diverging at right angles, one at either side. They expand slightly towards the free tips, which are thin and membranous and somewhat squarish. Each wing has two areas, a rather smaller triangular area at the base, widest posteriorly, and bearing cilia (referred to as the 'Wimperfeld' by Meisenheimer), and the longer free flaps which are colourless and translucent. The ciliated areas are thicker and firmer, and the contraction of their dorsal and ventral muscle sheets results in the movements causing locomotion. In front of the horseshoe-shaped fold bounding the mouth, the head bears a single azygous tentacle (AZ T) with a short terminal papilla inserted in a cylindrical base. Its unpaired condition is a peculiar and unexplained feature, which seems to be common to limacinids in general (Pelseneer, 1888).

The coiling of the visceral mass is sinistral so that the inhalant and exhalant openings of the mantle cavity are reversed from the position usual in gastropods. The pallial aperture is wide and unconstricted, but the chief point of entry of the water current is on the left side near to the posterior corner of the pallial opening. Here the rim of the mantle is encircled with dense cilia beating inwards; their action is so strong that the particles from the surrounding water from a distance of 5-10 mm. are rapidly cleared and drawn into the pallial cavity. Along the whole of the inhalant side, the inner surface of the mantle is clad with cilia. A short way behind the aperture it becomes glandular, with the appearance of a thick, broad shield of mucus-producing epithelium, which extends around the longer or concave side of the pallial cavity and reaches back to the pericardium on the right side. This is the 'shield' or pallial gland, mentioned by Pelseneer (1888) and the 'manteldruse' of Meisenheimer, and in its structure and histology it forms a very constant feature of all known Thecosomata. In position it has the same relations as the hypobranchial gland of prosobranchs, though in histology there seems to be no real similarity. Meisenheimer (1905) provides a simple figure of the 'manteldruse', and we may illustrate it again here for *Limacina* (Fig. 3, right side). The glandular cells are of uniform height ( $45\mu$ ) and width ( $5\mu$ ), and the superficial layer of the epithelium, to about one-fourth its depth, contains a row of ovoid mucous droplets, ready for secretion. The remaining cytoplasm after Bouin's fixation tends to break up into a number of vertical strands of darker staining secretion (brown in azan) while the large, binucleolate nuclei lie in a single



row at the base of the epithelium. Ciliated cells are smaller and less frequent, about one to each pair of glandular cells. They appear as tiny wedges with dark nuclei, inserted between the tips of the glandular cells, but they have evidently a narrow drawn-out connexion with the basement membrane and seem to remain intact during secretion.

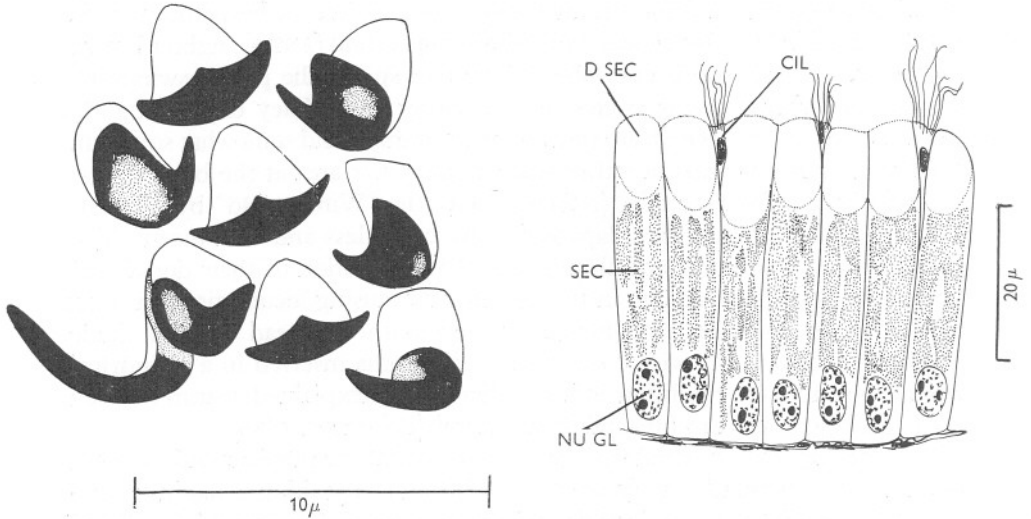


Fig. 3. *L. retroversa*. Left side. Three rows of radular teeth. A lateral mounted flat is shown in the lower row, left side. Right side. Epithelial cells of the pallial mucous gland. (Bouin's fluid, Heidenhain's azan). CIL, ciliated cell; D SEC, distal droplet of secretory material; NU GL, nucleus of gland cell; SEC, darker staining secretory material in the middle part of the cell.

The function of the pallial gland in *Limacina* is to provide mucus for compaction of the particles entering the inhalant side of the pallial cavity, and to carry forward a coherent string of collected particles along the right side of the cavity. Fig. 2 shows the course of the ciliary currents carrying particles into the pallial cavity. There is a strong inward stream on the left side to the tapered upper end of the cavity, with a sharp reversal at the top, and the return of collected material along the pallial glandular strip at the right side. This part of the pallial cavity in *Limacina*, extending far back alongside the stomach and anterior part of the digestive gland, calls to mind in a more elementary form the more elaborate pallial caecum of *Actaeon* (see Fretter, in press) and *Scaphander*, with inward and outward ciliary currents. *Limacina* itself would appear to require little help from ciliary currents in this region: the whole circulation is probably brought about chiefly by the action of the inhalant cilia lying farther forward. Only the lightest of particles seem to make the complete circuit of the pallial cavity. Coarser material, of the size of diatom

frustules, constantly appears to drop out of the stream on to the pallial mucous gland, and to become bound up into the string that constantly moves forward on the right side. This string is carried by cilia along the surface of the glandular sheet (FS) and issues from the mantle cavity on the right side at the anteriormost part of the aperture. Its contents are regularly ingested. They make their way to the funnel containing the mouth, across a tiny, saddle-shaped depression, between the right lateral lobe of the foot and the horseshoe lobe. There is a similar depression on the left side, which was however never seen to be crossed by a food string. At the bottom of the funnel the narrow radular ribbon is thrust from the mouth, and the tiny chitinous teeth take hold of the string and detach a small bolus, which is swallowed into the buccal mass by rapid thrusts of the odontophore. Around the mantle margin as a whole there is a region of strong ciliation. Here, to a smaller extent, particles may be carried directly across the rim and into the mantle cavity. Some particles too may alight upon the horseshoe-shaped anterior lip, where they are caught up by cilia and travel direct to the mouth. Others again, being rejected, find their way on to the ciliary tracts of the under surfaces of the wings and are carried outwards and cast off. These may include larger and looser particles breaking away from the pallial string.

In addition to the collection of food particles in the pallial cavity, a significant part is played also by the foot in *L. retroversa*. This, however, would appear to be much less important than is the case in the Cavoliniidae and Cymbuliidae. Particles alighting on the free surface of the median lobe receive a covering of mucus and are carried forward by cilia in small strings (P FS) directly to the mouth, along the channel between the lateral lobes. Especially strongly ciliated are the brown-pigmented lateral lobes. These have long, close-set cilia keeping up a swift current carrying further particles, embedded in mucus, to the mouth.

On the ciliated bases or 'Wimperfeld' of the wings, the food-collecting action seems much less important. The cilia are shorter than on the lateral lobes of the foot, the direction of current less decisive. So far as could be determined the cilia of the 'Wimperfeld' beat outwards, away from the foot, and their action on particles alighting on the wings is probably rejectory. The flapping of the wings in swimming also serves to dislodge particles from both the upper and lower surfaces; and it would not appear that particles collected on the wings could be easily transferred across the side lobes of the foot in the direction of the oral funnel. The dorsal surfaces of the bases of the wings, which lie closer against the shell, also possess rejectory currents carrying particles away from the mid-line.

A rejectory action seems also to be carried out by the short triangular lobe or 'balancer' (BAL), which Pelseneer has noticed as an outgrowth of the pallial skirt on the right side. Rather strong ciliary currents here proceed from the edge of the mantle, along the lobe to its tip.



## DIGESTIVE SYSTEM

The regions of the gut were described briefly by Pelseneer and Meisenheimer. There is a tiny buccal bulb with an odontophore bearing the radula and a small chitinous jaw. The radula is figured for *L. retroversa* (Fig. 3, left side). Tesch has already illustrated it (1946) for the larger *helicoides*, and the small differences apparent are due chiefly to variations in the inclination of the teeth in the preparations obtained. As stated by Pelseneer (1888) and by Tesch, *Limacina* possesses three teeth in each row, a single-cusped median, flanked by two laterals. The oesophagus leads backwards as a fine tube, lined with short cilia, beating rapidly backwards along its whole length. The salivary glands are minute, secreting mucus through the ciliated roof of the buccal bulb. The narrow part of the oesophagus widens greatly at its posterior end into a conical gizzard, broadest behind, and strongly invested with circular muscle. This organ differs somewhat in shape from that of *Cymbulia*, described by Howells (1936), but is so essentially similar, both in its histology and the arrangement of its teeth, as to need little further description. Each tooth forms a stout thickening of the cuticular lining of the gizzard, terminating in a backwardly curved claw. Four teeth fit very closely together, forming a strong mill filling the whole of the central part of the gizzard. A fifth tooth lies posteriorly, near the threshold where the stomach proper begins; it is smaller and less elevated, forming a flat, thickened sheet of cuticle.

The gizzard, as in all the tectibranchs, is oesophageal, and the stomach, properly so-called, opens widely from it behind. It forms a compact, thin-walled bag, finely ciliated throughout, and serves essentially as an annexe to the single digestive diverticulum. The stomach is illustrated in transverse section in Fig. 5, upper, from which it will be seen to have two exits, the digestive diverticulum and the narrower intestine. In addition, there is a small, more strongly ciliated pocket opening into the stomach opposite the digestive diverticulum. This corresponds, from its structural relations and the nature of its epithelium, to the style caecum which has been found to survive in each of the thecosomatous pteropods investigated. In *Cymbulia* it is a longer, blunt-tipped tube; in *Limacina* it is little more than a rounded pocket only  $25\mu$  in greatest diameter. The epithelium is extremely low, cuboidal or even squamous,  $1-2\mu$  in height, never glandular, and the cilia are long and slender, about twice the length of the cells and always difficult to activate in living material. The contents of the sac, after fixation in Bouin's, consist of a reticulum of mucus, rather denser than in the rest of the stomach. There was never a rotating rod visible in living animals; but from its characteristic ciliated lining, and from the presence in *Cymbulia* of a transparent 'style', it is hard to deny this sac, vestigial though it is, the character of a style caecum. Its function must be little if any at all; the mode of action of the stomach and gizzard is one in which the gentle rotation of a style can play no part. The

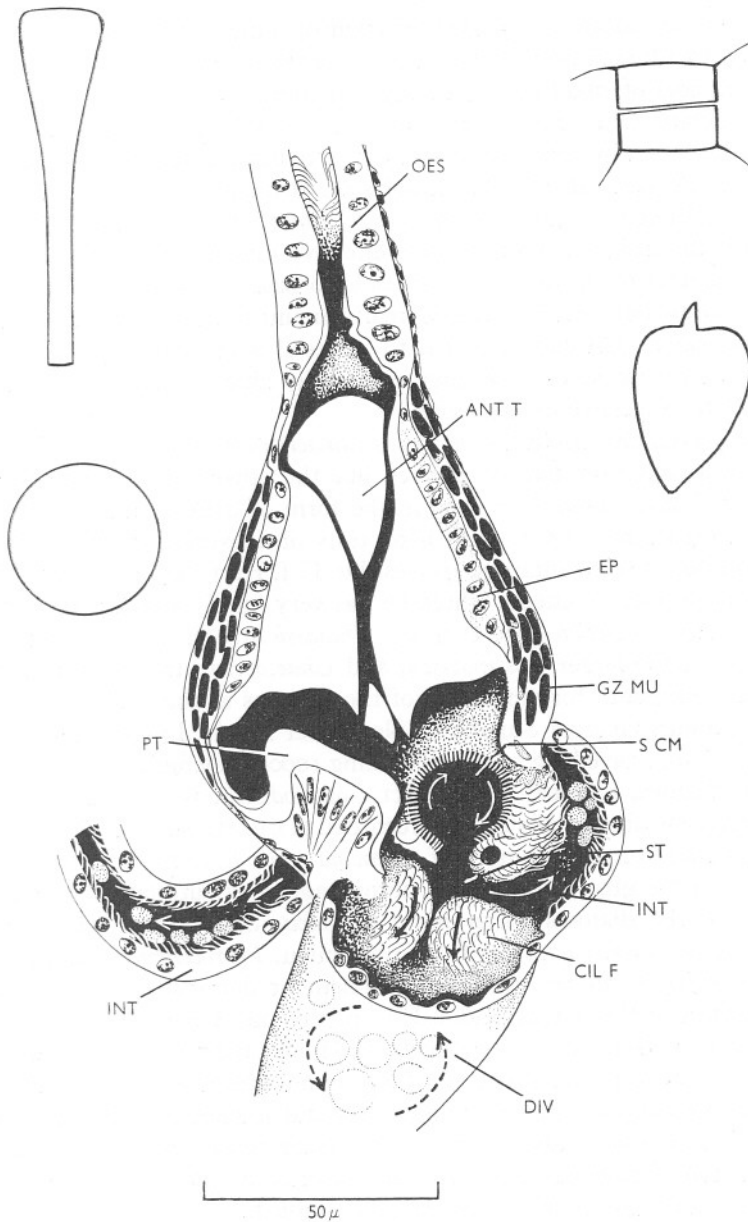


Fig. 4. *L. retroversa*. The gizzard and stomach represented in longitudinal section, chiefly as seen by transparency in the living animal, with structural details supplied from histological observations. The arrows show the directions of the principal ciliary currents. To the same scale are drawn outlines of three species of diatoms and one dinoflagellate, presumed to form part of the food supply of *L. retroversa* at Plymouth. (Left upper, *Asterionella japonica*; left lower, *Skeletonema mediterraneum*; right upper, *Chaetoceros constrictus*; right lower, *Prorocentrum micans*.) ANT T, one of the four anterior teeth of the gizzard; CIL F, ciliated fold at the entrance to the digestive diverticulum; DIV, digestive diverticulum; EP, epithelium underlying a gizzard tooth; GZ MU, circular muscles of the gizzard; INT, intestine; OES, oesophagus; PT, posterior tooth of gizzard; s CM, style caecum; ST, cavity of stomach.



digestive diverticulum is bounded by a pair of rounded folds, closely approximated and with cilia beating outwards from the stomach. Into the intestine, a uniform coat of cilia beats in an anal direction.

The gizzard is a structure perhaps unexpected in a ciliary and mucous feeder, but when looked at in action in the living animal, its presence is less difficult to explain. Its circular muscles are strongly contractile, and its four anterior teeth closely interlock across the lumen. They open apart when the gizzard is distended, to form a most effective mill, through which particles with a diameter of up to  $40-50\mu$  are able to pass. Expansion of the gizzard serves to draw between the teeth a small amount of mucus-bound food from the oesophagus. On contraction hard material is crushed and the contents released in the form of tiny fragments easily able to be received into the mouth of the digestive diverticulum.

Although diatoms could not be fed to *Limacina retroversa* in the laboratory, it is a fair assumption that this species, like the other members of the genus investigated, takes most of its food in the form of diatoms and other phytoplankton organisms, many with hard tests or frustules. For *L. helicoides*, one of the larger sized antarctic species, Dr T. J. Hart has made the following analysis of stomach contents which he has very kindly allowed me to quote: *Fragilariopsis antarctica* (size *c.*  $30\mu$ ), *Thalassiosira* (*c.*  $35\mu$ ), together with fragments of the larger *Coscinodiscus* and *Chaetoceros*, the latter being very large and dominant, forming 75% of the diatoms at the station referred to (St. 500, South Georgia Survey, 21 November 1930). Larger individuals of *Chaetoceros* spp. have long bristles, reaching  $1000\mu$  in length, and it is obvious that this diatom, together with intact *Coscinodiscus*, was being rejected as unwieldy. Few dinoflagellates were recorded at this station, and no specimens are mentioned from the stomach of *Limacina*. For *L. retroversa* at Plymouth, the figure of the gizzard (Fig. 4) is accompanied by outlines to similar scale of three of the diatoms, *Asterionella japonica*, *Skeletonema mediterraneum* and *Chaetoceros constrictus*, and one peridininian, *Prorocentrum micans*, stated by Lebour (1918) to be especially abundant or dominant in the plankton at the mouth of the Sound, and at E 1, in July and August. The oesophagus of *retroversa*, with its five or six longitudinal folds can be widely distended with the passage of larger particles, and there is no reason to believe any of the above phytoplankton organisms would be found unmanageable. In addition, no doubt, *Limacina* is able to exploit the large resources of nanoplankton, including naked flagellates, though the presence of these quickly digestible organisms in the gut is much less easy to establish.

The passage of carmine particles through the alimentary canal is relatively swift. Material administered in the laboratory and ingested from the pallial food string is received from the stomach into the intestine in 30 min. after ingestion. Carmine is never admitted to the digestive diverticulum, and having once reached the intestine it is discharged from the anus about 2 min.

later, being impelled through the intestine in a loose faecal rope, with the help of both cilia and peristalsis.

The muscular action of the gizzard in *Limacina* provides also a pumping action. There is a constant ebb and flow between the stomach and the digestive diverticulum, of a watery mucoid substance, containing suspended yellowish green droplets as well as black and dark brown particles extruded from the excretory cells of the digestive gland. This material is thoroughly circulated within the stomach, while the intestine is held closed, either by the approximation of its walls, or the presence of more solid faecal particles. At intervals, most of the particles from the digestive gland are forcibly ejected as a compact mass into the intestine. Fragments of diatom cases seldom appear to enter the diverticulum, but find their way straight through to the intestine. There is little muscular contraction by the wall of the stomach proper—most of the compression of the stomach is supplied from the gizzard. Neither are there any evident gland cells in any part of the oesophagus, or stomach. Mucus is provided from the mantle cavity and foot, and in smaller part probably from the salivary glands.

The digestive gland is a long, wide tube forming most of the visceral mass, anteriorly to the gonad. It is dark greenish yellow in colour, packed with particles which appear strongly refractile in the intact animal. The lining of the gland has no cilia—there is merely a tapered flange of ciliated cells from the stomach, running backward into the diverticulum, and soon giving way to digestive tissue.

The structure of the epithelium (Fig. 5, lower) is notable for its strong resemblance to that of *Cymbulia* as described by Howells (1936). The lumen is pocketed into small shallow lobules ( $50\mu$  across), set close together by their basement membranes, and lined with cells of three types. The most numerous are the digestive cells, which, at the phase of activity at which they were examined, tend to be squarish or broader than long. They contain two sorts of vacuoles, the basal ones filled with finely granular material staining blue in azan, the more superficial ones on the whole larger, and colourless. The digestive cell at its intact stage has the finely striated border referred to by the present writer (in the press) in the Ellobiidae, and there is throughout a general resemblance between the digestive cells in primitive pulmonates and opisthobranchs. No stages in the formation of 'fragmentation phagocytes' were observed in material of *Limacina*, and from the stage of activity at which the gland was sectioned these are probably not to be expected. The remaining two types of cell are to be regarded as excretory, and both have already been observed by Howells in *Cymbulia*. The first have large rounded nuclei, with prominent nucleoli and chromatin strands and their cytoplasm stains uniformly dark brown with azan or black with Heidenhain's haematoxylin. The second are rather smaller, very broad-based and triangular; their cytoplasm is filled with small distinct spherules staining deeply black with haematoxylin.



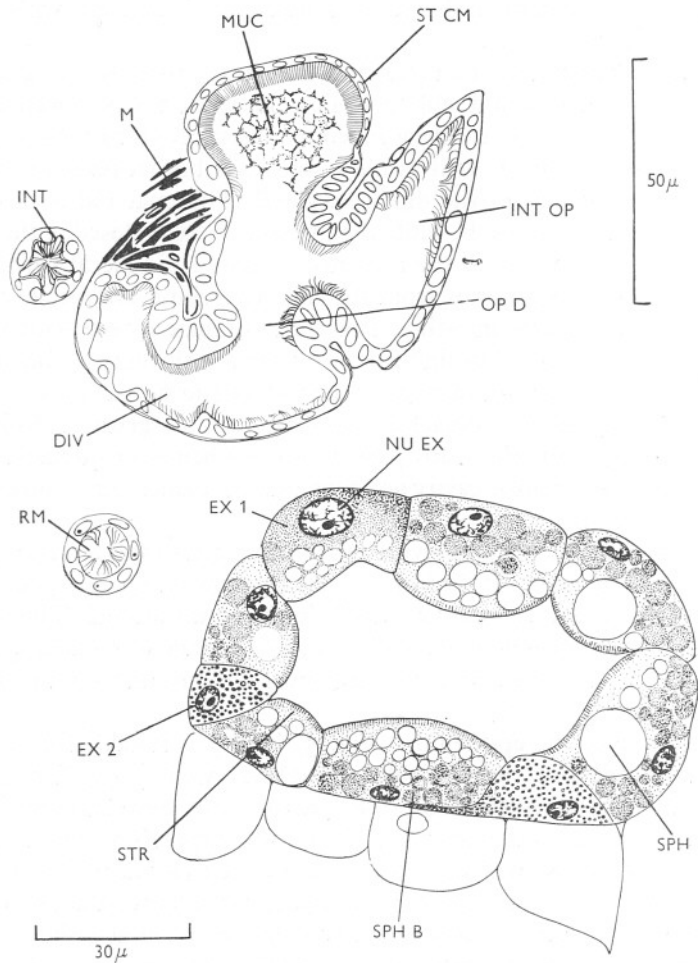


Fig. 5. *L. retroversa*. Upper. Transverse section passing through the stomach, intestine and rectum. DIV, digestive diverticulum; INT, intestine; INT OP, opening of the intestine from the stomach; M, hindmost part of gizzard muscles; MUC, mucous contents of style caecum; OP D, opening of digestive diverticulum from the stomach; RM, rectum; ST CM, style caecum. Lower. Section of a single lobule of the digestive gland. (Bouin's fluid; Heidenhain's azan.) EX 1, EX 2, two types of excretory cells; NU EX, nucleus of excretory cell; SPH, clear spherule of digestive cell; SPH B, blue staining vacuole of digestive cell; STR, striated border of digestive cell.

#### DISCUSSION

Pelseneer first provided a reliable anatomical account of the pteropods, and he settled the distinctness of the two groups Thecosomata and Gymnosomata. The collective name 'pteropods' survives now only as a convenient term of common

reference for pelagic tectibranchs. Thus the more recent classification adopted by Thiele (1931) and by Hoffmann (1939), reverting to a single order Pteropoda as a major division of the Opisthobranchiata, must be regarded as an altogether retrograde step. The Thecosomata and Gymnosomata are unlike in almost all features with the single exception of their habit of swimming. They are almost certainly separately derived from bottom-dwelling groups of tectibranchs. The Thecosomata are entirely ciliary feeding; the Gymnosomata are active carnivores, very often feeding on small thecosomes, and in the majority of their characters they are very much more specialized. It is hoped in a forthcoming paper to give a better account of the anatomy and reproductive history of the British gymnosome *Clione limacina*.

Our knowledge of small thecosomatous pteropods is rather better than for the Gymnosomata. Here we have the advantage of an animal with a hard shell, or a pseudoconcha, which suffers much less damage in collecting and survives longer in the laboratory. Of this group we now have an adequate knowledge of the feeding mechanism, digestive system, sexual succession and reproductive organs; and in oceanographic literature *Limacina* is well known for its ecological importance as a grazer on phytoplankton. All our evidence confirms Pelseneer's derivation of these animals from bullomorph tectibranchs; an instructive parallel is provided by *Acera* which has developed an ability to swim by the enlargement of its parapodial lobes, though this animal has probably little to do with the actual phylogeny of the pteropods. *Limacina*, which is still shelled, spirally coiled and operculate, is in all ways an excellent form for an understanding of the transition to pelagic pteropoda.

Pteropods have been previously looked at as living animals chiefly by Yonge (1926). He did not, however, examine *Limacina*, and in this generalized form there are now several differences to report from the Cavoliniidae and Cymbuliidae. Paradoxically, so small a creature as *Limacina* is much easier to examine intact and living than the larger pteropods; its mode of feeding and the action of the gut are readily observed through the transparent shell, and it remains happily alive in sea water in the laboratory for many days.

It is admitted by all that the three families of Thecosomata, the Limaciniidae, Cavoliniidae and Cymbuliidae, stand in that order of specialization; and within the Cymbuliidae it is probable that *Gleba*, as suggested by Yonge, is the most advanced genus. In food-collecting the greatest point of difference is the absence in the Cavoliniidae and Cymbuliidae, as reported on by Yonge, of any trace of pallial food-collecting with the passage forward of a mucous string. In *Cavolinia* and *Creseis* the whole of the food-collecting is done by the 'Wimperfeld' of the wings and by the side lobes and median lobe of the foot. In *Cymbulia* the ciliary action of the 'Wimperfeld' is lost, and the emphasis is on feeding by the foot. Food particles travel to the mouth along two obliquely transverse grooves, bounded in front by the two lateral lobes, forming raised margins, and behind by the unpaired median lobe. The mouth is

depressed into a funnel into which these ciliary currents lead downwards. The ciliary tracts on the wings are much reduced and there is little if any collection of food; the flapping of the wing dislodges alighted particles. In *Gleba* there is the same situation in essentials, but the parts of the foot are here drawn out into a long, spatuliform 'proboscis', lying parallel to the surface of the wings, with the mouth in a central cleft near the tip. Ciliated grooves carrying food particles proceed along the rounded edges of the 'proboscis', the lateral lobes of the foot forming the upper margin and the highly modified median lobe the lower.

A glance at Pelseneer's figures for *Cavolinia* and *Cymbulia* shows why pallial food-collecting cannot take place in these forms, at least by a food string emerging anteriorly to the head in the method used by *Limacina*. In these higher pteropods the visceral mass has turned through  $180^\circ$  to bring the anus to the left side of the mantle cavity of the now bilaterally symmetrical body. The position of the mantle cavity is now ventral, and its opening is separated from the mouth by the interposition of the foot and wings. In the floating and swimming position, especially in *Cymbulia*, which lies on its dorsal surface with the ventral mouth and foot uppermost, this change in orientation of the mantle cavity may be explained by reference to the requirements of the animal in flotation.

A food string from the mantle cavity would thus be unable to pass to the mouth in the same way as in *Limacina*, and it is difficult to visualize how *Cavolinia* or *Cymbulia* might make use of the mantle in feeding. But the problem remains of the relatively huge pallial gland developed in cavoliniids and cymbuliids just as in limacinids. In the latter its function is evidently wholly concerned with feeding, and its survival in the higher families is puzzling to explain in the absence of a mucous feeding role. From the histology of the pallial gland and from the evidence of simplification of the pallial complex, this gland is almost certainly not a direct survival of the hypobranchial gland of prosobranchs which is a different structure that was lost with the gill. The function of the hypobranchial gland—detritus removal and sanitation of the pallial cavity—is scarcely in the least likely to be important in a pelagic mollusc in clear water. Further, the histology of the pallial gland is identical in all the Thecosomata, and we are clearly dealing with the same structure in each of the three families. While the issue of a mucous string from the pallial cavity in Cavoliniidae is feasible, with a changed course along the foot, it is not easily possible in Cymbuliidae which have a wide spread of the wings almost entirely closing off the opening of the pallial cavity. It may yet be worth further investigation of these pteropods to see if the pallial cavity and its gland actually play any role in collecting food.

Whatever the role of the pallial cavity in the Cymbuliidae, the ciliary collecting fields on the wings are greatly reduced, and in *Gleba* they appear to be quite lost. In the Cavoliniidae, where they are probably best developed,

they are even here of much less importance than the lobes of the foot. In the Limacinidae they seem to be of minimal importance in feeding; more than any other family this group retains the use of the widely expanded median lobe or 'sole' of the foot.

Of other evolutionary trends connected with feeding in Thecosomata, we may mention the reduction of the buccal mass. The Cavoliniidae have this structure moderately well developed, with salivary glands, jaws and radula. The same is true of the less advanced Limacinidae. In *Cymbulia*, Yonge states that the buccal mass is 'much less developed than in the two species already described', and in *Gleba* there is 'no buccal mass, hence no jaws, radula or salivary glands'. It is lacking also in *Corolla*.

The essential structure of the stomach and gizzard is fairly constant in all these groups. The gizzard was regarded by Yonge as probably another structure 'handed down from carnivorous ancestors and clearly of little use to an animal which feeds by ciliary mechanisms'. In *Limacina*, however, the gizzard has only to be seen in action to realize its usefulness, both as a diatom mill and, by the action of its muscular wall, as a pumping device, regulating the inflow and outflow from the digestive diverticulum. The important part played by muscle, even in a ciliary feeder, is well emphasized in a creature small enough to view with its working stomach intact, and, as stressed by Yonge (1949) in connexion with the eulamellibranch Tellinacea, we should not undervalue the role of muscle in the digestive economy of ciliary feeders. Comparison may be made with *Scaphander* which uses its gizzard to crush the shells of small bivalves, and here differs only in its size and choice of food from *Limacina*.

The tiny style sac of *Limacina*, though of much smaller size, corresponds exactly to that described by Yonge in *Creseis* and by Howells (1936) in *Cymbulia*, and said by Meisenheimer (1905) to be retained in all Thecosomata. Here it can be little more than a vestige, as it is impossible to see of what use a style could be in its normal role of stirring and moving the stomach contents. Moreover, an amylolytic enzyme has never been demonstrated in Thecosomata, and from the nature of the food is likely to be little needed. The survival of the style sac in this group once again impresses us with the great unity in the pattern of the stomach shown among so many of the mollusca.

#### SUMMARY

The biology of the thecosomatous pteropod, *Limacina retroversa*, is discussed with special reference to the swimming mechanism, mode of food collection and structure and action of the alimentary canal. The ciliary currents of the foot and pallial cavity are described in detail, in relation to the collection of food particles, and it is shown that the pallial cavity, with its mucus-secreting pallial gland, plays the principal role in gathering food from the surrounding water. A mucous food string is compacted and carried forward to the mouth.



The structure of the gizzard and the stomach is described, and the toothed, muscular gizzard is shown to form a mechanism for crushing diatom frustules. The vestigial style sac, and the epithelium of the digestive diverticulum are described and compared with those of other Thecosomata.

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## NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

### III. A COMPARISON OF *DIDEMNUM MACULOSUM*, *D. CANDIDUM*, *D. HELGOLANDICUM*, AND *TRIDIDEMNUM ALLENI*

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(Text-figs. 1-5)

When Michaelsen (1923) described a species of didemnid from Heligoland, he stated that it was very similar to *Leptoclinum maculosum* Milne Edwards (1841), so far as may be judged by external appearance, but since no adequate description of this latter existed he proposed to call his Heligoland form by a new name, *Didemnum helgolandicum*, rather than use an old name which might well be wrong. Neither Michaelsen nor Hartmeyer, who described further details of *D. helgolandicum* (1924), was able to compare it directly with specimens of *D. maculosum* from the Channel coasts whence it was first described by Milne Edwards. Through the kindness of the directors of the Zoological Museum of Copenhagen and of the Biological Station of Roscoff I have been able to obtain specimens of both these forms and to compare them with specimens collected around Plymouth and on the coast of North Wales, and with specimens of *D. candidum* Savigny, collected at Naples, and from the Red Sea, and of *Trididemnum alleni* Berrill, collected around Plymouth.

#### MATERIAL EXAMINED

Three small colonies of *Didemnum helgolandicum* collected by Ørsted in 10-13 fathoms 5 'quarter-miles'<sup>1</sup> north-west of Hirtshals (one of Michaelsen's type localities) and determined by Michaelsen. This is part of the type material.

About twenty colonies of *D. maculosum* collected at Roscoff (one of Milne Edwards's type localities) in the *Laminaria* zone, and one specimen from the same locality determined by Pizon.

Six colonies of *Didemnum maculosum* from various points on the North Wales coast.

About 200 colonies of *D. maculosum* and 100 of *Trididemnum alleni* from the Plymouth area (Berrill's type locality) collected and determined by Miss P. Kott, and in the local collection of the Plymouth Laboratory.

About 600 colonies of *Didemnum* spp. from the Plymouth area collected by myself, the identification of which is discussed below, and about 300 colonies of *Trididemnum alleni*.

<sup>1</sup> If these are Danish miles, since Ørsted collected the specimen, this distance is c. 8.5 km.; if, however, they are German miles, since Michaelsen wrote the present label, then the distance is c. 9.4 km.

Twelve colonies of *Didemnum* sp. collected by me from the Secca di Benda Palummo in 40 m. and from other localities at Naples.

Two colonies of *D. candidum* in the local collection of the Naples Zoological Station determined by Traustedt.

One specimen of *D. candidum* from the Red Sea (Savigny's type locality) determined by Garstang.

Nine specimens of *D. candidum* from the Red Sea, four colonies from the Suez Canal, and thirteen colonies from the Great Barrier Reef (Australia), in the British Museum collection.

#### OBSERVATIONS

When examining specimens of '*D. maculosum*' collected in the Plymouth area I soon realized that I was dealing with two separate species. The difference was most obvious in the larvae, one of which was twice as long as the other. An examination of the specimens in the local collection of the Plymouth Laboratory showed that these too fell into two groups, though unfortunately none of the specimens of one group had any larvae. The larvae and adults of the other group of specimens agreed in general appearance with those described under the name of *D. maculosum* auctt. by Millar (1949).

Here, then, were two species under the name *D. maculosum*. One of them I have identified with *D. candidum* Savigny, the other with *D. helgolandicum* Michaelsen. About one-quarter of the specimens collected in the Plymouth area agreed with Michaelsen's description of *D. helgolandicum* and with the specimens identified by him which I have had the opportunity to examine. All the other specimens from Plymouth, those from North Wales, those from Roscoff, and those from Naples, agreed with Savigny's (1816), van Name's (1945) and Michaelsen's (1920) descriptions of *D. candidum* and with the specimens of this species which I have examined. These latter specimens, especially those from Roscoff, are presumably the *Leptoclinium maculosum* of Milne Edwards, so this name must be abandoned in favour of the older name *Didemnum candidum* Savigny (1816), the type species of the genus. *D. helgolandicum* is different from this, as Michaelsen well knew, for he had the opportunity of examining specimens from near Savigny's type locality (Michaelsen, 1920). Accordingly, Michaelsen's name stands since it is not a synonym of any earlier described species. It is fortunate that the name to be abandoned is that of Milne Edwards with its insufficient description.

The descriptions which follow are based primarily upon the Plymouth material.

The colonies of the two species are indistinguishable externally. Both are thin encrusting forms, 1-2 mm. thick, growing on *Laminaria* holdfasts, gorgonians, other ascidians, in fact on any organic substratum and even occasionally on rock. The spicules vary in abundance, but usually it is possible to see the zooids or at least their position. The colour is very variable, whitish, blue-violet, violet, grey, brownish grey, where there is much mud, white,

yellow ochre or orange in cleaner situations. The larger colonies are usually marked with lines of deeper colour, which are usually furrows running over the surface in an irregular reticulum. The deeper colour seems to be a result of the less density of spicules in these furrows. The pigment appears to be free in the test, and is not contained in special pigment cells. It rapidly fades in alcohol, more slowly in formalin. The surface of the colony of each species is marked with small granulations most commonly, though they may be missing; these are wart-like protrusions, one near each branchial opening. There may be a layer of bladder cells at one or both of the surfaces of the colony, but this is not constant in either species.

The spicules are most abundant in the upper layers of the colony, immediately below the layer of bladder cells if these are present, and especially clustered in the wart-like protrusions and around the buccal siphons. They diminish in abundance steadily towards the deeper layers, but in *D. helgolandicum* there is frequently a greater abundance in the basal layer itself. In *D. candidum* there is rarely any special density near the zooids, but in *D. helgolandicum* the test immediately surrounding the zooids and especially that part under the abdomen of each zooid is denser in spicules than the rest of the test. The stippling in Fig. 1 gives an indication of the relative density of spicules in the various areas of the test.

The spicules are rather similar in general form, but rather more variable in *D. candidum*. In this species the rays most commonly have a terminal angle of about  $45^\circ$ , but they may be rounded, almost square-tipped, or even rod-like. The most common formula (see Carlisle, 1953) is 1, 6, 9, but 1, 6, 12, 1, 4, 8, or even 1, 6, 12, 24 may occur. The variability in spicule form is greater at Plymouth than is illustrated by Van Name (1921) for West Indian colonies. In *D. helgolandicum* the most common terminal angle for spicule rays is  $60^\circ$  and variations from this form are few. The formula is most frequently 1, 4, 8, 14, but 1, 6, 12, 1, 4, 8 and 4, 8 occur, or even 1, 4 in the lateral thoracic organs, where smaller spicules tend to accumulate, and around the lobes of the buccal siphon. In both species the size range is 25–45  $\mu$  with a few smaller or larger (absolute limits: 4 and 75  $\mu$ ). Those of *D. candidum* tend to be on the average rather larger than those of *D. helgolandicum*.

In *D. candidum* the zooids are rarely arranged in definite systems except around the edges of young colonies. Where a system is distinguishable the zooids are arranged around a common cloacal aperture with the atrial apertures facing inwards. The ring of zooids thus presents an outer aspect (outer with respect to the ring) of ventral surfaces. In *D. helgolandicum*, in the Plymouth material especially, though also in the material from Hirtshals, systems of zooids are much more definite and more commonly developed. Here, however, they are arranged the other way round, with the atrial apertures turned outwards from the ring of zooids and the ventral sides inwards. The centre of such a ring of zooids is solid test; the common cloacal



system runs between the systems of zooids. I have never seen this type of system developed in any other species of ascidian.

The zooids of both species are arranged nearer to the vertical than is usual in didemnids. They are never quite vertical, and the abdomen is more inclined than the thorax, so that the neck is bent, especially in *D. candidum*. The total

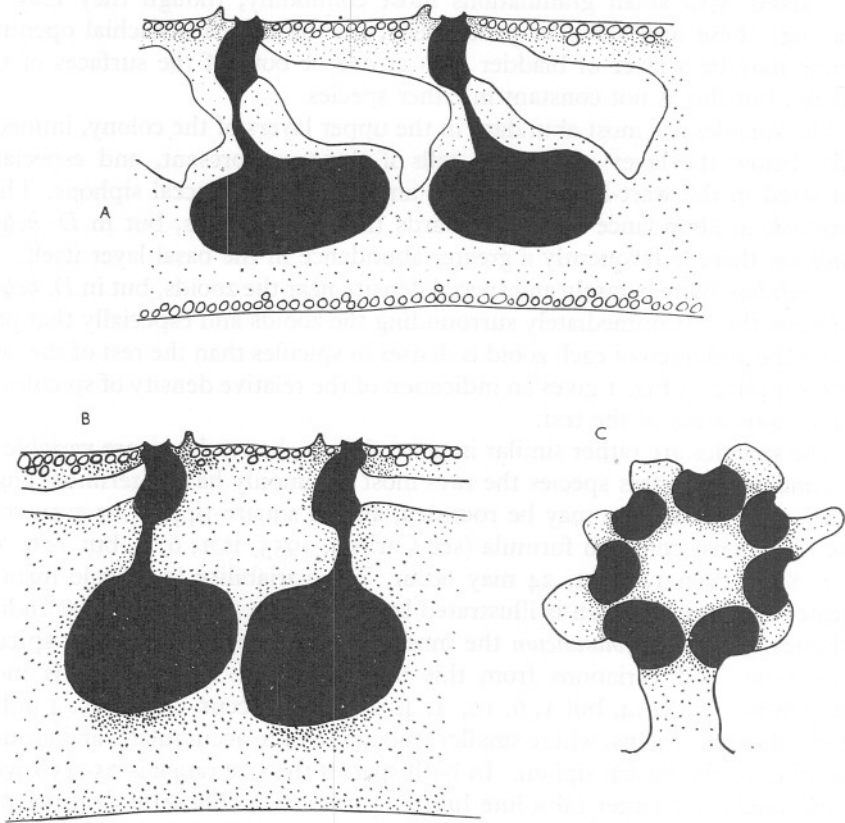


Fig. 1. Sections through colonies of *Didemnum candidum* and *D. helgolandicum*. A, vertical section through part of a colony of *D. candidum*; B, vertical; and C, horizontal section at the thoracic level, through part of a colony of *D. helgolandicum*. The stippling indicates the relative abundance of spicules in the various parts of the colony. Notice the association of the zooids of *D. helgolandicum* into systems with the ventral sides adposed.

length of a zooid of *D. candidum* in the usual contracted condition of preservation is about 1.1 mm.; in the expanded condition it may measure up to 1.6 mm. The corresponding figures for *D. helgolandicum* are 0.9 and 1.3 mm. The difference is chiefly a result of the longer neck and slightly larger abdomen of *D. candidum* (see Fig. 2).

The thorax of both species is smaller than the abdomen. There is a cline in *D. candidum* in this respect; the difference in size between the two parts, in European waters, is more pronounced farther north. It is most pronounced on the coast of North Wales, less so at Plymouth, still less at Roscoff, less again at Naples, while in the Red Sea the thorax is little smaller than the abdomen. This may be, at least partly, a result of temperature or nutrition differences. The difference is least marked in older, larger zooids, and colonies

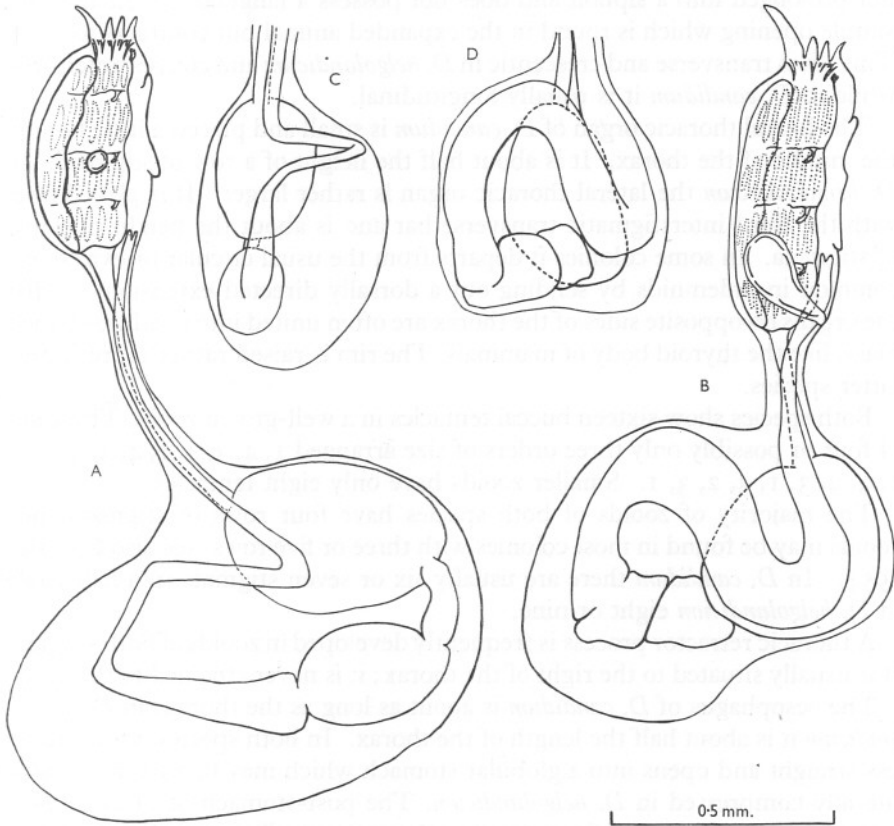


Fig. 2. Drawings from the left sides of zooids of (A) *Didemnum candidum*, and (B) *D. helgolandicum*; and from the ventral of sides of abdomina of (C) *D. candidum*, and (D) *D. helgolandicum*. The entire genital system and pyloric gland are omitted. The buccal lobes and tentacles are drawn only from one side, omitting those of the other.

grown in warmer waters grow faster to a given size so that there is a less proportion of small zooids. This is not, however, the whole explanation for differences in proportion exist even between zooids of the same size from the different localities. The thorax is separated from the abdomen by a long neck, which in *D. candidum* is about the same length as the thorax, sometimes

shorter, sometimes longer, while in *D. helgolandicum* it is always shorter than the thorax, usually less than half. The relative measurements refer especially to the uncontracted condition; the neck suffers more from shrinkage than most other parts of the body, and in a contracted preserved condition it is frequently much shorter.

The branchial siphons are six-lobed in both species though occasionally an eight-lobed individual of *D. candidum* may be found. The atrial aperture is not prolonged into a siphon and does not possess a languet. It remains as a simple opening which is round in the expanded animal but contracts to a slit. This slit is transverse and crescentic in *D. helgolandicum* and convex anteriorly, while in *D. candidum* it is usually longitudinal.

The lateral thoracic organ of *D. candidum* is small and placed a little behind the middle of the thorax. It is about half the height of a row of stigmata. In *D. helgolandicum* the lateral thoracic organ is rather larger. It is placed level with the third interstigmatic transverse bar and is about the height of a row of stigmata. In some colonies it departs from the usual circular or oval shape common in didemnids by sending out a dorsally directed extension, so that the organs on opposite sides of the thorax are often united into a saddle-shaped mass, like the thyroid body of mammals. The rim is raised rather more in this latter species.

Both species show sixteen buccal tentacles in a well-grown zooid. These are of four or possibly only three orders of size arranged 1, 4, 3, 4, 2, 4, 3, 4, 1 or 1, 3, 2, 3, 1, 3, 2, 3, 1. Smaller zooids have only eight tentacles.

The majority of zooids of both species have four rows of stigmata, but zooids may be found in most colonies with three or five rows (see also Carlisle, 1954). In *D. candidum* there are usually six or seven stigmata in a half-row; in *D. helgolandicum* eight or nine.

A thoracic retractor process is frequently developed in zooids of both species. It is usually situated to the right of the thorax; it is never attached to the neck.

The oesophagus of *D. candidum* is about as long as the thorax; in *D. helgolandicum* it is about half the length of the thorax. In both species it is more or less straight and opens into a globular stomach which may be rather strongly laterally compressed in *D. helgolandicum*. The post-stomach of *D. candidum* leaves the posterior end of the stomach and runs vertically to the globular mid-intestine, which is the most posterior part of the gut-loop and is sometimes nearly half as large as the stomach though usually no more than one-quarter. In *D. helgolandicum* the post-stomach leaves the ventral side of the stomach and runs horizontally; it is the most posterior part of the gut-loop. It is short and thick and opens into a short, thick mid-intestine which is much smaller than the stomach. From the mid-intestine the rectum, in *D. candidum*, runs ventrally with an enlarged proximal portion, then takes an abrupt turn anteriorly; another abrupt turn to the left produces an elbow which sticks out sharply to the left of the stomach before the rectum comes parallel to the

oesophagus to run up the narrow neck. The anus in this species is level with the middle of the last row of stigmata. In *D. helgolandicum*, on the other hand, all the curves in the course of the rectum are smooth and gradual. It leaves the mid-intestine, with no proximal swelling, in an anterior direction, curves round in a smooth S-shaped curve, lying partly to the left of the stomach, but without any pronounced elbow, and then runs up the neck beside the oesophagus. The anus is level with the hinder end of the third row of stigmata. The tight S-shaped curve of the rectum of *D. helgolandicum* is reminiscent of that of *Trididemnum niveum* (see Carlisle, 1953).

The epidermal ampullae are short and inconspicuous in both species, but rather variable in development, perhaps depending on the stage of growth.

In *Didemnum candidum* the vas deferens makes between seven and ten turns around the testis, with eight perhaps being the commonest number. Zooids may however be found, even within the one colony, with as many as twelve turns of spire, or as few as six. In *D. helgolandicum* there is similar variation with nine as the most common number of turns of spire. The testis itself in both species is a single spherical vesicle about as big as the stomach. The ovary is of the typical didemnid form with one predominant large ovum at a time and a series of progressively smaller ones.

The size of the larva of both species is somewhat variable: as in most species of didemnids the small peripheral zooids of the colony produce smaller ova and hence smaller larvae than do the larger central zooids. But the larvae from a zooid of *D. helgolandicum* is approximately twice the size of one from an equal-sized zooid of *D. candidum*. The body length, excluding tail, of a larva from a medium-sized zooid of *D. candidum* is approximately 230  $\mu$ , that from *D. helgolandicum* 630  $\mu$ .

The larva of *D. candidum* is unusual in possessing only two suckers set vertically, one above the other. They are cup-shaped with a conical cement gland set in the centre of each. They are flanked by three or four ampullae on each side. There are three suckers in *D. helgolandicum* and these are flanked by six or seven ampullae on each side. The cement glands in the centres of the suckers are rather more obtuse angled than is usual. The ampullae are relatively rather longer than in *D. candidum*. The endostyle is set more vertically in *D. candidum* than in *D. helgolandicum*, where it is inclined at about 45° to the axis of the larva. The branchial sac, at the moment of hatching, is perforated usually with four rows of stigmata, each of four to six stigmata per half row in *D. candidum*, or six to eight in *D. helgolandicum*. In smaller larvae from the periphery of the colony, however, there may be only three rows; in this case a fourth row does not perforate at least up to metamorphosis beyond which stage I have not observed them further. Below the sac is a mass of yolk-laden cells which persists far into free-swimming life. The gut remains as a solid rod, without lumen, up to metamorphosis. In *D. candidum* it lies in the normal position for the family, with a horizontal oesophagus



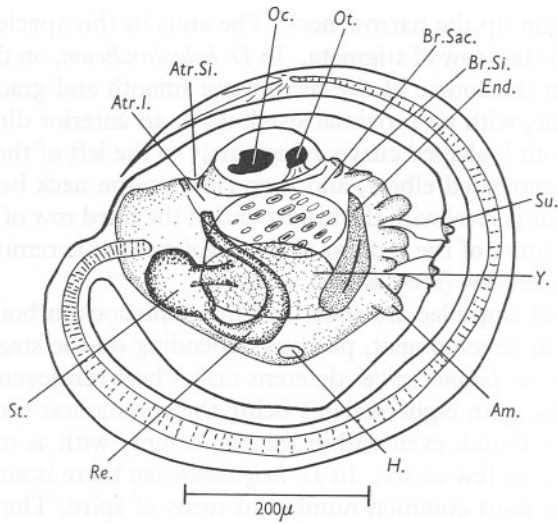


Fig. 3. Larva of *Didemnum candidum* from the right side. *Am.*, ampulla; *Atr.I.*, atrial invagination; *Atr.Si.*, atrial siphon; *Br.Sac.*, branchial sac; *Br.Si.*, branchial siphon; *End.*, endostyle; *H.*, heart; *Oc.*, ocellus; *Ot.*, otolith; *Re.*, rectum; *St.*, stomach; *Su.*, sucker; *Y.*, yolk.

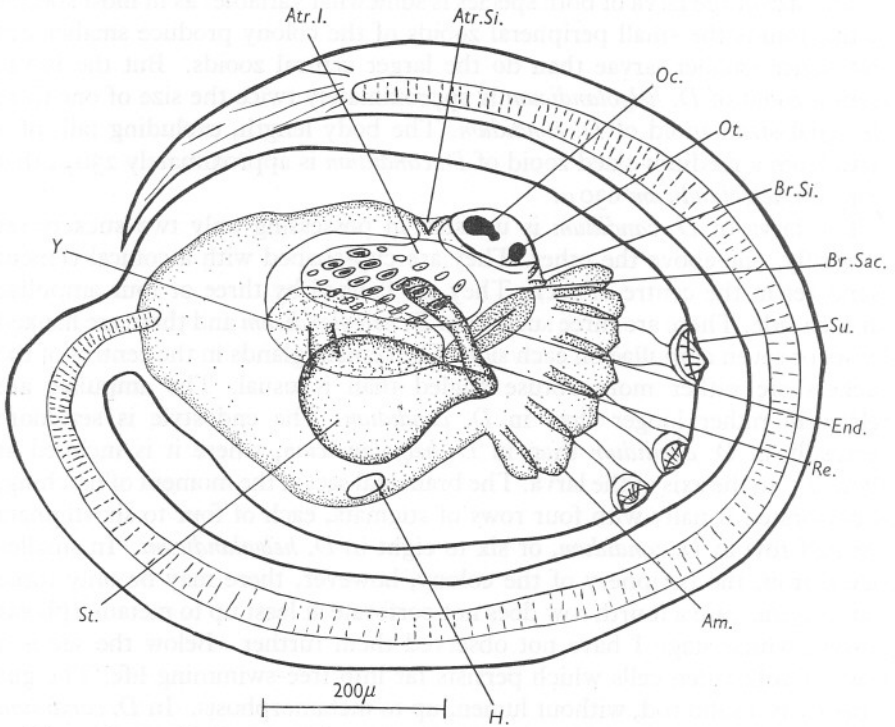


Fig. 4. Larva of *Didemnum helgolandicum* from the right side. Lettering as in Fig. 3.

projecting back from the branchial sac or from the yolk mass, leading to a posteriorly situated (posteriorly with respect to the swimming axis of the larva) globular stomach. The intestine-cum-rectum leaves from the antero-ventral corner of the stomach and sweeps round in a wide S-shaped curve to finish in the atrial cavity above the oesophagus. In *D. helgolandicum* the whole gut is much farther forward than is usual in the family. The oesophagus leaves the branchial sac or yolk mass in a downwards direction and runs into the stomach, which lies below the yolk mass, at its posterior end. The stomach is pyriform with the front end narrower. From this front end, even in front of the endostyle, the intestine-cum-rectum departs at right angles, lying transversely across the body. It then runs up at an angle of  $45^\circ$  almost straight to finish in the atrial cavity. The heart is in the usual position in both species, but in *D. candidum* the gut is entirely behind it, whereas in *D. helgolandicum* it lies beneath the stomach.

The sensory vesicle contains both ocellus and otolith, which are large and well developed in both species. The tail is of the same relative length in both, extending around the body, just before hatching, almost as far as its own base.

The free-swimming period is about 1 hr. in *D. candidum*, 4 hr. in *D. helgolandicum*.

*Leptoclinum maculosum* Milne Edwards is in some respects different from *Didemnum candidum* Savigny from the type locality, in particular in the relatively larger abdomen and in the swelling of the mid-intestine, but in all other respects they are alike. The 'elbow' which the rectum makes (which can be seen in Savigny's drawing, 1816, pl. XX, fig. 1) occurs in both; this character is quite unlike anything found in other species of *Didemnum*. The larvae of *D. candidum* from the Red Sea share with the specimens of '*D. maculosum* auctt.' the possession of only two suckers, a character which is shared only by *Trididemnum alleni* among ascidians. In fact the larvae from Red Sea specimens and from Plymouth specimens are indistinguishable. If we consider the two characters mentioned above, in which the two forms differ, the relative size of the abdomen and of the mid-intestine, we find that there is a cline in both these characters. Where specimens from Naples differ from those from the Red Sea in these two characters they do not differ enough to justify separate specific rank. Where those from Roscoff differ from those from Naples the difference is again not enough to justify specific separation. Again Roscoff and Plymouth specimens do not differ more than one expects to find within a species, nor do Plymouth and North Wales specimens. But direct comparison of specimens from North Wales with those from the Red Sea would suggest that they might conceivably be different but closely related species, or more likely, geographical subspecies. Since, however, intermediates are provided from the stations in between, I do not propose even subspecific rank for the English forms. I regard them as representatives of the extreme northern end of a cline with a complete range of intermediates down to the type-locality in the

Red Sea. They differ from the Red Sea specimens only in the relatively larger abdomina and in the greater degree of swelling of the mid-intestine. It is to be noted that the Red Sea specimens also show some degree of swelling of the mid-intestine, as may be seen in Savigny's drawing (1816, pl. XX, fig. 1<sup>4</sup>).

*T. alleni* Berrill (1947) is a form described from Plymouth. It seems to represent dwarf or young stages of *Didemnum candidum*. It has already been noted (above and in Carlisle, 1954) that the marginal zooids of *D. candidum*, and indeed of many species of *Didemnum*, have only three rows of stigmata and a simplified gut. Moreover, such zooids produce smaller larvae with only three rows of stigmata. In fact, the marginal zooids of *D. candidum* are identical with the zooids of *Trididemnum alleni*, and so also are the larvae of such zooids. I would suggest that *T. alleni* is the product of such a dwarf larva (or even occasionally of one of the larger larvae), which has settled in a place where there is either little food or little space for expansion. In such a situation the colony is bound to remain small, the zooids are likely to be smallish, hence the simplified gut, and unlikely to produce the larger branchial sacs which are characteristic of flourishing zooids when they replace a worn-out sac by a newly budded one. Direct observation in the field supports this hypothesis. I have on several occasions observed a scatter of young colonies evidently formed from simultaneously released larvae from one parent. Such a scatter settling around the base of a gorgonian or of a *Cystoseira*, some on the rock and some on the organism, consists of identical colonies. If one or two are removed they are usually found to consist of about six to ten zooids, of which one only may have four rows of stigmata; this doubtless is the oozoid. The other zooids are typical *Trididemnum alleni*. But in the course of 2 or 3 months, the colonies growing on the rock and on the larger expanses of substrate have developed into typical *Didemnum candidum* with four rows of stigmata in all but the marginal zooids. The colonies on restricted space have hardly grown at all and have remained in the *Trididemnum* condition with only very few zooids, if any, possessing four rows of stigmata. Such a colony transplanted to a position where feeding is better and where there is room for expansion soon develops into a typical *Didemnum candidum*. *Trididemnum alleni* is thus only one form of *Didemnum candidum*.

#### GEOGRAPHICAL DISTRIBUTION

*D. candidum* is a tropical and temperate species extending from the West Indies to the East Indies, New Zealand and Japan (Fig. 5). It does not apparently occur in the main body of the Pacific, or on the western shores of America. North and south it is bounded more or less exactly by the 15° C. isotherm.

*D. helgolandicum* is only known from Heligoland, the Skagerrak (Michaelsen, 1923), Faeroes (Hartmeyer, 1924) and Plymouth.

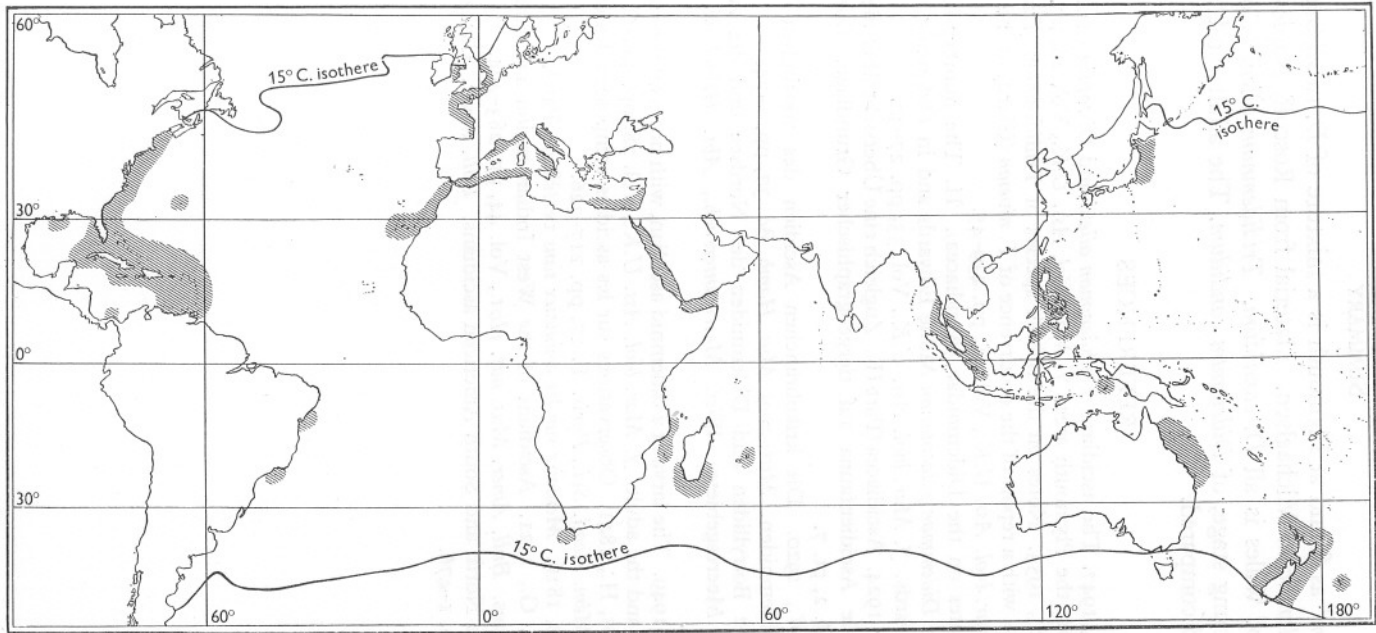


Fig. 5. Map of the distribution of *Didemnum candidum* (including *D. maculosum*, *D. candidum lutarium* and *Trididemnum allenii*).



## SUMMARY

'*D. maculosum*' as found at Plymouth is a mixture of *D. candidum* Savigny and *D. helgolandicum* Michaelsen. Material from Roscoff, Naples and the north coast of Wales is all *D. candidum*. *Trididemnum alleni* is one form, a dwarf or young stage, of *Didemnum candidum*. The adults and larvae are described and compared.

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## NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

### IV. THE INCREASE OF *TRIDIDEMNUM NIVEUM* (GIARD) IN THE PLYMOUTH AREA, AND THE STRUCTURE OF ITS LARVA

By D. B. Carlisle

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(Text-fig. 1)

Since I first found *Trididemnum niveum* (Giard) at Salcombe in the autumn of 1951 (Carlisle, 1953) this species has spread and increased greatly in the Plymouth area. I have found it in abundance at Salcombe (Castle rocks and the Salstone), Wembury, and especially at Looe around the Island. The favourite habitat at all these places is the same as that at Roscoff, on the fronds of *Cystoseira*. It occurs rather less frequently on the holdfasts of *Laminaria ochroleuca* and occasionally on other species of *Laminaria*, but very little on any other substrate. It is not truly intertidal; the majority of specimens are to be found just below low-water mark of spring tides, and very rarely are any seen above low-water mark of even the lowest tides of the year.

In March 1952 a few specimens of *Trididemnum niveum* were found at each of the localities listed above. By June the numbers were greatly increased and by October still further. The following March (1953) the numbers were about the same as in the previous June, greater, that is, than the previous March by a factor of about three times. By midsummer *T. niveum* was the most abundant didemnid, and possibly the most abundant ascidian, at Looe in the neighbourhood of the island. At Salcombe it lagged behind *T. tenerum* and *Didemnum candidum* in abundance; at Wembury it was between *D. candidum* and *Trididemnum tenerum* in numbers, but was perhaps more conspicuous than either. In September 1953 it was still the most abundant didemnid at Looe, the second most abundant at Wembury and the third at Salcombe. In March 1954 it is by far the most abundant ascidian at Looe, forming at least 75% of the total ascidian fauna at low-water mark.

The association with *Laminaria ochroleuca*, which species was first observed in Britain in the Plymouth area in 1946 (Parke, 1948), and which is still spreading steadily, might suggest perhaps that *Trididemnum niveum* has arrived from Brittany with this oar-weed and is spreading along with it, perhaps at a distance of 2 or 3 years behind.

Salensky (1895) described the embryology and the larva of a species which he called *Didemnum niveum* in the title of his paper. On the second page

(p. 289) he states, 'Nach meiner früheren Bestimmung glaubte ich, dass diese dem *D. cereum* angehörten; es waren zwar in der That einige Exemplare von dieser Species dabei, aber die Hauptmasse der Colonien, die ich studiert habe, gehören einer anderen Species an und stehen dem *D. niveum* am nächsten'. Apparently he was not altogether sure of his identification. Whatever his species was it was evidently the most abundant didemnid at Villefranche. Harant & Vernières (1933) state that *Trididemnum niveum* has only once been recorded in the Mediterranean, thus implicitly doubting Salensky's identification. Berrill (1950) includes Salensky's species under the synonyms

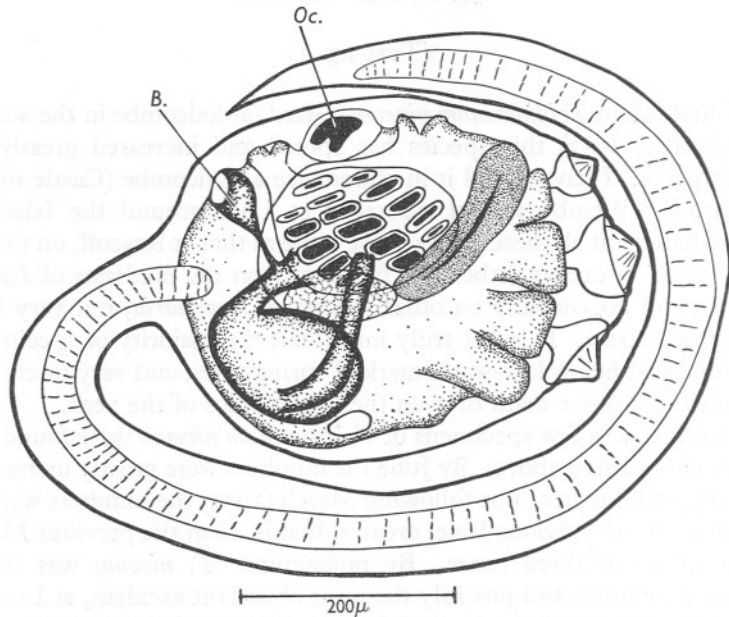


Fig. 1. Drawing from the left side of the larva of *Trididemnum niveum*. Note precocious budding and lack of otolith. B., bud; Oc., ocellus.

of *T. tenerum* (= *T.* or *Didemnum cereum*), but also gives it as a reference for *Trididemnum niveum* with a note (p. 141) suggesting that Salensky may be mistaken in his identification. The larva which Salensky describes is in fact unmistakably that of *T. tenerum* and differs in many points from that of *T. niveum*. Presumably he had two different colour varieties or forms of *T. tenerum*, one of which he recognized as such ('*Didemnum cereum*') while the other he failed to ascribe to its correct species.

Accordingly, the larva of *Trididemnum niveum* has not been described, an omission that can now be remedied.

The length of the body of the larva of *T. niveum* is slightly greater than that of *T. tenerum*, averaging about 480  $\mu$ , while that of *T. tenerum* averages about

430  $\mu$ . Mediterranean specimens of *T. tenerum* tend to have a smaller larva than northern ones, in my experience, and Salensky's figures indicate a length of about 400  $\mu$ . The tail is comparatively longer in *T. niveum*, extending round even past the sensory vesicle as far as the atrial siphon, whereas in *T. tenerum* it rarely passes the branchial siphon, when coiled around the body just before hatching. At the time of hatching the stomach of *T. tenerum* is hardly swollen beyond the thickness of the rest of the gut; in *T. niveum* it is globular. On the other hand, the gut of *T. tenerum* already has its lumen developed by the time of hatching, whereas that of *T. niveum* is solid even up to metamorphosis. There are three suckers in both species. The ampullae number about four pairs, but departures from this number are to three pairs in *T. tenerum* and to five pairs in *T. niveum*. I have been unable to distinguish any signs of the existence of an otolith in the *T. niveum*, a feature which is shared, so far as I know, only with *Pycnoclavella* (Berrill, 1947) and has not been observed in any other didemnid. Quite a number of specimens of larvae of *T. niveum* show precocious budding from the oesophageal region. The precocity is not nearly so pronounced as in *Diplosoma*.

The free-swimming period is about 20 min.

#### SUMMARY

*Trididemnum niveum* has increased in numbers at Plymouth between spring, 1951, and March 1954, until in some localities, the species is the most abundant of ascidians. The larva is described and figured; it lacks an otolith and shows precocious budding, but otherwise is typical for the family.

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## STYELA MAMMICULATA N.SP., A NEW SPECIES OF ASCIDIAN FROM THE PLYMOUTH AREA

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(Text-figs. 1 and 2)

*Styela mammiculata*<sup>1</sup> n.sp. is based on four specimens collected in the Plymouth area during the summer of 1953. The most noticeable external feature is the extensive mammillation of the test. The mammillae are much smaller than those of *Phallusia mammillata* and deserve to be called rather 'mammiculae'.

Of the four specimens, two were taken from Drake's Island buoy (National Grid Reference, 20/4753) in Plymouth Sound, growing about half a metre below the water line, and two from the oyster bed of the Lynher river estuary, near Antony Ferry (National Grid Reference, 20/4257), growing about 12 m. below low-tide mark attached to oyster shells. The largest specimen was from the latter locality and measured 111 mm. long in the contracted, fixed condition. The smallest, from Drake's Island buoy, was 54 mm. long.<sup>2</sup>

Each specimen (see Fig. 1) is attached by an enlarged plate of test substance from which arises a short stout pedicel, accounting for about one-third of the total length of the body. It is evident that the stalked condition is not dependent on habitat, as it is in *Styela plicata* (Lesueur), which develops the stalked condition only under conditions of crowding, for of the four specimens of *S. mammiculata* two were growing (on Drake's Island buoy) in crowded conditions among *Molgula* while the other two, from the Lynher oyster beds, were quite isolated, attached to oyster shells, and yet showed the same degree of stalk development. In all four specimens the stalk is bent through a right angle so that the body comes to lie parallel to the substrate with the ventrum outwards. The animals in the Lynher must therefore have been lying horizontally, and those on the buoy probably vertically. The curvature persisted in one specimen through 3 months in the aquarium, during which time the animal doubled in size. The basal 'holdfast' is rather sharply delimited from the pedicel which is transversely wrinkled at the base, even in living animals, but longitudinally wrinkled for the greater part of its length. The body is mammillated in the upper region and transversely wrinkled, when contracted, in the lower part. The entire test is pubescent. In colour the

<sup>1</sup> From Latin *mammicula*, a small breast or protuberance.

<sup>2</sup> Since writing this paper a fifth specimen, 47 mm. long, has been collected from a buoy in the Sound and a sixth, 129 mm. long, from West Wharf, Millbay docks.



animals are brown, with the mammillations a paler colour. The colour is retained in formalin for at least 3 months. The four-lobed atrial and buccal siphons are marked with longitudinal stripes of almost white and a rich chocolate brown, four of each. The brown stripes are rather wider than the white, and each one is divided by a central paler zone which does not quite reach to the edge of the siphon (see Fig. 1, inset). The same colour pattern is repeated on the inside of each siphon but does not continue very far in, fading to a whitish hue about 4-5 mm. down. When the siphons are open they are

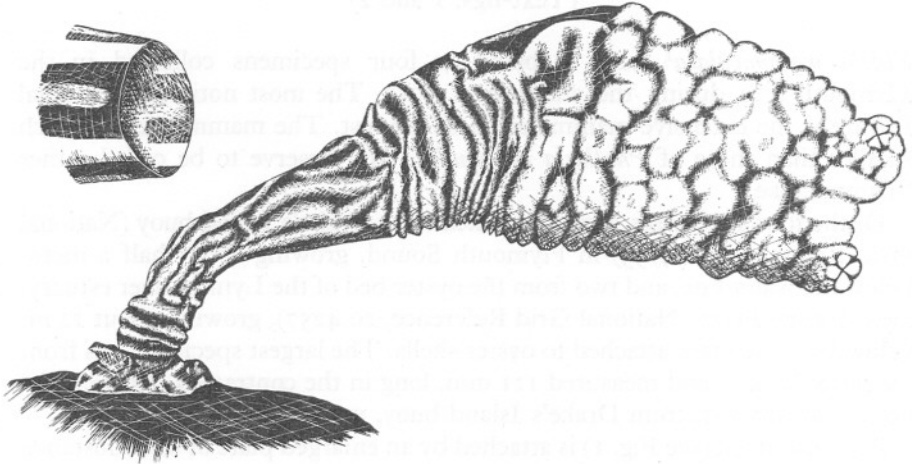


Fig. 1. Sketch of a contracted specimen of *S. mammiculata* from the left side; natural size. Inset: the buccal siphon open, showing the pattern of dark and light stripes;  $\times 3$ .

almost perfectly round, or at least there are no obvious angles. When they close the white stripes remain outwards, while the dark stripes bend to meet each other; the white stripes in the contracted condition show slight mammillations. An end view of a partly contracted siphon rim shows the pattern of a four-leafed clover with the sharpest angles inwards and rounded angles on the outside; this is the reverse of the more common condition in Styelidae and Pyuridae, where the partly closed siphon is frequently squarish in end view, with the most acute angles outwards.

The mantle separates readily from the test and is moderately stout and muscular. It extends the whole way down the pedicel, which is hollow, but not into the 'holdfast' which is solid. This character is shared with *S. montereyensis* (Dall) and *S. barnharti* Ritter & Forsyth, the stalked species of the Californian coast.

The branchial siphon has a single ring of about forty simple tentacles which are winged (Fig. 2D), but not distinctly keeled, and all of one order of size—about 4.5 mm. in the largest specimen. The dorsal tubercle is about 2 mm.

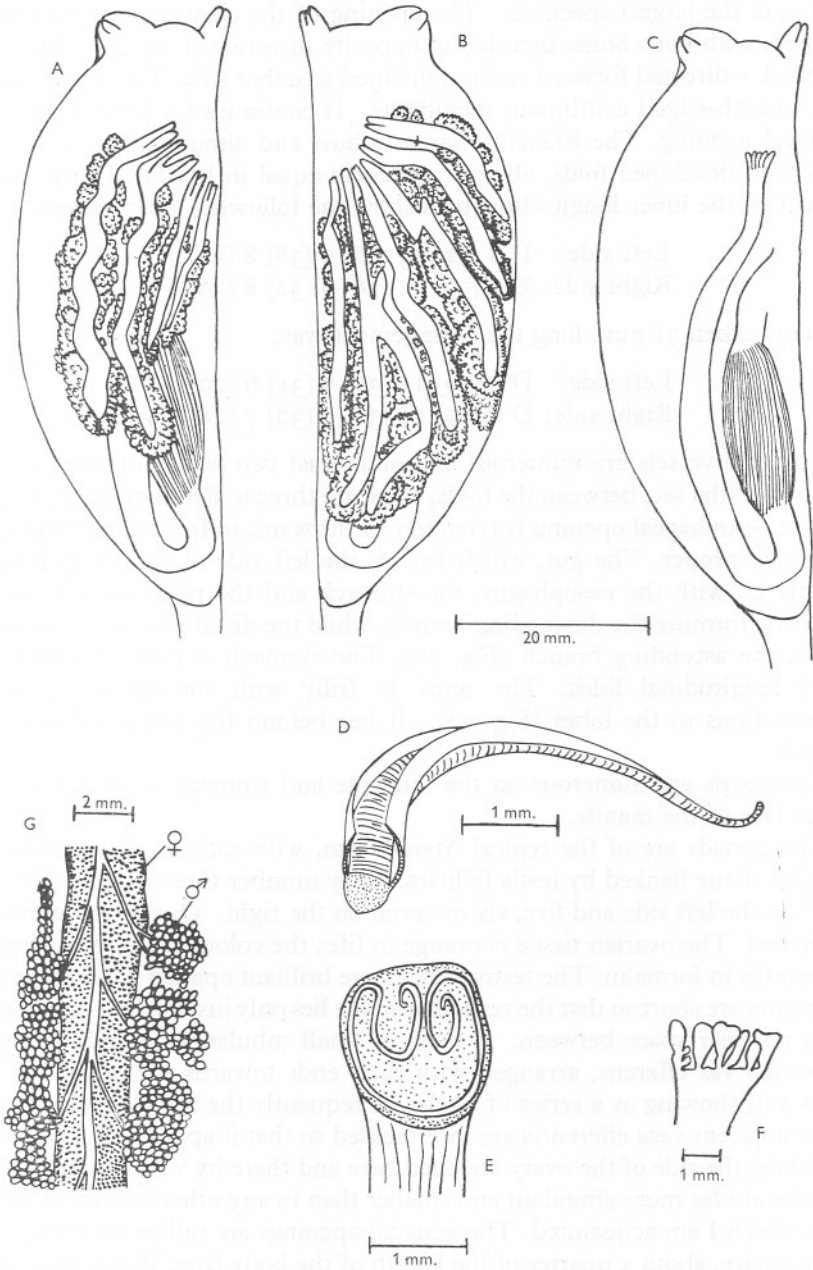


Fig. 2. Anatomical details of *S. mammiculata*. A, zooid removed from test, drawn from the left side; B, the same from the right side; C, the zooid from the left side with the mantle and gonads removed to show the course of the gut; D, an oral tentacle showing the 'wings'; E, the dorsal tubercle; F, the anus; G, a portion of a gonad.

across in the largest specimen. The opening of the ciliated pit is horseshoe-shaped, with both horns incurled in opposite directions (Fig. 2E). The open interval is directed forward and not inclined to either side. The dorsal lamina is a smooth-edged continuous membrane. It continues far behind the oesophageal opening. The branchial sac is robust and almost opaque. It bears four well-developed folds, all approximately equal in height. In the largest specimen the inner longitudinal bars show the following arrangement:

Left side: D 8 (32) 13 (36) 9 (36) 8 (30) 7 V  
 Right side: D 7 (30) 12 (36) 9 (32) 8 (30) 6 V

In a specimen 71 mm. long the arrangement was:

Left side: D 6 (29) 11 (30) 9 (31) 6 (29) 5 V  
 Right side: D 6 (29) 10 (31) 9 (30) 7 (28) 5 V

Transverse vessels are numerous and of at least two orders of size. On the flat part of the sac, between the folds, there are three to five stigmata per mesh.

The oesophageal opening is relatively far forward, in front of the middle of the body proper. The gut, which lies on the left side of the body, forms a simple U, with the oesophagus, the stomach and the proximal part of the intestine forming the descending branch, while the distal part of the intestine forms the ascending branch (Fig. 2C). The stomach is pleated with about forty longitudinal folds. The anus is frilly with sometimes secondary ramifications to the lobes (Fig. 2F). It lies behind the anterior end of the gonads.

Endocarps are numerous on the intestine and stomach as well as on the inner face of the mantle.

The gonads are of the typical *Styela* form, with each a central strand of ovarian tissue flanked by testis follicles. They number three or four (possibly five) on the left side and five, six or seven on the right. One or more may be branched. The ovarian tissue is orange in life; the colour fades over a period of months in formalin. The testis follicles are brilliant opaque white. The vasa efferentia are short so that the testicular tissue lies only just beyond the ovarian, with no clear space between. A mass of small tubular testis follicles opens into each vas efferens, arranged with their ends towards the outside of the mass and showing as a series of circles. Frequently the follicles from two or more adjacent vasa efferentia are intertangled so that it appears as if the testis ran along the side of the ovary attached here and there by vasa efferentia. The tubules are far more abundant and smaller than in any other species of *Styela* with which I am acquainted. The gonadal openings are rather far back in the atrial cavity, about a quarter of the length of the body from the atrial siphon, as an average. Those on one side are arranged along an arc of a circle.

There can be no doubt that this is a species of *Styela*. *S. mammiculata* is clearly different from any European species of *Styela* in the large number of

gonads on the left side. In certain respects it approaches the Mediterranean *S. plicata*, notably in the number and macroscopic form of the gonads of the right side, but it differs in the number of gonads of the left side (2, occasionally 3, in *S. plicata*), in the arrangement of bars on the branchial folds (*S. plicata* has fewer bars and the ventral fold is smaller than the rest), in the form of the tentacles (which are usually keeled in *S. plicata*, and of more than one order of size), in the smaller number of stigmata per mesh, in the smaller, more numerous testis follicles and in the stalk. *S. mammiculata* is nearest to Van Name's (1945) description of the South Californian species of *S. barnharti* Ritter & Forsyth, but rather more different from the description of this species given by Ritter & Forsyth (1917). It differs from van Name's description of this species in the following points:

(i) The pattern of furrowing of the body is distinct. (ii) *S. mammiculata* has only about forty oral tentacles compared with 'at least seventy in large examples' of *S. barnharti*. (iii) The fourth branchial fold has only two to four less bars than the others in *S. mammiculata*, while in a specimen of *S. barnharti* of about the same size as the specimen whose bar-numbers are given above the fourth bar has thirteen to fifteen less bars than the others. (iv) The dorsal tubercle is more complex in *S. mammiculata*. (v) *S. barnharti* tends to have rather more gonads on the right side than *S. mammiculata*. (vi) The vasa efferentia of *S. mammiculata* are relatively shorter. (vii) The testis follicles are smaller and more numerous in *S. mammiculata*. (viii) In *S. barnharti* the gonads have never been reported to branch, a character found in three of my four specimens. (ix) The course of the gut is more circuitous in *S. barnharti* and the stomach is the hindmost part of the loop, whereas in *S. mammiculata* part of the intestine forms the posterior end of the gut loop. (x) The gonadal apertures and the anus are farther back in *S. mammiculata* than in *S. barnharti*.

*S. mammiculata* does not approach the Japanese species *S. elsa* Hartmeyer (1906) which Ritter & Forsyth regarded as nearest to *S. barnharti*.

*S. mammiculata* appears to be a new arrival in Plymouth waters, for an animal so conspicuous and of such large size is not likely to have been overlooked. Moreover, Mr T. R. Tozer, the Senior Scientific Assistant in charge of the specimen room at this laboratory, brought the first specimen to my notice because he had never seen anything like it in the course of his 20 years in that department. It is of course possible but rather improbable, that it may have been overlooked. It is likely that it has been imported with oysters.

The type specimen has been deposited in the British Museum (Natural History).

I wish to thank Dr R. H. Millar for his helpful criticisms of the manuscript.

## SUMMARY

*Styela mammiculata* is a new species of ascidian from the Plymouth area. It is stalked and characterized from the remaining European species of the genus by possessing more than two gonads on the left side. Of previously described species of *Styela* it is nearest to *S. barnharti* Ritter & Forsyth from southern California.

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## NOTE ON THE PLYMOUTH 'NITZSCHIA' CULTURE

By N. Ingram Hendey

Royal Naval Scientific Service

(Plate I)

For nearly fifty years a small marine organism has been cultured at the Marine Biological Association's Laboratory at Plymouth, mainly to be used as a food supply in rearing marine larval forms.

The cultures were originated by the late Dr E. J. Allen in 1907. The first record of these cultures and the methods used to maintain them were published by Allen & Nelson (1910) in a paper describing methods for obtaining persistent cultures of eighteen species of plankton diatoms. One of these cultures flourished so successfully that subcultures have been distributed to many laboratories and institutions both in Europe and America. This organism was named *Nitzschia closterium* W. Sm. forma *minutissima*. The authors, however, did not describe the organism or produce an illustration, so the combination can be considered only as a *nomen nudum*.

In 1939 the care of the cultures passed to Dr Douglas P. Wilson who published the first detailed account of the organism together with illustrations (Wilson, 1946). Wilson found the organism to be pleomorphic and described ovoid, fusiform, triradiate, and cruciform forms. After discussing these forms in great detail Wilson concluded 'Both normals [fusiform] and triradiates produce ovals by division, and the ovals so produced can multiply to form further ovals, or can grow either two or three arms, generally two, to form normal or triradiate cells' (p. 268), and 'there is no doubt that as a general rule triradiates arise from normals only through the intermediary of oval cells' (p. 251).

As Allen & Nelson (1910) did not describe the organism there is no means of knowing whether the original cultures contained the varying forms or not.

It is certain that Allen & Nelson examined the organism under the microscope, and because of its small size must have used high-power objectives to do so. It is reasonable to conclude, therefore, that the original sample contained only fusiform specimens, or that these were strongly dominant, for had the original workers noticed the triradiate ones, it is almost certain that they would have commented upon them.

Wilson (1946) states that Dr Allen had seen occasional triradiate forms in the cultures, and that Mr Clifford Dobell, who examined some old exhausted cultures during the winter of 1910-11, noticed enormous numbers of triradiate

forms, and rightly concluded that they were present in the early cultures, and were not a product of long-continued artificial environment.

Recently considerable doubt has been expressed concerning the true taxonomic position of this organism and this paper describes efforts to clarify the problem. Hendey (1937) explained that *Nitzschia* Hassall (1845) is an absolute synonym of *Sigmatella* Kützing (1833), as both genera were based upon *Bacillaria sigmoidea* Nitzsch. The name *Nitzschia* was adopted until it was legally conserved, in the sense that most modern taxonomists have used it—that is, in the sense that W. Smith used it in his *Synopsis of British Diatomaceae* (1853)—taking *Nitzschia sigmoidea* W. Smith based on *Sigmatella Nitzschii* Kützing, which was *Bacillaria sigmoidea* Nitzsch, as the type of the genus.

Smith (1853) described *Nitzschia* as follows: 'Frustules free, elongated compressed; valves linear, keeled, with one or more longitudinal lines of puncta; keel frequently eccentric.' This definition differs in no material respect from Hassall's, and, in explanatory notes that follow it, the importance of the keel, usually eccentric and punctate, is stressed as the dominant generic character.

Wilson (1946), in his description of the three phases of the organism, makes it quite clear that he was unable, with certainty, to see any of the characteristic markings associated with the genus *Nitzschia*. He states (p. 237): 'I have not been able to satisfy myself that I have seen any of the usual valve markings of the Nitzschioideae, the keel and canal raphe or the carinal dots . . . Only in some gently incinerated specimens mounted in Sirax (a medium of high refractive index) were to be seen what might possibly be the keel and raphe with some slight suggestion of carinal dots, but it was impossible to be certain of the identity of the structures seen; they might very well have been artefacts.'

Commenting on the structure of the triradiate cell Wilson states (p. 237): 'It would be interesting to know how the keel and raphe, if present, are arranged, but it has not been possible to make them out.'

It is well known that some diatoms are very weakly siliceous and that definitive markings are difficult to observe, and, further, that some diatoms in culture have been induced to vacate their siliceous frustules and continue to live as naked bodies (Wiedling, 1941; Hendey, 1945, 1946), but these forms were derived from individuals possessing normal frustules. There can be no suggestion that the weakness of silicification and the consequent hyaline nature of the cell wall is attributable to culture conditions, or that a 'laboratory species' has, at some time, suddenly arisen due to conditions imperfectly known.

Wilson states (1946, p. 265), on the authority of Dr Mary Parke, also of the Plymouth Laboratory, that both the triradiate and fusiform phases were frequent in water samples from the Irish Sea off Port Erin.

It must be accepted, therefore, that there exists in nature a weakly siliceous organism having certain diatom characteristics, and that persistent cultures of it have been maintained over many years without producing any apparent change.

This polyphasic organism was ably described and illustrated by Wilson (1946) under the name of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen & Nelson. Wilson drew attention to the fact that a unicellular alga described by Bohlin (1897) as *Phaeodactylum tricorutum* agreed in all particulars with the triradiate of the Plymouth cultures. Bohlin had found this organism in Baltic rock pools; it had occurred with *Brachiomonas submarina* Bohlin and had multiplied rapidly in culture as the *Brachiomonas* died out.

Mr Michael Droop of the Marine Station, Millport, who is familiar with Bohlin's organism which he has himself collected in the Baltic, has intimated (in a private communication) that it commonly occurs there in rock pools on the skerries, and that he has found it within a few miles of Runmarö. Further, that in his experience, naturally occurring material always has been triradiate.

Mr Droop deposited a culture of the Baltic material at the Plymouth Laboratory, and comparisons made with it and the Plymouth specimens confirm beyond all possible doubt that both should be referred to Bohlin's species.

It should be noted that Allen & Nelson (1910) did not describe or illustrate their organism, but were content to list it as an undescribed variety of a well-known species. One of two explanations seem probable. First, that Allen & Nelson made a misidentification due to lack of information on the diatoms. The fusiform organism found by them in the original Plymouth material, whatever it was, was probably unknown to them. Its general appearance suggested a relationship with *Nitzschia closterium* Ehrenberg, but they satisfied themselves that it was not the type variety, and adopted forma *minutissima*, without describing it, merely as a matter of convenience. Secondly, that the original material cultured was predominantly a small *Nitzschia closterium* with a few specimens of *Phaeodactylum* which were, most likely, not noticed because of their small size. Subsequent subculturing at short intervals favoured the *Phaeodactylum* which replaced the *Nitzschia* very much in the same way as it replaced the *Brachiomonas* in Bohlin's cultures.

Dr D. P. Wilson (in private communication) favours the former view. He states that when Dr Allen spoke of the 'normals' in the cultures, he referred to those illustrated by Wilson (1946, p. 236, fig. 1, left-hand figures), which are fusiform specimens of *Phaeodactylum tricorutum* Bohlin, and that to the best of his knowledge and belief the original cultures had contained only 'normals', i.e. fusiform organisms. Dr Wilson also assures me that on some microscope mounts made by him in 1930 the triradiate form is present in small numbers but there are many more fusiform cells with them, and that no

true *Nitzschia* are there to be found. Whatever the cultures may have contained in the beginning, it is now clear that they have consisted only of *Phaeodactylum tricornerutum* Bohlin in several phases for very many years.

The varying phases of the organism, ovoid, fusiform, triradiate, etc., and the ability to change one into the other, have been dealt with by Wilson & Lucas (1942) and Wilson (1946). It is not clear what factors determine the shape of the cells, but no doubt age of culture, availability of nutrients, and light intensity all play a part.

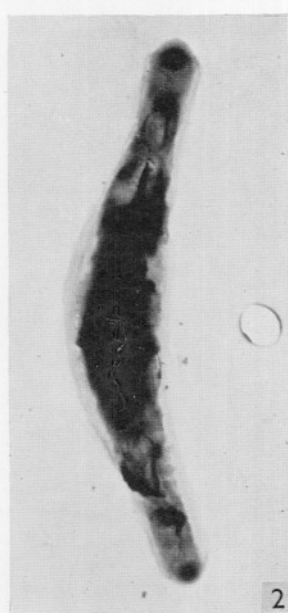
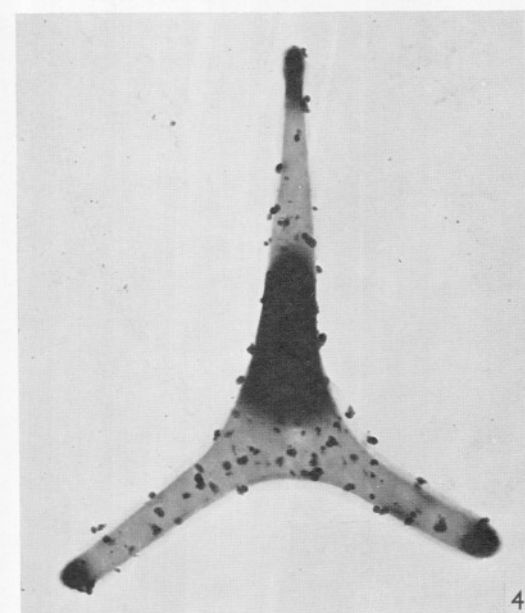
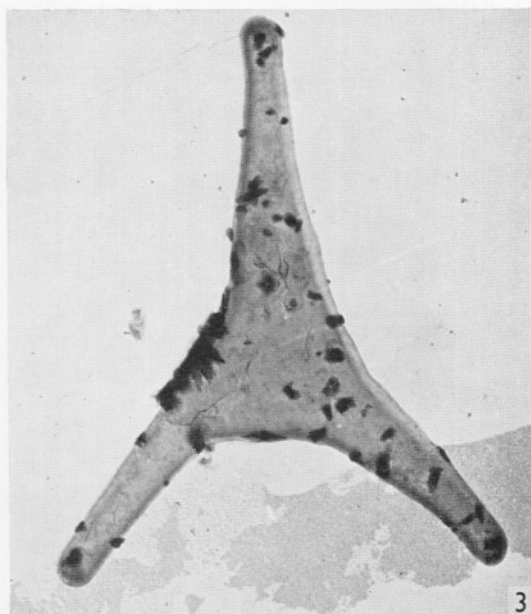
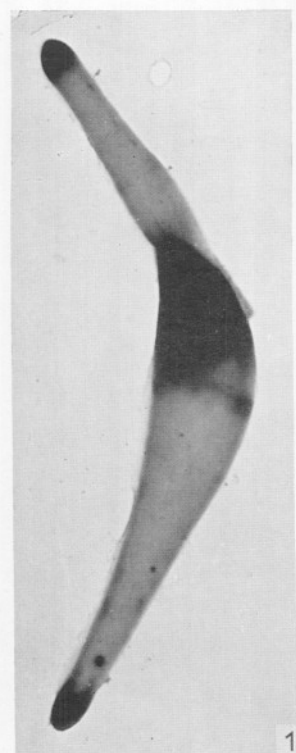
Bohlin (1897) described only triradiate specimens, Allen & Nelson (1910) indicated that only fusiform specimens were to be seen in their material. This does not exclude the possibility that some fusiform specimens were present in Bohlin's material, or that some triradiates were present in Allen & Nelson's, but only that they were not recorded. Droop intimated that his culture (on solid media) of the Plymouth organism has never given rise to triradiate forms, while a clone from the Baltic had been triradiate for most of the time since collection, but now, on solid media, contains only ovals which when sub-cultured into liquid media have given rise to fusiform cells but not to triradiates.

In an attempt to elucidate the problem, electron micrographs have been prepared. Pl. I, figs. 1 and 2 show the fusiform phase, and figs. 3 and 4 show the triradiate phase. The original prints of these were made at a magnification of  $\times 10,000$ . The micrographs show the cell-wall to be completely hyaline and entirely devoid of any structures. The organism, as interpreted by the electron microscope, adds little to that already observed by the high-power optical microscope.

The true taxonomic position of this organism is still in some doubt. There appears to be evidence that the cells divide longitudinally as do the diatoms, but the weakly siliceous nature of the cell-wall precludes any positive observations to determine whether or not the cell is frustular. Wilson (1946, p. 237) sectioned the cells, but was unable to see any evidence that would suggest the presence of valves and girdles characteristic of diatoms.

Recently Dr Mary Parke has shown (unpublished) that leucosin is present in the cell of *Phaeodactylum tricornerutum* from the Plymouth cultures. The presence of leucosin suggests that *Phaeodactylum* might be related to the Chrysophyceae, but too much importance must not be given to this suggestion without further research.

I wish to express my indebtedness to Mr F. W. Cuckow for the electron micrographs shown in Pl. I, and to Dr Mary Parke, Dr D. P. Wilson, and Mr Michael Droop for their kind assistance.





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## EXPLANATION OF PLATE I

*Phaeodactylum tricornutum* Bohlin

Figs. 1 and 2. Cell in fusiform phase.  $\times c. 6000$ .

Figs. 3 and 4. Cell in triradiate phase.  $\times c. 6000$ .

## PRELIMINARY NOTE ON A SURVEY OF STOKE POINT ROCKS WITH SELF-CONTAINED DIVING APPARATUS

By G. R. Forster

The Plymouth Laboratory

(Text-fig. 1)

It has long been recognized that a proper knowledge of the fauna of submerged rocks can be obtained satisfactorily only by direct study with diving apparatus (Gislen, 1930; Kitching *et al.*, 1934). Although the latest methods of indirect underwater observation, photography and television, may be useful on a smooth sea floor, they can give little information about a rocky area owing to their inability, as yet, to look underneath a boulder or an overhanging ledge. The superiority of the 'submarine biologist' over the indirect methods lies in the fact that he can readily collect samples for positive identification, or mark individual organisms for further study; though at present, unless very expert, he is limited to depths of under 20 fathoms. The successful use of free-diving apparatus for biological work is well established. Five years ago Prof. Drach with Cousteau's now famous 'aqualung' investigated the sublittoral fauna of various rocky shores, reaching a depth of 30 m. (Drach, 1948). Much additional work has been done in the Mediterranean and other warm seas, while farther north Bainbridge (1952) used a Siebe-Gorman apparatus to study the behaviour of plankton in the Clyde.

My training was carried out at the Portsmouth and Plymouth Royal Naval diving establishments. In 1952 a Siebe-Gorman 'aqualung' was purchased with the aid of a Royal Society apparatus grant. This breathing set, combined with a S.-G. light two-piece waterproof suit formed the equipment used during a survey in 1953.

The area of Stoke Point rocks was selected as being the most suitable locality for studying the fauna and flora which replace *Laminaria hyperborea* (Gunn) Fosl. at a depth of about 10 fathoms (see Chapman, 1944), since in less than 100 yards the depth increases from 6 to 12 fathoms. The exact position is: distance from Stoke Point, MHWST, 0.40 sea mile, bearing 146° T. After considerable practice, the sub-*Laminaria* zone was reached in August 1953, but only six dives were made during the period from August till October, owing to bad weather restricting the already limited opportunities to use the research vessels. The results are thus based on a total period of but 2 hours 'diving time' and refer only to the larger and more conspicuous organisms.

The results are shown diagrammatically in Fig. 1. *Laminaria hyperborea* is

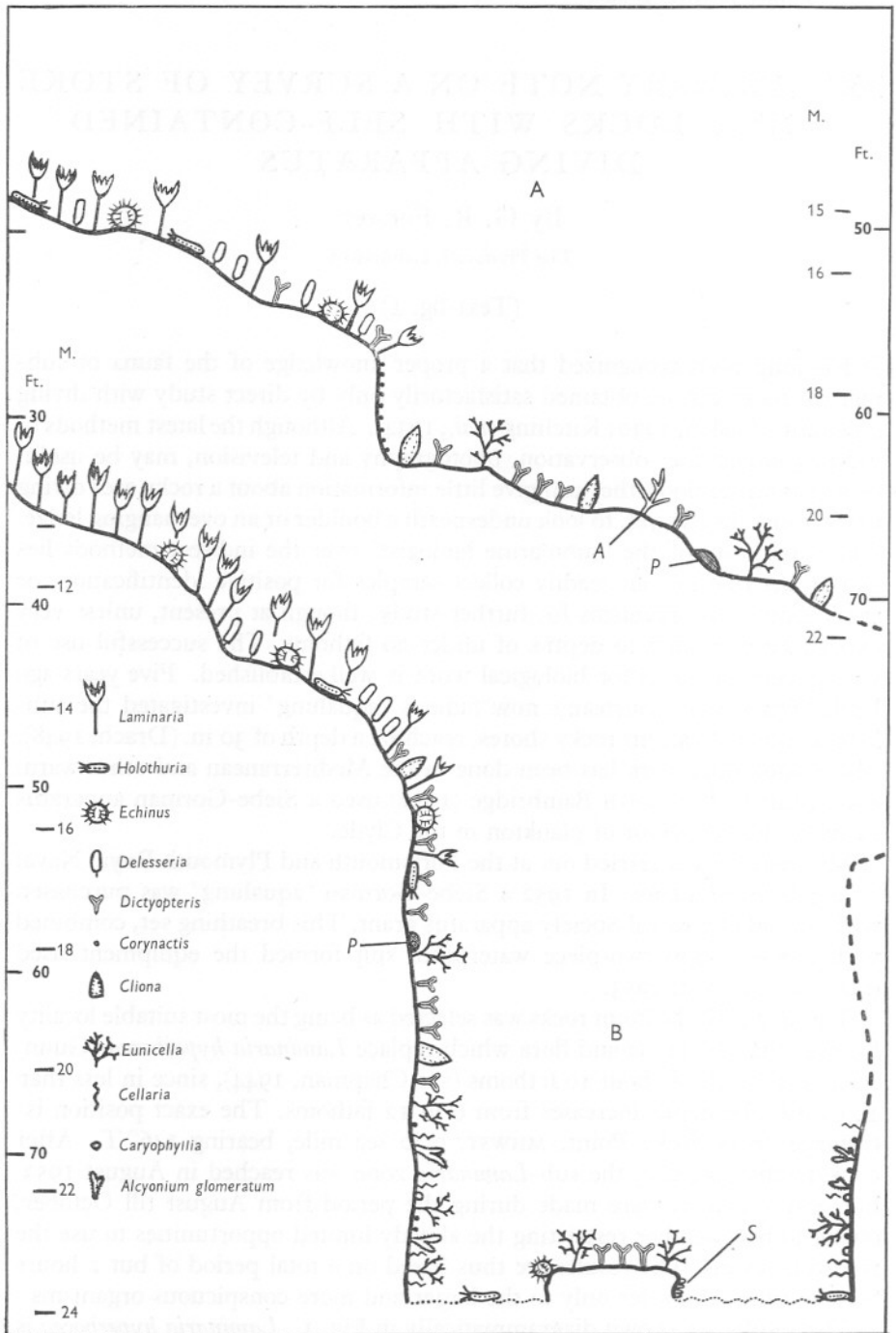


Fig. 1. Diagrammatic profiles of rocks at Stoke Point, S. Devon. The depths are given as feet and metres below chart datum, i.e. MLWST Devonport. The horizontal scale is approximately the same.  
 A, profile of reef 100 yards north-west of gully;  
 B, profile of the gully. Key inset above. A, *Axinella*; P, *Pachymatisma*; S, *Stolonica socialis*.

clearly the dominant form to a depth of 50 ft. below low water (C.D.). At 35-40 ft. a few *Echinus esculentus* L. appear, together with *Holothuria forskali* Delle Chiaje, though unlike *Echinus*, *Holothuria* is occasionally taken intertidally in the Plymouth area. Below 50 ft. the *Laminaria* is sparse and *Delesseria sanguinea* (Huds.) Lamour. becomes abundant, both plants being heavily encrusted with Bryozoa. The *Delesseria* is replaced at about 55 ft. by a thin carpet of the brown weeds, *Halopteris filicina* (Grat.) Kütz. and especially *Dictyopteris membranacea* (Stackh.) Batt., neither of which suffer from Bryozoa or epiphytes. At this depth large yellow colonies of the sponge *Cliona celata* Grant are seen, together with the smooth grey *Pachymatisma johnstoni* Bowerbank, which in shallower water is confined to crevices or shaded positions. The conspicuous pink or flesh-coloured colonies of *Eunicella verrucosa* (Pallas) appear at around 65 ft. These 'sea fans' were always orientated in a nearly vertical plane, thereby presenting the maximum area to the tidal stream. In the gully (Fig. 1 B) *Eunicella* reaches a maximum frequency of very roughly 2-4 per square metre, from 10 ft. above to 1 ft. above the bottom: the lowermost foot was, at least in several places, completely bare due presumably to abrasion by the coarse sand of the gully floor. In this area where the *Eunicella* flourishes, the *Dictyopteris* is distinctly sparse, the ground layer being composed largely of Bryozoa, especially *Cellaria* sp., some encrusting sponges and many *Corynactis viridis* Allman. Almost as numerous as the *Eunicella* is *Alcyonium glomeratum* (Hassal), whose snow-white polyps make a bright contrast against the otherwise deep red surface of the colony. The rarity of this species in dredgings is probably explained by the fact that it has not been seen outside the gully. At the same depth as the *Alcyonium* and *Eunicella*, a few large solitary ascidians *Phallusia mamillata* (Cuvier) appear, and occasional colonies of ross (*Lepralia pallasiana* (Moll.)), the tubicolous polychaete *Filograna implexa* (Berkeley) and the yellow branching sponge *Axinella dissimilis* (Bowerbank).

#### SUMMARY

A preliminary survey has been made, near Stoke Point, S. Devon, of the rock fauna at depths below the *Laminaria* belt, with self-contained compressed-air diving apparatus. By this method much more exact records of the distribution, and general abundance of the commonest species, have been obtained than would be possible with dredgings. It is hoped to make a greatly extended survey in the future.

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## WARM-WATER SPECIES IN THE PLANKTON OFF THE ENGLISH CHANNEL ENTRANCE

By J. H. Fraser, D.Sc.

Marine Laboratory, Aberdeen

Through the kindness of Dr L. H. N. Cooper of Plymouth I have been given the opportunity of examining some of the plankton samples taken by Surg.-Lt. P. Campbell, R.N.V.R., of H.M.S. *Challenger*. It is hoped that the results of these and later surveys will be published elsewhere in more detail, and in association with those made by the Scottish research vessels farther north, but two records of unusual interest are brought to notice here. The collections so far examined in detail were taken during April and May 1953, on a line of stations west of the English Channel, from 49° 39' N., 3° 30' W. to 47° 16' N., 17° 52' W. between 15 and 19 April, and on the return line from 47° 50' N., 17° 40' W. to 49° 28' N., 5° 52' W. between 25 and 28 May. They were made by 10 min. horizontal hauls at 30 fathoms depth.

On 17 April 1953, at 47° 31' N., 14° 21' W. the plankton contained a rich variety of oceanic species consisting mainly of various Siphonophora. Amongst them were found a single specimen of the solitary form of the salp *Ritteriella picteti* Apstein, 15 mm. long, and seven specimens of the aggregate form of *R. amboinensis* Apstein, 10-25 mm. long. No other salps were found there.

This specimen of the solitary form of *R. picteti* has sixteen body muscles, all interrupted on the ventral side, the range for this species being from thirteen to twenty-one or more. It is clearly distinguishable from the solitary form of the closely allied *R. amboinensis*, which usually has only eleven muscles of which the first three are continuous ventrally, but may have from ten to thirteen. There are also other differences, particularly in the form of the gut. The specimen is in excellent condition and was obviously thriving when caught; it is a young one as this species can reach at least 70 mm. in length.

The seven aggregate specimens of *Ritteriella* found by H.M.S. *Challenger* have muscle arrangements exactly corresponding to the published description of *R. amboinensis*, including the arrangement of muscle VI which, according to Thompson (1948), is not known in other *Ritteriella* species or in the *Cyclosalpa* group. The aggregate form of *Ritteriella picteti* is imperfectly known. One very small embryo taken from the stolon of a solitary form and figured by Apstein as *R. amboinensis* was later ascribed by Ihle to *R. retracta*, now thought to be synonymous with *R. picteti*. Three small (3-4 mm.) specimens from Australian waters were ascribed with some doubt to *R. picteti* by Thompson (1948), who also took embryos from the stolon of a solitary

form. It would appear from Thompson's description to have five dorsally approaching muscles, as in *Salpa cylindrica*, and would thus be sufficiently distinct from that of *Ritteriella amboinensis* to make recognition possible. These *Challenger* specimens are in quite good condition, and their identification as *R. amboinensis* and not *R. picteti* would, according to this, seem reliable, although more work and material is required before the relationship between these species can be adequately understood.

Both these species are considered to be equatorial and are regarded as rather rare even there, especially *R. picteti*. Both occur in the Pacific Ocean, particularly in the neighbourhood of the East Indies and Philippines, and in the Indian Ocean. *R. amboinensis* has been recorded in the equatorial and southern Atlantic, off the coast of Africa, but because of a confusion in synonymy it is doubtful if *R. picteti* has yet been recorded from the tropical Atlantic. Neither species has previously been recorded from the north-temperate Atlantic.

The same area was re-examined by H.M.S. *Challenger* towards the end of May, and in the interval the total plankton had decreased and only a few siphonophores were taken, with some *Salpa fusiformis*. However, at 47° 40' N., 13° 58' W. on 26 May, i.e. only a few miles from the earlier station referred to, a specimen of a very large *Coscinodiscus* type of diatom, 1.8 mm. in diameter, was taken. This was identified for me by Mr R. Ross of the British Museum (Natural History) as *Ethmodiscus gazellae* (Janisch) Hustedt, which is the largest diatom known and is recorded up to 1.9 mm. in diameter. He tells me that this species is widespread in tropical waters, being specially common in the Pacific, and that it has been recorded from the Mediterranean (Hustedt, 1930, pp. 374-6) and from the Cape Verde Islands (Castracane, 1886, as *Ethmodiscus gigas*). This is believed to be the first record of its presence in the north-east Atlantic. The specimen was in excellent condition and obviously alive when caught.

The abundance of 'Mediterranean' or 'Lusitanian' species in the plankton west of the British Isles has been remarkable in 1953, and will form the basis of a more detailed report elsewhere.

The specimens of *Ritteriella picteti* and *R. amboinensis* are being held at the Marine Laboratory, Aberdeen; the *Ethmodiscus gazellae* is deposited with the British Museum.

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## BIOLOGICAL DIFFERENCES BETWEEN SEA WATERS: EXPERIMENTS IN 1953

By Douglas P. Wilson, D.Sc. and F. A. J. Armstrong

The Plymouth Laboratory

(Text-fig. 1)

The experiments during the 1953 season were arranged to investigate certain criticisms and suggestions made to us after publication of the earlier work (Wilson, 1951; Wilson & Armstrong, 1952), and there were included tests of our own devising intended to narrow the field within which must be made any search for an explanation of the observed biological differences between sea waters from different localities. We were again unable to obtain water from the Celtic Sea and once more, by kind co-operation of the Millport Marine Station, used water from the Firth of Clyde instead. Both the E I and Clyde waters were collected on the same day and the fertilizations of *Echinus esculentus* were made and the experiments started 4 days later, which was the minimum time possible after collection of the Clyde water. Every experiment included a control comparison of the two waters.

One of the more interesting investigations last year was the effect on the larvae of *Echinus* of water passed through active carbon. Unfortunately further investigation, as detailed below (p. 351), showed that the sample of carbon used contained a significant amount of copper, and the results obtained may be therefore attributable to the presence of that metal. This was found by analysis of the carbon and checked by analysis of sea water passed through it and, later, by the addition of copper to sea water in a section of Exp. I below. Messrs Sutcliffe, Speakman and Co., on being informed of our observations, very kindly supplied a sample of active carbon substantially free from copper with which we were able to repeat last year's experiment with different results.

In a review (*J. Conseil*, Vol. 18, p. 249) of a paper by Loosanoff and others and also in private correspondence, Dr H. A. Cole pointed out that no attention has been paid to the possibility that differences between waters may be due to bacterial activity during the period between collection and use. He suggested that, to test this, water should be filtered free from bacteria at the time of collection. This we have endeavoured to do as recorded below.

That extracts from adult *Echinus* could supply substances needed by the developing larva was a possibility worth testing. Water in which larvae had decayed and presumably released materials into the water was filtered and tested.

From time to time it has been suggested to us by various people that vitamin B<sub>12</sub> would be likely to benefit larvae developing in water to which it had been added. We are grateful to Dr A. P. Orr for informing us that at Millport improvement in fertilization of *Echinus* had been effected by the action of E.D.T.A. in sequestering heavy metals and for referring us to work by Tyler (1953). Tyler claims a significant increase in the life-span of sperm pre-treated with versene (E.D.T.A.)—which he used amongst other sequestering agents—as well as an improvement of the fertilization reaction.

The stability to heat of the factor responsible for the differences between waters was tested by heating the waters at boiling-point and also by autoclaving.

Half a century ago Herbst (see list of papers in Needham, 1931) in a series of papers described the different effects obtained by rearing echinoderm larvae in artificial sea waters from which he omitted one normal constituent. We decided to observe the effect of rearing the larvae in an artificial sea water known to contain all normal inorganic constituents, because the factor we are seeking is most probably not one of these.

## METHODS

### *Cleaning of Apparatus*

The methods used in the earlier experiments were followed. Glassware was cleaned with hot 1:1 sulphuric acid, and the filtering apparatus was sterilized in an autoclave and washed with boiled distilled water before use.

### *Collection of Water*

Water from the International Hydrographic Station E1 was taken from the sea surface with a wooden bucket and was strained through 200-mesh bolting-silk into a cleaned carboy. Clyde water was taken in the Largs channel near Millport, being dipped from the sea in a glass breffit and strained through 200-mesh bolting-silk into a carboy cleaned and dispatched from Plymouth.

### *Collection of Bacteria-Free Water*

The most practicable method seemed to us to be one in which water was drawn through a bacteriological filter into a sterile bottle. The apparatus had to be portable and simple. The first arrangement tried was that shown in Fig. 1A. After cleaning, the Winchester bottle was evacuated at a water pump, the rubber tube being disconnected. The tap was then closed, the tube and filter candle attached, and the whole arrangement was sterilized by heating in an autoclave at 10 p.s.i. pressure for 30 min. The stopcock was lubricated with a silicone grease which did not run when heated. To collect water the filter candle was immersed in a bucket of sea water and the tap opened to allow the water to be drawn through the candle into the bottle. On test, the apparatus held a vacuum for a week in the laboratory and the sterilization was

satisfactory. In use, however, the stopcocks leaked, possibly because of vibration in transit, and the arrangement of a sealed breakable glass tube shown in Fig. 1 B was substituted. Although this avoided leakage, the samples were still not sterile. We think it likely that the Berkefeld filter candles were not of

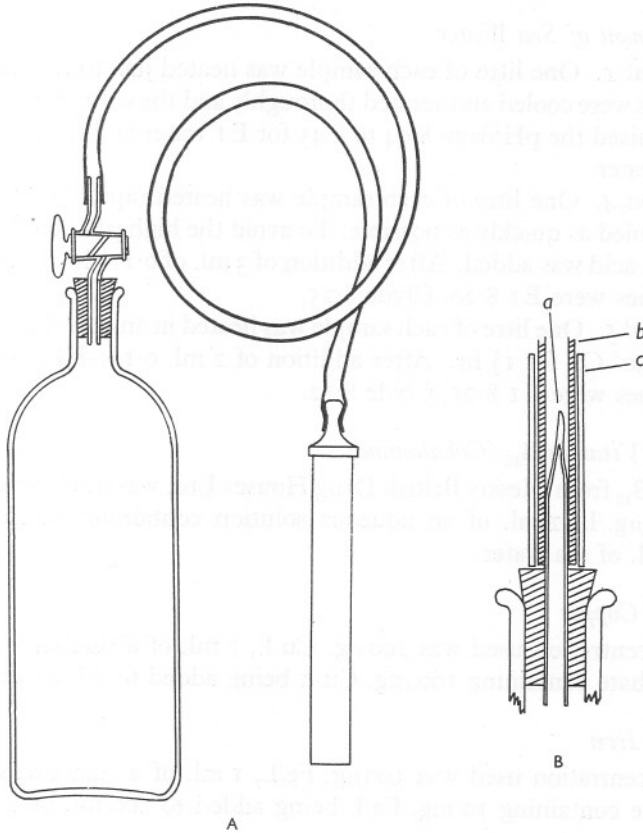


Fig. 1. A, Winchester bottle with filter candle attached for collecting filtered water. B, sealed tube arrangement to replace stopcock: (a) glass tube sealed after evacuation of bottle; (b) rubber tubing; (c) loosely fitting thick glass sleeve to protect tip of A. The apparatus is evacuated, sealed and assembled, and then sterilized. In use the sleeve c is slid up the rubber tube and the tip of a broken off inside b.

a sufficiently fine porosity. On one occasion, however, a sample was obtained which was sterile by our tests, and the experiments show that no obvious harmful effects are caused by the rubber or ceramic of the filter apparatus.

#### *Sterility Testing*

We are indebted to Dr C. H. Jellard, Public Health Laboratory Service, for his help and interest in this part of the work. Dr Jellard himself carried out most of the inoculations. On one or two occasions one of us (F.A.J.A.) did them.



The media used were: (i) peptone sea water, (ii) peptone sea-water agar, (iii) casein sea-water agar, as described by Spencer (1952).

Tubes and Petri dishes were incubated at 22° C. The sample considered satisfactorily sterile showed no bacterial growth after 3 weeks.

#### *Heat Treatment of Sea Water*

*Experiment 1.* One litre of each sample was heated just to boiling for 1 hr. The samples were cooled and aerated thoroughly and the volume adjusted. The treatment raised the pH from 8.04 to 8.45 for E1 water and from 7.99 to 8.48 for Clyde water.

*Experiment 4.* One litre of each sample was heated rapidly just to boiling, and then cooled as quickly as possible. To avoid the high pH noted in the first experiment, acid was added. After addition of 3 ml. of 0.1 N-HCl and aeration, the pH values were E1 8.20, Clyde 8.25.

*Experiment 5.* One litre of each sample was heated in an autoclave at 25 p.s.i. pressure (130° C.) for 1½ hr. After addition of 2 ml. 0.1 N-HCl and aeration the pH values were E1 8.01, Clyde 8.12.

#### *Addition of Vitamin B<sub>12</sub> (Cobalamine)*

Vitamin B<sub>12</sub> from Messrs British Drug Houses Ltd. was used in a concentration of 1.1 µg./l., 1 ml. of an aqueous solution containing 1.1 mg./l. being added to 1 l. of sea water.

#### *Addition of Copper*

The concentration used was 100 µg. Cu/l., 1 ml. of a standard solution of cupric sulphate containing 100 mg. Cu/l. being added to 1 l. of sea water.

#### *Addition of Iron*

The concentration used was 100 µg. Fe/l., 1 ml. of a standard solution of ferric citrate containing 10 mg. Fe/l. being added to 100 ml. of artificial sea water.

#### *Physiological Fluids*

A mixture of equal parts of coelomic fluids from male and female animals was used. Ovaries and testes were separately squeezed between glass plates, and the expressed liquids diluted with equal volumes of water. These fluids were filtered just before use, first on Whatman No. 41 (loose texture) filter-papers, and then on 'Gradocol' membranes, A.P.D. 0.49 µ.

#### *Water in which Larvae had Died and Decayed*

This was from the beaker of E1 water in Exp. 1. After filtration on No. 41 paper and on a membrane it had a strong fishy but not unpleasant smell. It was well aerated before use.

### *Carbon Extracts*

After the 1952 experiments an attempt was made to identify the material obtained by acetone extraction of active carbon through which sea water had been passed. Copper as cupric chloride was found, in an amount too large to have come from the sea water. On examining the carbon, it was found to contain some 1500 p.p.m. of copper, in a form which was insoluble in acetone, but which became partly soluble after the carbon had been wetted with sea water. Moreover, on passing sea water through a column of the carbon, the copper content of the water increased from  $6\ \mu\text{g./l.}$  (a normal value) to  $60\ \mu\text{g./l.}$  We consulted Messrs Sutcliffe, Speakman and Co., who very kindly supplied a sample of their carbon no. 315. On analysis it showed only a trace of copper, and no increase of the copper content was found when sea water was passed through this carbon.

This carbon was used to repeat the 1952 extraction experiments. Extracts of similar appearance were obtained. Copper was not detected in them.

### *Artificial Sea Water*

The salts listed in the formula of Lyman & Fleming (1940) for the hypothetical combination of ions in sea water were used. After aeration the pH was 8.02.

### *Ethylene Diamine Tetra-acetic Acid*

The disodium dihydrate (Versene, Trilon B, Sequestrine, SETate, etc.) referred to here as E.D.T.A. was used as an approximately 0.1M solution (37.2 g./l.). Addition of 10 ml. of this solution to 1 l. of sea water gave a concentration of approximately 0.001M or 370 mg./l., and decreased the pH to between 5 and 6. After addition of 18 ml. 0.1N-NaOH and thorough aeration the pH values were, in Exp. 4, E1 8.12, Clyde 8.17.

In Exp. 5, the procedure was altered slightly to minimize salinity changes. The 0.1M E.D.T.A. solution was made up in artificial sea water, which was then adjusted to pH 8.66 by adding sodium hydroxide. 10 ml. of this solution were added to 1 l. samples of the various sea waters, giving the same concentration (0.001M) of E.D.T.A. as in Exp. 4. Only 3 ml. of 0.1N-NaOH were then needed to adjust the pH to suitable values. These were E1 8.07, Clyde 8.06, artificial sea water 8.14.

### *Antibiotics*

Penicillin G (Benzyl Penicillin B.P.C.) Glaxo, and Streptomycin (calcium chloride) Glaxo were used, one mega-unit of each being dissolved in 5 ml. of distilled water. Small volumes of this solution were measured with a microburette into 100 ml. portions of Clyde water to give concentrations of 100, 300 and 500 units/ml.

*Fertilizations*

These were always made in a mixture of equal parts of Berkefeld filtered Clyde and E1 waters, one male and one female being used, both carefully selected from a number of recently trawled sea urchins (*Echinus esculentus* L.).

After fertilization the eggs were immediately divided equally between two beakers, and each portion washed six times with one of the two waters. Eggs were distributed equally among the experimental dishes by the dipper method already described (Wilson, 1951, p. 5).

## EXPERIMENTAL RESULTS

In every experiment at least four and often five dishes of each kind of water were tested. It should be emphasized that, unless noted to the contrary, larvae in all the dishes comprising a set of one kind of water were identical one with another. This uniformity within a set was very striking, and it was most unusual for any variation to appear before the fifth or sixth day. When differences between individual dishes within a set began to be noticeable the experiment was ended. This rarely happened before the larvae were obviously dying off.

*Experiment 1*

*E1 water* collected 16. iii. 53. Ship: R.V. *Sabella* with F.A.J.A. Salinity, 35.34; pH 8.04; temperature at time of collection, 8.5° C.

*Clyde water* collected 16. iii. 53. Small boat with Mr E. Latham. Salinity, 33.30; pH 7.99; temperature at time of collection, 7.1° C.

The sea-urchins were trawled on 19 March 1953 and kept under circulation until the fertilization was made on the following day. Out of about twenty sea-urchins opened none had completed spawning and only two were partially spent. A good fertilization was obtained from one ripe male and one ripe female, although a small percentage of the ova did not fertilize.

The following waters were tested. Each set comprised five dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water Berkefeld filtered at sea
IV	E1 water Berkefeld filtered at sea (bacteria-free until used)
V	Clyde water heated
VI	E1 water heated
VII	Clyde water + vitamin B <sub>12</sub>
VIII	E1 water + vitamin B <sub>12</sub>
IX	Clyde water + Cu
X	E1 water + Cu

The larvae did better in the Clyde water (Set I) than in the E1 water (Set II), but the difference was small compared with the more striking differences obtained in previous years. Nevertheless, it was clear that in the E1 water there were more abnormal and stunted larvae than in the Clyde water and that the best plutei in the latter were more finely developed than

the best in the E I water. When 2 days old the larvae in the Clyde water swam a little more strongly than those in the E I water, but there was never at any time a marked concentration up at the surface such as had often occurred in the better waters of previous experiments. On the sixth day (5-day-old larvae), when the larvae in all sets were dying, those in the E I water survived for a few hours in slightly better condition than those in the Clyde water.

It is worth mentioning that the two beakers, into which the eggs were divided after fertilization, had been kept as a check on the dishes. The concentration of larvae in these beakers was very much greater than in the dishes, and the difference between the two waters was here more marked. In the Clyde water the larvae swam more strongly and survived longer than those in the E I water.

Of the waters filtered through Berkefeld candles at sea (Sets III and IV) only that from E I proved to be bacteria-free when tested just before use. The Clyde water similarly filtered, and the waters in the carboys (which after filtration supplied Sets I and II) gave heavy bacterial growths on the test plates. The larvae in the E I water which had been bacteria-free between collection and use (Set IV) were no different from those in the other E I water (Set II) and were inferior to those in the Clyde waters. The larvae in the Clyde water filtered at sea (Set III) were no different from those in the control Set I and were, of course, better than those in both the E I waters (Sets II and IV). Incidentally the water in Set III had a thin oily film, derived presumably from the rubber connexions of the apparatus.

The Clyde water which was heated (Set V) was barely inferior as a rearing medium to that which had not been so heated, but the E I water after heating (Set VI) produced decidedly poorer larvae than did the unheated E I water.

The addition of vitamin B<sub>12</sub> to the waters (Sets VII and VIII) made no difference whatever to the appearance of the larvae.

The water with copper proved to be poisonous; in the E I water (Set IX) most deaths took place during early cleavage, but in the Clyde water (Set X) mainly during the blastula stage.

### Experiment 2

Particulars of E I and Clyde water as for Exp. 1.

Fertilization made 27 March 1953 from sea-urchins trawled the previous day. Of the twenty opened only one or two were partially spent. About 100% of the ova fertilized, but nearly half cleaved irregularly and gave misshapen blastulae.

The following waters were tested. Each set comprised three dishes.

Set	
I	Clyde water
II	E I water
III	E I water + filtered coelomic fluid from an adult <i>Echinus</i>
IV	E I water + filtered ovary juice from an adult <i>Echinus</i>
V	E I water + filtered testis juice from an adult <i>Echinus</i>
VI	75 ml. E I water + 25 ml. filtered E I water from Set IV of Exp. 1 after death and decay of the larvae

In the Clyde water (Set I) the larvae did a little better at first than in the E1 water, but by the fifth day they were a little better in the E1 water, repeating more or less the result in Exp. 1. Again the beakers showed the most marked difference, the Clyde water giving the most vigorous larvae and the longest survival.

In all instances the additions (Sets III-VI) had slightly unfavourable effects on the development of the larvae, and each kind of addition had an effect a little different from any of the others. It is possible that the experiment was to some extent adversely affected by the large proportion of eggs in all dishes which failed to develop properly and died and decayed early.

### Experiment 3

*E1 water* collected 13. iv. 53. Ship: R.V. *Sabella* with F.A.J.A. Salinity, 35.30; temperature at time of collection, 8.7° C.

*Clyde water* collected 13. iv. 53. Small boat with Mr N. Thomson. Salinity, 33.30.

In addition to the usual carboys of water strained through bolting-silk one Winchester of water filtered through a Berkefeld candle (see p. 348) was obtained from each locality. Unfortunately the glass stopper of the Clyde Winchester was broken on the journey back to Plymouth and half the water leaked away through a packing of sterilized cotton-wool. The water remaining in the Winchester from the Clyde proved to be heavily contaminated with bacteria, as did indeed also that from E1. These waters therefore can only be regarded as having been more efficiently freed from the larger plankton organisms at the time of collection than were the waters in the carboys.

The sea-urchins were trawled on 16 April and kept under circulation until a fertilization was made the following day. The following waters were tested. Each set comprised five dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water filtered when collected
IV	E1 water filtered when collected
V	Clyde water + Clyde extracts
VI	Clyde water + E1 extracts
VII	Clyde water + blank extract
VIII	Clyde water through carbon
IX	E1 water + Clyde extract
X	E1 water + E1 extract
XI	E1 water + blank extract
XII	E1 water through carbon

Apart from Sets III and IV the experiment was a repetition of Exp. III of 1952 (Wilson & Armstrong, 1952, pp. 344-7) in which an attempt had been made to extract a growth factor. As already explained (p. 351) the active carbon



used for this purpose was later discovered to contain a significant proportion of copper, which, while not appearing in the control blank extracts, affected the sea water passed through it and rendered certain conclusions invalid. In the present experiment an active carbon free from copper was used.

Throughout this experiment very little difference was discernible between the control Clyde and E1 waters, Sets I and II, but the Clyde water had slightly better plutei until the fourth day, after which the plutei in the E1 water were in a very slightly better condition. Some larvae in all dishes were dead on the fifth day and by the sixth day conditions generally were bad and the experiment was ended. At no time in either water were the plutei as well formed or as vigorously swimming as those in most previous experiments. It is not possible to be sure whether this was due to the condition of the waters or to unripeness of the eggs or sperm. About 100% of the eggs fertilized but a small proportion cleaved irregularly.

In the water collected through Berkefeld filters that from the Clyde (Set III) had a slightly milky appearance, but nevertheless produced larvae almost as good as those in the Clyde water control (Set I). The larvae in the E1 water filtered when collected (Set IV) were indistinguishable from those in the E1 control (Set II).

As in the similar experiment the previous year the carbon extracts (Sets V, VI, IX and X) proved poisonous, while the blank extracts (Sets VII and XI) made very little difference to the waters to which they were added, the larvae in these dishes being very little inferior to those in the controls (Sets I and II). The important deviation from the results of 1952 is that the larvae in the waters which had passed the active carbon (Sets VIII and XII) were indistinguishable from those in the controls (Sets I and II), whereas in 1952 they were mainly abnormally formed and most of them died early. The 1952 result must now be attributed to the presence of a toxic amount of copper, derived from the carbon, in the waters which had been passed through it.

#### *Experiment 4*

*E1 water* collected 27. iv. 53. Ship: M.F.V. *Sula* with F.A.J.A. Salinity, 35.17; pH 8.06; temperature at time of collection, 9.2°C.

*Clyde water* collected 27. iv. 53. Small boat with Mr E. Latham. Salinity, 34.01; pH 8.35.

Two Winchesters of water filtered through Berkefeld candles were taken at each locality.

The sea-urchins were trawled on 1 May and the fertilization was made on the same day. On this date less than a quarter of the urchins opened had shed their genital products. In comparison with previous years this indicated a late spawning season.

The following waters were tested. Each set comprised four dishes.

Set	
I	Clyde water
II	Clyde water, filtered when collected (Winchester A)
III	Clyde water filtered when collected (Winchester B)
IV	Clyde water heated for a short period
V	E1 water
VI	E1 water filtered when collected (Winchester C)
VII	E1 water filtered when collected (Winchester D)
VIII	E1 water heated for a short period
IX	Clyde water + E.D.T.A.
X	E1 water + E.D.T.A.
XI	Aquarium water
XII	Artificial sea water

The experiment was originally planned to contain sets of Clyde and E1 waters after heating in an autoclave for comparison with the same waters heated for a short period. Unfortunately the samples were accidentally spoilt during preparation and could not be used.

Throughout the 4 days' duration of the experiment (the larvae were everywhere dying when 5 days old) there was little to choose between larvae in the Clyde and E1 controls (Sets I and V). Blastulae in the Clyde water swam up a little in advance of those in the E1 water. At an age of 3 days there were more slightly abnormal larvae in the E1 water than in the Clyde. The difference was just sufficiently marked for an independent observer to distinguish between them after a careful comparison. Thus the two waters, so far as their effects on the larvae were concerned, were almost identical. Although almost 100% of the eggs developed with very few abnormalities the larvae never showed any concentration near the surface as is usual with good cultures in good water. The room temperature rose slowly from 15.2 to 19.0° C. during the experiment; for the first 2 or 3 days when surface concentrations normally take place it was not unduly high. In view of past experience the temperature is not regarded as having induced weakness in the larvae, in fact they swam most strongly when 3 days old at a temperature of 18° C. The lack of vigour must therefore be due either to eggs or to sperm in poor condition, or to the condition of the waters. As the fertilization appeared initially to be good (well-raised fertilization membranes, even cleavage of almost all the eggs) it is reasonable to suspect that neither water was a perfect medium for the development of *Echinus*.

All the waters filtered at the time of collection (Sets II, III, VI and VII) gave heavy bacterial counts and the larvae reared in them differed in no way from those in the controls (Sets I and V), except Set VI where all larvae were always in poor condition with many dying at an early age. The reason for this has not been discovered, but it is thought that this water must in some way have become contaminated. The Berkefeld filter candle used was observed to have a dark stain, which while defying analysis may have been due to some toxic substance. Heating the waters for a short period (Sets IV and VIII)

made no difference to the Clyde water but it very slightly worsened the E1 water.

The addition of E.D.T.A. (Sets IX and X) did not improve either water; in fact the plutei showed slight abnormalities compared with those in the controls (Sets I and V).

The plutei in the aquarium water (Set XI) were always structurally inferior, with shorter arms, to those in the controls (Sets I and V) but they swam higher in the water and on the last day had fewer arm rods protruding from the shrinking flesh.

The plutei in the artificial sea water (Set XII) were decidedly small and stunted, but in the final stages did not degenerate as quickly as those in the Clyde and E1 waters.

#### Experiment 5

*E1 water* collected 11. v. 53. Ship: R.V. *Sabella*, with F.A.J.A. Salinity, 35.16; pH 8.08; temperature at time of collection, 10.50° C.

*Clyde water* collected 11. v. 53. Small boat with Mr E. Latham. Salinity, 33.28; pH 8.07.

The sea-urchins were trawled on 12 May, and kept under circulation until a fertilization was made on the following day. It was found that the majority had spawned or were partially spent. A good fertilization with almost 100% normal cleavage was obtained.

The following waters were tested. Each comprised a set of four dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water + E.D.T.A.
IV	E1 water + E.D.T.A.
V	Artificial sea water
VI	Artificial sea water + Clyde water
VII	Artificial sea water + E1 water
VIII	Artificial sea water + E.D.T.A.
IX	Artificial sea water + Fe
X	Clyde water autoclaved
XI	E1 water autoclaved

The eggs for Sets V-IX were washed in artificial sea water before being placed in the dishes.

In addition to the above some tests with penicillin and streptomycin were made, each in strengths of 100, 300 and 1000 units/ml.

There was again very little difference between the controls, Sets I and II. For the first 2 or 3 days larvae in the Clyde water swam a little more strongly than in the E1 water, and in both were more strongly swimming than in the similar waters of Exp. 4, though again neither showed the marked concentration at the surface which had been a feature of the best cultures in previous years. Later there was no difference in swimming vigour to be observed. Structurally there was no difference to be observed between the two sets of larvae throughout the experiment.

The addition of E.D.T.A. made very little difference. When 2 days old the larvae in the waters with E.D.T.A. (Sets III and IV) swam a little more strongly than in the controls, but later they were not quite as vigorous and structurally became a little inferior to the larvae in the same waters without the sequestering agent.

Larvae in the artificial sea water (Set V) were a little inferior in swimming vigour and in structure to the larvae in the natural sea waters (Sets I-IV). They were better in the mixtures (Sets VI and VII), the mixture with Clyde water being slightly the better. The larvae were a little better in the artificial water to which E.D.T.A. had been added (Set VIII) except on the last day when they were worse. The addition of Fe (Set IX) to the artificial sea water made little or no difference to the larvae.

The larvae in the autoclaved Clyde and E1 waters (Sets X and XI) were inferior structurally to those in the controls though for a short time they swam a little more vigorously.

The antibiotics had little or no effect on the larvae.

None of the differences throughout this experiment was anything more than slight.

#### DISCUSSION

Throughout the 1953 experiments the two sorts of water were closely similar in their effect on the larvae and they never showed as marked a difference as had often, but not always, been a feature of the comparisons made in previous years. None the less the larvae were slightly better formed and a little healthier in the Clyde than in the E1 water, more especially during the early part of the season. Owing to the many variable factors involved, direct comparisons of one year with another are open to more than one interpretation, and cannot very profitably be made. The impression has been gained, however, that the E1 water tested in 1953 was on the whole a little more suitable for *Echinus* larvae, while the Clyde water was a little less so, than on most occasions when used in 1952. Also that in neither were the larvae ever as well formed, or as vigorously swimming, as on the best occasions in earliest years. Their condition was always not very dissimilar from that recorded of the larvae in Exp. III of 1952 (Wilson & Armstrong, 1952, p. 344) where there was also little to choose between the two waters.

We are indebted to Dr Sheina M. Marshall for the information that a tow-net haul made in the Clyde in mid-April 1953 contained forty-seven *Sagitta elegans* and thirteen *Sagitta* too young for certain identification. Mr P. G. Corbin informs us that the standard  $\frac{1}{2}$  hour oblique hauls made with the 2 m. stramin ring-trawl, at the Eddystone in March 1953 indicated a sparse plankton characterized by *Sagitta setosa*. In April the hauls both at the Eddystone and E1 (10 miles S.S.W. of the Eddystone) were again small in quantity with only very few *Sagitta*, both *S. setosa* and *S. elegans* being

seen. But on 11 May, at E1, when water for our last experiment was collected, the presence in the continuing sparse plankton of 20 *Aglantha rosea* showed an influx of western water, although no *Sagitta* or other indicator species were present. Silicate analyses made by F.A.J.A. also suggest a change in the water mass at E1 by 11 May. This change is paralleled by our results, in so far as such differences as were apparent between larvae reared in waters from E1 and the Clyde were more pronounced in the earlier experiments than in the later.

It was unfortunate that the unsuspected copper in the active carbon used in 1952 gave rise to a misleading result. The sea water passed through that particular sample of carbon must have picked up sufficient copper to have become poisonous to the larvae. The larvae in this year's carbon-treated sea water were similar to those in the untreated controls, but the carbon extracts once again proved poisonous to the developing eggs. These results seem to close what had seemed to be an encouraging approach to the solution of the problem through the use of activated carbon.

The attempts to filter the water free from bacteria at the time of collection were mainly unsuccessful but one quantity of E1 water proved to be bacteria-free when examined just before use. The results with it did not differ in any way from those obtained with the water collected at the same time and merely strained into a carboy. Since the bacterial content of this carboy water was high when tested this result is of interest. It is noteworthy, too, that bacteriostatic concentrations of penicillin and streptomycin were without effect.

The addition of various substances, notably the vitamin B<sub>12</sub> and the sequestering agent E.D.T.A., did not improve either of the waters: if anything they adversely affected the larvae. Heating the waters generally made them, especially the E1 water, worse as a medium for larval development. In artificial sea water the larvae did moderately well, though not quite as well as in the natural sea waters. It may be possible to use artificial sea water as a standard medium with which to compare natural waters and to which to add any substance the effect of which on the larvae it is desired to test.

#### SUMMARY

In all comparative tests made in 1953 developing *Echinus* larvae did only slightly better in sea water from the Clyde than in sea water from the English Channel at E1.

The activated carbon used in one of the 1952 experiments contained a significant amount of copper. The experiment was therefore repeated in 1953 with copper-free carbon, and this time the sea water passed through the carbon was not toxic although the carbon extracts were so, as previously found.

Larvae reared in a quantity of E1 water which had been filtered free from bacteria at the time of collection were no different from larvae reared in water collected at the same time in the usual way.

Antibiotics in bacteriostatic concentration had no effect on the waters as media for *Echinus* development.

The addition to the water of extracts of gonads and of filtered coelomic fluid from adult *Echinus* were detrimental to the larvae, as was also filtered sea water in which other larvae had died and decayed.

Additions of the vitamin B<sub>12</sub> and of the sequestering agent E.D.T.A. had little effect on the larvae, being slightly detrimental.

Heating the waters for shorter or longer periods generally had an adverse effect on larvae reared in them.

Larvae reared in artificial sea water, with or without the addition of iron, were in general inferior to those reared in natural sea waters.

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## THE ATTRACTIVE FACTOR IN THE SETTLEMENT OF *OPHELIA* *BICORNIS* SAVIGNY

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Largely as a result of experiments made in 1951, sands were classed as attractive, neutral or repellent (Wilson, 1953*a*); but only after the 1952 experiments had been completed did it become reasonably certain that the sands in which the larvae of *Ophelia bicornis* Savigny readily settle carry an attractive factor apparently distinct from a repellent one present in sands in which they will not settle (Wilson, 1953*b*). Whilst the precise natures of the factors were uncertain, it seemed probable that they both derive from organic activity. The 1953 breeding season was devoted to an attempt to obtain more information about the attractive factor.

### METHODS

Methods closely followed those used previously. All glassware was cleaned in hot strong sulphuric acid, and similar acid was used in the preparation of the acid-cleaned sands. These were always prepared from Bullhill Bank sand (collected 10 June 1952) generally a few days, and not more than a few weeks before use, being stored, after cleaning, in distilled water in stoppered bottles. Fertilizations and experiments were mostly in a mixture of Berkefeld-filtered sea waters from the Eddystone or E 1 and from the Clyde (obtained by the helpful co-operation of the Millport Marine Station, and Mr E. Latham in particular). Occasionally Clyde water was used alone but never Eddystone or E 1 water alone. Although all fertilizations were not equally successful there was no difficulty in obtaining healthy larvae for all the experiments, as there had been in 1952 on occasions when E 1 water only was used (Wilson, 1953*b*, p. 220). Throughout this paper 'sea water' means Berkefeld-filtered sea water.

The culture medium used was 'Erdschreiber', similar to that described by Gross (1937, p. 754). It was kindly supplied by Dr M. W. Parke from her stock, intended for the culture of diatoms and flagellates.

Sands were always tested in Pyrex crystallizing dishes by the free-choice method (Wilson, 1953*a*, p. 415) and sometimes also by conical-vessel tests (Wilson, 1952, p. 66). The former method distinguishes between attractive and neutral sands, the latter between repellent and neutral sands (Wilson, 1953*b*, p. 210). Very many tests were made, and when time did not allow of the settled larvae being counted the comparative sizes of the settlements were

recorded in words. The scale of words used to express number is the same as before, but for convenience it is reprinted here below Table I. All the un-metamorphosed larvae recorded were sticking to sand grains; this is a slight departure from previous practice when larvae seen to swim out of a sand sample were also counted.

Experiments are no longer described in the order in which they were made; the former method of numbering has therefore been discontinued. In this paper all experiments of the same type are grouped under one heading. The results were too numerous for all to be recorded in detail; typical results are given in the tables; others are described in the text. A few inconclusive tests are not mentioned; these did not in any way conflict with past or present findings.

Sands referred to as having been 'soaked', were kept for the period stated in sea water (or other medium) in covered Pyrex crystallizing dishes on a window bench shielded from the sun.

Each experiment was controlled with a small dish, the bottom of which was completely covered with fresh Bullhill Bank sand. This dish is not usually mentioned in the account of an experiment, or shown in the tables, but it was always employed to check the ability of the larvae to metamorphose during the actual period of the experiment. In these control dishes almost every larva was fully metamorphosed after 2 days.

In every free-choice dish, no matter what sands were tested, there were always at the end of the experiment many unmetamorphosed larvae swimming freely, or attached lightly to the surface film. Generally there were also a few sticking lightly to the glass bottom of the dish between the sand heaps. Occasionally one or two metamorphosing or metamorphosed worms were also to be seen on the glass; it was always practically certain that they had crawled out of a sand heap.

## THE EXPERIMENTS

### *Experiments with Filter-Paper*

In a preceding paper (Wilson, 1953*b*) it was mentioned (p. 200) that 'some tests based on filtering water in which fresh sands had been shaken gave promising, though imperfect results'. These tests had been included in experiments, carried out in late July 1952, which had been spoilt by the condition of the larvae. The results, so far as they had gone, suggested that whereas acid-cleaned Bullhill Bank sand is made attractive by soaking for some days in sea water in which fresh Bullhill Bank sand had been shaken, it is made even more attractive if the water, after the shaking, is filtered through Whatman No. 42 filter-paper (a fine-textured double acid-washed paper). This surprising result merited further investigation.

Some fresh Bullhill Bank sand, collected (11 June 1953) from the surface of the bank at a place where adult *Ophelia* worms were numerous, was

vigorously shaken (12 June 1953) in sea water. After the grains had settled the water was poured off (for convenience such water will in what follows, and in the tables, be referred to as 'B.B. water'), a portion being filtered through a piece of Whatman No. 42 filter-paper. Small quantities of acid-cleaned Bullhill Bank sand were placed in both the filtered and the unfiltered 'B.B. water', and were also placed on the piece of paper used for the filtration. Further quantities were placed on an unused sheet of the paper in sea water, and, for control, in a dish of sea water alone. Portions of these sands were tested with 5-day-old larvae after so soaking for 5 days and some of them again after 26 days. The tests after 5 days were further controlled with acid-cleaned sand straight from storage in distilled water, and the 5-day tests included both dish and conical vessel experiments, those after 26 days dish experiments only.

The results are shown in Table I. They do not confirm the result of last year's imperfect experiment. The acid-cleaned sand which had soaked in unfiltered 'B.B. water' had become definitely more attractive (especially after 26 days) than that soaked in sea water only, but that which had been in the filtered 'B.B. water' seemed to be even slightly less favourable than that from sea water alone. The acid-cleaned sand on the filter-paper through which the 'B.B. water' had been passed seemed to be unaffected (after 5 days), but that on the unused piece of filter-paper had become markedly attractive, inducing heavy settlements in both dish and conical vessel experiments. This result being the reverse of what had been expected it may be as well to note that there is no possibility of the two papers, or the sands from them, having been interchanged or confused during the experiment. It should be noted, however, that the paper used for the filtration of the 'B.B. water' unfolded itself when placed in a dish of water and much of the sand sprinkled over it may not have been on that part of the paper which had picked up most of the particles from the water filtered through it.

At the time of the second test, after 26 days, the acid-cleaned sands from both filtered and unfiltered 'B.B. waters' had minute growths, apparently algal, on the grains and contained some ciliates. These were most numerous in the sand from the unfiltered water, and this sand was, in addition, a little silty. The control sand, from sea water only, appeared clean. These observations were made with a dissecting binocular at a magnification not exceeding  $\times 100$ .

Some further experiments were made with filtered (No. 42 paper) and unfiltered 'B.B. water', and with the intention of obtaining improved results culture medium was added to equal quantities of the 'B.B. water' and this was controlled by diluting other portions of the 'B.B. water' with equal amounts of sea water, as well as using normal sea-water controls. The results of these more elaborate experiments were less definite than the one just described. The culture medium itself had little or no effect on the acid-cleaned sands soaked in it, neither did it appear to increase the potency of the 'B.B.

water' to which it was added. Dilution of the 'B.B. water' may have decreased its powers of making the acid-cleaned sand attractive, at least for the relatively short periods (not exceeding 16 days) the sand was soaked in it. The results, while not showing very marked differences between the variously soaked sands, are in general agreement with those of the earlier experiment. So far as they go they do indicate that acid-cleaned sand is made attractive by soaking in unfiltered 'B.B. water', but is little affected if the 'B.B. water' first be filtered through No. 42 paper.

The unexpected result in the first experiment (Table I) where an acid-cleaned sand became attractive by contact, in sea water, with unused Whatman No. 42 filter-paper led to a series of experiments to investigate the possibility that the filter-paper contained some substance which became adsorbed on the grains or which encouraged bacterial or other growths attractive to the larvae.

It is not proposed to consider all these experiments in detail. Some of them were negative, but the majority were slightly in favour of the filter-paper having some such favourable influence. The best result was obtained by soaking acid-cleaned sand in the first small quantity of sea water passed through a new piece of paper, especially (Table II) after soaking for several days. After much sea water had been passed through the paper the last quantity of water collected did not have as marked an effect. Never again was a really good settlement obtained with acid-cleaned sand allowed to lie on, or between, unused filter-papers in sea water. Indefinite results were obtained with concentrated extracts of several filter-papers, and with filter-papers washed in strong hydrochloric acid followed by distilled water. All the papers came from the same box and care was taken to avoid contamination during handling. The results are quite inconclusive and are of value only in indicating that results obtained with acid-cleaned sands soaked in filtered 'B.B. water' will either not be influenced at all, or if they are they will be in the direction of attractiveness. Thus the results with filtered 'B.B. water' already discussed can at least be accepted provisionally. It may be too, that the explanation of last year's imperfect experiment, which led to this investigation, can be explained by the favourable action of the particular piece of filter-paper then used.

*An Experiment with Sea Water in which Fresh Sand from the Salthouse Lake was Shaken*

From one of last year's experiments (Wilson, 1953*b*, Expt. 57B) it appeared that sea water in which fresh sand from the Salthouse Lake (Station II) was shaken was capable, like 'B.B. water' of making attractive an acid-cleaned sand soaked in it. To check this result a similar experiment was made this year. After washing away, with sea water, all the easily removable silt a quantity of surface sand from the Salthouse Lake (Station II) was, the day after collection, vigorously shaken in sea water. After settlement of the grains

the sea water, now rather dirty, was poured off. It was divided into two portions, to one an equal volume of clean sea water was added, to the other an equal volume of culture medium. To these and to controls, consisting of sea water only and sea water plus an equal volume of culture medium, acid-cleaned sand from storage in distilled water was added. The sand was soaked for 7 days and then tested with 5-day-old larvae, in a free-choice dish and in conical vessels. After 2 days the results were not very definite (see Table III) but it was noted that the larvae used in this experiment swam unusually strongly, keeping mainly to the surface of the water. It was therefore decided to continue the experiment for another 3 days, and the sands from the free-choice dish were carefully put back again and some more larvae from the original culture (now 7 days old) were added. The conical vessel tests were not repeated. After a further 3 days in the dark the free-choice dish sands were again examined, with the result shown in Table III. There seems no doubt that the acid-cleaned sand had been improved by soaking in the 'S.L. water' and that the addition of culture medium, as had been found in several other experiments, has little or no effect on acid-cleaned sands soaked in it.

*Acid-cleaned Sands Soaked in Sea Water in the Presence of Fresh Natural Sands*

The experiences with 'B.B. water' and 'S.L. water' led to another type of experiment in which an acid-cleaned sand was placed at one side of a dish with freshly collected Bullhill Bank or Salthouse Lake sand at the other. Such acid-cleaned sands when tested after only a few days showed no difference from the controls kept in sea water only, but after several weeks they markedly improved in attractiveness, but then so too did the controls, though usually not to quite the same extent. A series of tests is recorded in Table IV from which it appears that the fresh Salthouse Lake sand had an even more favourable influence on the acid-cleaned sand kept with it than had Bullhill Bank sand itself. This, however, was not so in the experiment recorded in Table V. It is unfortunate that the experiment being lengthier than originally anticipated the control acid-cleaned sand kept in sea water had all been used up before the last tests were made.

In another series of tests (see Table V) the fresh sands occupied most of the bottom of the dish, the acid-cleaned sand being placed in cleared areas in the middle. The cleaned sands were thus completely surrounded by the fresh sands. A mixture of equal parts of sea water and culture medium was used instead of sea water only. Fresh sand for a depth of 12-18 in. on the Bullhill Bank was tried as well as sand from the surface. The Salthouse Lake sand was, as usual, from the surface layer. The results, while not very definite, point to a slight improvement of the acid-cleaned sand surrounded by the fresh Bullhill Bank surface sand and little or no improvement by the deep sand or by the sand from the Salthouse Lake. It is possible that the soaking times were too



short for definite results. Moreover, the larvae used on 2 August 1953 (and this applies also to the previous experiment) were noticeably less ready to settle than usual for their age. This is deduced from their tendency to swim vigorously up against the surface film and from their behaviour in control dishes (not recorded in the tables) plentifully strewn with fresh Bullhill Bank sand. As has been pointed out before (Wilson, 1953*b*, p. 221), it is usual for some cultures of larvae to show less readiness to metamorphose than others of the same age.

*Acid-cleaned Sands Soaked in Sea Water, in Distilled Water and in Culture Medium*

Towards the end of the 1953 series of experiments it was found that acid-cleaned sands which had been soaked in sea water as controls for earlier experiments had become markedly attractive to the larvae. An acid-cleaned sand soaked for a few days in sea water had often induced a few more larvae to settle in it than had the same sand stored in distilled water and tested with it in the same free-choice dish. However, the increase in attractiveness had almost always been very small. With acid-cleaned sands in sea water for a varying number of days (from 1 to 26) there were only two occasions out of twenty-three when 5-day-old larvae settled in appreciable numbers (several metamorphosed and several metamorphosing) and on those two occasions (one after 5 days of soaking and one after 10) there were no tests of sands from distilled water storage with which to compare. It is possible that the larvae used in those particular experiments were more ready to settle than usual.

There were three tests with 6-day-old larvae with sands soaked for 20, 22 (see Table VII) and 25 days, and these all gave good settlements, but no comparative tests with acid-cleaned sands from distilled water were made. It must be remembered that 6-day-old larvae usually settle more readily than do 5-day-old.

Of three tests with acid-cleaned sand soaked for 37, 40 and 43 days, respectively, all induced good to heavy settlements while there were only light settlements in sand from distilled water storage tested with them. Of these three results one is recorded in Table IV (virtually with 6-day-old larvae), the other two in Table VI which shows the results of four tests of an acid-cleaned sand kept in sea water over a period of nearly 6 weeks. In the last test, on 25 July 1953, the larvae were in the free-choice dish for 3 days instead of the usual 2.

It will be realized that the experiments were not planned as they are here presented. This explains the lack of uniformity with regard to the age of the larvae used for testing and the occasional absence of control sands from distilled water. It was not until late in the season that there was any suspicion that soaking acid-cleaned sands in sea water for long periods improved them,



and it was then too late to arrange further experiments. None the less, from the data just given, it seems impossible to avoid the conclusion that soaking in sea water has this favourable effect.

Reference has frequently been made to using acid-cleaned sand stored in distilled water as a control. During the course of the 1953 experiments such sand was tested sixteen times in free-choice dishes. Only on one occasion (recorded in Table VI) was there even a moderate settlement; in all other instances there was either no settlement or a very small one. Acid-cleaned Bullhill Bank sand stored for over a year in distilled water was tested with 6-day-old larvae in a free-choice dish with sand stored for 50 days only. In neither, after 2 days, were any metamorphosed worms to be found, and only a few or very few metamorphosing ones, and about the same number unmetamorphosed. Thus acid-cleaned sand kept in distilled water does not increase in attractiveness, as it does when kept in sea water.

In several experiments culture medium was added to the sea water in which acid-cleaned sand was soaking, with the intention of encouraging the growth of autotrophic organisms already present in fresh sands. The effect of culture medium added to sea water only had therefore to be determined. In Tables III, V and VII comparisons between sands from sea water only and from sea water to which culture medium was added have already been given, and there were a number of others. Of eleven distinct tests there was no difference in four; in the remaining seven the sand from the culture medium was either slightly more attractive (three tests) or slightly more repellent (four tests, the most positive in the repellent direction being recorded in Table VII) than sand from sea water only. It may thus be concluded that for the relatively short periods of soaking employed (not more than 22 days) the addition of culture medium to plain sea water has no appreciable effect.

#### *Experiments with Diatom and Flagellate Cultures*

The possibility that the attractiveness of Bullhill Bank natural sand is due, partly at least, to the presence on the grains of living micro-organisms led to experiments in which acid-cleaned sand was kept for 2-3 weeks with living diatoms and flagellates. A mixture of several species of each (kindly provided by Dr M. W. Parke from the stock cultures kept at the Laboratory) was used for the first experiment, the results of which are recorded in Table VII. Complete counts were made of the larvae settled in the two controls (tests 1 and 2), but time did not allow of a complete count of the much larger number of larvae settled in the sand with the diatoms and flagellates (test 3), and after as many as possible had been removed and counted it was obvious that at least as many again were left in the sand. It should be noted that the sand was rinsed in plain sea water before testing and many diatoms and flagellates were then washed away. The sand grains were thus left relatively clean, but numbers of minute diatoms and flagellates could be seen on the grains while the count

of larvae was being made. Conical vessel tests (not shown in the table) of the same sands, run concurrently, gave fairly heavy and very similar settlements for all three sands, from which it should probably be inferred that the increased attractiveness of the sand from the diatom and flagellate culture was actually only slight. This seems to be confirmed by a later experiment (Table VIII) in which mixed diatoms and mixed flagellates were used separately as well as together. It was only in the sands which had been in the presence of flagellates that any noticeable increase in settlement was obtained and even here none had fully metamorphosed after 3 days in the free-choice dish (no conical vessel tests were made). In this experiment numerous flagellates were stuck to the sand grains but the diatoms seem to have been lost from the sand during the rinse in sea water immediately before testing.

Taking the two experiments together it seems reasonable to conclude that the presence on the acid-cleaned grains of flagellates, or possibly bacteria from their particular cultures, has some slight attraction for the larvae.

An attempt was made to culture organisms from fresh Bullhill Bank sand and transfer these to acid-cleaned sands. Diatoms and some other organisms multiplied in culture medium but the acid-cleaned sand kept with them was only slightly improved after 15 days. The grains were covered with adherent diatoms, singly and in clusters, in a manner not seen in fresh Bullhill Bank sand. There may have been too many of them.

#### *Experiments with Organic Extracts*

An experiment with acid-cleaned sand kept for half an hour in an extract of *Ulva*, and for the same period in starch dextrine gave almost negative results. The *Ulva* extract had no effect, but the sand with the starch was apparently slightly improved compared with the same sand from distilled water. However, a later test with sand kept in starch dextrine for 28 h was completely negative.

#### *Acid-cleaned Sand kept with Adult Ophelia bicornis*

In a previous experiment (Wilson, 1953*a*, Expt. 51) some washed Salthouse Lake sand kept with adult *Ophelia* worms for several days had been made even less attractive than before. It was considered desirable to repeat this experiment with acid-cleaned Bullhill Bank sand. Accordingly, a few spent worms were kept in a clean dish for 2 or 3 days until they had emptied their guts of all sand grains. They were then transferred to another dish with a fair quantity of acid-cleaned Bullhill Bank sand. It was noticeable that they would not burrow into this sand (as they normally do into fresh sand), but lay on the top. After 4 days a little of this sand was tested, both by free-choice dish and conical vessel tests, against sand from sea water only. It was found to attract slightly fewer larvae than the latter, but in both the settlements were small.

A similar result was obtained with more of the same sand after 16 days with the adult *Ophelia* worms. The presence of the adult worms had not improved the sand, neither had they markedly worsened it.

#### *Tests for Gregarious Settlement*

It has been shown that the larvae of the oyster (Cole & Knight-Jones, 1949), of the acorn barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950) and of the polychaete *Spirorbis borealis* (Knight-Jones, 1951) when ready to settle are attracted by the presence of newly metamorphosed young of their own species. There was therefore a distinct possibility that the larvae of *Ophelia bicornis* might show a preference for settling in sand in which others of their kind were metamorphosing or had recently metamorphosed. In order to test for this two experiments were arranged in which 5-day-old larvae were given a choice between acid-cleaned sand containing metamorphosing and recently metamorphosed larvae from an earlier fertilization and similar sand without. In order to distinguish the younger larvae from the older the former were lightly stained with methylene blue. The ability of the stained larvae to metamorphose was concurrently tested in another dish containing fresh Bullhill Bank sand, in this they settled and metamorphosed normally and as readily as did larvae from the same fertilization not stained and separately tested.

The result of the first of these experiments is given in Table IX from which it will be seen that unfortunately three of the unstained older and recently metamorphosed young worms had during the course of the experiment crawled into the pile of sand which was not supposed to contain them. There were, of course, very many more of these young worms in the other pile, the sand of which was spread by their activities until it occupied a greater area than it had done originally. On the clean glass bottom of the dish, particularly in the neighbourhood of this pile, were a number of metamorphosing and metamorphosed larvae, both stained and unstained, which had apparently crawled out of the sand. These were not counted, and the figures given for this pile in the Table are therefore lower than they should be, but only by a small fraction of the total possible. A curious feature of the figures for the stained younger larvae is that almost all found in the sand (there were many still swimming freely in the dish or lightly attached to the surface film of the water) were fully metamorphosed. This is unusual for this type of dish experiment, where newcomers normally settle in an attractive sand at any time throughout the duration. It seems that here settlement must have taken place mainly during the first or second day and very little afterwards, and it is not clear why this should be. The number settled in the sand is higher than what would normally be expected in a neutral acid-cleaned sand, but there is no significant difference between the pile with the three older unstained worms and the pile with the 114. Of course, if any stimulating soluble substance is given

off during metamorphosis, or for a short while afterwards, it would quickly diffuse throughout the dish and affect both sands alike. That this hypothetical substance, the actual existence of which is doubtful, did not of itself, without the presence of sand, bring about metamorphosis is obvious from the large number of younger stained larvae still swimming freely in the dish, or attached singly or in clusters, unmetamorphosed, to the surface film. The correct interpretation of these results is thus not clear, but they do not seem to offer much support for the existence of gregarious settlement in this species.

The second and later experiment, the results of which are recorded in Table X, are in general agreement with the conclusion from the earlier one. Conducted on similar lines, with 5-day-old larvae stained before use, it differed only in using acid-cleaned sand from distilled water as the control and fewer settling older and unstained larvae in the test sand. Some of these unstained larvae must have migrated to the control sand early in the experiment and quite a number were found, unmetamorphosed, on the surface film with stained larvae when the dish was examined immediately after removal from the dark box in which it had been kept for 3 days. A fair number of metamorphosed young worms, unstained, and one or two stained were crawling on the glass bottom of the dish, especially around both piles of sand. In comparison with the first experiment it is noticeable that only a small number of stained larvae settled in either pile.

#### *Experiments with an Almost Pure Charcoal*

In many previous experiments forms of activated charcoal have produced heavy settlements, but it has never been quite certain whether it was the carbon which attracted the larvae or some impurity such as copper (Wilson, 1953*b*, p. 224). I am indebted to Mr F. A. J. Armstrong for making from 'AnalaR' sucrose a charcoal almost totally free from impurity (analysis for copper, by F.A.J.A. gave less than 0.2 parts/million). That this charcoal had some absorptive properties was demonstrated by its power to decolorize weak dye solutions. The sucrose charcoal was, for testing, mixed with an acid-cleaned sand; the results are recorded in Table XI (two separate tests with different batches of larvae). The larvae were undoubtedly strongly attracted by it, and if the numbers settling seem fewer than in some earlier experiments with other forms of charcoal this is to be explained by failure to obtain an intimate mixture of the sucrose charcoal with the sand. This charcoal was much coarser than that manufactured for decolorizing purposes, so often used previously, and did not adhere to the grains at all. The particles were angular and the mixtures contained much more sand than charcoal.

From these results it is probably permissible to conclude that the carbon of the charcoal rather than the impurities attracts the larvae, but whether for its absorptive property or for some other reason is still unknown.

### *Other Experiments*

The possibility that the minute amount of copper contained in activated charcoal might stimulate the larvae to metamorphose (Wilson, 1953*b*, p. 224) led to an experiment in which acid-cleaned sand was soaked for an hour in 100  $\mu\text{g}$  Cu/100 ml. sea water. The sand was then well washed in filtered sea water and tested in a free-choice dish with similar sand not so soaked as a control. After 2 days the control sand contained one or two metamorphosing larvae and two or three unmetamorphosed ones, while the sand from the copper solution contained only a large number of unmetamorphosed larvae, many of them dead. A likely explanation is that larvae exploring the sand were quickly weakened by the copper and, unable to swim away, were eventually killed by it.

The 1952 experiments (Wilson, 1953*b*) included several tests in which neutral sands (contained in bolting-silk envelopes) buried in fresh sands acquired an attractive factor from fresh Bullhill Bank sand but not from fresh Salthouse Lake (Station II) sand. It has also been shown that heating Bullhill Bank sand in water to about 100° C (previously referred to as 'normal sterilization') destroys the attractive factor, but no check had been made in which a neutral sand, inside a bolting-silk envelope, had been buried in sand so heated. Thus among the present experiments were included tests of an acid-cleaned sand, contained within bolting-silk envelopes, buried in heated Bullhill Bank sand and in heated Salthouse Lake (Station II) sand, in sea water for 8 days. The buried sands were then presented to larvae in a free-choice dish and in conical vessels, using as control a similar sand kept for 8 days in sea water only. The sand exposed to the heated Bullhill Bank sand induced light settlements almost identical with those in the control, but that exposed to the heated Salthouse Lake sand induced smaller settlements, suggesting that it had become a little repellent.

### DISCUSSION

There have been few methods by which a neutral sand has been transformed into an attractive one. They have included the use of charcoal, a substance unlikely to be present in the Exe sandbanks, and extracts of filter-paper which gave the curious results already discussed. Almost the only other methods have all employed some form of soaking the neutral sand in sea water under conditions likely to encourage growths of micro-organisms too small to be readily visible during normal examination of the sands. It will therefore be well to consider what evidence there is from these experiments that such organic growths do indeed form on clean sand grains, immersed in sea water, and what evidence that the larvae react to their presence.

The first fact to note is that acid-cleaned sand stored in distilled water, in stoppered bottles, even for over a year, does not increase in attractiveness.



Distilled water, devoid of nutrients, does not encourage growths. On the other hand, it is improbable that anything kept in even Berkefeld-filtered sea water, contained in a perfectly clean dish covered only by a loose glass plate, could remain for long free from bacteria, etc. Such sea water must almost always contain some nutrient material sufficient for small growths to take place. After several weeks small organisms, motile and non-motile, have always been seen in such dishes, and a scratch on the bottom with a needle has generally revealed a very thin film of slime.

I am indebted to Dr C. H. Jellard (Public Health Laboratory Service) for endeavouring to demonstrate, by normal bacteriological techniques, that acid-cleaned sand does become coated by bacteria, etc., after days or weeks in filtered sea water. Unfortunately the irregularities, natural scratches, colourings and other markings on the sand grains made this an almost impossible task. In substitution for the sand grains we immersed acid-cleaned cover glasses in Berkefeld-filtered sea water for various periods, and it was then easy to see that they indeed became covered by bacteria and other organisms and that these increased in abundance for a time, never becoming excessively numerous. The control cover-glasses, kept in distilled water in stoppered bottles, remained clean, as was expected.

That the organisms which develop in dishes of filtered sea water are not always the same species is apparent when such dishes are compared after several weeks. Some will then contain minute diatoms not present in others, or rounded unidentified objects or flocculent growths absent from other dishes on the same bench close by. Occasionally minute ciliates or flagellates are seen. Acid-cleaned sand kept in these dishes for several weeks or even months looks clean until close inspection reveals that there are on the grains small numbers of the organisms which are to be seen more easily on the glass of the dish. It is likely that some species are more acceptable to the larvae than are others. This is to some extent indicated by the experiments with diatoms and flagellates from culture; the flagellates (or unseen organisms introduced with them) having some slight attraction for the larvae, the diatoms seemingly none at all, even diatoms cultured from Bullhill Bank itself. Differences in the kinds of organisms growing on the acid-cleaned sands after days or weeks in sea water could explain some of the small variations in intensity of settlement in sands from different dishes, or in sand from the same dish tested at different times. But on the whole it remains true that, within the period of time covered by the 1953 experiments, the longer the acid-cleaned sands were soaked in sea water, the more they increased in attractiveness.

The fact that acid-cleaned sand soaked in sea water in which fresh Bullhill Bank or Salthouse Lake sands had been shaken becomes more attractive than the same sand kept for an equal time in sea water only is very suggestive of living organisms. It may be assumed that many organisms are washed off the natural sands by the shaking and subsequently adhere to and grow on the



acid-cleaned grains.<sup>1</sup> Moreover, these are likely to be the species that are the natural food of the young worms. Even the presence of the fresh sands in the dish with the acid-cleaned sand eventually imparts to the latter a little more attractiveness than soaking for a like period in sea water alone. The addition, in the 1952 experiments (Wilson, 1953*b*), of a few grains of fresh sand, from either the Bullhill Bank or the Salthouse Lake, imparted a degree of attractiveness to acid-cleaned sands, but more markedly so after several days at room temperature during which organisms introduced on the fresh grains would have had time to multiply and spread. This happened both in the light and in the dark, and although other explanations are possible this suggestion that living organisms, multiplied after a lapse of time, were responsible for the increased attractiveness is at least reasonable. Some similar inoculation experiments, in 1952, using weak media instead of sea water only, and at a raised temperature in a warm dark oven, had similar though less definite results.

The burying of a neutral sand, enclosed within a bolting-silk envelope, in fresh Bullhill Bank sand has been shown to be a sure way of making that sand attractive (Wilson, 1953*b*). But if the Bullhill Bank sand is first heated to about 100° C in water no such increase of attractiveness is apparent. This again can be explained on the assumption that in the first instance living organisms pass through the bolting silk to mingle with the neutral sand, as indeed they were observed to have done, whereas in the second instance there would be no living organisms in the sand after heating. In all earlier experiments treatments of fresh Bullhill Bank sand which would have killed organisms living in it have always reduced its attractiveness to *Ophelia* larvae (for a summary of these treatments see Wilson, 1953*a*, pp. 423-4).

Occasionally, after treating a neutral sand by one or other of the methods by which micro-organisms would be encouraged to grow on it the sand has induced a settlement about half as large as that obtained in fresh Bullhill Bank sand, presented to the larvae under the same conditions. This is a decided advance on anything achieved during earlier breeding seasons when almost the only known method of ensuring, especially under free-choice conditions, a heavy settlement in sands other than fresh Bullhill Bank sand was to use activated charcoal, the attractiveness of which for the larvae is still unexplained.

The solution of the main problem, that of explaining how *O. bicornis* larvae recognize their own natural sand, now seems to be within reach. From the results of previous work it can be accepted that suitable grade is one of the factors influencing the larvae in their choice of a sand in which to settle. Cleanliness is another. But this cleanliness is only relative, for a sand of

<sup>1</sup> Filtration of the 'B.B. water' through No. 42 filter-paper generally reduced its efficiency in making attractive acid-cleaned sand soaked in it (see pp. 363-4) but in view of the inconsistent effects of filter-paper these results are not advanced in support of the present thesis.

suitable grade, if perfectly free from all organic matter, is neutral in the sense of neither encouraging nor discouraging larvae to settle. Only when the relatively clean grains carry living micro-organisms, or their organic products, are they really attractive to larvae in the exploratory phase prior to settlement. It seems most likely that these micro-organisms must belong to a limited, though perhaps fairly extensive range of types and species, and that there is an optimum abundance just as there seems to be an optimum size range of sand grains. If the two optima coincide then there will be produced the best possible conditions for settlement.

An explanation such as that just outlined would bring *O. bicornis* larvae more or less into line with the larvae of some sedentary animals which do not settle on solid surfaces until after the surfaces have become coated with bacteria or other organisms or with the slime films produced by them (for references and a short discussion see Wilson, 1952, pp. 52-3), although these are probably not sought for as food. It would also fit the many facts now known concerning the settlement reactions of *Ophelia* larvae.

Attraction is, however, only half the picture. During 1953 no attention was paid to a repellent factor which seems to co-exist with an attractive one on the Salthouse Lake sands in which adult *Ophelia* does not live. It has previously been suggested that the larvae may dislike some kinds of dead organic matter on the sand grains, or the presence of too many micro-organisms, or the wrong kinds. It is probable that variations in the quantity and quality of the organic matter, living or dead, on and among the sand induces corresponding variations in the intensity of the settlement of *Ophelia* larvae coming into contact with them.

#### SUMMARY

During the 1953 breeding season attention was paid to ways in which neutral acid-cleaned sand may be made attractive to the larvae of *Ophelia bicornis*. It was found that one of the most effective methods is to soak the sand for a long period in sea water, during which time there is evidence that the grains acquire a coating of living micro-organisms. Similar soaking in water in which fresh sands have been shaken, or in the presence of fresh sands in the same dish, are even more effective ways of rendering the neutral sand attractive. It is concluded that the presence on the grains of living micro-organisms, or products of their activity, in not too great an abundance, makes the grains attractive to the larvae.

Acid-cleaned sand soaked for long periods in distilled water is not made attractive to the larvae, neither does it acquire a coating of micro-organisms. This was demonstrated by substituting acid-cleaned cover-glasses for sand, and comparing the results of keeping them in distilled water and in sea water.

An acid-cleaned sand enclosed within a bolting-silk envelope and buried, in sea water, in Bullhill Bank sand, previously heated to about 100° C, does

not become attractive, as it does when the Bullhill Bank sand is fresh and unheated. The difference is regarded as being due to the absence of living organisms in the pre-heated sand.

Diatoms alone are ineffective in making a neutral sand attractive to the larvae, but the presence of flagellates appears to impart some slight attraction.

The addition of culture medium (Erdschreiber) to the sea water in which the sands are soaked is without significant effect. This type of culture medium may not be a suitable nutrient fluid for the kinds of organisms the larvae like.

It is shown that a very pure form of charcoal, made from sucrose, is as effective in inducing larvae to settle as were impure forms in earlier experiments. It is concluded that it is the carbon itself, and not contained impurities, which is attractive, although why this should be is still an unsolved problem.

It was found that acid-cleaned sand is sometimes made attractive by contact in sea water with Whatman No. 42 filter-paper, or in extracts from it. The results are, however, erratic and their significance obscure.

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TABLE I

Acid-cleaned Bullhill Bank sand soaked in

Tests beginning	Days of soaking		(1) Sea water	(2) 'B.B. water'	(3) 'B.B. water' through filter-paper	(4) Sea water on filter-paper used for no. 3	(5) Sea water on unused filter-paper	(6) Distilled water
Settlements after 2 days in free-choice dish								
17. vi. 53	5	Metd	2	3	1	None	35	1
		Meting	3	4	3	1	16	1
		Unmet.	7	12	12	7	12	5
Settlements after 2 days in conical vessels								
17. vi. 53	5	Metd	2 or 3	Several	2 or 3	1 or 2	Many	1 or 2
		Meting	Fair number	Good number	Several	Several	Many	Several
		Unmet.	Fair number	Many	Fair number	Several	Several	Fair number
Settlements after 2 days in free-choice dish								
8. vii. 53	26	Metd	1 or 2	Good number	None	—	—	—
		Meting	Few	Several	None	—	—	—
		Unmet.	Several	Several	Several	—	—	—

Metd = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed. 'B.B. water'—see text, p. 363.

Scale of words used to express number in Tables I–XI:

Very few (sometimes also expressed in numbers 1–4)	Good number
Few	Many
Several	Very many
Fair number	Multitude

TABLE II

Set A. Acid-cleaned Bullhill Bank sand soaked for 2 h in

	(1) Sea water	(2) Sea water and between 2 pieces of filter-paper	(3) Sea water and between 2 pieces of filter-paper previously washed with HCl	(4) First small volume of sea water passed through one filter-paper	(5) Small volume of sea water passed through the filter-paper used for (4) after previous passage of much sea water
Metd	None	None	None	None	None
Meting	Several	Several	Several	Several	Several
Unmet.	Several	Several	Several	Fair number	Several
Set B. Acid-cleaned Bullhill Bank sand soaked for 5 days as above					
Metd	Several	Few	Several	Many	Fair number
Meting	Several	Several	Fair number	Many	Fair number
Unmet.	Several	Several	Several	Fair number	Several

The larvae used for both sets were from the same culture and were 5 days old. A single free-choice dish was used for testing each set and the settlements were those obtained after 2 days. Different filter-papers were used for each set.

TABLE III

Acid-cleaned Bullhill Bank sand soaked for 7 days in

	(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) 'S.L. water' plus an equal volume of sea water	(4) 'S.L. water' plus an equal volume of culture medium
Settlements after 2 days in free-choice dish				
Metd	None	None	1	None
Meting	None	None	1	None
Unmet.	Very few	Very few	Few	Few
Settlements after 2 days in conical vessels				
Metd	1 or 2	None	Several	Few
Meting	Very few	Very few	Fair number	Good number
Unmet.	Good number	Good number	Several	Several
Settlements after 3 further days in free-choice dish				
Metd	3	5	24	29
Meting	3	3	6	14
Unmet.	5	5	5	12

TABLE IV

Acid-cleaned Bullhill Bank sand soaked in

Tests beginning	Days of soaking	(1) Sea water	(2) Sea water with fresh Bullhill Bank sand	(3) Sea water with fresh Salthouse Lake sand	(4) Distilled water
Settlements after 2 days in free-choice dish (except for tests beginning 25. vii. 53 which were after 3 days)					
17. vi. 53	5	Metd Meting Unmet.	1 or 2 1 or 2 Several	None 1 or 2 Several	None 2 or 3 Several
8. vii. 53	26	Metd Meting Unmet.	1 or 2 Few Several	— — —	— — —
25. vii. 53	43	Metd Meting Unmet.	Fair number Very few Very few	Good number Several Fair number	Many Many Fair number
2. viii. 53	51	Metd Meting Unmet.	— — —	10 7 5	47 16 8
					Very few Very few Very few None None Very few

TABLE V

Tests beginning	Days of soaking		Acid-cleaned Bullhill Bank sand soaked in				
			(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) Surrounded by fresh Bullhill Bank surface sand, in sea water plus culture medium as (2)	(4) Surrounded by fresh Bullhill Bank deep sand in sea water plus culture medium as (2)	(5) Surrounded by fresh Salthouse Lake surface sand, in sea water plus culture medium as (2)
Settlements after 2 days in free-choice dish							
22. vii. 53	9	Metd Meting Unmet.	1 or 2 Few Several	Few Several Several	Several Several Several	Very few Few Fair number	Very few Several Several
2. viii. 53	19	Metd Meting Unmet.	Few Very few Very few	1 or 2 Very few Few	Several Very few Very few	Very few Few Several	Few Very few Few

TABLE VI

Tests beginning	Days of soaking		Acid-cleaned Bullhill Bank sand soaked in	
			(1) Sea water	(2) Distilled water
Settlements after 2 days in free-choice dish (except for tests beginning 25. vii. 53 which were after 3 days)				
20. vi. 53	5	Metd Meting Unmet.	None 2 or 3 Fair number	— — —
23. vi. 53	8	Metd Meting Unmet.	None 1 or 2 Several	— — —
22. vii. 53	37	Metd Meting Unmet.	Many Many Several	Few Several Fair number
25. vii. 53	40	Metd Meting Unmet.	Good number Few Several	Very few Very few Very few

TABLE VII

	Acid-cleaned Bullhill Band sand 22 days in		
	(1) Sea water	(2) Culture medium	(3) Culture medium containing diatoms and flagellates
Settlements obtained with 6-day-old larvae after 2 days in free-choice dish			
Metd	22	16	(31)
Meting	35	21	(44)
Unmet.	76	45	(65)
			All × 2 at least



TABLE VIII

Acid-cleaned Bullhill Bank sand 15 days in

	(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) Medium as (2) containing diatoms	(4) Medium as (2) containing flagellates	(5) Medium as (2) containing diatoms and flagellates
	Settlements obtained with 5-day-old larvae after 3 days in free-choice dish				
Metd	None	None	None	None	None
Meting	Very few	Very few	Very few	Fair number	Fair number
Unmet.	Few	Few	Few	Many	Many

TABLE IX

Acid-cleaned Bullhill Bank sand 5 days in

	(1) Sea water		(2) Sea water with larvae (unstained) 6 days old at the beginning of the period	
	Stained larvae	Unstained larvae	Stained larvae	Unstained larvae
	Settlements after 3 days in free-choice dish			
Metd	23	3	28	114
Meting	2	None	2	1
Unmet.	6	None	10	1

TABLE X

Acid-cleaned Bullhill Bank sand

	(1) Stored in distilled water		(2) In sea water for 3 days with larvae (unstained) 5 days old at the beginning of the period	
	Stained larvae	Unstained larvae	Stained larvae	Unstained larvae
	Settlements after 3 days in free-choice dish			
Metd	None	Very few	None	Fair number
Meting	Very few	Several	Very few	Several
Unmet.	Several	Few	Several	Few

TABLE XI

	Acid-cleaned Bullhill Bank sand	
	(1) From storage in distilled water	(2) Sand as (1) mixed with charcoal made from sucrose
Settlements obtained with 5-day-old larvae after 2 days		
A 1 in free-choice dish		
Metd	None	Fair number
Meting	2 or 3	Fair number
Unmet.	Several	Fair number
A 2 in conical vessels		
Metd	1 or 2	Fair number
Meting	Several	Fair number
Unmet.	Fair number	Fair number
B in free-choice dish		
Metd	1	12
Meting	4	14
Unmet.	13	63

A 1 and A 2 ran concurrently and used larvae from the same fertilization. B was at a later date with larvae from a different fertilization.

## PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING THE YEARS 1950 TO 1953

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(Text-figs. 1-3)

The analyses reported here refer to water from International Hydrographic Station E1 (lat.  $50^{\circ} 02' N.$ , long.  $4^{\circ} 22' W.$ ). They extend the sequence begun in 1923 when sufficiently sensitive chemical methods were first applied at this station (Atkins, 1923*a*), and reported thereafter in a series of papers from this Laboratory (Atkins, 1923*a, b*, 1924, 1926*a, b*, 1928, 1930, 1953; Cooper, 1933*a, b*, 1937, 1938; Harvey, 1948, 1950; Armstrong, 1951; Armstrong & Harvey, 1950). This is a factual report and interpretation and comparison with earlier years are deferred.

Since early in 1948 analyses have been made at roughly monthly intervals by the methods listed below, for phosphate and 'total' phosphorus, and, since early in 1950, for silicate also.

I am obliged to Lt.-Cdr. C. A. Hoodless and the crew of R.V. *Sabella*, and to Capt. W. Creese and the crew of M.F.V. *Sula* for assistance at sea. I am also indebted to Dr L. H. N. Cooper for sometimes taking samples at sea, and to him and to Dr H. W. Harvey for their encouraging interest in this work.

### METHODS

Samples were taken at approximately monthly intervals, as nearly as the weather allowed. A Nansen-Pettersson insulated water-bottle was used, except occasionally in rough weather, when a wooden bucket was used for taking surface samples. Samples for silicate determinations were kept in polyethylene bottles. Analyses were completed within 48 h of sampling.

Phosphate and 'total phosphorus' were determined by the absorptiometric methods of Harvey (Harvey, 1948; Armstrong, 1949). The results are inherently free of 'salt-error', and are given in microgram-atoms of phosphorus per litre. The factor for conversion to milligrams P per cubic metre is 30.98, and for milligrams  $P_2O_5$  per cubic metre 70.98. Phosphate results include arsenate, 1  $\mu g$  atom of As as arsenate being determined as 1  $\mu g$  atom of P as phosphate. Arsenic in sea water at Station E1 *inter alia* has recently been determined by Smales & Pate (1952), by a neutron-irradiation method. 0.024-0.047  $\mu g$  atom As/l. were found, in confirmation

of figures from 0.013 to 0.041 got by an indirect chemical method by Armstrong & Harvey (1950). The fraction of the arsenic present as arsenate in these water samples is not known, so that the correction to be applied to the phosphate figures is uncertain. Some analyses by Gorgy, Rakestraw & Fox (1948) of Pacific water samples of arsenic contents from 0.43 to 0.53  $\mu\text{g}$  atom As/l. showed that about 10% of the arsenic was present as arsenate.

Arsenic is not included in the 'total' phosphorus figures. The validity of the 'total' phosphorus method has been tested by comparison with the perchloric acid digestion procedure of Hansen & Robinson (1953) which may reasonably be assumed to convert all organic phosphorus to phosphate. Analyses were done in sextuplicate on two samples of filtered sea water, by each of the two methods. The figures got by the autoclave method were 94 and 98% of those by the perchloric acid digestion. Dr B. H. Ketchum has stated (private communication) that he and his associates have also found satisfactory agreement between figures obtained by the autoclave method and by the sulphuric acid-peroxide digestion method of Redfield, Smith & Ketchum (1937).

Estimates of organic phosphorus may be somewhat low if they are computed from these figures by subtracting phosphate (which includes arsenate) from the corresponding 'total' phosphorus figures.

Silicate was determined by the molybdenum-blue method described previously (Armstrong, 1951). The results, which are again inherently not subject to 'salt-error', are given in microgram-atoms of silicon per litre. The factor for conversion to milligrams  $\text{SiO}_2$  per cubic metre is 60.06.

The salinities quoted were determined at the Government Laboratory.

It is hoped that the tabulated figures will appear elsewhere in due course. The presentation here is mainly graphical, and is arranged to show the vertical distribution of temperature, phosphate, and silicate for each year. In addition, the variations of the integral mean concentrations in the water column of phosphate, 'total phosphorus' and silicate are tabulated.

#### OBSERVATIONS

##### *Temperature 1950-53*

Temperature diagrams are included for comparison with those for phosphate and silicate (Fig. 1).

##### *Phosphate 1950-53 (Fig. 2)*

1950. The water column was homogeneous from January until the end of April, the decrease having been rapid during the latter month. Vertical discontinuity was thereafter apparent until December, though in October and November the decrease in the upper layers was confined to the top 5 m. In July and August there was a mass of water of phosphate-content greater than 0.3  $\mu\text{g}$  atom P/l., below 20 m.

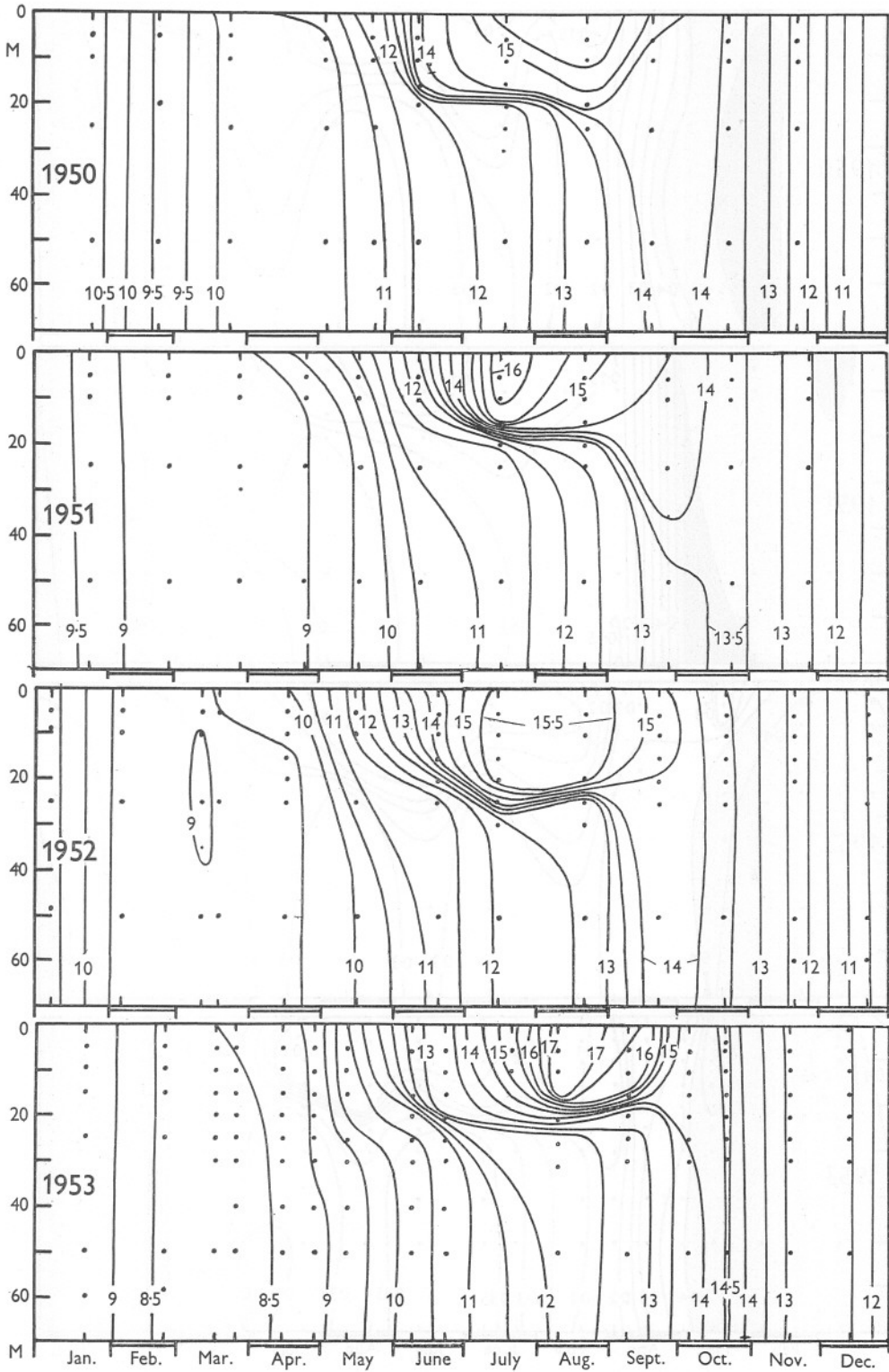


Fig. 1. Vertical temperature distribution at International Hydrographic Station E I, 1950-53. Contour lines at 0.5° C intervals.

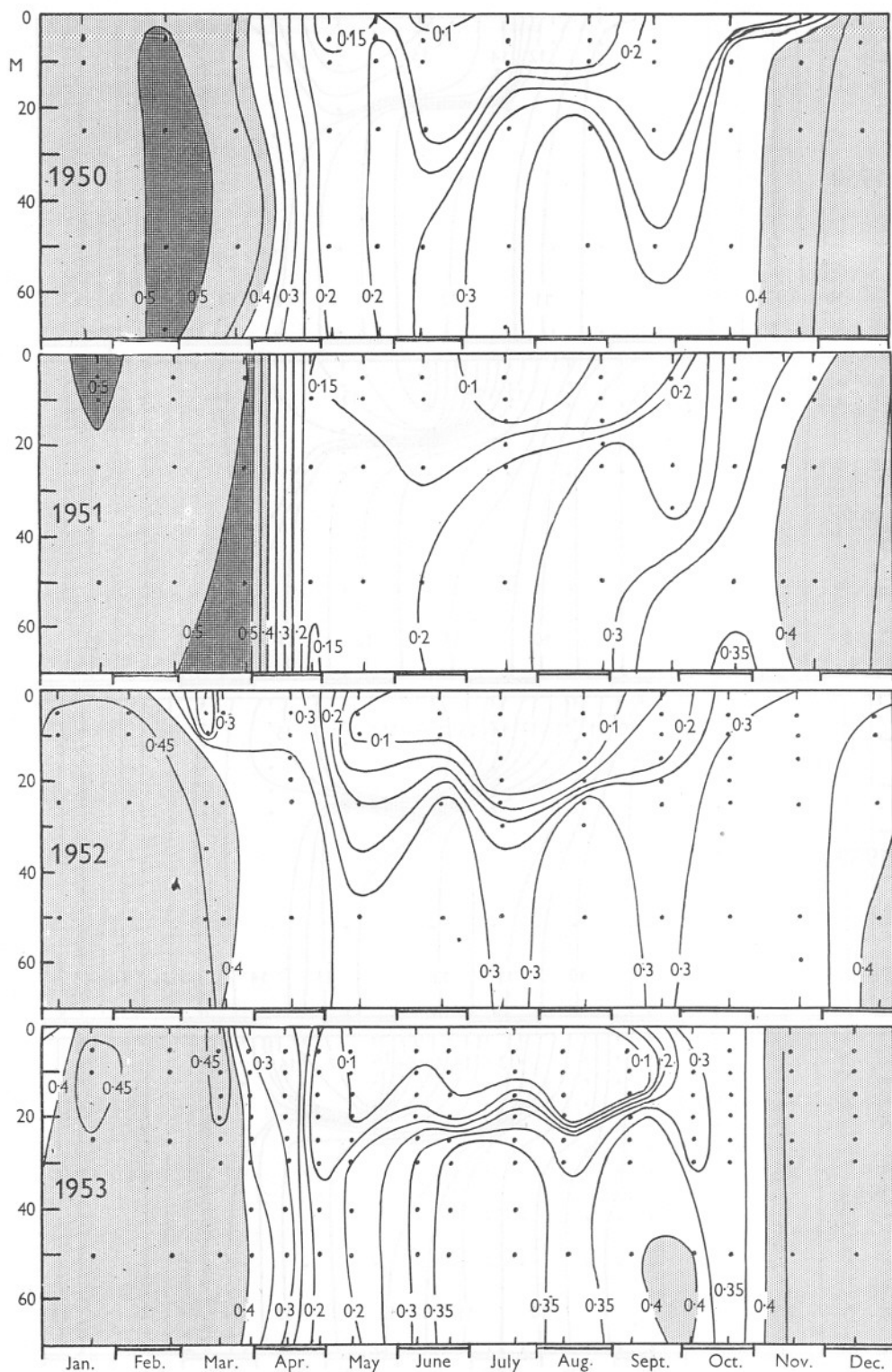


Fig. 2. Vertical distribution of phosphate, as  $\mu\text{g atom P/l.}$ , at International Hydrographic Station E1, 1950-53. Contour lines at  $0.05 \mu\text{g atom P/l.}$  intervals.



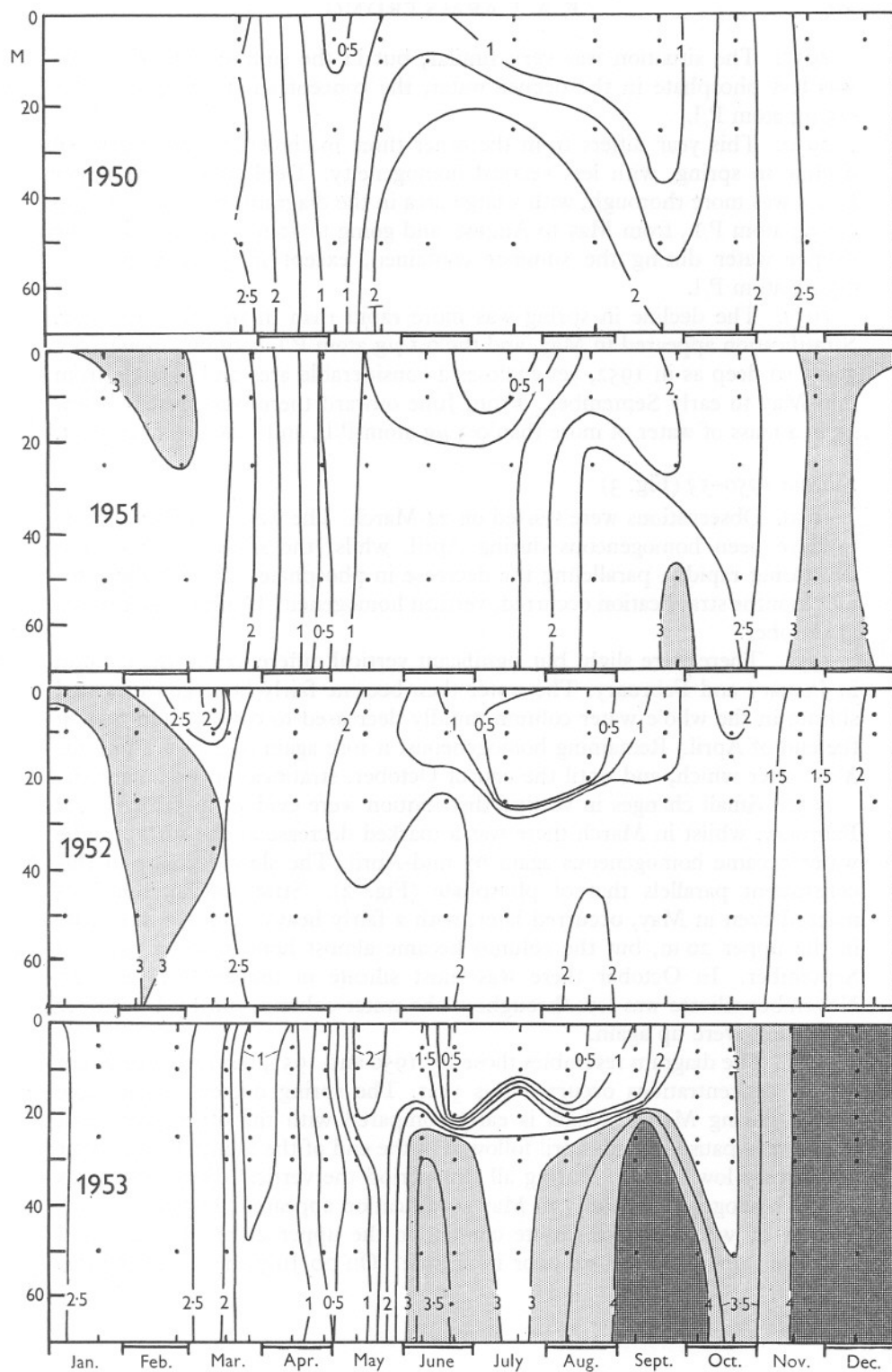


Fig. 3. Vertical distribution of silicate, as  $\mu\text{g atom Si/l}$ , at International Hydrographic Station E 1, 1950-53. Contour lines at  $0.5 \mu\text{g atom Si/l}$  intervals.

1951. The situation was very similar, but in the summer months there was less phosphate in the deeper water, the concentration being less than  $0.3 \mu\text{g}$  atom P/l.

1952. This year differs from the other three in showing a more gradual decline in spring, with less vertical homogeneity. Depletion of the upper layers was more thorough, with a large area in the diagram showing less than  $0.1 \mu\text{g}$  atom P/l., from May to August, and going to 20 m deep in July. The deeper water during the summer contained, except in July, more than  $0.3 \mu\text{g}$  atom P/l.

1953. The decline in spring was more rapid than in the previous year. Stratification appeared in May, and the  $0.1 \mu\text{g}$  atom P/l. contour, though not going so deep as in 1952, yet encloses a considerable area and extends from mid-May to early September. From June onward there was present below 25 m a mass of water of more than  $0.3 \mu\text{g}$  atom P/l., and much of nearly 0.4.

#### *Silicate 1950-53 (Fig. 3)*

1950. Observations were started on 24 March. The water column appears to have been homogeneous during April, whilst the silicate content was decreasing rapidly, paralleling the decrease in phosphate. During the summer months stratification occurred, vertical homogeneity being re-established in October.

1951. There were slight but significant vertical differences in the column in January and February. The water then became fairly homogeneous, and silicate in the whole water column rapidly decreased to  $0.5 \mu\text{g}$  atom Si/l. at the end of April. Remaining homogeneous, it rose again to over 1.0 in mid-May, after which, and until the end of October, stratification was marked.

1952. Small changes in vertical distribution were evident in January and February, whilst in March there was a marked decrease at the surface. The water became homogeneous again by mid-April. The slow decrease of this constituent parallels that of phosphate (Fig. 2). Stratification, not very marked even in May, occurred later, with a fairly heavy decrease in silicate in the upper 20 m, but the column became almost homogeneous again in September. In October there was most silicate in the *upper* layers. In November silicate was low throughout the water column, but by December the figures were up again.

1953. The diagram resembles those for 1950 and 1951, but rather different silicate concentrations occurred this year. The spring decrease took place mainly during March, which is early compared with the other years, but there was a pause in mid-April followed at the end of the month by a further fall to very low values. During all this period the vertical distribution was almost homogeneous. Early in May stratification appeared; however, it was marked by water of *high* silicate content in the upper 20 m. In June and after the upper layers were poor in silicate. On 20 July the lowest figures

recorded here so far occurred in the top 10 m. At 0.5 and 5 and 10 m silicate concentrations were below the minimum perceptible with certainty by the method, i.e. were less than 0.05  $\mu\text{g}$  atom Si/l.

In June, and from August onward notably high figures were observed in the deeper water.

#### *Changes in the Integral Mean Concentrations in the Water Column*

For each date on which analyses were made, the integral mean concentrations in the water column have been computed, using the formula

$$\frac{1}{2d_n} \{(a_1 + a_2)(d_2 - d_1) + (a_2 + a_3)(d_3 - d_2) + \dots + (a_{n-1} + a_n)(d_n - d_{n-1})\},$$

where  $a_1, \dots, a_n$  are observations at depths  $d_1, \dots, d_n$ . The results for phosphate, total phosphorus and silicate are given in Table I.

#### *Phosphate*

The seasonal variation is well shown; sharp declines in spring occurred in April, except in 1952 when, as noted above, the fall was less abrupt. The recoveries in summer and autumn show considerable irregularity, though that of 1951 is smooth. The difference between the maximum and minimum values for each of the four years in order were 0.34, 0.35, 0.27 and 0.32  $\mu\text{g}$  atom P/l. respectively.

#### *Total Phosphorus*

A seasonal variation is evident, although, as shown earlier (Armstrong & Harvey, 1950) it is less marked than that of phosphate. The differences between the maximum and minimum values for each of the four years in order were 0.27, 0.21, 0.27 and 0.15  $\mu\text{g}$  atom P/l. It is not clear where this considerable quantity of phosphorus has gone. Were it used in the growth of plant or animal matter, this would amount to some 1-3  $\text{g}/\text{m}^3$ , an amount so far unaccountable in estimates of annual production (Harvey, 1950).

#### *Silicate*

The fluctuations in this constituent were considerable, and the only general observation which may safely be made is that a decrease occurs in spring. Even so this decrease was not so steep nor rapid in 1952 as in the other years. There is a rough correspondence between changes in phosphate and silicate. It has been pointed out (Atkins, 1953) that the relative decreases in phosphate and silicate may give an idea whether diatoms or non-siliceous algae have been mainly responsible for a plankton outburst.

The most interesting of these figures were for 20 November 1952, and for 8 June and 8 September 1953 onward. Between 21 October and 20 November 1952 the mean value in the water column fell from 1.93 to 1.24  $\mu\text{g}$  atom Si/l.,

TABLE I. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN  
AT STATION E I

Date	Phosphate-P ( $\mu\text{g}$ atom P/l.)	'Total-P' ( $\mu\text{g}$ atom P/l.)	Silicate ( $\mu\text{g}$ atom Si/l.)
18. i. 50	0.46	0.57	—
22. ii. 50	0.51	0.63	—
24. iii. 50	0.46	0.54	2.53
3. v. 50	0.17	0.52	0.64
25. v. 50	0.20	0.36	1.91
12. vi. 50	0.20	—	1.60
19. vii. 50	0.28	0.42	1.88
23. viii. 50	0.32	0.48	1.68
20. ix. 50	0.28	0.46	1.07
23. x. 50	0.36	0.46	1.77
22. xi. 50	0.43	0.58	2.66
18. xii. 50	0.47	0.56	2.60
24. i. 51	0.49	0.58	2.78
26. ii. 51	0.49	0.59	2.92
28. iii. 51	0.51	0.62	1.94
25. iv. 51	0.16	0.45	0.43
17. v. 51	0.17	0.44	1.19
12. vi. 51	0.17	0.41	1.07
17. vii. 51	0.19	0.42	1.13
22. viii. 51	0.23	0.49	2.40
27. ix. 51	0.28	0.51	2.50
24. x. 51	0.33	0.46	2.39
15. xi. 51	0.40	—	—
27. xi. 51	0.40	0.53	3.19
7. i. 52	0.46	0.56	2.79
5. ii. 52	0.46	0.57	3.02
11. iii. 52	0.39	0.58	2.73
17. iii. 52	0.40	0.57	2.55
16. iv. 52	0.37	0.50	2.20
15. v. 52	0.23	0.48	2.01
19. vi. 52	0.28	0.43	1.88
15. vii. 52	0.19	0.31	1.07
20. viii. 52	0.26	0.35	1.41
22. ix. 52	0.25	0.39	1.52
21. x. 52	0.33	0.40	1.93
20. xi. 52	0.33	0.46	1.24
22. xii. 52	0.40	0.52	2.29
21. i. 53	0.45	0.56	2.69
23. ii. 53	0.43	0.56	2.72
16. iii. 53	0.44	0.53	1.96
25. iii. 53	0.36	0.53	0.86
13. iv. 53	0.30	0.51	1.13
27. iv. 53	0.15	0.41	0.26
11. v. 53	0.16	0.48	1.22
8. vi. 53	0.25	0.49	3.02
22. vi. 53	0.29	0.51	2.31
20. vii. 53	0.31	0.52	2.08
10. viii. 53	0.23	0.41	2.36
8. ix. 53	0.32	0.42	3.41
5. x. 53	0.34	0.49	3.50
20. x. 53	0.32	0.42	3.06
17. ix. 53	0.47	0.52	4.05
14. xii. 53	0.47	0.54	3.90

with no corresponding change in phosphate. This fall in silicate cannot readily be ascribed to a late autumn outburst of diatoms because at the same time the total amount of chlorophyll in the water column decreased (Atkins, Jenkins & Warren, 1954).

On 8 June and on and after 8 September 1953, silicate figures were notably higher than at the beginning of the year. These high integral mean concentrations do not of course include any silicate sequestered by diatoms, yet silicate in solution has increased in the column as a whole.

The salinity determinations give little support to the idea that these results are caused by changes in the body of water sampled at E1. It is, however, possible that enrichment (not the impoverishment noted in November 1952) could be caused by solution of siliceous matter suspended in the water. Proximate analyses of this matter have shown (Armstrong & Atkins, 1950) that at the surface it may account for 1.3–14.7  $\mu\text{g}$  atom Si/l., thus solution of a fraction only of this silica would account for the increases found. It has indeed been shown (Armstrong, 1950; Atkins, unpublished) that the soluble silicate content of sea water increases on standing (in non-siliceous bottles).

During 1953, such a process of solution would need to have been rapid to cause the changes seen between 11 May and 8 June and between 10 August and 8 September. It is odd too that this is the only year in which the effect is seen.

#### *Changes in Salinity*

Small changes in salinity occur frequently in the records for these four years, and do not necessarily show that different water masses have been sampled at different times. Since the water at E1 is only some 70 m deep, measurable changes in salinity throughout the water column may be caused by heavy rainfall.

At the end of 1952 and in the latter half of 1953, the integral mean salinities were as shown in Table II.

TABLE II. SALINITIES AT E1 IN 1952 AND 1953

Date	Integral mean salinity (%)	Number of observations for computation
21. x. 52	35.22 <sub>3</sub>	8
20. xi. 52	35.14 <sub>8</sub>	8
22. xii. 52	35.17 <sub>6</sub>	7
11. v. 53	35.15 <sub>9</sub>	10
8. vi. 53	35.16 <sub>5</sub>	10
22. vi. 53	35.14 <sub>2</sub>	10
20. vii. 53	35.12 <sub>8</sub>	10
10. viii. 53	35.12 <sub>1</sub>	9
8. ix. 53	35.16 <sub>1</sub>	9
5. x. 53	35.20 <sub>6</sub>	9
20. x. 53	35.21 <sub>0</sub>	9
17. xi. 53	35.21 <sub>0</sub>	9
14. xii. 53	35.26 <sub>1</sub>	9

It is probably justifiable to assume that the water mass sampled in November 1952 was different from that sampled in October. Evidence from salinity measurements in 1953 is inconclusive, but the increase in salinity from October onward was accompanied by a change in silicate content to values greater than at the previous winter maximum.

Attention has been given to the possibility of change in the water body at E1, because changes of water masses, possibly with different stocks of nutrient salts, may invalidate arguments based on the apparent utilization of these nutrients.

On 11 March 1952 (the year which was unusual in showing a slower decrease in phosphate and silicate in spring) there were plainly two water masses, of different densities, one above the other at the Station. This is shown by the figures of Table III.

TABLE III. SALINITY AND DENSITY AT E1 ON 11 MARCH 1952

Depth (m)	Temperature (°C)	Salinity (‰)	Density <i>in situ</i> ( $\sigma_t$ )
0.0	9.1	35.13	27.22
5	9.04	35.09	27.20
10	9.00	35.09	27.20
25	8.95	35.21	27.20
35	8.98	35.27	27.35
50	9.11	35.26	27.33
68	9.11	35.28	27.34

TABLE IV. ALTERNATE SILICATE OBSERVATIONS, E1, 1953

Date	$\mu\text{g atom Si/l.}$	Date	$\mu\text{g atom Si/l.}$
21. i. 53	2.69	21. i. 53	2.69
23. ii. 53	2.72	23. ii. 53	2.72
25. iii. 53	0.86	16. iii. 53	1.96
27. iv. 53	0.26	13. iv. 53	1.13
11. v. 53	1.22	11. v. 53	1.22
22. vi. 53	2.31	8. vi. 53	3.02
20. vii. 53	2.08	20. vii. 53	2.08
10. viii. 53	2.36	10. viii. 53	2.36
8. ix. 53	3.41	8. ix. 53	3.41
5. x. 53	3.50	20. x. 53	3.06
17. xi. 53	4.05	17. xi. 53	4.05
14. xii. 53	3.90	14. xii. 53	3.90

It is instructive also to consider an aspect of the silicate figures for 1953. Analyses were done more frequently than usual since samples were taken when the ships were at the Station for other purposes. It is possible to choose alternate dates at roughly monthly intervals, to make two series of observations (Table IV). Each series gives twelve observations in 12 months, but the conclusions drawn from the two series might be very different. Phosphate and 'total phosphorus' do not show such marked differences.

The desirability of making frequent and complete observations is obvious, even in so simple a task as logging the changes at a single station.



## SUMMARY

Samples taken at the International Hydrographical Station E I during the last four years have been analysed by recently developed methods.

The results for phosphate, 'total phosphorus', and silicate are presented graphically, showing the seasonal changes in vertical structure and integral mean concentration in the water column.

The figures are discussed, particular note being made of unusual silicate values in 1952 and 1953.

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## HATCHING IN *CALANUS FINMARCHICUS* AND SOME OTHER COPEPODS

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(Plate I and Text-figs. 1-5)

During observations on the development of the eggs of *Calanus finmarchicus* the process of hatching was watched on many occasions. Since it differs from that described by Ziegelmayr (1926) for cyclopids it seemed worth while examining the process in other groups of copepods as well.

Hatching can be followed very easily in *Calanus* (Pl. I). For some time before, the form of the nauplius can be clearly seen with its limbs folded close to the body (A); in many a red pigment spot develops at this time in the otherwise transparent egg. Shortly before hatching the limbs begin to twitch occasionally and a space is just visible round the body of the nauplius. At this stage the inner and the outer egg membranes cannot be distinguished, but when hatching begins, the outer membrane must crack for the delicate inner membrane bulges out (B). The bulge enlarges rapidly (C), and the nauplius extends into the bulge, sometimes leaving a clear space between itself and the membranes (D). Usually it is either the head or the tail end which pushes into the bulge, but occasionally it is a limb. Probably it is abnormal for a limb to extrude first since such eggs do not always hatch successfully. As the inner membrane expands, the outer slips back and the inner, enclosing the nauplius, pops out (E). This emergence often occurs suddenly, but sometimes it is gradual (as in E), when the outer membrane crumples up on the inner, regaining its original shape only when the inner has slipped out and the tension is released (F). The inner membrane now forms a perfect sphere of much greater diameter than the original egg.

During the first part of the hatching process the nauplius continues to twitch at irregular intervals. After it has emerged in its inner membrane, it has a considerable space round it, and it then begins to stretch out its limbs and become more active (G). Quite suddenly it tears the membrane and swims away (H). The diaphanous remains of the inner membrane are hardly visible. The whole process takes about 10-15 min at room temperature (15° C), but the time spent in each stage is variable. Sometimes longer is spent in hatching from the outer membrane and sometimes longer from the inner.

Since the diameter of the egg is 145  $\mu$  its volume is about 0.0016 mm.<sup>3</sup>. Two eggs, in which the inner membrane was measured immediately after slipping

out, had diameters of 191 and 209  $\mu$ , the volumes being now more than double, 0.0037 and 0.0048 mm.<sup>3</sup> respectively. The diameter increased and was measured again just before the nauplius escaped, when it was for both eggs 244  $\mu$ , giving a volume of 0.0076 mm.<sup>3</sup> or nearly five times the original. The surface area had therefore increased 2.8 times. One nauplius was measured a few minutes after hatching and was 174  $\mu$  long, i.e. larger than the original diameter of the egg.

Osmotic hatching has been suggested in a number of Crustacea (see Yonge, 1937), and we therefore examined the possibility in *Calanus* eggs. Twenty-nine eggs near hatching were used for the experiment; fourteen were kept in normal sea water as controls and fifteen in sea water to which a small quantity of fresh water was added. In the controls three bulged and hatched successfully; in the fifteen which were treated experimentally, eleven bulged although only seven of these hatched successfully. It is obvious, therefore, that osmosis may be an important factor in hatching. If fresh water is added some time before the nauplii are ready to hatch, the egg ruptures, extruding a mass of tissue, but the inner membrane does not appear at all.

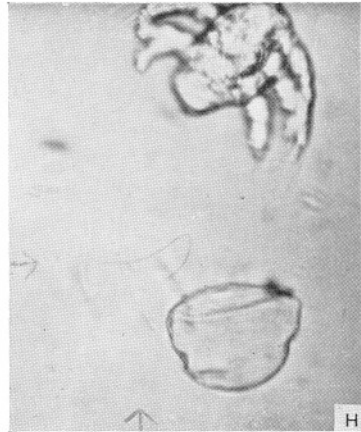
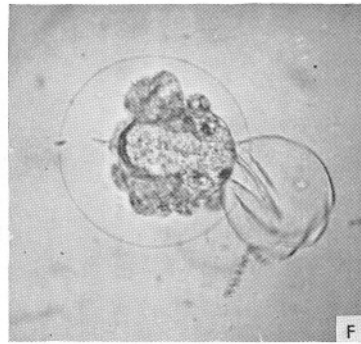
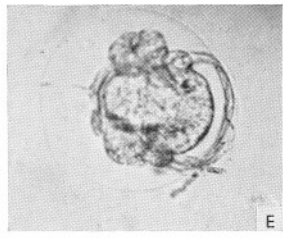
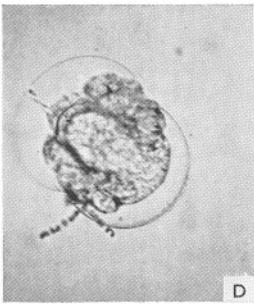
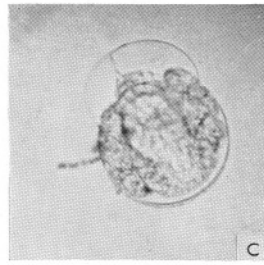
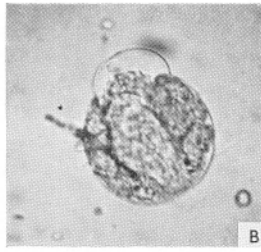
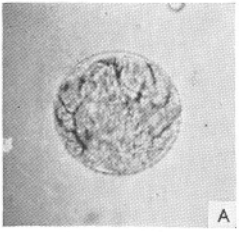
*C. helgolandicus* eggs hatch in exactly the same way. In another very similar form of *Calanus*, found at Tromsø (Marshall, Orr & Rees, 1953), eggs are laid which produce, shortly before the first division (possibly on fertilization), an extra, thin, irregular, membrane separated by a varying space from the original egg. Hatching of these takes place in the same way as in other *Calanus*.

The eggs of *Metridia longa* and *Acartia clausi* also hatch in this way. In *Acartia* the outer membrane seems to be relatively more rigid than in *Calanus*. It does not crumple, but splits and slips off by gaping wider. The inner membrane also seems relatively thicker since it is quite clearly visible after the nauplius has left it. Shortly before hatching a rosy tinge becomes visible over about half the egg, and in the hatched nauplius the tips of the antennules are pink. The eggs measure 77–78  $\mu$  before hatching begins, and in one the inner membrane just before the nauplius escaped had swelled to 129  $\mu$  thus increasing the volume four and a half times.

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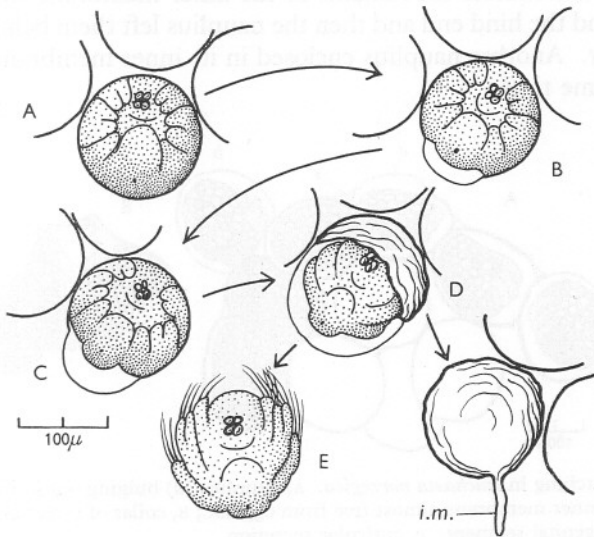
#### EXPLANATION OF PLATE I

- A. Egg of *Calanus finmarchicus* shortly before hatching showing the nauplius form.
- B. The inner membrane begins to bulge. The outer membrane has a diatom chain attached which can be seen throughout the rest of the series.
- C. The bulge of the inner membrane is growing rapidly.
- D. The nauplius inside the inner membrane is emerging from the outer.
- E. The outer membrane has crumpled up and the diameter of the inner membrane much exceeds that of the original egg.
- F. The outer membrane has sprung off and has partly regained its spherical form. There is a large space between the nauplius and the inner membrane.
- G. The nauplius stretches its limbs.
- H. The nauplius is swimming away and the remains of the inner membrane can be faintly seen, indicated by the arrows.



All these copepods liberate their eggs directly into the sea. Others, however, carry them in sacs attached to the genital segment, and several of these species were observed to see how hatching takes place.

The female of *Pseudocalanus minutus* carries a mass of eggs varying in number according to the season from about 10 to 28 (Marshall, 1949). As the eggs develop they get darker in colour, and in some a red pigment spot is seen. The process of hatching is much the same as that already described; the outer membrane cracks, the inner membrane bulges out and eventually the nauplius comes out (Text-fig. 1). Usually, however, the inner membrane does not come completely free from the outer before the nauplius struggles and breaks it.



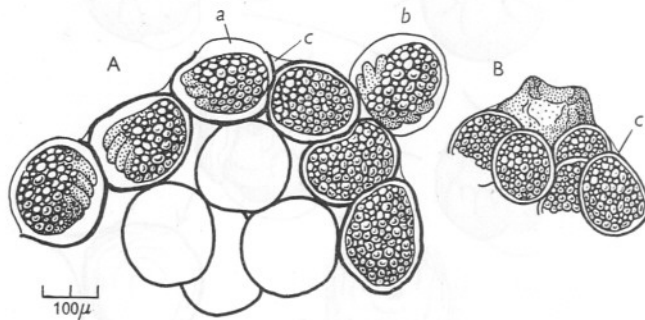
Text-fig. 1. Hatching in *Pseudocalanus minutus*. A, egg shortly before hatching; B, inner membrane beginning to bulge out; C, a later stage; D, outer egg-membrane crumples up and nauplius emerges in inner membrane; E, nauplius escapes and inner egg membrane (*i.m.*) is left attached to outer.

The torn inner membrane usually remains attached to the outer. Once the inner membrane enclosing the nauplius came right out and floated away. It swelled until there was a space round the nauplius but this egg failed to hatch. The eggs before hatching measured between 120 and 140  $\mu$  and the inner membrane increased up to 160–170  $\mu$  in diameter, an increase of a little more than twice the volume. The swelling is therefore not so great as in *Calanus*. Although the egg-mass often looks as if it were contained in an actual sac, when the nauplii have hatched out it seems to be composed only of individual outer membranes stuck together.

*Euchaeta norvegica* is a large copepod living in deep water and it does not survive well under laboratory conditions. The eggs when first laid are deep



blue in colour, and according to Nicholls (1934) a normal egg-sac contains about 50 eggs. Development is slow, and the interval between laying and hatching is at least 8 days compared with the 24 h normal for *Calanus*. It was therefore not unexpected that the process of hatching should be prolonged. As development goes on the eggs change in colour from dark blue to reddish brown, and the body of the nauplius is seen to be full of fat globules. As usual the outer membrane eventually cracks and the inner membrane bulges out (Text-fig. 2A). Nauplii may remain in this condition for many hours. On one occasion a nauplius had broken its inner membrane and was partly hanging out, twitching actively, when a sudden jerk on the part of the mother threw it right out. For a minute the remains of the inner membrane were to be seen clinging round the hind end and then the nauplius left them behind and swam actively away. Another nauplius enclosed in its inner membrane was thrown out at the same time.

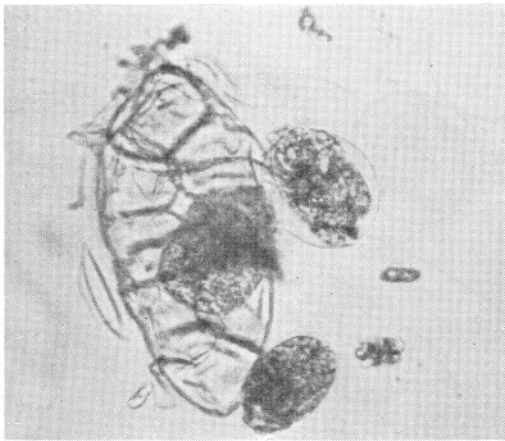


Text-fig. 2. Hatching in *Euchaeta norvegica*. A, nauplius (a) bulging out in inner membrane; and (b) in inner membrane almost free from egg-sac; B, collar of secretion which attaches egg-sac to genital segment. c, cuticular secretion.

One female in which the eggs were hatching was kept overnight. In the morning there were about twenty-two nauplii in the surrounding water, most free and active but several enclosed in their inner membranes. They remained like this for some time and actual hatching was not seen. In detaching an egg-sac from a dead female a number of nauplii, some in the inner membrane and some free, were shaken out, and it seems likely that the movements of the mother play an important part in helping the nauplii to hatch. The egg-sac, which is flattened in form, is attached to the genital segment by a projecting collar of secretion (Text-fig. 2B).

In the cyclopids the eggs are carried in paired sacs attached to the genital segment. In *Oithona similis* the egg is oval, the diameter varies from 70 to 95  $\mu$ , and each sac contains from five to eighteen eggs according to the season. The process of hatching is not so easily followed as in a larger egg, and there are several differences from *Calanus*. When the outer membrane cracks the inner

membrane cannot be seen bulging out. It envelops the nauplius much more closely than in *Calanus*, and the only sign of the outer membrane rupturing is a sudden stretch on the part of the nauplius up to  $105\ \mu$  or more. After a short time the nauplius slides forwards and pushes well out of the outer membrane, sometimes twisting round as it does so. A female has been seen swimming about with the nauplii hatching and hanging out in all directions round the egg-sac. The inner membrane cannot always be made out at this stage, but is seen most clearly when the nauplius is viewed from the side. After quite a long pause, during which the nauplius twitches frequently, it stretches out its limbs (which up till now have been close to the body) and struggles to get its spines loose. When it finally breaks free and swims off, the inner membrane is usually left attached to the outer.



Text-fig. 3. Photograph of the egg-sac of *Cyclops agilis*. Most eggs have hatched, leaving the inner membranes hanging on to the outer. A nauplius still in its inner membrane is seen on the right.

In the freshwater copepod *Cyclops agilis* the eggs measure  $90\text{--}110\ \mu$  in diameter. The nauplius was not seen to twitch before hatching. When the outer membrane cracks the inner membrane bulges out for an instant and then almost immediately slips right out, although still remaining attached to the outer. It fits the enclosed nauplius fairly closely; it is not spherical and can be seen most clearly when the nauplius is viewed from the side, for although touching the head and tail it is well separated dorsally and ventrally (Text-fig. 3). Very little swelling takes place, the maximum diameter being only  $120\ \mu$  and the volume is thus only doubled. After a few minutes, during which it twitches occasionally, the nauplius gives a violent movement, breaks out and swims away, almost always leaving the inner membrane attached to the outer. The inner membranes seem to be relatively thicker than in *Calanus*

and, after all the nauplii have hatched, they can be seen hanging out all round the egg-sac.

In *Cyclops viridis*, another freshwater copepod with eggs about  $130\mu$  in diameter, hatching takes place more slowly. The inner membrane bulges out (Text-fig. 4A) and, as it swells, the outer membrane can be seen crumpling up (Text-fig. 4B), as it sometimes does in *Calanus* (Pl. I E), springing back to its original shape when the nauplius pops out in its inner membrane. This stretches to contain nearly four times the original volume and, as it does so, the nauplius increases in size so as to fill it completely (Text-fig. 4C). When the nauplius breaks out and swims away, it usually leaves the inner membrane attached to the outer (Text-fig. 4D), but sometimes leaves it free in the water. All the eggs in one egg-sac hatch at practically the same time, and the violent movements of the hatching nauplii jerk the detached egg-sac about.

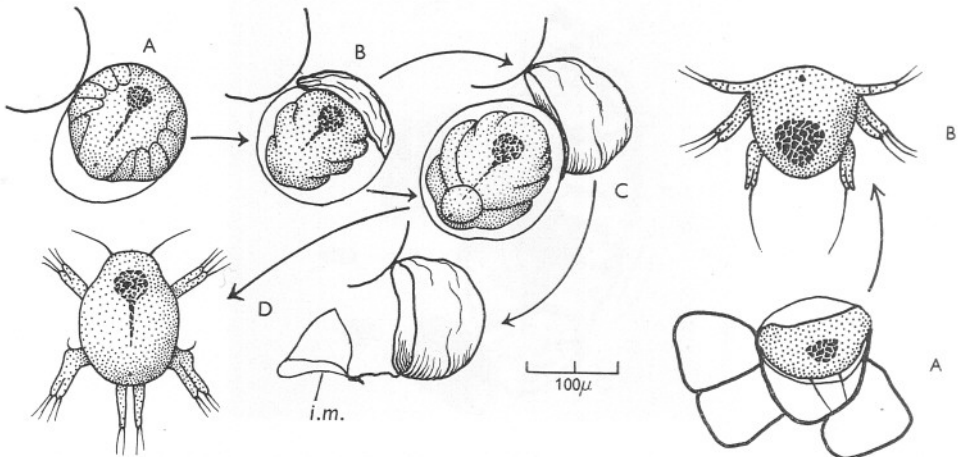


Fig. 4.

Text-fig. 4. Hatching in *Cyclops viridis*. A-C, stages in hatching; D, nauplius and egg-shell with inner membrane (*i.m.*) still attached.

Fig. 5.

Text-fig. 5. Hatching in *Tigriopus fulvus*. A, the nauplius bulging out in its inner membrane; B, nauplius showing the long mandibular spines.

A harpacticid *Tigriopus fulvus* was also observed. There is a single egg-sac and, as in *Cyclops viridis*, the eggs all reach the hatching stage at about the same time. The nauplii twitch occasionally for about an hour before emerging. The outer membranes crack, the inner membranes bulge out, and in a short time the whole sac is covered with nauplii hanging out in their inner membranes (Text-fig. 5A). A curious feature is that the long mandibular spines seem to extend beyond the inner membrane and remain attached to the inside of the outer membrane. The inner membrane disappears suddenly, but whether it bursts or whether the nauplius increases to fill it completely is uncertain. The

nauplius is still firmly attached by the mandibular spines and violent struggles are necessary to detach them. After all the eggs have hatched a few inner membranes are seen hanging on the outer membrane, but they are not so many nor so distinct as in *Cyclops*.

In the semi-parasitic copepod *Caligus rapax*, the egg-sacs are each formed of a long row of single eggs one behind the other, and hatching takes place in the same way. The eggs hatch successively from behind forward and each bulges out in the inner membrane for a few minutes before breaking out and swimming off. According to Mr B. T. Hepper of the Burnham-on-Crouch Laboratory (personal communication) the eggs of *Mytilicola intestinalis*, a copepod parasitic in mussels, hatch in a rather similar way.

#### DISCUSSION

It is clear that in all these copepods, belonging to several different groups, hatching takes place on the same general plan. As development goes on there is often a colour change in the nauplius (*Calanus*, *Pseudocalanus*, *Euchaeta*, *Acartia*), and this is sometimes the first sign of hatching. The rigid outer egg-membrane is first burst by the pressure inside the inner membrane. This inner membrane, enclosing the nauplius, then emerges from the outer membrane, but it may do so gradually as in *Calanus* or very quickly as in *Cyclops agilis*. The increase in volume which takes place inside the inner membrane varies with the species. In *Calanus* the volume increases about fivefold before the nauplius hatches, but in eggs which are carried in egg-sacs the increase is usually much smaller and sometimes may be little more than is necessary to crack the outer shell. In some, e.g. *Calanus*, there is a large clear space round the nauplius after it has slipped out, while in others such as *Oithona* the nauplius fills the inner membrane completely; the other cyclopids are intermediate in that they seem to fill it when viewed dorsally or ventrally but not when viewed laterally. In both types the nauplius stretches or imbibes water before tearing the inner membrane, but the first does so at a later stage than the second.

In those copepods which lay eggs freely in the sea (*Calanus*, *Metridia*, *Acartia*) the inner membrane separates completely from the outer, but in those hatching from egg-sacs it more commonly remains attached. This may help the nauplius to avoid entanglement. It is not clear how the inner membrane stays attached, for it often (see Text-figs. 3 and 4) seems to come right out of the outer. *Euchaeta norvegica* is a copepod which shows both methods. Some of the nauplii in their inner membranes come out quite clear of the egg-sac before hatching, while others leave their inner membranes attached. The movements of the female in this and other copepods may have some effect in helping nauplii to escape from the egg-sac.

A puzzling feature is that in *Oithona* and *Tigriopus* the spines seem to extend through the inner membrane. This follows the outline of the nauplius body

when the latter is hanging out from the outer shell, but the spines project beyond it and seem to be attached to the inside of the outer membrane (see Text-fig. 5A). The nauplii have a considerable struggle to release the spines before they escape.

Ziegelmayr's (1926) observations on the hatching of seventeen species of cyclopids differ considerably from ours. According to him it is the outer egg-membrane which swells. This process may take 14 h or more. A pressure develops in the space thus formed between the outer and the inner membranes. The outer membrane eventually bursts and the inner, in which the nauplius is tightly enclosed, emerges. After a short time the nauplius moves its antennae, tears the inner membrane, and escapes; it then remains still for 10–20 sec, begins to move its limbs, and finally swims off.

According to our observations, however, it is the inner membrane and not the outer which expands. It is difficult to decide whether Ziegelmayr was unable to see the bulging out of the inner membrane or whether the specimens he examined behaved in a different way. The eggs in his two photographs, taken after 3 and 10 h swelling, are, when we allow for the different magnifications, very much the same in diameter, and the second resembles a nauplius released in its inner membrane.

That hatching may be an osmotic process is suggested by the few experiments on *Calanus* eggs described above, and this has been found also in cyclopid copepods by Ziegelmayr (1926), and in several other Crustacea—e.g. in *Hemimysis* by Manton (1928), and in *Daphnia* and *Simocephalus* by Przylecki and Ramult (Needham, 1931).

A possible explanation of the mechanism of hatching might be that a sudden increase of excretion by the embryo leads to an increased content of salts and the imbibition of water. It is noteworthy that in nearly all species twitching of the nauplius begins shortly before hatching. It is difficult, however, to understand how osmotic control can be effective in *Tigriopus*, which lives in shore pools and is therefore subject to considerable variations in salinity.

In the eggs of decapods (Yonge, 1937, 1946) there are two membranes, a cuticular, secreted by glands on the pleopods of the female, and a chitinous, secreted by the oviduct. According to Herrick (1895), the cuticular membrane splits at hatching and completely separates from the underlying membrane except at one point. This underlying membrane (presumably the chitinous one of Yonge) is now greatly distended and from it the young lobster hatches.

In most copepods that bear egg-sacs only two membranes are visible. *Euchaeta norvegica* is the only species we have used that is large enough to give clear results with chemical tests. In it the mass of secretion joining the egg-sac to the genital opening, together with the material connecting the eggs (Text-fig. 2B, c), has been shown to be cuticular in nature. This cuticular secretion presumably also surrounds each egg. In *Caligus* (Wilson, 1905) cement glands are present which secrete the egg-sac and also a covering round each egg.

It seems most probable that in the copepods the two membranes we have described as outer and inner correspond to the cuticular and chitinous membranes in the lobster, the chitinous one being extensible.

An alternative explanation might be, however, that in copepods the extensible inner membrane is secreted by the developing embryo, and that the chitinous and cuticular membranes are so closely adherent that they are indistinguishable except possibly in *Euchaeta*.

We are grateful to Prof. C. M. Yonge, F.R.S., for his interest in the problems raised and especially for his help in the chemical tests. We should also like to thank Dr J. P. Harding of the British Museum for identifying the species of *Cyclops*, *Tigriopus* and *Caligus*, and Dr R. B. Pike for preparing the drawings for reproduction.

#### SUMMARY

The process of hatching is described for *Calanus finmarchicus* and other copepods. In *Calanus* the outer egg membrane breaks and the nauplius emerges enclosed in a very delicate inner membrane which swells to contain a volume as much as five times that of the original egg. The nauplius escapes by tearing this membrane. Other copepods which lay their eggs freely in the sea hatch in the same way, but in copepods that carry egg-sacs the swelling in the inner membrane is less, and when the nauplius emerges the inner membrane is usually left attached to the outer.

The mechanism of the hatching process and the nature and origin of the membranes are briefly discussed.

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## THE DEVELOPMENT OF THE ASCIDIAN *PYURA MICROCOSMUS* (SAVIGNY)

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(Text-figs. 1 and 2)

Several recent papers have dealt with the development of members of the ascidian family Pyuridae (Hirai, 1941; Berrill, 1948; Millar, 1951; Sebastian, 1953), but the total number of species with known larvae is still only five, in spite of the large size of the family.

The present paper deals with the embryonic development, the larva, and the post-larval development of *Pyura microcosmus* (Savigny).

### MATERIAL AND METHOD

On 30 July 1953 specimens of *Pyura microcosmus* were collected from stones about the level of zero tides, on the north shore of Loch Creran, Argyll, on the Scottish west coast. The animals were placed in a large vacuum jar of sea water and taken on the following day to the Marine Station, Millport. Artificial fertilizations were made by mixing eggs from the oviduct of one individual with sperm from the sperm duct of another. The development obtained from three separate pairs of animals was closely similar. Shortly after fertilization the water was changed to remove excess sperm, and during the course of development two more changes of water were made. The temperature of the water varied from 18.0 to 20.0° C.

### EMBRYONIC DEVELOPMENT

The ripe ovum is spherical and about 200  $\mu$  in diameter, with pink-grey cytoplasm and a clear germinal vesicle. The ovum is surrounded by a layer of irregular outer-follicle cells, each containing a few grains of rose-purple pigment, and by scattered inner-follicle cells. The two layers of follicle cells are separated by the thin chorion which in newly extracted eggs has a diameter of about 235  $\mu$ . After a short time the perivitelline space enlarges and the diameter of the chorion consequently increases.

After fertilization the germinal vesicle disappears and a grey-brown cap is formed on one side of the ovum, with a pale centre and a surrounding ring of clear cytoplasm. Before the first cleavage the grey-brown cap becomes crescentic and it is shared between the first two cells during cleavage (Fig. 1A). This grey-brown cytoplasm corresponds to the yellow crescent of *Styela*

*partita* originally described by Conklin (1905), and the variously coloured crescent of other ascidians (Hirai, 1941; Berrill, 1948; Millar, 1951; Sebastian, 1953). Subsequent cleavage is essentially the same as that of styelid and pyurid ascidians already studied. The larva, still within the chorion, is fully developed and showing muscular twitchings within 13 hours of the mixing of eggs and sperm. Hatching follows in the next few hours.

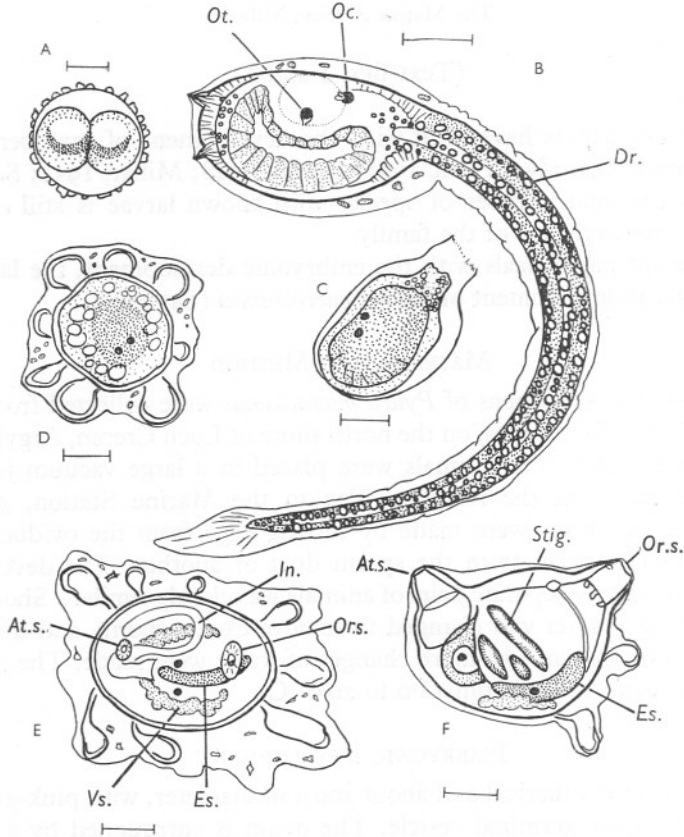


Fig. 1. Development of *Pyura microcosmus* (Savigny). A, two-cell stage, showing dark crescent; B, larva; C, larva during metamorphosis; D, dorsal view of metamorphosing larva, with ring of ampullae; E, dorsal view of young ascidian after differentiation of gut; F, lateral view of young ascidian. The scales represent  $100\ \mu$ . At.s., atrial siphon; Dr., refringent droplets; Es., endostyle; In., intestine; Oc., ocellus; Ot., otolith; Or.s., oral siphon; Stig., anterior of the first three stigmata of the branchial sac; Vs., crescent of vesicular cells.

#### LARVA

The larva (Fig. 1B) is divided into a trunk about  $285\ \mu$  long, and a tail about  $1000\ \mu$  long. A layer of clear test covers the surface of the larva and is developed into a vertical fin along the dorsal and ventral sides of the trunk and

tail. A leaf-like expansion of the fin projects beyond the end of the tail. At the anterior end of the trunk are two dorsal and one ventral adhesive papillae. The rudiment of the pharynx lies in the ventral half of the trunk. Near the dorsal surface of the trunk is the sensory vesicle, containing on its posterior wall the ocellus and on its ventral wall the otolith. The ocellus (Fig. 1 B, *Oc.*) has a black cup-shaped mass of pigment  $14\mu$  in diameter, behind which are the retinal cells. Clear lens cells project from the cavity of the pigment cup. The otolith (Fig. 1 B, *Ot.*) is a spherical black body  $13.2\mu$  in diameter contained in a single pear-shaped cell which projects upwards from the floor of the sensory vesicle.

Many small refringent droplets are present in the trunk, mainly in the posterior regions; it is not certain which cells contain these droplets.

In the tail are the usual structures of the ascidian larva: a central notochord, dorsal neural strand, and three bands of striated muscle on each side. A longitudinal row of refringent droplets (Fig. 1 B, *Dr.*) is associated with each of the muscle bands of the tail.

#### METAMORPHOSIS AND POST-LARVAL DEVELOPMENT

Within 24 hours of hatching some larvae have started to metamorphose, although others still show no sign of change. Attachment of larvae to a substratum is not necessary for metamorphosis, which occurs in attached and unattached larvae. The first change is reduction of the tissues of the tail (Fig. 1 C), but not the covering test which retains its original form and persists long after metamorphosis is complete. While the tail is shrinking the trunk produces a ring of anterior ampullae which are applied to the substratum (Fig. 1 D). Typically these ampullae number eight, but some variation is found. They grow outwards as long finger-like structures, and simultaneously a disk of expanding test spreads over the substratum. Meanwhile other changes occur in the body, and a central dark mass of cells appears, surrounded by large vesicular cells. The larval sensory pigment is still present within the body of the developing ascidian. The dark central mass now elongates and differentiates to form the rudiment of the pharynx and gut, and the surrounding vesicular cells become arranged in a crescent on each side of the pharynx. At an early stage in the formation of the pharynx the endostyle is recognizable, and shortly afterwards the oral and atrial siphons are formed (Fig. 1 E). The atrial siphon is, from the first, a single structure, and is not derived from the fusion of paired peribranchial openings. With the further differentiation of the gut to form the oesophagus and stomach, and the intestinal loop on the left of the pharynx, and with the perforation of one pair of protostigmata, the essential organization of the adult is accomplished. The original protostigmata divide to form two, then three, pairs of openings (Fig. 1 F). Up to about this stage the larval pigment of the ocellus and otolith remain unchanged within the body of the young ascidian, but now they are transported out of the body

and deposited either on the outer surface of the test, or, more usually, quite outside the body and often at some distance from it (Fig. 2). This remarkable movement of the pigment spots, which was observed in each of the young ascidians examined, is presumably accomplished by phagocytes which, having ingested the pigment, migrate out of the body.

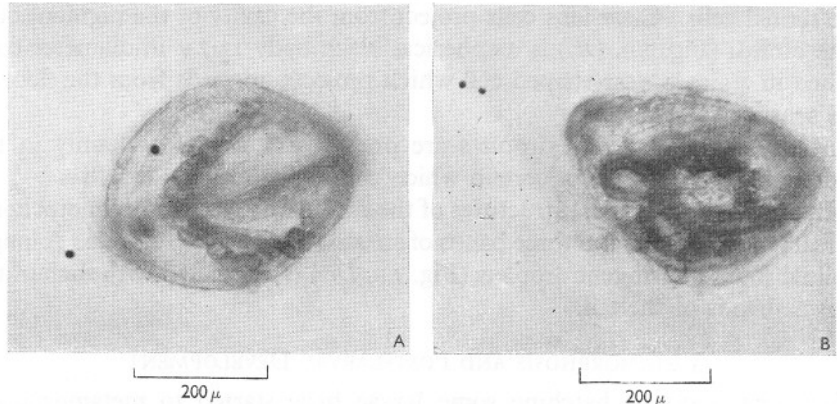


Fig. 2. Development of *Pyura microcosmus* (Savigny). A, dorsal view of young ascidian showing outward migration of one of the larval pigment spots. B, young ascidian after migration of both pigment spots out of the body.

The heart by now is differentiated, and starts to beat. It already shows the reversal characteristic of ascidians, although at this early stage it changes direction at irregular intervals.

Shortly after, a circle of spines develops round each of the siphons, this being the first sign of the elaboration of the test typical of the Pyuridae.

TABLE I. TIME-TABLE OF DEVELOPMENT

Stage	Time
Eggs and sperm mixed	11.00 a.m., 3. viii. 53.
First cleavage	12.20 p.m.
Second cleavage	12.45 p.m.
Gastrula	4.45 p.m.
Closure of blastopore	5.45 p.m.
Trunk and tail differentiated	8.00 p.m.
Larva complete, and active within chorion	11.45 p.m.
Larva hatched and active	9.00 a.m., 4. viii. 53.
Metamorphosis: tail reduced and ampullae forming	5. viii. 53.
Tail resorbed, ampullae enlarging	6. viii. 53.
Endostyle differentiated	10. viii. 53.
Siphons and gut differentiated	11. viii. 53.
One pair of stigmata present and active	12. viii. 53.
Three pairs of stigmata; removal of larval sensory pigment outside body; heart beating	14. viii. 53.
Elaboration of test spines on siphons	17. viii. 53.

Table I shows the times at which important stages were reached in development.

The general course and timing of development agree more closely with those of *Pyura squamulosa* (Alder) than with any of the other pyurid ascidians whose development has been studied. The egg of *P. microcosmus*, however, is larger than that of *P. squamulosa*, and the larva also correspondingly larger.

#### SUMMARY

The embryonic development, larva, and post-larval stages are described in the ascidian *Pyura microcosmus* (Savigny). The development is similar to that of *P. squamulosa* (Alder), but the egg and larva are larger. In the young ascidian the persisting pigment of the larval ocellus and otolith are transported out of the body, presumably by the action of phagocytes.

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## EXPERIMENTAL OBSERVATIONS ON THE VERTICAL MIGRATIONS OF PLANKTON ANIMALS

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(Plates I and II and Text-figs. 1-29)

### CONTENTS

	PAGE
The general principles of the method . . . . .	410
Limitations of the method . . . . .	412
Construction of the apparatus . . . . .	413
Examples of records . . . . .	417
Survey of results . . . . .	422
Types of swimming behaviour . . . . .	422
Speeds of swimming . . . . .	426
Differences between animals . . . . .	426
Changes in behaviour with time . . . . .	430
Correlation of behaviour with changes in light intensity . . . . .	437
Discussion . . . . .	446
Summary . . . . .	447
References . . . . .	448

Much information concerning the vertical migration of plankton animals has been gained by using nets at different levels in the sea and at different times of the day and night, and also by experimenting with animals in apparatus of various kinds both in the laboratory and in the sea itself. Such observations and experiments have been summarized in the extensive reviews by Russell (1927) and Cushing (1951) who give full lists of references.

In all this earlier work a limited knowledge of the migrations was obtained by inference. It had not been possible actually to see plankton animals making their extensive upward or downward journeys, so that little was known of how they accomplished them, whether for example by swimming directly and continuously upward or by making more random movements with a general upward tendency. There were no direct data regarding the speed or duration of their climbing and it remained an open question whether they descended passively by sinking or actively by swimming down. Such observations would normally require a vertical column of water of great length. The present paper describes the results of experiments and observations made with a new type of apparatus designed to overcome this difficulty and to give answers to some of these questions.



Before the war, with the help of a grant from the Leverhulme Trustees, Hardy & Paton (1947) began studies on vertical migration by using glass cylinders in the sea. This work was interrupted by the war and Neil Paton was killed in action. The Leverhulme Trustees kindly allowed the remaining portion of their grant to be used in the present investigation. The equipment about to be described is related in its origin to the long tube apparatus of the former investigation; it is in fact such a long tube bent in a circle to form a continuous channel of water. A paper describing the apparatus and some of the results obtained with it was given at the Edinburgh meeting of the British Association in 1951 and is briefly summarized in Hardy & Bainbridge (1951*b*).

#### THE GENERAL PRINCIPLES OF THE METHOD

We will first describe the method and the working of the apparatus in general terms: this will be followed in a later section by a more detailed specification of its structure. A curved transparent tube of Perspex, of 2 in. by 1½ in. cross-section, is made to form a complete circle of 4 ft. diameter and is then mounted vertically to be rotated as a wheel. At one point on the rim there is an opening, which may be closed by a lid, sealed with vaseline and bolted down; through this, when it is turned to the top, the tube may be filled with water and the experimental animal put in. After sealing the opening, the wheel is turned so that the animal is halfway up one side. The apparatus, as will presently be explained, is provided with an overhead lighting as nearly as possible equivalent to under-water conditions; by a series of screens the intensity of this light may be varied from bright daylight to darkness. The animal is now free to move upwards or downwards; as it so moves through the water, the wheel is rotated carefully by hand so as to keep the animal opposite a fixed point outside the wheel, i.e. stationary in relation to the observer. It will be seen that the animal is now swimming in an endless column of water; so gradual is the curve, it can go on swimming *straight* up or down for hundreds of feet provided that the wheel is turned at a corresponding speed (see Text-fig. 1, p. 414). Inside the tube are small doors, some with weights and some with floats, which automatically open and close as the wheel goes round; they are always wide open on the side at which the animal is swimming, and so do not interfere with it, but are closed on the other side to ensure that the water turns exactly with the wheel and that the movement of this corresponds exactly with that of the animal.

The wheel is mounted in a frame. Fixed to its axle, so as to turn with it, is a pulley having a circumference exactly one-tenth that of the wheel itself; another similar pulley is mounted on the frame below it and a cord, kept at tension by a spring, passes round the two. On this cord is mounted a needle which records on the smoked drum of an electric kymograph. As the animal swims upwards the wheel is turned downwards and so the pulley system

turns to move the needle upwards exactly one-tenth the distance moved by the animal. A graph at 1/10 vertical scale is thus traced on the drum, recording the upward and downward movements of the animal against a time base-line. If the wheel is skilfully turned so as to keep the moving animal always opposite the fixed position outside, its every little movement up or down is faithfully recorded for later analysis. It is in such cases, where the animal is jiggling up and down, that the two kinds of doors referred to above are so valuable; they ensure that there is no lag of the water behind the movement of the wheel: it is moved as sharply as the wheel itself, because in *each* direction the thrust of the water at once meets a door closed tight against a stop. Because of this, together with quickness in following the movements of the animal, the tracing on the smoked paper can be an exact record of the animal's vertical migrational behaviour at a scale of 1/10.

The first experiments were made at the Plymouth Laboratory in a room having a skylight under which the wheel was mounted, the light being varied by a system of screens. It was realized that this was a poor representation of the illumination to be met with in the sea, so that a small glass house (a miniature 'greenhouse') was used for all the subsequent experiments which were carried out at Millport. The sides of it were covered with different thicknesses of paper, to give a graded light from above downwards, and the intensity of the light as a whole could be varied by a series of white, grey and black sheets which could be drawn over the roof and sides. Fixed to the frame, against the wheel and side by side with it, is one quadrant of an exactly similar Perspex tube: this was filled with water and received just the same illumination as the wheel itself. At the lower end of this quadrant, which is exactly opposite the point at which the animal is kept swimming, is fixed a photo-electric cell connected to a sensitive galvanometer which measures the light intensity; these values may be recorded at any moment for correlation with the vertical migrational behaviour of the animal in the tube. When working in darkness, a small red lamp, placed on one side of the tube, enabled the outline of the swimming animal to be kept silhouetted against a dim red disk of light; and thus the movement of the wheel could still be controlled to correspond with the animal's behaviour.

This description of the method will be sufficient to explain in general how the records were obtained and how a single animal can be followed whilst it swims through a column of water corresponding to a vertical height of maybe 200 or 300 ft. (as with the large euphausiacean *Meganyctiphanes norvegica*). The full details of the structure and arrangement of the apparatus will be given later (pp. 413-17).

## LIMITATIONS OF THE METHOD

The ease of following an animal while swimming for such long vertical distances is achieved at the cost of certain disadvantages which must be recognized. There are three main limitations. First, conditions differ from those in nature in that as the animal swims upward or downward it does not experience the gradual reduction or increase in pressure that it would do in the sea. Some preliminary experiments have been done (Hardy & Bainbridge, 1951*a*) which appear to show that the behaviour of *Calanus finmarchicus* is not affected by pressure differences, although with decapod larvae a higher proportion of the population swims upward as the pressure is increased. It is unlikely that the small changes in pressure undergone as the animal swims a matter of a few feet will have an effect on its swimming behaviour, but clearly with forms like decapod larvae the *extent* of their migration in the apparatus may be very different from what it would be in the sea. Secondly, in the same way in the sea there would be a gradual increase or diminution in the light intensity as an animal swam up or down. This again does not occur in the apparatus, although it can be made to do so roughly either by adjusting screens overhead or by the use of artificial illumination with a dimming device; this, however, could not be in the continuous and gradual manner in which it is experienced in the sea. Thirdly, whilst it is swimming upward or downward in relation to the water passing it, the animal itself is not actually moving upwards because the wheel is turning against it to keep it stationary in relation to the outside world; consequently, if the wheel is turned exactly with the movement of the animal when it begins to swim, it will not experience any acceleration against gravity, i.e. the sensation we get when a lift starts to ascend or descend. This difference from nature will only occur at the moment of starting to rise or fall and will not be experienced once an animal is swimming at a more or less uniform speed. It is a difficulty which in practice is usually automatically overcome because the animal nearly always gets a slight start on the observer when it begins to swim, and the wheel is only subsequently turned to bring it opposite the pointer.

It may perhaps further be suggested that the movement of the animals is to some extent guided in an upward or downward direction by the proximity of the walls of the tube and that the path of swimming we have recorded is an artificial one. This might well be true if we were dealing with a large plankton animal such as a medusa which occupied a good deal of the cross-section of the tube; the plankton animals we are observing here however are so small in relation to the width of the tube that such a 'canalization' of movement does not occur. From time to time an upward swimming animal may converge on to and collide with the side of the tube; when this happens it may often dart away or turn a somersault so that the effect of the walls is rather to delay the upward swimming of an animal instead of guiding it in a vertical direction.

Animals which swim up diagonally will of course strike the sides more frequently than those which ascend more vertically.

Finally it may be thought that the wall of the tube may affect the swimming of the animals by offering a resistance to the movement of the water when they are swimming close against it. This indeed may have some effect when the animal is *very close* to the wall but, again because of the small size of the animal in relation to the cross-section of the tube, it will be against the wall only occasionally.

These various limitations must be kept in mind in the interpretation of results, but it is unlikely that they introduce a false representation either of the speed, duration or pattern of swimming behaviour of the animals considered. These factors will be referred to again in the discussion of results.

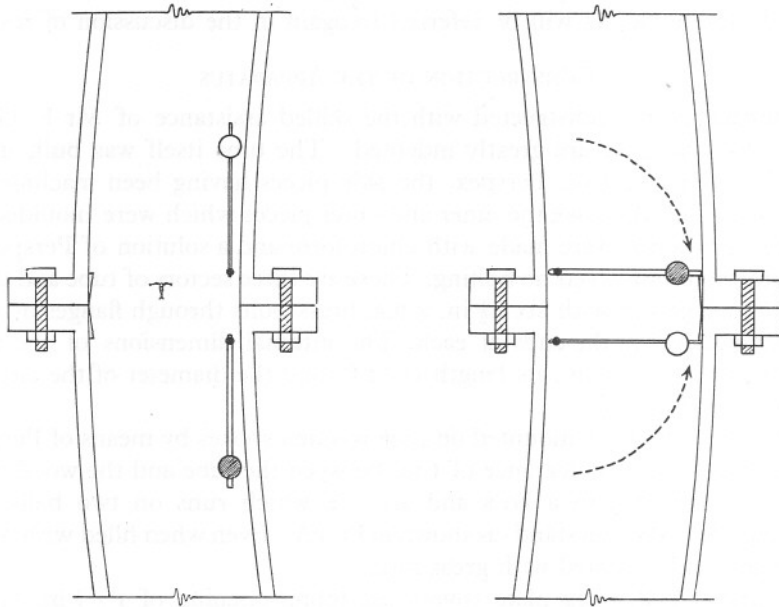
#### CONSTRUCTION OF THE APPARATUS

The apparatus was constructed with the skilled assistance of Mr F. G. C. Ryder, to whom we are greatly indebted. The tube itself was built up of shaped sections of  $\frac{1}{4}$  in. Perspex, the side pieces having been machined to shape and rebated to take the inner and outer pieces which were moulded by heating. The joints were made with chloroform and a solution of Perspex in chloroform and involved no bolting. There are three sectors of tube and these are bolted together with six  $2\frac{1}{2}$  in. 4 B.A. brass bolts through flanges of 1 in. Perspex attached to the ends of each. The internal dimensions of the tube so formed are  $1\frac{1}{2} \times 2$  in., its length is 12 ft. and the diameter of the circle it produces roughly 4 ft.

This circular tube is mounted on nine wooden spokes by means of Perspex clamps which allow a clearance of 1 in. between the tube and the wood. The spokes are attached to a boss and an axle which runs on two ball-races supported by a wooden stand (as shown in Pl. I A). Even when filled with water the wheel can be rotated with great ease.

A removable Perspex plate covers the filling opening of  $1 \times \frac{3}{4}$  in. in the outer curved part of the tube. This closing plate is secured by four brass nuts and bolts, the bolts being embedded in a shaped piece of Perspex attached to the main curved section of the tube. The doors or valves, which ensure that the water turns exactly as does the wheel, are fitted in three pairs—one pair at each of the three pairs of flanges. They consist of rectangles of 1 mm thick Perspex cut to fit the cross-section of the tube exactly, and arranged to swing freely on an axle placed as near as possible to the inner (curved) wall of the tube. One door of each pair has a float made from a hollow cylinder of Perspex (of 1 cm diameter) attached close to its outer (free) edge and the other member has a weight consisting of a cylinder of lead encased in a hollow cylinder of Perspex attached in a similar position. The automatic action of these weights and floats ensures that the doors are always wide open on the side of the wheel facing the observer, where the animal is swim-

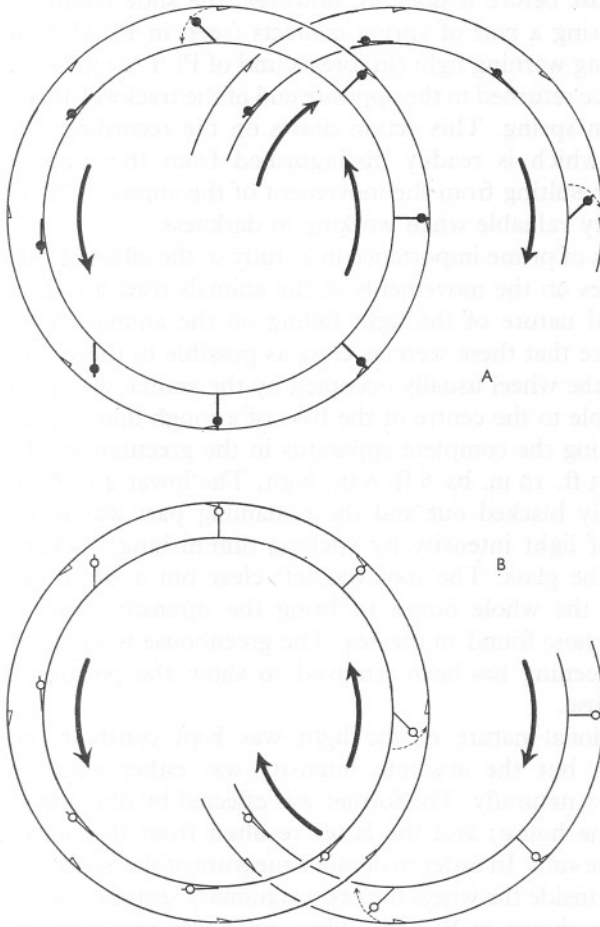
ming, and closed on the opposite side (Text-fig. 1). Their cycle of opening and of closing against small wedge-shaped Perspex stops is shown in Text-fig. 2. Originally only one set, of weighted doors, was fitted. These suffice when the wheel is being turned steadily in an anticlockwise direction (when viewed from the right, as in the figures). When the direction of swimming changes, however, and the wheel has suddenly to be turned in the opposite direction, then the inertia of the water causes the doors on the side opposite the observer to be forced open and the movement of the wheel no longer bears a direct relationship to the movement of the animal. The inclusion of a second set of doors working with floats and closing against stops facing the



Text-fig. 1. Diagram showing the opened and closed positions of the trapdoors at opposite sides of the wheel. A specimen of *C. finmarchicus* is drawn to scale swimming at the observation side.

opposite way ensures that there is always at least one door closed against a stop in whatever direction the wheel is turned. The efficiency of this method of locking the water to the wheel depends upon the elimination of leakage past the sides of the doors when the direction of rotation is being changed; and upon the free opening and closing of the doors by the forces of gravity. It is essential that the doors should be completely open opposite the observer in order to avoid obstructing the animal; in making the doors loose enough to do this, some slight leakage of water is necessarily entailed, but this is not sufficient to have an appreciable effect on the accuracy of the whole system.

The recording device consists, as already mentioned, of a large pulley wheel fitted rigidly to an extension of the axle of the wheel on the right-hand side with, below it, a second but free-running pulley of the same size fitted to the side of the wooden stand. The spring-tensioned cord connecting these two pulleys passes along a trackway of  $\frac{1}{2}$  in. brass angle, 12 in. long, on the side of



Text-fig. 2. Diagrams showing the cycle of the opening and closing of (A) the weighted doors, and (B) the buoyant doors according to the way the wheel is turned—either clockwise or anticlockwise.

the pulleys away from the observer, and is connected to a slide which runs in the trackway and carries a recording needle. The pulley wheels were made to be precisely one-tenth of the diameter of the Perspex tube (taken from the centre of the tube at one side to the centre at the other) so that the needle moves through one-tenth of the distance swum by the animal and in the same



direction; in doing so it impinges on an electric kymograph drum carrying smoked paper. By this means a graph representing the movement of the animal is obtained, with time along the horizontal axis and distance swum vertically along the vertical axis. Should the animal swim persistently in one direction then the pointer comes sooner or later to the top or the bottom of the paper. Just before it does so, however, the slide completes an electric circuit by closing a pair of spring contacts (seen in Pl. IIB) and so flashes a corresponding warning light (in foreground of Pl. IA); the slide and pointer are then at once returned to the opposite end of the trackway by pulling sharply on the tension spring. This action draws on the recording drum a straight vertical line which is readily distinguished from the more irregular and sloping lines resulting from the movement of the animal. The warning lights are particularly valuable when working in darkness.

Two factors of prime importance in a study of the effect of various environmental changes on the movements of the animals used are the intensity and the directional nature of the light falling on the animal. Every effort was made to ensure that these were as close as possible to those found in nature. The place in the wheel usually occupied by the animal was arranged to be as close as possible to the centre of the base of a rough dome of light. This was done by housing the complete apparatus in the greenhouse which measured 5 ft. 9 in. by 3 ft. 10 in. by 6 ft. 6 in. high. The lower 4 ft. 6 in. of the walls was completely blacked out and the remaining part was made to transmit a gradation of light intensity by sticking diminishing thicknesses of tissue paper on to the glass. The roof was left clear but a white linen sheet was thrown over the whole house to bring the intensity down to one comparable with those found in the sea. The greenhouse is shown in Pl. IB, but the paper screening has been removed to show the position of the wheel inside the house.

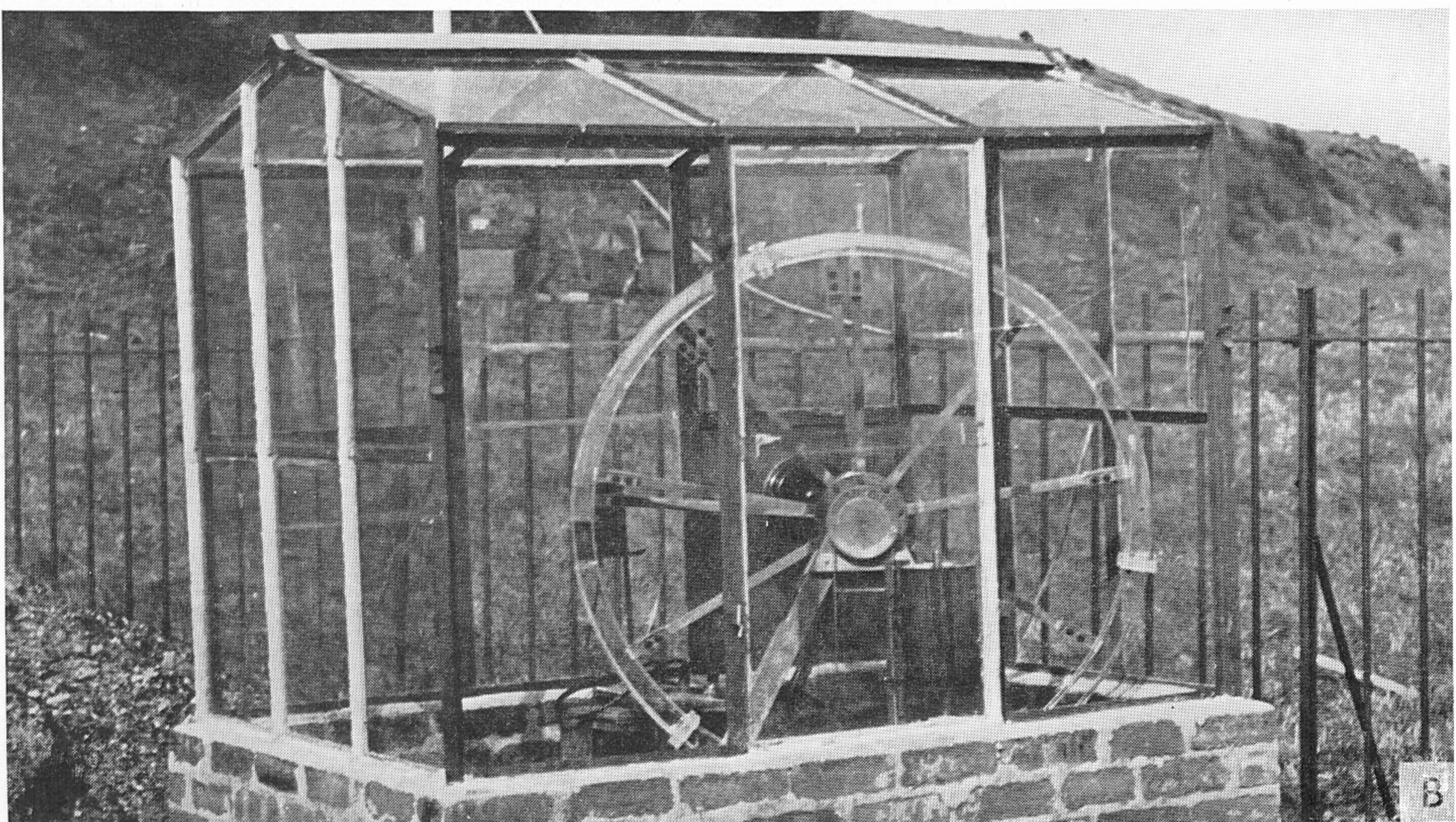
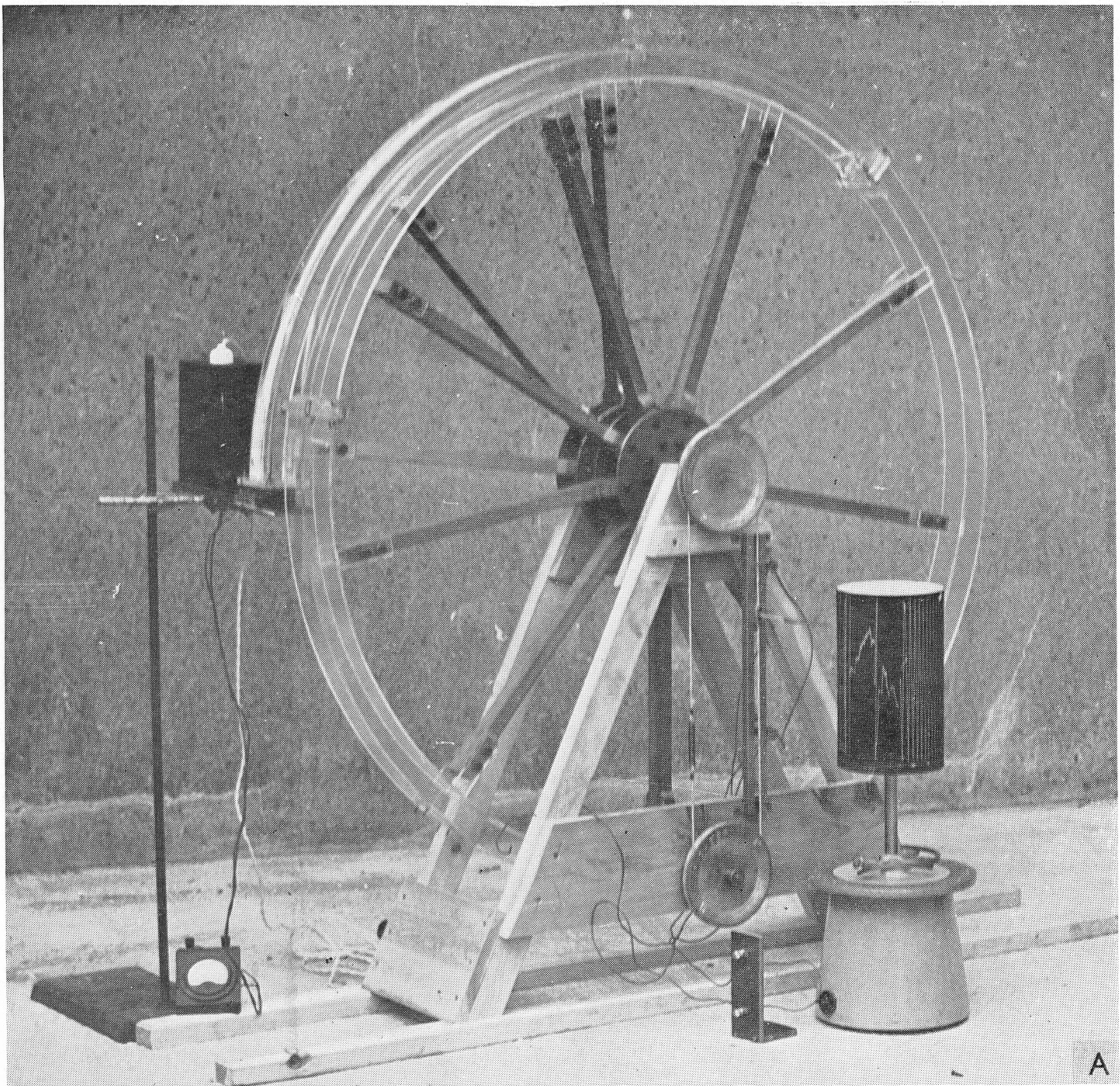
The directional nature of the light was kept constant throughout the investigations, but the absolute intensity was either varied artificially or allowed to vary naturally. The former was effected by drawing grey and black sheets over the house; and the latter resulted from the normal passage of clouds over the sun. In order to obtain a measure of the actual intensity falling on the animal inside the wheel the extra stationary sector of tube was mounted alongside it as shown in Pl. IIA. This sector was one-quarter of the whole circle and its lower end, closed with a 1 mm thick Perspex plate, was arranged to be directly opposite the position occupied by the animal. It was completely

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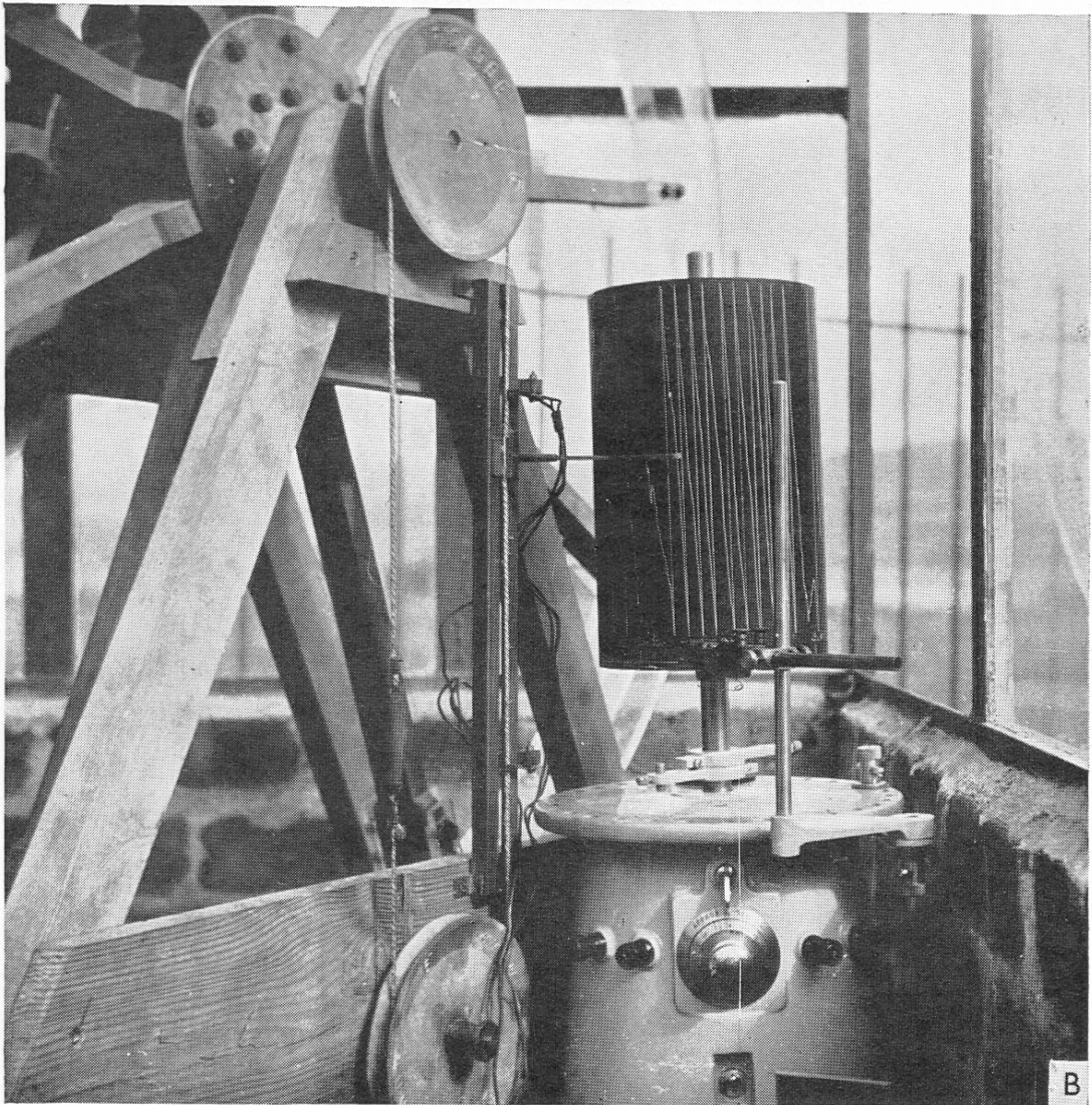
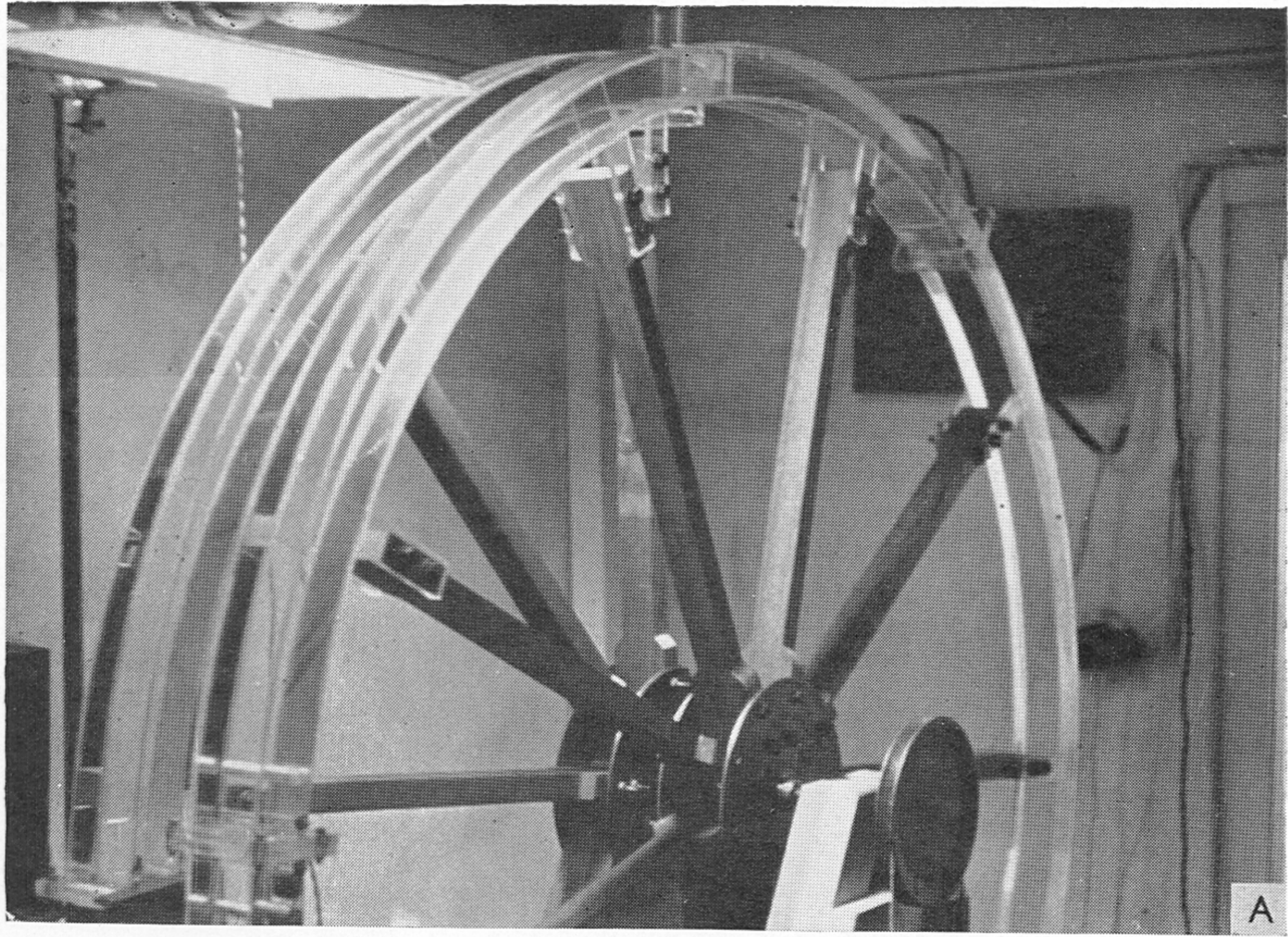
#### EXPLANATION OF PLATE I

- A. General view of the wheel apparatus for the study of the vertical migration of plankton animals.
- B. The apparatus mounted in the small greenhouse; the paper screening designed to give a gradation of light intensity has been removed to show the position of the wheel.











filled with water through a funnel at the top and an Electro-Selenium Photocell was bolted immediately below the Perspex plate and pointed directly upwards. The current produced by this photocell was measured at regular intervals throughout the course of an experiment with a galvanometer, and the current value was used to calculate the light intensity falling on the cell. This is assumed equal to that falling on the animal inside the wheel, because the stationary sector is identical in every respect to a sector of the wheel itself.

In order to use a photocell such as the E.E.L. cell used here for measurement of light intensities, it is necessary for it to be calibrated against a standard cell. This was most kindly and thoroughly done for us by Dr W. R. G. Atkins, F.R.S., of Plymouth, who also gave us valuable advice on the use of cells and circuits. The arrangement finally adopted consisted of an E.E.L. selenium rectifier cell mounted under an opal flashed glass and a Cambridge Unipivot type L galvanometer no. 65542. These were connected with shunts of various value depending upon the light intensity at the time. The galvanometer reads up to  $24 \mu\text{A}$ , which is equivalent to about 250 Lux of incident light. For light intensities higher than this it is necessary to use a shunt in order to reduce the amount of current passing through the galvanometer. Shunts of 100, 40, and  $20 \Omega$  were employed and, with the latter, light intensities up to about 10 kilolux could be measured. Unfortunately the current produced by this type of cell varies according to the resistance in the external circuit, and the cell had therefore to be calibrated separately for each shunt. When this was done graphs of scale reading of galvanometer against light intensity were drawn and the conversion of any subsequent experimental reading simply entailed inspection of these.

Use of a greenhouse to obtain the required type of illumination introduced the problem of keeping the apparatus cool. This was accomplished fairly satisfactorily by fitting a Vent Axia fan to draw out the warm air and by watering the roof at intervals with cold water from a watering can. Evaporation from the damp sheet then reduced the temperature considerably.

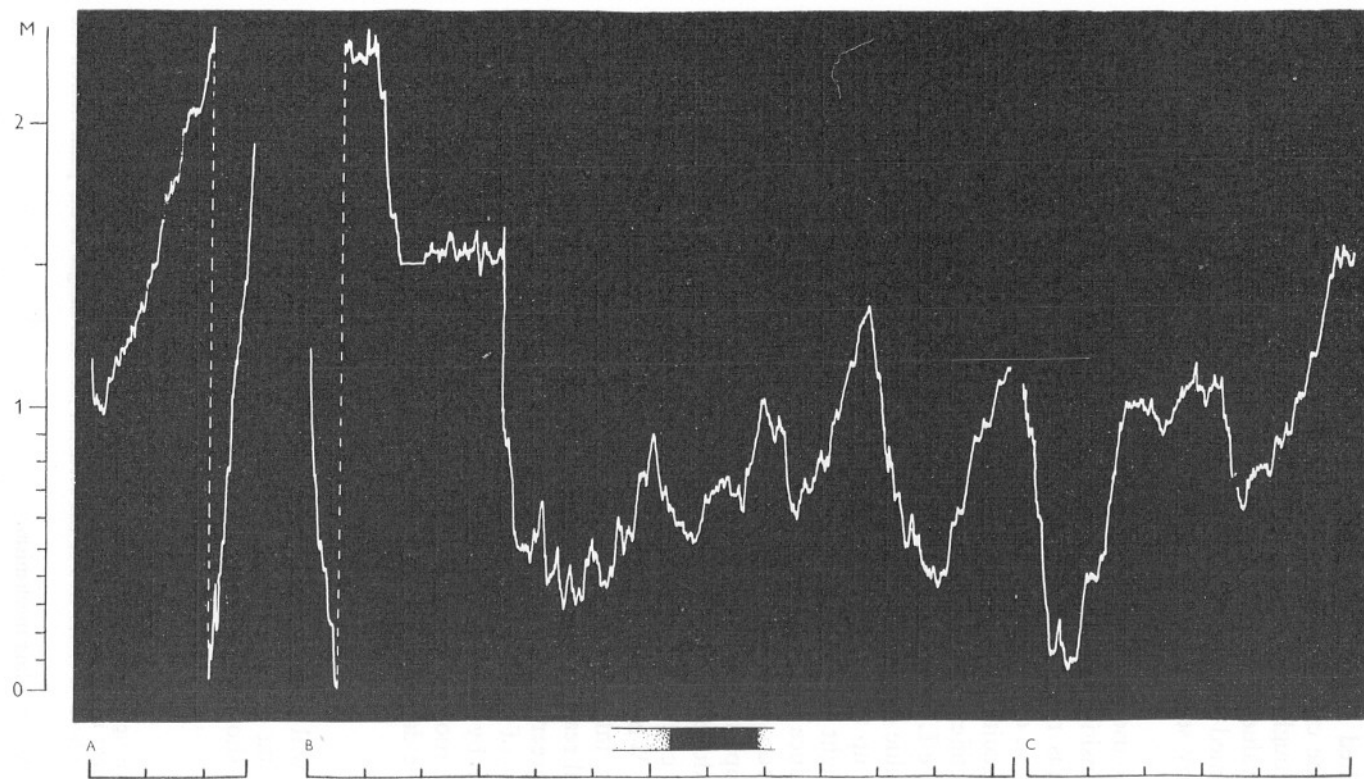
#### EXAMPLES OF RECORDS

The results will be understood more easily if they are introduced by showing and describing a few examples of the records made with the apparatus. Text-fig. 3 shows the first one to be made: that taken at Plymouth on

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#### EXPLANATION OF PLATE II

- A. Detail of the upper part of the wheel to show the extra parallel quadrant for the measurement of light intensity; note also the buoyant and weighted doors in the open position inside the wheel on either side of the sectional junction flanges at the point nearest the camera.
- B. Details of the recording mechanism.



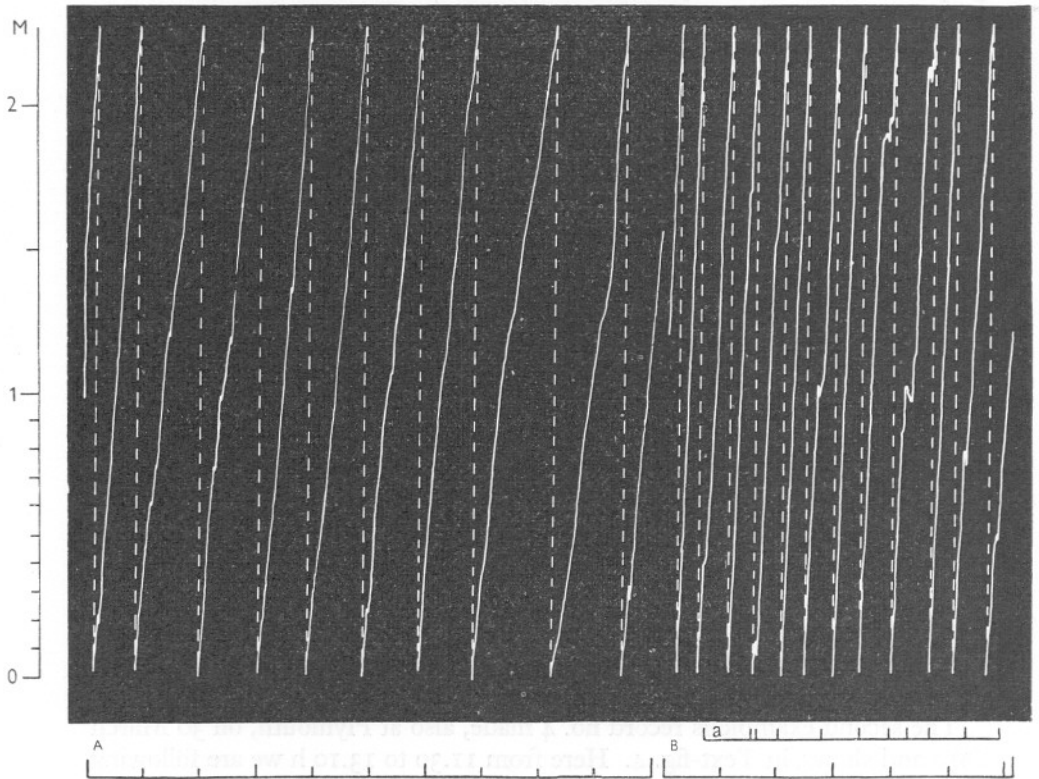
Text-fig. 3. Reproduction of the kymograph tracing obtained in record no. 1 experimenting with *C. finmarchicus*. A, a specimen observed from 18.05 to 18.40 h on 15 March 1950; B, a second specimen from 10.51 to 12.55 h on 16 March 1950; C, the same specimen from 14.54 to 15.51 h on the same day, the water in the wheel having been enriched with diatoms and flagellates. The time scale is divided into 10 min periods (except for period A when the kymograph was rotating more slowly). The blackened and dotted rectangle in B indicates a period of partial and complete blacking out. For full description see text opposite.

15-16 March 1950 with the copepod *Calanus finmarchicus*. It begins with a 35 min period from 18.05 to 18.40 h. The smoked paper, from the rotating kymograph drum, is moving to the left at a rate of 2 mm/min, and, as already explained, the graph traced by the recording needle represents exactly one-tenth of the distance travelled by the animal. In reproduction the graph is further reduced, but a scale shows the *actual* distance travelled in metres. During this first 35 min the *Calanus*, while jiggling up and down to a certain extent, is moving upwards with a gradual acceleration; it climbs 0.8 m in the first half of this period and 2.32 m in the second half. The broken vertical lines represent where the recording needle has been moved from the top to the bottom of the paper, or *vice versa*. The second part of the record is made from 10.51 to 12.55 h on the following day. *Calanus*, a different specimen, now descends rapidly 1.25 m then jigs up and down for 10 min, descends again 0.75 m, remains quite stationary for 5 min and then jigs again for another 18 min before diving down, swimming head first, for another metre. From now until this part of the record was stopped at 12.55 the *Calanus* rises and falls with little bursts of active swimming followed by short intervals of passive sinking or, occasionally, downward swimming; for alternating periods the movement is more upward than downward and *vice versa*, producing the main peaks and troughs shown on the graph. Halfway through this period the light from above was gradually blacked out to complete darkness for 15 min and then gradually restored again, as shown in the figure; this change in light intensity appeared to have no effect upon the behaviour of the animal. The last part of the record was taken for just under an hour, 14.54 to 15.51 h, with the *Calanus* in water rich in diatoms and flagellates (added from cultures): it swims downwards at first for nearly 1 m, then upwards, jigs for a time and then downwards again for 0.5 m followed by an irregular upward movement.

The second example is record no. 4 made, also at Plymouth, on 30 March 1950 and shown in Text-fig. 4. Here from 11.30 to 13.10 h we are following the movement of a group of *Balanus* nauplii which kept together as a cluster and swam upwards with great regularity. We are not following one individual all the time, for in the cluster it was difficult to be certain which one was which, as they swam near each other and passed and repassed one another. But because they kept so well together the graph does in fact closely represent the path taken by one individual. The speed of swimming and the energy displayed are surely remarkable for such small animals; for, during the 1 h 40 min they were observed, they never stopped swimming and averaged a climb of 14.9 m/h. The speed of ascent of the cluster as a whole varied somewhat as is indicated by the distances between the vertical broken lines: in each such interval the cluster has climbed approximately 2.25 m and the varying times in minutes taken for this distance are 7.5, 11, 10.5, 8.5, 9.5, 9.5, 9.5, 13.5, and 12. The wheel was now stopped and without emptying out

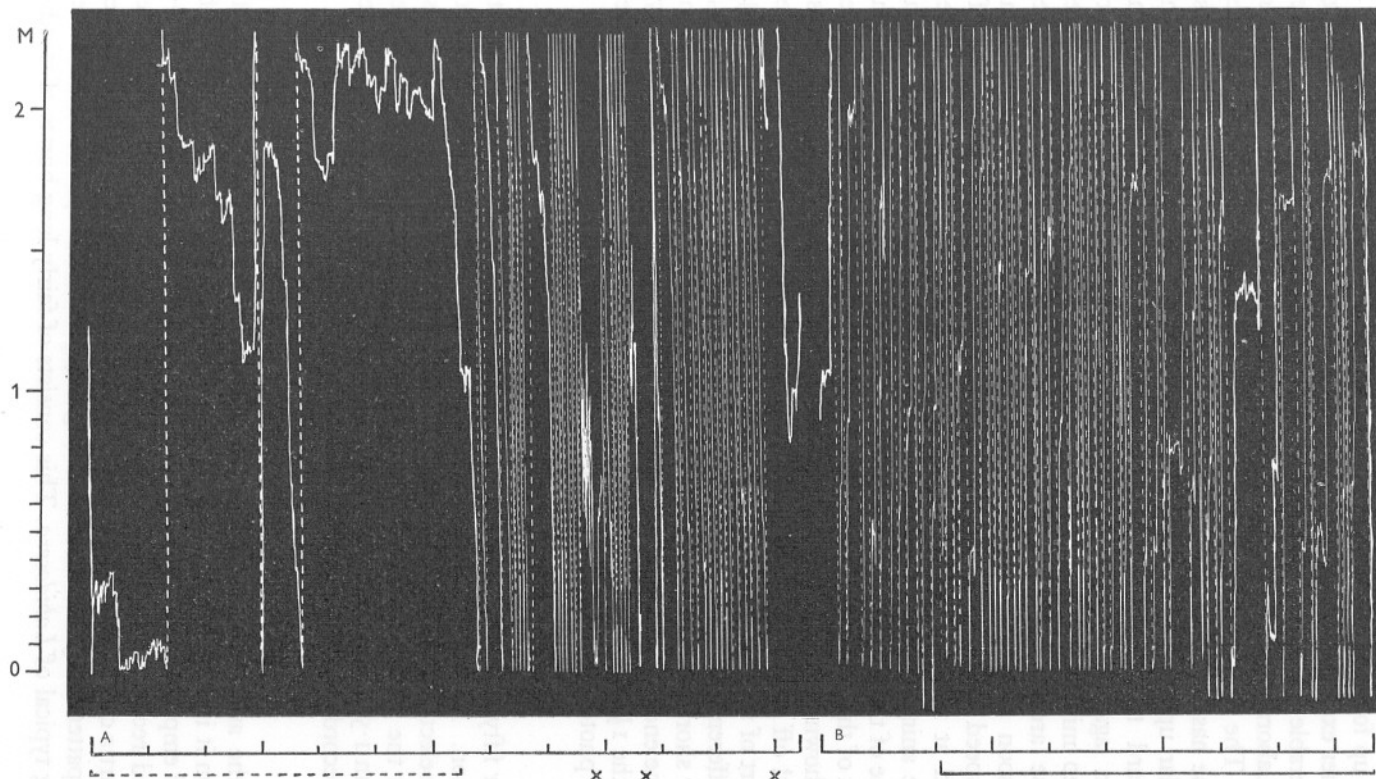


the water a small medusa of about 1 cm diameter, probably a late ephyra stage of *Aurelia*, was added; during the second part of the record, from 13.52 to 14.54 h, we followed this medusa and not the nauplii which remained in the water with it. The medusa, like the nauplii, was swimming steadily upwards but at a speed of 27.1 m/h, i.e. nearly twice as fast. Since we were



Text-fig. 4. Kymograph tracing from record no. 4. A, showing the upward migration of *Balanus* nauplii from 11.30 to 13.10 h; B, the upward migration of a small medusa from 13.52 to 14.54 h. The time scale is divided into 10 min periods. The line at *a* indicates pauses for the eating of nauplii: see text on this page.

now keeping pace with the medusa, the nauplii were carried round with the wheel and from time to time were overtaken by the medusa; each time this occurred the medusa stopped for a moment and with a few deft movements of the manubrium snatched up one or two nauplii before proceeding on its way. These pauses are clearly shown on the record as marked below the graph. The position of each nauplius must have been determined by the combined action of the tentaculocysts which presumably were picking up the vibrations in the water made by its swimming movements; each movement of the



Text-fig. 5. Kymograph tracing from record no. 10. A, showing a medium-sized *Meganyctiphanes norvegica* observed from 18.45 to 20.50 h; B, a large *M. norvegica* observed from 22.11 to 23.44 h. The dotted line under A indicates the period during which the ends of the greenhouse were shaded. The crosses indicate occasions when the animal was trapped behind a door. The unbroken line under B indicates the period during which the animal's photophores could be seen glowing. The time scale is divided into 10 min periods. See text, p. 422.

manubrium appeared to be made directly at the prey just as if it was a hand snatching up food in passing.

One other example, Text-fig. 5, will be given to show an animal swimming at considerable speed: record no. 10 made in the small greenhouse at the Millport Laboratory on 14 June 1950 with the euphausiacean *Meganyctiphanes norvegica*. The record starts at 18.45 h with a medium-sized animal and the greenhouse has each end shaded with a black cloth. At first the animal has an irregular up-and-down movement lasting about half an hour but with a downward tendency, then it swims rapidly upwards for about 3 m and down again for 3 m, after which it swims irregularly again for another 20 min or so until the dark sheets shading the ends of the greenhouse are removed leaving only the white sheet over the whole top. As soon as this change in lighting is made the animal swims down at great speed and continues to do so until the end of this first part of the record at 20.50 h, except at the points marked with an *X*; at these points the animal, in its headlong downward dash, has accidentally swum behind one of the doors of the wheel and some time has elapsed before it can be got out of this position by jerking the wheel to and fro. This jerking of the wheel is shown on the graph at the first *X*, but later the recording needle has been lifted off the drum whilst these artificial movements were made. The second part of the record was made on the same evening from 22.11 to 23.44 with a different and fully grown specimen of *M. norvegica*. This animal, except for short pauses which become more frequent and of longer duration towards the end, swam rapidly upwards covering a distance of approximately 132 m in the 1½ h. During part of the upward swimming, as indicated on the chart, the photophores could be seen to be shining.

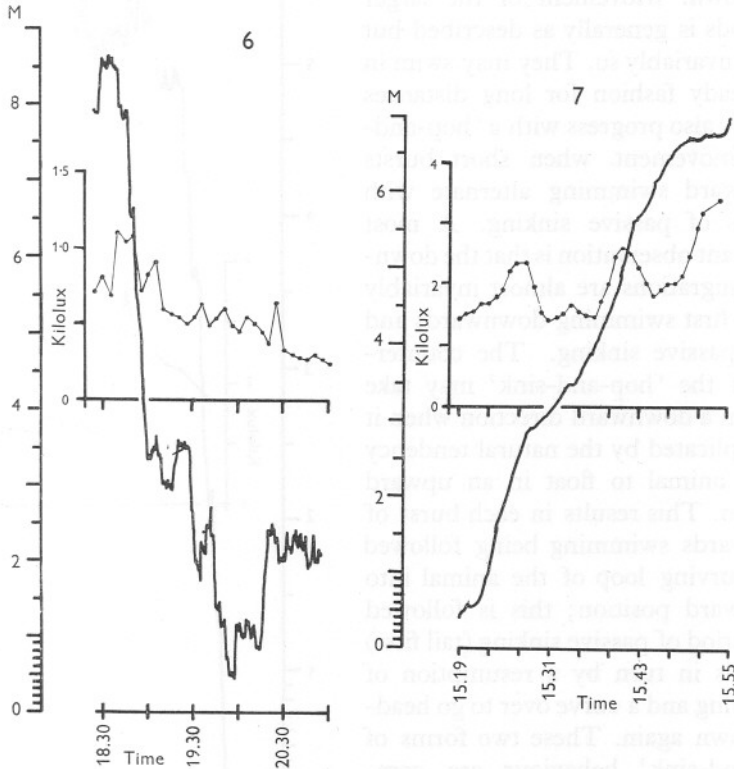
#### SURVEY OF RESULTS

Altogether fifty records were made with the apparatus, each averaging about 3 h duration. It is not proposed to reproduce and describe all of these but rather to select those which best illustrate the various kinds of observations made and the scope of the work as a whole. The kymograph speeds have varied from 1.5 mm/min in some records to 5 mm/min in others; a time scale for each record is provided below each tracing reproduced.

#### *Types of swimming behaviour*

While the automatic recording device is making a permanent record of a migration it is possible to make sustained observations on the behaviour of the animal employed. It can then be seen that some animals swim in a characteristic and recognizable fashion; and this movement may be so distinctive as to impart a characteristic pattern to the smoked drum record. An example of such a pattern may be seen in Text-fig. 6 (record no. 28) which shows the swimming typical of *Labidocera*. This consists of fairly long bursts of steady

swimming separated by roughly equally long periods of jiggling up and down. In contrast, record no. 22 shows, in Text-fig. 7, the steadier swimming of *Centropages* and record no. 10 (Text-fig. 5) the steady and much faster swimming of *Meganyciophanes*. A trace typical of a zoea larva is record no. 24, in Text-fig. 8, which shows steady swimming to be possible but also that this may be replaced by an indeterminate bobbing up and down which gives no resultant movement in either direction.



Text-fig. 6. Continuous tracing from part of kymograph record no. 28, showing the behaviour of *Labidocera wollastoni* ♂, together with a graph of changes in light intensity (25 July 1950).

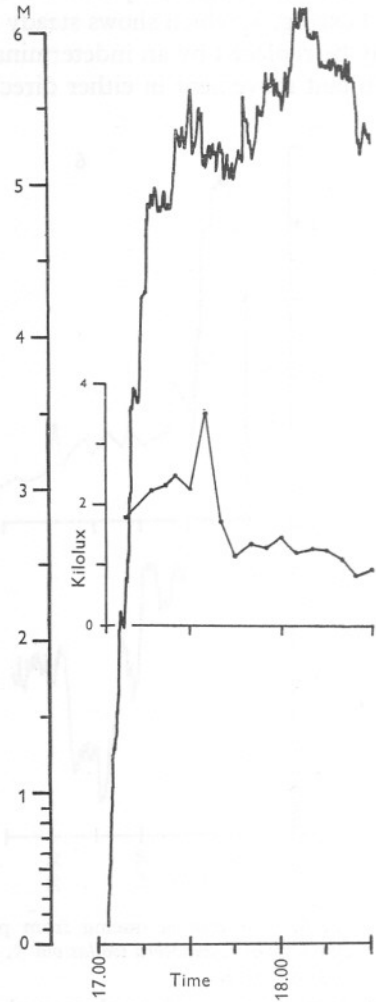
Text-fig. 7. The same of record no. 22, showing the behaviour of *Centropages* sp. ♀, but with different scales for time and light intensity (20 July 1950).

Generally, amongst the animals used, two distinct types of movement can be distinguished. The first of these is an up-and-down swimming which appears to be quite independent of the nature of the tube; and the second is one with a greater horizontal component which may in fact sometimes entirely replace up-and-down movement. The first type seems to embrace three subdivisions—the direct, the indirect, and the ‘hop-and-sink’. The larger



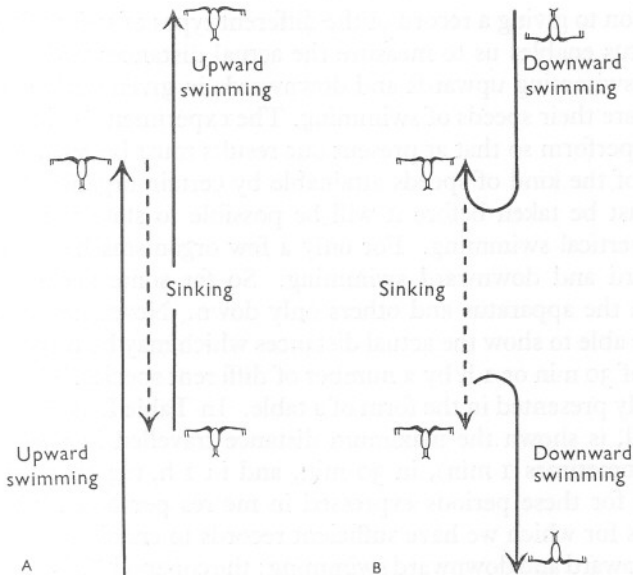
copepods such as *Calanus*, *Labidocera* and *Temora* swim up and down in a direct manner. Some of the smaller copepods such as *Acartia* and *Paracalanus* swim up and down with an indirect motion; i.e. while their resultant movement is in an upward or downward direction they follow an irregular zig-zag path, still keeping clear of the sides of the tube but not moving *directly* up and down. Movement of the larger copepods is generally as described but is not invariably so. They may swim in the steady fashion for long distances but may also progress with a 'hop-and-sink' movement, when short bursts of upward swimming alternate with periods of passive sinking. A most important observation is that the downward migrations are almost invariably a head-first swimming downwards and not a passive sinking. The counterpart of the 'hop-and-sink' may take place in a downward direction when it is complicated by the natural tendency of the animal to float in an upward position. This results in each burst of downwards swimming being followed by a curving loop of the animal into an upward position; this is followed by a period of passive sinking (tail first) and this in turn by a resumption of swimming and a curve over to go head-first down again. These two forms of 'hop-and-sink' behaviour are compared in Text-fig. 9.

The second type of swimming is indulged in by the euphausiids, decapod larvae, and by the larger copepod *Euchaeta*. These forms, while often making straight up-and-down excursions of the greatest length and speed, may also swim horizontally into the sides of the tube. When they do this it is impossible to make a recording of them although of course, if they were in the sea, they would be moving horizontally or at a large angle to the vertical.



Text-fig. 8. Continuous tracing from part of record no. 24 showing the behaviour of a zoea larva, together with a graph of changes in light intensity (21 July 1950).

Besides these large-scale movements of up-and-down or sideways swimming it is possible to detect by eye smaller-scale movements which are not recorded on the trace. Amongst the copepods it is clear that the steady and general type of propulsion involves the thoracic appendages and this forms the basis of the 'hop-and-sink' movement too. For short and much more rapid leaps, both violent movement of the antennae and flexing of the abdomen appear to be employed. Occasionally a violent caper may be indulged in when an animal leaps about in all directions with so great speed that it is not possible to determine which appendages are used.



Text-fig. 9. Diagrams illustrating the two types of 'hop-and sink' behaviour of *Calanus finmarchicus* during (A) upward swimming, and (B) downward swimming.

The underwater observations reported in Bainbridge (1952, 1953) were undertaken expressly to determine whether the swimming observed in apparatus of this type is similar to that occurring in the sea. The results reported make clear that it is so, and in general the observations made on animals in the wheel may be taken as applying also to behaviour in the sea. In particular the direct, the indirect, and the 'hop-and-sink' modes of up-and-down swimming have been observed in the sea, as has some amount of horizontal swimming and occasionally the violent caper mentioned above. The close parallel between all these movements in the wheel and in the sea makes it clear that reliance may be placed on the results and serves to refute the criticism that the 'canalizing' effect of the tube must make behaviour in the wheel unnatural.

None of the animals which have been fitted into the above categories must be taken as moving solely in the manner described there. All the species mentioned have been observed to swim, at one time or another, in several or all of the ways described. Behaviour is both individualistic and variable. The account (p. 420) of the change in swimming of the medusa in the presence of *Balanus* nauplii serves to demonstrate both how swimming movements can be altered to suit particular circumstances and also how useful the wheel may be in making such observations.

#### *Speeds of swimming*

In addition to giving a record of the different types of swimming behaviour, the apparatus enables us to measure the actual distances various animals are capable of swimming upwards and downwards in given periods of time, and so to compare their speeds of swimming. The experiments take a long time to set up and perform so that at present our results must be regarded only as an indication of the kind of speeds attainable by certain organisms; many more records must be taken before it will be possible to state the limits to their speeds of vertical swimming. For only a few organisms have we records of both upward and downward swimming. So far some species would only swim up in the apparatus and others only down. Nevertheless, for the first time we are able to show the actual distances which may be travelled vertically in periods of 30 min or 1 h by a number of different species. This information is most easily presented in the form of a table. In Table I, as far as possible for each animal, is shown the maximum distance travelled in a short period of 2 min (or sometimes 1 min), in 30 min, and in 1 h, together with the speed maintained for these periods expressed in metres per hour. There are only two animals for which we have sufficient records to enable us to compare the speeds of upward and downward swimming: the copepod *Calanus finmarchicus* and the euphausiacean *Meganyctiphanes norvegica*. For a short period of 2 min *Calanus* can swim upwards at a speed of 66 m/h and downwards at 107 m/h; maintained for a whole hour the speeds are only 15 and 47 m/h respectively. The copepod *Centropages* can climb 30 m in an hour. *Meganyctiphanes* can swim upwards at a speed 173 m/h for 2 min and downwards at 215 m/h for the same period; for a whole hour the respective speeds are 92.8 and 128.8 m/h. The remarkable speeds attained by so small a creature as a *Balanus* nauplius, as well as its powers of prolonged swimming, have already been commented on (p. 419).

#### *Differences between animals*

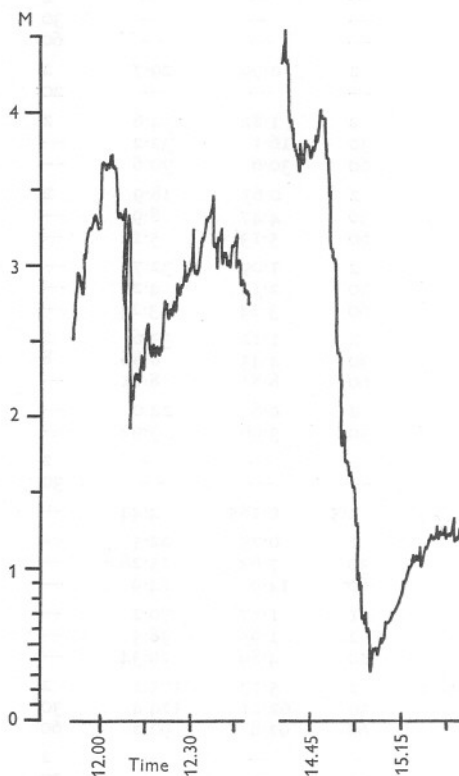
There are in general considerable differences in behaviour between animals which might be expected to react similarly. While the reaction—positive or negative—towards light may usually be uniform within a group of apparently identical animals caught at the same time, their speeds of swimming will differ

TABLE I. DISTANCES TRAVELLED AND SPEEDS OF VERTICAL SWIMMING OF VARIOUS PLANKTON ANIMALS OVER DIFFERENT PERIODS OF TIME

	Up			Down		
	Duration (min)	Distance (m)	Speed (m/h)	Duration (min)	Distance (m)	Speed (m/h)
<i>Calanus finmarchicus</i>	2	2.2	66	2	3.57	107
	30	17.8	35.6	30	29.9	59.8
	60	15	15	60	47	47
<i>Euchaeta norvegica</i>	—	—	—	2	4.52	135.6
	—	—	—	30	41.25	82.5
	—	—	—	60	70.4	70.4
<i>Labidocera wollastoni</i>	2	0.99	29.7	2	0.78	23.4
	—	—	—	20	7.97	23.9
<i>Centropages</i> sp.	2	1.82	54.6	2	0.7	21.0
	30	16.1	32.2	—	—	—
	60	30.6	30.6	—	—	—
<i>Temora longicornis</i>	2	0.63	18.9	2	0.57	17.1
	30	4.47	8.94	—	—	—
	60	5.13	5.13	—	—	—
<i>Paracalanus parvus</i>	2	1.09	32.7	—	—	—
	30	2.14	4.28	—	—	—
	60	3.24	3.24	—	—	—
<i>Acartia clausi</i>	2	1.12	33.6	2	0.57	17.1
	30	4.43	8.86	8	1.61	12.1
	60	8.81	8.81	—	—	—
<i>Oithona similis</i>	2	0.8	24.0	—	—	—
	30	3.96	7.92	—	—	—
Bopyrid larva	—	—	—	2	1.75	52.5
	—	—	—	30	10.95	21.9
<i>Calanus nauplius</i>	2.5	0.185	4.44	—	—	—
<i>Balanus nauplius</i>	2	0.75	22.5	—	—	—
	30	7.63	15.26	—	—	—
	60	14.9	14.9	—	—	—
Zoea (Brachyura)	1	1.17	70.2	—	—	—
	2	1.95	58.5	—	—	—
	10	4.89	29.34	—	—	—
<i>Meganctiphanes norvegica</i>	2	5.77	173.1	2	7.17	215.1
	30	62.21	124.4	30	68.2	136.4
	60	92.8	92.8	60	128.8	128.8
<i>Thysanoessa inermis</i>	—	—	—	2	4.15	124.5
	—	—	—	30	19.5	39.0
	—	—	—	60	40.4	40.4
<i>Tomopteris</i>	—	—	—	2	6.84	205.2
	—	—	—	28	47.46	101.7
<i>Sagitta elegans</i> (small, $\frac{1}{2}$ in)	1	0.44	26.4	2	0.3	9
	—	—	—	15	1.17	4.68
<i>Rossia macrosoma</i>	—	—	—	2	4.58	137.4
	—	—	—	30	44.65	89.3
Small medusa late Ephyra (1.0 cm diam.)	—	—	—	2	1.29	38.7
	—	—	—	30	15.06	30.12
	—	—	—	60	27.1	27.1



greatly and one or two will even behave in the opposite manner. In four separate experiments with four similar *Calanus* females each caught at 10.30 h and experimented with at 11.00 h on similar days, speeds for swimming down of 50.8, 6.7, 50.8 and 13.5 m/h were obtained. On another occasion, of two *Calanus* females caught at the surface at the same time, the first experimented with showed a somewhat uncertain, but slightly downward, movement from 11.52 to 20.45 h, finally swimming down at 13.7 m/h just before 20.45; the second, having been kept in a breffit during the day, was put in the wheel at

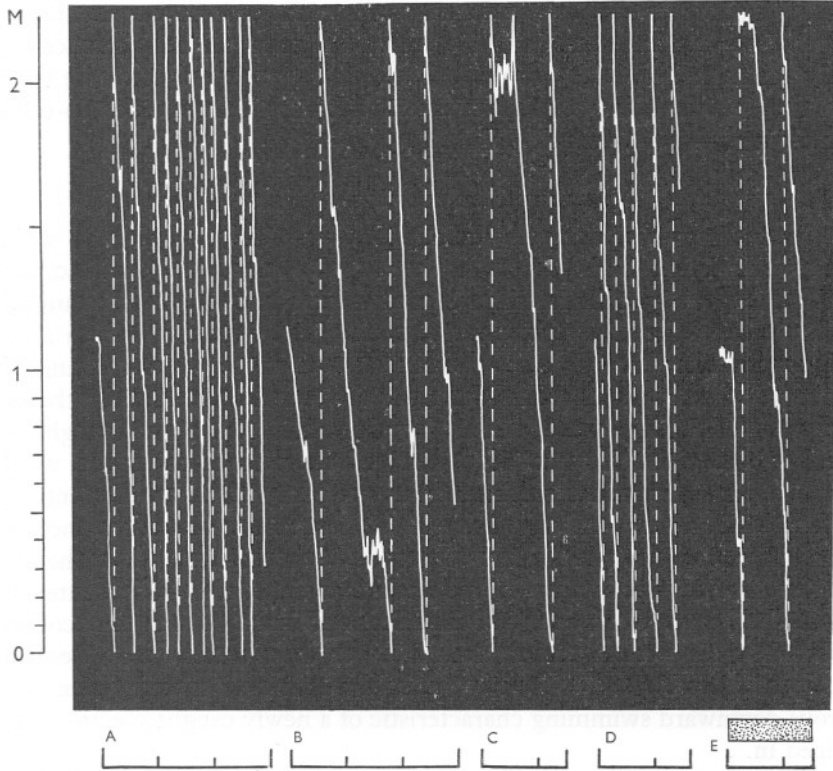


Text-fig. 10. Continuous tracings from kymograph record no. 46 showing the behaviour of *Calanus* in complete darkness (10 July 1951).

20.55 h and swam up consistently at 18.6 m/h. Even the same animal may show differences under conditions which must be considered identical. Record no. 46 shows, in Text-fig. 10, the swimming of a *Calanus* on two occasions separated by 2 hours. In one there is a slight upward resultant and in the other a downward one. At both times the greenhouse was completely blacked out. Record no. 43, which is discussed in the light intensity section (see Text-fig. 23 on p. 442), shows, further, how inconsistent the reaction of an

individual (*Cyclops*) may be to some regulated stimulus such as partial blacking out.

This occurrence of variation where uniformity might be expected makes all the more difficult an interpretation of those cases in which differences might be expected. Three recordings were made with *Calanus* taken from the surface and from 60 m. These were members of the spring brood which, in the Clyde



Text-fig. 11. Reproduction of kymograph tracing from record no. 40. A, *C. finmarchicus* ♂ from 60 m depth, observed from 11.25 to 11.55 h, 10 May 1951; B, *C. finmarchicus* ♀ from surface, observed from 12.10 to 12.40 h, 10 May 1951; C, same *Calanus* as in B but observed from 11.40 to 11.55 h on 11 May 1951; D, same *Calanus* as in A but observed from 11.57 to 12.12 h, 11 May 1951; and E, the same *Calanus* from 12.25 to 12.40 h. The stippled rectangle in E indicates partial blacking out of the greenhouse. The time scale is divided into 10 min periods. See text below.

area, is often to be found partly or almost wholly up at the surface. In the first example, record no. 40 given in Text-fig. 11, a *Calanus* male taken at 60 m swam down at 51.3 m/h and in a second test at 39.1 m/h; a *Calanus* female taken at the surface also swam down but at 14.9 and 17.2 m/h in separate tests. In a second example, not illustrated, a female from 60 m

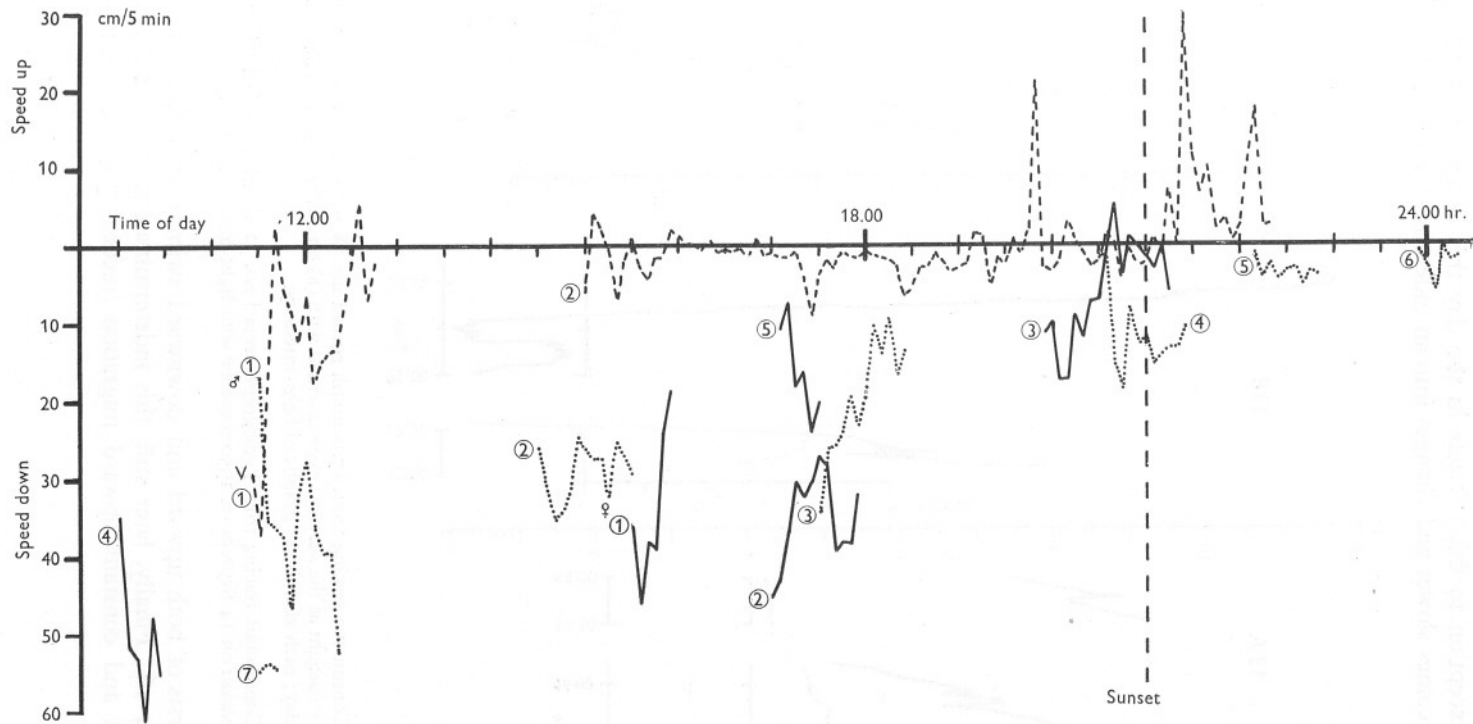
swam down at 1.4 m/h while one from the surface swam up at 35.6 m/h. In both these cases there is an understandable difference between the two types of animal, but in a third example the behaviour of stage V animals both from the surface and from 60 m was identical and consisted in swimming down at about 4 m/h. A further anomaly is revealed if the 12.10–12.40 period of swimming down in record no. 40 is compared with that of a precisely similar animal 2 days later from 12.10 to 12.25 in record no. 41 (p. 445); here a *Calanus* from the surface is found swimming up at 6 m/h. The light intensity on both these occasions was in the region of 6 kilolux. These differences are puzzling, but the possibility, suggested by Bainbridge (1952), must be borne in mind of there being no fundamental difference between the so-called 'surface' and 'deep' forms of *Calanus*.

#### *Changes in behaviour with time*

A series of experiments was performed with *C. finmarchicus* in which records were made each day for 1 h periods at 11.30, 14.30, 17.30 and 20.30 h, and also occasionally at intermediate times and later than 21.30 h. The animals used were caught immediately prior to testing and were taken from about 40 m depth. The aim of these experiments was to see if any change in behaviour occurred as the time for normal vertical migration in the sea approached. A change was generally clearly visible. When first caught the animals would swim down rapidly, but at later periods the speed would become less and less and sometimes an upward migration would eventually take place. There was much variation in the results but never was there an upward migration early in the day or a rapid downward one late in the day. This change of behaviour must be due either to the alteration in light intensity or to an alteration in the reaction of the animals rather than to their becoming moribund or adapted to the circumstances of their confinement, because, when it was possible to test the same animal on the next morning, the same vigorous downward swimming characteristic of a newly caught specimen was indulged in.

Text-fig. 12 shows some of the results of this series of observations depicted in a graphical form. The speed of swimming of each animal has been calculated for successive 5 min periods and expressed as cm/5 min. These values, termed positive if the animal was moving upwards, negative if downwards, are plotted with a scale representing time of day running horizontally. Those values pertaining to the same animal are joined by a distinctive line. Examination of this figure shows clearly the absence of upward migrations early in the day and the absence of marked downward ones late in the day.

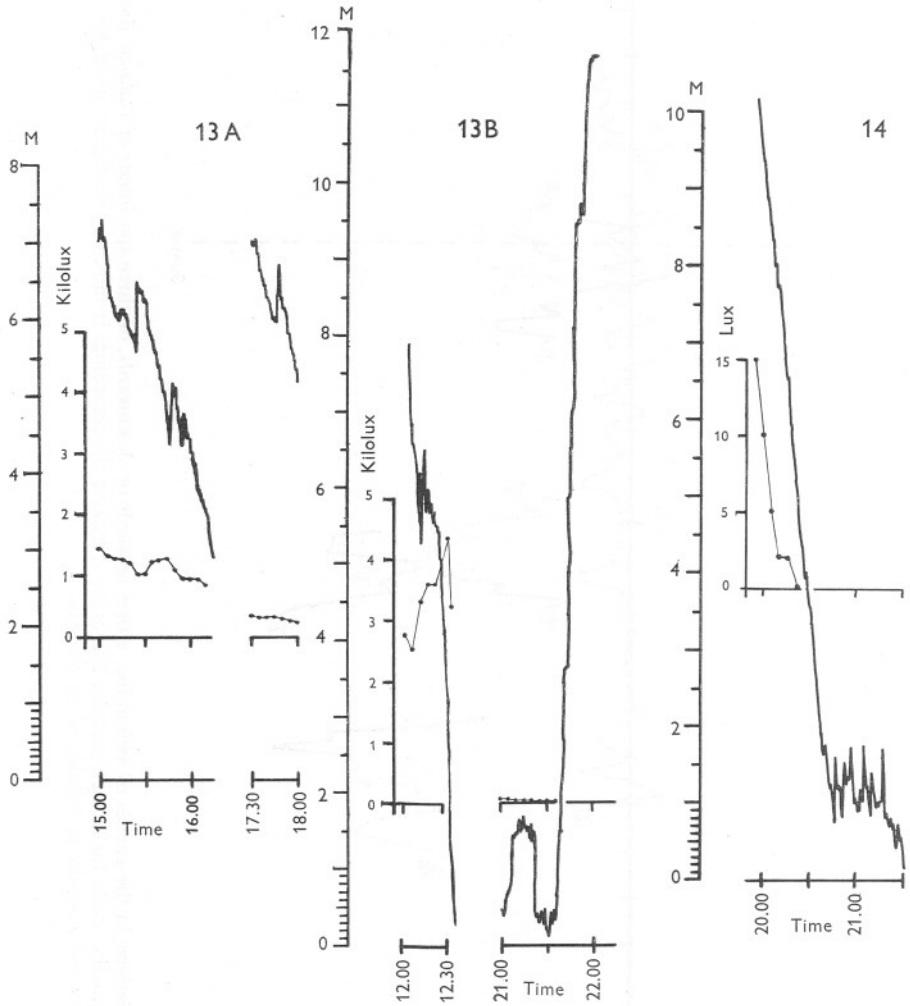
It is clear that the typical daytime behaviour, under the light intensities experienced in the wheel, is a downward swimming, and such is shown in the early parts of a number of records, for example as shown in Text-fig. 13. (The upward swimming of the 'surface' *Calanus* mentioned elsewhere is of



Text-fig. 12. Graph showing the variations in the speeds of swimming, either upwards or downwards, of three specimens of *Calanus finmarchicus* (♂, ♀, and stage V) at successive 5 min intervals during 14 h periods. The order of making the recordings is shown by the figures in circles; ♂ ⑦ and ♀ ④ and ⑤ being taken on the day following the remainder of the records.



course an exception to this.) Later in the day this downward movement generally becomes slower and changes into an indeterminate one comprising

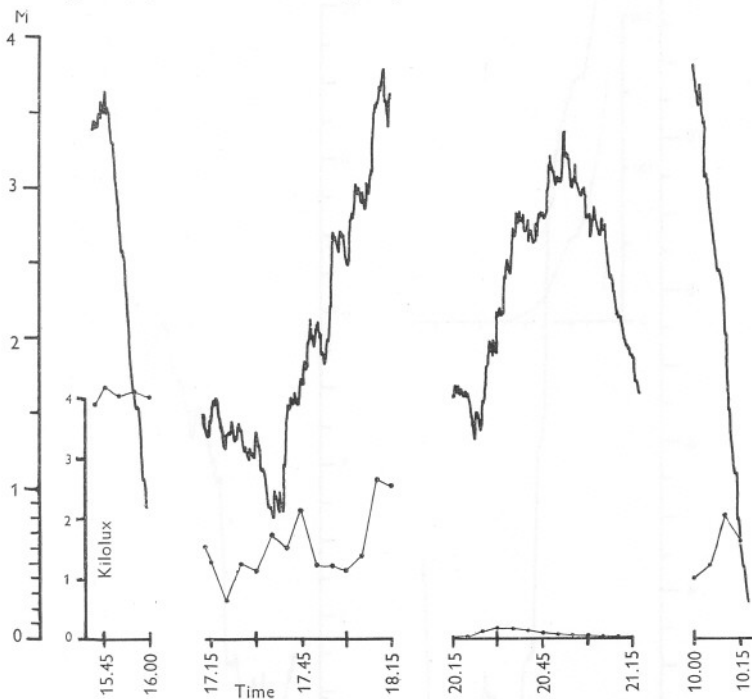


Text-fig. 13. Continuous tracing from kymograph record no. 38 showing the behaviour of a *Calanus* ♂ (caught at the surface) experimented with (A) on 4 May 1951 and (B) on the following day; each also shows graphs of light-intensity.

Text-fig. 14. Continuous tracing from kymograph record no. 32 showing the behaviour of *Calanus* on 14 September 1950 together with light-intensity graph.

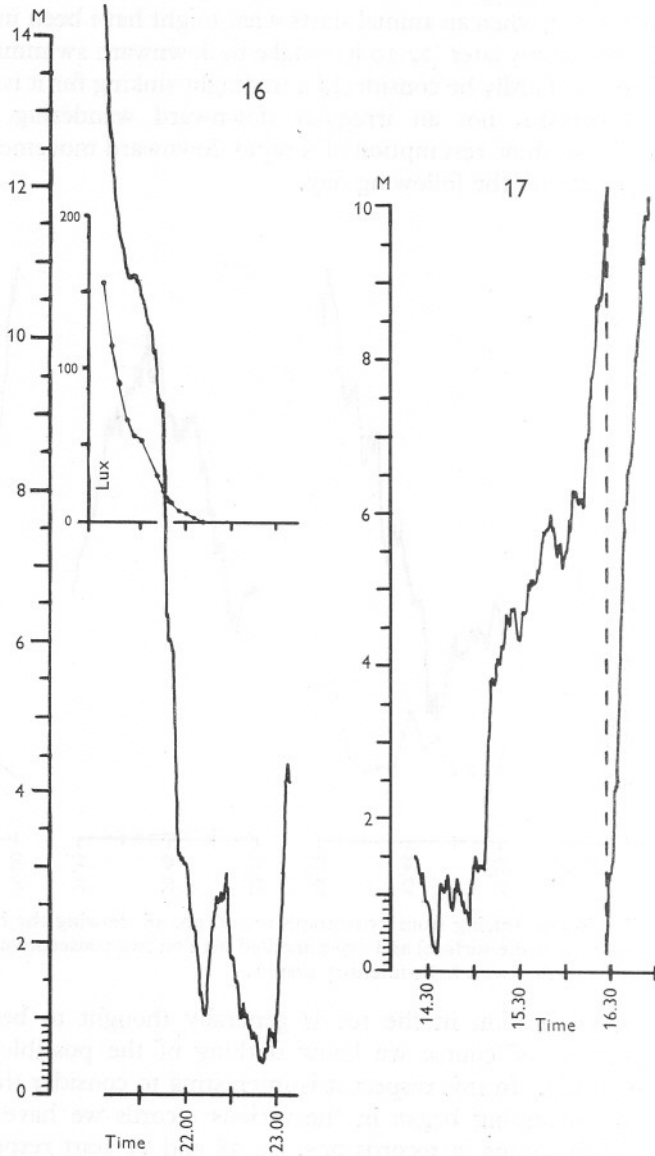
sporadic bursts of both upward and downward swimming, as is shown in Text-figs. 14-16. Finally, later still, this indeterminate movement changes into a rapid and constant upward migration (records 38, 11, and 24 in

Text-figs. 13, 17 and 18). This cycle is not always followed, however, as in no. 29 (Text-fig. 19), when an animal starts what might have been interpreted as the evening rise, only later (22.10 h) to take to downward swimming again. The latter here can hardly be considered a midnight sinking for it is a definite swimming downward, not an irregular downward wandering. Records nos. 25 and 26 also show resumption of a rapid downward movement but, as would be expected, on the following day.



Text-fig. 15. Continuous tracing from kymograph record no. 36 showing the behaviour of *Calanus*  $\delta$  (caught at the surface) and experimented with on two consecutive days (2 and 3 May 1951), together with light-intensity graphs.

The evening migration in the sea is generally thought to begin about sundown, although of course we know nothing of the possible variation between individuals. In this respect it is interesting to consider the times at which upward swimming began in the various records we have obtained. Clear upward migrations in records nos. 24, 38 and 11 start respectively at 21.15, 21.30 and 16.05 h, while the abortive rise in no. 29 is at 21.35 h. Of these times 16.05 is well before sundown at the time of the experiment; 21.15 is 10 min after; 21.30, 1 h; and 21.35, 1 h 20 min after. In no. 32 (Text-fig. 14) the downward migration changed to an indeterminate swimming at 20.45 h, again 1 h 20 min after sundown. In record no. 36 (Text-fig. 15) a similar change occurred at least at 17.15, although possibly earlier,

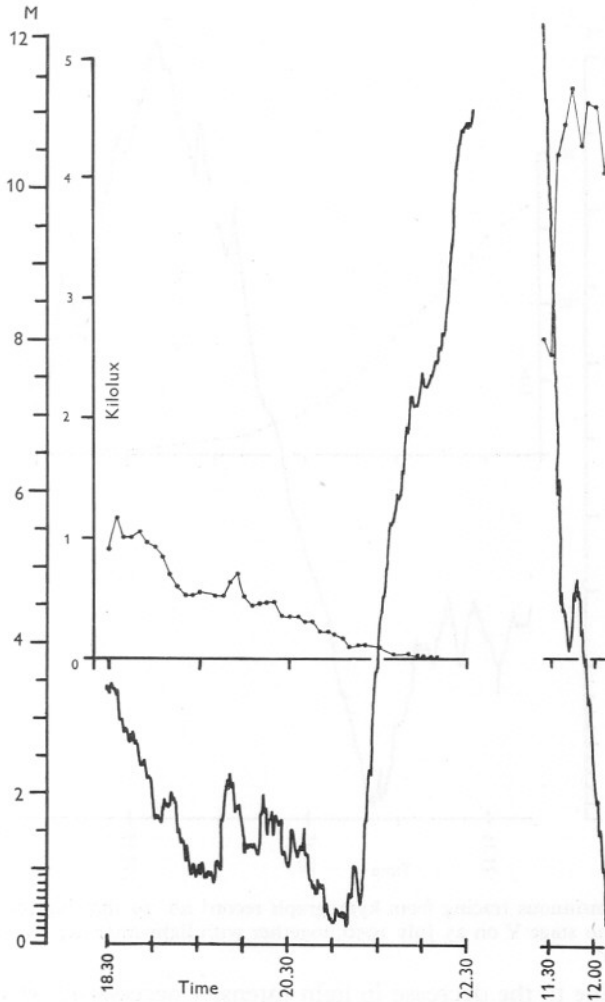


Text-fig. 16. Continuous tracing from kymograph record no. 27 showing the behaviour of *Calanus*, stage V, on 22 July 1950, together with light-intensity record.

Text-fig. 17. Continuous tracing from kymograph record no. 11 showing the behaviour of *Calanus* ♀ on 15 June 1950.

before the second part of the record began; but this particular *Calanus* was one of those found swimming near the surface.

Esterly (1917) gives evidence for the existence of a rhythm of upward migration, persisting even in continued darkness. It must therefore be considered whether those upward migrations obtained appear independent of the

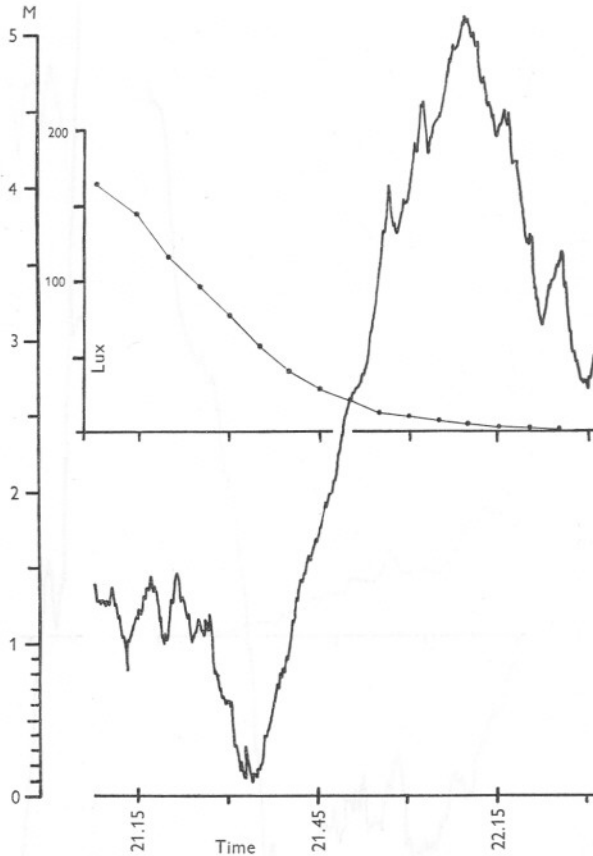


Text-fig. 18. Continuous tracings from kymograph records nos. 24 and 25 showing the behaviour of the same *Calanus* stage V on 21 July 1950 and on the following morning, together with light-intensity records.

light intensity existing at the time or whether migration sets in at a particular intensity rather than at a particular time. In no. 24 (Text-fig. 18), upward movement starts when the light intensity has fallen to 125 lux, in no. 29



(Text-fig. 19) when it is at 60 lux and in no. 38 (Text-fig. 13) not until a zero reading was obtained. In no. 32 (Text-fig. 14) also, indeterminate movement set in at a zero intensity but in no. 36 there was no upward movement even at zero lux. It cannot be said how far all the changes of behaviour enumerated in this section may be due to the passage of time, and how far



Text-fig. 19. Continuous tracing from kymograph record no. 29 showing the behaviour of *Calanus* stage V on 25 July 1950, together with light-intensity record.

they may be due to the decrease in light intensity necessarily associated with the advancing time of day. It seems possible, however, as upward migrations could not be induced by blacking out during the daytime, that there may be a rhythm in the capacity to respond to a great reduction in light intensity. This would account for the lateness of those migrations that were obtained, as the intensity just below the surface must reach that equivalent to the sundown intensity at 50 or 60 m depth, much later in time.

It is known from other experiments that the behaviour of *Calanus*, in particular, changes with prolonged captivity. This was again found to be so and a greater proportion of animals swimming up was always found amongst samples that had been obtained several days previously. For this reason animals were experimented with as soon as possible after the time of capture.

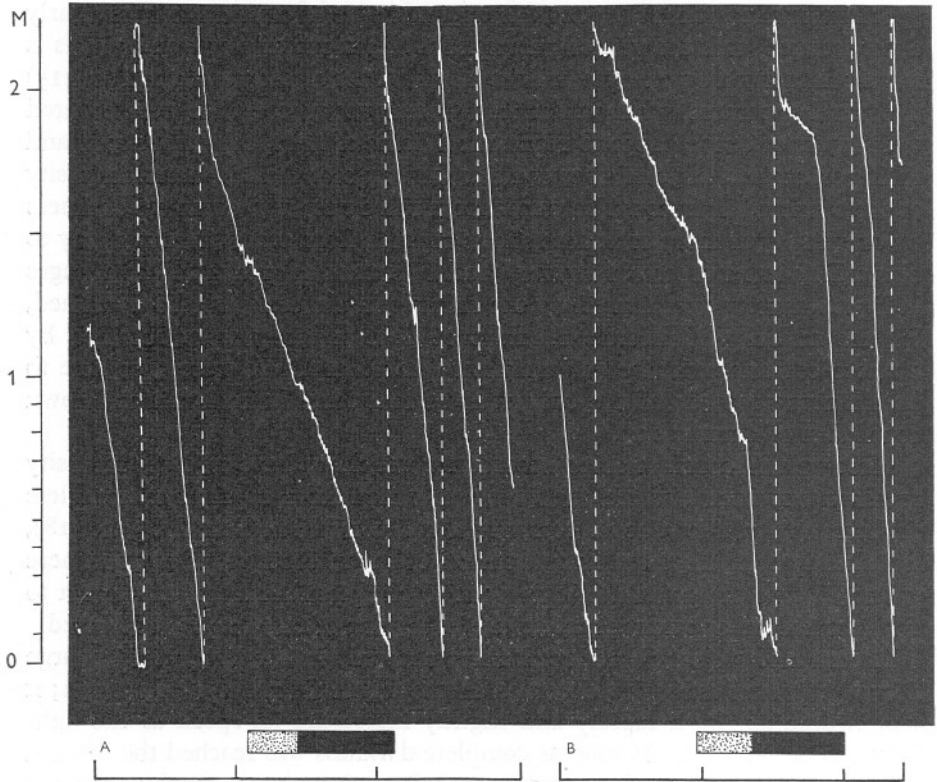
*Correlation of behaviour with changes in light intensity*

It has for a long time been considered, and indeed from time to time clearly demonstrated, that the vertical migrational behaviour of plankton animals is in part influenced by changes in light intensity. As already explained (p. 415) the wheel apparatus was mounted in a greenhouse whose sides were covered with layers of tissue paper to represent a dome of light, brightest above and falling off at the sides, similar to conditions in the sea; then the actual intensity of the overhead light could be varied from full daylight to complete darkness by drawing over more and more sheets ranging from white, through grey to black. This latter process, however, cannot be made as gradual as the changes in light intensity experienced by the animals in the sea. As also explained, for the majority of records, readings of light intensity were obtained by a photo-cell and sensitive galvanometer (see p. 415). We were thus able to obtain many records of the behaviour of individual animals under known conditions of illumination.

First we will consider seven records for which we have no light intensity readings but during which the light was varied from daylight to complete darkness as explained above. Record no. 1, illustrated in Text-fig. 3 (p. 418), has already been discussed as an example of the method on p. 419, and here we noted that the gradual blacking out to complete darkness and return to daylight had no apparent effect on the behaviour of the *Calanus* then used.

Record no. 9 (not reproduced here) showed, in the first part, a large *Meganyctiphanes norvegica*, observed in the wheel from 11.38 to 12.45 h; it was swimming down rapidly and slightly increasing its speed as the light began to be reduced. As soon as complete darkness was reached the descent was checked and then, after some up-and-down movement, continued but at a slower rate and with a number of pauses. A new part of the record was started at 14.42 with another specimen of *M. norvegica*; one of medium size. This time the record started in complete darkness and the animal began by making almost as rapid a descent as did the other specimen in full daylight, but after about 15 min its behaviour changed, its descent being broken by many short up-and-down movements. When full light was restored it descended again but less rapidly than in the dark, and it was seen to pause for a time at regular intervals which, from their distance apart, must have corresponded to the position of the doors at the junctions between the sections of the wheel; although transparent they may produce a slight shadow.

In no. 31 (also not reproduced), *Calanus finmarchicus* began by descending, then went up for a short distance and then down more steeply. Descent now continued till the end of the record at 16.00 h, but during the period in which the light was reduced—either to a partial blackout or to complete darkness—the speed of descent was also reduced; on full daylight being restored again the speed of descent increased once more.

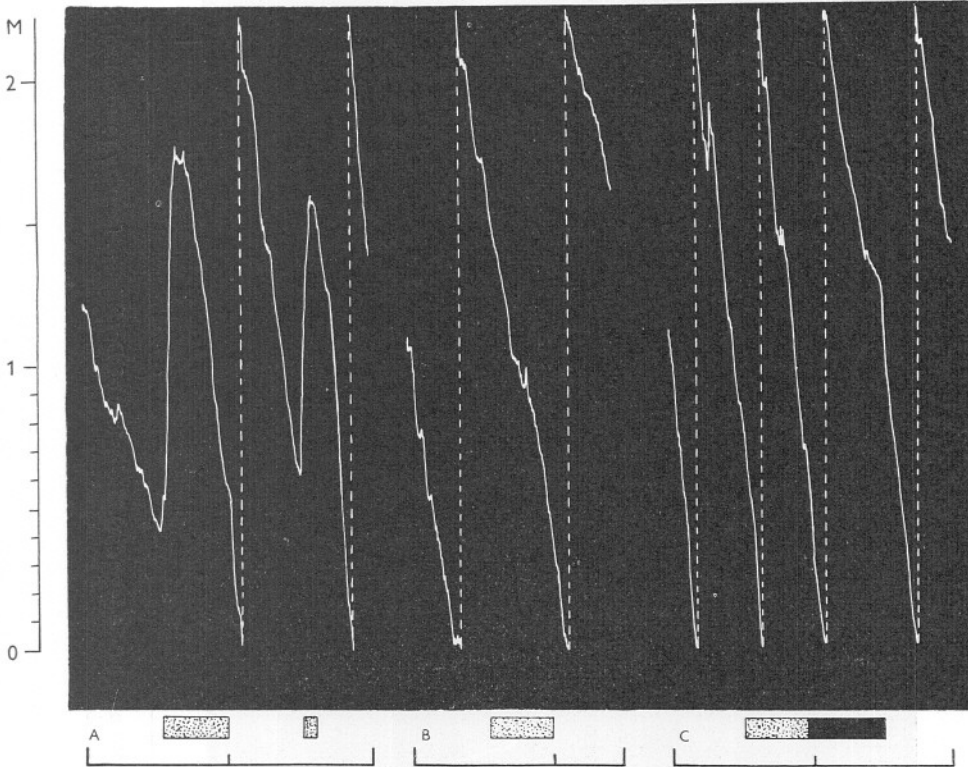


Text-fig. 20. Reproduction of kymograph tracing from record no. 42. A, behaviour of a specimen of *Cypris* sp. observed from 16.35 to 17.05 h on 15 May 1951. B, the same specimen from 10.20 to 10.45 h on 16 May 1951. The stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See text below.

Text-fig. 20 shows record no. 42 which follows the descent of a freshwater *Cypris* (species undetermined). Here the recording paper is moving at 5 mm per min. The first part, made from 16.35 to 17.05 h on 15 May 1951, shows a similar reduction in the speed of downward movement during the partial and complete blackout, as observed in the previous record for *Calanus*; the second part, however, 10.20–10.45 h on the following day, shows a reduction

of speed before the blackout and an increase in speed again before the daylight was restored.

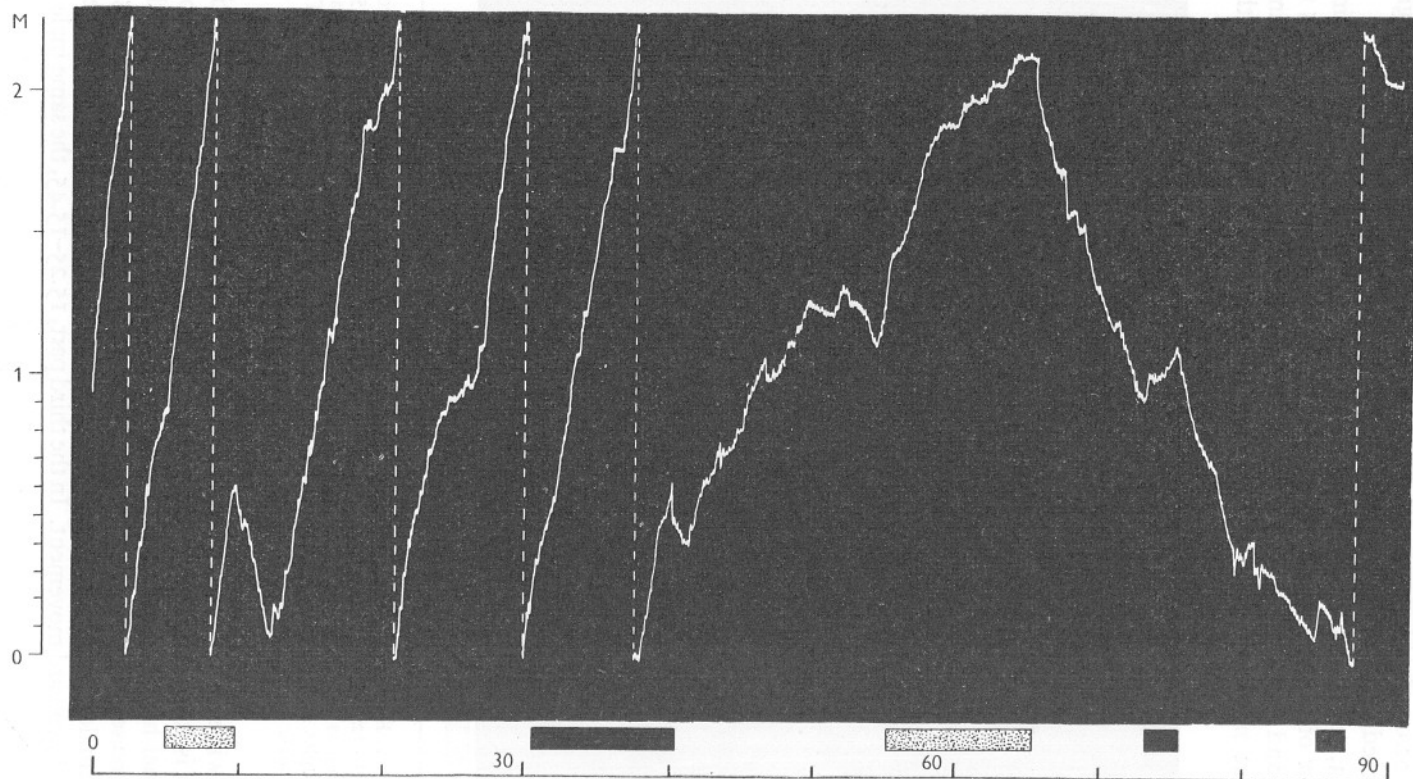
No. 43, made also on 16 May 1951, shows in Text-fig. 21 the varying behaviour of freshwater *Cyclops*. The first part is with a male from 12.25 to 12.45 h, and each time a partial blackout is introduced the downward swimming is at once changed to an upward movement; on the first occasion the



Text-fig. 21. Kymograph tracing from record no. 43. A, behaviour of *Cyclops* sp. ♂ from 12.25 to 12.45 h on 16 May 1951; B, *Cyclops* sp. ♀ from 15.10 to 15.25 h on 16 May 1951; C, same as in A from 15.25 to 15.45 h also 16 May 1951. The stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See adjacent text.

animal swims up 1.35 m and then begins to descend again, although the partial blackout is still maintained, while the next time the full light is restored before the animal has climbed more than 1 m and it descends again as soon as the light increases. The second part of the record is made from 15.10 to 15.25 h with a female *Cyclops*, but here the partial blackout appears to have no effect upon its downward movement. In the third part, 15.25–15.45, the same male as





Text-fig. 22. Kymograph tracing from record no. 44 (16 May 1951). Behaviour of *Daphnia* sp. from 17.10 to 18.40 h. Stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See text opposite.

was used in the first part is again subjected to a partial blackout which is then followed by complete darkness; this time its behaviour appears quite unaffected.

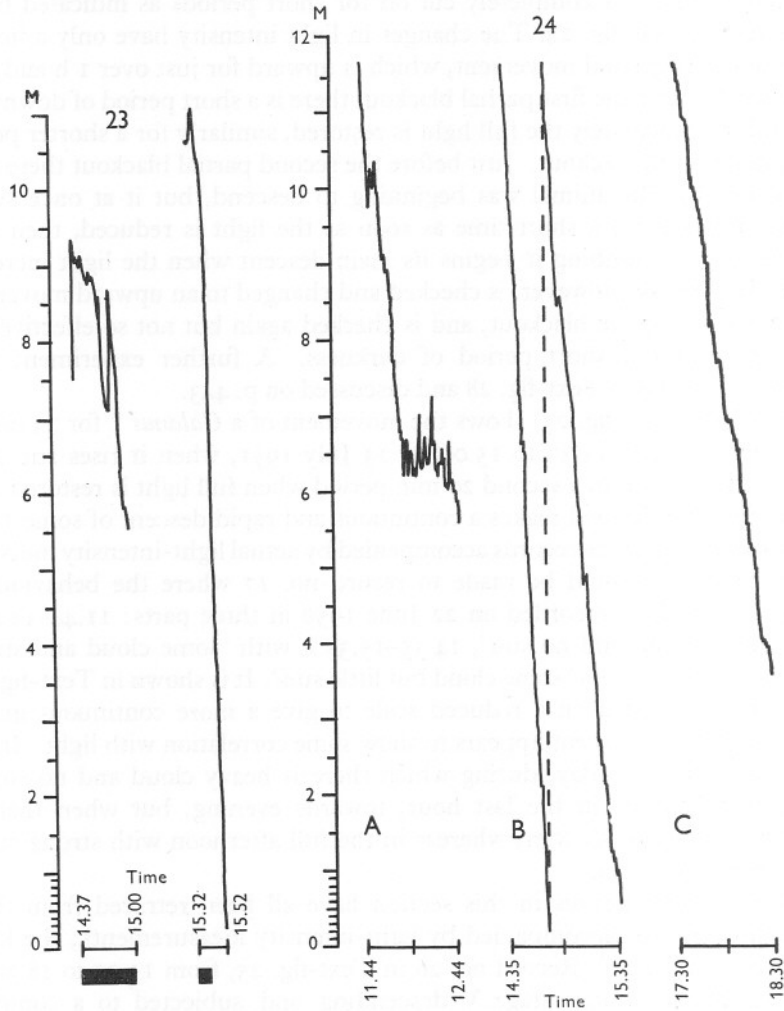
No. 44 shows the behaviour of *Daphnia* species from 17.10 to 18.40 h with the light reduced or completely cut off for short periods as indicated below the graph in Text-fig. 22. The changes in light intensity have only a limited effect upon its general movement, which is upward for just over 1 h and then downward. After the first partial blackout there is a short period of downward swimming immediately the full light is restored, similarly for a shorter period after the first full blackout. Just before the second partial blackout there is an indication that the animal was beginning to descend, but it at once swims quickly upward for a short time as soon as the light is reduced, then after a slackening in climbing it begins its main descent when the light increases again. Its descent, however, is checked and changed to an upward movement at a second complete blackout, and is checked again but not so effectively at the third and last short period of darkness. A further experiment with *Daphnia* is shown in Text-fig. 28 and discussed on p. 443.

Record 47 (Text-fig. 23) shows the movement of a *Calanus* ♀ for 23 min in complete darkness, 14.37 to 15.00 on 13 July 1951, when it rises and falls, rises and falls; then in a second 20 min period when full light is restored after 10 min the same animal makes a continuous and rapid descent of some 11 m.

Before passing to the records accompanied by actual light-intensity measurements reference should be made to record no. 17 where the behaviour of a *Calanus* female is recorded on 22 June 1950 in three parts: 11.44–12.44 h with 'heavy cloud and no sun', 14.35–15.35 h with 'some cloud and strong sun' and 17.30–18.30 h 'some cloud but little sun'. It is shown in Text-fig. 24, having been retraced on a reduced scale to give a more continuous graph. Here the speed of descent appears to show some correlation with light. In the first hour, about midday, during which there is heavy cloud and no sun, it descends only 6 m; in the last hour, towards evening, but when there is a little sun, it descends 8 m; whereas in the full afternoon with strong sun it descends nearly 23 m.

The remaining figures in this section have all been retraced from those kymograph records accompanied by light-intensity measurements; the latter are shown with them. Record no. 26 in Text-fig. 25, from 15.00 to 18.30 on 22 July, shows *Calanus* stage V descending and subjected to a complete blackout; its speed of descent is appreciably reduced during darkness and greatly increased again as soon as light is restored. Text-figs. no. 26 and 6 (pp. 443 and 423) are parts of the same record, no. 28, taken on 25 July 1950; the first part from 17.20 to 18.20 h recording *Calanus* stage V and the second part 18.30 to 21.00 h recording *Labidocera wollastoni* ♂. In the second part the light intensity is a good deal lower than in the first part and is shown on a different scale. In each case as the light declines so the downward movement of the animal is checked.

Text-fig. 27 shows the behaviour of a *Calanus* stage V in record no. 37, from 14.00 to 16.30 and 17.00 to 17.30 h on 3 May 1951, and from 10.00 to 10.50 h on the following day. In the first period it begins by climbing some



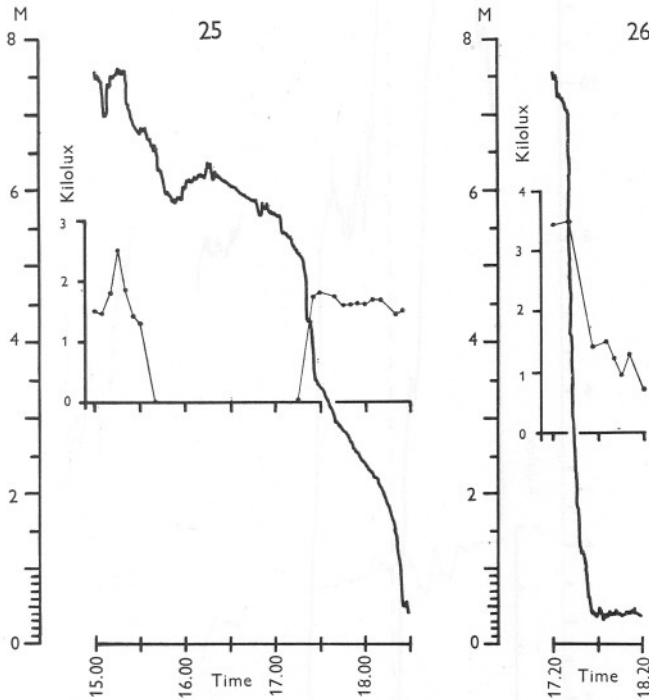
Text-fig. 23. Continuous tracing from kymograph record no. 47 (13 July 1951) showing behaviour of *Calanus* ♀ with artificial blackout.

Text-fig. 24. Continuous tracing from kymograph record no. 17 (22 June 1950) showing the behaviour of *Calanus* ♀ under different conditions of cloud and sunlight (see text, p. 441): A, heavy cloud, no sun; B, some cloud, strong sun; C, some cloud, little sun.

9 m and then makes an equally rapid descent of 18 m when there is a check for half an hour followed by a further rapid descent; its movement shows no correlation at all with the light intensity which falls from 5.6 to 4 kilolux with

fluctuations due to passing clouds. It is likewise impossible to see any obvious correlation in the other two periods.

Text-fig. 28 shows record no. 39 made with *Daphnia* sp. on 7 May 1951. In the first period of half an hour the light is raised from 4 to 8 kilolux and then down to darkness; in the second period of half an hour it is raised from darkness to 6 kilolux and back to darkness again, but on neither occasion does the downward movement of the animal appear to be affected. In the

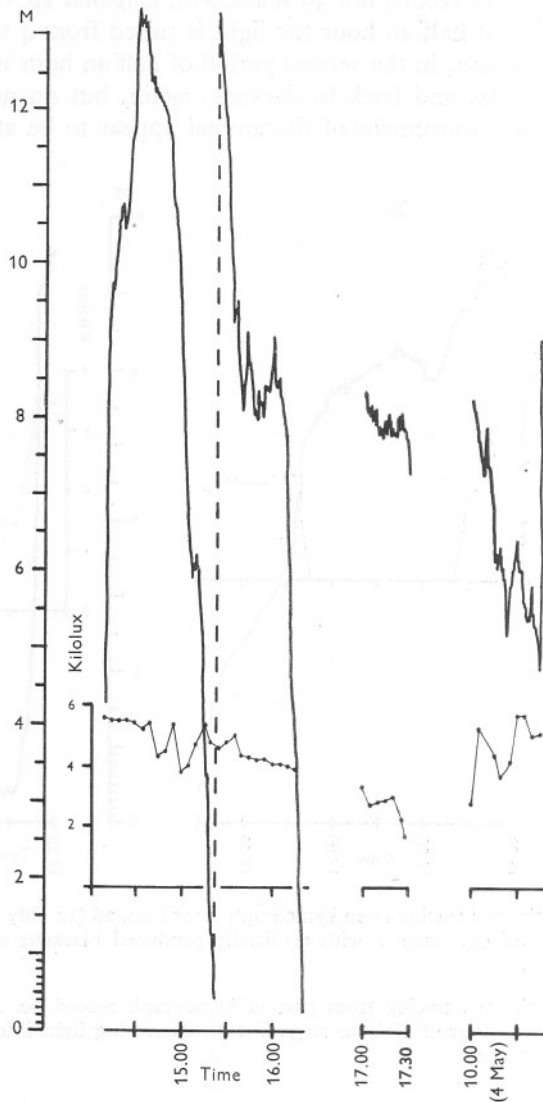


Text-fig. 25. Continuous tracing from kymograph record no. 26 (22 July 1950) showing the behaviour of *Calanus* stage V with artificially produced blackout as shown in light-intensity graph.

Text-fig. 26. Continuous tracing from part of kymograph record no. 28 (25 July 1950) showing the behaviour of *Calanus* stage V with decreasing light intensity as shown in accompanying graph.

third period, however, from 14.45 to 16.10, the behaviour of the same animal is markedly different; as soon as the light falls from 6 kilolux to zero the *Daphnia* climbs for some 6 m and then, when the light is brought back to 6 kilolux, it descends as steeply for nearly 15 m only to start climbing again as the light is again dimmed; finally it falls yet again as the light is once more increased.

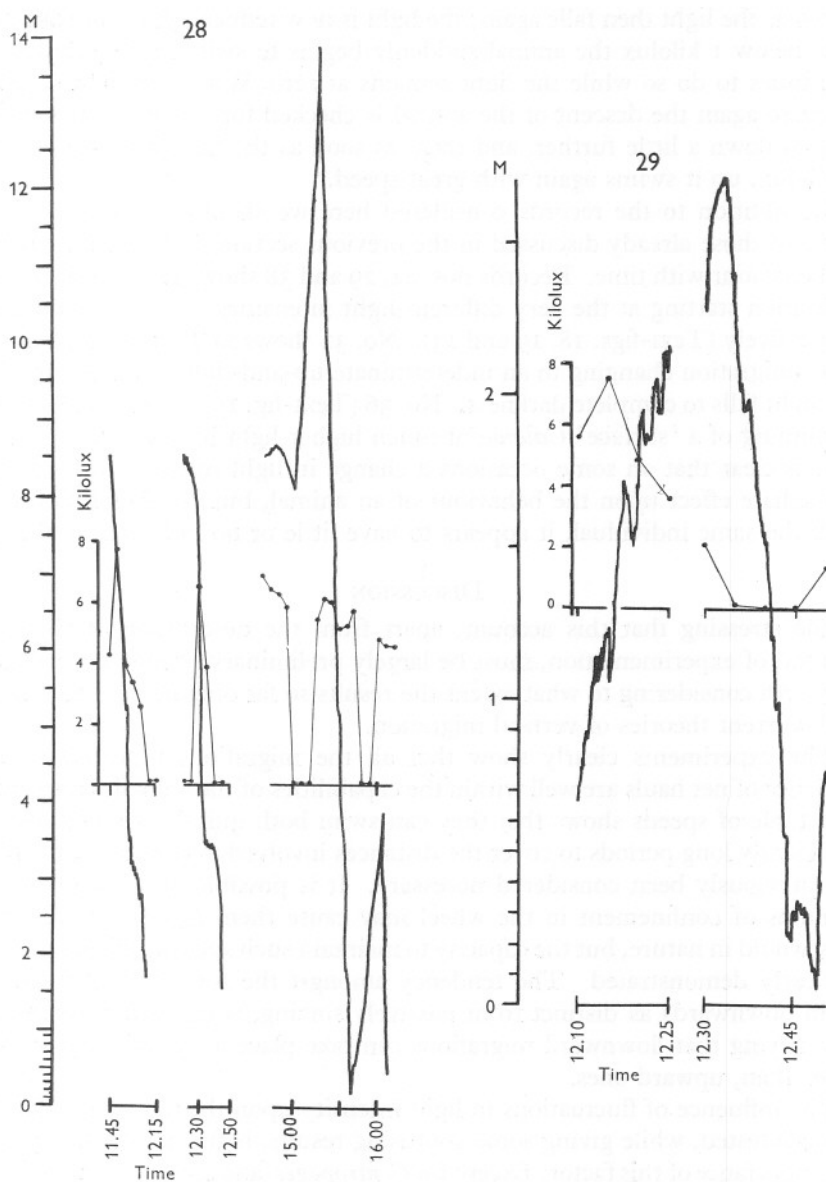
Record no. 41, illustrated in Text-fig. 29, is particularly interesting because it is made with one of the spring brood of *Calanus*, a female taken at the



Text-fig. 27. Continuous tracing from kymograph record no. 37 (3 and 4 May 1951) of *Calanus* stage V showing behaviour which appears to have no correlation with accompanying light-intensity graph.

surface in bright light at Millport on 12 May 1951. The record is from 12.10 to 12.50 with a 5 min break at 12.25. The animal starts by climbing steeply while the light is increased from 5.6 to 7.5 kilolux and continues to





Text-fig. 28. Continuous tracing from kymograph record no. 39 (7 May 1951) showing the behaviour of *Daphnia* sp. under artificial control of light as shown in light-intensity graph. See text, p. 443.

Text-fig. 29. Continuous tracing from kymograph record no. 41 (12 May 1951) showing the behaviour of *Calanus* ♀ caught at the surface and placed under artificially controlled light as shown in accompanying graph. See text, p. 444.

climb as the light then falls again; the light is now reduced gradually and as it falls below 1 kilolux the animal suddenly begins to swim rapidly down and continues to do so while the light remains at zero. When the light starts to increase again the descent of the animal is checked for a few minutes before it goes down a little further, and then, as soon as the light intensity reaches 1 kilolux, up it swims again with great speed.

In addition to the records considered here we should also refer back to some of those already discussed in the previous section dealing with changes of behaviour with time. Records nos. 24, 29 and 38 show an upward evening migration starting at the very different light intensities of 125, 60 and 0 lux respectively (Text-figs. 18, 19 and 13). No. 32 shows in Text-fig. 14 a downward migration changing to an indeterminate up-and-down movement when the light falls to complete darkness. No. 36 (Text-fig. 15) shows some upward swimming of a 'surface' *Calanus* at much higher light intensities.

It is clear that on some occasions a change in light intensity will have an immediate effect upon the behaviour of an animal, but at other times, even with the same individual, it appears to have little or no influence at all.

#### DISCUSSION

While stressing that this account, apart from the description of this new method of experimentation, must be largely preliminary, it may nevertheless be worth considering to what extent the results so far obtained are consonant with current theories of vertical migration.

The experiments clearly show that all the migrations reported on the evidence of net hauls are well within the capabilities of the animals concerned. The table of speeds shows that they can swim both quickly enough and for sufficiently long periods to cover the distances involved in even less time than has previously been considered necessary. It is possible of course that the stimulus of confinement in the wheel may cause them to swim faster than they would in nature, but the capacity to maintain such speeds for long periods is clearly demonstrated. The tendency amongst the forms investigated, to swim downwards as distinct from passively sinking, is especially interesting in verifying that downward migrations can take place as quickly as, or even faster than, upward ones.

The influence of fluctuations in light intensity upon the movements of the animals tested, while giving some confusing results, is further evidence as to the importance of this factor. Except for *Centropages* (and specimens of *Calanus finmarchicus* found at the surface in daylight during the spring), all the animals tested exhibited a negative reaction towards strong light and swam downwards and away from it at some of the highest speeds. A gradual reduction in intensity is usually accompanied by a diminution in this reaction and at low values in the evening it may be replaced by a migration upwards and towards the light. The artificial removal of all light during the daytime

does not on most occasions evoke an upward movement, and so it is clear that there is not a negative geotaxis which comes into play in the absence of the inhibiting effect of strong light; there is clearly demonstrated, again on most but not on all occasions, a definite positive migration towards the source of low light intensities. If these latter reactions should occur in the sea then most animals, if they were reacting in the same way, would automatically accumulate in regions of a certain light intensity (which may conveniently be termed the optimum) and move up to the surface as the level of this intensity rises with the setting sun; this indeed is as postulated in the hypotheses of Michael (1911) and Russell (1926). The movement towards the optimum conditions is shown generally not to be a random or 'indirect' one but, in most of the larger forms, to consist of sustained directional swimming either up or down; this appears to be replaced by a 'hop-and-sink' type of movement when the region of optimum conditions has been reached.

This simple picture of daily change is not always true, however, and sometimes (as with the upward swimming of some of the surface members of the spring brood of *Calanus*) it may be completely reversed. *Centropages* exhibited this reverse type of behaviour whenever we observed it, and the effect of this in the sea would be to keep individuals near the surface during the hours of daylight. Even apart from these regular exceptions, however, the variation met with among the different animals experimented with shows that their migrations cannot be governed by changes in light intensity alone. In record no. 43 (p. 439), for example, we have seen that a partial blackout at midday caused a *Cyclops* male to swim upwards at once, whereas in the afternoon of the same day both a partial and a complete blackout had no effect whatever on the same individual. Similarly, with *Daphnia* in record no. 39 (Text-fig. 28, p. 445) marked alterations in light intensity *in the morning* on this occasion had no effect on its behaviour, whereas *in the afternoon* a complete blackout caused the same individual to swim rapidly upwards on two occasions. These results clearly show that a great many more observations are required before we can determine the relative importance of light and other factors in the regulation of vertical migrational behaviour.

#### SUMMARY

An apparatus is described in which it is possible to observe and make continuous records of the vertical migrations of plankton animals, if necessary through hundreds of metres of water.

Records obtained with this apparatus are described and discussed.

It is considered that vertical migration in the sea is predominantly a direct up-and-down swimming with only a few smaller forms moving in an indirect way. Downward migration is shown to be an active swimming and not a passive sinking.

The speeds of vertical swimming of various animals are measured and listed.

Much variation and 'individuality' is shown amongst the animals studied but a pattern of behaviour consisting of a rapid movement downwards and away from the bright light during the day, followed by a diminution of this as the light intensity falls and an eventual movement upwards under low light intensities, appears to be usual. Upward migration cannot generally be induced by blacking out during the daytime and is assumed to be a positive migration towards low light intensities rather than a negative geotaxis.

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#### ADDENDUM

Since going to press a very interesting paper by Professor J. E. Harris has appeared, on the 'Physical factors involved in the vertical migration of plankton' (*Quart. J. micr. Sci.*, Vol. 94, pp. 537-50, Dec. 1953). We cannot agree that all his conclusions, based upon the study of *Daphnia magna*, for example those concerning a negative geotaxis, can apply to all plankton animals which migrate vertically. It may however be significant that the animals which we found at times swimming horizontally were mainly those having, like his *Daphnia*, compound eyes: euphausians and decapod larvae (see p. 424).

## THE VAPOUR PRESSURE AND OSMOTIC EQUIVALENCE OF SEA WATER

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Sea water is a complex solution in which the principal ions are sodium, potassium, calcium, magnesium, chloride and sulphate. The vapour pressure (V.P.) of such a solution can be calculated approximately by making the assumption that each salt contributes to the vapour pressure lowering in amount proportional to its concentration, but such a calculation would ignore the interactions between the various ions. The theory of these interactions has been worked out only for very dilute solutions and it is, therefore, better to rely on direct experimental determinations. Measurements have now been made by the isopiestic vapour-pressure method (Robinson & Sinclair, 1934), in which samples of sea water are equilibrated with sodium chloride solutions until they have the same vapour pressure. The results are expressed in terms of chlorinities of sea water and molalities (moles per kilogram of  $H_2O$ ) of sodium chloride solution which have the same vapour pressure. It is hoped that the results will be of use to physiologists who have occasion to make up salt solutions equivalent to sea water.

### EXPERIMENTAL

Three samples of sea water were used:

(1) Eau de mer normale, P17, 31 October 1948, % Cl = 19.386; found by gravimetric analysis, 19.408%\* (i.e. by precipitation as silver halide, calculated as silver chloride).

(2) An artificial sea water made up as follows:

	g/kg solution		g/kg solution
Sodium chloride	28.85	Calcium chloride	1.244
Potassium chloride	0.811	Magnesium sulphate	3.649
Magnesium chloride	2.633		

The composition is quoted in terms of anhydrous salt. Found by titration against Eau de mer normale: % Cl = 20.58; by gravimetric analysis: 20.62.

(3) Sea water taken from the Straits of Singapore. Found by titration against Eau de mer normale: % Cl = 17.27; by gravimetric analysis: 17.35.

\* This figure includes the weight of bromine in excess of the equivalent of chlorine. If allowance is made for this, and the new figure divided by 1.00045 to allow for change in atomic weights since 1937, the gravimetric chlorinity becomes 19.390%. Similarly the gravimetric chlorinities of samples 2 and 3 become 20.60% and 17.33%, agreeing with the titration chlorinities even better than the author claims.—(Ed.)



The densities of these three solutions were found to be  $d_4^{25} = 1.0233_4$ ,  $1.0249_8$  and  $1.0206_2$ , respectively. The results of this investigation are all expressed in terms of chlorinities as found by titration.

In the isopiestic method samples of sea water are weighed in two platinum dishes, and samples of a NaCl solution of known composition are weighed out into two other platinum dishes. The four dishes are then placed on a copper block in a desiccator which is evacuated and rocked gently in a thermostat at  $25^\circ\text{C}$  for 2 days. During this interval water distils from one solution to another until equilibrium is reached when the concentrations of all four solutions are such that the vapour pressures of all four are equal. The dishes are then weighed again and, from the loss or gain in weight, the final concentrations of the solutions are calculated. These solutions of equal vapour pressure are said to be isopiestic and the ratio,  $R$ , of the concentration of the sodium chloride solution to that of the sea water is called the *isopiestic ratio*. If the vapour pressures of solutions of sodium chloride are known as a function of their concentration, and tables of such vapour pressures have been published (Robinson, 1945; Stokes & Levien, 1946), then the vapour pressure of the sample of sea water can be calculated for a particular concentration. Thus, in one experiment, a sea-water solution of 20.02‰ chlorinity was found to have the same v.p. as 0.5889 M-NaCl solution; the relative molal v.p. lowering of NaCl,  $(p^0 - p)/mp^0$ , where  $p^0$  is the v.p. of pure water and  $p$  is the v.p. of NaCl solution of molality  $m$ , is 0.03290 at 0.5 M and 0.03292 at 0.6 M. It may be taken as 0.03292 at 0.5889 M and the relative vapour pressure lowering  $(p^0 - p)/p^0$  or  $\Delta p/p^0$ , as  $0.03292 \times 0.5889 = 0.01939$ . If the v.p. is required we put  $p^0 = 23.756$  mm at  $25^\circ$  so that  $(p^0 - p) = 0.461$  mm and  $p = 23.295$  mm. This is also the v.p. of 20.02‰ Cl sea water.

The experiment is repeated at a number of different concentrations to investigate the change in v.p. over a range of concentrations. Fourteen measurements were made using the three sea-water samples and the results are given in Table I. Over the range 9–22‰ Cl, the ratio of NaCl molality to sea-water chlorinity can be expressed as

$$R = 0.02782 + 0.000079 (\% \text{ Cl}),$$

a formula which expresses the results in Table I with an average deviation of 0.18%.

#### DISCUSSION

The above equation can be used to calculate values of  $R$  at round values of the chlorinity between 10 and 22‰. These are recorded in Table II. The third column of the table gives the molality of NaCl solution of the same v.p. as the sea water whose chlorinity is given in the first column. A very careful study has been made (Robinson, 1945) of the ratio of the molalities of NaCl and KCl solutions which are isopiestic (i.e. have the same v.p.), and it is therefore possible to give in the fourth column the molalities of KCl solutions

TABLE I. MOLALITIES OF SODIUM CHLORIDE SOLUTIONS AND CHLORINITIES OF SEA WATER OF THE SAME VAPOUR PRESSURE

Sample	M-NaCl	% Cl	R	
			Observed	Calculated
1	0.4296	14.79	0.02905	0.02899
	0.5454	18.62	0.02929	0.02929
	0.5847	19.90	0.02938	0.02939
	0.6185	21.01	0.02944	0.02948
2	0.2700	9.44	0.02860	0.02857
	0.3774	13.08	0.02885	0.02885
	0.4220	14.60	0.02890	0.02897
	0.4350	15.04	0.02892	0.02900
	0.4737	16.35	0.02897	0.02911
	0.5492	18.72	0.02934	0.02930
	0.5889	20.02	0.02942	0.02940
	0.6171	20.96	0.02944	0.02948
3	0.4628	15.87	0.02916	0.02907
	0.5753	19.52	0.02947	0.02936

$$R = \text{M-NaCl} / \% \text{ Cl}$$

TABLE II. VAPOUR PRESSURE AND OSMOTIC EQUIVALENCE OF SEA WATER AT 25° C

% Cl	R	NaCl	KCl	CaCl <sub>2</sub>	MgCl <sub>2</sub>	MgSO <sub>4</sub>	Na <sub>2</sub> SO <sub>4</sub>	Sucrose	Urea	V.P. lowering	Osmotic pressure (atm.)
10	0.02861	0.2861	0.2908	0.2039	0.2005	0.5056	0.2374	0.5065	0.5400	0.00946	12.87
11	0.02869	0.3156	0.3211	0.2240	0.2199	0.5597	0.2643	0.5560	0.5965	0.01042	14.19
12	0.02877	0.3452	0.3516	0.2441	0.2393	0.6138	0.2918	0.6053	0.6534	0.01139	15.51
13	0.02885	0.3751	0.3825	0.2642	0.2588	0.6675	0.3196	0.6546	0.7112	0.01237	16.85
14	0.02893	0.4050	0.4134	0.2841	0.2780	0.7206	0.3477	0.7040	0.7695	0.01334	18.19
15	0.02901	0.4352	0.4447	0.3043	0.2975	0.7738	0.3762	0.7534	0.8285	0.01433	19.55
16	0.02908	0.4653	0.4760	0.3243	0.3165	0.8264	0.4051	0.8025	0.8880	0.01532	20.91
17	0.02916	0.4957	0.5077	0.3445	0.3356	0.8786	0.4347	0.8516	0.9482	0.01631	22.28
18	0.02924	0.5263	0.5397	0.3645	0.3546	0.9300	0.4648	0.9008	1.010	0.01732	23.66
19	0.02932	0.5571	0.5719	0.3845	0.3738	0.9803	0.4954	0.9497	1.071	0.01833	25.06
20	0.02940	0.5880	0.6043	0.4044	0.3929	1.028	0.5264	0.9982	1.133	0.01936	26.47
21	0.02948	0.6191	0.6370	0.4243	0.4122	1.076	0.5578	1.047	1.197	0.02039	27.89
22	0.02956	0.6503	0.6698	0.4440	0.4313	1.123	0.5896	1.095	1.260	0.02142	29.33

The column headed v.p. lowering gives the relative pressure lowering  $\Delta p/p^0 = (p^0 - p)/p^0$ , where  $p$  is the vapour pressure of the sea water and  $p^0$  is the vapour pressure of pure water.  $p^0 = 23.756$  mm at 25° C.

isopiestic with sea water. Similar comparisons of CaCl<sub>2</sub> with NaCl (Stokes, 1945*a*), MgCl<sub>2</sub> with KCl (Robinson & Stokes, 1940; Stokes, 1945*b*), MgSO<sub>4</sub> with KCl (Robinson & Jones, 1936), Na<sub>2</sub>SO<sub>4</sub> with KCl (Robinson, Wilson & Stokes, 1941), sucrose with KCl (Robinson & Sinclair, 1934; Scatchard, Hamer & Wood, 1938; Robinson, Smith & Smith, 1942) and urea with NaCl (Scatchard *et al.*, 1938) have been made, enabling us to give in the next six columns of Table II, molalities of various solutions of the same v.p. as sea

water. The solutions whose concentrations are given in any one row of Table II have the same v.p. and the same (thermodynamic) water activity; it is not claimed that any of them can be mixed without change in v.p. We know little about the v.p. of mixed salt solutions but what information is available suggests that whilst solutions of NaCl, KCl, and perhaps  $\text{CaCl}_2$  and  $\text{MgCl}_2$ , can be mixed without significant change in v.p., the admixture of any one of these with  $\text{MgSO}_4$  may lead to a marked change in v.p.

In the last column but one of Table II are given the v.p. lowerings corresponding to each chlorinity. These can be expressed by the formula

$$(p^0 - p)/p^0 = 0.0009206 (\% \text{ Cl}) + 0.00000236 (\% \text{ Cl})^2,$$

where (% Cl) is the chlorinity given in the first column of Table II. The v.p. lowering is therefore not linear in the chlorinity as would appear from the equation of Witting (1908):

$$p/p^0 = 1 - 0.000969 (\% \text{ Cl}),$$

an equation which gives a good representation of the vapour-pressure lowering of sea water only in the vicinity of 20‰ chlorinity. Thus for standard sea water of 19.386‰ Cl, our formula gives  $\Delta p/p^0 = 0.01874$ , compared with 0.01879 by Witting's formula.

The osmotic pressure,  $\Pi$ , of these solutions can be calculated by the formula

$$\Pi = -(RT/V_1) \ln a_w,$$

where  $V_1$  is the partial molal volume of water in the solutions and  $a_w$  is the water activity or the relative v.p.,  $p/p^0$ . It can be assumed without significant error that  $V_1$  can be equated to the value in pure water; that is to say, it is put equal to the molar volume of pure water. Moreover, the osmotic coefficient,  $\phi$ , of the solution, defined by

$$\phi = -(55.51/2m) \ln a_w$$

enables us to make the transformation to

$$\Pi = (2mRT\phi)/(55.51V_1).$$

(The osmotic coefficients of these salt solutions have been tabulated and are easier to use in computations than the quantity  $\log a_w$ ; the factor 2 in the above equation is valid for salts dissociating into two ions such as NaCl; for salts like  $\text{CaCl}_2$  the factor is 3.)

Substituting numerical values at 25°, this equation becomes

$$\Pi = 48.8m\phi.$$

Substituting values of  $\phi$  corresponding to the molalities of NaCl in the third column of Table II and using the tables of osmotic coefficients already evaluated (Robinson, 1945; Stokes & Levien, 1946), the osmotic pressures given in the last column of Table II are calculated. They refer to a temperature of 25° C; at another temperature,  $t^\circ$  C, the osmotic pressure can be calculated *approximately* by multiplying by the factor  $[1 + (t - 25)/298]$ .

All these experiments refer to 25° C; none has been done at other temperatures and we can only estimate from other work what the temperature effect is likely to be. One way in which an estimate of the temperature effect can be made is as follows. Thompson (1932) has given a formula for the depression of the freezing-point of sea water:

$$\Delta T = -0.0966 (\% \text{ Cl}) - 0.000052 (\% \text{ Cl})^3,$$

from which the freezing-point at various chlorinities has been calculated and recorded in Table III. Scatchard & Prentiss (1933) have measured very accurately the freezing-point of NaCl solutions, and from their tables we can find by interpolation the molalities of NaCl solutions which freeze at the same temperature as these sea-water solutions. Solutions of the same freezing-point must have the same v.p. For each of the seven selected chlorinities these NaCl molalities are also given in the table as well as the corresponding NaCl molality at 25° C. It will be seen that the effect of a 26–27° C temperature difference corresponds to only a small change in the NaCl molality, a change of between 0.4 and 0.8% over a chlorinity range of 10–22‰.

TABLE III

% Cl	10	12	14	16	18	20	22
Freezing-point depression	0.971	1.168	1.366	1.567	1.769	1.974	2.180
M-NaCl at freezing-point	0.2851	0.3439	0.4028	0.4627	0.5230	0.5839	0.6450
M-NaCl at 25°	0.2861	0.3452	0.4050	0.4653	0.5263	0.5880	0.6503

Finally we may consider the accuracy which can be attained by calculating the v.p. lowering as the summation of the values for the component salts. We can try the assumption that all the chlorinity can be counted as NaCl and find the corresponding v.p. lowering. For example, the standard sea water of 19.386‰ chlorinity would contain 31.96 g NaCl per kg of solution calculated on this assumption, equivalent to 0.5648 M-NaCl. Such a solution has a v.p. lowering of  $\Delta p/p^0 = 0.01858$  compared with 0.01873 for this sea water (interpolated from Table II). Similarly, the artificial sea water (sample 2) of 20.58‰ chlorinity is calculated as 0.6008 M-NaCl which has  $\Delta p/p^0 = 0.01978$  compared with the observed (interpolated) value of 0.01996, a difference corresponding to only 0.004 mm of mercury pressure. Alternatively, we could assume that the contribution of each salt is determined by its relative molal v.p. lowering at the total ionic strength of the sea water. For example, the artificial sea water (sample 2), as made up, had the following composition in moles per kg of H<sub>2</sub>O:

NaCl	KCl	MgCl <sub>2</sub>	CaCl <sub>2</sub>	MgSO <sub>4</sub>
0.5125	0.0113	0.0287	0.0116	0.0315

By taking account of the valencies of these salts the total ionic strength can be calculated as 0.7707. At this ionic strength the relative molal v.p. lowering

of each salt can be interpolated from the tables to which reference has already been made, and  $\Delta p/mp^0$  found to be 0.03300, 0.03192, 0.04745, 0.04652, 0.02032 for the salts in the order listed above. Hence  $\Delta p/p^0$  for these salts is 0.01691, 0.00036, 0.00136, 0.00054 and 0.00064, and the total is 0.01981. The mixed solution had a chlorinity of 20.58‰, and by interpolation in Table II the relative v.p. lowering is 0.01996. The difference between 0.01981 and 0.01996 corresponds to only 0.003 mm of pressure. In the absence of direct measurements, therefore, the v.p. can be calculated with some confidence either from the v.p. lowering of the component salts or by assuming that sea water is a NaCl solution of equivalent chlorinity. It is worth while reiterating, however, that the  $MgSO_4$  in these solutions is present in comparatively small amount, and the simple additivity rule might not apply so well if this salt were present in large quantities.

I wish to thank Dr L. H. N. Cooper for a number of valuable suggestions and Mr R. W. Green and Mrs H. Tong for assistance with the analyses.

#### SUMMARY

Measurements have been made by the isopiestic method of the vapour pressure at 25° C of sea water of chlorinity between 10 and 22‰. A table is given of the concentrations of solutions of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulphate, sodium sulphate, sucrose and urea of equal vapour pressure to these sea waters. Their osmotic pressures are also tabulated.

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## A COMPARATIVE STUDY OF THE BRITISH SPECIES OF *NUCULA* AND *NUCULANA*

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(Plate I and Text-figs. 1-8)

The survey of the sublittoral fauna of the Clyde Sea Area from 1949 onwards has shown that five species of the Protobranchiata are abundant throughout this region on a variety of substrata. Pelseener (1891, 1899, 1911), Heath (1937), and Yonge (1939) have contributed much to the knowledge of the group as a whole, but little comparative work has been done at species level. Verrill & Bush (1897, 1898) studied the shell characters of the American Atlantic species. Moore (1931*a, b*) worked on the faecal pellets of the British Nuculidae and attempted to distinguish the species by this means, while Winckworth (1930, 1931), mainly in the light of the latter work, attempted to clarify the nomenclature of these species. Winckworth (1932) lists six British species of the family Nuculidae: *Nucula sulcata* Bronn, *N. nucleus* (Linné), *N. hanleyi* Winckworth, *N. turgida* Leckenby & Marshall, *N. moorei* Winckworth and *N. tenuis* (Montagu); and four species of the family Nuculanidae: *Nuculana minuta* (Müller), *Yoldiella lucida* (Lovén), *Y. tomlini* Winckworth and *Phaseolus pusillus* (Jeffreys). All species of *Nucula*, except *N. hanleyi*, were taken from the Clyde Sea Area, although the latter species is included in the Clyde fauna list (Scott Elliot, Laurie & Murdoch, 1901). Only *Nuculana minuta* of the Nuculanidae has been taken on the present survey. *Yoldiella tomlini* is included in the 1901 list but is noted as being 'insufficiently attested'. *Nucula hanleyi* was obtained from the Marine Station, Port Erin, but *Yoldiella* and *Phaseolus* were unobtainable.

Difficulty has been experienced in distinguishing the species of *Nucula*. Recent work (Allen, 1953*a*) shows no differences between *N. turgida* and *N. moorei*, these are now combined under the name *N. turgida* Leckenby & Marshall. Moore (1931*a*) has also suggested that *N. hanleyi* and *N. nucleus* do not differ from each other, but the present study does not support this view. The specific differences of the British Nuculidae have been investigated and, though no single character is diagnostic, it has been found that the species can be readily distinguished when several characters are considered. An attempt has been made also to correlate specific differences with mode of life. In addition, information on growth rates and length of life has been determined.

Measurements and observations on *Nuculana* have been included where they complete the ecological picture and where they indicate similarities at the generic level.

I wish to express my thanks to Prof. C. M. Yonge, F.R.S., for his criticism of the manuscript; and to my wife for her help and criticism. Thanks are due to the Director and Staff of the Millport Marine Station for their help, and to the skipper and crew of the R.V. *Calanus* for their assistance in obtaining the animals. I also wish to thank Dr N. S. Jones of the Port Erin Marine Station for his kindness in obtaining the sample of *Nucula hanleyi* without which this work would have been incomplete.

This work was carried out in the first place with financial assistance from the Browne Research Fund of the Royal Society and later by a Research Grant from the Development Commission.

#### ECOLOGY AND HABITAT

The animals were taken by means of the Agassiz Trawl and the Naturalist's Dredge. Much of the material was taken in 1949-50 from the samples collected in the course of the Clyde fauna survey. Further large samples were taken in 1951 which included *Nuculana minuta* from the Kilbrennan Sound, *Nucula sulcata* from the Cumbrae Deep, and *N. nucleus* from the Minard Narrows, Loch Fyne.

The habitat varies for the different species. They occur on all types of substrata ranging from sand to mud and from sandy-gravel to muddy-gravel.

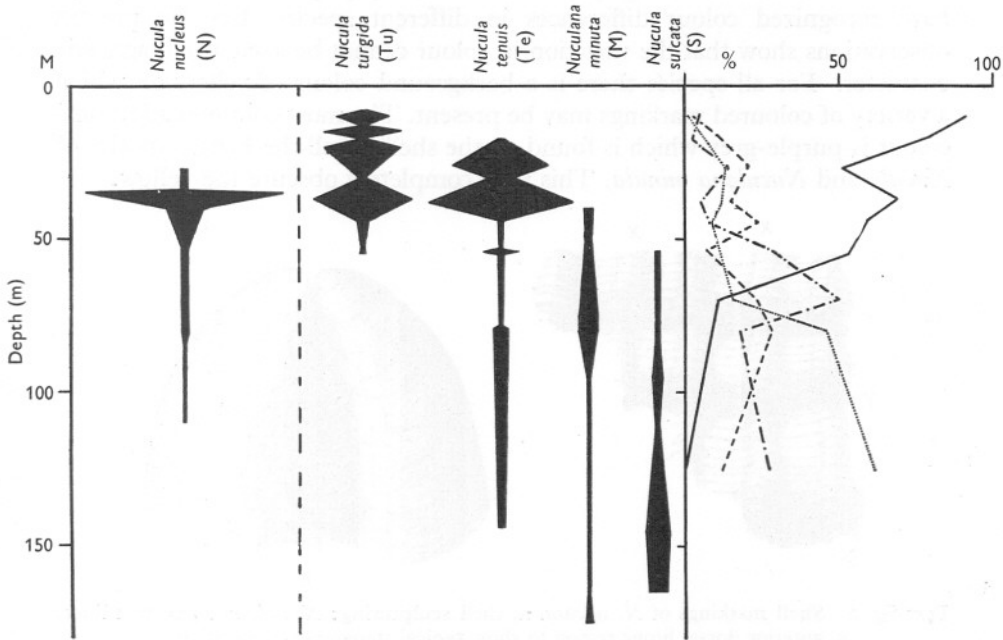
*Nucula turgida* was obtained from sands and sandy silts in water ranging in depth from 8 to 100 m, and very occasionally from muds at a maximum depth of 180 m. It is most abundant in sandy-mud (Text-fig. 1). It is present in large numbers in the Cumbrae Deep where there is a small amount of gravel and sand present in the mud. Where *N. turgida* is most abundant it is found together with *Pectinaria belgica*, *Cyprina islandica*, *Abra alba*, *Corbula (Aloidis) gibba* and *Amphiura filiformis*.

*N. sulcata* was taken in large numbers in floccular muds such as are present in the Arran Deep. It is found also in muds which contain small quantities of gravel and was taken from the Cumbrae Deep; however, there is evidence that animals from this locality differ in some respects from the rest of the Clyde specimens. It was taken in depths ranging from 60 to 200 m. It occurs together with *Lipobranchius jeffreysii*, *Glossus humanus*, *Amphiura chiajei* and *Brissopsis lyriformis*.

*Nucula tenuis* is the least common of the British species of *Nucula*. It is most abundant in soft sandy mud but extends into the less floccular muds and thus overlaps the ranges of both *N. turgida* and *N. sulcata*. It was taken from depths ranging from 20 to 150 m. Associated with it is *Thyasira flexuosa* and

some of the species above, e.g. *Abra alba*, which can tolerate a fairly wide range of particle sizes.

*Nucula nucleus* was taken in a few restricted areas within the Clyde such as the Fairlie Channel and the Minard Narrows, where the bottom is a coarse muddy gravel. The associated fauna is similarly restricted in habitat, and the dominant species are *Astarte sulcata*, *A. montagui* and *Dentalium entalis*.



Text-fig. 1. Distribution of species of *Nucula* and *Nuculana* along a traverse from Etterick Bay, Bute, to the Arran Deep compared with the particle size of the substratum. Particle size: > 62.5  $\mu$ , —; 62.5-31.2  $\mu$ , - - -; 31.2-15.6  $\mu$ , ····; < 15.6  $\mu$ , - · - ·.

*Nucula hanleyi* has not yet been taken in the present Clyde survey. It was obtained at Port Erin from a fine sandy gravel at a depth of between 16 and 20 m. It occurs in an offshore fine-gravel community described by Jones (1951).

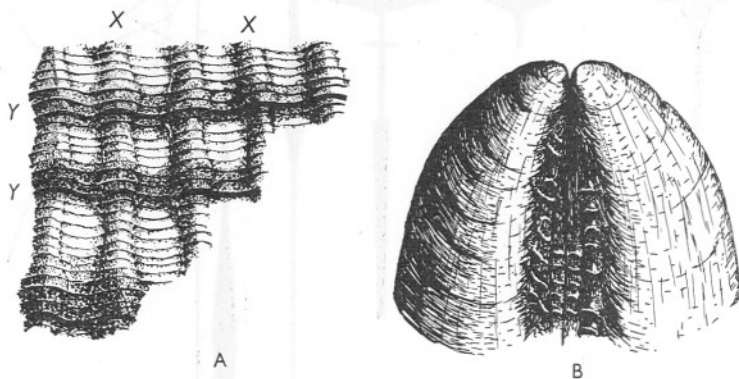
*Nuculana minuta* closely resembles *Nucula sulcata* in its habitat range, although it extends farther into the sandy mud grades and is most abundant in a less flocular mud. The associated fauna is the same as that of *N. sulcata*.

The faunal survey has shown that over considerable parts of the Clyde area there is a gradation of particle size with depth. Large particles—rocks, stones, sand—at shallow depths grade to flocular muds in the deeps (see Text-fig. 1). Correlated with this is a sublittoral zonation of the fauna. This is particularly obvious in the case of the Lamellibranchia. The Nuculidae show

such a zonation and thus resemble the intertidal Littorinidae. It is where the gradation of the particles is interrupted by shelves and banks of shell gravel that *N. nucleus* is found.

#### SHELL COLOUR AND CHARACTERISTICS

Shell colour has been variously described as ranging from yellow through various olivaceous hues to grey. Forbes & Hanley (1853) and Jeffreys (1863) have recognized colour differences in different species, but the present observations show that the variation in colour cannot be used as a diagnostic character. For all species there is a background colour of yellow on which a variety of coloured markings may be present. The most common additional colour is purple-grey which is found on the shells of all the British species of *Nucula* and *Nuculana minuta*. This may completely obscure the yellow.



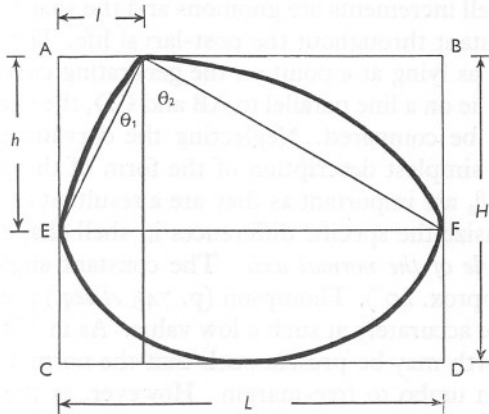
Text-fig. 2. Shell markings of *N. sulcata*. A, shell sculpturing: X, striae; Y, growth lines. B, anterior dorsal hinge region to show typical transverse corrugations.

The grey colour is rarely present in *Nucula tenuis*, this species usually being clear yellow. In *N. turgida*, as already described (Allen, 1953*a*), it is often in the form of radiate markings from the umbo to the free-margin (see Pl. I). Similar radiate markings occur on *N. hanleyi*, but these are red and, like those of *N. turgida*, vary in width and numbers. This red colour has been observed also on *N. sulcata* taken from the Cumbrae Deep, though here it is not as rays but in patches of varying size. Radiate markings have never been observed in *N. nucleus*, *N. tenuis* and *Nuculana minuta*.

There are differences in the surface of the periostracum, *Nucula turgida* and *N. tenuis* have a very glossy surface, while *N. sulcata* and *N. nucleus* have matt surfaces. *N. hanleyi* and *Nuculana minuta* are intermediate in this respect, although the former is more glossy than the latter. The species with matt surfaces collect a deposit of manganese on their shells while those with a glossy surface do not. *Nuculana* and *Nucula hanleyi* are again intermediate, the deposit never being so heavy.



There are few differences in shell sculpturing. *N. nucleus*, *N. hanleyi* and *N. turgida* have striae radiating from the umbo to the free-margin which may be very slightly decussated by an occasional strongly marked growth line. The growth lines of the latter three species are obvious and the inside edge of the free-margin is crenated. *N. sulcata* is finely decussated (Text-fig. 2A) and in the hinge area anterior to the umbo there are characteristic irregular transverse corrugations (Text-fig. 2B). The inside edge of the free-margin is also crenated. *N. tenuis* and *Nuculana minuta* have no radiating striae. The former shell has a smooth surface with very few growth lines showing while the latter is strongly ridged with growth lines, neither have a crenated inner shell margin.



Text-fig. 3. Diagram to show shell measurements taken. For explanation of symbols see text.

#### SHELL MEASUREMENTS

Apart from ridges on the gut wall which produce grooved faecal pellets (Moore, 1931*a*), examination of the anatomy did not show any significant differences between the species studied. Comparisons, therefore, have been made from shell features. Measurements were taken of 413 *Nuculana minuta*, 490 *Nucula sulcata*, 227 *N. nucleus*, 2500 *N. turgida* (large numbers taken for examination of the validity of *N. moorei*, see Allen, 1953*a*), 100 *N. hanleyi* and 106 *N. tenuis*.

Shells were placed on a grid ABCD (see Text-fig. 3) so that a line joining the points E and F is parallel to AB and CD (point F in *Nuculana* was taken as the dorsal corner of the posterior end of the shell). The distances *H*, *L*, *l* and *h* were then measured, and from these the angles  $\theta_1$  and  $\theta_2$  were calculated. In addition, the greatest width (*W*) was measured. All measurements were recorded to the nearest 0.1 mm and the angles to the nearest minute. Measurements were taken with the aid of a travelling microscope and vernier calipers.

The mathematical description of the growth and form of the molluscan shell has been discussed by Huxley (1932), Thompson (1942), Lison (1949), and Owen (1953). Quantitative measurement of the specific differences of the British Nuculidae has been considered in relation to the above work and with reference to: (i) growth and form of the generating curve, (ii) the constant angle of the normal axis, (iii) the form of the normal axis, and (iv) the angle of retardation.

*Growth and form of the generating curve.*<sup>1</sup> Although Owen states that the measurements of 'length', 'height' and 'breadth' do not alone provide a satisfactory means of comparing shell form in different species, they nevertheless provide useful information. In the majority of lamellibranchs, including *Nucula*, shell increments are gnomons and the shape of the generating curve remains constant throughout the post-larval life. The umbo of *Nucula* may be considered as lying at a point on the generating curve and, providing the points E and F lie on a line parallel to AB and CD, the shell measurements  $L$ ,  $H$ ,  $l$  and  $h$  can be compared. Neglecting the curvature, these measurements provide the simplest description of the form of the generating curve. The angles  $\theta_1$  and  $\theta_2$  are important as they are a resultant of  $l$  and  $L-l$  and  $h$  and further emphasize the specific differences in shell shape.

*The constant angle of the normal axis.* The constant angle of the lamellibranch is small (approx.  $40^\circ$ ). Thompson (p. 743 *et seq.*) points out that it is not easy to measure accurately at such a low value. As in *Nucula*, a tangential component of growth may be present such that the normal axis is no longer a straight line from umbo to free-margin. However, as the tangential component in *Nucula* is small and is of the same order in all species, the ratio  $H/\frac{1}{2}W$  has been used to compare the concavity of the valves, i.e. the constant angle.

*The form of the normal axis.* This is turbinate for all *Nucula* species. The measurement of the degree of turbination is difficult and no accurate determination was made. Observation of *N. turgida* and *N. hanleyi* with radial shell markings and of those species with striae indicates that the degree of turbination is of the same order in all.

*Angle of retardation.* As the umbo approximates to a point on the generating curve in all the Protobranchiata, the angle of retardation, i.e. the retardation of growth of the inner as compared with the outer whorl, approaches infinity and thus can be neglected.

In addition, age and growth rates and hinge teeth numbers are compared in the species studied.

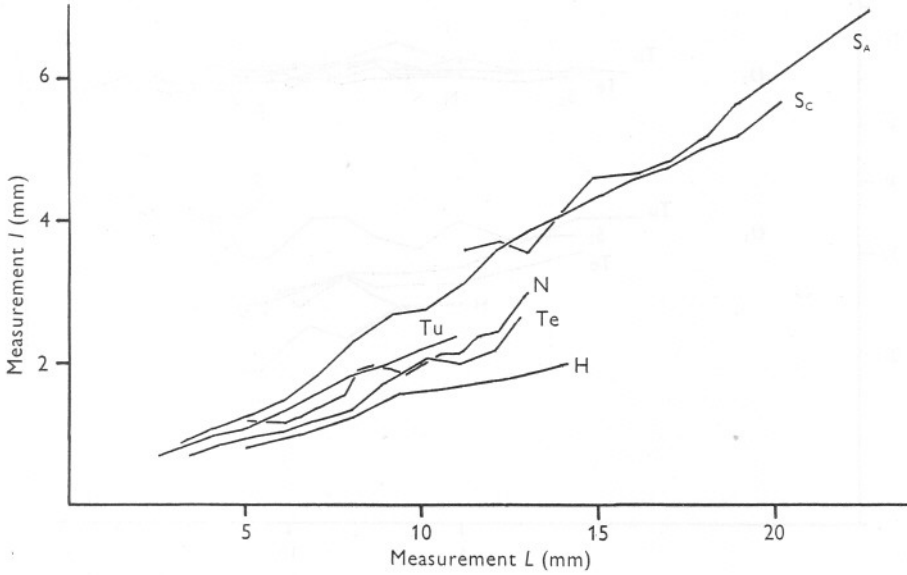
#### COMPARISON OF SHELL SHAPES

##### *Generating curve*

Comparison of the overall measurements of height ( $H$ ) and length ( $L$ ) shows there is very little difference between the five species of *Nucula*. Thus

<sup>1</sup> The nomenclature proposed by Owen (1952, 1953) has been used.

mean shell heights calculated for a length of 1 cm are as follows (length was chosen because the growth increments in length are almost constant for the British species of *Nucula*): *N. nucleus* 0.83 cm, *N. sulcata* (Cumbrae Deep) 0.81 cm, *N. hanleyi* 0.80 cm, *N. turgida* 0.78 cm, *N. sulcata* (Arran Deep) 0.78 cm and *N. tenuis* 0.76 cm. Comparisons of the individual variations in the overall measurements of height and length emphasize the fact that the measurements overlap each other too much to show any significant difference between the species. However at high values the measurements of *N. nucleus*



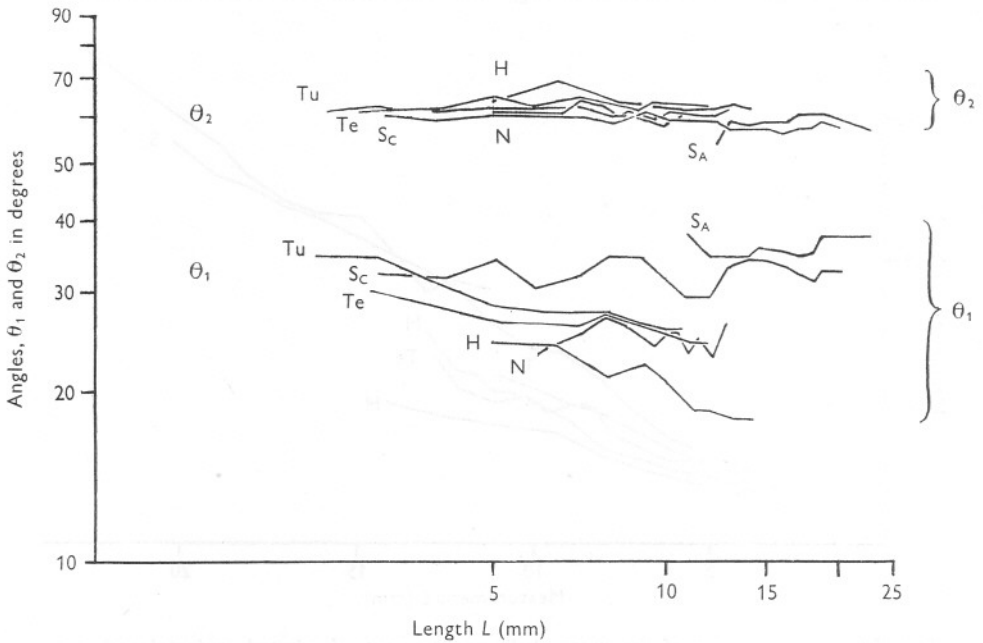
Text-fig. 4. Comparison of the mean shell measurements,  $l$ , plotted against length ( $L$ ). H, *Nucula hanleyi*; N, *N. nucleus*; S, *N. sulcata*; Tu, *N. turgida*; Te, *N. tenuis*; A, Arran Deep; c, Cumbrae Deep.

and *N. tenuis* (the highest and lowest respectively in relative height measurements) when considered by themselves are nearly distinct. There is also a distinction between *N. sulcata* from the Cumbrae Deep and the Arran Deep. This is also indicated in other measurements, and the possible explanation will be discussed later (see p. 465).

Although the overall measurements show few differences the angles  $\theta_1$  and  $\theta_2$  need not be the same for all species. Reference to Text-fig. 4 shows that specific differences for the value  $l$  are marked and that *N. hanleyi* has the lowest value for  $l$  and *N. sulcata* the highest. Here, the measurements of *N. nucleus* and *N. tenuis* lie much closer together, the latter having the lower values for  $l$ . These slight differences in  $l$  and  $H$  (*N. tenuis* having a lower value for both measurements) make the values of  $\theta_1$  for both these species approxi-

mately equal, while the values for  $\theta_2$  are distinct. This explains why *N. tenuis* appears elongate when compared with the other species (Text-fig. 5).

$\theta_1$  and  $\theta_2$  are the best measurements for comparing the generating curves as they take into account both measurements for height and length. Values of  $\theta_1$  in Text-fig. 5 further show that *N. sulcata* and *N. hanleyi* are distinct while values for the other species are nearly identical. Although differences in  $L-l$  will not be so clearly reflected in  $\theta_2$  when this is in the region



Text-fig. 5. Comparison of the mean shell angles  $\theta_1$  and  $\theta_2$ , double-log plot. Abbreviations as given under Text-fig. 4.

of  $60^\circ$ , Text-fig. 5 shows that values of  $\theta_2$  for *N. nucleus* approximate to those of *N. sulcata* while those of *N. tenuis* lie close to those of *N. hanleyi*. Again there are differences in the measurements of *N. sulcata* from the Cumbræ Deep and those from the Arran Deep. Whereas the species other than *N. sulcata* with low values for  $\theta_1$  have high values for  $\theta_2$ , *N. sulcata* from the Arran Deep with a larger angle  $\theta_1$  than *N. sulcata* from the Cumbræ Deep, also has a larger angle  $\theta_2$  than the shells from the Cumbræ Deep.

#### Constant Angle

Reference to Table I shows that the differences in the constant angle are very slight. However, they do bear out the field observations that *N. nucleus* has the greatest spiral angle and *N. tenuis* the smallest.

*Discussion and Conclusions on Shell Shape*

It is convenient at this stage to summarize the above data and attempt to correlate the differences in shell measurements with the differences in habitat. Table I summarizes the shell measurements of the species of *Nucula* which are calculated for a shell length of 1.0 cm. Although there are few differences in the overall shell measurements, the species can be distinguished by considering the measurements in relation to the position of the umbo. Studies on normal variation show that there is an overlap of the measurements, but consideration of all the measurements, particularly when these are taken in

TABLE I. MEAN SHELL MEASUREMENTS CALCULATED FOR  $L = 1.0$  CM

	$l$	$L-l$	$h$	$H$	$W$	$H/\frac{1}{2}W$	$\theta_1$	$\theta_2$
<i>Nucula tenuis</i>	0.19	0.81	0.40	0.76	0.41	3.76	26° 31'	63° 40'
<i>N. turgida</i>	0.23	0.77	0.43	0.78	0.45	3.46	26° 56'	60° 41'
<i>N. sulcata</i> (C.D.)	0.28	0.72	0.43	0.81	0.44	3.68	32° 54'	58° 45'
<i>N. sulcata</i> (A.D.)	0.30	0.70	0.41	0.78	0.43	3.63	36° 18'	59° 13'
<i>N. nucleus</i>	0.21	0.79	0.44	0.83	0.49	3.38	25° 13'	61° 06'
<i>N. hanleyi</i>	0.15	0.85	0.42	0.80	0.44	3.63	19° 42'	63° 26'

C.D., Cumbrae Deep. A.D., Arran Deep.

conjunction with other characters (see p. 471), show that the species of *Nucula* can be distinguished from each other. The most important shell measurements are the angles  $\theta_1$  and  $\theta_2$  for they are the resultant of  $l$  and  $L-l$  and any slight difference in height. The effect of the slightly smaller height measurements of *N. tenuis* has meant that while  $\theta_1$  for this shell is of the same order as that of *N. turgida*, its value for  $\theta_2$  is slightly larger than that of *N. hanleyi*. This explains the characteristically elongate appearance of *N. tenuis*. *N. turgida* and *N. nucleus* are the only species that cannot be readily separated by shell measurements, although *N. nucleus* has the highest measurement of height and the greatest spiral angle. *N. sulcata* shows differences in measurements at different localities. Similar differences have been shown for *Chlamys septemradiata* taken from different regions of the Clyde (Allen, 1953*b*). Differences between *Nucula sulcata* from different localities and the shell differences between the other species are apparently correlated with differences in their habitat. *N. sulcata* living in soft floccular mud has a large measurement for  $l$ , while *N. hanleyi* in firm sandy-gravel has a small measurement for  $l$ . The other species of *Nucula* are intermediate both in type of substratum and measurement  $l$ . The mud of the Cumbrae Deep is more compact than that of the Arran Deep and has a small amount of sand and gravel mixed with it. Thus some differences might be expected between *N. sulcata* taken from these localities. Thus differences in shell shape may have arisen because those with a small  $l$  value may be better adapted for movement in firm substrata.



## AGE AND GROWTH

The histograms in Text-fig. 6 show that the species of the Protobranchiata examined have definite size-groups which are in all probability year-groups (see Allen, 1953*a*). *N. turgida* and *N. sulcata* were found to have ripe sperm and eggs in January and February but no successful fertilizations were carried out. *N. nucleus* differs from the other species in that the size-groups indicate that it either grows at half the rate of other *Nucula* species or that there are two breeding periods annually. Lebour (1938) states that the breeding period is from spring to autumn (April to November). This is longer than the other species. There are probably two maxima, a view that is supported to some extent by the fact that Dr Lebour was unable to carry out artificial fertilization in August. Assuming that the size-groups are year-groups (in *N. nucleus* two size groups per year) then the maximum ages attained by the species are as follows: *N. turgida* 12 years, *N. tenuis* 13 years, *N. nucleus* 13 years, *N. hanleyi* 14 years, *N. sulcata* 20 years and *Nuculana minuta* 17 years.

The increments of total length and total height are nearly the same for all species, this being particularly true of the length increment (see Table II below).

TABLE II. MEAN YEARLY SHELL INCREMENTS

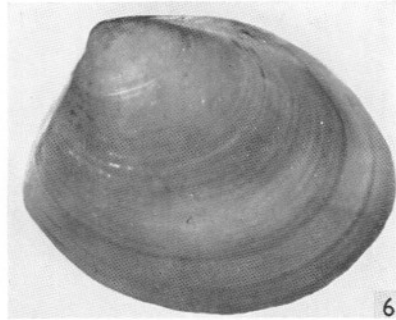
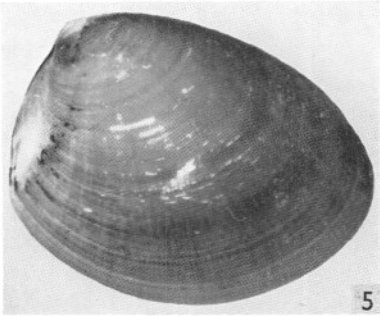
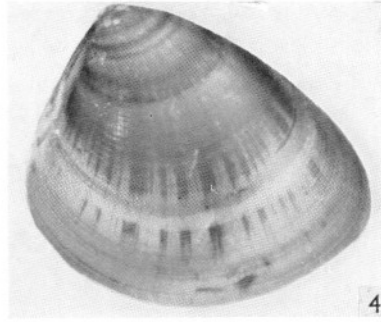
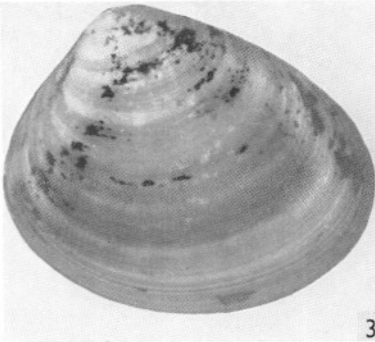
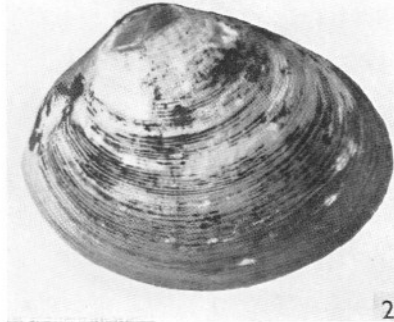
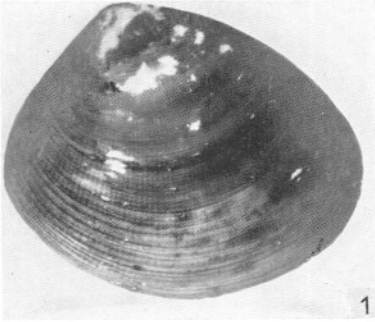
	(Measurements in mm)				
	<i>L</i>	<i>l</i>	<i>L-l</i>	<i>h</i>	<i>H</i>
<i>Nucula turgida</i>	0.94	0.19	0.75	0.43	0.75
<i>N. tenuis</i>	0.96	0.18	0.78	0.42	0.76
<i>N. hanleyi</i>	1.01	0.13	0.82	0.47	0.83
<i>N. sulcata</i> (C.D.)	0.96	0.27	0.69	0.40	0.76
<i>N. sulcata</i> (A.D.)	0.96	0.28	0.68	0.38	0.70
<i>N. nucleus</i>	0.94	0.21	0.73	0.41	0.80
<i>Nuculana minuta</i>	0.98	0.52	0.46	0.28	0.57

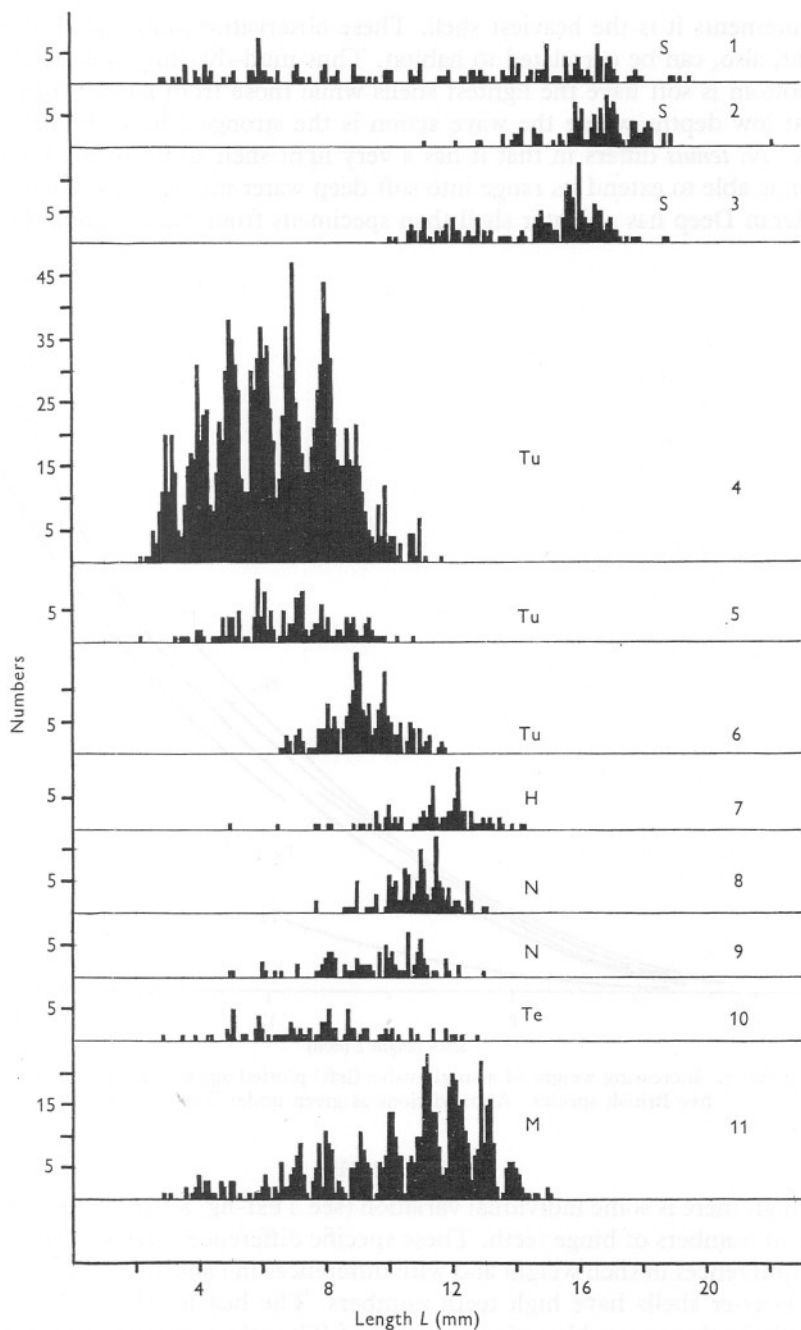
*Nucula hanleyi* is an exception but this is offset by a correspondingly high growth rate in height. *N. nucleus*, the other species living in gravel, also has a high height increment while *N. sulcata* from the floccular mud of the Arran Deep has the lowest. The growth rate remains practically constant throughout the life of the species. *Nuculana minuta*, although being a member of a different family with marked differences in shell shape, has the same growth increment of length with similar year-groups (see Text-fig. 6).

Shell weights have been compared and these show that the curves for increase of shell weight with length are similar (Text-fig. 7). *Nucula hanleyi* differs slightly in that the initial rate is slightly less than *N. turgida* and *N. nucleus*. This increases between 8 and 10 cm length so that at high

## EXPLANATION OF PLATE I

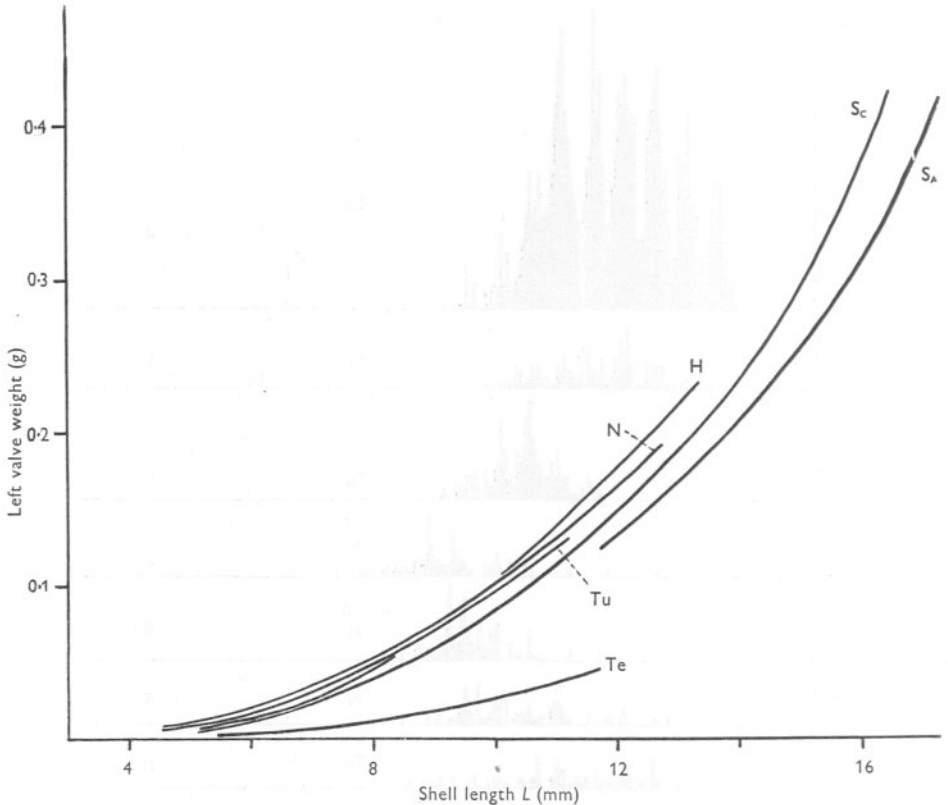
Lateral views of the right valve of the five British species of *Nucula*. 1, *N. sulcata* (Cumbrae Deep); 2, *N. sulcata* (Arran Deep); 3, *N. nucleus*; 4, *N. turgida*; 5, *N. hanleyi*; 6, *N. tenuis*.





Text-fig. 6. Population histograms of the largest samples taken. 1, *Nucula sulcata*, Cumbrae Deep, July 1951. 2, *N. sulcata*, Arran Deep, July 1951. 3, *N. sulcata*, Arran Deep, Aug. 1951. 4, *N. turgida*, Cumbrae Deep, Oct. 1950. 5, *N. turgida*, Cumbrae Deep, June 1951. 6, *N. turgida*, Minard Narrows, July 1951. 7, *N. hanleyi*, Port Erin, Jan. 1952. 8, *N. nucleus*, Fairlie Channel, Dec. 1948. 9, *N. nucleus*, Minard Narrows, Aug. 1951. 10, *N. tenuis*, Etterick Bay, July 1949. 11, *Nuculana minuta*, Kilbrennan Sound, Aug. 1951.

measurements it is the heaviest shell. These observations indicate that shell weight, also, can be correlated to habitat. Thus mud-dwelling species where the bottom is soft have the lightest shells while those from harder substrata and at low depths where the wave action is the strongest have the heaviest shells. *N. tenuis* differs in that it has a very light shell and possibly for this reason is able to extend its range into soft deep water muds. *N. sulcata* from the Arran Deep has a lighter shell than specimens from the Cumbrae Deep.



Text-fig. 7. Increasing weight of a single valve (left) plotted against length ( $L$ ) in the five British species. Abbreviations as given under Text-fig. 4.

#### HINGE TEETH

Although there is some individual variation (see Text-fig. 8) species of *Nucula* differ in numbers of hinge teeth. These specific differences may be correlated with differences in shell weight and with differences in  $l$  and  $L-l$ . The species with heavier shells have high teeth numbers. The heavier shells also come from hard substrata and have low values for  $l$ . Thus the posterior hinge region is shorter than in the species from soft substrata and the numbers of teeth in

this region will be restricted by lack of space. Specific differences in the length of the relatively long anterior hinge are not great enough to have much effect on teeth numbers. Shell weight rather than limiting space has the greater effect on teeth numbers (see Text-fig. 8). *N. sulcata* with the longest value of *l* has low numbers of teeth and it is only in the case of *N. hanleyi* that a low value for *l* appreciably lowers the number of posterior teeth. *N. tenuis* with the very fragile thin shell has the least number of teeth.

Hinge numbers increase with increasing age and there are specific differences in the rate at which they are laid down. This rate is not the same anterior and posterior to the hinge, the posterior teeth being laid down at a slower rate. *N. sulcata* from the Arran and Cumbrae Deeps are almost identical in teeth numbers, with a slight tendency for fewer in the Arran Deep shells.

TABLE III. AVERAGE INCREASE IN SHELL LENGTH (MM) FOR EACH ADDITIONAL TOOTH

	Anterior	Posterior
<i>Nucula sulcata</i>	1.20	1.65
<i>N. tenuis</i>	1.15	1.40
<i>N. turgida</i>	0.85	0.95
<i>N. nucleus</i>	0.60	0.95
<i>N. hanleyi</i>	0.70	1.15

#### DISCUSSION AND CONCLUSIONS

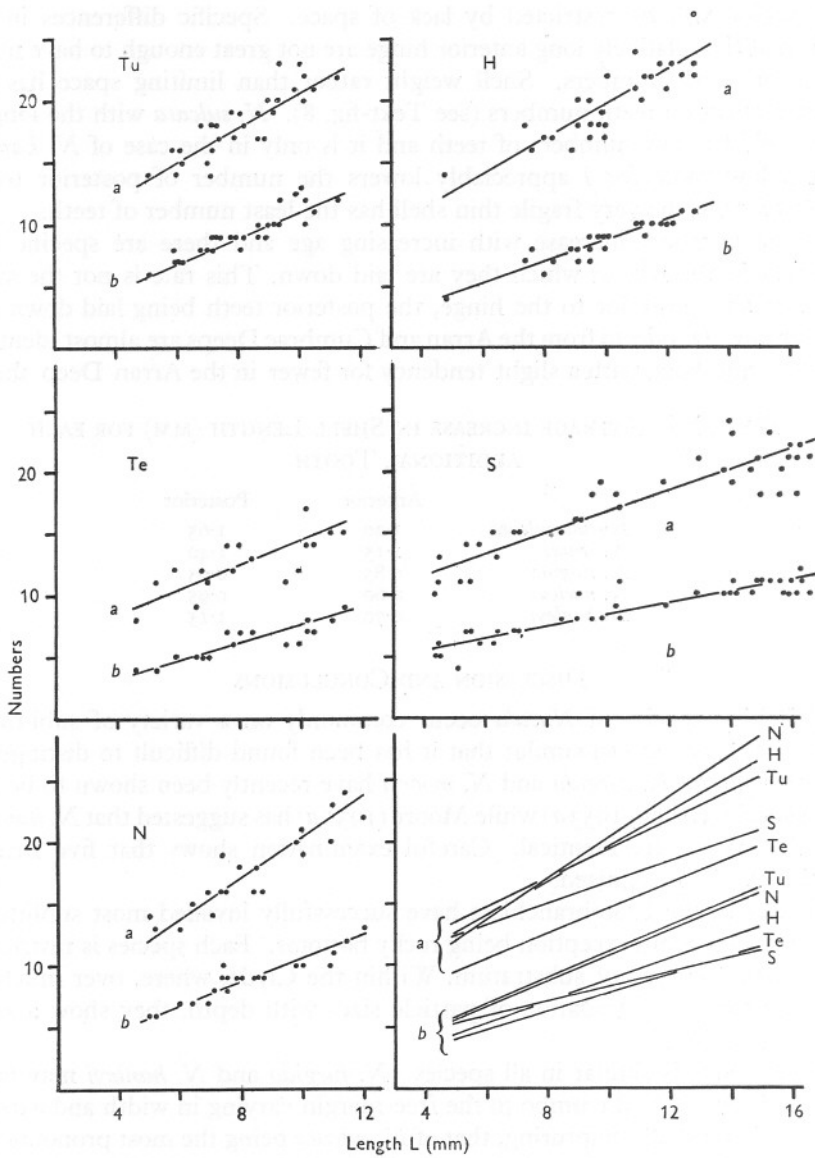
The British species of *Nucula* occur commonly on a variety of sublittoral substrata. They are so similar that it has been found difficult to distinguish between them. *N. turgida* and *N. moorei* have recently been shown to be the same species (Allen, 1953*a*) while Moore (1931*a*) has suggested that *N. hanleyi* and *N. nucleus* are identical. Careful examination shows that five British species can be recognized.

Species of the Protobranchiata have successfully invaded most sublittoral substrata, the main exception being rocky bottoms. Each species is restricted to a particular type of substratum. Within the Clyde, where, over much of the area there is a gradation of particle sizes with depth, they show a sublittoral zonation.

Shell colour is similar in all species. *N. turgida* and *N. hanleyi* may have coloured rays from the umbo to the free-margin varying in width and extent. There is little shell sculpturing, that of *N. sulcata* being the most pronounced. *N. tenuis* differs from the other species in that it has no crenations along the inner edge of the free-margin of the shell. There are differences in the degree of gloss of the surface of the periostracum ranging from *N. tenuis* with a high gloss to *N. sulcata* with a matt surface.

Shell measurements were taken and studied in the light of recent work on the growth and form of the lamellibranch shell. There is little difference in the overall shell measurements of length and height but the measurements





Text-fig. 8. The variation and comparison of the numbers of anterior (a) and posterior (b) teeth at different values of length (L). Abbreviations as given under Text-fig. 4.

$l$  and  $L-l$  are important in distinguishing between the species. The angles  $\theta_1$  and  $\theta_2$  are also important as they are the result of both differences in  $l$  and  $L-l$  and height. Thus the slightly lower measurements in height of *N. tenuis* and *N. sulcata* from the Arran Deep are reflected in the angles  $\theta_1$  and  $\theta_2$  and

emphasize differences already shown in  $l$  and  $L-l$ . Differences in shell form may be correlated with differences in habitat. Thus species in hard substrata with large particles (*N. hanleyi*) have smaller measurements for  $\theta_1$  and  $l$  than those from soft mud (*N. sulcata*). This may be correlated with the fact that a greater pull of the foot is necessary for movement through the harder ground. It has been shown that the weight of shell in the species living in mud is less than in species living in sand and gravel. This is reflected in the number of shell teeth present, i.e. fewer in species from mud. This is probably associated with the fact that shells from sand and gravel tend to be more robust, particularly in the Clyde Sea Area where they are in shallow water and will be affected more by wave action.

Not only can specific differences be related to differences in substratum but species themselves can differ in different habitats. This is very well shown in *N. sulcata*.

*N. nucleus* and *N. hanleyi* are not one and the same species as was suggested by Moore in his study on faecal pellets. On the other hand, *N. turgida* and *N. moorei*, recently shown to be the same species, were separated by Winckworth on supposed differences in the faecal pellets as demonstrated by Moore. It must be concluded that differences in pellets cannot be used by themselves for the identification of *Nucula* species.

The specific differences have been summarized in Table IV.

TABLE IV. COMPARISON OF THE SPECIES OF *NUCULA*

	<i>N. hanleyi</i>	<i>N. nucleus</i>	<i>N. turgida</i>	<i>N. tenuis</i>	<i>N. sulcata</i>
Habitat	Sandy gravel	Muddy gravel	Muddy sand	Sandy mud to mud	Mud
Shell colour, etc.	Red rays, periostracum fairly glossy, inner margin crenated	No rays, periostracum matt, inner margin crenated	Purple-grey rays sometimes present, periostracum glossy, inner margin crenated	No rays, periostracum glossy, inner margin not crenated	No rays, periostracum matt, inner margin crenated, slightly decussated with irregular transverse corrugations above ant. hinge
Shell shape, measurements, in parentheses in mm, mean values given	Lowest measurement for $l$ (0.15) and $\theta_1$ ( $19^\circ 42'$ )	Differs only slightly from <i>N. turgida</i> but has highest measurement of $H$ (0.83) and constant angle ( $3.38$ )	All measurements are intermediate to other species	Lowest measurement of $H$ (0.76) and largest angle $\theta_2$ ( $63^\circ 40'$ )	Highest measurement for $l$ (0.30) and $\theta_1$ ( $36^\circ 18'$ )
Maximum age, growth, etc.	14 years, greatest growth rate and heaviest at lengths above 1 cm. One breeding period/year	13 years, two breeding periods/year	12 years, one breeding period/year	13 years, very light and fragile shell with very low hinge teeth numbers, one breeding period/year	20 years, one breeding period/year

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## THE BREEDING OF *BALANUS PORCATUS* (DA COSTA) IN THE IRISH SEA

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(Text-figs. 1-10)

*Balanus porcatus* (da Costa) is a widely distributed northern species, being found in the Arctic Ocean as far as 80° N., and in the northernmost extensions of the Atlantic and Pacific Oceans. Its southern limits are closely related to water temperatures. Whereas on the east coast of America, where the Labrador current flows southward, it extends to Long Island (lat. 40° N., see Pilsbury, 1916), on the west European coast it scarcely penetrates the English Channel (lat. 51° N.). It is found in some abundance however in the North Sea, the Skagerrak and Kattegat (Krüger, 1927), the Irish Sea, and off the west coast of Scotland and Ireland. The present survey is probably representative of its breeding habits in regions near the southern limits of its range.

In the Irish Sea it is generally found from 3 to 20 fathoms, usually in association with *Modiolus modiolus* (L.) or *Pecten maximus* (L.). This association exists probably because these molluscs provide much of the suitable substrata offshore, for the barnacle is also found on stones, pieces of coal, etc., when these happen to be dredged in the vicinity of the *Modiolus* beds. It occurs occasionally on rocks at low-water mark and at higher levels in the intertidal zone: for example a few specimens have been encountered at mid-tide level on rocks thickly draped with *Ascophyllum nodosum*, and also on the fronds of *Ascophyllum* itself at Church Island Reefs, Menai Straits.

### COLLECTION OF SAMPLES

Most of the samples forming the basis of this study were obtained from a ground in Beaumaris Bay at the eastern entrance to the Menai Straits between Puffin Island and the east side of the Dutchman bank, at 3-5 fathoms. The samples were stripped off *Modiolus* shells collected in an otter trawl. Each haul swept a length of about 1 mile, and generally brought in about a hundred *Balanus porcatus*. Of these, usually about half were isolated specimens, one only occurring on each *Modiolus* shell, while the other half occurred in groups of two or more barnacles on each shell. The high proportion of isolated individuals indicated that the ground was not subject to heavy settlements of this species, but the large size of the barnacles showed that the conditions were favourable for survival and growth.

The only abundant forms whose presence was clearly deleterious to the barnacles were *Alcyonium digitatum* L. and *Cliona celata* Grant. Barnacles completely covered by *Alcyonium* were sometimes dead, or moribund, or, if alive, had very poorly developed ovaries and were therefore excluded from the survey. *Cliona* infected the shells of many older barnacles and completely filled the majority of dead shells. The only other barnacles observed in the samples were occasional individuals or small groups of *Balanus crenatus* Brug. and *Verruca stroemia* (O. F. Müller).

Samples were also kindly taken for me by the staff of the Marine Biological Station, Port Erin. These served as corroborative evidence. Most of them were taken south and west of Port Erin Bay and Chicken Rock, where *Balanus porcatus* occurs mainly on *Pecten* shells in association with *Balanus crenatus*. It is also occasionally found on *Modiolus* shells south-west of

TABLE I. DIFFERENCES BETWEEN SAMPLES OF *BALANUS PORCATUS* ON SHELLS OF *PECTEN MAXIMUS* TAKEN SOUTH-WEST OF PORT ERIN AND ON *MODIOLUS MODIOLUS* FROM BEAUMARIS BAY

	Port Erin samples	Beaumaris Bay samples
Number measured	61	271
Mean basal diameter (mm)	16.1	26.4
Percentage with basal diam. > 30 mm	0	29
Numbers examined for <i>Hemioniscus</i>	61	831
Number infected by <i>Hemioniscus</i>	4	0
Percentage infection	6.6	0

Port St Mary in association with *Balanus hameri*. The specimens from Port Erin differed from those from Beaumaris Bay in being smaller and more closely packed on the shells and, moreover, in being occasionally infected by the parasitic isopod *Hemioniscus*, presumably *H. balani* (Bate), which has been reported in several other cirripedes besides its normal host *Balanus balanoides* (L.) (Crisp, 1951; Sandison, 1954). These differences are shown in Table I. The absence of isopod parasites from *B. porcatus* dredged in Beaumaris Bay was not unexpected since the incidence of *Hemioniscus* in *Balanus balanoides* was also low on the surrounding shores.

The barnacles were removed from their substrate in the laboratory with a strong knife and were generally examined while fresh; a few samples were perforce examined after storage in 5% formaldehyde in sea water.

On opening the barnacle, it was immediately clear whether the ovary had been discharged. Fertilized eggs formed two conspicuous masses lying in the mantle cavity on either side of the body, as illustrated by Moore (1935) for *B. balanoides*. Unfertilized eggs, on the other hand, were found within the tubules of the ovary, immediately within the calcareous basis, and had the appearance of a diffuse, fatty tissue, creamy yellow in colour. As fertilization in this species occurs in each individual only once a year, during the month



of February, the frequency of sampling was intensified in January, and throughout the ensuing period when the embryos were developing in the paired egg sacs, until their release into the sea in April and May. The period from January to May not only witnesses the most significant changes in the female gonads, but striking changes take place in the male organs, which were also studied. Sampling was continued, but less frequently, throughout the rest of the year.

#### THE MALE REPRODUCTIVE ORGANS

The testes, like those of other cirripedes (Darwin, 1854), consist of a mass of club-shaped diverticula lined with germinal epithelium. They communicate directly with the paired vesiculae seminales and discharge spermatozoa into them. In an early state of development the lobes of the testis are cylindrical, few in number, and devoid of sperm, hence perhaps readily overlooked; but when fully developed they are extensive and clearly visible through the cuticle. The degree of development of testis tissue in each individual examined was assigned to one of the following classes: (i) absent; (ii) poorly developed, visible only on dissection or teasing out; (iii) moderately developed, occupying a small part of the body and just visible through the cuticle; (iv) well developed, occupying a considerable part of the body and clearly visible from outside. In order to pool the information in a given sample and to represent more simply the probable state of development of the whole population on that date, these classes were scored 0, 1, 2 and 3 respectively, so that the average score taken over all individuals of the sample could be used as an index.

In these investigations only individuals of diameter exceeding 10 mm were included, smaller ones being immature; most samples consisted of from ten to twenty mature individuals.

The vesiculae seminales are paired fusiform sacs lying close to the dorsal surface visible through the cuticle as two prominent white patches, except when they are atrophied. When fully developed they become extremely bloated and turgid because of the great quantity of seminal fluid which they contain, and their elastic walls readily cause the discharge of this fluid should the ducts uniting them with the intromittent organ or penis become severed. When they are full the seminal fluid can often be seen extending to the lumen of the penis. The development of the vesiculae seminales was assessed by a method similar to that used for the testes, the classes being: (i) absent, or present as thin strands of tissue; (ii) poorly developed, sacs small, shrivelled, with a little sperm; (iii) moderately developed, sacs cylindrical, of diameter about equal to that of the gut; (iv) well developed, sacs large and bloated, exceeding the gut in diameter.

The penis is a highly extensible and muscular organ, arising medially between the most posterior cirri. It bears annular thickenings in the cuticle,

and sensory setae at the tip. Its lumen is continuous with those of the vesiculae seminales, the ducts of which unite just before entering it. In a few individuals of each sample the contracted penis was measured, using a binocular microscope provided with a calibrated micrometer eye-piece. As the penis is readily stretched, great care was taken to treat all samples similarly during measurement. The length of the penis is a function of the

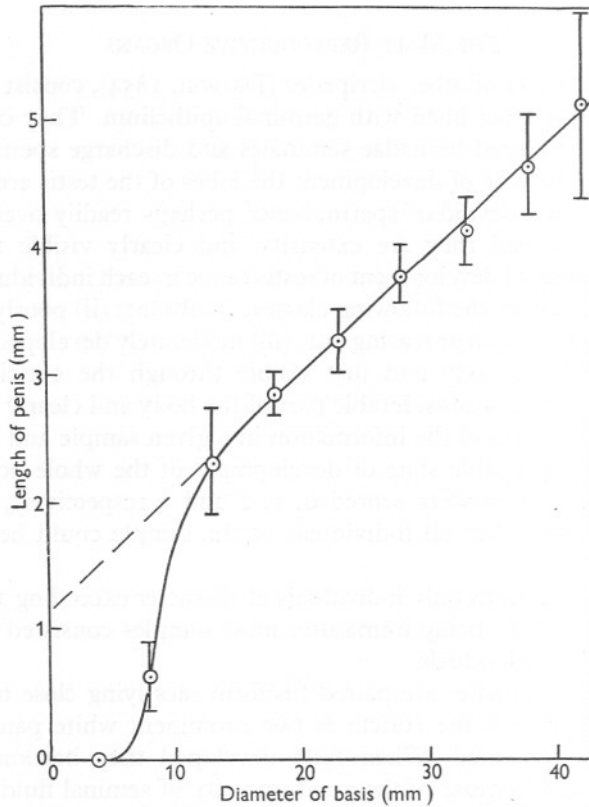


Fig. 1. Relation between the mean lengths of penis (vertical axis) and the basal diameter (horizontal axis) in *B. porcatius*. The vertical lines represent the standard error associated with each point.

size of the individual; therefore the diameter of the basis of each individual was recorded at the same time. If all the results of penis measurements throughout the year are grouped according to the size of the individual bearing the penis, the relation illustrated in Fig. 1 is obtained. The mean penis length is here plotted against the diameter of the basis, with upper and lower limits showing the standard error in the penis length associated with each point. Although there is a wide variation in length for individuals of each size-group,

it is clear that the growth of the penis is very rapid during the period in which the basal diameter increases from 6 to 10 mm. It is probably even more rapid than appears from the graph since this represents a mean of measurements on numerous individuals which will probably vary in the size-range over which the rapid growth of the penis occurs. If so, such variations would have the effect of spreading the rapid increase in mean length over a wider range of size than would be characteristic of the growth of an individual penis. The basal diameter at which the penis shows this heterogonic growth is attained towards the latter part of the first year of life, and probably takes place in the majority of individuals shortly before copulation is imminent. After the attainment of a basal diameter of 14 mm the mean growth of the penis in relation to basal diameter is approximately constant, the equation relating its length ( $l$ ) to the basal diameter ( $d$ ) being  $l = (1.10 + 0.095d)$  mm. In plotting seasonal changes the mean penis length was therefore corrected for variations in size of the individuals in the sample by means of the above equation, each record being reduced to a standard basal diameter of  $d = 20$  mm. The mean reduced length was then employed as an index of development.

#### SEASONAL CHANGES IN THE MALE REPRODUCTIVE ORGANS

Fig. 2 illustrates the seasonal changes in the testes, vesiculae seminales, and penis, the index of each being plotted on the vertical axis against the time of year on the horizontal.

Prior to copulation in early February the male reproductive organs attain their fullest development. The vesiculae seminales occupy a large part of the body space, having steadily increased in size since the preceding summer. They reach their maximum size at the time of copulation. The penis is also enlarged at this time, measuring in some individuals as much as 10 mm, and being capable of extension to some two or three times this length. The testes, which also occupy a prominent part of the body space, reach their fullest development a little earlier, and have already begun to decline by the time copulation takes place. After fertilization a marked recession, heralded by rapid degeneration of the testes, affects all the male organs. The penis shrinks, the vesiculae seminales wither into two faint tubes lying dorsal to the gut, and the testes become transparent and devoid of sperm before gradually disappearing.

During April, however, regeneration of the testes begins and within a few weeks these organs are almost reconstituted; indeed the new growth can often be seen to have commenced before the older tissue has been resorbed. Concurrently, the vesiculae seminales are restored and become gradually refilled with sperm. The size of the penis, however, does not increase until later in the year. Thus the slight expansion of the penis in mature individuals and the rapid initial growth in first-year barnacles occur at about the same time.

The decline in the male reproductive organs does not depend upon discharge of accumulated sperm, nor upon the discharge of ova following copulation with other individuals, for it occurs on an identical scale in isolated individuals which cannot inseminate and which retain an unfertilized ovary,

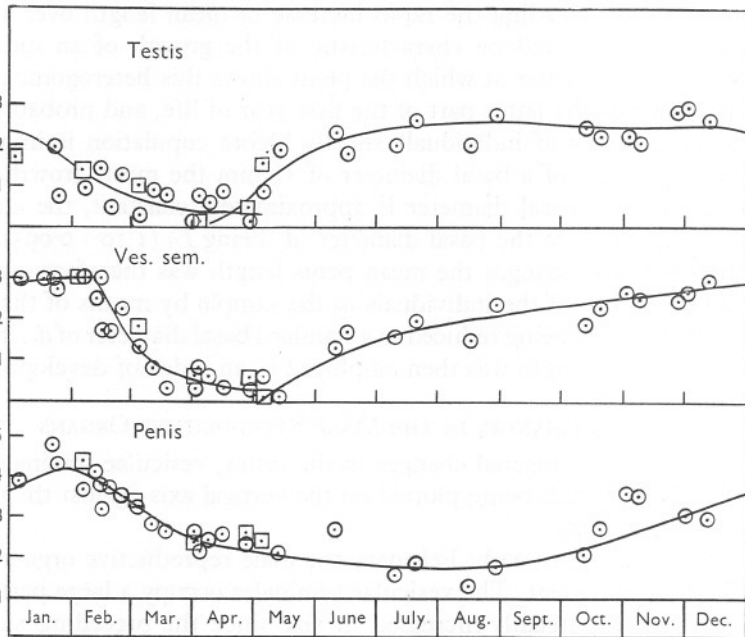


Fig. 2. Seasonal changes in development of penis (bottom curve), vesiculæ seminales (middle curve) and testis (top curve). Penis length is given in mm. For the methods of assessment of these organs see pp. 475-7.  $\odot$ , adjacent individuals;  $\square$ , isolated individuals.

TABLE II. PARALLEL DEGENERATION OF MALE ORGANS IN ADJACENT AND ISOLATED INDIVIDUALS DURING THE PERIOD FEBRUARY TO MAY

Date	Testis		Vesiculæ seminales		Penis	
	Adjacent	Isolated	Adjacent	Isolated	Adjacent	Isolated
6. ii. 52	0.95	1.4	3.0	3.0	3.65	4.4
4. iii. 52	0.3	1.1	1.3	1.8	3.25	3.4
1. iv. 52	0.1	0.1	0.3	0.6	2.6	2.4
5. v. 52	0.9	1.6	0.6	0.1	2.55	2.45

self-fertilization not taking place in this species (p. 483). Table II lists the indices of the development of the male organs for parallel sets of solitary unfertilized individuals and for normal adjacent individuals taken during this period and demonstrates that there is no appreciable difference in behaviour.

## THE FEMALE REPRODUCTIVE ORGANS

The gross development of the ovary of each individual in a sample was classified, scored, and pooled to give an index, following the same general procedure described above. The ovary was classed as: (i) absent, no ovary visible; (ii) poorly developed, only a thin layer of ovarian tissue in the basal membrane; (iii) moderately developed, ovary apparently filling about a third of the mantle cavity; (iv) well developed, ovary apparently filling the greater part of the mantle cavity. A small piece of ovarian tissue was taken where possible and teased out on a slide in a drop of sea water, the size of several of the largest ova present being then measured with a calibrated micrometer eyepiece.

During the breeding season the number of individuals bearing fertilized egg-masses was recorded as a percentage of the total number examined, somewhat larger samples being employed for this purpose than were used in determining ovarian development.

## DEVELOPMENT AND FERTILIZATION OF OVA

The ova are of large size compared with those of other British species (Table III), being exceeded only by those of *B. hameri*. In the ovarian tubules they are compressed to a polygonal shape, but when ripe they round off immediately upon release into sea water, where, as would be expected, they may increase somewhat in size if the water is hypotonic and decrease if it is hypertonic. When fertilized they are at first only slightly compressed into an ellipsoid by the egg-shaped membrane which is formed around them. Calculations of the relative volume prior to and after fertilization ( $ab^2/r^3$ ), treating the egg as an ellipsoid, show that there is a slight reduction in volume when the ripe egg becomes fertilized and is surrounded by an egg case. This appears to be general in several species of cirripedes (Groom, 1894) and is known to occur in other animals (Glazer, 1914; Okkelberg, 1914). It seems likely that just after copulation the eggs are discharged via paired oviducal openings at the bases of the first cirri; these openings become swollen and glandular at the breeding season and may be responsible for secreting the matrix which subsequently hardens and holds together the eggs within each of the egg masses. A moult probably occurs at the time of copulation, as in some other arthropods (Jurine, 1820; Höglund, 1943; Burkenroad, 1947; Schöbl, 1880) since at this time many cast skins appear in the plankton.

Fig. 3 shows the observed changes in the state of the ovaries of mature individuals taken during 1951-52 in Beaumaris Bay. The records are arranged to show the full annual cycle of ovarian development, which may conveniently be divided into three periods. These are demarcated on the graph by the letters A, B, C and D.

The ovary which will give rise to the subsequent year's brood begins to



develop whilst the mature ovary or the fertilized eggs are still present. Its rudiments are first clearly discernible in mid-February (A) as a diffuse fatty tissue containing minute ova. During March and April the ovary grows rapidly, and by the end of May (B) this new ovary is almost full size. During

TABLE III. SIZES OF OVA AND FERTILIZED EGGS IN BARNACLES

(All figures are means of measurements on eggs from several individuals. The measurements listed in the first three columns of figures are in  $\mu$ .)

Species	Diameter of ova when fully developed (2r)	Length of fertilized egg (2a)	Breadth of fertilized egg (2b)	Ratio of volume: $ab^2/r^3$	Ratio of length to breadth of fertilized egg: $b/a$	Volume of fertilized egg in ml. $\times 10^{-6}$
<i>Balanus hameri</i>	265	385	205	0.87	0.53	8.50
<i>B. porcatus</i>	225	307	168	0.76	0.55	4.52
<i>B. balanoides</i>	210	284	154	0.73	0.54	3.53
<i>B. crenatus</i>	170	237	120	0.69	0.51	1.78
<i>B. perforatus</i>	160	221	115	0.72	0.52	1.54
<i>B. improvisus</i>	123	161	93	0.75	0.58	0.73
<i>B. amphitrite</i> var. <i>denticulata</i>	120	150	90	0.71	0.60	0.64
<i>Elminius modestus</i>	125	190	93	0.84	0.49	0.86
<i>Chthamalus stellatus</i>	130	191	94	0.77	0.49	0.88
<i>Verruca stroemia</i>	140	205	100	0.75	0.49	1.07

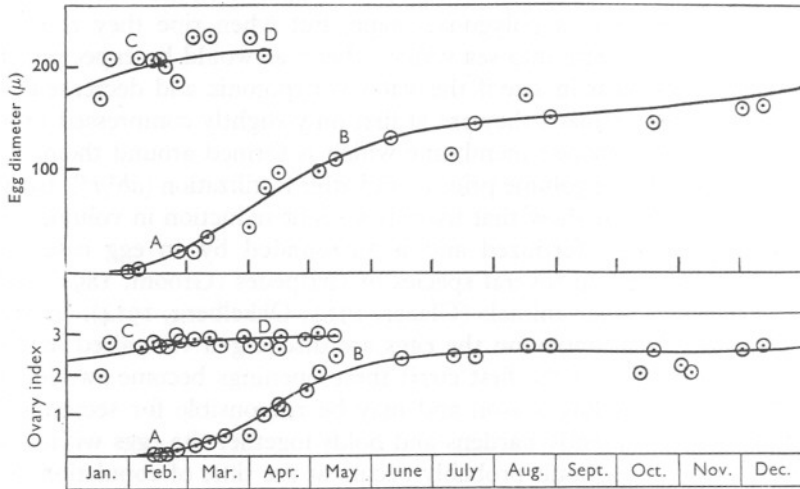


Fig. 3. Seasonal changes in the ovary. The upper curve illustrates the growth in size of ova, the lower curve variations in the ovarian index based on gross appearance (p. 479).

this same period the ova are swelling rapidly as indicated by measurements of the mean size of ova throughout the population. The size of the ova continues to increase, though more slowly, throughout the remainder of the year, until they are of mature size and age in early February (C) and remain so until fertilized (C-D). As there are individuals with varying stages of ovarian

development included in both the curve of the growth of the ovary and that of the growth of the eggs, the slope does not exactly represent the rate of development in a single individual; this probably takes place more rapidly than is indicated by the curves.

The eggs become fertilized and discharged into the mantle cavity immediately after copulation during February. Fig. 4 shows the progress of fertilization throughout a population during 1952 (upper rectangle), together with offshore and inshore temperature records (lower rectangle).

The vertical lines associated with each point in the upper graph give the 20% confidence limits based on Stevens' tables. Thus the curve representing the progress of fertilization throughout the population should pass through at least four-fifths of these lines, and should be as close to the points as possible. The full curve illustrated refers to the numerous samples taken in Beaumaris Bay, and is satisfactory in not passing outside these limits. The onset of fertilization at Port Erin, represented approximately by the broken lines, occurred at about the same time, but the number of samples available was lower, and it was not possible to compare the exact dates very closely. It will be noted that the percentage fertilized rose to an average maximum of about 80% in the Bangor samples, but almost 100% at Port Erin. This was almost certainly a reflexion of the lower density of the population at Bangor. Despite their greater size their sparse distribution probably resulted in a proportion being too distant from their neighbours for copulation. The lower fertilization maximum is unlikely to be due to innate sterility, for the Bangor individuals showed greater growth and gonad development, and never harboured the isopod *Hemioniscus balani*, which sometimes exercises a sterilizing or debilitating effect.

It is interesting to note that if in place of percentages in Fig. 4 the probits (Finney, 1952) are plotted against time, there is a reasonable approximation to a linear relationship over the period during which fertilization is taking place (Fig. 4, inset). This indicates that the frequency with which fertilization occurs is normally distributed about a central point of time. At this point, when the probit is 5.0, 50% of the population bear fertilized egg-masses. This fact offers a useful means of obtaining the central or mean date of fertilization using all the available data. This is shown to be 17 February for the Beaumaris Bay samples at which time the temperature was at its minimum. From a similar procedure the central date of liberation of nauplii is found to be 1 April. The interval of 44 days is thus an estimate of the embryonic life in this area, based on all samples taken. The curve representing the liberation of nauplii in the Port Erin samples was again close to that of the Beaumaris Bay material.

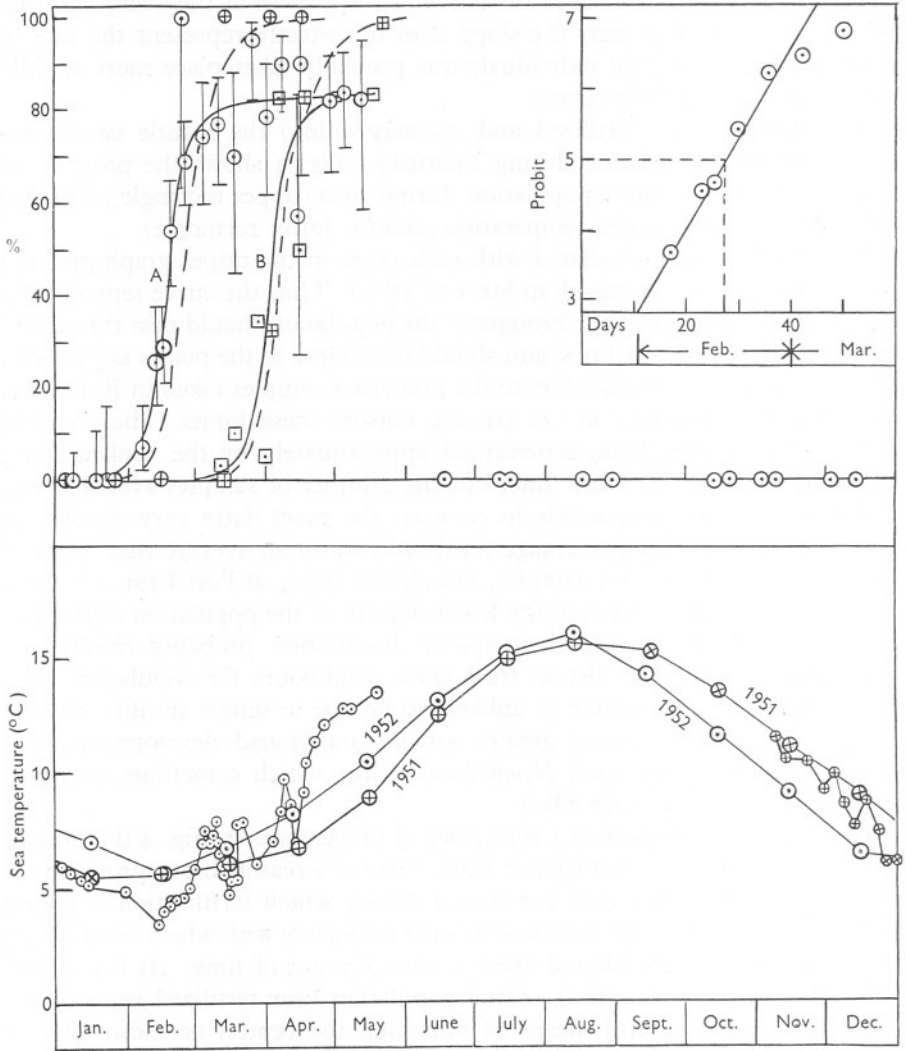


Fig. 4. Upper rectangle. Fertilization and liberation of nauplii in *Balanus porcatus* (1952).  $\odot$ , percentage of individuals bearing or having borne fertilized egg masses in Beaumaris Bay samples;  $\oplus$ , ditto, Port Erin samples;  $\square$ , percentage of individuals having liberated their nauplii, Beaumaris samples;  $\boxplus$ , ditto, Port Erin samples. To avoid confusion 20% confidence limits are shown only for the points giving the fertilization of the population in Beaumaris Bay. Lower rectangle, temperature records for 1951 and 1952.  $\otimes$ , monthly mean offshore temperatures, Beaumaris Bay, 1951;  $\odot$ , monthly mean offshore temperatures, Beaumaris Bay, 1952.  $\circ$ , inshore temperatures, Menai Straits, 1951;  $\ominus$ , inshore temperatures, Menai Straits, 1952. *Inset*. Probit transformation applied to fertilization records in Beaumaris Bay samples.

## EVIDENCE FOR OBLIGATORY CROSS-FERTILIZATION

Cross-fertilization is known to be obligatory in *Balanus balanoides* (Chipperfield, unpublished thesis), in *B. crenatus* and in *Elminius modestus* (Crisp, 1950). The present samples offered suitable material to investigate *Balanus porcatum*, since the individuals were often found singly on bivalve *Modiolus* shells. Such individuals, which were termed 'isolated', sometimes constituted more than half the catch. Where two or more individuals were found on a shell these were termed 'adjacent'. This division was rigidly adhered to even if the two individuals were at the extreme ends or on opposite valves of *Modiolus* and appeared to have little opportunity of inseminating each other, or if a minute and apparently sterile individual occurred on the same shell as an otherwise

TABLE IV. DIFFERENCES IN BREEDING BEHAVIOUR BETWEEN ADJACENT AND ISOLATED INDIVIDUALS

Date of sample	Numbers with fertilized egg masses		Numbers with full size ovaries	
	Adjacent	Isolated	Adjacent	Isolated
6. ii. 52	3/42	0/12	28/42	10/12
12. ii. 52	9/35	1/16	—	—
15. ii. 52	16/55	0/26	23/55	19/26
19. ii. 52	19/35	3/32	—	—
25. ii. 52	5/5	0/1	—	—
4. iii. 52	23/31	5/38	5/31	16/38
11. iii. 52	22/30	4/30	5/30	16/30
18. iii. 52	6/12	1/22	3/12	15/22
26. iii. 52	12/20	0/1	1/20	1/1
1. iv. 52	14/19	1/18	3/19	14/18
8. iv. 52	3/41	1/38	8/41	21/38
15. iv. 52	1/7	0/17	1/7	10/17
16. iv. 52	—	—	1/10	8/12
29. iv. 52	—	—	1/22	5/15
5. v. 52	—	—	11/32	3/4
13. v. 52	—	—	6/11	3/6
Total	133/332	16/251	* 96/332	141/239

isolated specimen. In both such cases the barnacles would be termed 'adjacent', since it was considered preferable to adhere to a definite criterion, rather than to attempt to make a judgement on the likelihood of cross-fertilization being possible, with a risk of introducing personal bias.

Table IV gives a series of parallel counts over the breeding period on samples which contained 'adjacent' and 'isolated' individuals. It will be seen that only a small fraction of the 'isolated' samples ever contained fertilized egg-masses, yet the adjacent samples showed a maximum of about 80% bearing them. Similarly, the fraction containing full-size ovaries fell to a minimum in the 'adjacent' samples, whereas the proportion of isolated individuals with large ovaries was maintained without significant change, since none had discharged egg-masses. These differences over the whole

set of samples are overwhelmingly significant. It is likely that before being dredged, a small number classed as isolated, might well have been in close proximity to individuals of the same species on other shells or objects nearby, whilst some of those classed as adjacent admittedly may have had little chance of insemination. The observed difference in behaviour of the two sets of samples is therefore slightly less than would be expected had an absolute division been possible into two classes, the one with and the other without mutual access for copulation. It is therefore clear that fertilization is at least greatly facilitated by individuals lying close together, and it is probable that this species also is an obligatory cross-fertilizing hermaphrodite. There is no indication that fertilization is merely delayed in isolated individuals, as would be expected if facultative self-fertilization were possible, and as apparently occurs in *Chthamalus stellatus* (unpublished data).

This study of the breeding cycle was therefore confined to adjacent individuals, except where otherwise stated.

#### DEVELOPMENT OF EMBRYOS AND RELEASE OF NAUPLII

The fertilized eggs develop rapidly and uniformly into embryos, which remain separately enclosed in egg-cases retained in the mantle cavity of the adult up to the time of their liberation as stage I nauplii.

The egg-cases are transparent, and details of development may be discerned simply by teasing one of the paired masses of eggs on a slide and examining under low power. After fixation in 5% formaldehyde in sea water the eggs retain their typical appearance, but most other fixatives render them opaque or cause cytolysis. Apart from small variations during the earliest stages, the development of all the eggs present in a given individual is uniform.

The development of the embryos was referred to a scheme of clearly distinguishable stages as outlined in Table V. Groom's (1894) description of the development of the eggs of *Balanus perforatus* has been found to be applicable to this and to other species of barnacle; reference is therefore included in the table to the appropriate illustrations in Groom's paper.

Unfortunately it has not been found possible to promote development *in vitro*; hence the rate of development has been ascertained only indirectly by means of population studies. In a population where the onset of fertilization is gradual, a range of several stages of embryonic development is to be found in different individuals at any one time. If enough samples of *equal* size are examined at *regular* intervals, throughout the whole period during which egg-masses are present, the percentage of the total number of individuals which are in a given stage will clearly be proportional to the time which this stage occupies in the whole developmental period. If sampling is irregular suitable adjustments for the variation in numbers of individuals in the samples and for the different periods of time between taking samples



are necessary. The proportionate time occupied by a given stage in the full term of development will then be given by the relation

$$t_n/T = \sum_i (S_n/S)_i \Delta t_i / \sum_n \sum_i (S_n/S)_i \Delta t_i,$$

where  $t_n$  is the time interval between stage  $n$  and  $n + 1$ ,  $(S_n/S)_i$  is the fraction of the total number in the  $i$ th sample lying between stages  $n$  and  $n + 1$ , and  $\Delta t_i$  is half the time interval between the  $(i - 1)$ th and the  $(i + 1)$ th sample.

TABLE V. THE DEVELOPMENTAL STAGES OF EMBRYOS IN THEIR EGG-CASES

		Corresponding stage from Groom (1894)			
		Figures illustrating stage in			
Stage	Description of development of embryo	Stage	<i>Lepas anatifera</i>	<i>Balanus perforatus</i>	<i>Chthamalus stellatus</i>
1	Unsegmented egg in oval or pyriform case	A	Pl. 14, figs. 1-7	Pl. 16, fig. 49	Pl. 18, figs. 83-86
2	Two simple blastomeres		Figs. 8-11	Fig. 50	Figs. 87-88
3	Upper blastomere divided, yolk not completely covered. 3 to 32 blastomeres	B	Figs. 12-19	Fig. 51; Pl. 17, fig. 64	Figs. 89-93
4	Yolk all undivided, completely or nearly completely covered by blastoderm cells		Pl. 15, figs. 20-23	Figs. 65-71	Fig. 94
5	Yolk cell divided in two by an oblique furrow, and completely covered by blastoderm cells	C	Figs. 24, 25	Fig. 72	Figs. 95, 96; Pl. 19, fig. 97
6	Yolk cell divided into 3 to 5 cells enclosed in blastoderm		Figs. 26, 27	Fig. 72 a, b	—
7	Six or more yolk cells. Posterior thickening of mesoblast present		Fig. 28	Figs. 73-75	—
8	Embryo divided by two or more constrictions between rudimentary swellings giving rise to the appendages	D, E	Figs. 29-35	Fig. 76	—
9	Appendages clearly visible as short bifid swellings, setae absent or not evident	F	Figs. 36-37	Fig. 77; Pl. 18, fig. 80	—
10	Appendages with distinct setae. No eye visible	G	Figs. 38-40	Fig. 81	—
11	Median eye red or poorly pigmented, mass of yolk cells present		Fig. 41	Fig. 82	—
12	Eyes darkly pigmented, black or reddish brown; endoderm forms a clearly defined gut. Not hatching within a few minutes of placing in sea water	H	—	—	—
13	As 12, but more strongly pigmented. Hatching within a few minutes of placing in sea water. In formalized material a few often found freed from egg-cases		Fig. 42	—	—

Since  $T$ , the total time of development, is known, the absolute intervals  $t_n$  between the various stages can be computed. In this way a developmental time-table can be compiled (Table VI). It will be seen that the earliest developmental stages are quickly over, but that at the end of development apparently fully developed embryos (Stage 13) are retained for some time before being liberated. This results in a wide variety of embryonic stages being present simultaneously in the early part of development, and giving place to

an appearance of greater uniformity towards the end. The degree of overlapping of embryonic stages is, however, limited, since each fertile individual produces one, but not more than one, annual brood.

During embryonic development the egg-masses change slowly in texture and appearance. When first discharged they appear bright creamy yellow and of the consistency of weak jelly, but gradually the egg-cases harden and the egg-masses become firmer. As the embryos develop more pigment, the colour of the egg-masses changes from yellow to ochre, then to fawn, and finally to dark brown. Each paired egg-mass is surrounded by an enveloping membrane in addition to the separate cases around each embryo. Immediately after fertilization spermatozoa may sometimes be seen between the fertilized eggs, but not within the substance of this enveloping membrane. Shortly

TABLE VI. DURATION OF EMBRYONIC STAGES IN *BALANUS PORCATUS*

Stage	Time of passing (days)	Duration (days)	Percentage of total time	Cumulative percentage
1	1.1	1.1	2.5	2.5
2	1.9	0.8	1.7	4.2
3	3.2	1.3	2.9	7.1
4	4.2	1.0	2.2	9.3
5	6.2	2.0	4.6	13.9
6	8.1	1.9	4.2	18.1
7	11.1	3.0	6.9	25.0
8	14.3	3.2	7.3	32.3
9	19.5	5.2	11.8	44.1
10	21.8	2.3	5.2	49.3
11	24.0	2.2	4.9	54.2
12	31.3	7.3	16.7	70.9
13	44.0	12.8	29.0	100

before liberation which, like copulation, appears to be accompanied by a moult of the adult barnacle, the egg-masses soften and break down, so that the egg-cases lie almost loose in the mantle cavity. Hatching occurs after liberation into the sea. The hatching movements of the embryos are inhibited in water of low oxygen tension. Possibly the oxygen tension in the mantle cavity of the adult is lowered by respiration, so that hatching is prevented until the eggs are individually expelled from the mantle cavity, and exposed to a greatly increased oxygen tension in the surrounding water.

Liberation of first-stage nauplii begins towards the end of March and continues through the early part of April. They moult almost at once, giving rise to second-stage nauplii. These are normally the earliest stage found in the plankton and may be distinguished from the second-stage nauplii of *B. balanoides* by their slightly larger size and longer forked process. The remaining stages are also larger than those of *B. balanoides*, with which they might otherwise readily be confused. There is no evidence that nauplii retained in the mantle cavity developed further as suggested by Barnes (1953*a*); in one instance abortive egg-masses were found still remaining towards the end of April, but, unlike those reported by Barnes (1953*b*), these

did not appear to be viable. Barnes (private communication) now considers that the larvae are invariably planktotrophic (Crisp & Knight-Jones, 1953).

The larvae have been found only in very small numbers in the plankton from Beaumaris Bay, but are much more common in samples taken in April and early May south of the Isle of Man, where the beds of *B. porcatus* are probably more extensive.

#### SETTLEMENT AND GROWTH

Not only have the larval stages been sparse in the Bangor area, but the settlement of the past two years has been correspondingly scanty compared with the heavy settlements of intertidal barnacles commonly observed. The proximity of the species to its geographical limits may explain its inability to produce heavy annual settlements as was also found in *B. balanoides* at Plymouth (Harris, 1946). Owing to the small numbers of spat found, it has not been possible to date settlement with great precision, but young spat have not been observed prior to the first week in May, and the main settlement appears to take place in the latter half of this month. By July considerable growth has occurred, and by August it is not easy to distinguish the settlement of the current year from the smaller individuals of the previous season. This difficulty results from the considerable individual variation in rates of growth, a feature which appears to be common in cirripedes, and is probably largely due to environmental differences.

Fig. 5 shows in histogram form the size-distribution of three samples of *B. porcatus*, the first taken in mid-April, a month before settlement; the second at the end of July, about  $2\frac{1}{2}$  months after settlement; and the third in December. The absence of well marked peaks indicating year-groups is due partly to variation in growth rate and partly to the small spatfalls of 1951 and 1952, which were scarcely sufficient to maintain the population at its present level and constituted only a small fraction of the total.

There can often be noted on the parietes of the shell of an old individual a series of slight concentric irregularities or ridges (Fig. 6), at which the angle of the shell changes slightly but abruptly. These discontinuities are not directly associated with moults, for animals kept in shallow dishes in the laboratory moult at frequent intervals; whereas no more than four such shell discontinuities are discernible on most large individuals. The histograms shown in Fig. 7 illustrate that the discontinuities measured from the apex of the barnacle form a regular series of frequency peaks. Slight changes in the thickness of the basis can also be observed, usually, but not always, agreeing in number with the discontinuities of the shell. It seems probable that both the parietal ridges and the basal growth bands are due to annual changes in the rate of growth. By equating the number of growth bands to the probable age, the individuals from which the histograms shown in Fig. 5 were derived were grouped, and the results of this grouping are shown as divisions within

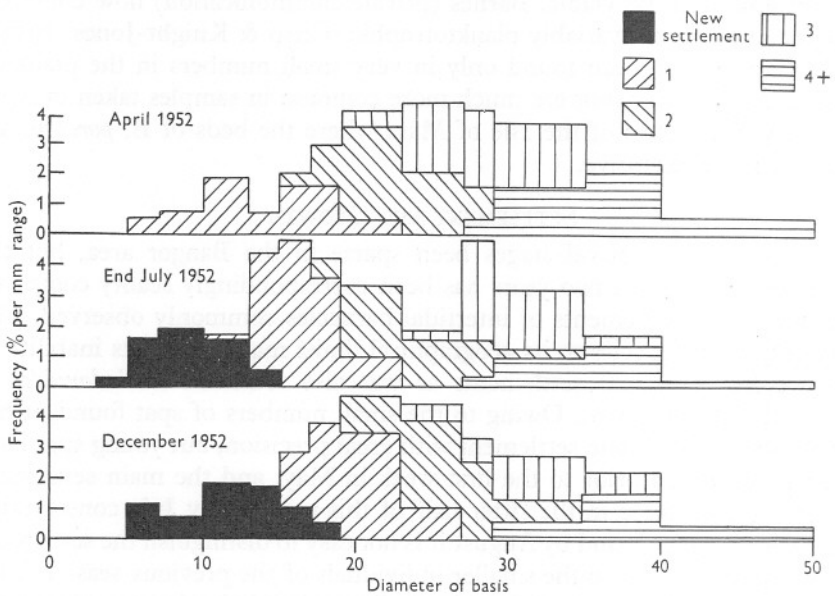


Fig. 5. Histograms of size frequency in *B. porcatulus*. The size is measured as mean basal diameter in millimetres. The histogram is divided internally to show year groups, 1 being those in their first year of growth in April 1952, 4+ being those in their fourth or later years.

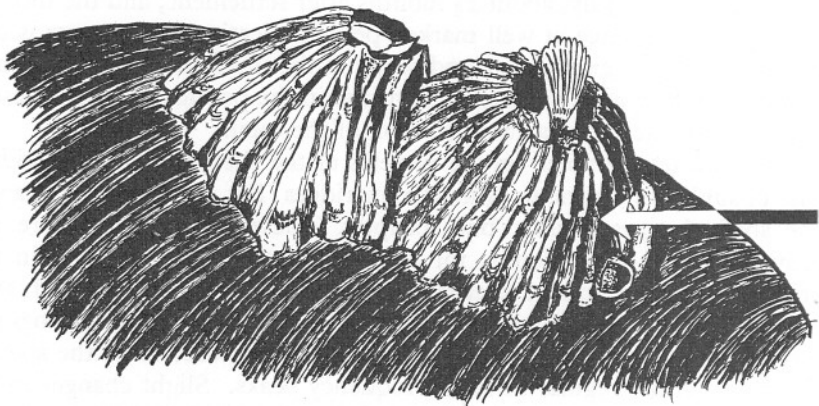


Fig. 6. Two individuals of *B. porcatulus* in their third year of growth. The arrow shows in the right hand individual the position of the rather prominent parietal growth ridges corresponding to the first year; the other growth ridges are present but are only just discernible in the original.

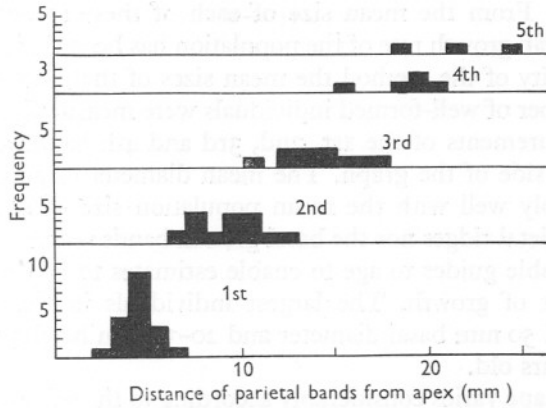


Fig. 7. Histograms relating to *B. porcatus* selected because they showed growth bands on the parietes. The frequency of occurrence of bands is plotted against the distance measured vertically (in mm) from band to apex. The horizontal scale therefore represents the probable height of the barnacle when the growth ring was formed, if increase in height is due solely to basal growth.

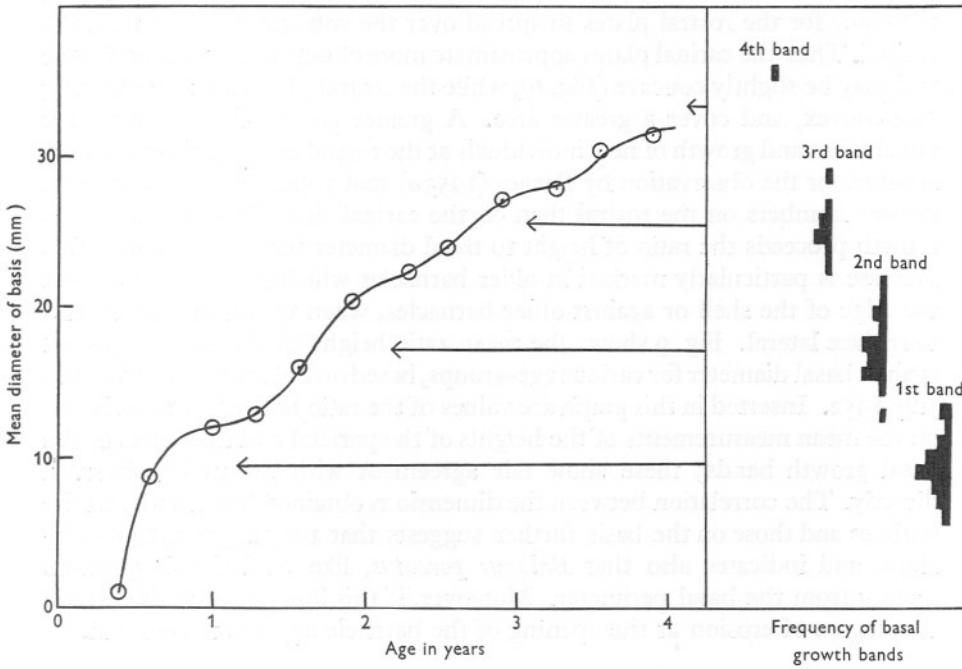


Fig. 8. Growth rate of *B. porcatus* in Beaumaris Bay. The horizontal axis gives the estimated age, the vertical axis the mean basal diameter from the histograms illustrated in Fig. 5. On the right-hand side are shown histograms of the basal growth marks of a few individuals in which they were clearly distinguishable; these correspond approximately to annual rings.



the histogram. From the mean size of each of these age-groups, a curve showing the mean growth rate of the population has been derived (Fig. 8). To check the validity of the method the mean sizes of the growth bands in the bases of a number of well-formed individuals were measured, and histograms based on measurements of the 1st, 2nd, 3rd and 4th bands are inserted on the right-hand side of the graph. The mean diameter of each growth band agrees reasonably well with the mean population size of each year-group. Neither the parietal ridges nor the basal growth bands seemed generally to be sufficiently reliable guides to age to enable estimates to be extended beyond the fourth year of growth. The largest individuals in the population, of between 40 and 50 mm basal diameter and 20–30 mm height, were probably some 5 or 6 years old.

Individual shape varies considerably according to the substratum on which the barnacle grows. In general, growth on the convex part of a *Modiolus* shell tends to give a tall individual, particularly if the edge of the barnacle reaches the edge of the molluscan shell and cannot grow further. Conversely, *Balanus porcatus* growing on a concave surface, as for example on the inside of a dead *Modiolus* shell, is often flatter than usual. There is also a marked tendency for the rostral plates to spread over the substratum more than the carinal. Thus the carinal plates approximate more closely to the perpendicular and may be slightly concave (Fig. 6), while the rostral plates are more sloping and convex, and cover a greater area. A greater area is thus available for attachment and growth of new individuals at the rostral end, which may partly account for the observation by Barnes (1953*a*) that young barnacles occur in greater numbers on the rostral than on the carinal side of the aperture. As growth proceeds the ratio of height to basal diameter increases slightly; this increase is particularly marked in older barnacles which have grown against the edge of the shell or against other barnacles, when upward growth tends to replace lateral. Fig. 9 shows the mean ratio height/basal diameter plotted against basal diameter for various age-groups, based on individuals collected in July 1952. Inserted in this graph are values of the ratio height/diameter based on the mean measurements of the heights of the parietal and diameters of the basal growth bands; these show fair agreement with the ratios observed directly. The correlation between the dimensions obtained from marks on the parietes and those on the basis further suggests that they represent growth-rings, and indicates also that *Balanus porcatus*, like *B. balanoides*, grows mainly from the basal perimeter. Moreover, if this interpretation is correct, the degree of erosion at the opening of the barnacle appears to be small.

#### DEVELOPMENT OF GONADS IN RELATION TO AGE

Fig. 10 illustrates the relation between size and gonad development, scored as described above (pp. 475–9). The data on which this graph is based are necessarily restricted to those seasons when the gonad is normally well developed in

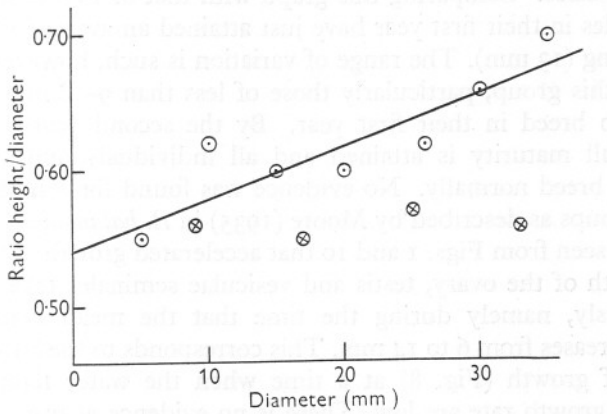


Fig. 9. Ratio of height to basal diameter in *B. porcatus*.  $\odot$ , mean ratios based on measurements of groups of individuals of a given size range;  $\otimes$ , ratios based on mean values for 1st, 2nd, 3rd and 4th parietal and basal growth marks respectively.

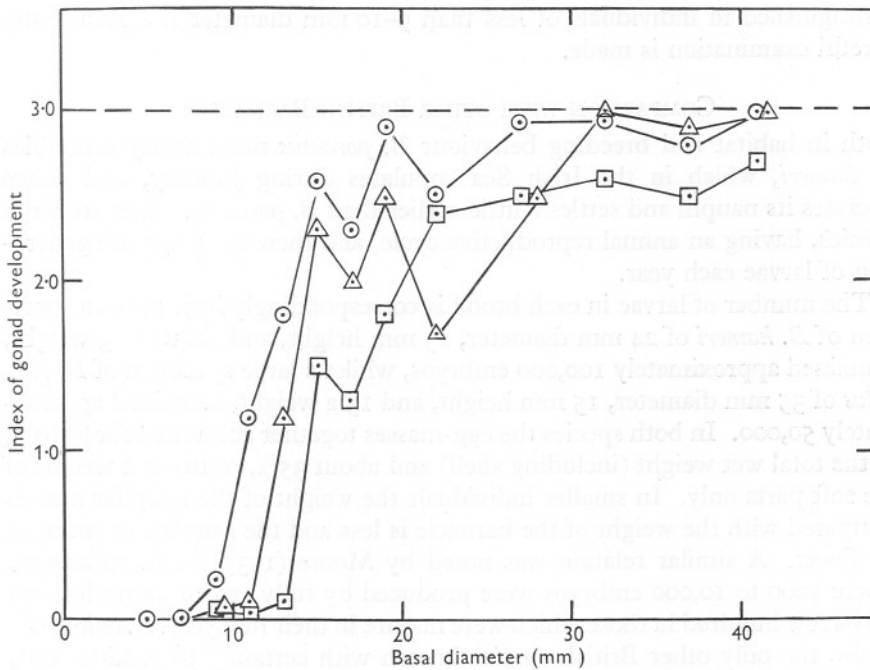


Fig. 10. Growth of gonads in relation to mean basal diameter (mm).  $\odot$ , Index of development of ovary (p. 479).  $\triangle$ , Index of development of vesiculae seminales (p. 475).  $\square$ , Index of development of testis (p. 475).

mature barnacles. Comparing this graph with that of Fig. 8, it will be seen that barnacles in their first year have just attained an average size consistent with breeding (13 mm). The range of variation is such, however, that a percentage of this group, particularly those of less than 9–10 mm in diameter, often fail to breed in their first year. By the second year and thereafter however, full maturity is attained and all individuals, unless isolated or parasitized, breed normally. No evidence was found for senility among the later age-groups as described by Moore (1935) in *B. balanoides*.

It will be seen from Figs. 1 and 10 that accelerated growth of the penis and initial growth of the ovary, testis and vesiculae seminales take place almost simultaneously, namely during the time that the mean diameter of the barnacle increases from 6 to 14 mm. This corresponds to the latter part of the first year of growth (Fig. 8) at a time when the water temperature and assimilative growth rate are low. There is no evidence at present to suggest that any one of the reproductive organs precede in development or initiate the growth of the others. The slightly earlier recorded appearance of the penis and ovary may be due only to the greater facility with which these structures can be observed in a rudimentary state, for the testes also can often be distinguished in individuals of less than 9–10 mm diameter if a sufficiently careful examination is made.

#### COMPARISON WITH OTHER BRITISH BARNACLES

Both in habitat and breeding behaviour *B. porcatus* most nearly resembles *B. hameri*, which in the Irish Sea copulates during January, and hence liberates its nauplii and settles a little earlier than *B. porcatus*. Both are large species, having an annual reproductive cycle, and therefore only one generation of larvae each year.

The number of larvae in each brood is correspondingly large; thus a specimen of *B. hameri* of 24 mm diameter, 25 mm height, and about 20 g weight, contained approximately 100,000 embryos, whilst a large specimen of *B. porcatus* of 35 mm diameter, 15 mm height, and 13 g weight, contained approximately 50,000. In both species the egg-masses together accounted for 8–10% of the total wet weight (including shell) and about 35% of the wet weight of the soft parts only. In smaller individuals the weight of the naupliar masses compared with the weight of the barnacle is less and the number of embryos far fewer. A similar relation was noted by Moore (1935) in *B. balanoides*, where 5000 to 10,000 embryos were produced by fully grown barnacles, but only a few hundred in those which were mature in their first year. *B. balanoides* is also the only other British species known with certainty to produce only a single annual brood.

*B. porcatus*, *B. hameri* and *B. balanoides* are all boreal-arctic forms reaching their southern limits in British waters. All three species therefore accord with the principle, enunciated by Appellöf (1912) and Orton (1919), that

animals living in the warmest parts of their geographical range breed in the coldest months of the year. Thus the approximate periods during which embryos are found in the mantle cavity in British waters are: *B. balanoides*, November–March (Moore, 1935); *B. hameri*, early January–early March (unpublished data); *B. porcatius*, February–March.

Orton (1919) quotes, in support of this general principle, *Antennularia ramosa* and *Bougainvillia ramosa* as being winter breeders in the Mediterranean, and early-summer breeders at Plymouth, pointing out that well-defined temperature limits control the period of breeding of such species and account for their latitudinal variations in the times of breeding. It seems unlikely, however, that boreal-arctic barnacles breed during the warmer months even in high latitudes. In *Balanus balanoides*, in particular, it is known that at higher latitudes the onset of breeding occurs earlier, but only sufficiently so to compensate for more prolonged development during the colder winter. The embryos are therefore still retained throughout the coldest months, although copulation occurs slightly earlier in the autumn and liberation slightly later in the spring.

It seems probable that in these species the annual cycle is so adjusted that over a wide range of latitude their larvae are liberated in the spring, when abundant phytoplankton is available, and when conditions are favourable for rapid and prolonged growth after settlement. The rapid ovarian development which occurs during early summer in all these species, but which does not lead immediately to reproduction, may be primarily an adaptation to arctic conditions when the growing season is short. Reproduction is postponed until winter, so that the planktonic stages will not be thrown on their own resources until the spring, when the adults too begin to assimilate reserves for the following winter's breeding cycle. This behaviour is in marked contrast to that of warm temperate barnacles such as *Elminius modestus*, *Chthamalus stellatus* and *Balanus improvisus*, which produce several broods during the summer months, but lie dormant and without obvious food reserves during the winter.

The marked recession of the testes and vesiculae seminales and the slight decrease in length of the penis, that occur in *B. porcatius* immediately after fertilization, occur also in *B. hameri* and are very pronounced in *B. balanoides*, where the male organs regenerate rapidly only a few months prior to copulation (Chipperfield, unpublished). Both in *B. balanoides* and *B. porcatius* it has been shown that this recession is seasonal and occurs whether or not copulation and discharge of eggs by the ovary have taken place. It is therefore improbable that the growth of the male gonad is controlled by any hormonal influence from the ovary, as might have been thought possible from the time relations of the development of these organs.

The onset of copulation corresponds to the coldest part of the year (Fig. 4), but it is not known how far it is directly controlled by temperature. In deep

turbid water temperature is probably the only reliable indicator of season; salinity, light and biological factors varying widely from year to year. A simple relation between the onset of breeding and the immediate temperature seems, however, rather unlikely, for there is a considerable variation in the minimum temperature reached, not only year by year but also at different depths, and this would make it very difficult to explain the regularity and uniformity in breeding shown in different seasons. A direct stimulus due to change of temperature is also improbable in this species since breeding commences when the temperature changes are minimal. It is considered more likely that the gradual fall in temperature throughout autumn and winter sets in motion metabolic changes causing gradual ripening of the gonads. If these changes were suitably geared to the falling temperature gradient, copulation could follow as a result of the maturation of individuals at the appropriate season without an immediate external stimulus. The normality of the distribution of fertilization times (p. 481) is probably a reflexion of a normal distribution in the development of the gonads, and therefore supports this view. Were an external stimulus responsible, one would expect a sudden onset of fertilization in which all the ripe individuals would take part, followed by a gradual tailing off as those developing later became mature.

The growth rates and longevities of *B. porcatus* and *B. hameri* are similar; their attainment of maximum size is very gradual, but mortality is much lighter than in the smaller species of barnacles. The thick shells of *B. porcatus* do not rapidly decay, yet relatively few dead ones are found among the early year-classes. This is probably related to the quieter conditions obtaining off-shore. It is somewhat surprising, in view of the remarkable similarity in the ecology of these two large species, that their general shapes are entirely different, and that the shell of *B. porcatus* is extremely thick and the compartments well knit together, whereas that of *B. hameri* is rather fragile.

My thanks are due to Prof. F. W. Rogers Brambell, F.R.S., for his continued hospitality during the period that the Marine Station was still unready for occupation, to Dr J. S. Colman for arranging the collection and sending of numerous samples of *B. porcatus* from the grounds near Port Erin, and to Dr E. W. Knight-Jones, who made a number of collections on my behalf before my arrival in Bangor, and who kindly read the text and suggested a number of improvements.

#### SUMMARY

*Balanus porcatus* is a cross-fertilizing hermaphrodite which breeds once a year, producing some 50,000 embryos in each brood.

In the Irish Sea copulation and discharge of eggs into the mantle cavity takes place in February and liberation of nauplii in March. During copulation



spermatozoa are injected into the mantle cavity and ova are discharged simultaneously. The spermatozoa become enclosed with each mass of eggs in an enveloping membrane which softens only when the embryos are fully developed and ready to hatch. Hatching occurs after liberation into sea water and is probably induced by the higher external oxygen tension. After a larval development lasting 6–8 weeks in the plankton, the cyprids settle in May and June.

In mature individuals the testes develop and the vesiculae seminales gradually fill during summer and autumn. The penis, though present throughout the year, becomes somewhat enlarged in mid-winter just before copulation. Prior to copulation the testes begin to degenerate, and just after copulation the vesiculae seminales shrivel and the penis becomes somewhat shortened.

The ovaries re-develop during spring and summer, the bulk of ovarian tissue being assimilated by autumn. The ova, however, increase in size steadily throughout the year, attaining maximum size and ripeness by mid-winter.

The rate of growth has been measured by noting the numbers of growth marks on the parietal plates and bases of the shell. The parietal marks are more distinct and are due to the angle of growth of the shell varying slightly during each season. Individuals commonly live for 3 or 4 years reaching a basal diameter of 30–40 mm: some probably survive for as long as 5 or 6 years. Some mature in their first year. Both male and female gonads appear when a basal diameter of about 10 mm has been attained, and the penis simultaneously undergoes rapid heterogonic growth.

It is suggested that the winter breeding of this species, of the closely similar *B. hameri*, and of *B. balanoides*, is an adaptation enabling the nauplii to be liberated as early as possible in the spring. They thus have the maximum available time to establish themselves during the short summer of higher latitudes. The main period of assimilation of the adult also falls during spring and summer, a long period with full gonads intervening before the postponed onset of breeding.

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## THE SUSPENDED MATTER IN SEA WATER AND ITS SEASONAL CHANGES AS AFFECTING THE VISUAL RANGE OF THE SECCHI DISC

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(Text-figs. 1 and 2)

In a previous paper (Armstrong & Atkins, 1950), 20 l. samples from International Hydrographic Station England No. 1 (E1), about 10 miles south-west of the Eddystone in the English Channel, were filtered through collodion membranes of  $1.09\mu$  average pore diameter (A.P.D.).

Between June 1948 and November 1949 the residue from these surface samples, when dried and ignited, amounted to  $0.45-2.77\text{ g/m}^3$  (or parts per million). These deposits consisted of 55-17% silica, 28-3% ferric oxide, 20 to under 1% of alumina, and 70 (or, excluding one high value, 29) to 9% calcium carbonate. A few determinations of insoluble organic matter gave to 1.15-1.77 p.p.m. when dried at  $100^\circ\text{C}$ . Pettersson (1934*b*), using the glass filters of Schott (Jena), found 1.4 p.p.m. for the total organic and inorganic matter similarly dried, from surface water in the Gullmar Fjord during the spring outburst.

The suspended matter, organic, and inorganic, is responsible for by far the greater part of the scattering of light (Atkins & Poole, 1952), the extinction of which is due to absorption by the water and suspended matter and to scattering.

The difference in the optical properties of water samples depends upon the amount and properties of the suspended matter and the 'yellow substance', which is supposed to be in solution. Furthermore, as the scattering produced by very fine particles affects the short wave end of the spectrum more than the rest of it, it acts in a manner similar to a yellow filter, and as far as we know 'yellow substance' has never been isolated. In the red and the deep red the much increased absorption reduces the relative importance of the scattered light.

### THE COMPARISON OF SUSPENDED MATTER ON FILTER DISCS

As shown by Atkins & Jenkins (1953) a good comparison can be made visually and in photographs; it is easy to place the discs in order of increasing deposit, when 1 l. or 2 l. of water are passed through a collodion membrane. Ordinarily the discs are of a greyish mud colour, tinged sometimes with faint yellowish

green. On 21 October 1952, and on 25 March 1953, the deposit was a light buff darkening with quantity to chocolate. This happened again on 5 October 1953.

The discs were photographed to get a permanent record. This places them in relative order for each series, but it is well known to be very difficult to get an accurate measurement of intensity of grey from a photograph.

Accordingly we determined the diffuse reflexion relative to that of an unused disc, namely the relative albedo. For comparison we also determined this in the photograph for 21 January 1953, taking the white filter-paper background as 100. Actually its reflexion may be higher or lower than that of the filter disc, but, as shown in the print reproduced in Fig. 1, the two were in some places indistinguishable, or in others the filter disc was very slightly the lighter. The effect would be to give a relative albedo 1% or 2% too high.

The relative albedo was found as follows. A selenium rectifier cell was mounted in the place of an eye-piece on an old microscope stand, on the stage of which the sample discs were placed inside a ring immediately beneath the tube, which was devoid of lenses. The discs were illuminated using a 36 W 12 V car headlight, at about 45° angle of incidence. The cell was racked up till its field of view embraced the greater part of the disc. With this arrangement a dry collodion disc gave 160–200 scale divisions when the cell was connected to a galvanometer of sensitivity  $0.946 \times 10^{-9}$  A/mm and resistance 686  $\Omega$ . Even with such a high resistance the selenium cell showed a rectilinear relation between current and light for such small currents, under 0.2  $\mu$ A. It was found that, with the depths of tint given on the discs by 2 l. of water from station E 1, the relation between quantity of suspended matter and tint was rectilinear. Thus the line for a sample as obtained and when diluted with distilled water was rectilinear for 75, 50, 25, 20, 15, 10, 5% sea water plotted against the albedo. The inorganic suspended matter was thus studied along with the phytoplankton.

It had been shown by Atkins & Poole (1952) that the scattering of light by surface samples of sea water was usually greater than that by the deeper water. To ascertain the variation with depth of the residue of the suspended matter, after extraction with 80% aqueous acetone to remove chlorophyll and carotenoids, the discs were ignited and weighed. The samples were taken at Station E 1, 19 June 1952. The photograph of these discs before extraction is shown in Atkins & Jenkins (1953). The ignited residue was 1.6 g/m<sup>3</sup> for surface water; 1.0 for 5 and 10 m, and 0.4 g/m<sup>3</sup> for 15, 20, 25 and 50 m. It is clear that an estimate based upon surface samples only may be much too high. The change in amount of residue did not coincide with the thermocline. The aqueous acetone extraction probably left some sea salt behind, but its major constituent, sodium chloride, would have been volatilized during the ignition, so it is unlikely that sea salt introduced an error. Under the microscope the residue appeared to be mostly amorphous, but with numerous crystalline mineral particles.

Taking the above values for mineral suspended matter and interpolating suitably, the 0–20 m column for 19 June was found to contain 18.0 g, which may be taken as having sp.gr. 2.8, and so a volume 6.4 cm.<sup>3</sup> If the suspended matter consisted of little cubes of 1  $\mu$  sides, a limit that might not pass the collodion filter, there would be  $6.4 \times 10^{12}$  of them in a 20 m<sup>3</sup> column, a depth at which one might see a Secchi disc in clear water. The number given is equivalent to having 32 in a haemocytometer field, 1 mm<sup>2</sup> in area and 0.1 mm deep. Of this area the cubes would obstruct 32  $\mu^2$ , out of the total 10<sup>6</sup>  $\mu^2$ . Thus were the cubes entirely opaque and—quite impossible of course—arranged so as not to overlap—one would have a complete screen cutting off all light in 3.12 m. It so happened that on this date, 19 June 1952, the Secchi disc could be seen to 23 m, the maximum for the years 1948 to 1953 inclusive. The date also gave a minimum for the chlorophyll for that summer, and with the assumptions made in the earlier paper the 37 mg of chlorophyll found in the 20 m column would indicate 6.4 g for the wet weight of the phytoplankton. Obviously this would give the same number of 1  $\mu^3$  organisms as there were mineral particles, and the size is a possible one. These, however, would be nearly transparent and the mineral particles would have a higher refractive index and would produce a greater effect optically. The coincidence in the numbers of particles is quite fortuitous, and occurred when the phytoplankton was at a minimum.

It is obvious that if the particles (or organisms) had sides 2  $\mu$  in length, the volume would have been 8  $\mu^3$  with  $0.8 \times 10^{12}$  particles in a 20 m column, and only four, with area 16  $\mu^2$ , in the volume of the haemocytometer field. Thus as the size increases the scattering and obscuration occasioned by the same mass of particles is much reduced. It follows that the optical effects produced by equal numbers of different species of the phytoplankton may be very different.

#### MICROSCOPIC EXAMINATION OF THE SUSPENDED MATTER

Dr A. G. Lowndes kindly examined some of the filter discs, which were handed to him after the plant pigments had been removed by 80% aqueous acetone in the cold. They were examined first of all by means of strong reflected light under a low-power binocular microscope, magnification 20 diameters, and presented a ground mass of varying colour which could not be resolved even under much higher powers. The ground mass appeared to be a fine mud or clay. A number of black specks and fibres were also present. These were removed, mounted in Canada balsam and observed with crossed nicols. The fibres were from wood and the remains of rope or twine. The wood fibres consisted of both oak and pine or woods closely allied to them. The other fibres consisted of such things as manila and coir and possibly a little jute. The black or dark specks consisted of carbonaceous matter, probably to be associated with soot, pitch or tar. These were readily volatilized when heated on the corner of the slide over a bunsen. Other dark specks consisted of oxides



of iron, possibly from the stokeholds of ships. Fragments of shell were always present. There was very little actual mineral matter, other than the clay, except for an occasional grain of quartz or cordierite one of which was about 0.1 mm in diameter. There was also a small amount of kaolinite. There were occasional sponge spicules, also diatoms and copepods.

Some incinerated residue from the discs of 19 June was also examined. This consisted of the minerals quartz and cordierite, also some micaceous matter, probably sericite. Broken grains of feldspar (orthoclase) were also present, as well as a small fragment of tourmaline. Most of these grains were under 40  $\mu$  in diameter. Dark grains of iron oxides were fairly abundant.

Cordierite (syn. iolite) may be found in minute crystals in granite and is not very common. It is a silicate of the oxides of aluminium, magnesium and iron. Orthoclase feldspar is typically a silicate of aluminium and potassium and is common in granite in Cornwall and in most granites everywhere. Tourmaline also is common in granite and varies in composition, it usually consists of silicates of aluminium, sodium and boron.

#### THE VARIATION IN SUSPENDED MATTER THROUGHOUT THE YEAR

The greater part of the organic suspended matter arises from the phytoplankton and a quantitative study of this, based upon the chlorophyll extracted from the filter discs, was made for 1951-52 by Atkins & Jenkins (1953).<sup>1</sup> But the collodion discs do not appear to alter greatly in depth of colour when extracted with 80% acetone, so it seems that the greater part of the residue, organic and inorganic, remains on them. From June 1952 to January 1953 photographs of the discs were obtained. Those for June and July 1952 have already been published and may be compared with Fig. 1 which shows the discs for 22 September 1952 and 21 January 1953. In September the surface disc is the darkest, and Table I shows that this is quite usual, but it is not usual to have the other discs so like the surface; from 15 to 50 m the water seemed uniform, with 5 and 10 m slightly poorer in suspended matter. This uniformity is the result of the vertical mixing of the water, the whole column having just reached the isothermal state. Such a column is less stable than one with an upper warm layer, but in spite of this and the fact that the water had cooled from 14.24 to 12.31° C in the 8 weeks' interval, the November column gave a very dark surface disc, and, after some mixing in December, so did that of January, as shown in Fig. 1. With very little plankton at this season, the effect must be almost entirely due to lifeless matter, much of which rapidly re-accumulates near the surface. It is not known how thick this layer is, but it is remarkable that photoelectric measurements of submarine light intensity have often shown a surface loss for which a satisfactory explanation has been

<sup>1</sup> In this paper the following *corrigenda* should be noted. At the bottom of p. 501 and top of p. 502, 'g.' (in three places) should read 'kg'. In the Summary (6th and 7th lines) 'wet weight of phytoplankton' should read 'chlorophyll'.

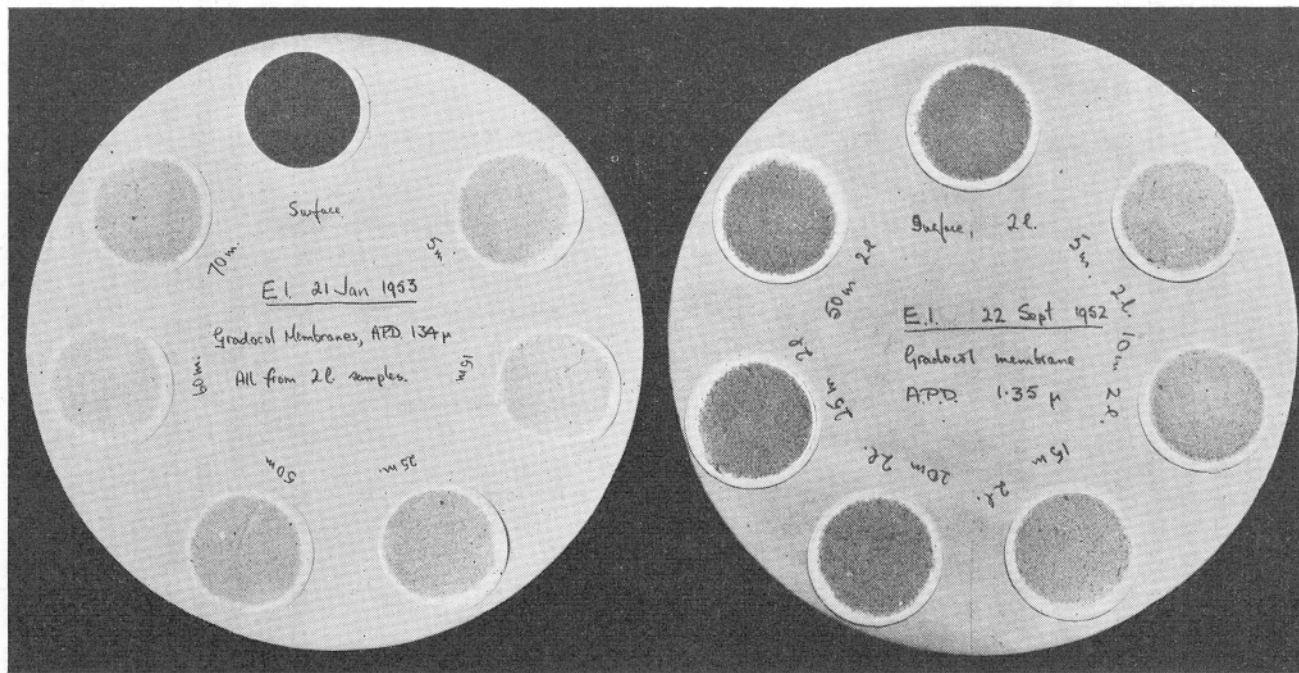


Fig. 1. Gradocol membranes after filtering 2 l. of water from Station E1 September 1952 and January 1953.

difficult. But when the suspended matter is much reduced the phytoplankton portion may cause a maximum at a considerable depth, 25 m, as on 15 July 1952, on 16 March and on 10 August 1953.

As previously explained, in order to get quantitative results, recourse was had to determinations of the relative albedo of the discs. Table II shows that the albedos of the photograph tend to increase the contrast somewhat. But the agreement of the visual examination and albedo methods can be very good

TABLE I. ORDER OF AMOUNT OF DEPOSIT ON COLLODION FILTER DISCS

The depths are placed in ordinal rank according to the amount of deposit on the collodion filter discs, the darkest colour ranking first, from June 1952 to November 1953. Two litres of water filtered through each from depths shown, at International Hydrographic Station, E I.

Depth (m)	19. vi.	15. vii.	20. viii.	22. ix.	20. xi.	22. xii.	21. i.	24. ii.	16. iii.
0	1	2	1	1	1	1	1	1	2
5	3	4	4	7	6	4	5	3	6
10	6	7	5	6	—	—	—	—	4
15	6	4	1	2	2	4	6	2	2
20	5	4	3	2	—	—	—	—	4
25	3	1	5	2	6	4	2	3	1
30	—	—	—	—	—	—	—	—	9
40	—	—	—	—	—	—	—	—	—
50	2	2	5	2	2	2	2	3	8
60	—	—	—	—	—	4	6	—	—
70	—	—	—	—	—	2	2	—	7

Depth (m)	25. iii.	27. iv.	22. vi.	20. vii.	10. viii.	8. ix.	5. x.	20. x.	17. xi.
0	5	1	1	7	5	7	2	1	1
5	2	5	2	2	5	1	9	2	2
10	7	—	—	—	4	3	1	6	4
15	6	5	5	7	8	5	8	3	3
20	7	8	4	5	2	6	4	5	5
25	9	8	3	6	1	9	5	4	5
30	10	5	5	7	3	4	7	7	9
40	2	2	—	4	—	—	—	—	—
50	2	3	5	2	8	2	6	7	7
60	—	—	—	—	—	—	—	—	—
70	1	3	5	1	7	8	3	7	8

as far as the placing in decreasing order is concerned. Thus for 16 March the two agree exactly save that the albedo places the 5 m as seventh and the 70 m as sixth, whereas the visual comparison reversed the order. Here, however, the albedo was 74 for 5 m and 73 for 70 m and errors larger than this could easily occur. On 10 August, however, with very pale discs, agreement was not at all as good, but five discs had albedos of 82–84%.

Going back to the re-establishment of layering as indicated by the photographs for the November and January discs, there is evidence of long standing that this is due to the presence of plankton or to organic matter. It is shown by a study of the 'respirable carbon' (Atkins, 1922), for on storing water taken from E I on 9 November 1921 and re-determining its pH value at intervals,

it was seen that the production of carbon dioxide was markedly greater between 20 and 25 m than elsewhere, with a minor peak in the graph at 5 m as seen in the 1921 figure.

TABLE II. RELATIVE ALBEDO OF COLLODION DISCS DURING 1953

The discs had filtered 2 l. of sea water taken at various depths from Station E 1 during 1953. The second column shows the relative albedo of the photographic print of the January series, alongside of the direct test on the disc as in column 3.

Depth (m)	21. i.	21. i.	23. ii.	16. iii.	25. iii.*	27. iv.	22. vi.†	10. viii.	8. ix.	5. x.*	20. x.	17. xi.
0	29	25	64	66	73	55	37	84	73	49	60	58
5	88	68	81	74	64	66	49	83	54	61	64	64
10	—	—	—	71	81	—	—	84	61	41	76	62
15	95	81	73	66	78	67	55	94	67	59	70	62
20	—	—	—	71	82	74	52	82	70	59	72	70
25	75	57	82	61	86	74	50	66	79	57	72	70
30	—	—	—	82	88	66	54	89	69	54	79	80
40	—	—	—	—	72	61	—	—	—	—	—	—
50	75	63	82	75	70	63	55	74	68	55	81	73
60	95	81	—	—	—	—	—	—	—	—	—	—
70	88	62	—	—	66	64	55	82	73	55	82	79

\* The colour was not grey or grey brown but light buff to chocolate, qualitatively similar to the October 1952 sample.

† The samples are comparable among themselves, but were wet and so more transparent and appeared too dark.

#### VISUAL RANGE OF THE SECCHI DISC IN RELATION TO THE PHYTOPLANKTON

It is known that the visual range of an object is affected by the illumination received by the intervening medium, air or water, as with strong illumination more light is scattered in the line of sight. We are indebted to Dr H. H. Poole for pointing out that determinations of visual range, when the line of sight is shaded from sunlight by the hull of a ship, are of necessity higher than when not thus shaded. However, the shading of the range is here not from direct sunlight, as in air, but from sunlight much reduced in intensity by passage through the water and with some surface loss. There is, on the other hand, a large reduction in visual range when the sunlit surface of the sea is interposed between the eye and the object. It seems preferable, therefore, to accept readings of the range made through a shaded water surface.

Thus on 12 April 1948, the sky was cloudless and the sea surface glassy; the disc was seen to 17 m in the sun, but to 21 m in the shade. Under the same conditions on 10 May the depths were, respectively 19 and 22 m. In more opaque water at the same station, E 1, the sun and shade readings were 6.5 and 7.7 m. Care was taken to have several youthful observers. It is obviously more difficult to see this small disc when the water is rough, but with patience consistent readings can be got. The colour seen here is greenish for 9 m and a lovely blue for 21 m.

The visual range of the Secchi disc has been determined for many years. The results using a 20 cm disc at Station E 1 from 1948 to 1953 are shown in Table III.

TABLE III. VISUAL RANGE OF 20 CM SECCHI DISC AT STATION E1

D, date; M, range in metres. Observations in shadow of ship.

	1948		1949		1950		1951		1952		1953	
	D	M	D	M	D	M	D	M	D	M	D	M
Jan.	—	—	—	—	18	11	24	11	7	12	21	16
Feb.	2	8	—	—	—	—	26	13	5	14.5	23	15
Mar.	3	16	1	18	—	—	—	—	11	12	16	13
Mar.	10	17.5	—	—	24	13	28	10	—	—	25	16
Apr.	12	21	13	13	—	—	—	—	16	13	13	14
Apr.	—	—	—	—	—	—	25	14	—	—	27	11
May	10	22	9	14.5	3	13	17	20.5	15	9	11	7.5
June	9	9	6	19.5	—	—	—	—	—	—	8	22
June	15	10	—	—	—	—	—	—	19	23	22	11
July	—	—	4	21	19	15	17	22	15	18	20	11
July	—	—	8	16	—	—	—	—	—	—	—	—
Aug.	16	7.5	17	18	23	14	22	14	20	11	10	17
Aug.	—	—	29	17	—	—	—	—	—	—	—	—
Sept.	—	—	—	—	20	13	27	9	22	18	8	11
Oct.	5	12	6	11	23	14	24	18	21	15	5	12*
Oct.	—	—	—	—	—	—	—	—	—	—	20	18
Nov.	—	—	8	9	22	11	27	13	20	14.5	17	17
Dec.	—	—	—	—	—	—	—	—	22	14	14	16

Depths are shown in italic, save maxima, in bold, and minima, in roman type.

\* 1 m added to correct for observation having been made in weak sunshine.

Several workers have presented results for phytoplankton and visual range of the Secchi disc which indicate an inverse relation between the two sets of results. Thus during the Great Barrier Reef expedition Marshall (1933) showed the 20 cm disc depths and number of diatoms per litre. The figures relate to hauls with the fine silk tow-net and wind action on the shallow water was a serious disturbing factor. Nevertheless, in her 1-year curve seven marked decreases in the disc readings coincided with high diatom numbers.

During the same expedition Russell & Colman (1934) found that the curve for total copepods taken in oblique hauls of the silk net follows closely that for the depth at which the disc disappeared. The peaks in the plankton curve occurred when the water was clearest. They considered that this was due to the copepods, etc., having moved down when the light passing through very clear water became too bright. This seems very probable, but the absence of the animals may in itself have contributed to the clearness, though the larger the animal is, the less is its obscuring effect over a large area, since finely divided matter has a greater surface, weight for weight.

Pettersson, too (1934*a*), cited phytoplankton figures to support a direct relation with the scattering of light, determined with his new scattering meter. The diatoms were centrifuged, identified, and counted by H. Högländ, who in later work instead of centrifuging allowed the cells to settle. Atkins & Poole (1933) measured the extinction coefficient in spring just when phosphate consumption indicated that a great increase in the phytoplankton had already occurred. They found it low. Evidence has since been obtained which shows



that such clearing of the water may be due to the sinking of the diatoms and to their consumption by animals. Further, the extinction coefficient is not largely affected by scattering when it is determined by measuring the illumination falling on horizontal plates, since light scattered out of the direct beam is compensated for by light scattered in.

There is no doubt that water masses may be characterized by the extinction coefficient. This has been shown by Jerlov & Koczy (1947) and in much detail by Jerlov (1950, 1951) also by Krey (1952). Joseph (1950) has recorded changes in extinction while the ship steamed ahead. When, however, we are dealing with a stable water mass, or one the movements of which are within an area experiencing closely similar meteorological conditions, it is then profitable to study the changes in visual range as a rapid and simple indication of changes in phytoplankton. Riley (1941) recorded this inverse relation, a rich plankton with Secchi disc 5.5–8.5 m, even down to 3.5 in April, and a lesser amount of plankton with 12.5 to 19 m. Riley followed the changes in the chlorophyll content of the matter filtered out, as has since been done by Gessner (1944) and others.

Fig. 2 shows the visual range in metres of the 20 cm Secchi disc at Station E 1 from September 1951 to November 1953, the scale being on the right-hand ordinate. On the left is the scale for chlorophyll in milligrams per  $m^2$  in the surface to 20 m water column,  $1 m^2$  in area. Very strikingly the minimum visual range, 7.5 m, was observed on 11 May 1953, when the chlorophyll from 0 to 20 m was almost at a maximum. On 16 August 1948, 7.5 m was also recorded, but nothing is known of the chlorophyll or plankton for that day. But on 8 June 1953 the disc was seen to 22 m with the minimum amount of chlorophyll. The clearest water for the years 1948 onwards was observed on 19 June 1952, with 23 m and only 37 mg chlorophyll in the column. But by 22 September 1952 the chlorophyll had risen to 231 mg as a result of the growth consequent upon vertical mixing which brought up phosphate, etc. The visual range had also risen, after having been down to 11 m in August, up to 18 m in September; it would not have been so high but for the recent mixing with the deeper water poor in plankton. One cannot therefore say that an increase in the visual range necessarily indicates a decrease in phytoplankton.

There was always watchfulness to see whether such changes in visual range were due to clearer oceanic water having moved into or across the English Channel. But salinity determinations gave no evidence in favour of such movements at the dates concerned. Thus on 27 April and 11 May with high chlorophyll the 0–20 m salinity averaged 35.18 and 35.16, whereas with minimum chlorophyll on 8 June the salinity averaged 35.13 at Station E 1. The very slight decrease in salinity is accounted for by dilution with river water.

Again, between 5 and 20 October 1953, the whole isothermal water column at E 1 rose  $0.50^\circ C$ . This might be taken to show that warmer water had come

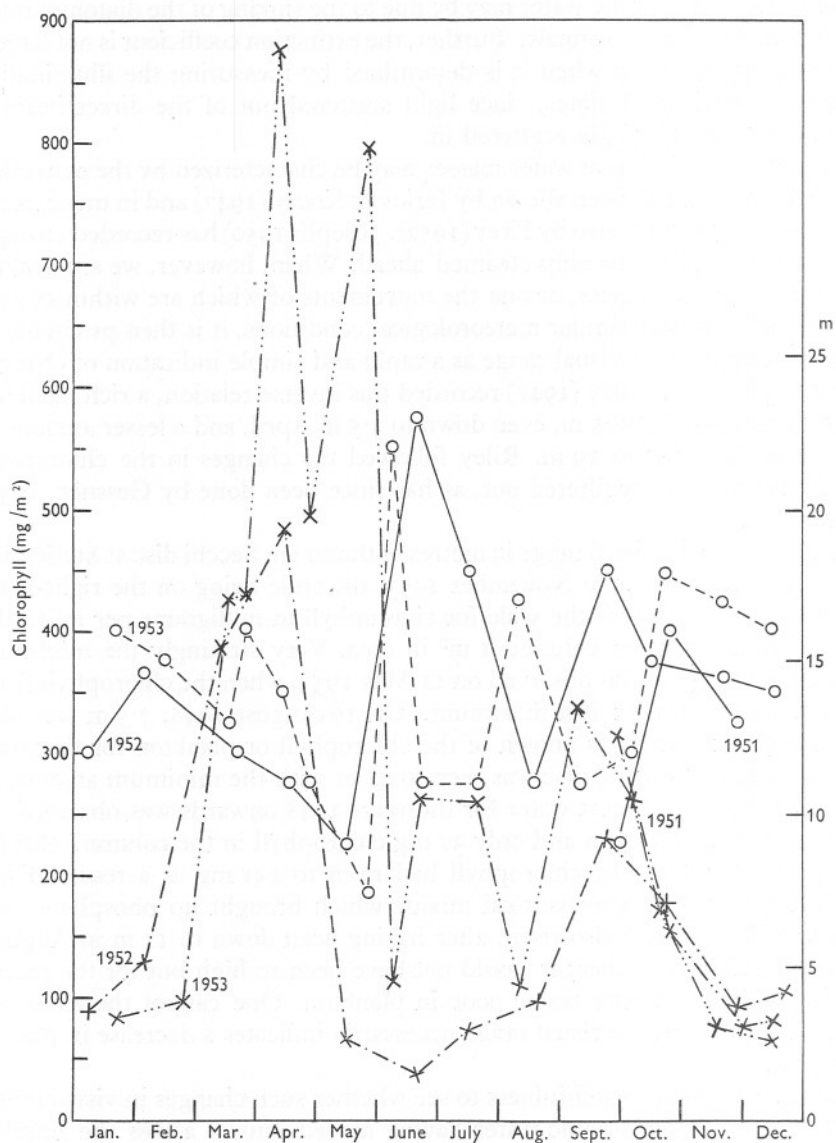


Fig. 2. The left-hand ordinates show chlorophyll in milligrams, the content of a column of sea water at Station E1, of area  $1 \text{ m}^2$  and depth 20 m. The right-hand ordinates show the visual range of the 20 cm diameter Secchi disc. The abscissae show months. Chlorophyll is shown by a dash and two dots for 1951 September to November; for 1952 by a dash and dot line; for 1953 by a dash and three dots. Visual ranges for 1951 September to November and for 1952 are shown by full lines, for 1953 by a broken line.

up from the south. But the occurrence was probably due to a local warming during a very sunny period, 4.78 h sunshine daily average, as against 2.62 for the rest of October, the monthly averages for which have been (1950) 3.47 h, (1951) 4.38 h, (1952) 3.19 h, and (1953) 3.73 h. Furthermore, the salinity at E1 was, as average 0-20 m, 35.22‰ on 5 October and 35.21‰ on 20 October with identical surface values nearer shore at Station L4 (about 5 miles from Plymouth Sound). The effect of the Sound water had pushed out farther during the summer months, but its influence upon visual range appears to have been negligible as compared with that of phytoplankton increase or decrease.

A low visual range in winter, or in exceptionally stormy weather, may be merely an indication of the stirring up of bottom deposits. Judging by the filter-disc photographs and albedo determinations, turbidity arising from the bottom must be very rare at E1, in weather when it is possible for the ship to work, save possibly during the isothermal mixing period. The bottom gave a relatively dark disc on 25 March 1953, due to a rich phytoplankton, but did so on 20 July because the whole column was remarkably poor in suspended matter. Marshall & Orr long ago (1928) reported that 'during the spring months it was not uncommon in shallow water to find more plankton near the bottom than anywhere else'. They also found that during the spring outburst the phytoplankton caused such a great reduction in submarine illumination that the compensation point (or depth) was at times raised to 5 m or less.

We are indebted to the captains, crews and naturalists in charge of the Research Vessels *Sabella* and *Sula* for the samples, temperatures and visual range observations, also to Mr F. A. J. Armstrong for filtering some of the samples, and to Dr H. H. Poole for valuable criticisms.

#### SUMMARY

When sea water of Station E1 is filtered through membranes of average pore diameter about  $1\mu$  the suspended matter, organic and inorganic, is usually seen to be greatest at the surface, occasionally at 25 m or other intermediate depths, and but rarely at the bottom, 70 m. High amounts, elsewhere than at the surface, are due to phytoplankton. The suspended matter, after ignition, was 1.6 parts per million at the surface in a June sample, with 1.0 at 5-10 m and 0.4 p.p.m. below. Values given previously, based on surface samples only, are too high.

Microscopic examination of the discs, carried out by Dr A. G. Lowndes, shows that the ground mass is fine clay. Fibres from woods, ropes and twines were also seen and black carbonaceous specks volatilized by heating the slide. There were also diatoms, copepods, sponge spicules, a small amount of kaolinite, specks of iron oxide, quartz grains, cordierite, micaceous matter probably sericite, tourmaline and broken grains of feldspar (orthoclase).

Fragments of shell were common. Most of the grains were under  $40\mu$  in diameter.

Comparison between successive months can be made from photographs of the discs, but more accurately by determining the relative albedo with a photo-electric cell. The lowest albedo was 25%, for 21 January. The lowest column values of the albedo were for 5 October, when the water had just become isothermal, and the 5 m interval mean, with appropriate interpolation, gave relative albedo 54, with similarly 64 for January and 81 for August. But the effects for mineral matter and plankton are not truly additive.

There is an inverse relation between the amount of phytoplankton determined by spectrophotometric analysis of chlorophyll extracts, and the visual range of the 20 cm Secchi disc, though admixture of suspended inorganic matter may at times disturb the relation. Very clear water is always poor in phytoplankton. Water unusually turbid for the locality is probably rich. The conclusions are based on a study of Station E I for two years and a quarter.

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## A NOTE ON THE ISOLATION OF SMALL MARINE ALGAE AND FLAGELLATES FOR PURE CULTURES

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Increasing interest in the comparative biochemistry of marine algae and protozoa is creating a demand for experimental material in the form of pure, i.e. bacteria-free, cultures. For nutritional and biochemical studies pure cultures are, of course, obligatory; but the number of marine strains available for this sort of work is lamentably small. Indeed, none of the so-called  $\mu$ -flagellates has hitherto been grown in pure culture as far as I know, although there exist pure strains of some of the larger and hardier forms.

Hardier species of motile or non-motile algae can be plated out by normal bacteriological techniques, provided that the material is growing sufficiently vigorously for there to be cells without attached bacteria. Marine examples of this class are species of *Chlorella*, *Chlamydomonas*, *Brachiomonas*, *Platymonas*, *Nannochloris*, *Phaeodactylum*, etc., many of which can be maintained on agar. For plating, I have found that test-tube slants (1% agar) are preferable to Petri dishes, since drying out of the medium is then delayed; but slants require more skill for the actual isolation than do plates.

The use of antibiotics seems, except with filamentous or gelatinous algae, to be applicable only to species which can be handled by simpler methods. This has recently been demonstrated by Spencer (1952).

Allen & Nelson's (1910) time-honoured 'dilution' method has been used with great success by Gross (1937), Parke (1949) and Butcher (1952) for obtaining unialgal cultures of many interesting marine species; but it is doubtful if pure cultures could ever be obtained by serial dilutions, since bacteria usually occur in far greater numbers than algae.

A method of purifying flagellates which is widely used among freshwater workers is the 'pipetting' or 'washing' method as developed by Pringsheim (1946). I want to show that, with very little modification, Pringsheim's technique can be used with success for some  $\mu$ -flagellates.

The washing method has two advantages over all other methods; one is of principle, that the organism to be isolated is selected by the worker and not by the medium; the other is a practical one, that the ratio of bacteria to algae is reduced by astronomical proportions at each manipulation, which is not the case with serial dilutions. Like the plating method it depends for its success on the cells being free from attached bacteria in the first place. Motile forms

are mostly clean and therefore present no problem. With non-motile or mucilagenous species it is necessary to employ vigorously growing populations. Then, even such gelatinous algae as the freshwater *Paulschulzia pseudovolvox* may be purified by washing, and indeed, I have also washed the diatom *Phaeodactylum tricorinitum* successfully.

Pringsheim's technique has been described in some detail in his book (1946) and in various articles (1950, 1951). The apparatus consists of a binocular dissecting microscope with a cardboard breath-guard; a store of sterilized Petri dishes, each with a watch-glass inside; micro-pipettes; and an appropriate variety of sterile culture media in test-tubes. Micro-pipettes are made simply by: (i) plugging a 9 in. length of  $\frac{3}{8}$  in. glass tubing at both ends with cotton-wool; (ii) drawing this out in the centre to make two long-nosed pipettes with a 1 mm bore at the narrow end, these to be sterilized and stored sterile; (iii) immediately before use, further drawing out the thin part of the pipette to a bore of about 0.1 mm in a very narrow flame, and breaking the capillary tube cleanly at a convenient length by a sharp longitudinal tug with flamed forceps, an operation to be repeated before every manipulation to ensure sterility; and (iv) attaching a piece of rubber tubing, glass-stoppered at the free end, which serves to eject liquid from the pipette, and also has the necessary strength to overcome the strong capillary force in the tube.

Into the first of a series of six of the watch-glass combinations, each containing about 3 ml. of sterile culture medium, is placed a drop of material containing the desired organism. One normally starts with the highest powers of the binocular for identification, but once the look of the organism and its characteristic movement have been learned it is possible to go to the lower powers for isolation. Single cells are selected and drawn into the pipette by capillarity and transferred to the second glass of the series. For small organisms it is convenient to watch them being ejected into the new medium, as this may save a little time in searching. In any case they should be allowed to swim around for a while before they are picked up again, so that they become washed of the old medium. This treatment is repeated, until after the final washing they are transferred, one to each culture tube.

This method, as it stands is applicable down to about  $15\mu$ ; but for smaller cells it becomes too tedious. I have used it in purifying cultures of *Oxyrrhis*, *Chlamydomonas*, *Brachiomonas*, *Platymonas*, *Syracosphaera*, etc.

It is possible to make use of phototactic responses for the purification of some  $\mu$ -flagellates. This avoids most of the difficulties arising from their small size. For example, an organism of  $5\mu$  can be washed with less trouble with the aid of phototaxis than one of  $50\mu$  handled in the normal way; but of course success depends on one's being able to induce phototaxis. Luckily, many species seem to exhibit a very strong negative taxis when they are transferred from the wild to a culture medium. All that is needed is to put a drop of the material gently at the window side of the watch-glass and wait 5 min (or

perhaps 10) when the flagellates will have congregated at the side away from the light, leaving most of the unwanted bacteria on the other side. From here the flagellates are transferred *en masse* with a rather large-bore micro-pipette to the window side of the next watch-glass, and so on until the last. Then under medium power of the binocular the flagellates, mere specks, are picked up singly with a fine-bore micro-pipette and placed in culture tubes. The last operation is very rapid: fifty isolations can be made in half an hour or so. The proportion of contaminated to clean cultures obtained with six washes is about 1:10.

I do not know what the size limit of this method is; but provided an organism is visible, if only as a speck, it is possible to pick it up. The essentials are that there is a preponderance of the desired flagellate in one's material to begin with, and secondly that it is motile and phototactic.

My successes during last summer with this method include two members of the Chrysophyceae, *Monochrysis lutheri* and *Prymnesium parvum*; one of the Cryptophyceae, *Hemiselmis* sp., and several Chlorophyceae, all below 10 $\mu$ . As an example of the efficiency of the procedure, *Monochrysis lutheri* (5-7 $\mu$ ) was found at a cell concentration of 16,000,000 per ml. in a pool of salinity 25‰. One day was devoted to preparing the media and isolating the organism. Forty-eight isolations were put into twelve different combinations of medium; and out of this forty-eight, ten flagellate cultures and three bacterial cultures resulted. All the successful ones contained glucose and liver extract. *Hemiselmis* (6 $\mu$ ), on the other hand, was more difficult. The natural material was at a cell concentration of about 30,000,000 per ml. from a pool of low salinity. I made three attempts on the same scale as for *Monochrysis*. In the first I ended up with a large number of pure cultures of a minute heterothallic *Chlamydomonas*; not until the third attempt did I obtain any growth of *Hemiselmis*. Then only three cultures resulted of which two were bacteria-free.

In general there is no difficulty in ridding a motile organism of bacteria; it is getting it to grow by itself which often presents problems. Synthetic media are inappropriate for preliminary studies, even for the few species which are known to grow in such media. It is often necessary and always safer to use natural extracts, natural sea water, soil extract, etc. Moreover, since the aim is bacteria-free cultures there is nothing to prevent the use of yeast, beef and liver extracts as well (Pringsheim, 1946).

The flagellate is probably phototrophic, but there is a strong possibility that it has one or more heterotrophic requirements in addition; these are best met by including extracts of organic materials, liver and yeast being the most efficient.

I find it convenient to prepare all stock media with an artificial sea water, sterilize them, then mix them with equal proportions of separately autoclaved natural sea water. In this way many combinations of yeast, beef, soil extracts,

peptones, etc., and other nutrients having various pH's can be quickly prepared, while at the same time ensuring a high proportion of natural sea water. Unautoclaved, but Seitz-filtered, media can also be included. The idea is to use a large range of media and isolate a large number of cells on the chance that one of the combinations will be suitable.

One word about sterility testing. As a routine, four tests are made: (i) fresh-water liquid, (ii) fresh-water agar, (iii) salt-water liquid, and (iv) salt-water agar. The medium contains yeast, beef, and soil extracts, glucose and acetate. In addition, all cultures are maintained, as far as possible, in organic media so that chance contamination becomes visible.

All the species which have been mentioned here are supra-littoral or neritic forms, and it is probable that they are easier to handle than the pelagic ones. Nevertheless, the  $\mu$ -flagellates among them are delicate: they need natural sea water for their growth and show the usual tendency to burst at the least provocation. It is possible that pelagic species could be handled in the same way as the littoral forms. I have no doubt that, wherever feasible, physical manipulation is the surest and quickest means of obtaining pure cultures, and in my view it is also the easiest. The object of this note has been to record that its range of application has been extended to include some organisms as small as  $5\mu$ .

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## THE ABUNDANCE OF *OCTOPUS* IN THE ENGLISH CHANNEL

By W. J. Rees and J. R. Lumby

(Text-figs. 1-5)

During 1950, the Common Octopus (*Octopus vulgaris* Lamarck) was to be found along the south coast of England in greater numbers than at any time since Garstang (1900) reported on the 'plague' on the coasts of Devon and Cornwall in 1899-1900.

In earlier papers (Rees, 1950, 1952) the distribution of the octopus in our northern waters was reviewed, and it was demonstrated that this species is an immigrant which breeds on our south coast only rarely. It reaches these coasts by being brought there as a planktonic larva by the water circulation in the English Channel and by migrations of the adult. The most important factor in controlling the movements of the adult, however, might be expected to be the water temperature in the English Channel—where the species is at the northern limit of its breeding range and might therefore be extremely sensitive to slight changes in temperature.

The earlier paper (1950) was completed in January of that year, at a time when fishermen in the Channel Islands were complaining of the abundance of octopus in their area. It then seemed likely (a surmise which proved correct) that the octopus might also be more abundant on the English coast in the summer, if events followed the same course as in 1899-1900. This paper deals primarily with its abundance in 1950 and subsequently. It is now possible to present a much better picture of the distribution of larvae based on the pilchard-egg investigations of the Ministry of Agriculture and Fisheries. More information is also available on water temperature as a controlling factor, and the possible seasonal migration of the adult in normal and abnormal years is considered.

### THE ABUNDANCE OF THE ADULT

Early in 1950 there was no evidence that *Octopus* would be abundant on the English coast, but the presence of the species in numbers in the Channel Islands in January was an indication that a repetition of the events of 1899-1900 was a distinct possibility. In March 1950, however, an occasional juvenile *Octopus* was caught by the research vessels of the Marine Biological Association off Plymouth. One of these, a specimen with an arm span of 70 mm and a ventral mantle length of 14 mm, was kindly sent to us by



Mr F. S. Russell. These can be assumed to have been spawned in the previous July or August and to be about 7-8 months old.

Apart from juveniles, the first indication of real abundance came from the Devon area. The trawler *Girl Vine* (Skipper T. Harvey), working out of Torquay in the third week of May in a position 20 miles S.S.E. of Berry Head, reported that in one haul the cod-end of the trawl was full of *Octopus*. These were large with a span of about 3 ft. A few octopus were also taken in other hauls made during the same fishing trip. Fourteen days later on the same grounds octopus were not noticeably abundant; later events proved that they had moved inshore.

During the last week in May an octopus with an arm span of about 30 in. was taken in a crab pot at Bexhill by a fisherman (Mr J. Easton). In the first half of June octopus began taking lobsters and crabs from baited pots in Start Bay and Babbacombe. As the season advanced Babbacombe fishermen were taking 30-40 a day in their pots. At Beer and Seaton, the shell-fishery was almost abandoned for the same reason. The area between Seaton and Brixham appears to have been affected the most, but farther west some Plymouth fishermen abandoned shell-fishing because the octopus were taking their catches. For the first time in many years the Plymouth Laboratory was able to obtain octopus in sufficient numbers for experimental work.

During August a large number of octopus entered Dartmouth Harbour. Most of these were large with an arm-span of 4 ft.; some were caught by hook and line. Fishermen reported seeing young octopus of very small size during the autumn in the south Devon area.

On the north coast of Devon *Octopus* were not noticeably abundant, but a few were caught off Lundy Island. Towards the middle of October few octopus were being caught on the south Devon coast.

On other parts of the south coast of England *Octopus* was also common. In mid-August specimens 2½-3 ft. in arm-span were being caught in some numbers by anglers at Eastbourne and Brighton, and during the first week of September octopus of all sizes up to 4 ft. 6 in. in arm-span were being caught in lobster pots on Hoo Bank off Selsey Bill. There was an interesting record of an octopus (30 in. in arm-span) from Deal on 2 September; this specimen, now in the British Museum (Natural History), is one of the few ever recorded from the North Sea.

Octopus were being caught throughout the winter (1950-51) by the R.V. *Sabella* operating within 16 miles of Plymouth Breakwater (see Appendix I by Lieut.-Commander C. A. Hoodless, D.S.C., R.N.R.). It is interesting to note that this ship caught its first octopus on 4 September 1950, the totals for September, October, and November being 2, 3 and 2. These small numbers probably give an accurate impression of the situation on offshore grounds and serve to emphasize that the main body of octopus was close inshore. One Plymouth fisherman, who had seen only four octopus in 35 years of fishing,

caught no fewer than fifty in and on his lobster pots by the first week in October. The increase in catches in winter and spring suggests that the octopus, like *Eledone*, moves into deeper water after the summer.

In Jersey, too, the octopus was numerous in 1950, and there appears to have been no reduction in numbers in 1951 and in 1952. On the French coast also, during the summer of 1950, *Octopus* appears to have been very abundant both in the neighbourhood of Cherbourg and on the north coast of Brittany.

Prof. J. Z. Young, F.R.S., has kindly allowed us to publish some notes on the situation in the Cherbourg area in September 1950. He states: 'There were considerable signs of a plague, at least on the stretch from Cherbourg to the tip of the Hague peninsula. On 4 September I saw a great many of them lying about in the port at Cherbourg; they were being caught by boys and were apparently unsaleable, the fishermen were baiting their lines with pieces of tentacle. On 11 September I was still more struck with their abundance at Omonville. There is a little harbour there and the jetty runs out among the rocks. Looking down among these we could see a great number of *Octopus* moving about in the water at high tide. For instance, at one moment we counted no less than twenty-five in sight, even though the sea was rough and vision was limited to a few yards. Of course I cannot say whether they would be equally abundant away from the jetty, perhaps they had come in for food. They were certainly very hungry and attacked pieces of dead octopus as soon as lowered into the water; in fact the boys were fishing for them in this way and once the dead octopus had been seized the live ones would hang on to it and could be drawn out of the water on to the jetty. In this way one could pull them out one after the other almost as fast as the bait could be lowered into the water.'

'Elsewhere along the coast there were signs of abundance though I did not actually see such large numbers anywhere else. However at low water they were caught by prodding under rocks at Urville on several days.'

Some additional information published in *La Pêche Maritime*, Nos. 868, 869 and 870 (1950) is quoted below:

*May 1950*: Portsall. 'La pêche aux crustacés (homards et langoustes) a été d'un rendement à peu près nul et d'ailleurs la côte à un tel point infestée de pieuvres qu'il devient difficile de préserver le crustacé dans les casiers et viviers où ces mollusques parviennent toujours à s'infiltrer.'

*July 1950*. 'Les pieuvres très nombreuses, certaines d'assez grandes dimensions, pénétrant jusque dans les concessions ostréicoles, font des sensibles ravages. Les baies ont été visitées par de nombreux crabes-araignées d'un calibre respectable, mais se déplaçant rapidement, au grand dam des pêcheurs.'

*August 1950*: Paimpol. 'Les pieuvres sont plus nombreuses que jamais; on les trouve même dans les concessions ostréicoles. Les crabes-araignées

ont été parfois abondants dans les baies, se déplaçant jusque dans les chenaux.<sup>2</sup>

It is thus evident that the octopus was even more abundant in 1950 on the French coast than on the English side.

#### PLANKTONIC LARVAE IN 1948, 1949 AND 1950

In an earlier report the Channel larvae from cruises in 1948 and 1949 were plotted on a single chart (Rees, 1950, fig. 2). The results for these two years have been re-plotted on separate charts, and results from the pilchard-egg cruises of the Ministry of Agriculture and Fisheries research vessel *Sir Lancelot* have been added for 1949. The results for 1950 are based almost entirely on the catches of the *Sir Lancelot*.

*The year 1948.* In this year only the collections made by the M.Y. *Manihine* in the eastern Channel and over the eastern end of the Hurd Deep were available (Fig. 1, 1948). Young larvae found at five stations at the end of August are noted in Table I.

TABLE I. CATCHES AND LENGTHS OF YOUNG *OCTOPUS* LARVAE

Station	Catch	Ventral mantle length in mm
47	4	2.55, 2.85, 3.15, 3.15
48	7	1.5, 1.65, 1.8, 2.25, 2.4, 3.0, 3.0
49	5	2.25, 2.4, 2.85, 3.0, 3.0
50	4	1.65, 2.4, 2.7, 2.85
51	4	2.10, 2.55, 2.85, 3.0

The positions of these stations have already been given (Rees, 1950, p. 368). There was no information on distribution to the west and no larvae were taken at St. 45 (the southernmost of a line running across the Channel from south of the Isle of Wight towards the Cherbourg peninsula). Two larvae at St. 48 and another at St. 50 were at or just about hatching size; they could not have been many days old,<sup>1</sup> and it could be inferred that the breeding grounds were not far away.

*The year 1949.* During this year plankton hauls were again taken in the central area of the Channel by the *Manihine* in August and *Octopus* larvae obtained both to the east and the west of the previous positions (Rees, 1950, p. 368). However, a much more complete picture of distribution during June and September was made possible by an examination of the plankton hauls taken by the *Sir Lancelot*. These cruises covered the whole of the Channel and, although intended for another purpose, yielded valuable information on where larvae could be found during these particular months (Fig. 1, 1949). Larvae were found at three *Sir Lancelot* stations (39, 45 and 46) in June off the north coast of Brittany. No records are available for July and only those

<sup>1</sup> It is assumed here that larvae would hatch at about 1.5 mm (in ventral mantle length) as at Naples.

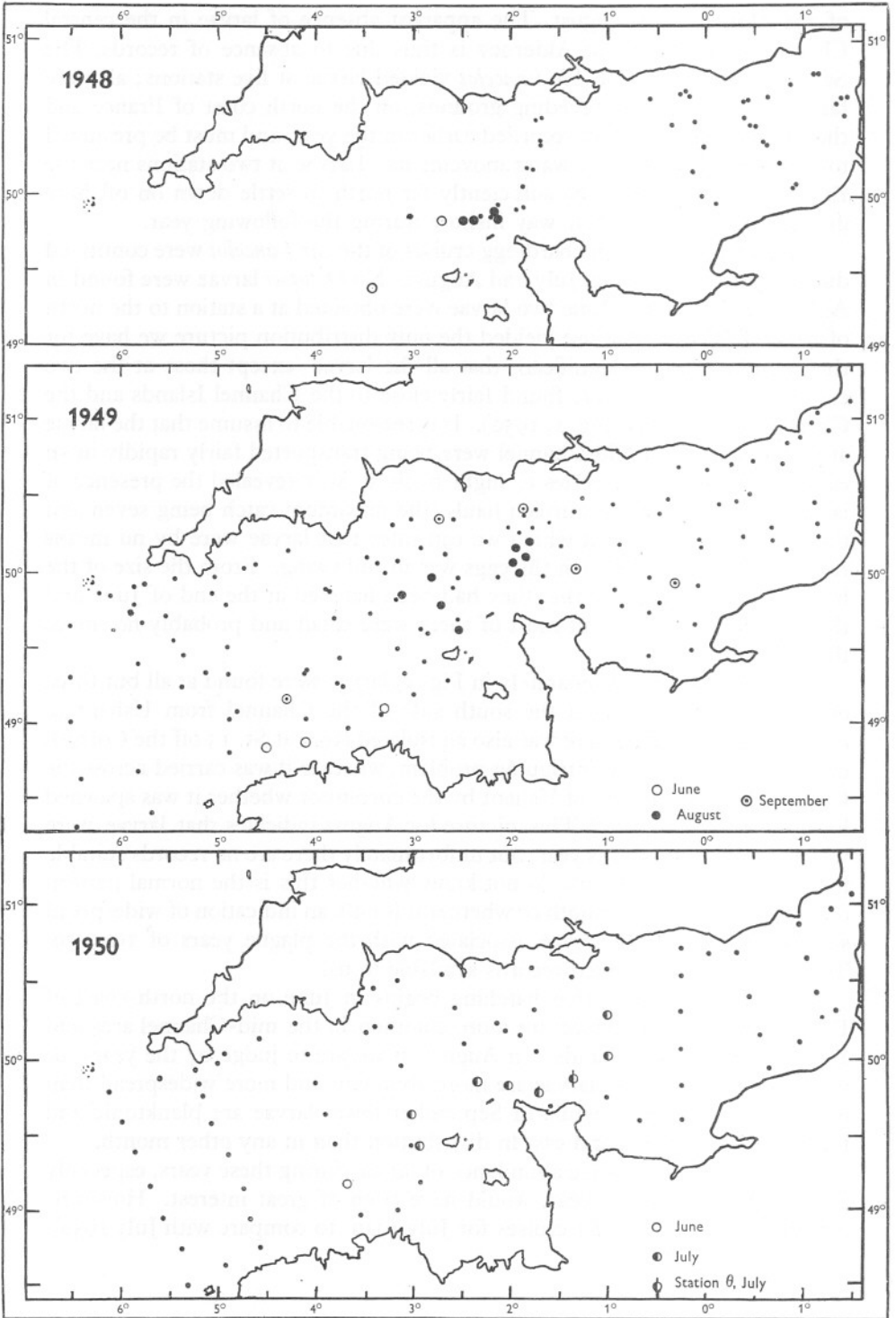
of the *Manihine* for August. The apparent absence of larvae in the central Channel to the north of Alderney is thus due to absence of records. The September cruise of the *Sir Lancelot* yielded larvae at five stations; all were farther away from the breeding grounds, on the north coast of France and the Channel Islands, than recorded earlier in the year, and must be presumed to have been dispersed by water movements. Larvae at two stations near the English coast appear to be sufficiently far north to settle down on offshore grounds and to work their way inshore during the following year.

*The year 1950.* The pilchard-egg cruises of the *Sir Lancelot* were continued during April, May, June, July and August. No *Octopus* larvae were found in April and May, but in June two larvae were obtained at a station to the north of Roscoff. The July cruise yielded the only distribution picture we have for this month, and it is significant that all the larvae (except those at the two easternmost stations) were found fairly close to the Channel Islands and the Cherbourg Peninsula (Fig. 1, 1950). It is reasonable to assume that the larvae at Sts. 33 and 34 in mid-Channel were being transported fairly rapidly in an easterly direction. The series of night hauls at St.  $\theta$  revealed the presence of larvae in no fewer than fourteen hauls (the maximum catch being seven and the minimum one), from which we can infer that larvae were by no means rare and that hatching from the eggs was in full swing. From the size of the larvae it can be assumed that they had been hatched at the end of June and the beginning of July; but most of them were small and probably not more than 7-14 days old.

In August (as shown separately in Fig. 1) larvae were found at all but three of the stations worked on the south side of the Channel from Ushant to approximately  $1^{\circ}$  W. There was also an isolated larva at St. 15 off the Cornish coast which presents an intriguing problem, whether it was carried across the Channel from the region of Ushant by the current or whether it was spawned locally (but see p. 522). This picture for August indicates that larvae were widely spread during this year, but unfortunately there are no records suitable for comparison, so that we do not know whether this is the normal pattern of distribution for this month or whether it is only an indication of widespread successful hatching of larvae associated with the plague years of 1949-50. These are the last detailed records available to us.

The records indicate that hatching begins in June on the north coast of Brittany and by July larvae are more common in the mid-Channel area and around the Channel Islands. In August (if we are to judge by the years for which we have records) larvae are more abundant and more widespread than during earlier months, while in September fewer larvae are planktonic and they are farther north and east in distribution than in any other month.

A comparison of relative abundance of larvae during these years, especially as 1950 was a 'plague' year, would have been of great interest. However, there were no *Sir Lancelot* cruises for July 1949 (to compare with July 1950),





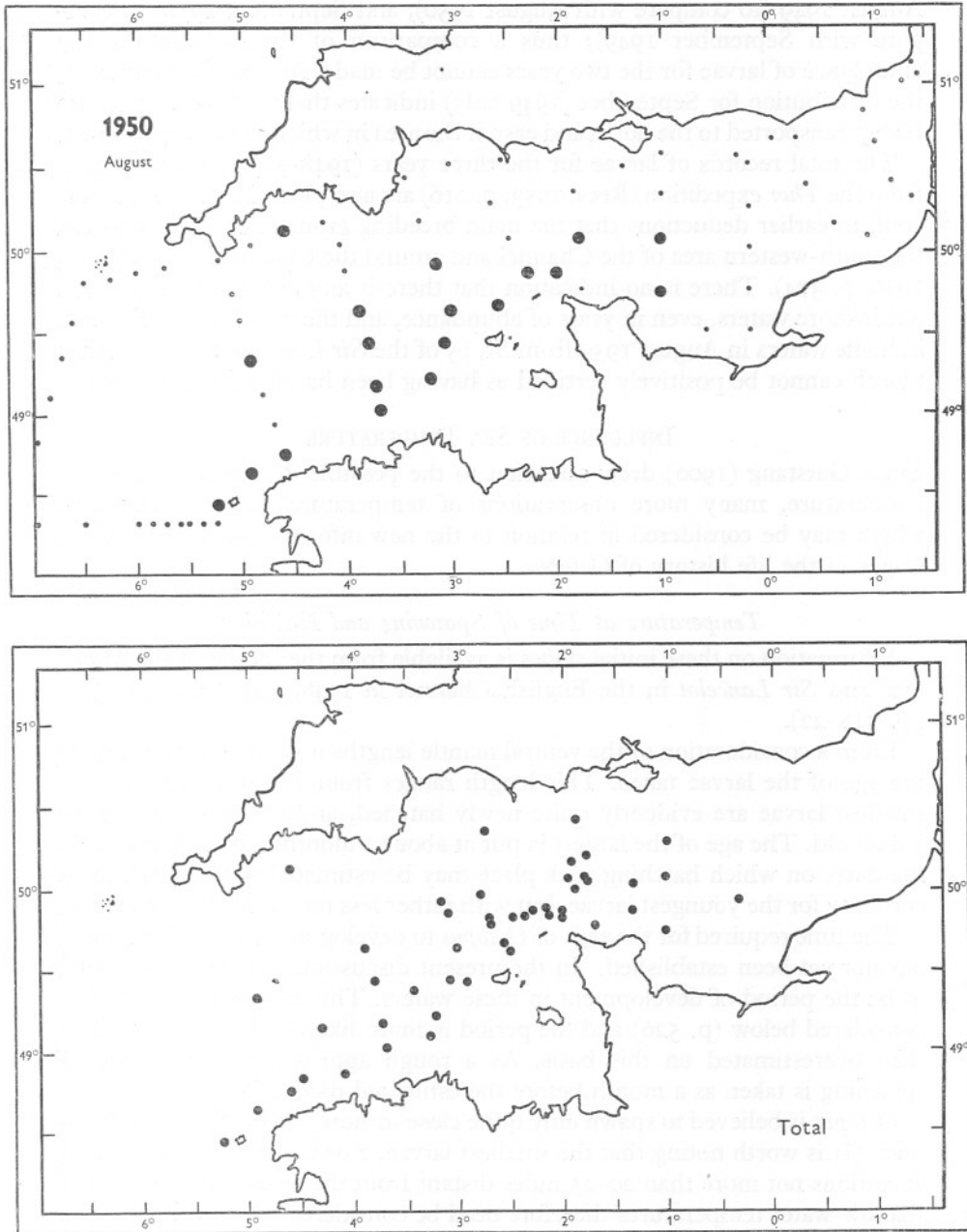


Fig. 1. The distribution of *Octopus* larvae 1948-50. Stations worked during the period in question are marked with a dot. Circles denote stations at which larvae were taken, as specified on the separate maps. The two open circles on the 1948 map denote positions at which the Danish research vessel *Thor* took larvae in 1906 and 1910. These positions are also included in the last map which gives an aggregate of positions at which larvae were taken.

August 1949 (to compare with August 1950), and September 1950 (to compare with September 1949); thus a comparison of the distribution and abundance of larvae for the two years cannot be made. As already mentioned, the distribution for September (1949 only) indicates that these late larvae are being transported to the north and east of the area in which they were hatched.

The total records of larvae for the three years (1948-50) and two records from the *Thor* expedition (Rees, 1953, p. 216) are also plotted in Fig. 1). These confirm earlier deductions that the main breeding grounds of *Octopus* are in the south-western area of the Channel and around the Channel Islands (Rees, 1950, p. 374). There is no indication that there is any extensive breeding in our inshore waters, even in years of abundance, and the only record of a larva in home waters in August 1950 from St. 15 of the *Sir Lancelot's* cruise in that month cannot be positively certified as having been hatched in that area.

#### INFLUENCE OF SEA TEMPERATURE

Since Garstang (1900) drew attention to the possible role played by water temperature, many more observations of temperature have accumulated, which may be considered in relation to the new information on the various stages in the life history of *Octopus*.

#### *Temperature at Time of Spawning and Hatching*

Information on these initial stages is available from the cruises of the *Manihine* and *Sir Lancelot* in the English Channel in 1948, 1949 and 1950 (see pp. 518-22).

From a consideration of the ventral mantle lengths it is possible to estimate the age of the larvae taken. This length ranges from 1.5 to 6 mm, and the smallest larvae are evidently quite newly hatched, and not more than 2 or 3 days old. The age of the largest is put at about 2 months. From these data, the dates on which hatching took place may be estimated with considerable certainty for the youngest larvae, but with rather less for the larger specimens.

The time required for the eggs of *Octopus* to develop in the English Channel has not yet been established. In the present discussion a month is assumed to be the period of development in these waters. This assumption is further considered below (p. 526) and the period is more likely to have been under- than overestimated on this basis. As a rough approximation the date of spawning is taken as a month before the estimated date of hatching.

*Octopus* is believed to spawn only quite close inshore, within the 10 m depth line. (It is worth noting that the smallest larvae, 2 or 3 days old, were found at stations not more than 20-25 miles distant from the nearest coasts.) Only shallow-water temperatures therefore need be considered. Unfortunately the temperature data available for such inshore waters, particularly on the French side, are decidedly meagre. We are indebted to Mr H. J. Baal, President of the Société Jersiaise, for a series of monthly average temperatures at Jersey.

These are based on observations made at 9 a.m. daily at 1 m depth in the shade at the end of the Albert Pier, St Helier. For the western part of the Brittany coast, the only data available consist of the surface observations made near Ushant by commercial vessels crossing the mouth of the Channel, between Ushant and Land's End. The position to which the data used relate is approximately  $48^{\circ} 27' N.$ ,  $5^{\circ} 15' W.$ , admittedly some distance off the mainland. In addition, there are the routine observations made by the commercial vessels on the Southampton-Channel Islands service, and by the research vessels of the Marine Biological Association off Plymouth. Except those for Jersey, these data are published in the *Bulletins Hydrographiques* issued by the International Council for the Exploration of the Sea, Copenhagen. They are illustrated, together with those for Jersey, for the relevant period, in Fig. 2.

The use of surface temperatures is justified in present circumstances, since at least in April and the early part of May, the waters of the English Channel may be expected to be homothermal (see Dietrich, 1950). Surface observations are thus reasonably reliable guides to the conditions on the sea bottom at this season, especially in the shallow waters inshore. Certain disadvantages may arise, however, from the fact that most of the data employed for this inquiry are in the form of monthly averages. In shallow water, the fluctuations of temperature are likely to be much greater than in the deeper water well clear of the land. It can be seen, for example, in Fig. 2 that the annual range tends to be considerably greater at Jersey than off Ushant, or even off Plymouth. Short-period fluctuations, on the other hand, are not to be discerned in the curves based on monthly averages. One or two very cold days in an otherwise mild month may have a disproportionately adverse effect on biological processes, while a few bursts of sunshine in an otherwise gloomy month may give a lot of encouragement to them. Such effects are likely to be masked by the use of monthly averages. However, other uncertainties involved in, for example, the dates of spawning and hatching and the places at which they occurred, are so numerous that it is doubtful whether we could profit by a closer examination of the temperature conditions.

During the greater part, if not all, of the season in which spawning, development, and hatching are taking place, the water temperature is in general rising. By estimating the dates, in the way described, on which the largest, and therefore the oldest, larvae taken were hatched and spawned, and reading the temperatures for these dates from the appropriate curves, we arrive at an estimate of the lowest water temperatures at which these events occurred. The values so obtained are shown in Table II.

The table indicates that in the years 1949 and 1950 spawning began in the first half of May on the western part of the Brittany coast, and in the latter half of the month in the Channel Islands district. For 1950, these dates are further supported by the fact that larvae were absent from the catches in

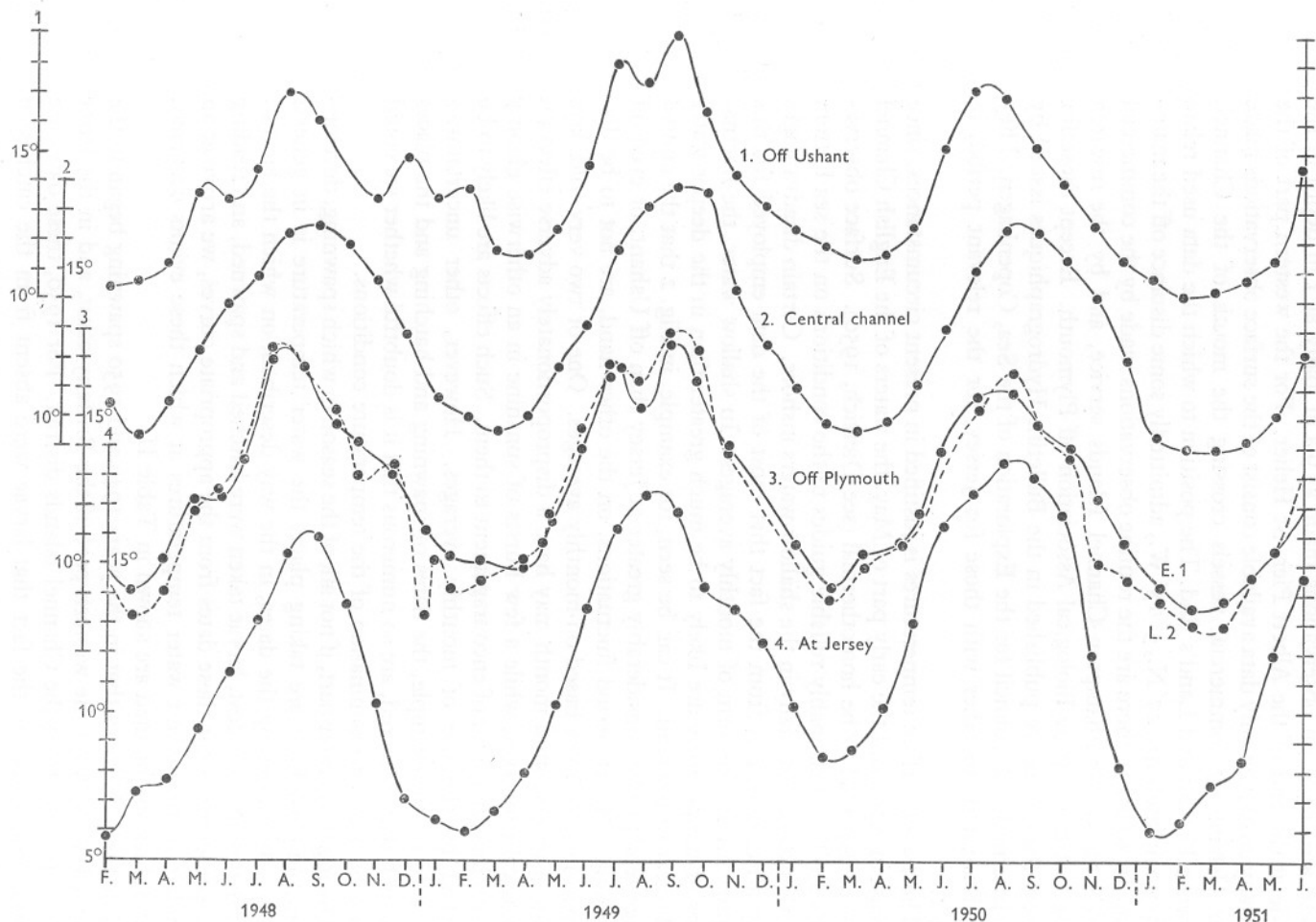


Fig. 2. The sea surface temperature ( $^{\circ}\text{C}$ ) at four localities in the English Channel from February 1948 to June 1951. The curves shown for the two positions off Plymouth are based on single observations; the other three curves are based on monthly mean values. The locality off Ushant (1) is Region 10 (see Lumby, 1935); the Central Channel (2) is a combination of Regions 20, 24 and 25; the observations off Plymouth (3) were made at St. L. 2, close to the Breakwater, and at St. E. 1, close to the Eddystone; for Jersey (4) the observations were taken off the Albert Pier, St Helier.

April and May that year, and first appeared in June. The water temperature at first spawning is found to be about  $11\frac{1}{2}^{\circ}$  or  $12^{\circ}$  C.

TABLE II. THE ESTIMATED DATES AND PRESUMED LOCALITIES OF SPAWNING AND HATCHING, WITH THE APPROPRIATE SURFACE WATER TEMPERATURES ( $^{\circ}$  C)

Cruise (1)	Locality (2)	Spawning		Hatching		
		Date (3)	$T^{\circ}$ C (4)	Date (5)	$T^{\circ}$ C (6)	
M., Aug. 1948	C.I.	7 July	12.6 (J.)	12½	7 Aug. 14.8 (J.)	15
S.L., June 1949	B.	18 May	10.5 (J.) 12.5 (U.) 11.8 (C.)	11½	18 June 13.7 (J.) 14.8 (U.) 13.4 (C.)	14
M., Aug. 1949	C.I.	1 June	12.0 (J.)	12	1 July 15.0 (J.)	15
S.L., Sept. 1949	B. & C.I.	7 July	15.5 (J.) 17.3 (U.) (C.)	16	7 Aug. 17.2 (J.) 17.3 (U.)	17
S.L., June 1950	B.	5 May	12.2 (J.) 12.1 (U.)	12	5 June 15.8 (J.) 14.2 (U.)	15
S.L., July 1950	C.I.	22 May	13.6 (J.)	13½	22 June 16.7 (J.)	16½
S.L., Aug. 1950	B. & C.I.	12 June	16.6 (J.) 15.1 (U.)	16	12 July 17.5 (J.) 17.1 (U.)	17½

In column 1, *M.* indicates *Manihine*; *S.L.*, *Sir Lancelot*. In column 2, C.I. indicates the Channel Islands region; B., the western part of the north coast of Brittany. In columns 4 and 6, J. indicates at Jersey; U., off Ushant; and C., the central Channel between the Isle of Wight and the Cotentin peninsula. The temperatures in heavy type are the round values considered acceptable. Spawning and hatching refer to the oldest larvae taken on each cruise.

#### *Effect of Temperature on Date of Spawning*

As regards the effect of temperature on the time when spawning begins, the possibility must not be overlooked that *Octopus* may start to spawn in the English Channel some time in May, mainly in response to an innate rhythm, with only very little regard to water temperature. The average temperature both at Jersey (for the years 1935-39, 1946-52) and also off Ushant (for the years 1928-51) reaches  $11\frac{1}{2}^{\circ}$  C at about the end of the first week in May. In separate years this value may be reached at Jersey as early as mid-April (e.g. 1933) or may not occur until mid-June (1948). If spawning is closely controlled by temperature, fluctuations of a similar order of magnitude must be expected in the dates on which the process starts. It is true that the results of the August cruise in 1948, a year with particularly low spring temperatures at Jersey, point in this direction, as they give a date for first spawning as late as July. But as the table shows, in 1949 and 1950, when there were sequences of cruises, the later the cruise, the later is the estimated date of first spawning. The explanation of this is not apparent, unless it be that by the time of the later cruises, the largest larvae may have already been so close to the bottom that they were not taken by the *Sir Lancelot's* nets.<sup>1</sup>

<sup>1</sup> The *Manihine* made horizontal hauls as close to the bottom as practicable, whereas the *Sir Lancelot's* were vertical hauls.



There is, however, another feature which suggests that spawning began later in 1948 than in 1949 or 1950, namely, that the distribution of the larvae in August 1948 was not quite so wide as in the same month in the other two years. But not much reliance can be placed on this, since the western half of the Channel was not sampled in 1948. For the same reason it is doubtful whether we may conclude that the eggs spawned and larvae hatched in 1948 were fewer than in 1949 or 1950, as might at first sight appear to be the case. Moreover, these numbers may depend less upon temperature than upon the numbers of adults present. Thus the evidence at present available does not permit a decision to be reached on the question whether spawning may be expected to be earlier or later, or more or less intense, according as the water temperature is higher or lower.

Off Plymouth, the temperature of  $11\frac{1}{2}^{\circ}$  C is reached on the average (1928-51) only some 2 weeks later than on the French coasts. If *Octopus* can tolerate as wide a variation in the date of commencement of spawning as 2 months, it is surprising that it spawns so very rarely on the English coast. This suggests that in regard to choice of spawning ground some factor other than temperature determines the animal's preference for the southern side of the Channel over the northern.

#### *Accumulated Temperature and Development of the Egg*

In the foregoing discussion, the assumption is made that hatching occurs 1 month after spawning. No direct confirmation of this, however, is obtainable from the evidence at hand. In so far as 'accumulated temperature' (Tait, 1951) is of importance to development, the requirements of *Octopus* estimated on this basis appear to be somewhat less in the English Channel than in the Mediterranean. For the English Channel, taking the temperature at spawning as  $11\frac{1}{2}^{\circ}$  C and at hatching as  $14\frac{1}{2}^{\circ}$  C, with 30 days for development, we obtain a figure of 390 day-degrees. For the Mediterranean, Portmann's figures (Portmann, 1933) give a value of 450 day-degrees, and if it is assumed that the temperature at Naples was between 15 and  $20^{\circ}$  C at the time of his experiments, Naef's figures (Naef, 1921, 1923, 1928) give values between 420 and 560 day-degrees. It is not considered that these figures conflict unduly with the assumption of at least a month for development in the English Channel.

#### *Temperature and Abundance of Octopus*

For the later stages of the life history of *Octopus*, we may consider the reports on its abundance in the English Channel at different periods. After about 1885 octopus were scarce on both French and English coasts, until in 1899 their numbers assumed plague proportions on the French side, and began to rise appreciably on the English side. In the following year, 1900, the octopus were so numerous as to form a nuisance on both sides of the Channel.

They were noted as plentiful on the Sussex coast in 1913, 1922 and 1948, and they were a plague on the Finistère coasts in 1922. These latter, except possibly that in 1922, may be regarded as more or less minor occurrences, not comparable in magnitude with those of 1899 and 1900. In 1950 a plague sprang up anew on the French coasts, and the number of octopus began to rise on the English side also during the same year. There were very large numbers of octopus present on both sides of the Channel in 1951, and on the French side in the following year also. Whether they had in fact disappeared from the English coasts in 1952 is not certain. Official records of the size of the octopus population are not kept and the information available cannot be claimed as complete and precise. Accordingly, the absence of reports of octopus in the newspapers, for example, may be due to the animal having lost its news value in 1952.

In his report on the plague that occurred at the turn of the century, Garstang (1900) suggested that the warm summers and mild winters which, he states, were experienced during the few years preceding the plague provided conditions suitable to a warm-water animal. He remarked further that 'the plague of octopus may be traced to the influence on the reproduction of this species of the exceptionally favourable conditions which prevailed in 1893'. With these points in mind, we may examine the considerable sequence of temperature data now available for the English Channel, in relation to the further records of octopus abundance. Surface observations are representative of the whole column at all seasons in the English Channel, except in the westernmost part. Here a thermocline develops at times during summer, with the result that the upper 30 m or so of water may be up to 5° C warmer than the water below (see Dietrich, 1950). The height of 'summer', the season of highest water temperature, falls in July, August or September in the English Channel. Similarly, the depth of 'winter', the season of lowest temperature, occurs in January, February, March or April.

Fig. 3 shows for each year the maximal monthly mean temperature for three districts of the English Channel, at the mouth, in the centre, and towards the Straits of Dover.<sup>1</sup> The rings immediately below the upper scale of years indicate the years in which the octopus was recorded as notably abundant. We see that, as far as sea temperature is concerned, the summer of 1893 (as judged by the highest monthly mean temperature) was indeed warm, at least in the western part of the Channel, but it was followed immediately by a distinctly cool summer. Thereafter succeeding summers became gradually warmer, culminating in an outstandingly hot one in 1899. Turning to the 1950 plague, we observe that although the summer of 1949 was very hot, in

<sup>1</sup> The figures for the western district for the years after 1904 are based on the data available for the Regions 5, 8, 9 and 10 used by Lumby (1935); for 1904 and earlier years they are based on observations at the *Seven Stones* lightship alone (see E. C. Jee, 1919). Similarly, the central district comprises Regions 17, 18, 21, 22, 23, 24 and 25; and the eastern district, Regions 30, 31, 32, 33, 35, 38 and 40.

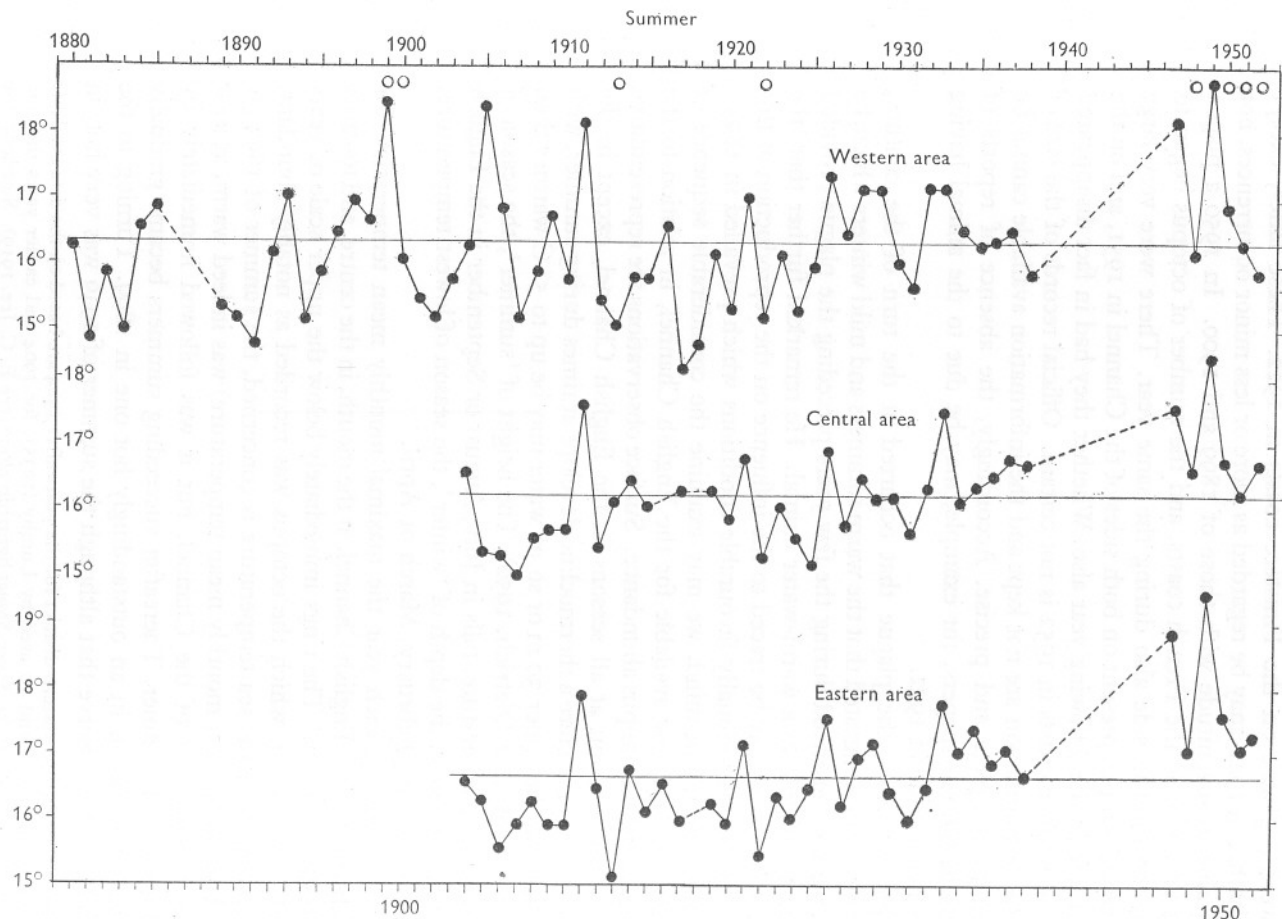


Fig. 3. The *maximal* monthly mean surface water temperature ( $^{\circ}$ C) for each year in three districts of the English Channel. The western area comprises Regions 5, 8, 9 and 10 (see Lumby, 1935); the central area, Regions 17, 18, 21, 22, 23, 24 and 25; and the eastern area, Regions 30, 31, 32, 33, 35, 38 and 40. For the years before 1905, the figures for the western area are based on observations at the Seven Stones lightship only. The rings beneath the scale of years indicate years in which *Octopus* was reported as notably abundant in the English Channel.

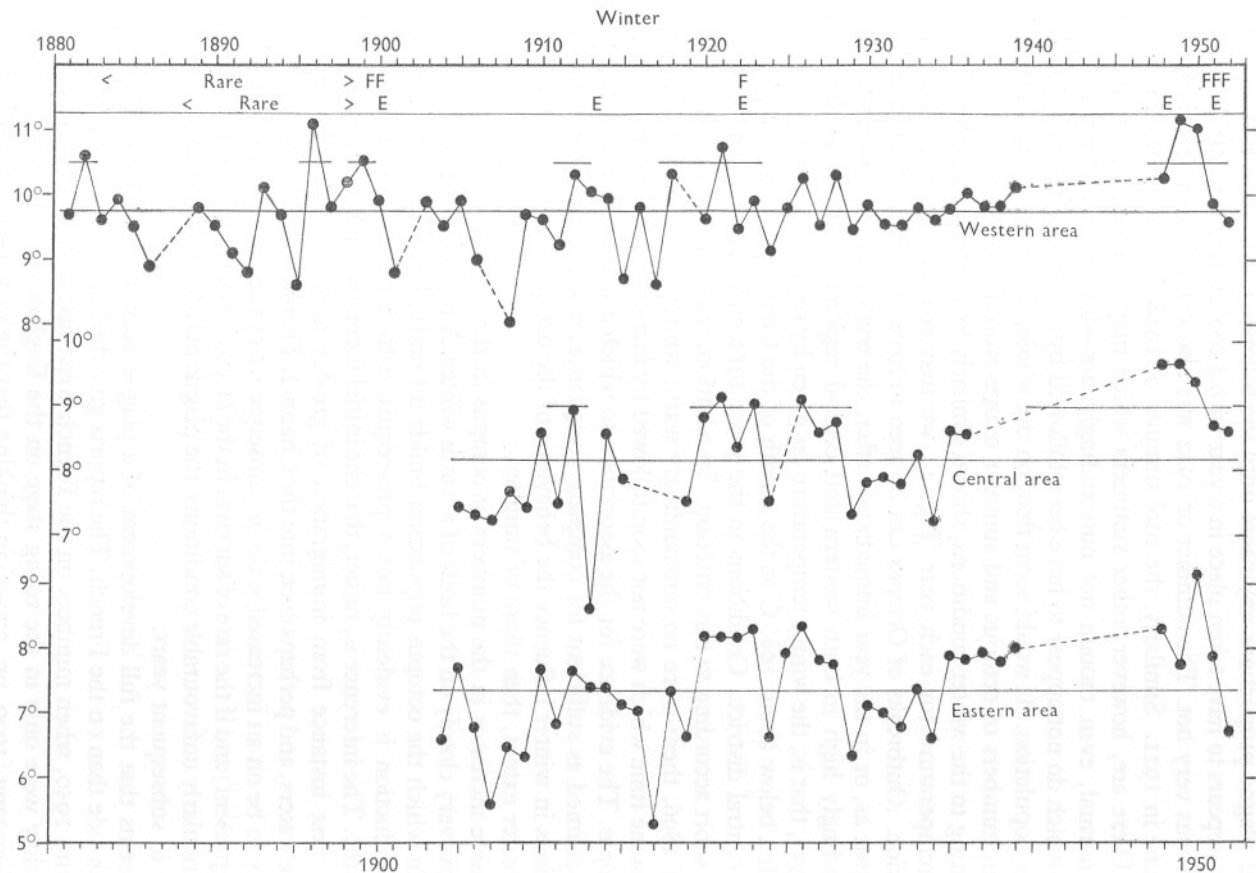


Fig. 4. The *minimal* monthly mean surface water temperature ( $^{\circ}$  C) for each year in three districts of the English Channel. For details, see legend to Fig. 3. For the western area, the figures for the years before 1906 are based on observations at the Seven Stones lightship only. The lettering beneath the scale of years indicates the years in which *Octopus* was reported as notably abundant on the French coast (F), and on the English coast (E).

all parts of the Channel, the numbers of octopus do not appear to have reached plague proportions anywhere until the following year. The outbreak in 1913 appears to have taken place in a year with a cool summer, though that of 1911 was very hot. The summer of 1922 was also cool, following a fairly warm one in 1921. Similarly, the cool summer of 1948 follows a hot one in 1947. There are, however, other summers which may be classified as well above normal, even though not outstandingly hot—for example 1926 and 1933—which do not appear to have been followed by notable increases in the octopus population. It would seem that, on the whole, the association, if any, between numbers of octopus and summer temperature is not close.

Turning to the winter conditions, shown similarly by the minimal monthly mean temperature for each year (Fig. 4), we may see a somewhat clearer connexion. Outbreaks of *Octopus* can be seen to have occurred either in the same year as, or in the year immediately after, the winter temperatures were outstandingly high in both western and central regions at the same time; whenever, that is, the winter temperature (as given by the monthly mean) has not fallen below about  $10\frac{1}{2}^{\circ}\text{C}$  at the mouth of the Channel, nor below  $9^{\circ}\text{C}$  in the central district. Conditions in the years 1912 and 1948 appear to fall a little short according to this criterion. In addition, with the possible exception of 1896, there were no outstandingly warm winters in the two regions at the same time which were not soon followed by increases in the abundance of *Octopus*. The evidence for the association to which attention is drawn here is not claimed as sufficient for complete acceptance, but it seems likely that conditions in winter influence the behaviour of the octopus more directly, or to a greater extent, than those of summer.

Notable increases in the numbers of octopus in the inshore waters appear to follow very closely on the heels of a warm winter. A period of several warm years in which the octopus population builds up under improved conditions of reproduction is evidently not a pre-requisite to a plague, as Garstang suggested. The inference is, rather, that the initial increase in numbers springs in the first instance from immigration of greater numbers of adults into inshore waters, and perhaps even into the Channel. Thereafter, since spawning is likely to be on an increased scale (in consequence of the greater numbers of adults present) and if the rate of survival in the larvae is not unduly diminished by particularly unfavourable conditions, the plague may be intensified by these means in subsequent years.

It seems that the full development of a plague occurs a year later on the English side than on the French. The reports give the impression that in both 1899 and 1950, when numbers on the French coast had already become very high, they were only in the rising stage on the English side. We may agree with Garstang (1900, pp. 270-1) in thinking that the explanation for this lies in the pressure of population on the French side becoming so great as to drive the animals to emigrate to the English coasts. It is doubtful whether water



temperature plays any part in this, since, although the summer of 1899 was exceedingly hot, that of 1950 was only slightly in excess of the average. In 1949, when the summer temperature was even higher than in 1899, the plague had not yet developed on the French side, so far as the records show.

#### *Lowest Tolerable Temperature and Migration*

As a rule the octopus appear to move away from the inshore regions when the height of summer has passed, and to spend the winter in the deeper waters offshore. After the winter, they return inshore for spawning. There is some slight indication that shoreward movement is discouraged by very severe winters, not only in the spring immediately following, but for a period of a year or more. The severity of the winter of 1895, for instance, may account for the absence of an outbreak of *Octopus* after the very mild winter of 1896. The winter of 1947 was extremely cold; the monthly mean temperature for February 1947 was  $4.8^{\circ}$  C at Jersey and  $4.9^{\circ}$  C off Plymouth. This may have delayed the outbreak of the plague, perhaps, but the evidence for this point is too indefinite to allow reliable conclusions to be drawn.

On the other hand, in some years octopus are known to remain inshore throughout the winter. An occasional juvenile octopus was caught off Plymouth by the research vessels of the Marine Biological Association in March 1950, and throughout the following winter (1951) *Octopus* were being caught by the *Sabella* within 16 miles of Plymouth Breakwater (see Appendix). During March 1950 the sea temperature near Plymouth Breakwater (Station L. 2) was between  $9$  and  $10^{\circ}$  C, and about  $\frac{1}{2}^{\circ}$  C higher near the Eddystone (St. E. 1). In winter 1951 the sea temperature at St. L. 2 fell to  $8^{\circ}$  C, possibly a little below, and to about  $8\frac{1}{2}^{\circ}$  C near the Eddystone during this period. The average winter temperature—the monthly mean for February—in Region 11 (see Lumby, 1935) is estimated at  $8.7^{\circ}$  C for the period 1903–27 and  $8.2^{\circ}$  C for the period 1928–51 (these latter figures are not yet published). On the other side of the Channel, as Mr Baal informs us, *Octopus* remained inshore at Jersey during three successive winters, those of 1950, 1951 and 1952. They did not leave these waters until October 1952. The lowest monthly mean temperatures in these waters were  $8\frac{1}{2}^{\circ}$ ,  $6^{\circ}$  and  $7\frac{1}{2}^{\circ}$  C, for these three winters respectively. These compare with an average value of  $7^{\circ}$  C over the years 1933–39, 1946–52. In October 1952, when the animal left these waters, the average temperature was  $13\frac{1}{2}^{\circ}$  C; in November,  $10\frac{1}{2}^{\circ}$  C.

From this we may conclude with assurance that *Octopus* tolerates temperatures as low as  $6^{\circ}$  C. However, in view of the fact that this value is well below the average winter temperature of the water both off Plymouth and at Jersey, and since the animal is not normally present in these waters in winter, it must be concluded that the occurrence of *Octopus* at this season is governed by temperature only to quite a limited extent. It is clearly not true that the animal may always be expected to be present when the water temperature is

above 6° C, and there is thus no justification for inferring from the absence of *Octopus* from waters of a certain temperature that the animal is intolerant of that temperature. We therefore cannot yet determine from the evidence before us, what is the lowest temperature *Octopus* can tolerate; for a warm-water animal, however, it is evidently surprisingly low.

At the mouth of the English Channel the lowest monthly mean temperature recorded, that of 1908, was just above 8° C (Fig. 4). Thus only very infrequently indeed would the octopus find a water temperature below 6° C in this part of the English Channel. Moreover, as the chart of average winter

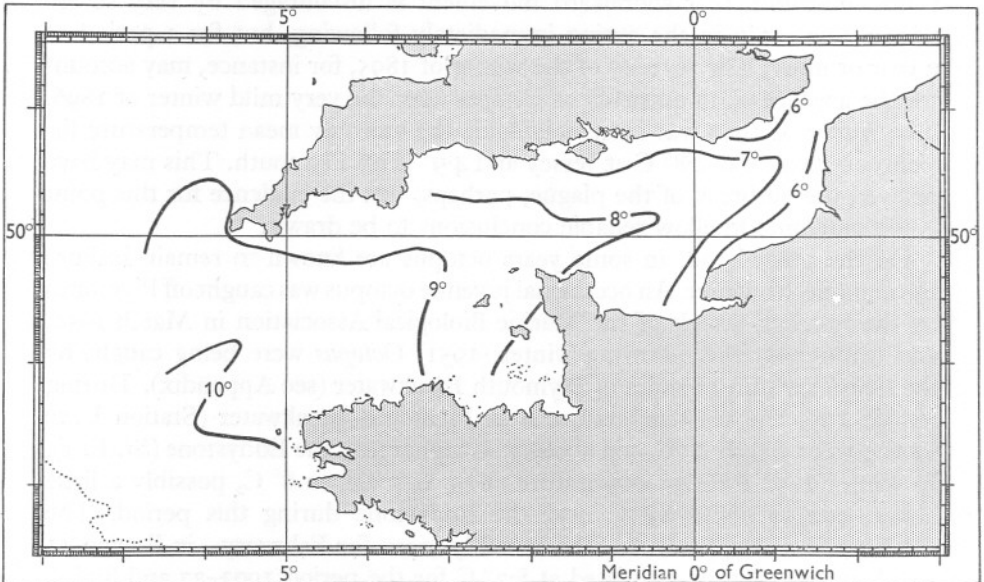


Fig. 5. The *minimal* monthly mean temperature (° C) of the surface water in the English Channel, based on observations over the period 1903-27.

temperature shows (Fig. 5), the octopus can find, on the average, water of a tolerable temperature almost everywhere in the English Channel. In view of this, and also of the apparent ability of the animal to increase its numbers inshore very quickly after a warm winter, it would not be surprising to find that there is almost always quite a large population of *Octopus* inhabiting the Channel, which is not noticed except in some special seasons.

#### DISCUSSION

The disturbances caused by the abundance of *Octopus* in 1950-51 followed the same general pattern as in 1899-1900. Recently, as in the earlier 'plague', shell-fishing had to be temporarily abandoned at some points on the Devon coast and on the French coast. In so far as it has been possible to get authentic

records for 1950-51, most of the octopus were of medium size with an arm span of  $2\frac{1}{2}$ -3 ft., but specimens reaching 5-5 $\frac{1}{2}$  ft. were reported. They were therefore smaller on the average than those mentioned by Garstang (1900).

We believe that the distribution of larvae shown in Fig. 1 gives conclusive evidence that the main breeding grounds of *Octopus* in the south-western Channel are in coastal waters, from Ushant eastwards along the north coast of France, and around the Channel Islands, to as far east as the neighbourhood of Grandcamp-les-Bains. Occasional spawning on the English side appears to be insufficiently frequent to be represented in the catches.

We have not been able to add much to greatly needed information on the growth of this species. It appears, however, that larvae hatched the previous year reach a size at least 14 mm in mantle length by March of the following year (see p. 515). This particular specimen may have hatched very late in the breeding season and may have lived through the winter without much growth. In size it may be comparable with young octopus of very small size reported by Devon fishermen in the autumn of 1950. From scattered reports received from the whole south coast most of the octopus seen in July and August 1950 were about  $2\frac{1}{2}$  ft. in arm span, and, on this point, we think it extremely likely that they were not more than two years old, and although we have no evidence on this point, some may have been only just over a year old.

During the recent years of numerical abundance of *Octopus* this species has been present in inshore waters the whole year round on the English coast and in the Channel Islands. Even in Jersey, as Mr H. J. Baal informs us (*in litt.*), in normal years *Octopus* disappears from inshore waters in winter but since the winter of 1949-50 it has been quite common throughout the period but disappeared into deeper water in October 1952 (Baal, 1953). Sinel (1906, p. 215) implies that it is present only in summer at Jersey in normal years in the words: 'it is constant during the summer months'. In another paragraph (p. 222) dealing with the capture of *Octopus*, he indicates his belief in a southerly migration of *Octopus* in the autumn: 'Yet another plan, much used in the autumn on the north coast of Jersey: in this particular locality, and at this time, they sometimes swarm on the sea surface, swimming in the method last described, no doubt to some more congenial shore. Men armed with long bamboo rods, with large hooks at the end, station themselves on outlying rocks, and simply hook them out as they pass. I have seen many tons' weight caught in one locality by this method, and being used to manure the land.' Mr Baal also informs us that he has seen this southerly movement at this place in the autumn but there is as yet no real evidence of large-scale migratory movement other than the migration across the Channel in plague years and seasonal retreat into deeper water (see p. 516). In *Eledone* these seasonal movements, from offshore fishing grounds to inshore waters, which take place in the summer, followed by a retreat to deeper water in winter, have been reported by Isgrove (1909). *Eledone cirrhosa* is much rarer on the

south side of the Channel than in English waters, but after the octopus became more abundant, *Eledone* became scarcer, presumably because it is eaten by the larger species.

The virtual disappearance of the lobster (*Homarus vulgaris*) on offshore fishing grounds with the arrival of *Octopus* (see Appendix I) was to be expected. The increase in numbers of crawfish (*Palinurus vulgaris*) on the trawling grounds implies that they were being driven out of their usual haunts to areas where *Octopus* was less abundant. There is no information available to suggest that the spider crab (*Maia squinado*) was being driven ashore on the English coast as in 1899-1900. Baal (1953), however, notes that in the autumn of 1952 spider crabs were numerous in shallow lagoons exposed at spring tides in Jersey. He observed that 'the spider crabs had collected into large heaps, about two feet and three feet in diameter, with their legs so entangled as to make it difficult to separate a crab from the heap. The octopuses captured some from the outside of the masses but the greater number survived, and day after day the heaps remained.' As Baal implies, this curious behaviour may have a survival value for the spider crab.

We wish to thank the Director of Fishery Investigations, Lowestoft, for readily placing the plankton samples of the *Sir Lancelot* cruises at our disposal. We are also greatly indebted to Mr H. J. Baal, Lieut.-Commander W. H. Batten, Lieut.-Commander C. A. Hoodless, D.S.C., and Prof. J. Z. Young, F.R.S., for information on local abundance of octopus.

Thanks are also due to Dr H. W. Parker (for larvae collected by him off the Casquets in 1950), Mr F. S. Russell, F.R.S. (for a juvenile octopus from Plymouth), and Alderman W. P. D. Stebbing for an adult octopus from Deal.

#### SUMMARY

The great abundance of *Octopus vulgaris* on the English coast of the Channel in 1950-51 recalls the previous 'plague' in 1899-1900 when, as now, Devon and Cornwall seemed affected the most.

It has been possible to demonstrate from plankton surveys of the Channel in 1948, 1949 and 1950 that planktonic larvae of *Octopus* originate on the south side of the Channel in the area between the Cotentin peninsula, the Channel Islands, and Ushant (Fig. 1). There is no indication of substantial breeding on the English coast.

Spawning commences in May in the extreme south-west, but the main spawning occurs in June and July, larvae becoming numerous in July and August. Larvae still planktonic in September are in process of dispersal to the north and east. The water temperature when spawning begins is estimated to be about  $11\frac{1}{2}$ -12° C.

Apart from a suggestion that mild winter conditions encourage the species

to become very abundant almost immediately on the French coast, *Octopus* appears to be influenced very little by sea temperature. Dearth of food seems to be the cause of subsequent trans-Channel migration of adults. From the evidence available *Octopus* tolerates temperatures as low as 6° C.

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## APPENDIX I

By Lieut.-Commander C. A. HOODLESS, D.S.C., R.N.R.,  
Master, R.V. *Sabella*

Records of *Octopus vulgaris* and *Eledone cirrhosa* trawled off Plymouth  
between 1 June 1948 and 30 June 1951 by R.V. *Sabella*

All the trawl hauls were made within a 16-mile radius of Plymouth Breakwater with a standard otter trawl with a head line of 56 ft.

1948. In the 7 months of this year covered by the record 2 *Eledone* were taken, both in October.

1949. 1 *Eledone* taken in October.



1950. Up to the commencement of the accompanying table single specimens of *Eledone* were taken in January, July and August.

During the whole of the above periods *Sabella* was making an average of 11 trawl hauls a month.

The first record of *Octopus* was on 4 September 1950. From that date *Homarus vulgaris* almost completely disappeared from the trawling grounds but *Palinurus vulgaris* was taken in much larger numbers than before.

The table below shows the numbers of *Octopus* and *Eledone* taken between September 1950 and June 1951, with the number of trawl hauls and the average number of specimens per haul:

Month	No. of trawl hauls	<i>Eledone cirrhosa</i>		<i>Octopus vulgaris</i>	
		No. of specimens	Average per haul	No. of specimens	Average per haul
September 1950	20	1	0.05	2	0.1
October	15	—	—	3	0.2
November	16	—	—	2	0.1
December	9	—	—	9	1.0
January 1951	13	3	0.2	3	0.2
February	13	—	—	29	2.2
March	3	—	—	4	1.3
April	16	1	0.1	13	0.8
May	20	—	—	17	0.9
June	10	—	—	2	0.2

## A PRELIMINARY CHECK-LIST OF BRITISH MARINE DIATOMS

By N. Ingram Hendey  
Royal Naval Scientific Service

The present list of diatoms may be considered an extension of the recent check-list of British marine algae by Parke (1953). It includes not only truly marine species, both sessile and planktonic, but also species inhabiting brackish waters with a few fresh-water species that manage to live, though somewhat precariously, in estuarine conditions.

It has been compiled for the following reasons: (1) to indicate the extent of the diatom flora around British coasts, so that comparisons may be made with that of the other countries; (2) to assist workers in the identification of material by indicating the size of any genus known to inhabit our shores; (3) to check the arrival of new entrants to the British flora; and (4) to provide a basis for a more detailed work, now in preparation. The check-list does not presume to lay down finalized decisions on the taxonomic rank ascribed to any organism or to consider the criteria upon which such ranks are based.

The vexed question of species, varieties and forms and the characters appertaining to them, as well as the degree to which such characters are possessed, is no part of the work at its present stage. Most of the varieties and forms cited are used in the accepted sense, and exceptions are explained in the notes.

As before, genera are listed alphabetically under the family, and species alphabetically under the genus. Synonyms are in brackets, and notes are appended at the end of each suborder: the number in brackets following a name in the list refers to these notes.

*Planktonic species are marked with an asterisk.*

Diatoms are considered as a Class of Algae, BACILLARIOPHYCEAE, comprising one order, BACILLARIALES, which is divided into ten suborders (Hendey, 1937, 1951), as follows:

- |                    |                    |
|--------------------|--------------------|
| 1. Discineae       | 6. Araphidineae    |
| 2. Aulacodiscineae | 7. Raphidioidineae |
| 3. Auliscineae     | 8. Monoraphidineae |
| 4. Biddulphiineae  | 9. Biraphidineae   |
| 5. Soleniineae     | 10. Surirellineae  |

The flora consists of 771 species arranged in 104 genera. The list includes all marine and brackish-water species that have been *recorded*, with the

omission of some that are doubtful or imperfectly described. It has not been possible to check all those listed.

The compiler will be grateful to hear of any errors or omissions, inevitable in a preliminary survey such as this, as well as to receive material or mounted slides that may help in advancing knowledge of our diatom flora.

Finally, it is hoped that this list will provide a stimulus for field-work of which a vast amount is yet necessary.

## BACILLARIOPHYCEAE

### BACILLARIALES

#### Suborder 1. DISCINEAE

#### Coscinodiscaceae

- ACTINOCYCLUS Ehrenberg, 1838. (1)  
*fulvus* (W. Sm.) Ralfs ex Pritch.\* (2)  
 (*Eupodiscus fulvus* W. Sm.)  
*octonarius* Ehrenb.\*  
 (*A. ehrenbergii* Ralfs ex Pritch.)  
 var. *octonarius*  
 var. *crassus* (W. Sm.)\*  
 (*A. crassus* (W. Sm.) Ralfs ex Pritch.)  
 (*A. ehrenbergii* var. *crassus* (W. Sm.)  
 Hust.)  
 (*Eupodiscus crassus* W. Sm.)  
 var. *ralfsii* (W. Sm.)\*  
 (*A. ehrenbergii* var. *ralfsii* (W. Sm.)  
 Hust.)  
 (*Eupodiscus ralfsii* W. Sm.)  
 (*Coscinodiscus fuscus* Norm.)  
 var. *sparsus* (Greg.)\*  
 (*A. sparsus* (Greg.) Rattray)  
 (*Eupodiscus sparsus* Greg.)  
 var. *tenella* (de Bréb.)\*  
 (*A. ehrenbergii* var. *tenella* (de Bréb.)  
 Hust.)  
 (*A. moniliformis* Ralfs ex Pritch.)  
 (*Eupodiscus tenellus* de Bréb.)  
*roperii* (de Bréb.) Grun. ex Van Heurck\*  
 (*ovalis* Grun.)  
 (*Eupodiscus roperii* de Bréb.)  
*subtilis* (Greg.) Ralfs ex Pritch.\*  
 (*falsus* W. Sm. ?)  
 (*subtilis* var. *disjuncta* Ratt.)  
 (*Eupodiscus subtilis* Greg.)  
 (*E. gregoryanus* de Bréb.)
- COSCINODISCUS Ehrenberg, 1838.  
*anguste-lineatus* A. Schm.\*  
*asteromphalus* Ehrenb.\*  
 var. *asteromphalus*  
 var. *hybrida* Grun.\*  
*centralis* Ehrenb.\* (3)  
*concinus* W. Sm.\*  
*curvatus* Grun. ex Schm.\*  
*divisus* Grun.  
*excentricus* Ehrenb.\*  
*fimbriatus* Ehrenb.\*  
*gigas* Ehrenb.\*  
*grani* Gough\*  
*joergenseii* Ostenf.\*
- jonesianus* (Grev.) Ostenf.\*  
 var. *jonesianus*  
 var. *commutatus* (Grun.) Hust.\*  
*kützingii* A. Schm.\*  
*leptopus* Grun. ex Van Heurck\*  
*lineatus* Ehrenb.\*  
*marginatus* Ehrenb.\*  
*nitidus* Greg.\*  
*nodulifera* Jan. ex Schm.\*  
*obscurus* A. Schm.\*  
*oculus-iridis* Ehrenb.\*  
*perforatus* Ehrenb.\*  
 var. *perforatus*  
 var. *pavillardii* (Forti) Hust.\*  
*radiatus* Ehrenb.\*  
*rothii* (Ehrenb.) Grun.\*  
 var. *rothii*  
 var. *normanii* (Greg.) Grun.\*  
 (*C. subtilis* var. *normanii* Van  
 Heurck)  
 (*C. normanii* (Greg.) Brockm.)  
*stellaris* Roper\*  
*sub-bulliens* Jörg.\* (3)  
*subtilis* Ehrenb.\*  
*woodwardii* Eulens.\* (4)
- COSCINOSIRA Grun, 1900. (5)  
*oestrupii* Ostenf.\*  
*polychorda* Grun.\*
- CYCLOTELLA (Kützing) de Brébisson, 1838.  
*kützingiana* Thwaites (6)  
*striata* (Kütz.) Grun.
- DRURIDGEA Donkin, 1861.  
*compressa* (West) Donk.\*  
 (*geminata* Donk.)  
 (*Podosira compressa* West)
- ENDICTYA Ehrenberg, 1845.  
*oceanica* Ehrenb.\*
- HYALODISCUS Ehrenberg, 1845. (7)  
*radiatus* (O'Meara) Grun.\*  
 (*Pyxidicula radiata* O'Meara)  
*scoticus* (Kütz.) Grun.\*  
 (*Cyclotella scotica* Kütz.)  
 (*Podosira smithiana* Grun.)  
*subtilis* Bail.\*  
 (*franklini* (Ehrenb.) Cleve)  
 (*Podosira subtilis* Mann)  
 (*Craspedodiscus franklini* Ehrenb.)

- MELOSIRA Agardh, 1824.  
*arctica* (Ehrenb.) Dick.  
 (*hyperborea* Schütt)  
*jurgensii* Agardh  
*moniliformis* (O. F. Müll.) Agardh  
 (*borreri* Grev. in Hook.)  
*nummuloides* (Dillw.) Agardh  
*westii* W. Sm.
- PARALIA Heiberg, 1863. (8)  
*sulcata* (Ehrenb.) Cleve  
 (*marina* Heib.)  
 (*Gaillionella sulcata* Ehrenb.)  
 (*Orthosira marina* W. Sm.)  
 var. *sulcata*  
 f. *coronata* (Ehrenb.) Grun.  
 f. *radiata* Grun.  
 var. *biseriata* Grun.
- PHACODISCUS Meunier, 1910.  
*punctulatus* (Grev.) Meun.\*  
 (*Coscinodiscus punctulatus* Greg.)
- PLANKTONIELLA Schütt, 1893.  
*sol* (Wall.) Schütt\*  
 (*wolterecki* Schimp.)  
 (*Coscinodiscus sol* Wall.)
- PODOSIRA Ehrenberg, 1840. (9)  
*montagnei* Kütz.\*  
*stelliger* (Bail.) Mann\*  
 (*maculata* W. Sm.)  
 (*Hyalodiscus stelliger* Bail.)  
 (*Melosira maculata* Lagerst.)
- POROSIRA Jörgensen in Nordgaard, 1905.  
*glacialis* (Gran) Jörg.\* (10)  
 (*Podosira glacialis* Cleve)  
 (*P. hormoides* var. *glacialis* Grun.)  
 (*Lauderia glacialis* (Grun.) Gran)
- PYXIDICULA Ehrenberg, 1833.  
*cruciata* Ehrenb.\* (11)
- ROPERIA Grunow ex VanHeurck, 1885. (12)  
*tessellata* (Roper) Grun.\*  
 (*Eupodiscus tessellatus* Roper)  
 (*Actinocyclus tessellatus* (Roper) Ralfs  
 ex Pritch.)
- SKELETONEMA Greville, 1865.  
*costatum* (Grev.) Cleve\*  
 (*Melosira costata* Grev.)  
*mirabile* Grun. ex Van Heurck\*
- STEPHANODISCUS Ehrenberg, 1845.  
*astraea* (Ehrenb.) Grun.\* (13)  
*dubius* (Fricke) Hust.\*  
*hantzschii* Grun.\*
- STEPHANOPYXIS Ehrenberg, 1844.  
*palmeriana* (Grev.) Grun.\*  
*turris* (Grev.) Ralfs ex Pritch.\*
- THALASSIOSIRA Cleve, 1873. (5)  
*baltica* (Grun.) Ostenf.\*  
*bioculata* (Grun.) Ostenf.\*  
*condensata* Cleve\*  
*decipiens* (Grun.) Jörg.\*  
*fallax* Meun.\*  
*fluviatilis* Hust.\*  
*gravida* Cleve\*  
*hyalina* (Grun.) Gran\*  
*nordenskioldii* Cleve\*  
*rotula* Meun.\*  
*subtilis* (Ostenf.) Gran\*

**Hemidiscaceae**

- HEMIDISCUS Wallich, 1860. (14)  
*cuneiformis* Wall.\*  
 (*Euodia inornata* Castr.)  
 (*E. cuneiformis* Schütt)

**Actinodiscaceae**

- ACTINOPTYCHUS Ehrenberg, 1843.  
*amblyoceros* (Ehrenb.) Schm.\*  
*senarius* Ehrenb.\*  
 (*undulatus* Bail.)  
*splendens* (Shad.) Ralfs ex Pritch.\*
- ARACHNOIDISCUS Deane ex Pritchard, 1852.  
 (15)  
*ehrenbergii* Bail. ex Ehrenb. (16)
- ASTEROMPHALUS Ehrenberg, 1844.  
*flabellatus* (de Bréb.) Grev.\*  
*heptactis* (de Bréb.) Ralfs ex Pritch.\*  
*hookerii* Ehrenb.\*

*Notes on Discineae*

- (1) *Actinocyclus* Ehrenb. is placed in the *Coscinodiscaceae* (Hendey, 1937) because the general valve structure resembles that of *Coscinodiscus* Ehrenb. and the presence of a marginal ocellus is regarded as of secondary importance. The genus is obviously related to *Roperia* Grun. and to the *Eupodiscaceae*, where it is placed by Hustedt and Cleve-Euler, but in that position it assumes affinities with *Auliscus* Ehrenb. which stretches relationships beyond reasonable bounds.
- (2) Probably a form of *Actinocyclus octonarius* or intermediate between it and *A. subtilis* (Grev.) Ralfs ex Pritch.
- (3) Cleve-Euler (1951) makes this a variety of *Coscinodiscus asteromphalus* Ehrenb.
- (4) Rattray makes this a synonym of *C. apiculatus* Ehrenb., but Mann (1907) points out that it is nearer to *C. radiatus* Ehrenb. Hustedt unites it with *C. perforatus* Ehrenb.
- (5) Cleve-Euler (1951) makes this a subgenus of *Coscinodiscus* Ehrenb. This is not in the least useful, as *Coscinosira* Gran and *Thalassiosira* Cleve have characteristic colony formation and are genera well recognized by plankton biologists, who make most of their determinations upon either living or formalin-preserved material.

- (6) Cleve-Euler (1951) credits this combination to Chauvin.
- (7) Hustedt's classification is followed here in that *Hyalodiscus* is reserved for those species that possess an umbilical line at about half the valve radius. More work is required on British material.
- (8) *Paralia* Heiberg is separated from *Melosira* on account of the punctate-areolate structure of the valve margin and mantle. Lebour (1930), Gran (1905), and Cleve-Euler (1951) accept *Paralia*; Hustedt gives it subgeneric rank. Often found in the plankton, particularly after winter storms.
- (9) Cleve-Euler (1951) makes *Podosira* Ehrenb. a subgenus of *Melosira* Agardh.
- (10) Gran (1905) and Lebour (1930) place this species in *Lauderia*. Hustedt separates them on account of the spinulae, which cover the valve in *Porosira* but are marginal only in *Lauderia*.
- (11) A very doubtful species. Three species only are known from European waters, and more work is required on this group.
- (12) *Roperia* Grunow connects *Actinocyclus* Ehrenb. with the Eupodiscaceae. Some authorities place it there, but it has closer affinities with *Coscinodiscus* Ehrenb.
- (13) Normally considered as a fresh-water species, but is often found living in marine conditions.
- (14) Very variable in outline and size, with a world-wide distribution.
- (15) The generic name *Arachnoidiscus* Deane was accepted for conservation against *Hemiptrychus* Ehrenb., *Ber. Akad. Wiss., Berl.*, 1848, p. 7, and *Arachnoidiscus* J. W. Bailey ex Ehrenb., *Ber. Akad. Wiss., Berl.*, 1849, p. 63, by the Special Committee for Diatomaceae at the 7th International Botanical Congress, Stockholm, July 1950 (*Int. Code bot. nomencl.*, 1952, p. 70, Utrecht).
- (16) This record is doubtful. Two records are given in the literature of the nineteenth century, both for the west coast. The species has been recorded (unpublished) from the Thames Estuary; but it would be unwise to consider the species as other than an alien in the British flora. With such a characteristic species there can be no case for misidentification, so it can be assumed only that the species was carried to our shores mechanically. The species is common on the Californian coast and around Cape Town.

## Suborder 2. AULACODISCINEAE

**Eupodiscaceae**

AULACODISCUS Ehrenberg, 1844. (1)

*argus* (Ehrenb.) Schm.\*  
 (*Eupodiscus argus* (Ehrenb.) W. Sm.)  
*sollitianus* Norman\*

EUPODISCUS Rattray, 1888. (2)

*radiatus* Bail.\*  
 (*Aulacodiscus radiatus* (Bail.) Brightw.)

*Notes on Aulacodiscineae*

- (1) *Aulacodiscus* Ehrenb. (*Ber. Akad. Wiss., Berl.*, 1844, p. 73) based on *A. crux* Ehrenb. was accepted for conservation by the Special Committee for Diatomaceae at the 7th International Botanical Congress, Stockholm, July 1950 (*Int. Code bot. nomencl.*, 1952, p. 70, Utrecht) against *Tripodiscus* Ehrenb., *Tetrapodiscus* Ehrenb., *Pentapodiscus* Ehrenb. and *Eupodiscus* Ehrenb.
- (2) The name *Eupodiscus* was accepted for conservation in the sense that Rattray used it (*J.R. micr. Soc.*, 1888, p. 909), i.e. for *E. radiatus* Bail. (*Int. Code bot. nomencl.*, 1952, p. 71)—see note (1).

## Suborder 3. AULISCINEAE

**Auliscaceae**

AULISCUS Ehrenberg, 1843.

*sculptus* (W. Sm.) Ralfs ex Pritch. (1)*Note on Auliscineae*

- (1) This species occurs but infrequently around British coasts, and it is thought that a more intensive search, particularly along the south and west coasts, would reveal a more general distribution of it, also that *Auliscus caelatus* Bail., a species favouring warmer water, might be encountered. *A. caelatus* is considered by some authorities to be identical with *A. sculptus*. Further researches on this species are required.



## Suborder 4. BIDDULPHIINEAE

**Biddulphiaceae** (1)

- BELLEROCHEA Van Heurck, 1885.  
*malleus* (Brightw.) Van Heurck\*  
 (*Triceratium malleus* Brightw.)
- BIDDULPHIA Gray, 1821. (2)  
*alternans* (Bail.) Van Heurck  
 (*Triceratium alternans* Bail.)  
*antediluviana* (Ehrenb.) Van Heurck  
 (*vesiculosa* (Agardh) Boyer)  
 (*Amphitetras antediluviana* Ehrenb.)  
*aurita* (Lyngb.) de Bréb.\* (3)  
 var. *aurita*  
 var. *obtusa* (Kütz.) Hust.\*  
 (*B. obtusa* (Kütz.) Ralfs ex Pritch.)  
 (*B. roperiana* Grev.)  
 (*Odontella obtusa* Kütz.)  
*granulata* Roper\*  
*laevis* Ehrenb.\*  
*mobilienensis* (Bail.) Grun. ex Van Heurck\*  
 (*baileyi* W. Sm.)  
 (*Zygoceros mobilienensis* Bail.)  
*pulchella* Gray  
 (*biddulphiana* (Smith) Boyer)  
*regia* (Schultze) Ostenf.\*  
*rhombus* (Ehrenb.) W. Sm.\*  
 f. *rhombus*  
 (*Zygoceros rhombus* Ehrenb.)  
 f. *trigona* Hust.\*  
*sinensis* Grev.\* (4)  
*spinosa* (Bail.) Boyer\* (5)  
 (*Triceratium spinosum* Bail.)  
 (*T. armatum* Roper)  
 (*T. serratum* Wall.)  
 (*T. setigerum* Bail.)  
*striolata* (Ehrenb.)\* (6)  
 (*Triceratium striolatum* Ehrenb.)  
 (*Trigonium striolatum* (Ehrenb.)  
 Mann)
- CERATAULINA Peragallo & Schütt, 1896.  
*pelagica* (Cleve) Hendey\*  
 (*bergonii* (Perag.) Schütt)
- CERATAULUS Ehrenberg, 1843 (1844). (7)  
*smithii* Ralfs ex Pritch.\*  
 (*Biddulphia smithii* Van Heurck)  
*turgidus* Ehrenb.
- DITYLUM L. W. Bailey, 1862.  
*brightwellii* (West) Grun. ex Van  
 Heurck\*  
 (*trigonum* Bail.)  
 (*undulatum* (Brightw.) Mann)  
 (*Triceratium brightwellii* West)  
 (*T. undulatum* Brightw. e.p.)
- EUCAMPIA Ehrenberg, 1840.  
*zodiacus* Ehrenb.\*  
 (*groenlandica* Cleve) (8)  
 (*britannica* W. Sm.)  
 (*nodosa* Schm.)
- HEMIAULUS Ehrenberg, 1844.  
*hauckii* Grun. ex Van Heurck\*  
 (*delicatulus* Lemm.)
- ISTHMIA Agardh, 1832.  
*enervis* Ehrenb.

- (*obliquata* Agardh e.p.)  
 (*Isthmiella enervis* Cleve) (9)  
 var. *enervis*  
 var. *subcylindrica* Taylor  
*nervosa* Kütz.  
 (*obliquata* Ehrenb.)  
 (*obliquata* Agardh e.p.)
- LITHODESMIUM Ehrenberg, 1840.  
*undulatum* Ehrenb.\*  
 (*victoriae* Karsten)  
 (*Triceratium undulatum* Brightw.)  
 (*T. intricatum* West)  
 (*Ditylum intricatum* Grun.)  
 (*D. undulatum* Mann.)
- STREPTOTHECA Shrubsole, 1890.  
*thamesis* Shrub.\*
- TRICERATIUM Ehrenberg, 1840.  
*favus* Ehrenb.\* (10)  
 (*muricatum* Brightw.)  
 (*fimbriatum* Wall.)  
 (*ferox* Castr.)  
 (*sarcophagos* Castr.)  
 (*Biddulphia favus* (Ehrenb.) Van  
 Heurck)
- TRIGONIUM Cleve, 1868.  
*arcticum* (Brightw.) Cleve (11)  
 (*Triceratium arcticum* Brightw.)

**Chaetoceraceae**

- CHAETOCEROS Ehrenberg, 1844. (12)  
*adhaerans* Mangin\*  
*affine* Laud.\*  
 (*schuttii* Cleve in Gran)  
 var. *affine*  
 var. *willei* (Grun.) Hust.\*  
*armatum* West\*  
*atlanticum* Cleve\*  
*boreale* Bail.\*  
*breve* Schütt\*  
*ceratosporum* Ostenf.\*  
 (*gracilis* Apstein)  
*cinctus* Gran\*  
*coarctatum* Laud.\*  
*compressum* Laud.\*  
 (*contortum* Schütt)  
*concavicornis* Mangin\*  
 (*criophilus* Gran)  
*constrictum* Gran\*  
*convexicornis* Mangin\*  
 (*peruvianus* Gran)  
*convolutum* Castr.\*  
*coronatum* Gran\*  
*crinitus* Schütt\*  
*curvisetum* Cleve\*  
*danicum* Cleve\*  
*debile* Cleve\*  
*decipiens* Cleve\*  
*densum* Cleve\*  
*didymum* Ehrenb.\*  
*difficile* Cleve\*  
*diversum* Cleve\*  
*eibenii* (Grun.) Meun.\*

## CHAETOCEROS (cont.)

*exospermum* Meun.\*  
*externum* Gran\*  
*furca* Cleve\*  
*glandazi* Mangin\*  
*gracile* Schütt\*  
*holsaticum* Schütt\*  
 (balticus Cleve)  
*imbricatum* Mangin\*  
*ingolfianum* Ostenf. in Gran\*  
*janischianum* Castr.\*  
 (dichaeta Ehrenb.)  
*lacinosum* Schütt\*  
*lauder* Ralfs in Laud.\*  
 (weissflogii Schütt)  
*lorenzianum* Grun.\*  
*mitra* (Bail.) Cleve\*  
 (Dieladia mitra Bail.)  
*neapolitanum* Schroder\*  
*perpusillum* Cleve\*  
*polygonum* Schütt\*  
*pseudocrinium* Ostenf.\*  
*pseudocurvisetum* Mangin\*  
*radians* Schütt\*  
*scolopendra* Cleve\*  
*seriacanthus* Gran\*  
*septentrionale* Oestrup\*

*simile* Cleve\*  
*simplex* Ostenf.\*  
*skeleton* Cleve\*  
*sociale* Laud.\*  
*subsecundum* (Grun.) Hust.\*  
 (distans var. *subsecunda* Grun.)  
 (paradoxus Schütt)  
 (paradoxus var. *ludersii* Engler)  
 (paradoxus var. *subsecunda* Van  
 Heurck)  
 (ralfsii Schütt)  
 (groenlandicus Cleve)  
 (diadema Grun.)  
 (Syndendrium *diadema* Ehrenb.)  
*subtile* Cleve\*  
*teres* Cleve\*  
*tetrastichon* Cleve\*  
*tortissimum* Gran\*  
*wighami* Brightw.\*

## BACTERIASTRACEAE

BACTERIASTRUM Shadbolt, 1854.

*delicatulum* Cleve\*  
*elongatum* Cleve\*  
*hyalinum* Laud.\*  
*solitarium* Mangin\*  
*varians* Lauder\*

## Notes on Biddulphiineae

- (1) This family is very ill defined and, despite the lengthy monograph by Boyer (1927), contains a wide range of forms whose only connexion is that they fail to fit reasonably into any other group of diatoms. In outline the cells may be circular, biangular or polygonal, and the valve surface is usually furnished with spines or processes. In such a family the synonymy is necessarily chaotic. The treatment adopted here is that of most modern taxonomists, and set out in Hendey (1937).
- (2) Van Heurck (1896) and Mann (1907) include *Odontella* Agardh, *Lampriscus* Bail., *Zygoceros* Ehrenb., *Denticella* Ehrenb., *Pseudo-stictodiscus* Grun., *Amphitetras* Ehrenb., *Amphipentas* Ehrenb., *Cerataulus* Ehrenb., and *Triceratium* Ehrenb. The last two are recognized here (in part) as separate genera.
- (3) Usually considered as a plankton species but frequently found in dense masses attached to a substratum.
- (4) This species made its first appearance in the North Sea sometime between 1903 and 1907, and by 1909 had spread to the English Channel and the Irish Sea. It is now firmly established in British coastal waters.
- (5) Not *Biddulphia spinosa* Grev.
- (6) Placed here tentatively. The valve is without horn-like processes, and is not a true member of *Biddulphia* Gray. Mann (1907) placed it in *Trigonium* Cleve, but that is giving *Trigonium* a meaning not intended by its author; neither is it a *Triceratium* as I interpret that genus. This species is different from *T. striolatum* Roper.
- (7) Included in *Biddulphia* by Van Heurck, but Hustedt separates it on account of the torsion of that cell in girdle view.
- (8) *Eucampia groenlandica* Cleve is considered to be distinct from *E. zoodiacus* by Lebour (1930) and Boyer (1927). I follow Hustedt here in considering them synonymous. It has been suggested that they are seasonal variations of the same organism.
- (9) Cleve (1873) created a new genus for this species on account of the absence of costae, a distinction not generally accepted.
- (10) Hendey (1937) interprets the genus *Triceratium* Ehrenb. strictly in terms of *T. favus* the type of the genus, and excludes all species that do not possess cornutate processes and hexagonal areolation. This treatment reduces a very unwieldy genus to less than thirty species.
- (11) *Trigonium* Cleve is reserved for species that have an areolate surface but whose angles are furnished with rounded bosses of fine pores.
- (12) *Chaetoceros* is here considered as a neuter noun, and all specific epithets have been treated accordingly. All members of the genus are planktonic.

## Suborder 5. SOLENIINEAE

**Corethronaceae**

- CORETHRON Castracane, 1886. (1)  
*criophilum* Castr.\*  
 (*hystrix* Hensen)

**Leptocylindraceae**

- BACTERIOSIRA Gran, 1900. (2)  
*fragilis* Gran\*  
 DACTYLIOSOLEN Castracane, 1886.  
*antarcticus* Castr.\*  
*mediterraneus* Perag.\*  
 DETONULA Schütt, 1893. (2)  
*confervacea* (Cleve) Gran\*  
 (*cystifera* Gran)  
 (*Lauderia confervacea* Cleve)  
 LAUDERIA Cleve, 1837. (2)  
*borealis* Gran\*  
 (*annulata* Cleve)  
 LEPTOCYLINDRUS Cleve, 1889.  
*danicus* Cleve\*  
*minimus* Gran\*  
 (*belgicus* Meun.)  
 SCHROEDERELLA Pavillard, 1913. (2)  
*delicatula* (Perag.) Pav.\*  
 (*Detomula delicatula* Gran)  
 (*Lauderia delicatula* Perag.)  
*schroderi* (Berg.) Pav.\* (3)

**Rhizosoleniaceae**

- GUINARDIA Peragallo, 1892.  
*flaccida* (Castr.) Perag.\*

- (*baltica* Schütt)  
 (*Rhizosolenia* ? *flaccida* Castr.)  
 (*R. castracanei* Cleve)  
 (*Hensemella baltica* Schütt)  
 RHIZOSOLENIA Brightwell, 1858. (4)  
*acuminata* (Perag.) Gran  
*alata* Brightw.\*  
 var. *alata*  
 var. *indica* Ostenf.\*  
 var. *inermis* (Castr.) Hust.\*  
*bergonii* Perag.\*  
 (*amputata* Ostenf.)  
*calcar-avis* Schultze\*  
*castracanei* Perag.\*  
*cylindrus* Cleve\*  
*delicatula* Cleve\*  
*fragilissima* Berg.\*  
 (*faeroeensis* Ostenf.)  
*hebetata* Bail.\*  
*imbricata* Brightw.\*  
 (*striata* Grev.)  
*robusta* Norm. ex Pritch.\*  
 (*sigma* Schütt)  
*setigera* Brightw.\*  
 (*japonica* Castr.)  
 (*hensemii* Schütt)  
*shrubsolei* Cleve\*  
*stolterfothii* Perag.\*  
 (*Eucampia striata* Stolt.)  
 (*Pyxilla stephanos* Hensen)  
*styliformis* Brightw.\*

## Notes on Soleniineae

- (1) Hendeby (1937) was able to show that all the published species of this genus should be considered as phases of one species. *Corethron hystrix* Hensen is therefore given as a synonym of *C. criophilum* Castr. The variation of this organism depends upon environmental factors of which probably salinity is the chief.
- (2) Hendeby (1937) placed this genus in Discineae; its transference to its present position is on account of its peculiar girdle formation, and follows Hustedt (1927-30) and Cleve-Euler (1951).
- (3) Hustedt (1927-30) unites with *Schroderella delicatula* (Perag.) Pav. More information is required on British material.
- (4) The genus *Rhizosolenia* Brightwell was accepted for conservation against *Rhizosolenia* Ehrenb., 1843, and *Monoceros* van Goor, 1824, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).

## Suborder 6. ARAPHIDINEAE

**Fragilariaceae**

- ASTERIONELLA Hassall ex W. Smith, 1856.  
 (1)  
*bleakeleyii* W. Sm.  
*formosa* Hass.\* (2)  
*japonica* Cleve & Müller ex Gran\* (3)  
*kariana* Grun. in Cleve & Grun.\* (4)  
*notata* Grun. ex Van Heurck\*  
 CAMPYLOSIRA Grunow, 1882.  
*cymbelliformis* (Schm.) Grun. ex Van  
 Heurck  
 (*Synedra cymbelliformis* Schm.)

- CYMATOSIRA Grunow, 1862.  
*belgica* Grun.  
 DIMEREGRAMMA Ralfs ex Pritchard, 1861.  
*fulvum* (Greg.) Ralfs ex Pritch.  
*marinum* (Greg.) Ralfs ex Pritch.  
 var. *marinum*  
 var. *lanceolatum* (Perag.) Hust.  
*minor* (Greg.) Ralfs ex Pritch.  
 var. *minor*  
 var. *nana* (Greg.) Van Heurck  
 FRAGILARIA Lyngbye, 1819.  
*bicipitata* Mayer

## FRAGILARIA (cont.)

- capucina* Desmaz. (2)  
*construens* var. *pusilla* Grun.  
 var. *venter* (Ehrenb.) Grun.  
*cylindrus* Grun.  
*hyalina* (Kütz.) Grun.  
*islandica* Grun. ex Van Heurck  
*leptostauron* (Ehrenb.) Hust.  
*oceanica* Cleve  
*pinnata* Ehrenb.  
 var. *pinnata*  
 var. *trigona* (Brun. & Hérib.) Hust.  
*striatula* Lyngb. (3)  
*virescens* var. *oblongella* Grun.  
 GLYPHODESMIS Greville, 1862.  
*distans* (Greg.) Grun.  
 OPEPHORA Petit, 1888.  
*marina* (Greg.) Petit  
 (*Meridion marinum* Greg.)  
*martyi* Hérib.  
*pacifica* (Grun.) Petit  
 (*Fragilaria pacifica* Grun.)  
*parva* (Grun.) Krasske  
 PLAGIOGRAMMA Greville, 1859.  
*brockmanni* Hust.  
*gregoryanum* Grev.  
*interruptum* (Greg.) Ralfs ex Pritch.  
*laeve* (Greg.) Ralfs ex Pritch.  
*stauraphorum* (Greg.) Heib.  
*vanheurckii* Grun.  
 RHAPHONEIS Ehrenberg, 1844.  
*amphiceros* Ehrenb. (5)  
 (*rhombus* Ehrenb.)  
 (*lanceolata* Ehrenb.)  
 (*amphiceros* var. *rhombica* Grun.)  
*belgica* Grun.  
 var. *belgica*  
 var. *intermedia* Grun.  
*minutissima* Hust.  
*nitida* (Greg.) Grun.  
*surirella* (Ehrenb.) Grun.  
 (*Zygoceros surirella* Ehrenb.)  
 SCEPTRONEIS Ehrenberg, 1844. (6)  
*caduceus* Ehrenb.  
 TRACHYSPHENIA Petit, 1857.  
*australis* Petit  
*communis* (Heib.) Grun.  
*dalmatica* (Kütz.) Grun.  
*debilis* (Kütz.) Grun. ex Van Heurck  
*ehrenbergii* (Kütz.) Grun.  
*flabellata* (Carm.) Agardh (7)  
*gracilis* (Ehrenb.) Grun. (7)  
 var. *gracilis*  
 var. *elongata* (Kütz.) de Toni  
*hyalina* (Kütz.) Grun.  
*juergensii* Agardh  
*lyngbyei* (Kütz.) Grun. ex Van Heurck  
 (7)  
*ovata* (W. Sm.) Grun.  
*paradoxa* (Lyngb.) Agardh  
 RHABDONEMA Kützing, 1844.  
*adriaticum* Kütz. (7)  
*arcuatum* (Lyngb.) Kütz. (7)  
*minutum* Kütz.  
 STRIATELLA Agardh, 1832.  
*delicatula* (Kütz.) Grun. ex Van Heurck  
*interrupta* (Ehrenb.) Heib.  
 (*Tessella interrupta* Ehrenb.)  
*unipunctata* (Lyngb.) Agardh  
 SYNEDRA Ehrenberg, 1830.  
*affinis* Kütz.  
 var. *affinis*  
 var. *fasciculata* Van Heurck  
 var. *gracilis* Grun.  
 var. *hybrida* Van Heurck  
 var. *parva* Kütz.  
*barbatula* Kütz.  
*crystallina* (Agardh) Kütz.  
*formosa* Hantzsch  
*fulgens* (Grev.) W. Sm. (7)  
*gaillonii* (Bory) Ehrenb.  
 var. *gaillonii*  
 var. *macilenta* (Grun.) Perag.  
*gracilis* Kütz.  
*hennedeyana* Greg.\* (8)  
*hyperborea* Grun.  
*investiens* W. Sm.  
*pulchella* Kütz.  
*robusta* Ralfs ex Pritch.  
 (*superba* Kütz.)  
*tabulata* (Agardh) Kütz. (9)  
 (*fasciculata* Kütz.)  
 (*affinis* var. *tabulata* (Kütz.) Grun.)  
 var. *tabulata*  
 var. *acuminata* (Kütz.) Grun.  
*ulna* (Nitzsch) Ehrenb.  
*undulata* Bail.  
 THALASSIONEMA Grunow ex Hustedt, 1932.  
*nitzschioides* Hust.\*  
 (*Thalassiothrix nitzschioides* Grun.)  
 THALASSIOTHRIX Cleve & Grunow, 1880.  
*frauenfeldii* Grun. in Cleve & Grun.\*  
*longissima* Cleve & Grun.\*

## Tabellariaceae

GRAMMATOPHORA Ehrenberg, 1840.

- angulosa* Ehrenb.  
*marina* (Lyng.) Kütz.  
*oceanica* Ehrenb. (7)  
 var. *oceanica*  
 var. *macilenta* (W. Sm.) Grun.  
 var. *subtilissima* (Bail.) De Toni  
*serpentina* Ehrenb.  
 LICMOPHORA Agardh, 1827.  
*anglica* (Kütz.) Grun.

## Notes on Araphidineae

- (1) Hassall (1850) first used the name *Asterionella* for what he described as a 'stelliform Diatoma'. A figure labelled *Asterionella formosa* was given but, as no generic description was provided, Hassall's name must be regarded as illegitimate. The authority for the genus is therefore given to Wm. Smith.

- (2) Usually considered as a freshwater species, but frequently found in estuarine conditions.
- (3) This species enjoys almost world-wide distribution, but usually favours warmer waters than those around our coasts.
- (4) An arctic form. Further details are required about the southern limits of its distribution.
- (5) The outline of this species is very variable.
- (6) A doubtful record.
- (7) Commonly epiphytic upon red algae, e.g. *Polysiphonia* and *Ceramium* spp.
- (8) Usually favours warm water and a high salinity.
- (9) Given by some authorities as a synonym of *Synedra affinis* Kütz. A more detailed examination of British material is required.

## Suborder 7. RAPHIDIOIDINAE

Not represented.

## Suborder 8. MONORAPHIDINEAE

**Achnanthaceae**

ACHNANTHES Bory, 1822.

*affinis* Grun. in Cleve & Grun.*angustata* Grev. (1)*brevipes* Agardh (2)var. *brevipes*var. *intermedia* Kütz.var. *parvula* (Kütz.) Cleve(A. *parvula* Kütz.)*clevei* var. *rostrata* Hust.*coarctata* var. *sinoensis* Hust.*delicatula* (Kütz.) Grun. in Cleve & Grun.*hauckiana* Grun. (2)var. *hauckiana*var. *rostrata* Schulz*lanceolata* (Bréb.) Grun.var. *lanceolata*f. *ventricosa* Hust.var. *elliptica* Clevevar. *rostrata* (Ostenf.) Hust.*latissima* Cleve*lemmermanni* Hust.var. *lemmermanni*var. *lineata* Salahvar. *obtusata* Hust.*lilljeborgei* Grun.*linkei* Hust.*longipes* Agardh*microcephala* (Kütz.) Cleve (3)*pseudobrevipes* Aleem*saxonica* Krasske*similis* McCall*subsessilis* Kütz. (2)*taeniata* Grun. in Cleve & Grun.

CAMPYLONEIS Grunow, 1862. (4)

*grevillei* (W. Sm.) Grun.(Cocconeis *grevillei* W. Sm.)

COCONEIS Ehrenberg, 1838.

*clandestina* Schm.*costata* Greg.*diaphana* W. Sm.*dirupta* Greg.*distans* Greg.*excentrica* Donk.*fluminensis* (Grun.) Perag.*grata* Schm.*guttata* Hust. & Aleem*molesta* var. *crucifera* Grun. ex Van

Heurck

*norvegica* Grun.*notata* Petit*pelta* Schm.*peltoidea* Hust.*placentula* Ehrenb.*pseudomarginata* Greg.*scutellum* Ehrenb.var. *scutellum*var. *ampliata* Grun.var. *distans* (Greg.) Schm.var. *ornata* Grun.var. *parva* Grun. in Clevevar. *stauroneiformis* W. Sm. in Cleve*sublittoralis* Hendey (5)*tenuis* Hust.

RHOICOSPHENIA Grunow, 1860. (6)

*curvata* (Kütz.) Grun.*marina* (W. Sm.) Schm.*pullus* Schm. M.*Notes on Monoraphidineae*

- (1) Considered by some authorities as a variety of *Achnanthes brevipes* Agardh.
- (2) This species and its varieties is seldom found in waters of full salinity, and in common with most brackish diatoms is liable to much variation in outline and size.
- (3) Usually considered as a fresh-water species, but has been found in marine waters having a salinity of 33‰.
- (4) Considered by some authorities to belong to *Cocconeis* Ehrenberg, but separated from that genus on account of a peculiar internal plate which is attached to the raphe-bearing valve.
- (5) More information concerning the distribution of this species is required.
- (6) Species of this genus were originally described as geniculate forms of *Gomphonema*. Further research, however, showed that the similarity with *Gomphonema* was superficial, and that the genus is correctly placed in the Monoraphidineae.



## Suborder 9. BIRAPHIDINEAE

## Naviculaceae (1)

- AMPHIPLEURA Kützing, 1844. (2)  
*micans* Lyngb.  
   var. *micans*  
   var. *fragilis* (Grev.) Grun.  
   *rutilans* (Trent.) Cleve  
     (*Schizonema rutilans* Trent.)  
 AMPHIPRORA Ehrenberg, 1843.  
   *alata* (Ehrenb.) Kütz.  
     (*Navicula alata* Ehrenb.)  
     (*Amphicampa alata* Rab.)  
   *constricta* Ehrenb.  
   *didyma* W. Sm.  
   *gigantea* var. *sulcata* (O'Meara) Cleve  
   *hyalina* Eulen. ex Van Heurck  
     (*paludosa* var. *hyalina* Eulen. ex Van  
       Heurck)  
   *hyperborea* Grun. in Cleve & Grun.\*  
   *lata* Grev.  
     var. *lata*  
     var. *angustior* McCall  
   *ornata* Bail.  
   *paludosa* W. Sm.  
     var. *paludosa*  
     var. *duplex* Donk.  
   *pulchra* var. *pulchella* Perag.  
   *robusta* McCall  
   *sulcata* Perag.  
   *surirelloides* Hendey  
   *vitrea* W. Sm.  
 ANOMOEONEIS Pfitzer, 1871. (3)  
   *exellii* Salah  
   *sculpta* (Ehrenb.) Cleve  
     (*Navicula sculpta* Ehrenb.)  
     (*N. tunens* W. Sm.)  
     (*N. rostrata* Kütz.)  
 AURICULA Castracane, 1873. (4)  
   *complexa* (Greg.) Cleve  
     (*Amphiprora complexa* Greg.)  
   *decepiens* Grun.  
   *dubia* Perag.  
   *ostrea* Brun & Temp. (5)  
 BREISSONIA Grunow, 1860. (2), (6)  
   *boeckii* W. Sm.  
 CALONEIS Cleve, 1894. (3)  
   *amphisboena* (Bory) Cleve  
     (*Navicula amphisboena* Bory)  
     var. *amphisboena*  
     var. *fenzlii* (Grun.) Cleve  
     var. *liburnica* (Grun.) Cleve  
     var. *subsalina* (Donk.) Cleve  
       (*N. subsalina* Donk.)  
   *bicuneata* (Grun.) Boyer  
     (*N. bicuneata* Grun.)  
   *brevis* (Greg.) Cleve  
     (*N. brevis* Greg.)  
   *formosa* (Greg.) Cleve  
     (*N. formosa* Greg.)  
     var. *formosa*  
     var. *holmiensis* Cleve  
   *fusioides* (Grun.) Heid. & Kolbe  
     (*N. fusioides* Grun.)  
     (*N. inornata* Grun.)  
     (*N. subula* Grun.)  
   *hustedtii* Aleem in Aleem & Hust.  
   *lepidula* (Grun.) Cleve  
     (*N. lepidula* Grun.)  
   *liber* (W. Sm.) Cleve  
     (*N. liber* W. Sm.)  
   var. *liber*  
   var. *linearis* (Grun.) Cleve  
   var. *umbilicata* (Grun.) Cleve  
 DIPLONEIS Ehrenberg, 1840. (3), (7)  
   *advena* (Schm.) Cleve  
     var. *advena*  
     var. *sanssegana* (Grun.) Cleve  
   *aestiva* (Donk.) Cleve  
     (*N. aestiva* Donk.)  
   *aestuari* Hust.  
   *bomboides* (Schm.) Cleve  
     (*N. bomboides* Schm.)  
     (*N. williamsonii* Van Heurck)  
   *bombus* Ehrenb.  
     (*N. bombus* Greg.)  
     (*N. abnormis* Castr.)  
     var. *bombus*  
     var. *egena* (Schm.) Cleve  
   *chersonensis* Grun.  
     (*N. apis* Ehrenb.)  
   *coffaeiformis* (Schm.) Cleve  
   *constricta* (Grun.) Cleve  
     (*N. constricta* Grun.)  
     (*N. donkimii* Schm.)  
   *contigua* var. *eudoxia* (Schm.) Cleve  
     (*N. mediterranea* Schm.)  
     (*N. eudoxia* Schm.)  
     var. *eugenia* (Schm.) Cleve  
       (*D. sejuncta* Jörg.)  
       (*N. eugenia* Schm.)  
   *crabro* Ehrenb.  
     (*N. crabro* Kütz.)  
     var. *crabro*  
     var. *expleta* (Schm.) Cleve  
     var. *pandura* (de Bréb.) Cleve  
       (*N. pandura* de Bréb.)  
   *cynthia* (Schm.) Cleve  
     (*N. cynthia* Schm.)  
   *didyma* (Ehrenb.) Cleve  
     (*Pinnularia didyma* Ehrenb.)  
   *divergens* (Schm.) Cleve  
     (*N. divergens* Schm.)  
     (*N. pfitzeriana* O'Meara)  
   *elliptica* (Kütz.) Cleve  
     (*N. elliptica* Kütz.)  
   *entomon* (Ehrenb.) Cleve  
   *fusca* (Greg.) Cleve  
     var. *fusca*  
     var. *delicata* Schm.  
     var. *gregorii* Cleve  
     var. *norvegica* Cleve  
   *hyalina* (Donk.) Cleve  
     (*N. hyalina* Donk.)  
   *incurvata* (Greg.) Cleve  
     (*N. incurvata* Greg.)

- DIPLONEIS (cont.)  
*incurvata* (cont.)  
 f. *incurvata*  
 f. *stricta* Hust.  
*interrupta* (Kütz.) Cleve  
 (N. *interrupta* Kütz.)  
*lineata* (Donk.) Cleve  
 (N. *lineata* Donk.)  
 (N. *adriatica* Grun.)  
*littoralis* (Donk.) Cleve  
 (N. *littoralis* Donk.)  
 (N. *ovulum* Grun.)  
*marginestriata* Hust.  
*nitescens* (Greg.) Cleve  
 (N. *nitescens* Greg.)  
 var. *nitescens*  
 var. *candida* McCall  
*notabilis* (Grev.) Cleve  
*oamaruensis* (Cleve) Mills  
 (adonis var. *oamaruensis* Cleve)  
 (N. *oamaruensis* (Cleve) Mann)  
*papula* (Schm.) Cleve  
 (N. *papula* Schm.)  
 var. *papula*  
 var. *constricta* Hust.  
*parca* (Schm.) Boyer  
*pseudovalis* Hust.  
*puella* (Schum.) Cleve  
*smithii* (de Bréb.) Cleve  
 (N. *smithii* de Bréb. in W. Sm.)  
*splendida* (Greg.) Cleve  
 (N. *splendida* Greg.)  
 (N. *entomon* Donk.)  
*stroemi* Hust.  
*subcincta* (Schm.) Cleve  
 (N. *subcincta* Schm.)  
*suborbicularis* (Greg.) Cleve  
 (N. *smithii* var. *suborbicularis* Greg.)  
 var. *suborbicularis*  
 var. *constricta* Hust.  
*subovalis* Cleve  
*vacillans* (Schm.) Cleve  
 var. *vacillans*  
 var. *delicatula* Cleve  
 var. *minuta* Grun.  
*weissflogii* (Schm.) Cleve  
DONKINIA Ralfs, 1860. (8)  
*angusta* (Donk.) Ralfs ex Pritch.  
 (Pleurosigma *angustum* Donk.)  
*carinata* (Donk.) Ralfs ex Pritch.  
 (P. *carinatum* Donk.)  
*recta* (Donk.) Grun. ex Van Heurck  
FRUSTULIA Agardh, 1824.  
*rhomboides* var. *amphipleuroides* (Grun.)  
 De Toni (9)  
MASTOGLIOIA Thwaites in W. Smith, 1856.  
*apiculata* W. Sm. (10)  
*binotata* (Grun.) Cleve  
 (Cocconeis *binotata* Grun.)  
*danseii* (Thwaites) W. Sm.  
*elliptica* (Agardh) Cleve  
 (*danseii* var. *elliptica* Van Heurck)  
 (Frustulia *elliptica* Agardh)  
*exigua* Lewis  
*lanceolata* Thwaites in W. Sm.  
*ovata* Grun.  
*ovulum* Hust.  
*portierana* Grun.  
*pumila* (Grun.) Cleve  
*smithii* Thwaites in W. Sm.  
 var. *smithii*  
 var. *lacustris* Grun.  
NAVICULA Bory, 1822.  
*abrupta* (Greg.) Cleve (11)  
 (Lyra var. *abrupta* Greg.)  
 var. *abrupta*  
 var. *rattrayi* Pant.  
*abstrusa* Hust.  
*aequorea* Hust.  
*aleemi* Hust.  
*algida* Grun.  
*ammophila* var. *flanatica* Grun.  
*approximata* var. *niceaensis* (12)  
 (*hennedyii* var. *niceaensis* Perag.)  
*arenicola* Grun.  
*avenacea* de Bréb. (13)  
 (*viridula* var. *avenacea* (de Bréb.) Van  
 Heurck)  
*bacillum* Ehrenb.  
*bahusiensis* Grun.  
 var. *bahusiensis*  
 var. *istriana* Grun. ex Van Heurck  
*biskanteri* Hust.  
*bottnica* Grun.  
*bremeyeri* Hust.  
 var. *bremeyeri*  
 var. *rostrata* Hust.  
*britannica* Aleem  
*bulnheimii* var. *belgica* Grun.  
*cancellata* Donk.  
 var. *cancellata*  
 var. *gregorii* Ralfs  
 var. *retusa* (de Bréb.) Cleve  
 (N. *retusa* de Bréb.)  
 var. *subretusa* (Ehrenb.) Van Heurck  
*carinifera* Grun.  
*cincta* Ehrenb.  
 var. *cincta*  
 var. *heufleri* Grun.  
 var. *leptocephala* (de Bréb.) Grun. ex  
 Van Heurck  
 (N. *leptocephala* de Bréb.)  
*clamans* Hust.  
*clavata* Greg.  
 var. *clavata*  
 var. *indica* (Grev.) Cleve  
 var. *wrightii* (O'Meara) Perag. (14)  
*clavicus* Greg.  
*clementis* Grun.  
*cluthensis* Greg. (15)  
*complanata* Grun. in Cleve & Grun.  
 var. *complanata*  
 var. *subinflata* Grun.  
*consentanea* Hust.  
*corymbosa* (Agardh) Cleve  
 (*Schizonema corymbosum* Agardh)  
*crucicula* (W. Sm.) Donk.  
 (*Stauroneis crucicula* W. Sm.)

## NAVICULA (cont.)

*crucicula* (cont.)var. *crucicula*var. *obtusata* Grun.*crucifera* Grun.(N. *rostellata* Schm.)*crucigera* (W. Sm.) Cleve(Schizonema *cruciger* W. Sm.)(Stauroneis *crucigera* (W. Sm.) Heib.)*cryptocephala* Kütz.var. *cryptocephala*var. *veneta* (Kütz.) Cleve*cryptostriata* Salah*cuspidata* Kütz.*cuspis* O'Meara (16)*cyprinus* (W. Sm.) Boyer (17)

(digito-radiata (Greg.) Ralfs)

(digito-radiata var. *cyprinus* (Ehrenb.)

Van Heurck)

(Pinnularia *digito-radiata* Greg.)var. *cyprinus*var. *linearis* (Hust.)var. *rostrata* (Hust.)(N. *digito-radiata* var. *rostrata* Hust.)*debilissima* Grun.*detersa* Grun.*dilucida* Hust.*diploneoides* Hust.*directa* (W. Sm.) Cleve (18)(Pinnularia *directa* W. Sm.)var. *directa*var. *subtilis* Cleve(N. *acutiuscula* Greg.)(Pinnularia *subtilis* Greg.)*diserta* Hust.*distans* (W. Sm.) Cleve (19)(Pinnularia *distans* W. Sm.)*dunstonii* Salah*elegans* W. Sm.var. *elegans*var. *cuspidata* Cleve (20)*favus* Salah*flagellifera* Hust.*flanatica* Grun.*forcipata* Grev.var. *forcipata*var. *densistriata* Schm. (21)var. *punctata* Cleve*formenterae* Cleve*fortis* (Greg.) Ralfs ex Pritch.(Pinnularia *fortis* Greg.)(P. *constricta* O'Meara)*fraudulenta* Schm.*fuchsii* Pant. (22)*gastrum* Ehrenb.var. *gastrum*var. *exigua* Greg.*gelida* Grun.(kariana var. *curta* Cleve)*gotlandica* Grun.*gracilis* var. *schizonemoides* Van Heurck*granii* Jörg.\*(Stauropsis *granii* (Jörg.) Meun.)*granulata* Bail. (23)*gregaria* Donk.*gregorii* Ralfs ex Pritch.*grevillei* (Agardh) Cleve (24)(Schizonema *grevillei* Agardh)var. *grevillei*var. *comoides* Aleem*groschopfi* Hust.*halophila* (Grun.) Cleve(cuspidata var. *halophila* Grun.)*hamulifera* Grun.*henmedyii* W. Sm. (25)var. *henmedyii*var. *manca* Schm.var. *nebulosa* (Greg.) Cleve (26)(N. *nebulosa* Greg.)*humerosa* de Bréb. in W. Sm.var. *humerosa*var. *arabica* Perag.var. *constricta* Clevevar. *minor* Heid.*hungarica* var. *linearis* Östrup*hyalosira* Cleve*incerta* Grun. ex Van Heurck*inflexa* (Greg.) Ralfs ex Pritch.(Pinnularia *inflexa* Greg.)*integra* (Smith) Ralfs(P. *integra* Smith)*jamaicensis* Cleve*kryophila* Cleve*lanceolata* (Agardh) Kütz.var. *lanceolata*var. *arenaria* (Donk.) Cleve(N. *arenaria* Donk.)var. *phyllepta* (Kütz.) Cleve(N. *phyllepta* Kütz.)*latissima* Greg.*libellus* Greg.*litoris* Salah*longa* (Greg.) Ralfs ex Pritch.(P. *longa* Greg.)*lucens* Hust. ex Salah*lunatapicalis* Salah*lyra* Ehrenb. (27)var. *lyra*var. *atlantica* Schm.var. *constricta* Perag.var. *elliptica* Schm.var. *granulata* Perag.var. *subcarinata* Grun.*maculosa* Donk.*margino-nodularis* Salah*marina* Ralfs ex Pritch. (28)

(punctulata W. Sm.)

*mediterranea* Cleve & Brun*membranacea* Cleve\*(Stauropsis *membranacea* (Cleve) Meun.)*mollis* (W. Sm.) Cleve (24)(Schizonema *molle* W. Sm.)*monilifera* Cleve (29)

(granulata de Bréb.)

var. *monilifera*var. *heterosticha* Cleve*moniliformis* Cleve

## NAVICULA (cont.)

- mutica* Kütz.  
*northumbrica* Donk.  
*obsidialis* Hust.  
*octavosignata* Salah  
*ostrearia* Turp. (30)  
 (fusiformis Grun.)  
*palpebralis* de Bréb. in W. Sm. (31)  
 var. *palpebralis*  
 var. *angulosa* (Greg.) Cleve  
 var. *barclayana* (Greg.) Cleve  
 var. *minor* (Greg.) Grun.  
 var. *obtusata* Van Heurck  
 var. *protracta* Perag.  
 var. *robusta* Heid.  
 var. *semiplena* (Greg.) Cleve  
 f. *vahliana* (Grun.) Hust.  
*pavillardii* Hust.  
*pelagica* Cleve\*  
 (*Stauropsis pelagica* (Cleve) Meun.)  
*pennata* Schm.  
*peregrina* (Ehrenb.) Kütz.  
 (*Pinnularia peregrina* Ehrenb.)  
 var. *peregrina*  
 var. *kefvingensis* (Ehrenb.) Cleve  
 var. *meniscus* (Schum.) Cleve  
*plicata* Donk.  
*praetexta* Ehrenb.  
*producta* McCall  
*protracta* Grun.  
*pseudo-bacillum* Grun.  
*pusilla* W. Sm.  
 var. *pusilla*  
 var. *lanceolata* Grun.  
*pygmaea* Kütz.  
*ramosissima* (Agardh) Cleve (24)  
 (*Schizonema ramosissimum* Agardh)  
 var. *ramosissima*  
 var. *amplia* Grun.  
 var. *mucosa* Aleem  
*retusa* de Bréb.  
*rhombica* Greg. (32)  
*rhyncocephala* Kütz.  
 var. *rhyncocephala*  
 var. *amphicerus* (Kütz.) Cleve  
 (*N. amphicerus* Kütz.)  
*salinarum* Grun.  
*salinicola* Hust.  
*scandinavica* Lagerst.  
*scopulorum* de Bréb. ex Kütz.  
 (*Pinnularia johnsonii* W. Sm.)  
 var. *scopulorum*  
 var. *belgica* (Van Heurck) Cleve  
 var. *perlonga* Brun  
*septentrionalis* (Östrup) Cleve\*  
 (*Libellus septentrionalis* Östrup)  
 (*Stauropsis septentrionalis* Meun.)  
*solaris* Greg.  
*spectabilis* Greg.  
*spicula* (Hick.) Cleve  
 (*Stauroneis spicula* Hick.)  
*spuria* Cleve  
*subinflata* Grun.  
*supralittoralis* Aleem & Hust.

- ulvacea* (Berk.) Cleve  
 (*Dickieia ulvacea* Berk.)  
*unilaterarea* Salah  
*vanhöffeni* Gran\*  
 (*Stauropsis vanhöffeni* (Gran) Meun.)  
*viridula* Kütz.  
 var. *viridula*  
 var. *rostellata* Kütz.  
 (*N. rostellata* Kütz.)  
*weissflogi* Schm.  
*zohdyi* Salah  
*zostereti* Grun.  
 OESTRUPA Heiden, 1906. (33)  
*musca* (Greg.) Hust.  
 (*Navicula musca* Greg.)  
 (*Caloneis musca* (Greg.) Cleve)  
 PINNULARIA Ehrenberg, 1843. (3)  
*ambigua* Cleve  
*clavicularis* (Greg.) Cleve  
 (*Navicula clavicularis* Greg.)  
*cruciformis* (Donk.) Cleve  
 (*N. cruciformis* Donk.)  
*ergadensis* Greg.  
 (*Navicula blanda* Schm.)  
 (*Caloneis blanda* (Schm.) Cleve)  
 var. *ergadensis*  
 var. *minor* (Perag.)  
 (*N. blanda* var. *minor* Perag.)  
*fritschii* Salah  
 var. *fritschii*  
 var. *lata* Salah  
*quadratarea* (Schm.) Cleve  
 (*Navicula quadratarea* Schm.)  
 (*N. pinnularia* Cleve)  
 var. *quadratarea*  
 var. *subproducta* (Grun.) Cleve  
 (*N. pinnularia* var. *subproducta*  
 Grun.)  
*rectagulata* (Greg.) Cleve  
 var. *rectangulata*  
 var. *subundulata* Grun.  
*stauntonii* (Grun.) Cleve  
 (*Alloioneis stauntonii* Grun.)  
*trevelyana* (Donk.) Rab.  
 (*Navicula trevelyana* Donk.)  
 PLEUROSIGMA W. Smith, 1852. (34)  
*acuminatum* var. *brebissonii* (Grun.)  
 Mills  
*aestuarii* (de Bréb. ex Kütz.) W. Sm.  
 (*Navicula aestuarii* de Bréb. ex Kütz.)  
*affine* Grun.  
 (*normanii* Ralfs ex Pritch.)  
 var. *affine*  
 var. *fossile* Grun.  
*angulatum* (Quekett) W. Sm. (35)  
 (*Navicula angulata* Quekett)  
 var. *angulatum*  
 var. *finmarchica* Cleve  
 var. *quadratum* (W. Sm.) Cleve  
 (*P. quadratum* W. Sm.)  
 var. *robustum* McCall  
*attenuatum* (Kütz.) W. Sm.  
 (*Frustulia attenuata* Kütz.)  
 var. *attenuatum*

## PLEUROSIGMA (cont.)

- attenuatum* (cont.)  
 var. *scalprum* (Gaill. & Turp.) Cleve  
 (*Navicula scalprum* Gaill. & Turp.)  
*balticum* (Ehrenb.) W. Sm.  
 (*Navicula baltica* Ehrenb.)  
 (*Gyrosigma balticum* (Ehrenb.) Rab.)  
*cuspidatum* Cleve  
*decorum* W. Sm.  
*delicatulum* W. Sm.  
 var. *delicatulum*  
 var. *gracile* McCall  
*diminutum* Grun.  
 (*Gyrosigma diminutum* (Grun.) Cleve)  
 var. *diminutum*  
 var. *constrictum* Grun.  
 (*P. reversum* Greg.)  
*distortum* W. Sm.  
 (*Gyrosigma distortum* (W. Sm.) Cleve)  
 var. *distortum*  
 var. *undulatum* McCall  
*elongatum* W. Sm.  
*eximium* (Thwaites) Cleve  
 (*Colletonema eximia* Thwaites)  
 (*Gyrosigma eximium* (Thwaites) Boyer)  
*fasciola* (Ehrenb.) W. Sm.  
 (*Ceratonis fasciola* Ehrenb.)  
 var. *fasciola*  
 var. *closterioides* (Grun.) Perag.  
 var. *prolongatum* (W. Sm.) Grun. ex  
 Van Heurck  
 var. *sulcatum* Grun.  
*formosum* W. Sm.  
 (*australicum* Witt)  
 (*tahitense* Witt)  
*hippocampus* (Ehrenb.) W. Sm.  
 (*Navicula hippocampus* Ehrenb.)  
 (*Gyrosigma hippocampus* Hass.)  
*intermedium* W. Sm.  
 var. *intermedium*  
 var. *nubecula* (W. Sm.) Grun. ex Van  
 Heurck  
 (*P. nubecula* W. Sm.)  
*lanceolatum* Donk.  
 (*transversale* Roper)  
*litorale* W. Sm.  
*longum* var. *subrigidum* (Grun.) Perag.  
*macrum* W. Sm.  
 (*Gyrosigma macrum* (W. Sm.) Cleve)  
*marinum* Donk.  
*maroccanum* Cleve (36)  
 (*Rhoicosigma maroccanum* Perag.)  
*naviculaceum* de Bréb.  
 (*japonicum* Castr.)  
 (*transversale* W. Sm.)  
*normani* Ralfs (37)  
*obliquum* Grun. in Cleve & Grun.  
 (*Gyrosigma obliquum* (Grun.) Boyer)  
*obscurum* W. Sm.  
 (*macilentum* Perag.)  
*parkerii* Har.  
 (*Gyrosigma parkerii* (Har.) Cleve)  
*rectum* Donk.  
*rigidum* W. Sm.

- var. *rigidum*  
 var. *giganteum* (Grun.) Cleve  
 (*P. giganteum* Grun.)  
*rhombeum* Grun. in Cleve & Grun.  
*speciosum* W. Sm.  
*spencerii* W. Sm.  
 (*Gyrosigma spencerii* (W. Sm.) Cleve)  
 var. *spencerii*  
 var. *exile* Grun.  
 var. *smithii* Grun. in Cleve & Grun.  
*strigilis* W. Sm.  
 (*Gyrosigma strigilis* (W. Sm.) Cleve)  
*strigosum* W. Sm. (38)  
 (*angulatum* var. *strigosum* Cleve)  
*subhyalinum* Hust. & Aleem  
*sulcatum* Grun.  
*tenuissimum* W. Sm.  
 (*Gyrosigma tenuissimum* (W. Sm.)  
 Cleve)  
*wansbeckii* Donk.  
 (*balticum* var. *wansbeckii* (Donk.) Van  
 Heurck)  
 SCOLIOPLEURA Grunow, 1860.  
*tumida* (de Bréb. ex Kütz.) Rab.  
 (*Navicula jemmerii* W. Sm.)  
 (*N. tumida* (de Bréb.) Cleve)  
 var. *tumida*  
 var. *adriatica* (Grun.) Cleve  
*westii* (W. Sm.) Grun.  
 (*N. westii* W. Sm.)  
 SCOLIOTROPIS Cleve, 1894.  
*latestriata* (de Bréb. ex Kütz.) Cleve  
 (39)  
 (*Amphipora latestriata* de Bréb. ex  
 Kütz.)  
 (*Navicula convexa* W. Sm.)  
 STAURONEIS Ehrenberg, 1843. (40)  
*acuta* W. Sm.  
 (*kochii* Pant.)  
*africana* var. *acuminata* Grun.  
*amphioxys* Greg. (41)  
 (*gregorii* Ralfs ex Pritch.)  
*amphorooides* Grun.  
*salina* W. Sm.  
*septentrionalis* Grun.  
 STENONEIS Cleve, 1894. (42)  
*inconspicua* (Greg.) Cleve  
 (*Navicula inconspicua* Greg.)  
 (*N. fistula* Schm.)  
 TOXONIDEA Donkin, 1858. (43)  
*gregoryana* Donk.  
*insignis* Donk.  
 var. *insignis*  
 var. *undulata* Norm.  
 TRACHYNEIS Cleve, 1894. (3)  
*aspera* (Ehrenb.) Cleve  
 (*Navicula aspera* (Ehrenb.) Donk.)  
 (*Stauroptera aspera* Ehrenb.)  
 var. *aspera*  
 var. *intermedia* (Grun.) Cleve  
 var. *pulchella* (W. Sm.) Cleve  
 (*Stauroptera pulchella* W. Sm.)  
 (*S. pygmaea* Castr.)  
*clepsydra* (Donk.) Cleve



## TRACHYNEIS (cont.)

*clepsydra* (cont.)*(Navicula clepsydra* Donk.)var. *clepsydra*var. *scotica* Schm.

## TROPIDONEIS Cleve, 1891.

*elegans* (W. Sm.) Cleve*(Amphiprora elegans* W. Sm.)*gibberula* Grun.*lepidoptera* (Greg.) Cleve*(A. lepidoptera* Greg.)*maxima* (Greg.) Cleve*(A. maxima* Greg.)var. *maxima*var. *dubia* (Cleve & Grun.) Cleve*(A. maxima* var. *dubia* Cleve & Grun.)*pusilla* (Greg.) Cleve*(A. pusilla* Greg.)*recta* (Greg.) Cleve*(A. recta* Greg.)*vanheurckii* (Grun.) Cleve*(Plagiotropis vanheurckii* Grun.)*vitrea* (W. Sm.) Cleve*(Amphiprora vitrea* W. Sm.)*(Plagiotropis vitrea* Grun.)

## Cymbellaceae

AMPHORA Ehrenberg, 1831.

*acuta* Greg.*acutiscula* Kütz.*(lineata* Greg.)*angularis* Greg.*angusta* Greg.*arcus* Greg. (44)*arenaria* Donk.var. *arenaria*var. *donkii* Rab.var. *rattrayi* Cleve*arenicola* Grun.var. *arenicola*var. *minor* McCall*bacillaris* Greg. (45)*binodis* Greg.*coffeaeformis* (Agardh) Kütz.*(aponina* Kütz.)*(Frustulia coffeaeformis* Agardh)var. *coffeaeformis*var. *borealis* (Kütz.) Cleve*(A. borealis* Kütz.)var. *perpusilla* (Grun.) Cleve*costata* W. Sm. (46)*crassa* Greg. (47)*cymbifera* Greg. (48)*decussata* Grun.*dubia* Greg. (49)*elliptica* (Agardh) Kütz.*ergadensis* Greg. (50)*eunotia* Cleve*(cymbifera* Cleve)*excisa* Greg. (51)*exigua* Greg. (52)*fluminensis* Grun. (53)*graeffi* (Grun.) Cleve (54)var. *graeffi*var. *minor* Perag.*granulata* Greg.*grevilleana* Greg.*(fasciata* Greg.)*(complexa* Greg.)*(sulcata* Greg.) ?*hyalina* Kütz.*(hemisphaerica* Grun.)*laevis* Greg.*(nobilis* Flögel)*laevissima* Greg. (55)*lineolata* Ehrenb. (56)*lyrata* Greg. (57)*macilenta* Greg.*marina* W. Sm. (58)*membranacea* W. Sm. (59)*milesiana* Greg. (60)*monilifera* Greg. (61)*nobilis* Greg. (62)*oblonga* Greg. (63)*obtusa* Greg. (64)var. *obtusa*var. *rectangularis* Perag.*ocellata* Donk.var. *ocellata*var. *singulata* Cleve*ostrearia* de Bréb. (65)*(littoralis* Donk.)var. *ostrearia*var. *lineata* Clevevar. *vitrea* Cleve*ovalis* Kütz.*(affinis* Kütz.)*(lybica* (Ehrenb.)*(ovalis* var. *lybica* (Ehrenb.) Cleve)*(Navicula amphora* Ehrenb.)*proboscidea* Greg. (66)*proteus* Greg.*(hexagonalis* Witt) ?*(speciosa* Castr.) ?var. *proteus*var. *oculata* Perag.*pusilla* Greg. (67)*pusio* Cleve*quadrata* Greg. (68)*rhombica* Kitt.*robusta* Greg. (69)*salina* W. Sm. (70)*spectabilis* Greg.*sulcata* de Bréb. (71)*tenera* W. Sm. (72)*tenerrima* Aleem & Hust.*turgida* Greg. (53)*valida* Perag.*veneta* Kütz.*ventricosa* Greg.

OKEDENIA Eulenstein ex De Toni, 1891.

(73)

*inflexa* (de Bréb.) De Toni*(Amphipleura inflexa* de Bréb. ex

Kütz.)

*(Amphora inflexa* (de Bréb.) Cleve)

## Gomphonemaceae

GOMPHONEMA Husted in Pascher, 1930.

*exiguum* Kütz.

**Epithemiaceae**

- EPITHEMIA de Brébisson, 1844.  
*adnata* var. *proboscidea* (Grun.)  
 (*zebra* var. *proboscidea* Kütz.)  
*sorex* Kütz.  
*turgida* var. *westermanii* (Ehrenb.) Grun.  
 RHOPALODIA O. Müller, 1895.  
*gibba* (Ehrenb.) Müll.  
 (*Navicula gibba* Ehrenb.)  
 (*Epithemia gibba* Kütz.)  
 var. *gibba*  
 var. *ventricosa* (Kütz.) Grun.  
*gibberula* var. *producta* (Grun.) Müll.  
*musculus* (Kütz.) Müll.  
 (*Epithemia musculus* Kütz.)  
 var. *musculus*  
 var. *constricta* (de Bréb.) Müll.

**Bacillariaceae**

- BACILLARIA Gmelin, 1778. (74)  
*paxillifer* (Müll.) Hendey  
 (*paradoxa* Gmelin)  
 (*Nitzschia paxillifer* (Müll.) Heib.)  
 (*Vibrio paxillifer* Müll.)  
*socialis* Ralfs  
 (*Nitzschia socialis* Greg.)  
 var. *socialis*  
 var. *baltica* Grun.  
 CYLINDROTHECA Rabenhorst, 1859. (75)  
*gracilis* (de Bréb.) Grun.  
 HANTZSCHIA Grunow in Cleve &  
 Grunow, 1880. (76)  
*amphioxys* var. *minor* Perag.  
*hyalina* Grun.  
*marina* (Donk.) Grun. in Cleve & Grun.  
*rigida* McCall  
*virgata* (Roper) Grun.  
 var. *virgata*  
 var. *gracilis* Hust.  
 NITZSCHIA Hassall, 1845. (77)  
*acicularis* (Kütz.) W. Sm.  
 (*Synedra acicularis* Kütz.)  
*acuminata* (W. Sm.) Cleve in Cleve &  
 Grun.  
 (*Tryblionella acuminata* W. Sm.)  
 var. *acuminata*  
 var. *subconstricta* Grun.  
*aequorea* Hust.  
*affinis* Grun.  
*amphibia* Grun.  
*angularis* W. Sm.  
*apiculata* (Greg.) Grun. in Cleve &  
 Grun.  
 (*Tryblionella apiculata* Greg.)  
*bilobata* W. Sm.  
 var. *bilobata*  
 var. *minor* Grun.  
*calcicola* Aleem & Hust.  
*calida* var. *salinarum* (Grun.) Freng.  
 (*N. tryblionella* var. *salinarum*  
 Grun.)  
*capitellata* Hust.  
*circumsuta* (Bail.) Grun. in Cleve &  
 Grun.  
 (*Surirella circumsuta* Bail.)  
*clausii* Hantzsch  
*closterium* (Ehrenb.) W. Sm.\* (78)  
 (*Ceratoneis closterium* Ehrenb.)  
*commutata* Grun.  
*constricta* Ralfs ex Pritch.  
 var. *constricta*  
 var. *subconstricta* Grun.  
*debilis* (Arnott) Grun.  
 (*Tryblionella debilis* Arnott)  
*delicatissima* Cleve\*  
 (*Pseudonitzschia delicatissima* (Cleve)  
 Heiden)  
*denticula* var. *delognei* Grun. ex Van  
 Heurck  
*distans* Greg.  
*dubia* W. Sm.  
*dubiformis* Hust.  
*epithemioides* Grun. in Cleve & Grun.  
*filiformis* (W. Sm.) Schütt  
 (*Homoeocladia filiformis* W. Sm.)  
*fonticola* Grun.  
*frigida* Grun. in Cleve & Grun.  
*frustulum* (Kütz.) Grun. in Cleve & Grun.  
 (*Synedra frustulum* Kütz.)  
*gotlandica* A. Cleve-Euler  
*granulata* Grun. in Cleve & Grun.  
*habirshawii* Feb.  
*hungarica* Grun.  
*hustedtiana* Salah  
*hybrida* Grun.  
*incurva* Grun. in Schneider  
*insignis* Greg.  
 var. *insignis*  
 var. *smithii* (Ralfs) Pell.  
 (*N. spectabilis* W. Sm.)  
*irregularis* Ross & Abidin  
*laevis* Hust.  
*lanceolata* W. Sm.  
 var. *lanceolata*  
 var. *incrustans* Grun.  
*linkei* Hust.  
*littoralis* Grun. in Cleve & Grun.  
 var. *littoralis*  
 var. *slesvicensis* Grun.  
*longissima* (de Bréb.) Ralfs ex Pritch.  
 (*birostrata* W. Sm.)  
 (*Ceratoneis longissima* de Bréb.)  
*lorenziana* Grun.  
 var. *lorenziana*  
 var. *subtilis* Grun.  
*macilenta* Greg.  
*marginulata* var. *didyma* Grun. in Cleve  
 & Grun.  
*marina* Grun. in Cleve & Grun.  
*martiana* (Agardh) Schütt  
 (*Homoeocladia martiana* Agardh)  
*microcephala* Grun. in Cleve & Grun.  
*navicularis* Grun. in Cleve & Grun.  
 (*Surirella navicularis* de Bréb.)  
 (*Tryblionella marginata* W. Sm.)  
*obtusa* W. Sm.  
 var. *obtusa* [Grun.  
 var. *scabelliformis* Grun. in Cleve &

## NITZSCHIA (cont.)

*panduriformis* Greg.var. *panduriformis*var. *minor* Grun.*paravasa* Grun.*parvula* W. Sm.*petitiana* Grun. ex Van Heurck*plana* W. Sm.*polaris* Grun.*punctata* (W. Sm.) Grun.var. *punctata*var. *coarctata* Grun.*pungens* Grun.*reversa* W. Sm. (78)*salinicola* Aleem & Hust.*seriata* Cleve\*(Pseudonitzschia *seriata* (Cleve) Perag.)*sigma* (Kütz.) W. Sm.(Synedra *sigma* Kütz.)var. *sigma*var. *intercedens* Grun.var. *rigida* Grun.var. *rigidula* Grun.var. *sigmatella* Grun.*sigmoidea* (Nitzsch) W. Sm.(Bacillaria *sigmoidea* Nitzsch)(Navicula *sigmoidea* Ehrenb.)*spathulata* de Bréb. ex W. Sm.var. *spathulata*var. *hyalina* (Greg.) Grun. in Van

Heurck

*spectabilis* (Ehrenb.) Ralfs

(brebissonii W. Sm.)

*subcapitellata* Hust.*subfrustum* Hust.*tenuissima* Perag.*thermalis* var. *littoralis* Grun.var. *minor* Hilse*tryblionella* Hantz.var. *tryblionella*var. *levidensis* (W. Sm.) Grun.var. *maxima* Grun.var. *recta* McCall*valdestriata* Aleem & Hust.*vasta* Hust.*vivax* W. Sm.

## Notes on Biraphidineae

- (1) The Naviculaceae is the largest family of diatoms and the genera recognized here follow the review of the naviculoid diatoms by Cleve (1894-5). Cleve instituted several new genera and recognized older genera created by Ehrenberg in order to reduce a very large genus to a more manageable size. In the following year Van Heurck (1896) ignored Cleve's work, and absorbed Cleve's genera into the genus *Navicula* Bory, which he divided into twenty-two groups. De Toni (1891-4) and Mann (1907) to a large degree followed Van Heurck, but most modern taxonomists follow Cleve.
- (2) Mann (1907) includes in *Frustulia* Agardh. Van Heurck and De Toni consider it a separate genus.
- (3) Included in *Navicula* by Van Heurck, see (1) above. The generic name *Pinnularia* Ehrenb., 1843, was accepted for conservation against *Pinnularia* Lindl. & Hutt., 1833, and *Stauroptera* Ehrenb., *Ber. Akad. Wiss., Berl.*, 1843, p. 45, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 71).
- (4) Seldom found in large quantities. A critical review of the genus is required to define specific differences and geographical ranges. The generic name *Auricula* Castracane, 1873, was accepted for conservation against *Auricula* Spach, 1840, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 70).
- (5) Recently observed in Chichester Harbour (Hendey, 1951), but originally described from a fossil deposit in Japan.
- (6) Appears to be more common along the north and west than other coasts of Great Britain. The name *Brebissonia* Grunow was accepted for conservation against *Brebissonia* Spach, 1835, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 70).
- (7) It is accepted to-day by all diatomists that *Diploneis* Ehrenb. is sufficiently distinct to be recognized as a separate genus, despite the fact that Van Heurck and Mann included it in *Navicula* Ehrenb. However, a certain amount of confusion exists in the genus, largely because so many of the species were described in early literature and, by modern standards, poorly illustrated. Many of Ehrenberg's species are unidentifiable and the synonymy is complicated. Most of the species vary greatly in size and appearance, and this has led to an unwarranted multiplicity of names. The genus requires a critical review and the distribution of each species defined.
- (8) The sigmoid diatoms are dealt with here after the manner of Peragallo in 'Monographie du Genre *Pleurosigma*' published in *Le Diatomiste* (1890-1). In *Donkinia* the raphe is keeled above the valve surface and usually strongly sigmoid, while the valve outline is seldom sigmoid or only weakly so.
- (9) Usually recognized as a fresh-water species, but this variety is found not infrequently in fully saline waters.

- (10) The genus *Mastogloia* is recognized by most diatomists to-day, although Mann (1907) grouped it under *Navicula*. It is distinguished from the latter by the presence of a loculiferous rim which is attached to the inside of the girdle. This internal system often becomes detached during cleaning operations and the valves without it differ in no way from those of *Navicula*. The genus is not well represented in British waters, and further research is needed on the distribution of the species.
- (11) Common around the west coasts of Britain, and usually considered distinct from *N. lyra*.
- (12) *N. approximata* Greville is sharply separated from other members of the Naviculaceae Lyratae by the foreshortening of the median striae around the raphe at the central nodule. This character is clearly shown in Peragallo's figure of *N. hennedyii* var. *niceaensis* (*Diat. Mar. France*, 1897-1908, pl. 24, fig. 19, 15).
- (13) Connected by intermediate forms to *N. viridula* Kütz.
- (14) Connected by intermediate forms to *N. lyra* Ehrenb.
- (15) Cleve makes this a variety of *N. punctulata* W. Sm.
- (16) Doubtful species.
- (17) The shape of the valve apex and the gibbous sides of this organism are very variable characters, and much confusion in the synonymy has arisen by creating varietal names to accommodate the differences seen. Most of these are here included under the earliest valid name of *N. cyprinus* (*Pinnularia cyprinus* Ehrenb., 1842).
- (18) A very variable species, the exact limits of which should be more accurately defined.
- (19) This species is a cold-water form, and the southerly limits of its distribution require defining.
- (20) A large gathering of *N. elegans* shows much variation of the valve apices, and var. *cuspidata* with rostrate apices is often linked to the type by a series of intermediate forms.
- (21) Usually more finely striate than the type, but the var. is of doubtful value.
- (22) A small form of this species (about half the size of specimens found on American coasts) was found at Anglesey. This is the first record for the British Isles. It is closely allied to *N. humerosa* de Bréb.
- (23) Owing to Bailey's poor illustration, this species frequently has been confused with *N. brasiliensis* Grun. and other allied species. *N. granulata* Bail. has rounded apices and interrupted striation. It is probably a cold-water form.
- (24) This species lives in large frondose colonies of mucous tubes in conditions of reduced salinity.
- (25) A very variable species, under which has been gathered a host of forms in which the marginal striae vary greatly in width. Smith did not publish a figure, but in his type specimen the marginal striate portion is less wide than the lateral area. Specimens from British coasts usually conform very well to the type.
- (26) It is possible that this should be regarded as a distinct species.
- (27) The type specimen was described from washings of seaweeds from the Falkland Islands in 1843, and since that time over thirty varietal names have been published. Some authors refuse to accept these names and refer all varieties to the type. The species is very variable, and a competent monograph on it is badly needed. It is not numerous around British coasts and, although the varieties listed are fairly readily recognized, their inclusion here is tentative.
- (28) First described by Wm. Smith in 1853 as *N. punctulata*, a name previously used by Ehrenberg in 1842 to describe another species.
- (29) First described by de Brébisson as *N. granulata*, a name previously used by Bailey in 1854 to describe another species.
- (30) This species is commonly found where oysters are cultured. The cytoplasm towards the apices of the cell frequently assumes a blue colour.
- (31) A widely distributed species, showing many varieties of somewhat doubtful value. More information required on distribution and ecology.
- (32) Should be compared with *N. grevillei* (Agardh) Cleve.
- (33) The genus *Oestrupia* Heiden, based upon *Caloneis powelli* (Lewis) Cleve, is connected to *Pinnularia* Ehrenberg and *Caloneis* Cleve. Hustedt (1935) compares and contrasts the valve structure of these genera, and shows that the transverse costae of *Oestrupia* are interrupted by a longitudinal siliceous band upon either side of the raphe.
- (34) The genus *Pleurosigma* W. Sm. here includes species whose valves have striae crossing at right angles as well as those crossing obliquely. Some authorities refer the former to *Gyrosigma* Hassall; no useful purpose is however served by this. The generic name *Pleurosigma* W. Sm. was accepted for conservation against *Scalprum* Corda, 1835, *Gyrosigma* Hassall, 1845, and *Endosigma* de Bréb. ex D'Orb., 1849, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).

- (35) All varieties of this species are of doubtful value, as intermediate forms connect them, producing a continuous series.
- (36) A doubtful species.
- (37) Probably *P. affine* Grun.
- (38) Electron micrographs of this species show that its ultimate structure is entirely different from that of *P. angulatum*, and that Cleve was in error in relating them.
- (39) More information is required about the distribution of this species.
- (40) *Stauroneis* Ehrenberg was included in *Navicula* Ehrenberg by Van Heurck and Mann but most modern taxonomists separate them.
- (41) Gregory's epithet must replace the later one of Ralfs which has been normally used for this species.
- (42) In *Stenoneis* Cleve the raphe is enclosed between two stout ribs. One species is represented in the British flora. More information is required concerning its distribution.
- (43) Included by some authorities in *Pleurosigma* but separated here on account of its bow-shaped raphe.
- (44) Several varieties of this species have been described but intermediate forms appear to connect them. The published figures are of poor quality and more information is required concerning the whole group.
- (45) The description provided by Gregory is not precise and the species requires a more accurate examination.
- (46) This may be identical with *A. monilifera* Greg.
- (47) Several varieties of this species are known but intermediate forms connect them. More information is required.
- (48) Cleve (1895) makes this a synonym of *A. terroris* Ehrenberg.
- (49) Doubtful species.
- (50) Cleve (1895) makes this species a variety of *A. macilenta*, but the identification from the figures provided is doubtful. This species is akin to *A. acutiuscula* but its striae are coarser.
- (51) This may be the same as, or related to, *A. laevis*.
- (52) Gregory's figure does not admit of trustworthy identification. This species is related to *A. acutiuscula*.
- (53) According to Cleve (1895) *A. fluminensis* Grun. agrees with *A. turgida* Greg.
- (54) This species is probably the same as *A. grevilleana* but the striae are closer and not distinctly punctate.
- (55) Considered by some authorities to be a variety of *A. laevis*.
- (56) There seems to be some doubt as to the identity of this species. The figures provided by Ehrenberg and Kützing are not very satisfactory. *A. lineolata* in Donkin represents another species.
- (57) This may be identical with *A. cuneata* Cleve.
- (58) A doubtful species, probably a form of *A. proteus*.
- (59) Probably the same as *A. ostrearia* de Bréb.
- (60) This species is closely allied to *A. exsecta* Grun.
- (61) Probably identical with *A. costata*.
- (62) This may be the same as *A. acuta*.
- (63) Seems to be a form of *A. robusta*. More work is required on this.
- (64) Very variable species. More work is required on the distribution of this species and its variety.
- (65) The varieties at first sight appear very dissimilar, but intermediate forms connect them to the type to such a degree that some authorities think they should be united.
- (66) Cleve (1895) considers this nearly akin to *A. graeffi*.
- (67) A doubtful species, very similar to *A. bacillaris* Greg.
- (68) Doubtful species, allied to *A. truncata* Greg.
- (69) Related to *A. proteus* but differs from it principally by coarser striae and larger size.
- (70) May be the same as *A. coffaeiformis*.
- (71) A doubtful species, probably connected to *A. elongata* Greg.
- (72) Cleve (1895) considers this may be a small form of *A. lineolata*. More information is required on this.
- (73) The generic name was originally attached to specimens by Eulenstein, and did not receive valid publication until 1891 when it appeared in De Toni's *Sylloge Algarum*. Cleve included *Okedenia inflexa* in *Amphora* but the organism bears no relation to that genus. More information is required on its distribution.
- (74) The genus *Bacillaria* has been accepted by most taxonomists because of the characteristic mode of colony formation adopted by the type species *B. paxillifer*. The cells join together by their valve faces to form packets and move by sliding one on the other



somewhat like a slide rule. As colony formation and methods of movement are no longer considered as valid characters on which to base genera, *Bacillaria* is retained here on account of the symmetry of the frustule. It is distinguished from the genus *Nitzschia* Hassall in that the raphe is central and in the apical axis of the valve, whereas in *Nitzschia* it is marginal and the two raphes are placed diagonally upon the frustule.

- (75) Not commonly found in marine waters. A spindle-shaped nitzschoid diatom in which the frustule is twisted about the apical axis so that the raphes appear helical.
- (76) The genus *Hantzschia* is separated from *Nitzschia* because the raphe-bearing keels occupy adjacent margins of the frustule (see note 74).
- (77) *Nitzschia* Hassall (1845) is an absolute synonym of *Sigmatella* Kützing (1833), as both genera were based on *Bacillaria sigmoidea* Nitzsch. The genus *Nitzschia* is accepted here in the sense that most modern taxonomists have used it, that is, in the sense that Wm. Smith (1853) used it taking *N. sigmoidea* Wm. Smith based on *Sigmatella nitzschii* Kützing, which was *Bacillaria sigmoidea* Nitzsch, as the type of the genus. Grunow in Cleve & Grunow (1880) divided the genus into twenty-four groups, most of which are generally accepted. British material requires much more detailed examination and revision. The generic name *Nitzschia* was accepted for conservation at the 7th Int. bot. Congr., Stockholm (*Int. Code bot. nomencl.*, 1952, p. 71).
- (78) Some authorities make this a variety of *Nitzschia longissima*. The group is a difficult one as the diatoms are not strongly siliceous. More information is required on the British material.

#### Suborder 10. SURIRELLINEAE

##### Surirellaceae (1)

CAMPYLODISCUS Ehrenberg, 1841.

- bicostatus* W. Sm.  
*clypeus* Ehrenb. (2)  
 (*ovatus* Ralfs)  
 (*Cocconeis clypeus* Ehrenb.)  
 (*Surirella clypeus* Ehrenb.)  
*decorus* de Bréb.  
*echineis* Ehrenb. (2)  
 (*argus* Bail.)  
 (*cribrosus* W. Sm.)  
 (*Coronia echineis* Ehrenb.)

*fastuosus* Ehrenb.  
 (*parvulus* W. Sm.)  
 (*thuretii* de Bréb.)

*hodgsonii* W. Sm.  
 (*eximius* Greg.)

*hypodromus* Brun & Temp.  
 (*gregoryii* Perag.)

*innominatus* Ross & Abdin  
*ralfsii* W. Sm.

PODOCYSTIS Bailey ex Wm. Smith, 1856. (3)

- adriatica* (Kütz.) Boyer  
 (*americana* Bail.)  
 (*Surirella adriatica* Kütz.)  
 (*Euphyllodium spathalatum* Shad.)

SURIRELLA Turpin 1828. (4)

- caspia* Brun  
 (*cardaria* Brockm.)  
*constricta* Ehrenb.  
*crumena* de Bréb. ex Kütz.  
 (*brightwellii* W. Sm.)  
*fastuosa* Ehrenb.  
 (*hohenackerii* Rab.)  
 (*cuneata* Schm.)  
 var. *fastuosa*  
 var. *cuneata* Witt.  
*gemma* (Ehrenb.) Kütz.  
*hispida* Ross & Abdin  
*lata* W. Sm. (5)  
 (*hybrida* Grun.)  
*minima* Ross & Abdin  
*ovalis* de Bréb.  
*ovata* Kütz.  
 var. *ovata*  
 var. *salina* (W. Sm.) Hust.  
*pyriformis* Kitt.  
*striatula* Turp. (6)  
 (*Navicula striatula* (Turp.) Ehrenb.)  
*strigosa* Hust.  
*subsalsa* W. Sm.

#### Notes on Surirellineae

- (1) Some authorities regard the genera described here as belonging to the Biraphidineae, but the type of raphe and its peripheral position on the valve separate them most definitely from those genera that possess a single raphe in the axial area of each valve.
- (2) Not abundant in British waters, more information is required concerning distribution and ecology.
- (3) This genus is sometimes ascribed to Kützing (Kützing, 1844, p. 62), but the name was not validly published there. The generic name *Podocystis* Bailey ex W. Smith was accepted for conservation against *Euphyllodium* Shadbolt, 1854, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).
- (4) The smaller species of this genus lack precise descriptions, and it is likely that a thorough review of the group would considerably reduce the number of names now in use.
- (5) Some authorities consider this to be identical with *Surirella fastuosa*.
- (6) This species is very variable and several worthless varieties have been made.

## NEW COMBINATIONS

During the course of this work it became necessary to make a number of new combinations. In order to validate them they are set out below together with synonyms and references.

*Actinocyclus octonarius* var. *crassus* (W. Smith) Henvey, *comb.nov.*

*Eupodiscus crassus* W. Smith, *Syn. Brit. Diat.*, 1, 24 (1853).

*Actinocyclus crassus* (W. Smith) Ralfs, in Pritchard, *Hist. Infus.*, ed. 4, 835 (1861).

*Actinocyclus ehrenbergii* var. *crassus* (W. Smith) Hustedt, in Rabenhorst, *Krypt.-Flora*, 7 (1), 529 (1929).

*Actinocyclus octonarius* var. *ralfsii* (W. Smith) Henvey, *comb.nov.*

*Eupodiscus ralfsii* W. Smith, *Syn. Brit. Diat.*, 2, 86 (1856).

*Actinocyclus ralfsii* (W. Smith) Ralfs, in Pritchard, *Hist. Infus.*, ed. 4, 835 (1861).

*Actinocyclus ehrenbergii* var. *ralfsii* (W. Smith) Hustedt, in Rabenhorst, *Krypt.-Flora*, 7 (1), 528 (1929).

*Actinocyclus octonarius* var. *sparsus* (Gregory) Henvey, *comb.nov.*

*Eupodiscus sparsus* Gregory, in *Trans. micr. Soc. Lond.*, N.S. 5, 81 (1857).

*Actinocyclus ralfsii* var. *sparsus* (Gregory) Ralfs, in Pritchard, *l.c.*: 835 (1861).

*Actinocyclus sparsus* (Gregory) Rattray, in *J. Quekett micr. Cl.*, ser. 2, 4, 170 (1890).

*Actinocyclus ehrenbergii* var. *sparsus* (Gregory) Hustedt, in Rabenhorst, *Krypt.-Flora*, 7 (1), 528 (1929).

*Actinocyclus octonarius* var. *tenellus* (Brébisson) Henvey, *comb.nov.*

*Eupodiscus tenellus* Brébisson, in *Mém. Soc. Sci. nat. Cherbourg*, 2, 257 (1854).

*Actinocyclus tenellus* (Brébisson) Grunow, in *Hedwigia*, 6, 31 (1867).

*Actinocyclus ehrenbergii* var. *tenellus* (Brébisson) Hustedt, in Rabenhorst, *Krypt.-Flora*, 7 (1), 530 (1929).

*Navicula cyprinus* var. *linearis* (Hustedt) Henvey, *var.nov.*

*Navicula digito-radiata* f. *linearis* Hustedt, in *Abh. naturw. Ver. Bremen*, 31, 626 (1939).

*Navicula cyprinus* var. *rostrata* (Hustedt) Henvey, *comb.nov.*

*Navicula digito-radiata* var. *rostrata* Hustedt, in *Abh. naturw. Ver. Bremen*, 31, 627 (1939).

*Epithemia adnata* var. *proboscidea* (Kützing) Henvey, *comb.nov.*

*Epithemia proboscidea* Kützing, *Die kies. Bacill.*, 35 (1844).

*Epithemia zebra* var. *proboscidea* (Kützing) Grunow, in *Verh. zool.-bot. Ges. Wien*, 12, 329 (1862), 'proboscoidea'.

## ACKNOWLEDGMENTS

I am much indebted to the British Phycological Society for inviting me to undertake this work, and to numerous persons who have been kind enough to send me records of species. Particularly I wish to thank Dr C. L. Odam, Rev. R. Fraser Bastow, and Mr H. G. Barber for lists of species found in Wales and the west country. My especial thanks are due to Mr R. Ross of the British Museum (Natural History) for making available to me his unpublished work on the diatoms of Loch Sween which added numerous new

records to the flora, and for undertaking the onerous task of checking the proofs. I am indebted also to Dr Mary Parke, without whose encouragement this list never would have been completed.

#### ABBREVIATIONS OF AUTHORS' NAMES

Bail.	J. W. Bailey	Jörg.	E. G. Jørgensen
Bail. L. W.	L. W. Bailey	Kitt.	F. Kitton
Berg.	P. Bergon	Kütz.	F. T. Kützing
Berk.	M. J. Berkeley	Lagerst.	N. G. W. Lagerstedt
de Bréb.	A. de Brébisson	Laud.	H. S. Lauder
Brightw.	T. Brightwell	Lemm.	E. Lemmermann
Brockm.	C. Brockmann	Lindl.	J. Lindley
Carm.	D. Carmichael	Lyngb.	H. C. Lyngbye
Castr.	A. F. Castracane	Meun.	A. Meunier
Desmaz.	J. H. B. J. Desmazières	Müll.	O. Müller
Dick.	G. Dickie	Norm.	G. Norman
Dill.	L. W. Dillwyn	Ostenf.	C. H. Ostefeld
Donk.	A. S. Donkin	Pant.	J. Pantocsek
D'Orb.	A. D'Orbigny	Pav.	J. Pavillard
Ehrenb.	C. G. Ehrenberg	Pell.	J. Pelletan
Eulen.	T. Eulenstein	Perag.	H. Peragallo
Feb.	H. Febiger	Pritch.	A. Pritchard
Freng.	J. Frenguelli	Rab.	L. Rabenhorst
Gaill.	M. Gaillon	Ratt.	J. Rattray
Greg.	W. Gregory	Schimp.	A. J. W. Schimper
Grev.	R. K. Greville	Schm.	A. Schmidt
Grun.	A. Grunow	Schm. M.	M. Schmidt
Har.	R. Harrison	Schum.	J. Schumann
Hass.	A. H. Hassall	Shadb.	G. Shadbolt
Heib.	P. A. C. Heiberg	Shrubs.	W. H. Shrubsole
Heid.	H. Heiden	Smith	J. E. Smith
Hérib.	J. Héribaud	Stolt.	H. Stolterfoth
Hick.	W. J. Hickie	Temp.	J. Tempère
Hook.	J. Hooker	Trent.	J. F. Trentepohl
Hust.	F. Hustedt	Turp.	P. Turpin
Hutt.	W. Hutton	W. Sm.	William Smith
Jan.	C. Janisch	Wall.	G. C. Wallich

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## INDEX TO GENERA

- Achnanthes, 545  
 Actinocyclus, 538  
 Actinoptychus, 539  
 Amphipleura, 546  
 Amphiprora, 546  
 Amphora, 551  
 Anomooneis, 546  
 Arachnoidiscus, 539  
 Asterionella, 543  
 Asteromphalus, 539  
 Aulacodiscus, 540  
 Auliscus, 540  
 Auricula, 546  
 Bacillaria, 552  
 Bacteriastrum, 542  
 Bacteriosira, 543  
 Bellerochea, 541  
 Biddulphia, 541  
 Brebissonia, 546  
 Caloneis, 546  
 Campylodiscus, 556  
 Campyloneis, 545  
 Campylosira, 543  
 Cerataulina, 541  
 Cerataulus, 541  
 Chaetoceros, 541  
 Cocconeis, 545  
 Corethron, 543  
 Coscinodiscus, 538  
 Coscinosira, 538  
 Cyclotella, 538  
 Cylindrotheca, 552  
 Cymatosira, 543  
 Dactyliosolen, 543  
 Detonula, 543  
 Dimeregramma, 543  
 Diploneis, 546  
 Ditylum, 541  
 Donkinia, 547  
 Druridgea, 538  
 Endictya, 538  
 Epithemia, 552  
 Eucampia, 541  
 Eupodiscus, 540  
 Fragilaria, 543  
 Frustulia, 547  
 Glyphodesmis, 544  
 Gomphonema, 551  
 Grammatophora, 544  
 Guinardia, 543  
 Hantzschia, 552  
 Hemiaulus, 541  
 Hemidiscus, 539  
 Hyalodiscus, 538  
 Isthmia, 541  
 Lauderia, 543  
 Leptocylindrus, 543  
 Licmophora, 544  
 Lithodesmium, 541  
 Mastogloia, 547  
 Melosira, 539  
 Navicula, 547  
 Nitzschia, 552  
 Oestrupia, 549  
 Okedenia, 551  
 Opephora, 544  
 Paralia, 539  
 Phacodiscus, 539  
 Pinnularia, 549  
 Plagiogramma, 544  
 Planktoniella, 539  
 Pleurosigma, 549  
 Podocystis, 556  
 Podosira, 539  
 Porosira, 539  
 Pyxidicula, 539  
 Rhabdonema, 544  
 Rhaphoneis, 544  
 Rhizosolenia, 543  
 Rhoicosphenia, 545  
 Rhopalodia, 552  
 Roperia, 539  
 Sceptroneis, 544  
 Schroderella, 543  
 Scolioleura, 550  
 Scoliotropis, 550  
 Skeletonema, 539  
 Stauroneis, 550  
 Stenoneis, 550  
 Stephanodiscus, 539  
 Stephanopyxis, 539  
 Streptotheca, 541  
 Striatella, 544  
 Surirella, 556  
 Synedra, 544  
 Thalassionema, 544  
 Thalassiosira, 539  
 Thalassiothrix, 544  
 Toxonidea, 550  
 Trachyneis, 550  
 Trachysphenia, 544  
 Triceratium, 541  
 Trigonium, 541  
 Tropiconeis, 551



## ABSTRACTS OF MEMOIRS

### RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

#### A MODIFICATION OF TEORELL'S METHOD FOR DETERMINING OF SMALL QUANTITIES OF AMMONIA

By M. Buljan

*Arh. Hem. Farm.*, T. 23, 1951, pp. 119-22

The use of Teorell's method (*Biochem. Z.*, Bd. 248, 1932, p. 246) is not satisfactory, since subsequent titrations of the hypobromite solution by means of a solution of naphthyl-red do not produce reproducible results. This is due to the interference of oxidation products of naphthyl-red. A way of eliminating this difficulty is given.

If hypobromite is not added in sufficient quantity to destroy the ammonium salt to be determined, another difficulty is encountered. This is a change in the direction of the titration curve, and owing to this the consumed volume of naphthyl-red is no longer in a direct relation to the quantity of ammonia actually present. An attempt is made to explain the behaviour of hypobromite when there is a surplus of ammonia by the production of hyponitrous acid radical, which, in an acid medium, has oxidizing properties and decolorizes the solution of naphthyl-red.

In order to eliminate these difficulties encountered when applying the original method, it is proposed to use a solution of potassium-indigo-disulphonate for the titration of the surplus hypobromite in an alkaline medium, instead of a naphthyl-red solution in an acid medium, as recommended by Teorell. M.B.

#### SULLA PRESENZA DI UN ORMONE D'ACCRESIMENTO IN UN CROSTACEO DECAPODE, LA *LYSMATA SETICAUDATA* RISSO (ON THE PRESENCE OF A GROWTH HORMONE IN A DECAPOD CRUSTACEAN, *LYSMATA SETICAUDATA* RISSO)

By D. B. Carlisle and P. F. R. Dohrn

*Ric. sci. suppl.*, 1953, pp. 95-100

In this paper, read before the Convegno di genetica e di scienze affini, held in the Genetics Institute of the University of Naples in June 1952, the discovery of a moult-accelerating or growth hormone in a decapod crustacean was first announced. A fuller account was published later in the *Pubbl. Staz. zool. Napoli*, Vol. 24, 1953, pp. 69-83 (abstracted in Vol. 32, No. 2 of the *Journal*), which, however, appeared in print before this account. D.B.C.

STUDIES OF THE MESOGLOEA OF COELENTERATES.  
I. HISTOLOGY AND CHEMICAL PROPERTIES

By Garth Chapman

*Quart. J. micr. Sci.*, Vol. 94, 1953, pp. 155-76

Evidence of the collagenous nature of the mesogloea of coelenterates is provided by histological and chemical methods.

A description is given of the arrangement of the fibrous material forming the mesogloea in various medusae and in the actinians *Calliactis* and *Metridium*, where the crossed fibrillar structure is regarded as being determined by mechanical forces acting on the tissue.

The role of the mesogloea in the life of the animal is discussed; in particular its viscous-elastic properties are thought to be well adapted to its skeletal functions.

G.C.

STUDIES ON THE MESOGLOEA OF COELENTERATES.  
II. PHYSICAL PROPERTIES

By Garth Chapman

*J. exp. Biol.*, Vol. 30, 1953, pp. 440-51

The physical properties of the isolated mesogloea of *Calliactis* and *Metridium*, which are mentioned in another paper (see above), are described in greater detail and the behaviour of the tissue on loading is recorded.

It is shown that the viscous-elastic properties of the body-wall which have been ascribed by previous authors to the muscles are the attributes of the mesogloea.

The thermal contraction of the mesogloea of *Calliactis* is compared with that of vertebrate collagen.

It is shown that the physical behaviour of the material is consonant with the crossed fibrillar collagenous nature of the mesogloea described in the other paper.

G.C.

UPWELLING IN THE BENGUELA CURRENT

By Ronald Currie

*Nature, Lond.*, Vol. 171, 1953, pp. 497-500

In 1950 the R.R.S. *William Scoresby* carried out two surveys of the Benguela Current, which is one of the major features of the circulation of the South Atlantic Ocean. This current is a region where cool, nutrient-rich subsurface

water is upwelled to the surface and gives rise to a great production of planktonic plants and animals.

The observations indicate that the current is composed of a series of eddies, similar to those which Gunther had postulated in the Peru Coastal Current. In those eddies the upwelled water moving offshore converges sharply with interlocking tongues of warmer offshore water. In March upwelling was mainly in abeyance, but in September–October there was an active offshore transport of upwelled water. This is related to the more active coastal winds in the latter months. Analysis of the distribution of specific volumes of the water suggests that the mechanism of the upwelling is of a similar nature to that which occurs on the Californian coast.

The waters on the continental shelf have a very high inorganic phosphate content. It is suggested that this is partly due to local regeneration, as at some 'stations' the phosphate content was higher than that of the water being upwelled.

A deposit of diatomaceous mud, extending for some 400 miles along the coast, is populated with sulphate-reducing bacteria. It is overlain by water of a very low dissolved oxygen content. In March the oxygen depletion was much greater than in spring, and this is probably associated with the more quiescent state of the current in March. It is suggested that the O<sub>2</sub> depletion may to some degree be associated with mass mortalities of fish which frequently occur in the summer months. R.C.

#### MICRODETERMINATION OF PHOSPHORUS IN BIOLOGICAL MATERIAL

By H. W. Harvey

*The Analyst*, Vol. 78, 1953, pp. 110–14

An absorptiometric method for determining the phosphorus content of small quantities of material within the range of 1–70  $\mu\text{g}$  of phosphorus has been devised and tested for precision. The material, collected by centrifuging, is decomposed in the centrifuge tube with sulphuric acid and hydrogen peroxide; pyrophosphoric acid is converted to orthophosphoric and any residual hydrogen peroxide decomposed with sulphite. The contents of the tube are diluted and the orthophosphoric converted to phosphomolybdic acid which is determined by the molybdenum blue method after controlled reduction with stannous chloride.

Experimental data are given to show the effect upon the blue colour formed due to (i) the concentration of stannous chloride, (ii) the temperature, (iii) acidity, and (iv) the time of reaction with stannous chloride. The effect of interfering substances is considered and experimental data are given which show the effect of traces of copper on the formation of the molybdenum blue.

H.W.H.

## THE DENSITIES OF SOME COMMON AQUATIC MOLLUSCA FROM PLYMOUTH

By A. G. Lowndes

*Ann. Mag. Nat. Hist.*, Ser. 12, Vol. 6, 1953, pp. 950-2

The density, sinking factor, and load carried when swimming or crawling are given for sixteen species of common Mollusca from the Plymouth district. The greatest density theoretically possible is something just short of that of aragonite, 2.95, while the lowest theoretical density is that of protoplasm, 1.05 (in the absence of fat).

*Ocenebra erinacea* L. attains a density of 2.07 while *Aplysia punctata* Cuvier has a density of 1.04. There is no apparent connexion between swimming ability and density. *Chlamys opercularis* (L.), whose density is 1.49, swims freely while *Aplysia* shows no sign of doing so. When *Chlamys opercularis* does swim it carries a load of 31% of its weight. The load carried by a typical teleost with a swim bladder is less than 0.2%.  
A.G.L.

OBSERVATIONS ON CERTAIN MECHANICAL PROPERTIES OF THE  
LIGAMENT OF *PECTEN*

By E. R. Trueman

*J. exp. Biol.*, Vol. 30, 1953, pp. 453-67

The conditions of the opening and the closing of the valves of *Pecten maximus* and *Chlamys opercularis* have been illustrated by drawing stress-strain curves for the intact ligament and for isolated parts of the inner layer of the ligament. These curves form a hysteresis loop, the area enclosed in that of *Pecten* being markedly less than that of other lamellibranchs, indicating the greater efficiency of this ligament. This is attributed to the inner layer of the ligament, calcified uniformly in most lamellibranchs, but which in *Pecten* (and associated forms) has a large central non-calcified region. The modulus of elasticity in compression of this region is approximately one-seventh of that of the inner layer of *Ostrea* or *Lutraria*, which are calcified structures. Thus the inner layer of the ligament of *Pecten* has less resistance to compression but greater efficiency than that of most lamellibranchs. These properties are important in relation to swimming, in which the valves of *Pecten* open and close rapidly and frequently.  
E.R.T.



# THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. xxvii (p. 761) and Vol. xxxi (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

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Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the *Journal* of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.



# CONTENTS

	PAGE
<b>J. E. Morton.</b> The biology of <i>Limacina retroversa</i> . . . . .	297
<b>D. B. Carlisle.</b> Notes on the Didemnidae (Asciacea). III. A comparison of <i>Didemnum maculosum</i> , <i>D. candidum</i> , <i>D. helgolandicum</i> , and <i>Trididemnum alleni</i> . . . . .	313
<b>D. B. Carlisle.</b> Notes on the Didemnidae (Asciacea). IV. The increase of <i>Trididemnum niveum</i> (Giard) in the Plymouth area, and the structure of its larva . . . . .	325
<b>D. B. Carlisle.</b> <i>Styela mammiculata</i> n.sp., a new species of ascidian from the Plymouth area . . . . .	329
<b>N. Ingram Hendey.</b> Note on the Plymouth 'Nitzschia' culture . . . . .	335
<b>G. R. Forster.</b> Preliminary note on a survey of Stoke Point rocks with self-contained diving apparatus . . . . .	341
<b>J. H. Fraser.</b> Warm-water species in the plankton off the English Channel entrance . . . . .	345
<b>Douglas P. Wilson and F. A. J. Armstrong.</b> Biological differences between sea waters: experiments in 1953 . . . . .	347
<b>Douglas P. Wilson.</b> The attractive factor in the settlement of <i>Ophelia bicornis</i> Savigny . . . . .	361
<b>F. A. J. Armstrong.</b> Phosphorus and silicon in sea water off Plymouth during the years 1950 to 1953 . . . . .	381
<b>S. M. Marshall and A. P. Orr.</b> Hatching in <i>Calanus finmarchicus</i> and some other copepods . . . . .	393
<b>R. H. Millar.</b> The development of the ascidian <i>Pyura microcosmos</i> (Savigny) . . . . .	403
<b>A. C. Hardy and R. Bainbridge.</b> Experimental observations on the vertical migrations of plankton animals . . . . .	409
<b>R. A. Robinson.</b> The vapour pressure and osmotic equivalence of sea water . . . . .	449
<b>J. A. Allen.</b> A comparative study of the British species of <i>Nucula</i> and <i>Nuculana</i> . . . . .	457
<b>D. J. Crisp.</b> The breeding of <i>Balanus porcatus</i> (da Costa) in the Irish Sea . . . . .	473
<b>W. R. G. Atkins, Pamela G. Jenkins and F. J. Warren.</b> The suspended matter in sea water and its seasonal changes as affecting the visual range of the Secchi disc . . . . .	497
<b>M. R. Droop.</b> A note on the isolation of small marine algae and flagellates for pure cultures . . . . .	511
<b>W. J. Rees and J. R. Lumby.</b> The abundance of <i>Octopus</i> in the English Channel . . . . .	515
<b>N. Ingram Hendey.</b> A preliminary check-list of British marine diatoms . . . . .	537
<b>Abstracts of Memoirs.</b> Recording work done at the Plymouth Laboratory . . . . .	561

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