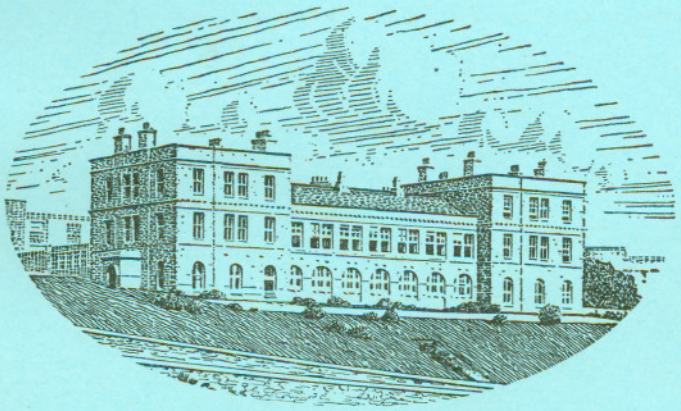
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



THE PLYMOUTH LABORATORY

VOLUME 38, No. 2

(issued June 1959)

CAMBRIDGE
AT THE UNIVERSITY PRESS
1959

Price Forty-six shillings net

(U.S.A. $7.75)

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

PATRON

H.R.H. The Prince Philip, Duke of Edinburgh, F.R.S.

OFFICERS AND COUNCIL

President: Prof. A. V. Hill, C.H., O.B.E., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, K.G., C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., LL.D., F.R.S.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.
G. M. Graham, C.M.G., O.B.E.

Honorary Members

Dr H. B. Bigelow
Dr R. Dohrn
Prof. Louis Fage
E. H. E. Havelock, C.B., C.B.E.
Dr A. G. Huntsman
Prof. Hans Pettersson, For. Mem. R.S.
Dr A. C. Redfield

COUNCIL

Elected Members

Prof. E. Baldwin, Ph.D.
H. A. Cole, D.Sc.
G. E. R. Deacon, C.B.E., D.Sc., F.R.S.
G. E. Fogg, Ph.D.
Prof. J. E. Harris, Ph.D., F.R.S.
M. N. Hill, Ph.D.
O. D. Hunt
C. E. Lucas, C.M.G., D.Sc.
C. H. Mortimer, Dr.phil., D.Sc., F.R.S.
Prof. R. J. Pumphrey, Sc.D., F.R.S.
Prof. J. E. Smith, Sc.D., F.R.S.
S. Smith, Ph.D.
Prof. G. P. Wells, Sc.D., F.R.S.
H. G. Vevers, M.B.E., D.Phil.
Prof. C. M. Yonge, C.B.E., D.Sc., F.R.S.

Governors

R. G. R. Wall (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers: The Prime Warden
Major E. G. Christie-Miller
Harrison S. Edwards
Prof. Sir Alister C. Hardy, Kt., D.Sc., F.R.S. (Oxford University)
C.F. A. Pantin, Sc.D., F.R.S. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
N. B. Marshall (Zoological Society)
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

Hon. Treasurer: Harrison S. Edwards, Westhumble Lacey, Nr. Dorking, Surrey

Secretary: F. S. Russell, C.B.E., D.S.C., D.F.C., LL.D., F.R.S., The Laboratory, Citadel Hill, Plymouth, Devon

SCIENTIFIC STAFF

Director: F. S. Russell, C.B.E., D.S.C., D.F.C., LL.D., F.R.S.

D. P. Wilson, D.Sc., F.R.P.S. (*Zoologist*)
L. H. N. Cooper, D.Sc., F.R.I.C. (*Chemist*)
G. M. Spooner, M.B.E., M.A., F.R.E.S. (*Zoologist*)
Mary W. Parke, D.Sc., Ph.D. (*Botanist*)
P. G. Corbin, B.A. (*Zoologist*)
J. A. C. Nicol, B.Sc., M.A., D.Phil. (*Experimental Zoologist*)
E. J. Denton, B.A., B.Sc., Ph.D. (*Physiologist*)
D. B. Carlisle, M.A., D.Phil. (*Bursar and Endocrinologist*)
N. A. Holme, M.A. (*Zoologist*)
G. R. Forster, B.Sc. (*Zoologist*)
F. A. J. Armstrong, A.R.I.C. (*Chemist*)
E. D. S. Corner, M.Sc., Ph.D. (*Biochemist*)
A. J. Southward, B.Sc., Ph.D. (*Zoologist*)
T. I. Shaw, M.A., Ph.D. (*Physiologist*)
J. V. Howarth, B. Sc. (*Physiologist*)
Q. Bone, M.A. (*Zoologist*)
G. W. Bryan, B.Sc. (*Special Appointment: Zoologist*)

June 1959

J. mar. biol. Ass. U.K. (1959) 38, 225–238
Printed in Great Britain

THE HABITS AND FEEDING ORGANS OF *DENTALIUM ENTALIS*

By J. E. MORTON
Department of Zoology, Queen Mary College, University of London

(Text-figs. 1–5)

The Scaphopoda or tusk-shells are the smallest and most uniform class of molluscs. They burrow in sand of medium to coarse shelly grade, and specialize on a diet of hard-shelled microbenthos, particularly Foraminifera. *Dentalium entalis* L. is the commonest West European species, and may become locally dominant. Le Danois (1948) has described a 'Dentalietum' in deeper water sandy ground at about 100 fathoms in the southern Celtic Sea. Stephen (1933) discusses the distribution of *D. entalis* in the northern North Sea, where it is largely restricted to the 'Offshore zone', the southern limit being marked by the 60 m line. Low salinity is probably responsible for its absence from the southern North Sea; and in general it seems limited more by hydrographic conditions than by the relative abundance of foraminiferan food. For example, in Stephen's *Thyasira* + Foraminifera Zone, it is no more than sparsely present. Holme (1953) finds *Dentalium entalis* rare or absent near Plymouth, though it was relatively common at the Eddystone Grounds at the end of last century (Allen, 1899). It has today receded to the mouth of the Channel, in coarse deposits resulting from fairly strong scour, and Holme suspects it to be one of the list of species sensitive to recent hydrographic changes in the area.

For the living material used in this study I am greatly indebted to Mr N. A. Holme, who collected it in deeper water in the Celtic Sea on a 1953 cruise of the R.R.S. 'Discovery II'. With it were collected *Chlamys opercularis*, *Caryophyllia* and in places *Astropecten irregularis*; there were also many of the tusk-shaped serpulid tube worm *Ditrupa arietina* which as I found can impose a neat deception on the uncritical hunter for scaphopods.

Dentalium entalis survived for some months in fairly clean sand of medium grade under circulation in the laboratory, settling happily only when it was able to burrow obliquely into the substrate with about a centimetre of the narrow tip exposed. As Yonge (1937) has shown, both the inhalant and exhalant pallial currents pass through the small pallial tube that projects at that end of the shell. Apart from Yonge's paper on the ciliary currents and water circulation of the pallial cavity, little has been published in detail on the mode of life of scaphopods, though it has been known for many years, at least

since Clark (1849), that they feed on Foraminifera. The purpose of this paper is to give a more detailed account, from living material, of the organs responsible for capturing and digesting food.

The concavely curved side of the shell is dorsal and in burrowing—whatever way it begins—the animal will soon twist until the dorsal side is uppermost in the obliquely buried position (Fig. 1 F). When *Dentalium* is withdrawn into its shell the mantle cavity is closed in front except for a narrowly constricted central pore; within this, the tongue-shaped foot is retracted,

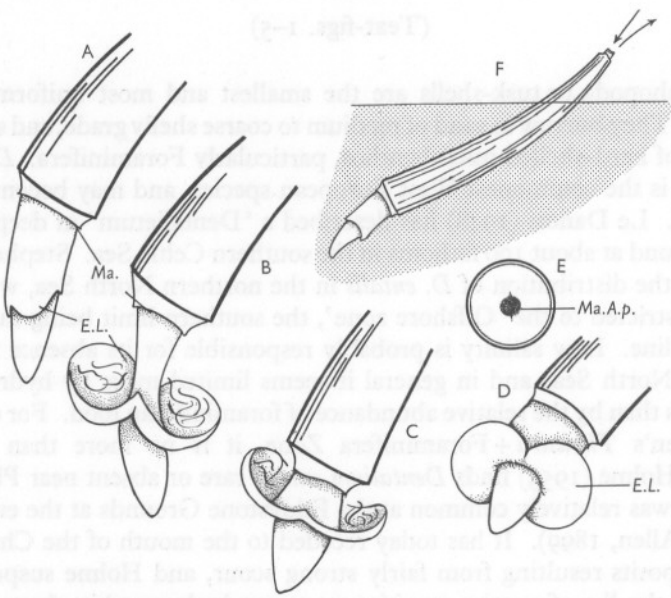


Fig. 1. *Dentalium entalis*: A, B, C, successive stages in the burrowing of the animal, showing the action of the foot and its extensible lobes. D, the extended foot viewed more anteriorly. E, the anterior end with the foot retracted showing the contracted pallial aperture. F, the shell and animal in natural posture in the sand. *E.L.*, extensible side lobes of the foot; *Ma.*, edge of the mantle with its aperture distended; *Ma.A.p.*, contracted pallial aperture.

forming a slightly curved muscular column lying ventrally to the head and filling most of the anterior third of the mantle cavity. As burrowing begins, the foot is protruded through the mantle pore as a firmly pointed lobe, which extends until it is fully external, encircled by the widely distended mantle orifice around its base. The foot has an erectile fold of skin, narrowest in the mid-line above and below and expanding into broad rounded lobes at either side. As the tip of the foot is plunged into the ground at each burrowing stroke these side lobes are retracted, until of small size, pressed close to the foot and forced into the sand with it. The extended foot is then engorged with blood from the general haemocoele, and the lobes are strongly erected, their edges being recurved to form a wide circular flange, anchoring the animal firmly in

the loose substrate. The stalk of the foot is now shortened as the shell is drawn up behind, and it is usually at the first such move that a twist is made through up to 90° as the animal orients dorsal side up. When the animal is suitably embedded the foot is partly retracted through the dilated pore of the mantle, and the animal puts out large numbers of the delicate cephalic appendages or captacula, which thrust their way in all directions through the surrounding sand.

THE CAPTACULA

These filiform prehensile tentacles arise in a dense bunch, from a single lobe (which may be partly divided) at the outer base of the proboscis on either side. Each is enlarged at its tip into a small ovoid or flattened bulb. They can elongate greatly as they are protruded and show a great deal of autonomous writhing movements when detached, but when retracted they lie in a compact cluster at either side between the proboscis and the mantle wall. They vary somewhat in number (in a typical cluster I counted about 135 on one side) and also in size; some, in the resting state, reach to the tip of the foot, others—probably recently regenerated—are short and their bulbs almost sessile. Extrusion takes place by the inflow of blood, and the captacula radiate in large numbers like the finest of strands through the substrate; rapid withdrawal is achieved by the contraction of their longitudinal muscle fibres. Though their action has never been described fully, they constitute the only food-catching organs, searching out and skilfully locating Foraminifera: there is seldom any admixture of detritus or of other living animals with the diet, and the *Dentalium* seems a strongly specialist feeder, though—as Clark (1849) has mentioned—it may sometimes vary its diet with bivalve spat or large diatoms. Once or twice I found the broken-off tip of a captaculum among the contents of the proboscis, still attached to a foraminiferan; and Dell (1957) has for a New Zealand species found Foraminifera entangled in the extruded captacula, as well as in the proboscis. *Dentalium* is nevertheless so shy in captivity that I could never once observe it in the act of taking food, and I can find no account in the literature bearing the stamp of actual observation.

For some understanding of the way the captacula work we need an account of their anatomy. From the size of these organs in relation to the bulk of a foraminiferan it seems a fair inference that a group of captacula comprising some numbers must attach together to each organism to be hauled in. Fol (1889) has given an account of the structure of the captacula with which I must in some points disagree. Each tentacle—as he showed clearly—is provided with means of extension and retraction and contains at its tip secretory glands, a ganglion and sensory receptors. Through the filament runs a central blood-filled cavity connected with the haemocoele of the head and surrounded by a layer of strong and regular longitudinal muscle fibres, usually ten in number. The central cavity has no internal epithelium as figured by Fol: the small

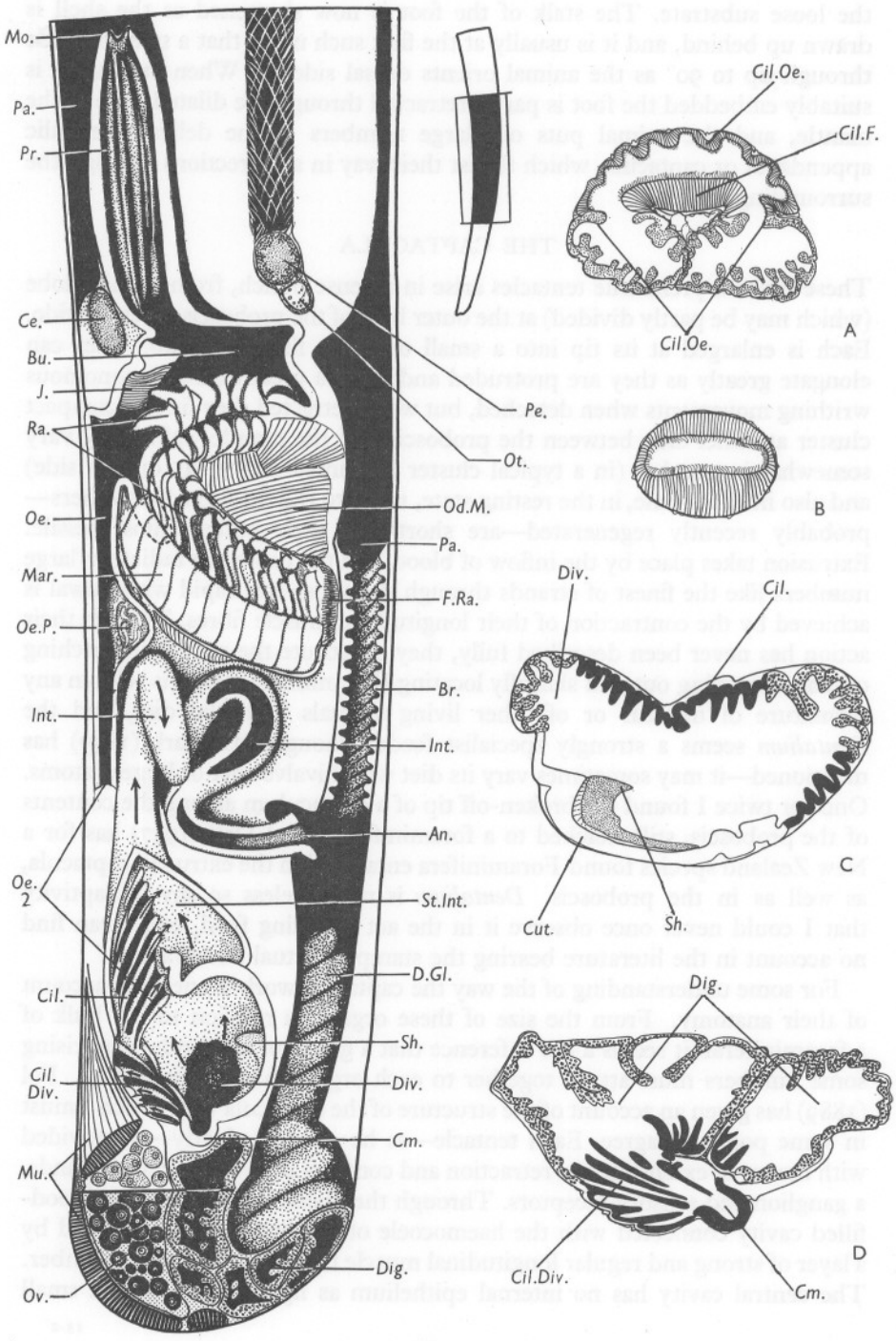

Mo.
Pa.
Pr.
Ce.
Bu.
J.
Ra.
Oe.
Mar.
Oe.P.
Int.
Oe.
2
Cil.
Cil.
Div.
Mu.
Ov.
Pe.
Ot.
Od.M.
Pa.
F.Ra.
Br.
Int.
An.
St.Int.
D.Gl.
Sh.
Div.
Cm.
Dig.
Cil.Oe.
Cil.F.
Cil.Oe.
A
B
Div.
Cil.
C
Cut.
Sh.
Dig.
D
Cil.Div.
Cm.

Fig. 2

dark-staining ovoid nuclei appearing there belong to blood amoebocytes or to a thin reticulum of connective tissue, especially concentrated near the slender nerve that runs through one side of the cavity. No circular muscles can be found; instead the filament is invested at close intervals by extremely slender connective tissue fibres. These are overlaid by a delicate squamous epidermis whose elongate nuclei run transversely to the filament. When the filament is contracted these cells stand out as regularly spaced annulae along its surface. The captaculum shows a rather close parallel to the echinoderm tube foot, now well understood from the work of Smith (1947), and forming essentially a tentacle with a hydroskeleton, longitudinal muscles and an outer circular investment of collagen. During elongation (in *Dentalium* by blood, in echinoderms by ambulacral fluid) increase in diameter is moderated by the limited elasticity of the circular elements while the longitudinal muscles are extended. When the longitudinal muscles contract to bring about retraction, the circular fibres must be sufficient to prevent distention of the wall by fluid under pressure, the attenuated cylinder having a minimal ratio of volume to restraining wall surface.

In none of the numerous sections examined could I find any trace of Fol's 'bandelette vibratile longitudinale' on one flattened side of the filament. The epidermal cells run right round the filament and it is difficult to see how such

Legend to Fig. 2

Fig. 2. *Dentalium entalis*. *Left*. Somewhat schematic view of the middle part of the body, displaying the left half in approximate sagittal section to illustrate the course of the alimentary canal and its relation to the surrounding structures. The dorsal side is to the left. At the posterior end, the left half of a transverse section is revealed, with the retractor muscle and ventral mantle wall continuing a little to the right of the middle line. A portion of the oesophagus is omitted, where it inclines to the right before entering the stomach. × 10. *Inset*. The shell at natural size, showing the extent of the parts illustrated. *Right*. Transverse sections of successive parts of the alimentary canal. A, the oesophagus, through the region of the oesophageal pouches; B, the oesophagus behind the pouches; C, the stomach, near the anterior limits of the digestive diverticulum; D, the posterior end of the stomach passing through the caecum and adjacent digestive gland and cutting tangentially the ciliated tracts associated with the caecum and diverticula. *An.*, anus; *Br.*, folds of the mantle wall serving as the respiratory organ; *Bu.*, buccal cavity; *Ce.*, cerebral ganglion; *Cil.*, ciliated fold of the roof of the stomach leading to the intestine; *Cil.Div.*, ciliated folds associated with the caecum and digestive diverticula; *Cil.F.*, ciliated fold of the glandular region of the oesophagus; *Cil.Oe.*, ciliated lining of the oesophagus; *Cm.*, caecum of the stomach; *Cut.*, cuticulated epithelium of the stomach; *D.Gl.*, digestive gland tubules spreading round the side of the mantle cavity; *Dig.*, digestive gland in section; *Div.*, digestive diverticulum leading from stomach; *F.Ra.*, formative cells of the radula at the bottom of the radular sac; *Gl.Oe.*, limits of the glandular region of the oesophagus; *Int.*, intestine; *J.*, jaw; *Mar.*, marginal teeth of left side within the radular caecum; *Mo.*, mouth; *Mu.*, retractor muscles attached posteriorly to the shell; *Od.M.*, muscles of the odontophore; *Oe.*, oesophagus; *Oe.P.*, oesophageal pouch; *Oe.2*, extent of interrupted section of the oesophagus; *Ot.*, otocyst; *Ov.*, ovary; *Pa.*, pallial cavity; *Pe.*, pedal ganglion; *Pr.*, proboscis, *Ra.*, radula (lateral teeth of left side and median teeth); *Sh.*, vestige of gastric shield; *St.Int.*, anterior part of stomach, tapering forward to intestine.

a ciliated tract could be maintained with continual alterations of length, or what function it could perform.

The tip of the captaculum (Fig. 3A)—unlike the filament—is ciliated all over, but especially densely in a subterminal alveolus or shallow depression lying on one side, where the cilia are twice as long as elsewhere. The interior of the terminal bulb is filled mostly by a close-packed mass of ovoid dark-staining epidermal nuclei, surrounding near the centre a small ganglion presumably connected with the captacular nerve. Associated with this ganglion

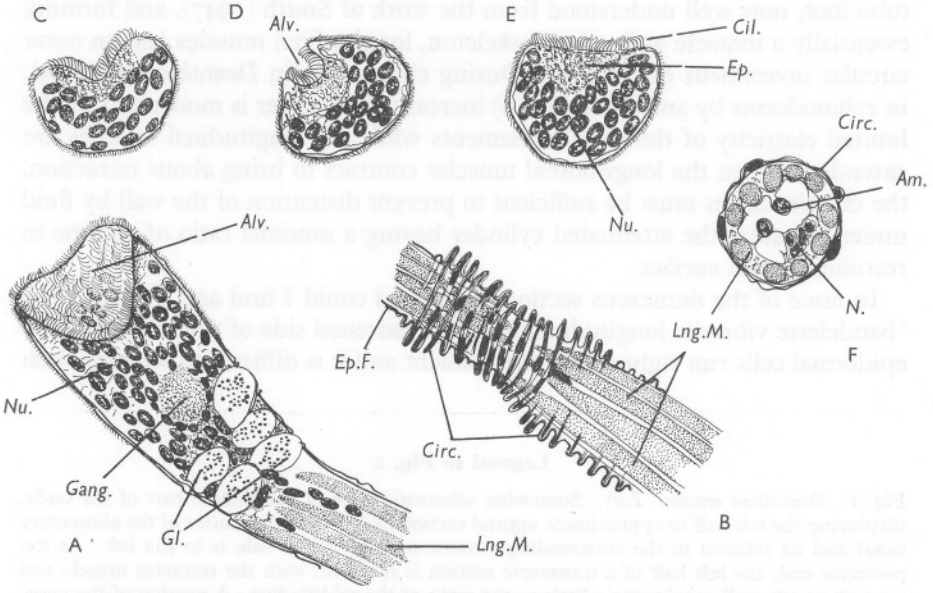


Fig. 3. *Dentalium entalis*. Histology of the captacula, from sections cut at 5 μ and stained in iron haematoxylin or in Heidenhain's azan. A, the tip of a captaculum in tangential section, showing part only of the ciliated alveolus, the ganglion and the nest of gland cells; B, tangential longitudinal section of part of a filament showing the arrangement of longitudinal muscles and circular connective tissue; C, D, E, transverse sections of the terminal bulb with different conditions of the ciliated alveolus, from contraction to the flatly expanded state; F, transverse section of a filament. *Am.*, amoebocyte in lumen; *Alv.*, alveolus; *Cil.*, cilia; *Circ.*, circular connective tissue; *Ep.*, epithelium of the alveolus; *Ep.F.*, epithelium of the filament; *Gang.*, ganglion; *Gl.*, glands; *Lng.M.*, longitudinal muscle; *N.*, nerve; *Nu.*, nuclei of epithelial and sensory cells of terminal bulb.

Fol clearly figures rod-like end-organs between the columnar cells lining the alveolus. At the base of the terminal bulb where the longitudinal muscles taper off, lies a nest of plump-bodied epidermal glands, with basal nuclei and their contents non-staining save for clusters of granules which become red in azan and black in iron haematoxylin. Some of these glands were seen in sections to be discharging at the surface of the epidermis.

By what means can the captacula take hold of a foraminiferan test? It is unlikely from their situation that the gland cells help in prehension; it is

tentatively suggested these may produce a toxin disabling living Foraminifera, or even a repugnatorial secretion protecting the animal's own widely deployed and otherwise vulnerable captacula. There are no obvious prehensile structures, in the form of organised suckers. A viscous secretion seems unlikely, from the histological structure of the bulbs, and although the filament can sometimes coil, it is impossible to visualize it as an entwining tentacle. As shown in Fig. 3, the subterminal depression of the bulb may be contracted to a deep cup, or be opened out shallowly, or perfectly flat in its full state of expansion. These changes of shape must be secured by a fine intrinsic musculature, which was, however, quite obscured by the crowded nuclei in the preparations illustrated. Closely applied to the foraminiferan test, when flattened out, the depression could provide a temporary but quite effective suction cap. Several captacula acting in concert could obviously exert a significant pull quite adequate to bring the foraminiferan to the mantle cavity and thence to the mouth. By its coat of cilia the tip of the captaculum might be moved over the surface of the test, exploring it for position before taking up its attachment. A parallel to such a mode of attachment is already known, in the tentacles of the terebellid polychaete worms, as carefully described by Dales (1955). These though much larger have a somewhat comparable structure, each forming a long, very extensible fluid-filled appendage traversed by longitudinal muscles. Instead of a terminal depression they have a ciliated groove all the way along; this can be applied locally to the substrate and creep along by its cilia, and, when the groove is locally flattened out, the tentacle can take a firm hold of the ground at any point along its length.

ALIMENTARY CANAL

The gut begins with a flattened muscular-walled proboscis, contractile but not inversible, projecting from the head dorsally to the foot and lying in front of the buccal mass between the bunches of captacula. The mouth is a horizontal slit running across the anterior end of the proboscis, and its sides are drawn out to give it a wide crescentic form. It has thickened, crenulated lips, and when the captacula are withdrawn into the mantle cavity, the tips of the longest come to lie about level with the mouth. From the position of some captacula in fixed material, it seems they may be drawn through or between the parted lips, as their attached load is ingested. After feeding, the muscular wall of the proboscis is then stretched quite thin, and its cavity is crammed with the tests of living Foraminifera, which bulge out all over its surface like small close-set pustules or sometimes angular projections. When full, it was found to contain a dozen or so large tests (up to 350 μ), three or four times as many small ones, and almost nothing more. Such fine power of selectivity is a testimony to the searching efficiency of the captacula. An occasional diatom frustule may be picked up, and at times empty foraminiferan tests, but the

great bulk of the meal is of living Foraminifera, all perfectly intact in the proboscis, trituration taking place further back in the gut. A specimen from the Celtic Sea, with the proboscis full, had for example collected the following haul:

Elphidium sp. (=*Polystomella*), the largest and commonest species, about six tests up to 350 μ, and many smaller ones.

Quinqueloculina sp., a miliolid, two large tests (200 μ) and a number of small.

Discorbis (?) sp., a trochoid rotaliid.

Globigerina test, apparently empty when picked up and not recorded living in this area.

A Millport preserved specimen examined for comparison had the proboscis filled with a single species of *Bulimina*.

I am indebted to Dr R. H. Hedley for identifying the Foraminifera as accurately as possible after decalcification resulting from fixation.

Behind the proboscis lies a buccal mass, of about the same length but much deeper and more muscular. The proboscis is very contractile and may empty its contents by peristalsis, while removal of Foraminifera may be helped by the radula which is so situated that its lateral teeth when protracted could haul whole frustules, perhaps several at a time into the buccal mass. Fig. 4 shows the relative size and proportions of the radula, typical foraminiferan tests, and captacular bulb. The radula is relatively immense by comparison with the rest of the gut, and is proportionately larger than in any other mollusc I have seen. It has approximately 18 rows of teeth, each bearing 5 teeth. The central tooth is a crescentic transverse plate with no sharp cusps. It is flanked at each side by a strongly falcate lateral tooth, and outside that by a smooth marginal tooth, little more than a broad plate of chitin paving the side of the odontophore. The radula has the usual morphological relations: it lies in a narrow groove at the centre of the odontophore, and is spread out for protection, and afterwards retracted, by muscles attached to an odontophoral skeleton of two 'cartilages'. The laterals are the main functional teeth, and their distal expansions are wide and hood-shaped behind the sharp tip. A foraminiferan test grasped by them and lifted up towards the roof of the buccal mass might then be engaged between the strong bar of the central tooth and the chitinous jaw. This is a median, bow-shaped plate, running across the front wall of the buccal mass. As well as transferring Foraminifera from the proboscis to the oesophagus, the radula may thus also have acquired the task of fracturing and triturating them. This would accord with its uniquely large size in *Dentalium*, the lack of other hard triturating surfaces farther back, and absence, in my material, of any intact frustules in later parts of the gut. Clark, however, mentions finding them in the stomach, and the very contractile stomach wall must play some part as a gizzard when distended by the food mass.

Of the rest of the gut the chief features for remark are the absence of mucus

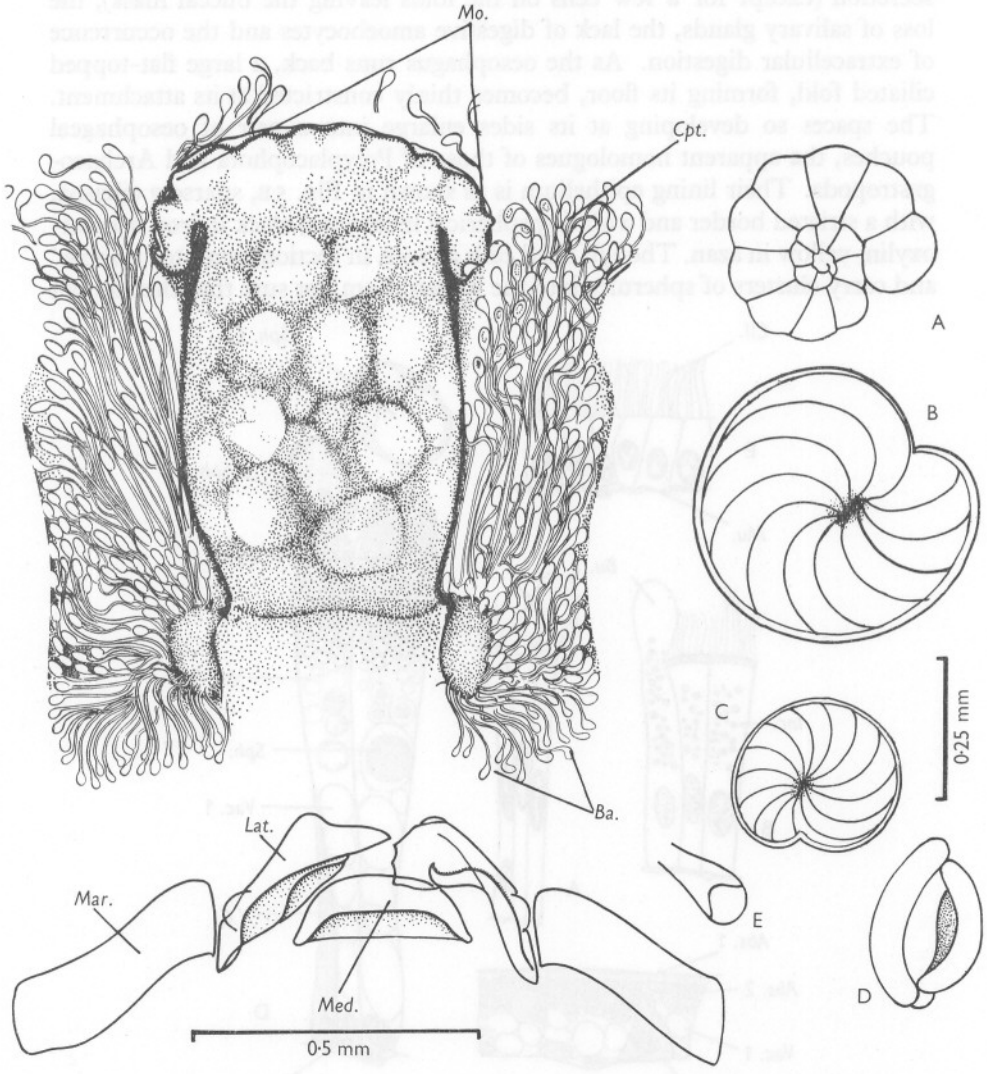


Fig. 4. *Dentalium entalis*. *Upper left*. The proboscis and the bunches of retracted captacula viewed from above, after the removal of the dorsal mantle wall. Ingested foraminiferans bulge through the wall of the proboscis. *Lower left*. A single row of teeth from the radula. *Right*. To the same scale as the radula are shown outlines of representative foraminiferans found in the proboscis: A, *Discorbis* sp.; B, C, *Elphidium* sp. of different sizes; D, *Quinqueloculina*. The tip of a captaculum, E, is also represented to the same scale. *Ba.*, short captacula at the base of the cluster; *Cpt.*, captacula; *Lat.*, lateral tooth; *Mar.*, marginal tooth; *Med.*, central tooth; *Mo.*, extent of mouth.

secretion (except for a few cells on the folds leaving the buccal mass), the loss of salivary glands, the lack of digestive amoebocytes and the occurrence of extracellular digestion. As the oesophagus runs back, a large flat-topped ciliated fold, forming its floor, becomes thinly constricted at its attachment. The spaces so developing at its sides enlarge into a pair of oesophageal pouches, the apparent homologues of those of Polyplacophora and Archaeogastropoda. Their lining epithelium is as shown in Fig. 5B, sparsely ciliated, with a striated border and with tiny spherical inclusions, black in iron haematoxylin, yellow in azan. Though fixed cells as seen in sections tend to 'bubble' and carry clusters of spherules into the lumen, I am not sure that they are an

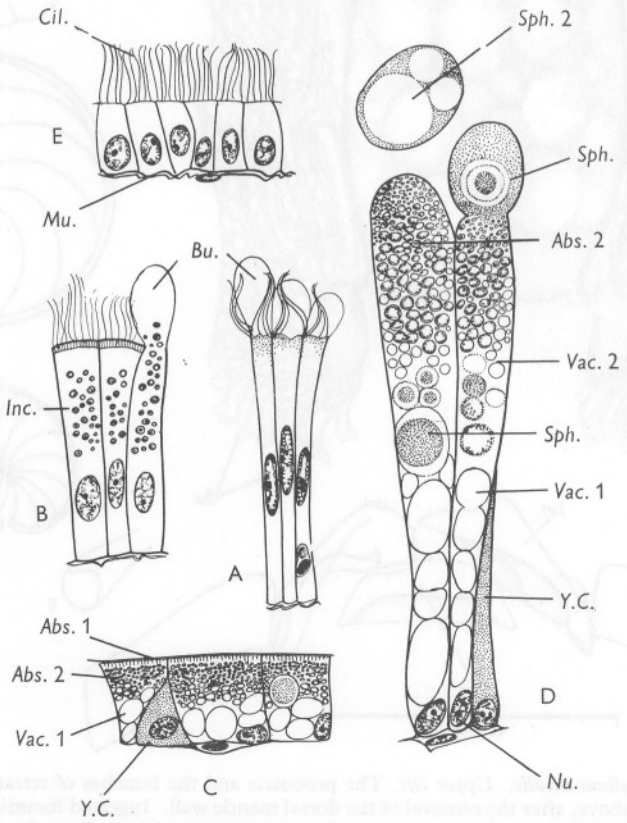


Fig. 5. *Dentalium entalis*. Histology of the alimentary canal. A, ciliated cells of the oesophagus; B, glandular cells of oesophageal pouch; C, cells of digestive gland at early absorbing stage; D, digestive cells of maximum size at late stage, producing 'secretory' spheres; E, cells of intestine. *Abs.* 1, finely striated flat absorbing surface of the digestive cell; *Abs.* 2, absorbed material not yet enclosed in cell vacuoles; *Bu.*, 'bubbling' cytoplasm of oesophageal cell; *Cil.*, cilia; *Inc.*, granular inclusions in oesophageal cell; *Mu.*, muscle fibre; *Nu.*, nucleus; *Sph.* 1, sphere rounding off at tip of mature digestive cell; *Sph.* 2, sphere cut off from cell; *Vac.* 1, colourless vacuoles towards base of cell; *Vac.* 2, digestive vacuoles with absorbed contents; *Y.C.*, young cell.

important source of secretion in life—similar types of inclusions may indeed be seen in the non-glandular cells beneath the cuticle of the stomach. Behind these pouches the oesophagus narrows to run as a ciliated tube to the right side of the stomach, close to where the intestine emerges towards the left.

The stomach, though thin-walled, has a muscular tunic and performs vigorous contractions in life. It probably serves chiefly as a gizzard into which open posteriorly at either side the very wide, almost symmetrical, apertures of the paired digestive diverticula. The stomach is a globular or pyriform sac, with little more than half the volume of the buccal mass, and tapers towards the oesophagus and intestine in front. Its structure, viewed in Fig. 2, shows disappointingly little of the primitive architecture of the molluscan stomach, though there are two features that would pass unnoticed but for an awareness of the past history of this organ. A small conical papilla, ciliated inside, and projecting behind the digestive diverticula is evidently a relic of the posterior gastric caecum. Immediately to the right and left of it begin the apertures of the respective digestive diverticula; and from the caecum as a centre point a series of ciliated ridges radiate over the posterior end of the stomach. Some of these extend to the thresholds of the diverticula, while most run up to the dorsal side of the stomach, slanting towards the intestine and terminating at the summit of a flat triangular fold (Fig. 2C, D). Their ciliation is simple, producing a set of currents leading along the grooves towards the intestine. These are the only ciliary movements within the stomach: cuticle covers the floor and such part of the side-walls as is not perforated by the two diverticula. The floor is raised at one point into a small spur of cuticle, the second relic of the primitive stomach, which represents a vestige of the gastric shield.

No trace of style-sac ciliation survives, and material from the stomach is periodically squeezed into the intestine by peristalsis, in a loosely compacted rope formed of the remains of Foraminifera, held together by the viscous outflow from the digestive gland. The intestine describes a double loop in the space between the stomach and buccal mass, the rectum then descending to open in the ventral mid-line into the pallial cavity immediately behind the region of the respiratory folds. The faeces are not bulky and are not compacted into separate firm pellets. The intestinal lining cells (Fig. 5E) are shortly columnar with sparse longish cilia; and I could find no secretory cells.

DIGESTIVE GLAND

The digestive gland clearly secretes enzymes for extracellular digestion, as can be judged by the bulky nature of the food, its complete breakdown in the stomach, and the lack of any other enzyme sources with the very doubtful exception of the small oesophageal pouches. The gland is also the site of absorption. It is built up of tubules closely adpressed with little or no intervening connective tissue. Each common diverticulum opens straight out of

the stomach, to break into a row of thick, finger-shaped tubules on either side. The right and left lobes of the digestive gland so formed unite in a median dorsal bridge over the mantle cavity and beneath the gonad. In either lobe, the series of tubules becomes smaller towards the front and the back of the lobe, and spreads round the mantle cavity, except for a median ventral strip which is thin and transparent.

The digestive cells absorb fluid and fine suspended material carried into the tubules by muscular contractions in the stomach. They show in local regions all stages between the two extremes of shape and size in Fig. 5C–D. The first stage is low and squarish, just beginning to absorb, with large clear basal vacuoles and finely granular vacuoles in the distal cytoplasm, whose contents stain blue or grey in azan, identically with the contents in the lumen. Later cells become greatly attenuated, widening and bulging at the loaded tips. The vacuoles in the basal half are still mostly clear, but distally they are loaded with ingested material. The tips are now rounding into compact spheres that will be nipped off and returned to the stomach, entering in large numbers and easily detectable in the faeces. They rarely contain a nucleus and such 'secretion' is merocrine rather than holocrine. This suggests that the cells may again become absorptive, but the digestive gland is also provided with young 'replacing cells' that have not yet begun to absorb. These are broad-based (5C) or narrowly trangular (5D) with rather large nuclei and uniformly dark-staining cytoplasm. As well as bluish vacuoles (azan) the fragmenting tips of the mature cells occasionally contain a vacuole filled by a single large greenish yellow droplet, probably excretory. The contents of all the extruded vacuoles must represent the residual waste returned to the stomach after digestion and assimilation of food. It must be further assumed that the abstricted spheres also provide, either in their vacuoles or in the residual cytoplasm, such enzymes as are necessary for extracellular digestion. The cells thus pass through a life-history widespread in mollusc digestive cells (see Morton, 1956, and Owen, 1955, for lamellibranchs, where there is usually no secretion, and Bidder, 1950, for cephalopods). In *Dentalium* the cycle includes absorption, assimilation, extrusion of indigestible and excretory residues, and the provision of extracellular enzymes in advance of the next meal.

The Scaphopoda must have early left the main road of advance, being known as a separate group since Silurian times. They retain a primitive simplicity of the renal organs, absence of separate genital ducts and external fertilization, together with a much more complete gut than in lamellibranchs. Even more than the lamellibranchs they have become deeply committed to burrowing life, and—as in the bivalves—the mouth is raised out of direct contact with the substratum. Feeding, which in most modern lamellibranchs has devolved on elaborate gills and in Nuculacea upon palp proboscides, depends in Scaphopoda upon the unique head appendages, the captacula, and on the

immensely developed radula, which in the bivalves is entirely lost. The lamellibranch gills and mantle cavity equipped their possessors for lavish and long-continued radiation; the scaphopod captacula, by contrast, at once restricted the possible range of diet and variety of habits—in evolutionary terms they are the badge of failure. *Dentalium* has no gills; the annular pallial folds are adequate for its respiratory needs, and their powerful cilia sufficient to maintain the inhalant and exhalant posterior currents. With a simplified respiratory system, the circulation is in turn rudimentary; there is no heart, but a simple contractile area of the haemocoele wall near the anus. The rhythmic expansion and contraction of the foot and its periodic charging with blood must have far more influence upon blood circulation than could the relatively feeble action of a heart.

SUMMARY

The scaphopod *Dentalium entalis*, studied with specimens from the Celtic Sea, is a specialized burrower in medium to coarse sands and its chief or only diet is of Foraminifera. Burrowing is performed by a muscular, very extensible, tongue-like foot, protruded through the anterior pore of the mantle cavity, and anchored in the sand at each distensible burrowing thrust by erectile side lobes. The foraminiferans are captured by very numerous extensible head tentacles or captacula. These radiate in the substrate and terminate in an expanded bulb, with gland cells, receptors and a ciliated alveolus which is able to be flattened out to provide—it is suggested—an attachment organ. Collected Foraminifera are hauled from the cavity of the proboscis into the buccal cavity by a large, strong radula, and pass along the oesophagus to the stomach. This is simplified in structure with a thin but muscular wall which serves the function of a gizzard. There is a vestigial caecum and gastric shield. The digestive diverticula open very widely from the stomach. Digestion appears to be wholly extracellular, and the digestive tubules are compared in structure with those of other Mollusca. The intestine is a simply coiled tube and the faeces are small and uncompacted.

REFERENCES

ALLEN, E. J., 1899. On the fauna and bottom deposits near the thirty fathom line from the Eddystone to Start Point. *J. mar. biol. Ass. U.K.*, Vol. 5, pp. 365–542.

BIDDER, ANNA M., 1950. The digestive mechanism of the European squids. *Quart. J. micr. Sci.*, Vol. 91, pp. 1–43.

CLARK, W., 1849. On the animal of *Dentalium tarentinum*. *Ann. Mag. nat. Hist.*, Ser. 2, Vol. 4, pp. 321–30.

DALES, R. P., 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

DANOIS, E LE., 1948. *Les Profondeurs de la Mer*. Paris: Payot.

DELL, R. K., 1957. A revision of the recent scaphopod Mollusca of New Zealand. *Proc. roy. Soc. N.Z.*, Vol. 84, pp. 561–76.

FOL, H., 1889. Sur l'anatomie microscopique du Dentale. *Arch. Zool. exp. gén.*, T. 7, pp. 91–148.

HOLME, N. A., 1953. The biomass of the bottom fauna in the English Channel off Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 1–49.

MORTON, J. E., 1956. The tidal rhythm and action of the digestive system in *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 563–86.

OWEN, G., 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia. I. The Anisomyaria and Eulamellibranchia. *Quart. J. micr. Sci.*, Vol. 96, 517–37.

SMITH, J. E., 1947. The activities of the tube feet of *Asterias rubens*. *Quart. J. micr. Sci.*, Vol. 88, pp. 1–14.

STEPHEN, A. C., 1933. Studies on the Scottish Marine Fauna: The natural faunistic divisions of the North Sea as shown by the quantitative distribution of the molluscs. *Trans. roy. Soc. Edinb.*, Vol. 57, pp. 601–16.

YONGE, C. M., 1937. The circulation of water in the mantle cavity of *Dentalium*. *Proc. malacol. Soc. Lond.*, Vol. 22, pp. 333–8.

J. mar. biol. Ass. U.K. (1959) 38, 239–248
Printed in Great Britain

FEEDING IN NUDIBRANCH LARVAE

By T. E. THOMPSON
Marine Biological Station, Port Erin, Isle of Man

(Text-figs. 1–7)

No account exists of the mechanism of food collection of nudibranch larvae, and, in fact, it has been considered unlikely that feeding does take place in these forms. Thorson (1946, p. 275) states 'Nudibranch larvae will normally take no—or only little—food from the plankton'.

Observations by the present author on larvae of the dorid nudibranch *Adalaria proxima* (Alder & Hancock) showed clearly that, although further development with metamorphosis will occur in this species regardless of whether food is provided for the larvae, a feeding mechanism is present and functions throughout pelagic life (Thompson, 1958). Moreover, sections through the larval digestive gland revealed the presence of food vacuoles, proving the ability to assimilate planktonic micro-organisms. These observations provided a stimulus to further work in order to ascertain whether the feeding mechanism described for *Adalaria* was present in other nudibranchs which possess a free-swimming larval phase.

Accounts of the anatomy of nudibranch veligers are few. Pelseneer (1911) figured many species, but made little attempt to ascribe functions to the various organs. Rasmussen (1944, 1951) gives more detailed figures of the larvae of a few nudibranchs, but again goes into little functional detail, and fails to orientate the veliger correctly (he repeatedly states that it is the *right* midgut diverticulum which is the larger whereas his figures leave no doubt that this is incorrect).

MATERIAL AND METHODS

Vestergaard & Thorson (1938) and Thorson (1946) divide nudibranch larvae into three types, according to the shape of the larval shell. In the present investigation, only species whose larvae belong to Thorson's shell-types B and C have been treated. There can be little doubt that the type A does not constitute a natural division, but represents a type B shell at an early stage of development (own unpublished observations on *Tritonia hombergii* Cuvier). The differences in structure between larvae of shell-types B and C, however, are far more striking and significant. Fig. 1 shows these two nudibranch larval shell types. The type C shell is confined to the families Dendronotidae, Eubranchidae, Tergipedidae and Fionidae; six families, Calmidae, Proctonotidae, Arminidae, Scyllaeidae, Hancockiidae and Lomanotidae have yet to

be investigated; members of all the other British families which have been studied (see below, and Thorson, 1946, p. 269) possess larval shells of type B. (For the reason stated above, the family Tritoniidae is here considered to consist of forms with shells of type B.) In only one instance does it appear that a family contains species with both type B and type C shells: *Coryphella rufibranchialis* is said by Thorson (1946) to have a larval shell of type C, whereas *C. lineata* (see below) has a shell of type B. It seems probable that subsequent work will show one or the other of these observations to be incorrect.

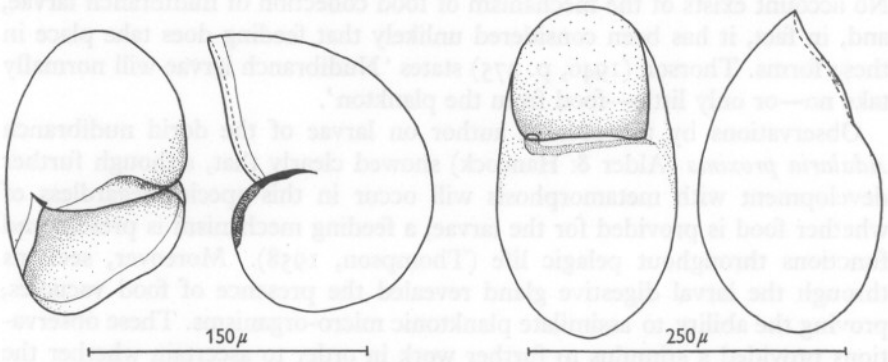


Fig. 1. Nudibranch larval shells of Thorson's types B and C: left, postero-ventral and lateral views of *Jorunna tomentosa* (type B); right, ventral and lateral views of the larval shell of *Trinchesia aurantia* (type C).

As a representative of the nudibranch larvae with shells of type B, *Archidoris pseudoargus* (Rapp) (Glossodorididae) has been investigated in detail, while less detailed observations were made on the following: *Jorunna tomentosa* (Cuvier) (Glossodorididae), *Onchidoris fusca* (Müller) (Limaciidae), *O. muricata* (Müller) (Limaciidae), *Polycera quadrilineata* (Müller) (Limaciidae), *Goniodoris nodosa* (Montagu) (Limaciidae), *Acanthodoris pilosa* (Müller) (Limaciidae), *Hero formosa* (Loven) (Heröidae), *Doto coronata* (Gmelin) (Dotöidae), *Facelina auriculata* (Müller) (Facelinidae), *Coryphella lineata* (Lovén) (Coryphellidae), *Limapontia depressa* Alder & Hancock (Limapontiidae), *Alderia modesta* (Lovén) (Stiligeridae).

As representatives of the shell-type C, observations were made on larvae of *Trinchesia aurantia* (Alder & Hancock) (Tergipedidae) and *Eubranchus exiguus* (Alder & Hancock) (Eubranchidae).

The nomenclature and classification employed are those advocated by Winckworth (1932, 1951).

All larvae were obtained from egg masses laid and reared in the laboratory. Feeding currents were observed in suspensions of fine carmine particles or of the diatom *Phaeodactylum tricornutum* Bohlin (from a culture kindly provided by Dr J. M. Kain). Larvae were fixed in hot Perényi's fluid, cleared with

amyl acetate (Barron, 1934) and sectioned at 5–10 μ. Stains employed included Mayer's haemalum, Heidenhain's iron haematoxylin and, as counterstains, eosin and alcian blue 8GS (Steedman, 1950).

THE LARVA OF *ARCHIDORIS PSEUDOARGUS*

The figures display most of the results of this investigation. Figs. 2 and 3 show the extended veliger larvae of the species, Fig. 4 the cephalopedal ciliary apparatus and Figs. 5 and 6 the alimentary canal. Only those features which are not immediately apparent from these figures will be described.

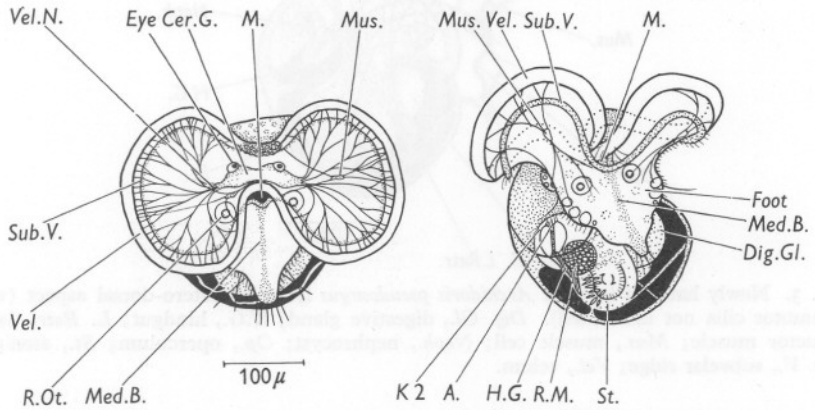


Fig. 2. Eyed larva of *Archidoris pseudoargus*, 3 days after hatching: left, viewed from the anterior; right, right latero-ventral aspect (velar locomotor cilia not illustrated). *A.*, anus; *Cer. G.*, cerebral ganglion; *Dig. Gl.*, digestive gland; *H.G.*, hindgut; *K* 2, larval kidney; *M.*, mouth; *Med. B.*, median pedal band of strong cilia producing the main rejection current; *Mus.*, muscle cell; *R.M.*, right midgut diverticulum; *R.Ot.*, right otocyst; *St.*, stomach; *Sub.V.*, subvelar ridge or subvelum; *Vel.*, velum; *Vel.N.*, nerve fibres from the cerebral ganglia to the velar cells.

The cephalopedal ciliary apparatus (Fig. 4)

The long velar cilia serve a dual purpose in both imparting a forward motion to the larva and bringing a constantly renewed supply of sea water within the influence of the feeding apparatus. Particles brought within the influence of the cilia arranged along the subvelar ridges (Figs. 2–4, *Sub.V.*) are rapidly conducted towards the mouth. The borders of the mouth (Figs. 2, 4, *M.*) are strongly ciliated and particles of sufficiently small size (less than *ca.* 15 μ in diameter) are impelled into the foregut.

The long velar cilia can be stopped and started, being under the control of the nervous system (Carter, 1926). All the other cilia of the larval body beat continuously, and a feeble feeding current is manifest even when the larva sinks passively with the velum inactive.

The external surface of the foot is ciliated, these cilia being particularly strongly developed along a narrow zone leading from the ventral border of the mouth to the papillate tip of the foot (Figs. 2, 4, *Med.B.*). This narrow band forms the main rejection current; particles which are too large to enter the mouth appear to be tipped over its ventral border and are then removed rapidly by this strong stream.

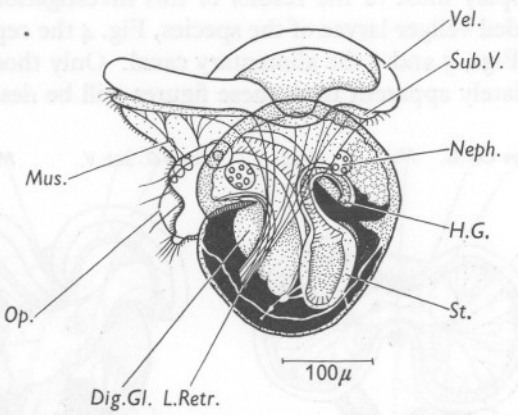


Fig. 3. Newly hatched larva of *Archidoris pseudoargus* from left latero-dorsal aspect (velar locomotor cilia not illustrated). *Dig. Gl.*, digestive gland; *H.G.*, hindgut; *L. Retr.*, larval retractor muscle; *Mus.*, muscle cell; *Neph.*, nephrocyst; *Op.*, operculum; *St.*, stomach; *Sub. V.*, subvelar ridge; *Vel.*, velum.

The foregut (Fig. 5, *F.G.*)

The foregut is strongly ciliated internally, these cilia creating a current which conveys particles from the mouth into the stomach.

The stomach and its diverticula (Fig. 5)

The stomach is internally ciliated throughout. Short, fast-beating cilia are arranged on a raised band (Figs. 5, 6, *Cil.B.*) which causes a rapid rotation of particles in the lumen. The direction of rotation was the same in all the individuals observed. Ventrally, close to the openings of the foregut and the midgut diverticula into the stomach (Fig. 6A, *F.G.op.*, *R.M.op.*, *Dig.Gl.op.*), the cilia are longer and more slow moving. They induce an apparently aimless particle agitation, which may on occasion be seen to become more co-ordinated and take the form of a rotatory movement in the ventral stomach. A small zone of hyaline, rod-like bodies is present in the ventral stomach wall on the right side (Fig. 6B, *Hy.Rods*).

The midgut diverticula are a pair, lying symmetrically against the antero-lateral walls of the stomach. Both possess lumina; this is more marked in the case of the left diverticulum which is by far the larger. The right diverticulum (Fig. 2, *R.M.*) consists of a few large yolk-laden cells at hatching; these cells

decrease in size during later larval life as, presumably, the yolk is utilized. This right diverticulum, as far as could be ascertained, plays no part in digestion.

The left midgut diverticulum or digestive gland (Figs. 2, 3 and 5, *Dig.Gl.*) is pale green-brown in life, and has a wall of single-cell thickness. It has a crescentic shape, following the line of the whorl of the shell. The cells composing its wall are ciliated and are of two kinds; the majority are cubical digestive cells but there are a few larger cells (mainly lying close to the opening of the organ into the stomach) which have yolk-laden cytoplasm. The nuclei are peripherally placed within all the cells of the digestive gland. Sections through larvae which have been allowed access to *Phaeodactylum* show large numbers of ingested diatoms enclosed in cytoplasmic food vacuoles

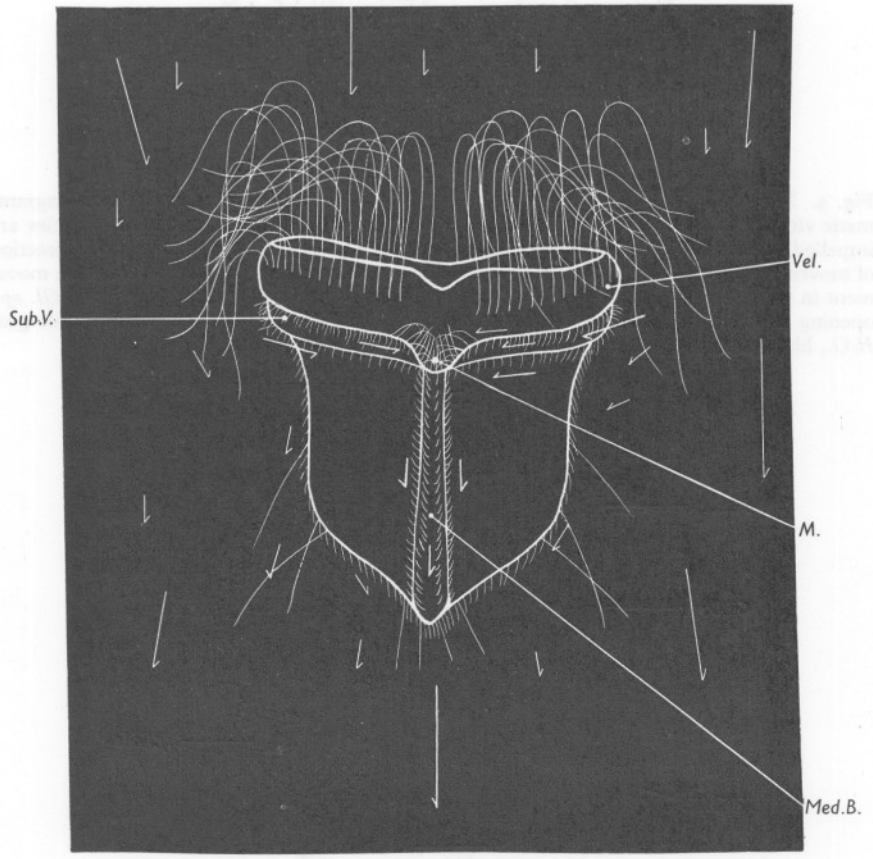


Fig. 4. Cephalopedal ciliary apparatus of larval *Archidoris pseudoargus*: semi-diagrammatic view from ventral aspect. The arrows indicate the direction in which particles are impelled. *M.*, mouth; *Med. B.*, median pedal band; *Sub. V.*, subvelar ridge; *Vel.*, velum.

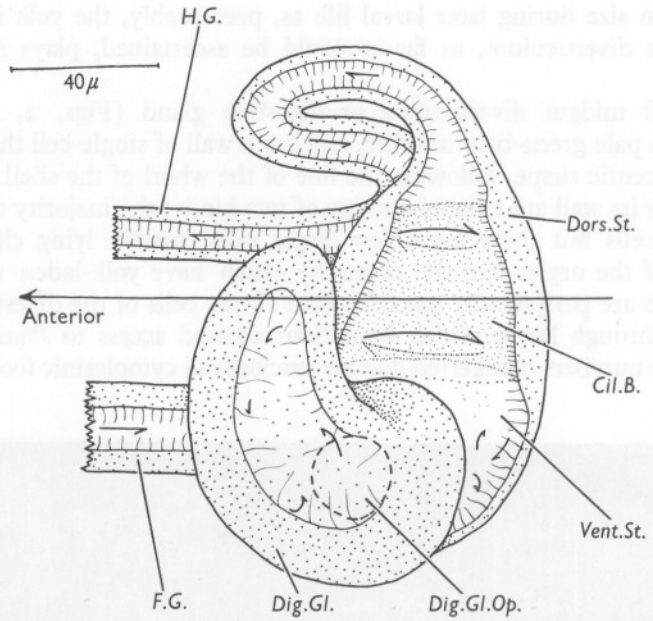


Fig. 5. The stomach and associated organs in larval *Archidoris pseudoargus*: semi-diagrammatic view from left lateral aspect. The arrows indicate the direction in which particles are impelled. In the region of the raised band of stomach cilia, the solid arrows show the direction of movement of particles in the higher focal levels whereas the dotted arrows show the movement in the lower levels. *Cil. B.*, raised band of cilia; *Dig. Gl.*, digestive gland; *Dig. Gl. op.*, opening of digestive gland into stomach; *Dors. St.*, dorsal region of stomach; *F.G.*, foregut; *H.G.*, hindgut; *Vent. St.*, ventral region of stomach.

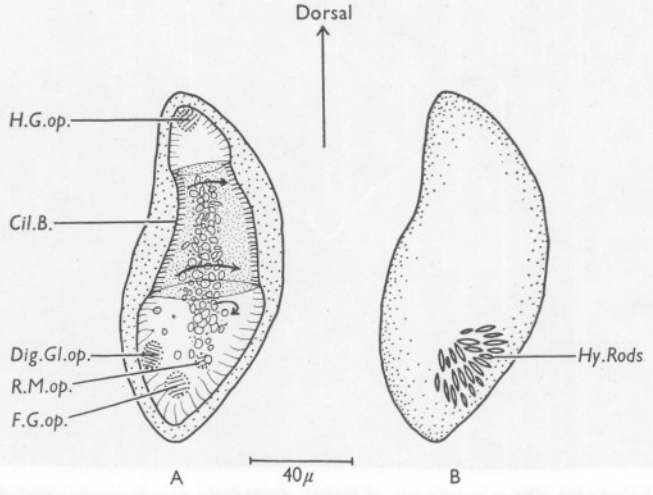


Fig. 6. The stomach in larval *Archidoris pseudoargus*: semi-diagrammatic view from the posterior aspect. A, in optical section; B, surface view only. *Cil.B.*, raised band of cilia; *Dig. Gl. op.*, opening of digestive gland into stomach; *F.G. op.*, opening of foregut into stomach; *H.G. op.*, opening of hindgut into stomach; *Hy. Rods*, zone of hyaline rod-like bodies; *R.M. op.*, opening of right midgut diverticulum into stomach.

in the digestive cells. The food vacuoles are nearly always situated close to the nuclei. Particles of carmine powder were not taken up by the digestive gland cells, as far as could be ascertained.

The hindgut (Figs. 2, 5, *H.G.*).

This is an internally ciliated tube, leaving the midgut dorsally and passing to the anus, situated in the mantle fold, latero-ventrally on the right side. Metachronal waves passing towards the stomach are always clearly evident. The anal opening is immediately ventral to the opening of the larval kidney (Fig. 2, *K*2). A pair of colourless vesicles of unknown function lie embedded in the mantle fold close to the anus (Fig. 2, *A.*). The cilia of the mantle fold and a zone of especially strong cilia on the right side of the foot (see Fig. 2) disperse the faeces as they emerge from the anus. The faeces are not cemented together into strings or pellets as they are in post-larval stages.

Course of particles entering the mouth

Particles on entering the mouth are rapidly transported by the foregut cilia into the stomach. In the stomach the raised ciliary band brings about a rapid rotation, a loose rod of particles soon being formed. The ventral extremity of this rod always is close to the patch of hyaline rod-shaped bodies in the stomach wall (see Fig. 6); it seems likely that this patch is the site of some enzymatic activity. Particles apparently haphazardly leave the rod of food particles and are impelled by the more sluggish cilia of the ventral stomach into the digestive gland. When in the lumen of the digestive gland, a proportion of them are ingested.

Millar (1955), working on larvae of *Ostrea edulis*, states that 'it is a matter of chance whether material drawn off into the midgut and thence passed to the rectum has been in the stomach for a short or a long time'; although this may be true in the larval oyster, and although no definite proof to the contrary can be provided by the present investigation, the impression was gained that a definite pattern of treatment for each new incoming particle was present, and that no particle of food value was passed directly into the hindgut unless a superabundance of such particles was present.

Passage of particles from the stomach into the digestive gland is brought about by ciliary means; nevertheless, contraction of muscle fibres in the membranes enclosing the visceral organs (Thompson, 1958) can bring about partial contraction of the organs and probably aids this interchange. Millar (1955) described a similar phenomenon in *Ostrea*.

OTHER SPECIES

In all the other nudibranch larvae examined, with the partial exceptions of *Trinchesia aurantia* and *Eubranchus exiguus*, the feeding mechanism is as described for *Archidoris pseudoargus*. Minor specific differences occur, such

as the black pigmented mouth of the larva of *Limapontia depressa* or the hyaline velar granules of *Hero formosa*, but the functions of the various organs and even their relative state of development is closely similar in all. Even the direction of rotation of particles within the stomach was in all cases the same as that shown for *Archidoris* in Fig. 5. (Yonge, 1926, found that the direction of rotation of particles within the stomach of larval *Ostrea* varied from one individual to another.)

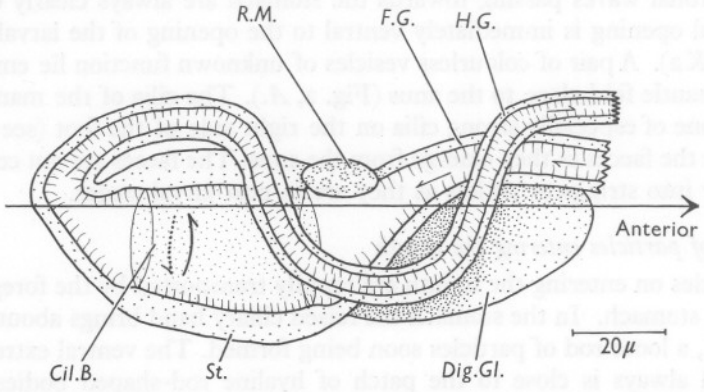


Fig. 7. The stomach and associated organs in larval *Trinchesia aurantia* from right lateral aspect. The arrows indicate the direction in which particles are impelled. In the region of the raised band of stomach cilia, the solid arrow shows the direction of movement of particles in the higher focal levels whereas the dotted arrow shows the movement in the lower levels. *Cil.B.*, raised band of cilia; *Dig. Gl.*, digestive gland; *F.G.*, foregut; *H.G.*, hindgut; *R.M.*, right midgut diverticulum; *St.*, stomach.

The larval feeding mechanism of *Trinchesia aurantia* and *Eubranchus exiguus* in functional essentials differs not at all from that found in larvae of shell-type B, but significant differences are manifest in the arrangement of the visceral organs. Fig. 7 shows the larval gut of *Trinchesia aurantia*; it is obvious that the long axis of the stomach in this species lies along the antero-posterior axis of the larva, while in *Archidoris* (see Fig. 5) and all the other nudibranchs of shell-type B, it lies at right angles to this. The significance of this is not known; in any event the mechanism of food capture in *Trinchesia aurantia* and *Eubranchus exiguus* does not differ from the general pattern, nor could any difference in the treatment of ingested particles be detected. The absence of hyaline rods in the stomach wall of these two species, however, may be of importance.

DISCUSSION

There can be no doubt that nudibranch larvae feed during planktonic life. That this planktonic period is a short one is highly probable (Thorson, 1946) but to dismiss the group as of no importance in the plankton would be inaccurate. All British nudibranchs (leaving aside the Ascoglossa) have a planktonic

larval phase and each individual adult produces very large numbers of eggs during the spawning season.

The mechanism of feeding was very similar in all the species investigated, some difference, however, existing between the orientation of the stomach of the larval shell-types B and C of Thorson (1946). Thorson's contention that nudibranch larvae with shells of type C are more fitted for planktonic life than those of type B finds no support from this investigation: larvae of the two types possess the same organs developed to the same degree.

The mechanism of particle capture in nudibranch larvae is similar in many respects to that described by Yonge (1926) for larvae of *Ostrea*, except that in nudibranchs the subvelar cilia are placed on a pair of prominent ridges. Movement of particles between the lumina of the visceral organs in larval nudibranchs is accomplished chiefly by ciliary means, assisted probably by the muscular movements which have been observed to occur; in *Ostrea* larvae, however, Millar (1955) states that particles pass into and out of the midgut diverticula chiefly or solely as the result of muscular action. The absence of the crystalline style and gastric shield and the unimportant role of the right midgut diverticulum are further differences from the larvae of lamellibranchs. It is possible, however, that the numerous hyaline rods in the nudibranch larval stomach wall (Fig. 6B, *Hy.Rods*), occupying a similar position in all those species in which it is present, serve a function similar to that of the style. The rotation of the particle mass against the site of these hyaline rods suggests strongly this interpretation of their function.

Cellular ingestion of food particles occurs solely in the digestive gland. In nudibranch larvae the right midgut diverticulum plays no part in the digestive process and the foregut and hindgut are concerned simply with the conduction of particles. The contents of the stomach and digestive gland in sections stain lightly with mucus stains and with haematoxylin, suggesting that intracellular digestion, which undoubtedly is the chief method of particle assimilation, may be aided by the production of extra-cellular enzymes. The presence of diffuse organic matter in the lumen of the gut is difficult to interpret otherwise.

The author is indebted to Mr J. S. Colman for helpful criticism and for the provision of laboratory facilities. The work was done while the author was the holder of a Leverhulme Fellowship in the University of Liverpool. The specimens of *Limapontia depressa* were most kindly brought by Mr A. Hopson from Flatford Mill, and those of *Alderia modesta* sent by Dr T. Gascoigne from Anglesey.

SUMMARY

The mechanism of food capture and the movement of food particles within the gut are described from observations on live and sectioned veliger larvae of the glossodorid nudibranch *Archidoris pseudoargus*.

Less detailed observations on the larvae of twelve other nudibranch species with shells of Thorson's type B, and of two species with shells of type C, showed similar mechanisms to exist in all.

Ingestion and assimilation of food particles occur solely in the left midgut diverticulum or digestive gland; some indirect evidence of the presence of extra-cellular enzymes is described.

REFERENCES

BARRON, D. H., 1934. Amyl acetate: a useful solvent for embedding masses. *Anat. Rec.*, Vol. 59, pp. 1–3.

CARTER, G. S., 1926. On the nervous control of the velar cilia of the nudibranch veliger. *Brit. J. exp. Biol.*, Vol. 4, pp. 1–26.

MILLAR, R. H., 1955. Notes on the mechanism of food movement in the gut of the larval oyster, *Ostrea edulis*. *Quart. J. micr. Sci.*, Vol. 96, pp. 539–44.

PELSENEER, P., 1911. Recherches sur l'embryologie des gastropodes. *Mém. Acad. R. Belg. Cl.Sci.*, Ser. II, 3, livr. 6, pp. 1–167.

RASMUSSEN, E., 1944. Faunistic and biological notes on marine invertebrates I. The eggs and larvae of *Brachystomia rissoides* (Hanl.), *Eulimella nitidissima* (Mont.), *Retusa truncatula* (Brug.) and *Embletonia pallida* (Alder & Hancock), (Gastropoda marina). *Vidensk. Medd. dansk naturh. Foren. Kbh.*, *Bd.* 107, pp. 207–33.

—— 1951. Faunistic and biological notes on marine invertebrates. II. The eggs and larvae of some Danish marine gastropods. *Vidensk. Medd. dansk naturh. Foren. Kbh.*, Bd. 113, pp. 202–49.

STEEDMAN, H. F., 1950. Alcian blue 8 GS: a new stain for mucin. *Quart. J. micr. Sci.*, Vol. 91, pp. 477–9.

THOMPSON, T. E., 1958. The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder & Hancock) (Gastropoda Opisthobranchia). *Phil. Trans.* B, Vol. 242, pp. 1–58.

THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Komm. Havundersøg, Kbh.*, Ser. Plankt, Bd. 4, No. 1, 523 pp.

VESTERGAARD, K. & THORSON, G., 1938. Über den laich und die larven von *Duvaucelia plebeja*, *Polycera quadrilineata*, *Eubranchus pallidus* and *Limapontia capitata* (Gastropoda, Opisthobranchiata). *Zool. Anz.*, Bd. 124, pp. 129–38.

WINCKWORTH, R., 1932. The British marine Mollusca. *J. Conchol.*, Vol. 19, pp. 211–52.

—— 1951. A list of the marine Mollusca of the British Isles: additions and corrections. *J. Conchol.*, Vol. 23, pp. 131–4.

YONGE, C. M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. mar. biol. Ass. U.K.*, Vol. 14, pp. 295–386.

J. mar. biol. Ass. U.K. (1959) 38, 249–261
Printed in Great Britain

ECOLOGY OF THE GENUS *ACANTHOCHONDRIA* OAKLEY (COPEPODA PARASITICA)

By Z. KABATA

The Marine Laboratory, Aberdeen

(Text-figs. 1–4)

Among the parasites of the branchial chamber of the flatfishes of the Eastern North Atlantic, those belonging to the genus *Acanthochondria* (fam. Chondracanthidae, tribe Cyclopiformes) occupy a conspicuous place. Both their relatively large size and their abundance, render them easily noticeable even to a casual observer, in spite of the protective nature of their environment. It is a reflexion on the state of our knowledge of marine parasites that, notwithstanding their accessibility, very little is known about the animals of this genus.

The genus *Acanthochondria* was erected by Oakley (Leigh-Sharpe and Oakley, 1927) for the chondracanthids, which until then were included in *Chondracanthus* Delaroche, 1811, and which differed from the other members of that genus in the absence of the cephalic barbs and the dorsal and ventral processes. They were also characteristic in their preference for the flatfish hosts.

The workers who studied the family Chondracanthidae in the past were chiefly concerned with its taxonomy. A short résumé of the work on the family was given by Oakley (1930), who contributed greatly to our understanding of the relationships of the chondracanthids, but did not go beyond their systematics. Of the more recent workers Heegaard (1945, 1947) studied the development of *Acanthochondria*, and Deboutteville (1951*a*, *b*) the general biology of the chondracanthids by observation, among others, of living animals. Their work, however, was also intended to bring out the taxonomic and phylogenetic relationships, both within the family and within the Arthropoda as a phylum. Similar purposes were served by the work of Illg (1948).

It is intended in this paper to consider the genus *Acanthochondria* from the ecological point of view, by discussing the distribution, abundance and the details of the habitats of its members in the Eastern North Atlantic. The latter term is used here to cover three areas: the northern North Sea and the seas around the Faroe Islands and Iceland. The great majority of data obtained in the course of this work were collected by the Fishery Research Ships "Scotia" and "Explorer" of the Scottish Home Department's Marine Laboratory,

Aberdeen. Some of the specimens of *A. cornuta* were collected by the Laboratory's earlier vessel "Goldseeker" in Scottish waters in 1921 and those of *A. solae* came from the collection of the late Dr T. Scott, now in the Royal Scottish Museum, Edinburgh.

ACANTHOCHONDRIAN FAUNA OF THE AREA

It is unfortunate that no agreement seems to exist at present as to the number and identity of the species of *Acanthochondria* occurring in the area under investigation. Apart from three undoubtedly valid species (*A. clavata*, *A. limandae* and *A. depressa*) there exist three more (*A. cornuta*, *A. flurae* and *A. solae*), the identity of which has been doubted by some authorities. Brian (1906) suggested that *A. flurae* and *A. solae* are merely varieties of *A. cornuta*. Hansen (1923) also regarded *A. cornuta* and *A. flurae* as being phenotypes of the same species. Hansen's opinion was accepted by Stephensen (1940) but not followed by Leigh-Sharpe & Oakley (1927), Oakley (1930) and Oorde-de Lint & Stekhoven (1936).

A. solae has not been found by the author. It is very rare in the area and will not be considered in detail in this paper. The morphology of the specimens from Dr T. Scott's collection was so distinct, that the author found it impossible to agree with Brian's opinion concerning this species. Brian's and Hansen's objections to the separation of *A. flurae* from *A. cornuta* cannot, however, be easily dismissed. Those who believe these two parasites to be distinct, regard the shape of the trunk as the main discriminant. The trunk of *A. cornuta* is taken to be long and slender, that of *A. flurae* short and squat. Another distinguishing feature used is that of specificity, *A. cornuta*, apparently occurring on the plaice, *Pleuronectes platessa* L., while *A. flurae* occurs on the long rough dab, *Drepanopsetta platessoides* Malm. According to Hansen, however, all the intermediate stages between the two typical shapes of the trunk existed and could be found on both flatfishes.

To check quantitatively the range of variation in the shape of the trunk of both parasites, the author compared the length: width ratios of 106 specimens collected from the long rough dab with those of 31 specimens from the plaice. The ratios of the former ranged from 1·9 to 3·3 (mean ratio 2·5) and those of the latter from 2·8 to 5·0 (mean ratio 3·6). Statistical analysis shows the significance of the difference between these groups of ratios to be at 0·001 level. It would appear, therefore, that in spite of some overlap of ratios the retention of the specific status for both these parasites is correct. The position is made complicated, however, by the existence of a population of *Acanthochondria*, superficially resembling those on the plaice (*cornuta* type), on the witch, *Glyptocephalus cynoglossus* (L). This flatfish has been previously recorded as a host of *A. flurae*. The examination of 10 specimens from it has shown their length: width ratios to range between 3·8 and 6·0 (mean ratio 4·8). The level of significance of the

difference between the parasites of the plaice and those of the witch is also at 0·001 level. We have thus three significantly different populations, one (from the long rough dab) of *flurae* type and two of *cornuta* type.

The mouthparts of *Acanthochondria* have been studied by Oakley (1930) and were used by him as diagnostic features in identifying various species of the genus. His valuable work is now in some respects outdated and the mouthparts require re-examination. Because of the scarcity of material, Oakley (personal communication) was unable to dissect his specimens and had to rely on the examination of whole mounts. Examined from one aspect only,

TABLE 1. SPECIES OF *ACANTHOCHONDRIA* OCCURRING IN THE EASTERN NORTH ATLANTIC AND THEIR HOSTS

Species of *Acanthochondria*	Host species: According to previous reports	Host species: As found by the author
A. cornuta (O. F. Müller, 1777) (Fig. 1)	*Platichthys flesus* (L.), *Pleuronectes platessa* L., *Scophthalmus maximus* (L.), *Lepidorhombus whiff-iagonis* (Walbaum)	*Pleuronectes platessa* L., *Glyptocephalus cynoglossus* (L.)
A. solae (Krøyer, 1838)	*Solea vulgaris* Day, *Platichthys flesus* (L.), *Pleuronectes platessa* L.	
A. flurae (Krøyer, 1863) (Fig. 1)	*Drepanopsetta platessoides* Gill, *Glyptocephalus cynoglossus* (L.)	*Drepanopsetta platessoides* Gill, *Lepidorhombus whiff-iagonis* (Walbaum), *Platichthys flesus* (L.)
A. limandae (Krøyer, 1863) (Fig. 1)	*Limanda limanda* (L.) *Platichthys flesus* (L.)	*Limanda limanda* (L.)
A. clavata (Basset-Smith, 1896) (Fig. 1)	*Microstomus kitt* (Walbaum)	*Microstomus kitt* (Walbaum)
A. depressa (T. Scott, 1905)	*Platichthys flesus* (L.)	

the shape of the mandibular palp,* as shown by Oakley, bears only a general resemblance to this appendage as examined by the author. In our present state of knowledge this structure cannot be used in diagnosing specific differences.

No final decision can as yet be reached on morphological grounds as to the distinctness of the two species. Ecological differences, however, encountered by the author in his study of *Acanthochondria*, tended to separate the parasites of the long rough dab on the one hand and those of the plaice and the witch on the other. In view of those differences the author decided to treat them as distinct and regard those from the long rough dab as *A. flurae* and those from other two hosts as *A. cornuta*.

Bearing the foregoing remarks in mind we can now postulate that six species of *Acanthochondria* occur in the investigated area. They are listed in Table 1, which shows also their host species, both as they are recorded in literature and as found by the author.

* Regarded by Heegaard (1947) as the first maxilla.

Numerous records of the occurrence of *Acanthochondria* in the eastern North Atlantic have been quoted by Oorde de Lint & Stekhoven (1936). According to these records, *A. cornuta* (Fig. 1A) occurs in various parts of the North Sea and at Faroe and Iceland. *A. clavata* (Fig. 1D) is said to occur in the North Sea (Firth of Forth), Firth of Clyde, and at Faroe. *A. depressa* occurs in the North Sea, *A. flurae* (Fig. 1B) on the Scottish coast and at Faroe and Iceland, *A. limandae* (Fig. 1C) in Moray Firth and Firth of Clyde and at Faroes, *A. solae* in the North Sea.

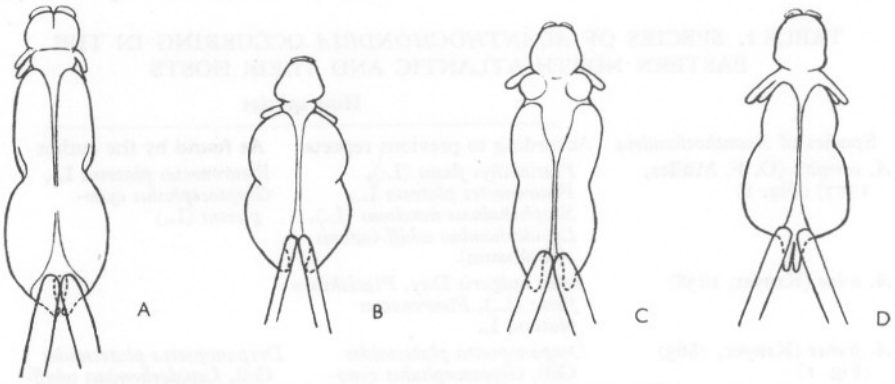


Fig. 1. General appearance of (A), *Acanthochondria cornuta*, (B) *A. flurae*, (C) *A. limandae* and (D) *A. clavata*.

To study the incidence of *Acanthochondria* the author examined 3961 flatfishes: 2553 from the North Sea, 419 from Faroe and 990 from Iceland. No specimens were found of *A. solae* or *A. depressa*. The latter species infests the flounder, *Platichthys flesus* (L.), and this fish was not extensively examined by the author, as the majority of the samples were collected from the open sea. The remaining four species, according to the data collected, are distributed quite widely throughout the localities inhabited by the host populations and show no distinct pattern of distribution within the separate areas.

The genus *Acanthochondria* shows a decrease in the number of species in the northerly direction. Six species are present in the North Sea, four off the Faroes and only two off Iceland. *A. depressa* and *A. solae* are absent from the acanthochondrian fauna of Faroes and Iceland. Only *A. flurae* and *A. cornuta* have been found in Icelandic waters. The explanation of this pattern in terms of limits to the distribution of the host populations is not quite satisfactory. For example, the lemon sole, *Microstomus kitt* (Walbaum), is found throughout the entire area, but is not infested at Iceland.

Consideration of distribution of *Acanthochondria* invites speculation on the way in which this genus spread throughout the area. The spread seems to have proceeded northwards from a southern centre, probably the environmental

conditions acting as the chief limiting factors. A similar way of colonizing the northern grounds has been suggested by the author (1957) for the genus *Lernaeocera*.

ABUNDANCE

The abundance of *Acanthochondria* varies quite considerably both from species to species and from area to area.

In the North Sea *A. flurae* and *A. clavata* infest more than 20% of their host populations, *A. cornuta* 11% and *A. limandae* 2% (Table 2). A similar relative abundance is shown by the two species present at Iceland, *A. flurae* and *A. cornuta* (Table 3).

TABLE 2. ABUNDANCE OF *ACANTHOCHONDRIA* IN THE NORTHERN NORTH SEA

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	155	8	5·2	*A. cornuta*
Platichthys flesus	19	2	10·5	*A. flurae*
Microstomus kitt	407	96	23·6	*A. clavata*
Drepanopsetta platessoides	1001	202	20·2	*A. flurae*
Limanda limanda	704	17	2·4	*A. limandae*
Glyptocephalus cynoglossus	98	11	11·2	*A. cornuta*
Lepidorhombus whiff-iagonis	159	—	—	—
Hippoglossus hippoglossus	9	—	—	—

TABLE 3. ABUNDANCE OF *ACANTHOCHONDRIA* OFF ICELAND

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	118	9	7·6	*A. cornuta*
Microstomus kitt	131	—	—	—
Drepanopsetta platessoides	410	96	23·2	*A. flurae*
Limanda limanda	105	—	—	—
Glyptocephalus cynoglossus	101	3	2·9	*A. cornuta*
Lepidorhombus whiff-iagonis	96	1	1·0	*A. flurae*
Hippoglossus hippoglossus	29	—	—	—

TABLE 4. ABUNDANCE OF *ACANTHOCHONDRIA* OFF THE FAROES

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	26	10	38·5	*A. cornuta*
Microstomus kitt	150	59	39·3	*A. clavata*
Drepanopsetta platessoides	62	11	17·7	*A. flurae*
Limanda limanda	70	25	35·7	*A. limandae*
Hippoglossus hippoglossus	111	—	—	—

The abundance of acanthochondrians off the Faroes is, however, quite different (Table 4). With the important exception of *A. flurae*, which shows an infestation incidence of the same order as in the other areas, all the three remaining species are much more abundant. Most surprising is the increase in abundance in comparison with the North Sea of *A. limandae* and *A. cornuta*, both of which infest over 30% of their host populations.

The abundance of a parasite cannot be fully measured by the percentage of the host population infested. It is also important to consider the numbers of parasites on an individual host. The details of this aspect of abundance are shown in Table 5.

TABLE 5. NUMBERS OF *ACANTHOCHONDRIA* ON AN INDIVIDUAL FISH

Species	North Sea		Faroes		Iceland	
	Nos. on one fish	Host / Parasite	Nos. on one fish	Host / Parasite	Nos. on one fish	Host / Parasite
A. flurae	1–5	3·5	1–3	4·1	1–7	2·7
A. cornuta (witch)	1–5	5·3	—	—	1–2	25·2
A. cornuta (plaice)	1–2	16·6	1–6	1·3	1	10·7
A. clavata	1–9	2·3	1–23	0·98	—	—
A. limandae	1–2	26·1	1–2	2·0	—	—

HABITAT

The members of the genus *Acanthochondria* are described by various authors as parasites of the gill cavity, of the gill arches or the gills. No study has been made, to the knowledge of the author, to determine the exact character of their habitat. Even less is known of the possible differences of the habitat between the species.

The numbers of both parasites and hosts studied in the course of this work have been sufficiently large to learn something of the habitats of *Acanthochondria*. No two species of this genus were identical in the manner of distribution within the gill cavity of their corresponding hosts. There existed also differences in the manner of association of *A. cornuta* with its two different hosts.

To examine habitats available to *Acanthochondria* on potential hosts, the opercular cavities of seven flatfish species were examined, by dissection and by making casts of subopercular spaces in latex rubber. The latter method is especially useful, as the shape of the cast remains unaffected by any strains during its removal.

The study of the respiratory apparatus of flatfishes has shown differences between the species, which may be said to form a range between two extremes, referred to here as "open" and "closed" types. The difference between the two types depends on the degree of development of the branchiostegal membranes, their relationship in space to the gills, on the amount of free space under the opercula and on the size and the shape of the mouth gape. The most typical representatives of the "open" type are the halibut, *Hippoglossus hippoglossus* (L.) and the megrim, *Lepidorhombus whiff-iagonis*. It will be seen from Fig. 2A that the branchiostegal membrane of the megrim is narrow, forming only a shallow pocket with the operculum, along the posterior border of which it is missing altogether. With the operculum *in situ* the inner

rim of the membrane is situated at some distance from the tips of the gill filaments, especially in the posterior part of the cavity. The gills, in other words, are not enclosed in the branchiostegal pocket. The membranes of the opposite opercula are not fused to any great extent. The gape of the mouth is wide. It appears that this type of the respiratory apparatus is associated with active mode of life, since the halibut shows an essentially similar type of the gill cavity. These two fishes are not infested by *Acanthochondria* (149 of the examined halibut were found to be free of infestation and on 254 megrims only one specimen of *A. flurae* was found attached to the gill raker, a quite atypical habitat).

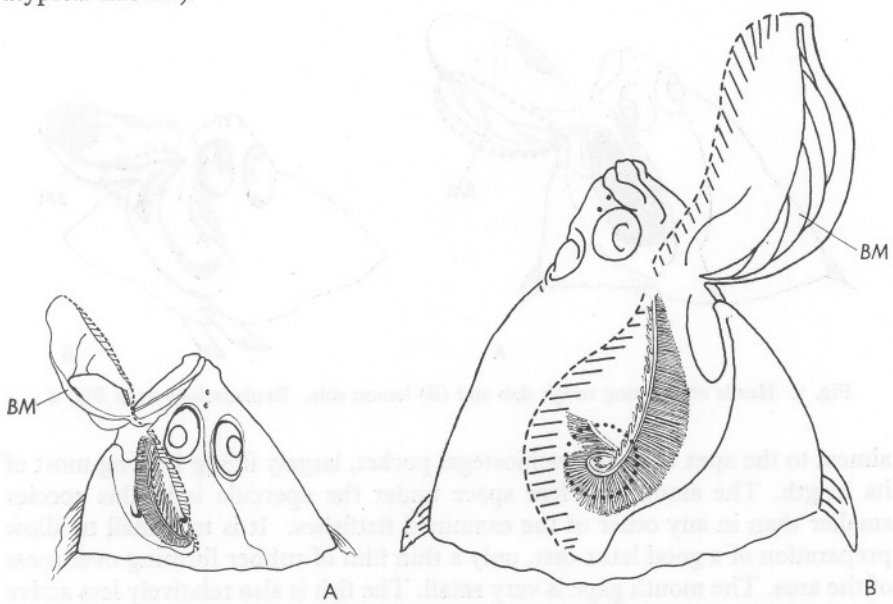


Fig. 2. Heads of (A) megrim and (B) plaice, with operculum dissected away to show spatial relations between various elements of the respiratory mechanism. Area of attachment of parasites indicated by dotted ring. *BM*, branchiostegal membrane.

Three further species of flatfish: the plaice, *Pleuronectes platessa* (Fig. 2B), the common dab, *Limanda limanda*, and the long rough dab, *Drepanopsetta platessoides* (Fig. 3A) are also of the 'open' type, although more towards the other end of the range. Their branchiostegal membranes are better developed, the tips of the gill filaments being just within the branchiostegal pockets. The gapes of the mouths are smaller than in the two preceding species, but still relatively well developed. These fishes, as far as we know, differ among themselves to some extent in their modes of life, the long rough dab being predatory and most active of the three. Not enough is known about the differences between the other two species, but their inclusion into the same group is not contrary to anything we know about their lives.

Nearer still towards the 'closed' type is the witch, *Glyptocephalus cynoglossus*, in which the branchiostegal pocket is more strongly developed, the membranes enclosing more of the gills and fusing with each other for a longer distance than in the preceding species. The gape of the mouth is smaller also.

At the end of the range is the extreme example of the 'closed' type, the lemon sole *Microstomus kitt* (Fig. 3B). In this species the membranes are fused quite a long way, enclosing the urohyal element of the pectoral girdle. The membranes are well developed, although narrowing posteriorly, and extend right round the opercular opening. The tips of the gill filaments project

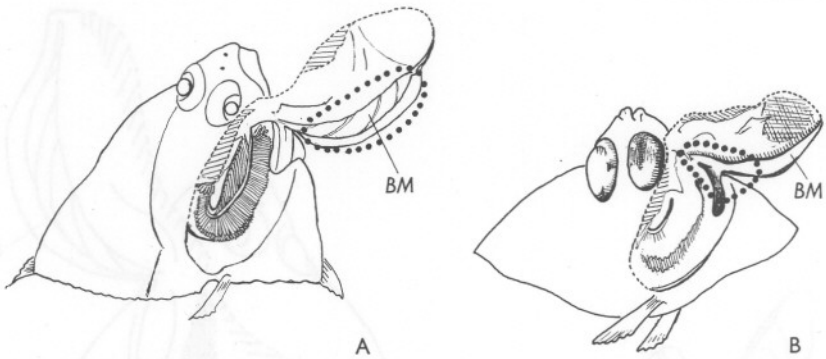


Fig. 3. Heads of (A) long rough dab and (B) lemon sole. Explanations as in Fig. 2.

almost to the apex of the branchiostegal pocket, largely filling it along most of its length. The amount of free space under the opercula is in this species smaller than in any other of the examined flatfishes. It is too small to allow preparation of a good latex cast, only a thin film of rubber forming over most of the area. The mouth gape is very small. The fish is also relatively less active than other flatfishes, as indicated by its diet.

As mentioned before, the first two species of the 'open' type are not infested by *Acanthochondria*. The next group of three shows two different ways of association with it. The first kind is illustrated by the distribution of *A. flurae* on the long rough dab (Fig. 4A). For the purpose of recording the distribution of the parasites within the opercular cavity, it was divided into four sections: three of roughly equal length along the edge of the operculum, the fourth comprising the urohyal. (The double line in the diagram indicates the extent of fusion of the branchiostegal membranes.) It will be seen that the great majority of the parasites (a sample of 331 *A. flurae*) are attached to the two posterior sectors of the opercula, all being within the branchiostegal pocket. Relatively few are found in the anterior sector of the opercula, where the membranes are fused. About one-third of the total number are attached to the urohyal. There is no significant difference between the two sides of the

gill cavity, the ocular side harbouring 44·4% and the blind side 55·6% of the parasites.

The distribution of the parasites on the other two members of this 'open' group is entirely different. *A. cornuta* on the plaice is found in a very definitely circumscribed and small locality (Fig. 2B). It is attached usually in the narrow space between the end of the first branchial arch and the pseudobranch. Only very few exceptions were found in the anterior end of the opercular cavity, all firmly wedged between the bases of the branchial arches. *A. limandae* on the common dab is found only in the latter place. No parasites were found in the branchiostegal pocket of either plaice or common dab.

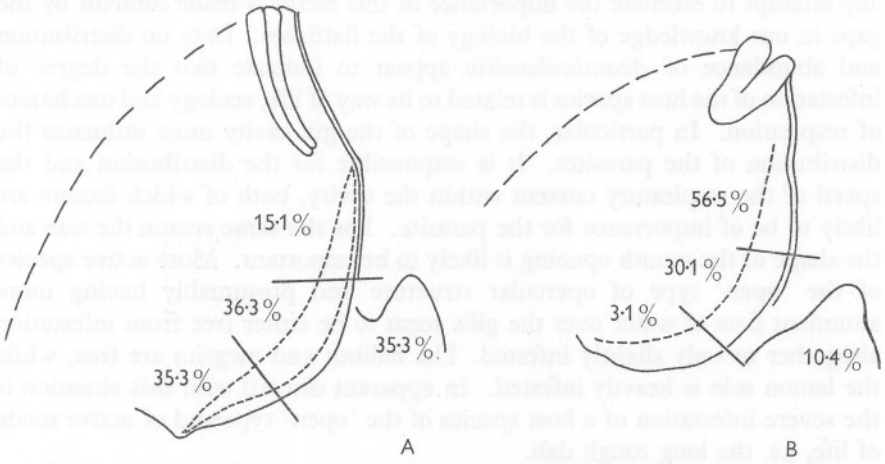


Fig. 4. Diagrams of the heads of (A) long rough dab and (B) lemon sole, showing the distribution of *Acanthochondria* in the opercular cavity.

The witch, more closely resembling the 'closed' type of the opercular structure is also infested with *A. cornuta*, which is so strikingly limited in the distribution on the plaice. Only few parasites were found on the witch, but those found were distributed along the branchiostegal pocket, not showing special preference towards any particular sector. None were present in the cleft between the pseudobranch and the base of the first gill arch and none either between the anterior bases of the arches.

The last of the hosts, the typically 'closed' type lemon sole, is heavily infested with *A. clavata*. The distribution of this species on the lemon sole is indicated by the dotted ring in Fig. 3B and diagram in Fig. 4B. The latter figure shows that more than a half of the sample of 193 parasites were distributed in the anterior sector of the branchiostegal pocket. The numbers of parasites in that pocket decreased posteriorly, only few being found in the posterior sector, where the membranes are most narrow. In this species there is also no appreciable difference between the ocular and the blind side of the cavity, the former carrying 52·3% and the latter 47% of the parasites.

DISCUSSION

The form of the host-parasite relationship is the outcome of the interaction of three factors: the host, the parasite and the external environment. All three factors contribute influences responsible for the final equilibrium. In the association between *Acanthochondria* and its flatfish hosts the operation of all these influences seems to be indicated by the facts related in the preceding sections of this paper.

The ecology of the host is undoubtedly responsible for the development of the specific relationship between it and the parasitic species. Unfortunately, any attempt to estimate the importance of this factor is made difficult by the gaps in our knowledge of the biology of the flatfishes. Data on distribution and abundance of *Acanthochondria* appear to indicate that the degree of infestation of the host species is related to its way of life, ecology and mechanics of respiration. In particular, the shape of the gill cavity must influence the distribution of the parasites. It is responsible for the distribution and the speed of the respiratory current within the cavity, both of which factors are likely to be of importance for the parasite. For the same reason the size and the shape of the mouth opening is likely to be important. More active species of the 'open' type of opercular structure and presumably having more abundant flow of water over the gills seem to be either free from infestation altogether or only slightly infested. The halibut and megrim are free, while the lemon sole is heavily infested. In apparent discord with this situation is the severe infestation of a host species of the 'open' type and of active mode of life, i.e. the long rough dab.

It is useful at this point to consider the role of the parasite in establishing host-parasite equilibrium. It seems that, on the whole, *Acanthochondria* favour habitats which are well protected and rather limited in the amount of free space. *A. cornuta* on the plaice appears to ignore the expansive areas of possible foothold offered by the broad branchiostegal membranes and lives only in the narrow gap, probably out of the way of the main respiratory current. The same species on the witch is found in the branchiostegal pocket, which in this fish is much narrower and has less free space, being filled by the tips of the gill filaments. It might be expected that the branchiostegal pocket of the witch has less flow of water and affords more protection than that of the plaice. *A. limandae*, found on the common dab, a fish of the same type as plaice, is also attached in a well protected and narrow space, this time between the bases of the gill arches. *A. clavata*, so abundant on the lemon sole, also appears to be most common in the best protected part of the branchiostegal pocket. Apart from the sensitivity to the conditions of the current, this might be related in some way to the well-developed tactile sense of the chondracanthids (Stekhoven, 1934; Goggio, 1927). It is not unlikely that a habitat affording optimal thigmotropic conditions might be chosen by a parasite

which appears to possess some power to change the place of attachment (Stekhoven, 1934).

Structural similarity of various species of *Acanthochondria* is not necessarily paralleled by biological uniformity. It is probable that they differ among themselves both in demands put on their hosts and in the range of tolerance of the conditions of the external environment. One fact, pointing to possible biological differences is, for example, the difference in fecundity. According to the production of eggs per individual, the four species investigated in this paper can be divided into two groups:

	No. of eggs	Diameter of eggs (mm)
A. flurae	1,427	0·15
A. clavata	1,328	0·15
A. limandae	860	0·13
A. cornuta	759	0·14

The individuals sampled for egg counts were chosen so as to avoid possible variations due to the size of the animal, the locality and the season of the year. Statistical analysis of these numbers shows the difference between the two groups to be significant at the 0·001 level. The two less fecund species are also less abundant in two out of three areas examined. Both are also restricted to a very small locality in distribution on their hosts. Their eggs are slightly smaller, perhaps due to the lesser quantity of yolk contained and this might be of biological significance. It is also interesting that the only available specimen of the very rare *A. solae* had only 328 eggs, 0·12 mm in diameter, both values being lowest for any individual of any species examined.

Summing up the above considerations we might say that: (i) the more active the flatfish species, the less it is used as a host by *Acanthochondria*; (ii) this may be due to the parasites of the genus preferring environments well protected and restricted in space; (iii) the species which are less fecund, and more restricted in distribution on the host, tend to be less abundant. There are two important exceptions to these general principles. One active host of the 'open' type, the long rough dab, is heavily infested, the incidence of infestation being of the same order in all areas (Tables 2, 3, 4). At the same time the distribution of *A. flurae* on the long rough dab is consistent with the distribution of the space available within the gill cavity, irrespective of the degree of protection. It is suggested that this is possibly due to the lesser need of protection and wider range of tolerance of the environmental conditions on the part of *A. flurae*. Such tolerance would tend to eliminate to some extent the environmental influences from the interplay of the three factors involved in the host-parasite equilibrium, leaving it undisturbed over the large area. Some differences in the environmental conditions undoubtedly exist between the North Sea, Faroe and Iceland. The absence of *A. clavata* from Icelandic waters inhabited by an abundant population of the lemon sole, indicates the possibility of environmental limiting factors. In face of such possible

differences *A. flurae* maintains a fairly constant degree of abundance in all areas.

In contrast to this uniformity of abundance of *A. flurae*, two other species, *A. limandae* and *A. cornuta*, are highly abundant on their respective hosts, the common dab and the plaice, at the Faroes (Table 3) but not at Iceland or in the North Sea, where they are less abundant than *A. flurae* and *A. clavata* (Tables 2 and 4). It is suggested that this might be due to their greater susceptibility to the environmental conditions, which favour their abundance at the Faroes but oppose it in the North Sea and at Iceland. That the environment at the Faroes is particularly favourable to *Acanthochondria* seems to be indicated by the fact that all the species of this genus, with the exception of *A. flurae*, are more abundant in that area than elsewhere.

All the biological differences between *A. flurae* and the remaining species, including *A. cornuta*, seem to lend support to those who believe in the distinctness of *A. flurae* from *A. cornuta*.

The author wishes to express his gratitude to Dr J. H. Fraser of the Marine Laboratory, Aberdeen, for his criticism of the manuscript and suggestions for its improvement.

SUMMARY

Four species of *Acanthochondria* are discussed in relation to their hosts, the flatfishes. The more active flatfishes, with less protected opercular cavities, appear to be less suitable as hosts for this genus of parasites.

A. flurae (found on the long rough dab) is equally abundant in the North Sea and at Faroe and Iceland. *A. clavata* (found on the lemon sole) is abundant in the North Sea, even more so at Faroe, but is absent from Icelandic waters. *A. limandae* (found on the common dab) is scarce in the North Sea, abundant at Faroe and absent from Iceland. *A. cornuta* (found on the plaice and the witch) is scarce in the North Sea and at Iceland, but very abundant at Faroe.

The four species differ in distribution on their respective hosts, all with the exception of *A. flurae* choosing well protected environments, which vary with the host. *A. flurae* appears to be less sensitive to the environment and directed in its choice more by the amount of space available for attachment. The abundance of the parasitic species in the area depends probably also on the environmental conditions.

The difference in biology between *A. flurae* and other species supports the opinion that it is a separate species.

REFERENCES

BRIAN, A., 1906. *Copepodi parassiti dei peschi d'Italia.* 187 pp. Genova.

DEBOUTTEVILLE, C. D., 1951*a*. Présence de la sous-famille des *Pharonidae* Illg (Copepodes, *Chondracanthidae*) en Méditerranée et remarques sur la classification et la biologie des Chondracanthidae. *Arch. Zool. exp. gén.*, T. 87, pp. 139–55.

—— 1951*b*. Sur la biologie des Chondracanthidae (Crustacés Copépodes). *C.R. Acad. Sci., Paris*, T. 232, pp. 117–9.

GOGGIO, E., 1927. Richerche sulla vita, sulla conformazione esteriore e sulla struttura del *Chondracanthus lophii* Johnst. *Pubb. Staz. zool. Napoli*, Vol. 8, pp. 427–74.

HANSEN, H. J., 1923. Crustacea Copepoda. II. Copepoda parasita and hemiparasita. *Dan. Ingolf-Exped.*, Vol. 3, (7), 92 pp.

HEEGAARD, P., 1945. Remarks on the phylogeny of the arthropods. *Ark. Zool.*, Vol. 37. No. 3, 15 pp.

—— 1947. Contribution to the phylogeny of the arthropods. Copepoda. *Spolia zool. Mus. Hauniensis*, Vol. 8, 236 pp.

ILLG, P. L., 1948. *Pharodine*, a new subfamily of *Chondracanthidae* (Crustacea-Copepoda) and a description of *Pharodes biarensis* n.sp. from New Guinea. *J. Wash. Acad. Sci.*, Vol. 38, pp. 404–12.

KABATA, Z., 1957. *Lernaeocera obtusa* n.sp., a hitherto undescribed parasite of the haddock (*Gadus aeglefinus* L.). *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 569–92.

LEIGH-SHARPE, W. H. & OAKLEY, C. L., 1927. *Lernentominae*, a new subfamily of *Chondracanthidae*, with a description of *Orialen triglae* (Blainville, 1822). *Parasitology*, Vol. 19, pp. 455–67.

OAKLEY, C. L., 1930. The Chondracanthidae (Crustacea: Copepoda); with a description of five new genera and one new species. *Parasitology*, Vol. 22, pp. 182–201.

OORDE DE LINT, G. M. VAN & STEKHOVEN, J. H. S., 1936. Copepoda parasitica. *Tierw. N.- u. Ostsee*, Teil 10C, pp. 13–198 (Lief 31).

STEKHOVEN, J. H. S., 1934. Zur Sinnesphysiologie der parasitären Copepoden *Lepeophtheirus pectoralis* (O. F. Müller) und *Acanthochondria depressa* (T. Scott). *Z. Parasitenk.*, Bd. 7, pp. 336–62.

STEPHENSEN, K., 1940. Parasitic and semiparasitic Copepoda. *Zoology of Iceland*, Vol. 3, Pt. 34, 24 pp.

J. mar. biol. Ass. U.K. (1959) 38, 263–266
Printed in Great Britain

RESPIRATION IN *CHONDRACANTHUS ZEI* DE LA ROCHE (COPEPODA)

By S. KRISHNASWAMY[1]

Department of Zoology, University, Southampton

(Text figs. 1–3)

In spite of the keen interest evinced in the taxonomy of the parasitic copepods, there is little information on their metabolic rate, though the respiration of free-living forms has been studied by such workers as Marshall & Orr (1955, 1957), Clarke & Bonnett (1939), Zeuthen (1947), Raymont & Gauld (1951), Gauld & Raymont (1953) and Conover (1956). Since the rate of oxygen consumption is a reflexion of the metabolic rate of an animal, it was felt that a study of the rate of oxygen consumption of a copepod parasite would be of interest. *Chondracanthus zei* De la Roche, 1811, is easily available and large.

C. zei is found on the gills and gill-arches of *Zeus faber* and is 'almost always present' (Oakley, 1930). Usually two to three parasites are found in each fish. It is a relatively large parasite, about 1·3 cm, and weighing up to 200 mg. It shows little movement in life except for occasional twitches, and is so deeply embedded in the tissue as to form a deep wound.

MATERIAL AND METHODS

The work was carried out at the Marine Biological Laboratory at Plymouth. The respiratory rate was followed in a Warburg manometer. Two to four animals, depending on their size, were used in each flask with 3 ml. of sea water; the CO_2 was absorbed by a roll of starch-free filter-paper dipping into 0·2 ml. of 20% KOH contained in the central wall of the flask. The manometers were shaken at sixty oscillations per minute. It was found that shaking did not damage the animal nor apparently in any way depress the respiratory rate. Pressures were corrected every 15 min and each experiment lasted for 3 h approximately. In all thirty-seven experiments were performed, but on occasions the same animals were used in more than one experiment owing to the difficulty in procuring enough material. All the experiments were carried out at 20° C. After each experiment, the animals were transferred on to filter-paper and weighed, and were then dried to constant weight in an oven at 100° C.

RESULTS AND DISCUSSION

The overall respiration rate varied from 19 to 30 μl. per copepod per hour. The same results expressed as oxygen consumed per mg dry weight per hour varied from 0·19 to 1·8 μl. depending on the size of the animal. There is a wide scatter (Fig. 1), especially in the smaller animals (50–60 mg range), which may

[1] Colombo Plan Research Fellow.

partly be due to individual differences in respiratory rate. Nevertheless, the rate of oxygen consumption evidently shows a fall with increase in weight as has been shown in many poikilotherms by Zeuthen (1947). It is interesting to find a similar relationship for parasitic forms also. Von Brand (1953) mentions a similar trend in many endoparasitic animals, but unfortunately no information is available for comparison on external parasites.

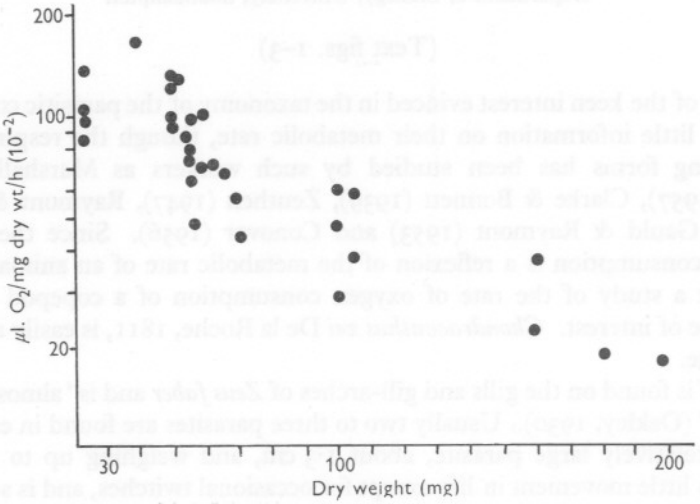


Fig. 1. Variations in the rate of oxygen consumption with body weight.

Marshall & Orr (1955, p. 115) observed that immediately after capture *Calanus* has a considerably higher respiration rate than some hours later, the fall being marked in the first few hours and not noticeable after about one day. Raymont & Gauld (1951) also found a similar fall in respiratory rate with time. During the present study the same trend was observed: the respiratory rate remained steady over a period of 3 h, but thereafter decreased. The results of a few experiments run continuously for 8 h clearly show that at the end the respiratory rate had fallen to a sixth of the initial rate (Fig. 2). This fall in respiratory rate probably explains much of the wide scatter in Fig. 1, since the same set of animals was used in more than one experiment. Experiments using the same animals on the second and third days also revealed a similar fall in respiratory rate (Fig. 3). However, on transfer to fresh sea water after each experiment, there appeared to be recovery in the rate, as evident from the gradual slope of Fig. 3. It is therefore probable that the decline in respiration was partly dependent on the oxygen tension in these long-term experiments.

The rate of oxygen uptake in *Chondracanthus* appears to be low as compared with a free-living copepod such as *Calanus finmarchicus*. A *Calanus*

weighing 0·30 mg respires 0·75 μl. O_2, a relatively very large parasite weighing 200 mg requires only 0·19 μl./mg dry wt/h. This difference is presumably correlated with the parasite being less active.

A calculation shows that a *Chondracanthus* weighing 200 mg will require 3·85% of its own body weight of food for its maintenance according to the O_2 uptake. In his review of Chondracanthidae Oakley (1930) states that these parasites probably live on a mixed diet of flesh and blood, and therefore the damage to the host must be considerable.

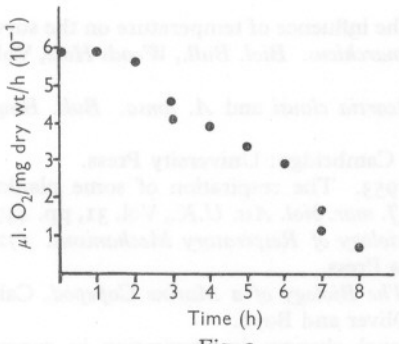


Fig. 2

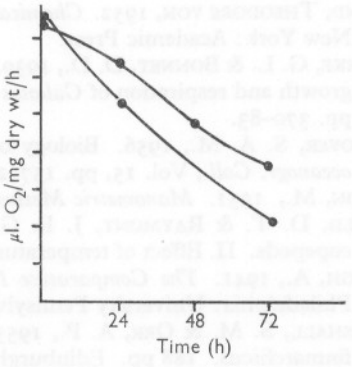


Fig. 3

Fig. 2. Fall in respiratory rate with time, in experiments run for 8 h continuously.

Fig. 3. Fall in respiratory rate with time, in experiments done on successive days.

In many animals a relationship has been established between surface area and respiration (Zeuthen, 1947, 1953). In a large parasite, like *Chondracanthus*, which lacks an efficient circulatory system, most of the oxygen required will have to be obtained by diffusion. Krogh (1941) pointed out that mere diffusion through the body surface may be insufficient to supply all the oxygen required in animals larger than 2 mm. It is therefore possible that the number of projections from the body surface helps to increase the surface area. Furthermore, *Chondracanthus* being located on the gill-arches is thereby exposed to a constant flow of fully oxygenated water so that the rate of diffusion of oxygen is maximal.

The author is indebted to Prof. J. E. G. Raymont of Southampton University for suggesting this problem and for helpful suggestions and criticisms. He also thanks the Director and Staff of the Marine Biological Laboratory, Plymouth, for the unfailing courtesy shown to him and for help and facilities. He is grateful to Dr E. D. S. Corner and Miss J. Lance for discussions and suggestions.

SUMMARY

The rate of oxygen consumption in *Chondracanthus zei* varied from 0·19 to 1·8 μl./mg dry wt/h depending on the body weight. There is a fall in respiratory rate with time. The metabolic rate as reflected by O_2 requirements shows that the animal may consume about 3·85% of its own body weight of food per day.

REFERENCES

BRAND, THEODORE VON, 1952. *Chemical Physiology of Endoparasitic Animals*. 339 pp. New York: Academic Press.

CLARKE, G. L. & BONNET, D. D., 1939. The influence of temperature on the survival, growth and respiration of *Calanus finmarchicus*. *Biol. Bull., Woods Hole*, Vol. 76, pp. 370–83.

CONOVER, S. A. M., 1956. Biology of *Acartia clausi* and *A. tonsa*. *Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 157–233.

DIXON, M., 1951. *Manometric Methods*. Cambridge: University Press.

GAULD, D. T. & RAYMONT, J. E. G., 1953. The respiration of some planktonic copepods. II. Effect of temperature. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 447–60.

KROGH, A., 1941. *The Comparative Physiology of Respiratory Mechanisms*. 172 pp. Philadelphia: University Pennsylvania Press.

MARSHALL, S. M. & ORR, A. P., 1955. *The Biology of a Marine Copepod*, Calanus finmarchicus. 188 pp. Edinburgh: Oliver and Boyd.

—— 1957. A preliminary note on seasonal changes in respiration in copepods. *Année. Biol.*, Vol. 33, pp. 221–6.

—— 1958. On the biology of *Calanus finmarchicus*. X. Seasonal changes in oxygen consumption. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 459–72.

OAKLEY, C. L., 1930. The Chondracanthidae (Crustacea: Copepoda) with a description of five new genera and one new species. *Parasitology*, Vol. 22, pp. 182–201.

RAYMONT, J. E. G. & GAULD, D. T., 1951. Respiration of some planktonic copepods. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 681–93.

SCOTT, T. E. A., 1913. *The Parasitic Copepoda*, Vol. 1, 252 pp. London: Ray Society.

ZEUTHEN, E., 1947. Body size and metabolic rate in the animal kingdom. *C.R. Lab. Carlsberg*, Ser. Chim, T. 26, pp. 1–165.

—— 1953. Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, Vol. 28, pp. 1–12.

J. mar. biol. Ass. U.K. (1959) 38, 267–275
Printed in Great Britain

APPLICATION OF TOXIC AGENTS IN THE STUDY OF THE ECOLOGICAL RESISTANCE OF INTERTIDAL RED ALGAE

By A. D. BONEY
Department of Chemistry and Biology, Plymouth Technical College
and E. D. S. CORNER
The Plymouth Laboratory

(Text-figs. 1 and 2)

Biebl (1939*a*, *b*) has found that algae from various ecological groups show marked differences in their resistance to changes in the temperature of the surrounding sea water. He regards this temperature effect as an important factor influencing the distribution of different intertidal algae and points out that a change of only 2° C may prove critical with certain species. One of us (A.D.B.) has studied the resistance of sporelings of various common intertidal red algae to changes in environmental temperature and has obtained results similar to those of Biebl.

The present study is an attempt to assess the importance of the lipid moiety of the cell membrane in determining the degree of this resistance. Evidence of the amount of lipid material has been obtained by measuring the relative rates of penetration into the plants of substances of different lipid solubilities, it being assumed that if the cell membrane contains a large quantity of lipid material then substances of high lipid solubility will penetrate far more rapidly than those of low lipid solubility; whereas, if it has little then the relative rates of penetration of substances of high and of low lipid solubility will be much closer in value. Analysis of the contents of the plant cells for traces of the penetrating substance was considered too complex a task; therefore poisons were used, for their rates of penetration can readily be estimated by examining the toxic effects of the compounds on the growth and viability of the test organisms. The poisons chosen were *n*-propylmercuric chloride (n-C_3H_7HgCl) and mercuric chloride, compounds which have markedly different lipid solubilities (Corner & Sparrow, 1957) and are toxic to red algal sporelings (Boney, Corner & Sparrow, 1959).

In general, the results obtained support the view expressed by Blinks (1951) that lipids do, in fact, play an important role in determining the resistances of intertidal red algae to increased sea-water temperatures; moreover, the present findings indicate that these lipids are located in the cell membrane.

METHODS

Test species

The plants used were (1) *Polysiphonia lanosa* (L.) Tandy; (2) *Plumaria elegans* (Bonnem.) Schm.; (3) *Spermothamnion repens* (Dillw.) K. Rosenv.; (4) *Ceramium flabelligerum* J. Ag.; (5) *Ceramium pedicellatum* (Duby) J. Ag.; (6) *Antithamnion plumula* (Ellis) Thur. *var. plumula*. Most of these were collected from Church Reef, Wembury, where their shore habitats cover a fairly wide range. Thus, (1) grows on *Ascophyllum nodosum* (L.) Le Jol.; (2) and (3) are found in the subflora below *Ascophyllum*; (4) grows prolifically on *Laurencia pinnatifida* (Huds.) Lamour. in the landward crevices and overhangs from just above M.T.L. down to M.L.W.N.T.; (5) is found in tide pools and is particularly abundant at M.T.L.; (6) is generally sublittoral but, for the present study, spores were obtained from a persistent growth of the plant in the 'Drake's Island' tank at the Plymouth Laboratory.

Settlement of spores

Tufts of each plant, bearing tetrasporangia, were floated on to glass slides lying in an enamelled dish containing filtered sea water from outside Plymouth breakwater. The fruiting tufts were left overnight and the spores released were allowed to remain undisturbed for 2 days at 16° C before being used in the experiments. Usually, settlements of 150–200 sporelings were obtained on each slide.

Toxicity studies

Stock solutions of *n*-C_3H_7HgCl (5 mg Hg/l.) and $HgCl_2$ (50 mg Hg/l.) in sea water were prepared freshly for each experiment.

The methods used were those described by Boney *et al.* (1959) for experiments with sporelings of *Plumaria elegans*.

Estimations of lipid content

A known dry weight of the plant under test was extracted with absolute ethanol by maceration in a Waring blendor. The alcohol extract was then evaporated to dryness and the residue was extracted with warm petroleum ether (b.p. 40–60° C). The quantity of lipid material was then estimated by evaporating off the petroleum ether and weighing the dried residue.

RESULTS

Resistance to increased sea water temperatures

Estimates were made of the times for which 50% of a crop of sporelings of each plant survived in sea water at different temperatures. The results are shown in Table 1, from which it will be seen that sporelings of plants that

grow in intertidal habitats where there is some exposure to air show a greater susceptibility to increased water temperatures, the one exception being *Ceramium flabelligerum*. Table 1 also includes the critical temperatures of the plants and these results correspond with those of Biebl (1939*a*) in that only three intertidal plants could tolerate 35° C and all were killed at 42° C.

TABLE 1. VIABILITIES OF SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE IMMERSED IN SEA WATER AT DIFFERENT TEMPERATURES

Time (h) for which 50 % of sporelings survived

	Species readily withstanding exposure to air				Species with low resistance to exposure to air	
Temp (° C)	*Plumaria elegans*	*Polysiphonia lanosa*	*Spermothamnion repens*	*Ceramium flabelligerum*	*Ceramium pedicellatum*	*Antithamnion plumula*
25	32	—	—	—	—	—
27	17·5	27·5	37	—	—	—
30	0·33	10	13	36	32	32
32	0·25	0·49	—	8	—	15
34	0·08	0·16	1·0	—	2·5	2·0
36	—	—	0·50	1·0	1·5	1·0
38	—	—	—	0·16	—	—
40	—	—	—	0·08	0·08	0·08
42	0	0	0	0	0	0

Lipid contents of selected species

Experiments were carried out to test if the lipid contents of selected algae showed any marked differences, and if these differences correlated with corresponding ecological resistances. The results of this study are shown in Table 2 and demonstrate that although the total lipid contents of the three plants are very low, nevertheless the species which have a higher lipid content are those which are more susceptible to increased water temperature. However, these results did not give any indication of the distribution of lipid material within the plant cells, and to investigate this point experiments with poisons were made.

TABLE 2. TOTAL LIPID CONTENTS OF CERTAIN INTERTIDAL RED ALGAE

Species	Lipid content as % dry wt.
Plumaria elegans	0·91
Polysiphonia lanosa	0·55
Ceramium flabelligerum	0·34

Resistance to poisons

The toxicities of $HgCl_2$ and n-C_3H_7HgCl were compared with 6 species of algae as the test organisms. The results are shown in Table 3, from which it will be seen that a very high ratio of LD_{50} values was obtained with those species that are most susceptible to increased sea-water temperatures (1, 2 and 3). The findings are therefore consistent with the view that these species possess a considerable amount of lipid material in the cell membrane.

TABLE 3. RELATIVE TOXICITIES OF N-C_3H_7HgCl AND $HgCl_2$ TO SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE

	Low resistance to raised sea-water temperature			High resistance to raised sea-water temperature		
	(1)	(2)	(3)	(4)	(5)	(6)
Species	*Plumaria elegans*	*Polysiphonia lanosa*	*Spermothamnion repens*	*Antithamnion plumula*	*Ceramium flabelligerum*	*Ceramium pedicellatum*
LD_{50} in $HgCl_2$ (mg Hg/l.)	6·7	8·0	3·0	5·0	3·2	4·2
LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	0·021	0·030	0·0125	0·045	0·080	0·15
Ratio of above	319	266	240	111	40	28

Effect of temperature

It seemed probable that the lipid moiety of the cell membrane would be so distorted by increased temperature as to become more readily penetrable. For this reason a study was made at different temperatures of the effects of a poison of low lipid solubility on *Antithamnion* and *Plumaria*, species with markedly different resistances to temperature changes. The results obtained (see Table 4) showed that the enhanced toxicity of $HgCl_2$ conferred by increased temperature was far more marked in experiments with *Plumaria* than in those with *Antithamnion*, and these findings provided further evidence in support of the view that the lipid moiety of the plasma membrane is closely involved in temperature sensitivity.

TABLE 4. LD_{50} VALUES FOR $HgCl_2$ AT DIFFERENT TEMPERATURES

Sporelings immersed in the toxic solutions for 0·5 h at each temperature.

	LD_{50} (mg Hg/l.)	
Temp ° C	*Antithamnion plumula*	*Plumaria elegans*
5	—	9·0
16	5·0	6·5
25	—	0·75
29	1·5	—
34	<0·5	—

Speed of toxic effect

It was considered likely that if the lipid moiety of the cell membrane was more developed in a species that was particularly temperature sensitive, this species would be only slowly penetrated by a poison of low lipid solubility. Accordingly, sporelings of several species were treated with $HgCl_2$ and examined for signs of toxic effects after 1 and 7 days. The results (Fig. 1) demonstrated that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* showed considerable resistance to poisoning by $HgCl_2$ when examined after 24 h, but that after 7 days the cumulative toxic effects of the poison had become

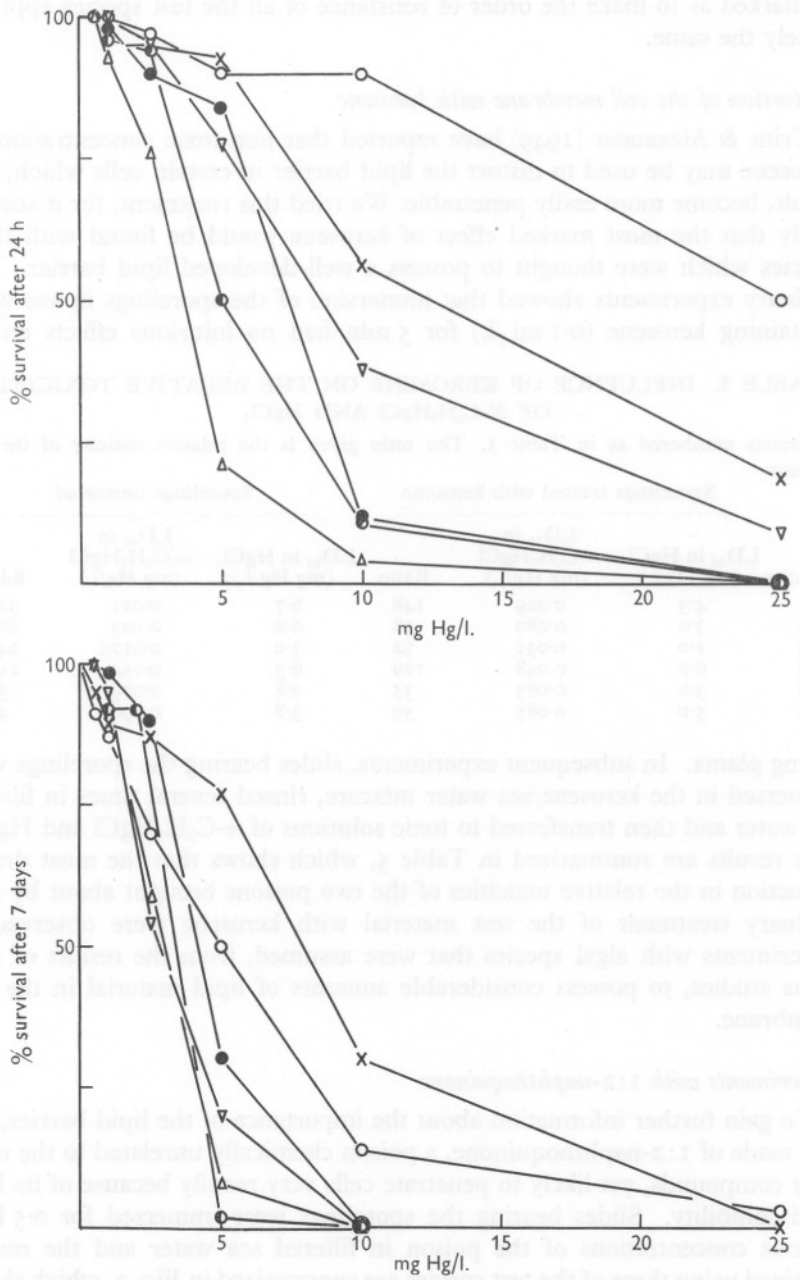


Fig. 1. Sporelings immersed 0·5 h in sea water containing various concentrations of mercury (as $HgCl_2$) then transferred to fresh sea water and examined 24 h (top figure) and 7 days (bottom figure) afterwards. ○—○, *Plumaria*; ×—×, *Polysiphonia*; ●—●, *Ceramium pedicellatum*; ◐—◐, *Antithamnion*; ▽—▽, *Spermothamnion*; △—△, *Ceramium flabelligerum*.

so marked as to make the order of resistance of all the test species approximately the same.

Distortion of the cell membrane with kerosene

Trim & Alexander (1949) have reported that non-toxic concentrations of kerosene may be used to distort the lipid barrier in certain cells which, as a result, become more easily penetrable. We tried this treatment, for it seemed likely that the most marked effect of kerosene would be found with those species which were thought to possess a well-developed lipid barrier. Preliminary experiments showed that immersion of the sporelings in sea water containing kerosene (0·1 ml./l.) for 5 min had no injurious effects on the

TABLE 5. INFLUENCE OF KEROSENE ON THE RELATIVE TOXICITIES OF N-C_3H_7HgCl AND $HgCl_2$

Species numbered as in Table 3. The ratio given is the relative toxicity of the two poisons.

	Sporelings treated with kerosene			Sporelings untreated		
Species	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio
1	4·3	0·029	148	6·7	0·021	319
2	3·0	0·080	38	6·0	0·023	260
3	1·0	0·031	32	3·0	0·0125	240
4	6·2	0·048	129	6·3	0·054	115
5	3·0	0·085	35	2·8	0·079	35
6	5·0	0·085	59	3·8	0·090	42

young plants. In subsequent experiments, slides bearing the sporelings were immersed in the kerosene/sea water mixture, rinsed several times in filtered sea water and then transferred to toxic solutions of n-C_3H_7HgCl and $HgCl_2$. The results are summarized in Table 5, which shows that the most drastic reduction in the relative toxicities of the two poisons brought about by preliminary treatment of the test material with kerosene were observed in experiments with algal species that were assumed, from the results of previous studies, to possess considerable amounts of lipid material in the cell membrane.

Experiments with 1:2-naphthoquinone

To gain further information about the importance of the lipid barrier, use was made of 1:2-naphthoquinone, a poison chemically unrelated to the mercury compounds, yet likely to penetrate cells very readily because of its high lipid solubility. Slides bearing the sporelings were immersed for 0·5 h in various concentrations of the poison in filtered sea water and the results obtained using three of the test species are summarized in Fig. 2, which shows the percentage of the sporelings that survived 24 h after their immersion in the poison. It will be seen that these results accord well with those obtained

using n-C_3H_7HgCl, in that 1:2-naphthoquinone is more toxic to sporelings of the species known to be highly sensitive to increased water temperature.

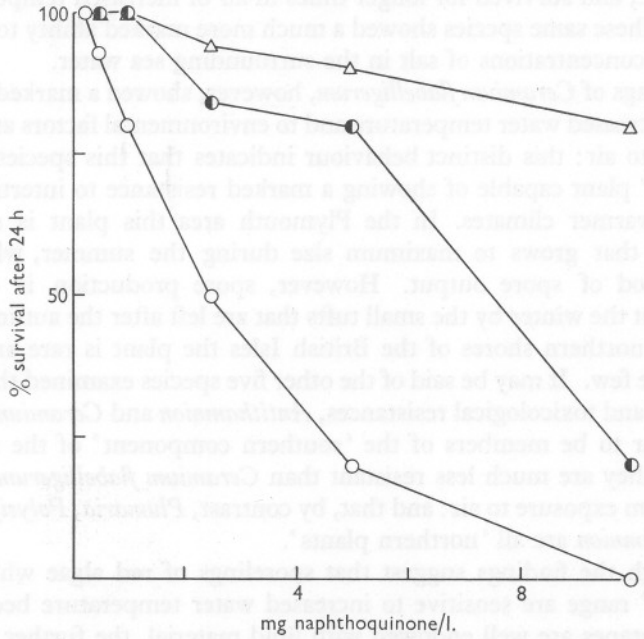


Fig. 2. Sporelings immersed 0·5 h in sea water containing various concentrations of 1:2-naphthoquinone then transferred to fresh sea water and examined 24 h later. △—△, *Ceramium flabelligerum*; ◐—◑, *Antithamnion*; ○—○, *Plumaria*.

DISCUSSION

Macpherson & Young (1949), examining the chemical composition of certain marine plants, found measurable quantities of lipid material in the red algae *Rhodymenia palmata* (3·78% of dry weight) and *Halosaccion ramentaceum* (2·82% of dry weight); but none in *Gigartina stellata*, *Chondrus crispus* and *Ahnfeltia plicata*. In the present study it was found that although three of the test species had only small lipid contents (0·34–0·9% of dry weight), nevertheless the alga most sensitive to temperature (*Plumaria elegans*) had the highest content and the species least sensitive to temperature (*Ceramium flabelligerum*) had the lowest. Furthermore, the use of toxic agents provided a good deal of evidence in support of the view that the cell membranes of species that were temperature-sensitive contained more lipid material than those of species that were temperature-resistant.

A complementary investigation of the resistances of sporelings of the test species to a number of environmental factors showed that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* were able to withstand exposure to air in the intertidal zones more readily than sporelings of *Ceramium pedi-*

cellatum and *Antithamnion*. Thus, sporelings of the first three species, when enclosed in a water film, were more resistant to prolonged exposure to air of 100% R.H., and survived for longer times in air of increased temperature. In addition, these same species showed a much more marked ability to withstand increased concentrations of salt in the surrounding sea water.

Sporelings of *Ceramium flabelligerum*, however, showed a marked resistance to both increased water temperature and to environmental factors arising from exposure to air: this distinct behaviour indicates that this species may be a 'southern' plant capable of showing a marked resistance to intertidal conditions in warmer climates. In the Plymouth area this plant is a common perennial that grows to maximum size during the summer, which is its main period of spore output. However, spore production is carried on throughout the winter by the small tufts that are left after the autumn defoliation. On northern shores of the British Isles the plant is rare and fruiting records are few. It may be said of the other five species examined that by their ecological and toxicological resistances, *Antithamnion* and *Ceramium pedicellatum* appear to be members of the 'southern component' of the algal flora, although they are much less resistant than *Ceramium flabelligerum* to factors arising from exposure to air: and that, by contrast, *Plumaria*, *Polysiphonia* and *Spermothamnion* are all 'northern plants'.

Although the findings suggest that sporelings of red algae which have a 'northern' range are sensitive to increased water temperature because their cell membranes are well endowed with lipid material, the further possibility cannot be excluded that temperature sensitivity may also be due to denaturing of proteins (*vide* Blinks, 1951). In fact it is possible that both these changes may occur and together account for subsequent inhibition of metabolic processes.

There seems no reason why the methods used in the present work should not be applied to many problems of ecological interest and further studies along these lines are planned with sporelings of several 'southern' and 'northern' species of red algae. For example, it would be of considerable interest to examine *Ceramium acanthonotum* Carm. ex Harv. and *Callithamnion arbuscula* (Dillw.) Lyngb. for, although these two species are clearly 'northern' plants, whereas the former also occurs on 'southern' shores the latter does not. In addition, similar studies on ecological resistances might be made using material collected from different localities within the geographical range of a particular species to supplement information about the form range of that species. For, as Parke (1953) has indicated, this is one of the most pressing problems in marine algal ecology.

One of us, A.D.B., is indebted to the Board of Governors of Plymouth Technical College for a grant enabling him to work at the Plymouth Laboratory. Both are grateful to Dr Mary Parke for valuable discussions.

SUMMARY

Sporelings of the intertidal red algae *Plumaria elegans*, *Polysiphonia lanosa* and *Spermothamnion repens* readily withstand exposure to air but have low resistance to increased sea-water temperature; those of *Antithamnion plumula* and *Ceramium pedicellatum* readily withstand increased sea-water temperature but have low resistance to exposure to air; those of *Ceramium flabelligerum* are resistant to both factors.

Poisons of high lipid solubility (*n*-C_3H_7HgCl and 1:2-naphthoquinone) are far more toxic than one of low lipid solubility ($HgCl_2$) to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion*; but when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material the relative toxicities of the poisons are much closer in value.

The toxic effects of $HgCl_2$ to sporelings of *Plumaria* develop slowly at normal temperature and much more quickly when the temperature is raised. However, when sporelings of *Antithamnion* are used, toxic effects develop rapidly at both normal and raised temperatures.

The relative toxicities of *n*-C_3H_7HgCl and $HgCl_2$ to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* are markedly reduced when the test material is first treated with sub-toxic amounts of kerosene, a substance known to distort the lipid moiety of the cell membrane. However, when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material, the effect of the kerosene is negligible.

These results are consistent with the view that the proportion of lipid material present in the cell membrane is important in determining the susceptibility of an intertidal red alga to increased sea-water temperature and have been discussed with especial reference to the geographical distributions of the various species examined.

REFERENCES

BIEBL, R., 1939*a*. Über Temperaturresistenz von Meeresalgen verschiedener Klimazonen und verschieden tiefer Standorte. *Jb. wiss. Bot.*, Bd. 88, pp. 389–420.

—— 1939*b*. Protoplasmatische Oekologie der Meeresalgen. *Ber. dtsch. bot. Ges.*, Bd. 57, pp. (78)–(90).

BLINKS, L. R., 1951. Physiology and biochemistry of algae. *Manual of Phycology*. Waltham, Mass.

BONEY, A. D., CORNER, E. D. S. & SPARROW, B. W. P., 1959. The effects of various poisons on the growth and viability of sporelings of the red alga *Plumaria elegans* (Bonnem.) Schm. *Biochem. Pharmacol.* (in the Press).

CORNER, E. D. S. & SPARROW, B. W. P., 1957. The modes of action of toxic agents. II. Factors influencing the toxicities of mercury compounds to certain crustacea. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 459–72.

MACPHERSON, M. G. & YOUNG, E. G., 1949. The chemical composition of marine algae. *Canad. J. Res.*, Vol. 27, pp. 73–7.

PARKE, M., 1953. A preliminary check list of the British marine algae. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 497–520.

TRIM, A. R. & ALEXANDER, A. E., 1949. Surface activity and permeability as factors in drug action. *Symp. Soc. exp. Biol.*, No. 3, pp. 111–42.

J. mar. biol. Ass. U.K. (1959) 38, 277–290
Printed in Great Britain

ON THE STRUCTURE, BIOLOGY AND SYSTEMATIC POSITION OF *PHARUS LEGUMEN* (L.)

By C. M. YONGE, D.Sc., F.R.S.
University of Glasgow

(Text-Figs. 1–7)

The species with which this paper deals was originally included by Linné in the genus *Solen* and was there retained to be included under that name in the magnificently illustrated account of this genus by Deshayes (1845–48). It was later placed in a new genus *Ceratisolen* by Forbes & Hanley (1853) who considered that it formed 'the connecting link between the Solenidae and Solecurtidae'. This procedure was followed by Jeffreys (1865). More recently the Solecurtidae were shown by Graham (1934*b*) having 'regard to such features as shape, presence of a cruciform muscle and the structure of the foot, siphons, adductor and pedal muscles, supra-axial extension of the outer demibranch, palps, and style-sac and intestine', to belong to the Tellinacea, a view fully supported by this author (Yonge, 1949). Assigned later to the genus *Pharus* which has priority over *Ceratisolen*, this 'connecting link' of Forbes & Hanley continued to be associated with the Solenacea by Thiele (1935) in his great systematic account of the Mollusca.

Personal observations on the Tellinacea (Yonge, 1949) and later on *Siliqua patula* and other members of the Solenacea (Yonge, 1952) have indicated that *Pharus legumen* should also be transferred to the Tellinacea. This paper is primarily concerned with the reasons for this change in classification, and this has involved some description of structure and habits in this little known member of the British marine fauna. Nothing can supersede the magnificent plates in Deshayes (1845–48) but his account, although detailed, is not, by present standards, critical, while the very short description by Bloomer (1903) is of little value.

This work has been made possible largely through the kindness of Prof. E. W. Knight-Jones who, from Anglesey and later from the Swansea area, sent living specimens with information about the conditions under which they were found. Preserved specimens from Brittany were also kindly supplied by Mr N. A. Holme of the Plymouth Laboratory, while Prof. A. Graham and Dr V. Fretter of Reading have supplied preserved specimens and given information about habitat. Acknowledgements are also due to Dr H. F. Steedman and Dr G. Owen of this Department for technical help and also, in the case of Dr Owen, who is investigating allied problems, for much profitable discussion.

EXTERNAL APPEARANCE AND SHELL

As Deshayes and other early workers pointed out, *P. legumen* (Fig. 1) may be distinguished from other species of '*Solen*' by the situation of the ligament, in the middle instead of at the anterior end of the long flat dorsal surface of the shell. There is also greater lateral compression of the shell than in species of *Ensis* or *Solen* while the valves taper somewhat anteriorly (in his figure of the shell with foot and siphons projecting, Jeffreys (1865) shows this the wrong way round). The shell attains a length of up to some 10 cm and is about four times longer than it is deep. It is pale yellow and glossy with a characteristic difference in texture and with a paler colour over the posterior sector (indicated in Fig. 1). Hinge dentition, which may here be mentioned, consists of two cardinals in the left valve (see Fig. 2) and one on the right with, in each valve, a long and low anterior lateral and a short, projecting posterior lateral. There is nothing here of value in determining the systematic position of *Pharus*.

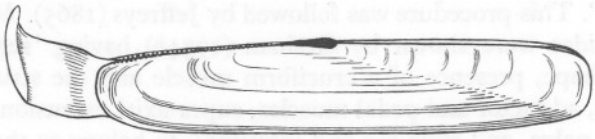


Fig. 1. *Pharus legumen*, viewed from left with foot partly extended. $\times \frac{3}{4}$.

The elongated and very compressed foot (Figs. 1, 2 and 4) issues through a restricted anterior gape. It is truncated terminally and when observed in a bowl of water makes very sudden extensions to a length as great as that of the shell, at the same time opening out terminally. It is then as quickly withdrawn. It is clearly fully as effective in rapid vertical burrowing as the foot of *Ensis* or *Solen*, described by Fraenkel (1927), but it differs in being less bulbous terminally. Siphons longer than those of species of *Solen* or *Ensis* (although shorter than those of *Siliqua patula* (Yonge, 1952)) project posteriorly and to a length of 5 mm in an animal of shell length 3·9 cm. But, unlike all true members of the Solenacea, the inhalant and exhalant siphons are separate throughout (see Fig. 2) and are also, as shown later, fundamentally different in structure, a fact which initially indicated the affinity of this species to the Tellinacea.

Although first recognized as a British species by Martin Lister in the seventeenth century, *Pharus legumen* is relatively seldom encountered. It is a southern species (hence its description by Deshayes in an account of the marine molluscs of Algeria) and is here near the northern end of its range. It appears to be confined to the western end of the south coast, to the north coasts of Devon and Cornwall, to the shores of Wales and to all but the north coast of Ireland. It is not recorded from the Isle of Man or from the Clyde

Sea Area. It ranges in the south from Brittany, around the shores of Spain and Portugal into the Mediterranean where it appears to be common. In Great Britain it is certainly only abundant around the shores of Wales and there, judging from information received from Prof. Knight-Jones, is commoner along the south coast than around Anglesey. It lives in relatively clean sand, although with some admixture of organic silt. To quote from a letter from Prof. Knight-Jones dealing with the Swansea area: 'The sand at Oxwich Bay where *Pharus* occurs near here is fine and fairly clean. The water above it is generally very turbid and there is a lot of silt washing about on the surface of the sand.' At Roscoff (1951) it is stated as occurring 'A très basse mer, dans du sable plus ou moins pur'. It appears to inhabit a restricted zone on the margin of the lower shore and in the shallow sublittoral. Hence it can only be dug at low water of the greater spring tides while its capacity for rapid vertical burrowing makes it difficult to obtain by dredging which in any case is never easy in very shallow water. These restrictions, both in horizontal and vertical distribution around the shores of Great Britain, go far to explain the prevailing ignorance about this species.

PALLIAL STRUCTURES

Muscles. As shown in Fig. 2, the pallial (orbicular) muscles are attached at some distance from the margin of the shell. Both adductors, the anterior being much the longer, are extended longitudinally. The posterior adductor (*PA*), as recently indicated by Owen (1958) in work concerned partly with the Solenacea, does not represent the termination of the pallial muscles at that end. For functional reasons, as in the Solenacea and in other bivalves where the shell has elongated, the region of local muscular hypertrophy represented by the adductor has migrated posteriorly but pallial attachment continues, as indicated in Fig. 2, up to the posterior end of the ligament. The shell is strengthened in the region of the hinge by a ventrally extending rib, the anterior surface of which bounds the posterior margin of attachment of the anterior adductor. Such strengthening of a relatively delicate extended shell is not unusual, occurring also in *Siliqua patula*. It has certainly no systematic value. The siphonal retractors are well developed and the pallial sinus is correspondingly capacious.

Mantle folds and fusion. The mantle margins are united along the greater part of the ventral surface and, except for the openings of the siphons, posteriorly right round to the posterior end of the ligament. Fusion is of Type B (Yonge, 1957), i.e. of inner (muscular) folds of the mantle edge together with the inner surfaces of the middle lobes. The outer surfaces of the middle lobes form a narrow ridge (*MF*) between the outer lobes the innermost margin of which constitutes the periostracal groove (Fig. 3). The outer lobes are broad with periostracum extending for some distance within the

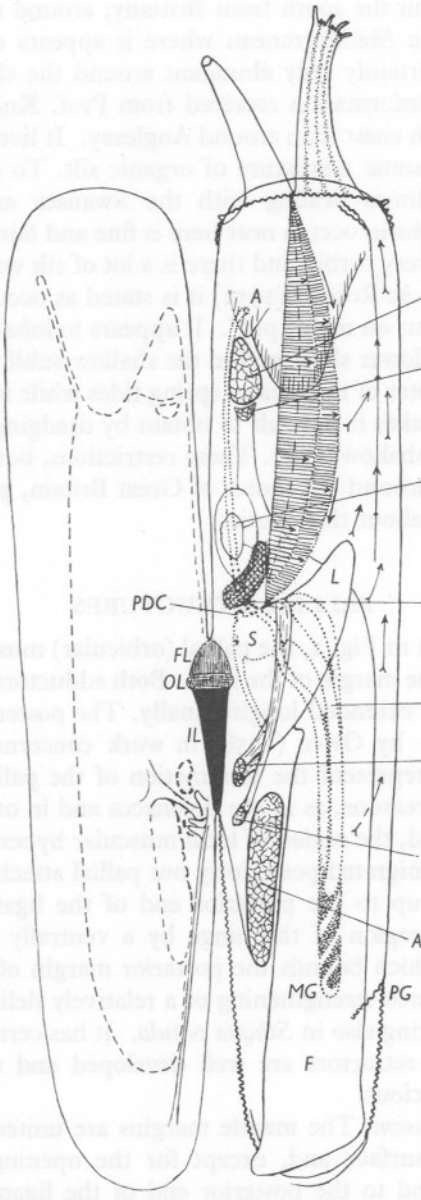


Fig. 2. *Pharus legumen*, animal lying in right valve with siphons extended, left valve turned back to show ligament, hinge teeth and muscle insertions (indicated by broken lines). × 1½. *A*, anus; *AA*, anterior adductor; *AR*, anterior pedal retractor; *F*, foot; *FL*, fusion layer of ligament; *H*, heart; *IL*, inner layer of ligament; *K*, kidney; *L*, labial palp; *MG*, coiled region of mid-gut extending into foot; *OL*, outer layer of ligament; *P*, pedal protractor; *PA*, posterior adductor; *PDC*, postero-dorsal caecum of stomach; *PG*, posterior end of pedal gape; *PR*, posterior pedal retractor; *S*, Stomach; *SS*, style-sac. Plain arrows indicate direction of food collecting currents, feathered arrows of rejection currents.

margin of the outer calcareous layer of the shell which is secreted by the outer surface of the outer fold (Yonge, 1957). On the dorsal surface anterior to the ligament, the middle mantle folds are nowhere united, their free margins being bounded by short tentacles as indicated in Figs. 1 and 2. But formation and anterior extension of the anterior adductor does represent extensive fusion of the inner mantle folds in this region. It should be noted that there is *no* cuticular fusion of the anterior regions of the mantle margins ventrally which is so characteristic a feature in the Solenacea where it occurs in *Solen*, *Ensis* and *Cultellus*; previously overlooked in *Siliqua patula* (Yonge, 1952), it has been found there by Owen (1959).

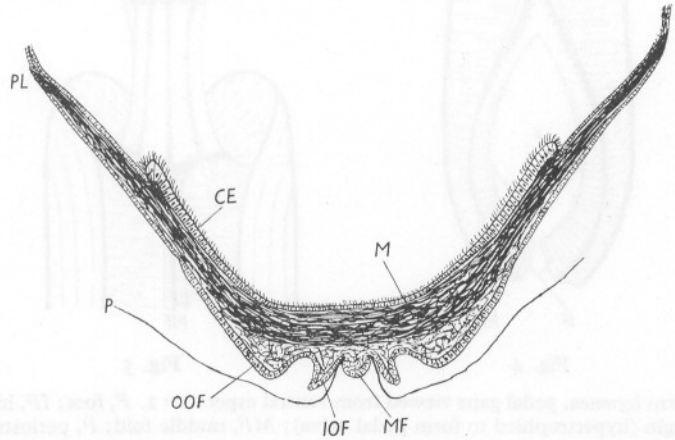


Fig. 3. *Pharus legumen*, transverse section through fused, mid-ventral region of mantle lobes posterior to palps (see Fig. 2). ×90. *CE*, ciliated epithelium; *IOF*, inner surface of outer fold of mantle margin (secretes periostracum); *M*, pallial muscle; *MF*, fused middle mantle folds; *OOF*, outer surface of outer mantle fold (secretes outer calcareous layer of shell); *P*, periostracum (other shell layers not shown); *PL*, pallial line.

The pedal gape extends from the anterior end of the ligament dorsally to a point some one-fifth of the distance back along the ventral surface. The single row of tentacles which fringe the middle mantle fold is well developed both ventrally and dorsally but anteriorly the tentacles are reduced over an area where the inner mantle folds (*IF*) increase greatly in depth and become frilled (Fig. 4). They form a pedal valve resembling that found in Solenacea such as *Ensis* and having presumably the same function, namely wiping the surface of the foot as it is being withdrawn.

Siphons. Separate to the base, these are formed exclusively from the inner folds of the mantle margin. The small middle folds with the adjacent periostracal groove (this representing the innermost margin of the outer fold) extend, as shown in Figs. 2 and 5, around the base of the siphons, uniting above and below. The exhalant siphon bears no marginal tentacles and curves slightly dorsalwards; the inhalant siphon is fringed with a series of eight large

and sixteen very small tentacles, the latter arranged in pairs between the larger ones. The surface of this siphon carries eight pigmented lines which run to each of the pairs of small tentacles. In their complete separation and formation exclusively from inner mantle folds, these siphons are identical with those of the Tellinacea; siphons in the Solenacea are largely united, the common outer covering with its tentacles being formed from the middle

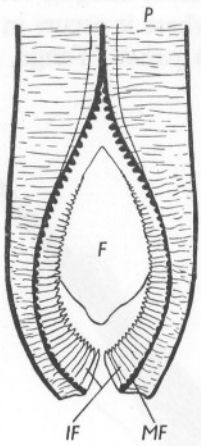


Fig. 4

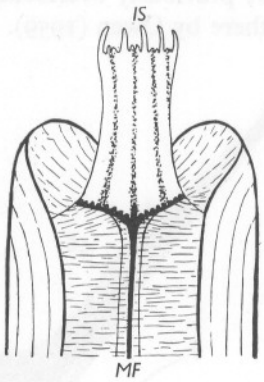


Fig. 5

Fig. 4. *Pharus legumen*, pedal gape viewed from ventral aspect. × 2. *F*, foot; *IF*, inner fold of mantle margin (hypertrophied to form pedal valve); *MF*, middle fold; *P*, periostracum.

Fig. 5. *Pharus legumen*, posterior end of shell with inhalant siphon viewed from ventral aspect. × 2. *IS*, inhalant siphon; *MF*, middle fold of mantle margin.

mantle folds. Furthermore, in transverse section (Fig. 6), the siphons of *P. legumen* reveal the highly characteristic structure of the siphons of the Tellinacea as described and figured by Rawitz (1892), Hoffmann (1914), Graham (1934*b*), Yonge (1949) and Chapman & Newell (1956). Between the inner and the outer epithelia there lie alternating circular layers of collagen fibres (C_1–C_4)—originally described by all workers as circular muscle fibres but correctly interpreted by Chapman & Newell—and bundles of longitudinal muscle (L_1–L_3). There are four layers of the former and three of the latter, the outermost of these muscle layers being poorly developed. Radiating strands (*R*) of probably mixed muscle and collagen fibres traverse these concentric layers. In the zone occupied by the innermost muscle layer run the symmetrically disposed nerves (*N*), four in the exhalant and eight in the inhalant siphon. Haemocoels (*H*) occur on the inner side of the middle of the three muscle layers.

The entire picture is precisely that seen in cross-sections through the siphons of species of such characteristic genera of the Tellinacea as *Tellina*,

Macoma, *Abra*, *Scrobicularia*, *Donax*, *Gari* and *Solecurtus* (Yonge, 1949). Only in the number of nerves is there some difference, this being six in species of all of the above genera where the inhalant siphon bears that number of fringing lobes. Rawitz, however, found that there were occasionally eight nerves in *Gari depressa* (presumably in association with that number of marginal lobes), while in *Tagelus dombeyi* the number is normally eight (Hoffmann, 1914). In the Solenacea the histological picture is completely different. Longitudinal, circular and radial muscles are all well developed but apart from a tendency (noted in sections of both *Ensis* and *Solen*) for longitudinal muscle to be most developed under the outer and inner epithelia,

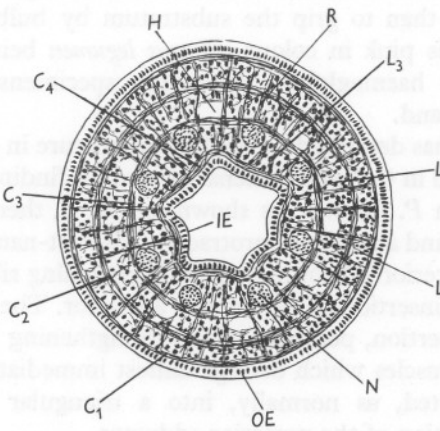


Fig. 6. *Pharus legumen*, transverse section through inhalant siphon. ×90. C_1–C_4, circular layers of collagen fibres; *H*, haemocoel; *IE*, epithelium lining cavity of siphon; L_1–L_3, layers of longitudinal muscle; *N*, nerve (one of eight); *OE*, epithelium of outer surface of siphon; *R*, radial muscle.

there is no trace of the organization into sharply defined zones so highly characteristic of the Tellinacea and associated, as demonstrated by Chapman & Newell, with an intrinsic and highly effective mechanism of extension and withdrawal.

Ligament. This requires little comment; it was not considered necessary to examine or describe it in detail. It is opisthodetic, elongate and external (Fig. 1). There is a long inner ligament layer secreted by the mantle isthmus where the epithelial cells are notably lengthened. Posterior to this is a broader but narrow region where the outer ligament layer is formed (anterior secretion of this layer is necessarily slight) and behind this again a broad fusion layer. This extends back under the level of the calcareous substance of the valves with which it becomes incorporated. These three major regions are indicated in Fig. 2. Conditions are thus essentially similar to those described in detail (although with superseded terminology) for *Tellina tenuis* by Trueman (1949)

and more generally for *Scrobicularia plana* and *Abra alba* (Trueman, 1953). The only differences in *Pharus* are the greater length of the ligament and its greater secondary attachment, by way of the fusion layer posteriorly, to the shell valves.

ORGANS IN THE MANTLE CAVITY

Foot and pedal muscles. The size of the foot has already been noted, even when fully contracted it is some half the length of the shell. Although obviously resembling that of the Solenidae in general form, to some extent it differs slightly from that organ and resembles the foot of the Tellinacea in the presence of a more defined and rather frilled lateral margin. In action it tends to open out rather than to grip the substratum by bulbous dilation as in *Ensis* or *Solen*. It is pink in colour, *Pharus legumen* being one of the few bivalves to possess haemoglobin. In young specimens there is a well-developed byssus gland.

Graham (1934*b*) has described the pedal musculature in a variety of species of the Tellinacea and in *Cultellus* (Solenacea) and his findings have been helpful in this work. In *P. legumen*, as shown in Fig. 2, there are anterior and posterior retractors and an anterior protractor. The last-named is inserted into the valves on the anterior side of the ventrally extending rib and dorsal to the posterior end of the insertion of the anterior adductor. The anterior retractors have one area of insertion, posterior to the strengthening shell rib, although consisting of two muscles which diverge almost immediately. The posterior retractors are inserted, as normally, into a triangular area immediately anterior to the insertion of the posterior adductor.

The anterior retractor has a single insertion as it does in the Tellinacea but this cannot be considered of systematic value because although there is usually a double insertion of this muscle in the Solenacae, in *Solen*, as recently shown by Owen (1959) it is single. However, conditions in *Pharus* do certainly resemble those in the Tellinacea the only difference being that the muscle usually divides further from the point of insertion than it does in *P. legumen*. In this species also the anterior retractors are the most internal of the extrinsic muscles of the foot whereas the opposite is the case in *Cultellus* (Graham, 1934*b*). But, despite earlier views to the contrary, this also has no systematic value, conditions varying greatly within the Solenacea as shown by Owen (1959).

Palps and Ctenidia. The palps are large, there being no such long proximal oral groove between them and the mouth as there is in Solenacea such as *Ensis siliqua* (Graham, 1934) or *E. arcuatus* (Yonge, 1952). They are much-ridged on the opposed faces and are clearly most efficient organs of selection.

With reference to the ctenidia, Ridewood (1903) states that, '*Ceratisolen* (i.e. *Pharus*) *legumen* has a non-plicate, homorhabdic gill, in striking contrast with those of other genera of the Solenidae'. However, the ctenidia of

Cultellus, undoubtedly a member of that family, are homorhabdic (Atkins, 1936) while in the Tellinacea, the ctenidia are homorhabdic in *Tellina*, *Macoma*, *Scrobicularia*, *Abra* and in *Donax vittatus* (Atkins, 1937*a*), although Ridewood (1903) found a variety of conditions in different species of *Donax*, some being homorhabdic and others heterorhabdic. In all species of *Gari* and *Solecurtus* examined by Ridewood (1903) and Atkins (1937*a*), the ctenidia were plicate, i.e. heterorhabdic.

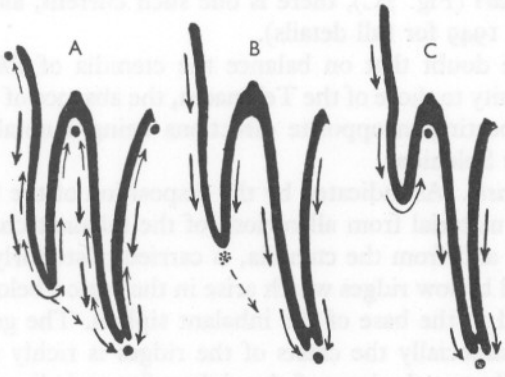


Fig. 7. Diagrams showing arrangement of ciliary currents on ctenidia of A, *Cultellus pellucidus*; B, *Pharus legumen*; C, *Donax vittatus* or *Gari* spp. (A and C after Yonge, 1949.)

A further point is the well-marked supra-axial extension of the outer lamellae of the outer demibranch. This is shown in Fig. 7B and the same condition is found very generally throughout the Tellinacea (Yonge, 1949, Fig. 22) where indeed it is much further developed in the Tellinidae (e.g. *Tellina*, *Macoma*) and Semelidae (e.g. *Scrobicularia*, *Abra*). Conditions in *Donax*, *Gari* (Fig. 7C) or *Solecurtus* most resemble those in *Pharus*. However, a slight supra-axial extension does also occur in *Cultellus* (Fig. 7A) and is well developed in a wide variety of other Eulamellibranchia. There remains for final consideration the ciliation. Here a real difference exists. In all Solenidae studied, i.e. *Ensis*, *Solen*, *Cultellus* (Atkins, 1936, 1937*a*) and *Siliqua* (Yonge, 1952), the ordinary filaments (in *Cultellus*, which is homorhabdic this means all filaments) bear both large and small frontal cilia (Fig. 7A). The former beat towards the free margins of the demibranchs and the latter in the opposite direction, i.e. towards the axis or the outer extremities of the demibranchs. The effect is to select the finer particles for conveyance oralwards along these three dorsal channels while the larger particles are conveyed to the marginal (so-called 'food') groove on the inner demibranch from which the greater part will fall off on to the mantle surface and be removed with the pseudofaeces.

In *Pharus legumen* (Fig. 7B) there are *no* small frontal cilia, all cilia beating marginally. There is a resemblance to the foregoing conditions in the presence of a marginal groove on the inner demibranch only but this is also true of all

Tellinacea (e.g. *Donax* or *Gari* as shown in Fig. 7C). The margin of the outer demibranch, however, bears a series of coarse marginal cilia precisely as described and figured in that region in *Gari fervensis* by Atkins (1937*b*, fig. 8). Also significant is the absence in *Pharus legumen* of any dorsal oralward current, conditions here differing from those in the Solenacea with three such dorsal currents (see Fig. 7A) and resembling those in the Tellinacea where in some species there are no such currents while in others, including species of *Donax* and *Gari* (Fig. 7C), there is one such current, along the ctenidial axis (see Yonge, 1949 for full details).

There is little doubt that on balance the ctenidia of *Pharus legumen* do reveal closer affinity to those of the Tellinacea, the absence of the double series of frontal cilia beating in opposite directions being a notable point of difference from the Solenidae.

Disposal of Waste. As indicated by the disposition of the feathered arrows in Fig. 2, waste material from all regions of the inhalant chamber, from the tips of the palps and from the ctenidia, is carried posteriorly along a ventral channel bounded by low ridges which arise in the region below the tips of the palps and extend to the base of the inhalant siphon. The general surface of this region and especially the crests of the ridges is richly ciliated (Fig. 3). Pseudofaeces collect at the base of the siphon for periodic expulsion. Much deeper ridges, better termed membranes, occur in *Siliqua patula* (Yonge, 1952) amongst the Solenacea and also in many Tellinacea (Yonge, 1949), although not in *Donax*, *Gari* or *Solecurtus*. Certainly no systematic value can be placed on this structural feature of obvious functional convenience.

ALIMENTARY CANAL

The general course of the gut in *Pharus legumen* is shown in dotted outline in Fig. 2. There is an unusually long oesophagus, the stomach is extended somewhat dorso-ventrally, the mid-gut is separated from the long style-sac, extending alongside and then for some distance beyond this well forward into the foot. It is thrown terminally into a series of tight coils (*MG*). It returns to pass dorsally to the right of the stomach and so, through the ventricle, to open at the anus behind the posterior adductor. The stomach easily dissects out in fresh material: it has a well-developed muscular wall with the contents, including sizable sand grains, in some degree of tension. There is a small postero-dorsal caecum (*PDC*), much as in *Solecurtus* (Yonge, 1949). The style is extremely stout and bears against a conspicuous gastric shield with a conspicuous tooth.

Significant matters are the character of the stomach and the deep penetration into the foot by the style-sac (*SS*) and mid-gut (*MG*). The stomach in the Tellinacea is highly characteristic having, in association with the deposit feeding habit, acquired many of the characteristics of a gizzard. These include

an unusually stout style, very extensive gastric shield, muscular walls and a conspicuous postero-dorsal caecum peculiar to the group and apparently serving as a safety valve wherein excess particles are temporarily stored prior to trituration between style and gastric shield (Yonge, 1949). All of these features are present in *Pharus*, though not to so marked an extent as in genera such as *Tellina* and *Scrobicularia*. There is no resemblance to the stomach of the suspension feeding Solenacea. Although in *Pharus*, as in the Solenacea, style-sac and mid-gut are separate whereas they are united in the majority of the Tellinacea, this is not a feature of any phylogenetic significance and indeed the two are separate in *Donax* which certainly belongs to the Tellinacea. Deep penetration of the foot by style-sac and mid-gut does not occur in the Solenacea (Graham, 1931, 1934*b*; Owen, 1959).

SYSTEMATIC POSITION

Confining this discussion to essentials, of the various features discussed in this account of *P. legumen* the following may be regarded, from the standpoint of its affinities, as crucial:

Siphons. Their complete separation and formation exclusively from the inner folds of the mantle margin being ringed (inhalant siphon only) with a single row of simple tentacles; their complex internal structure of the type highly characteristic of the Tellinacea.

Ctenidia. Supra-axial extension but much more significant the absence of any dorsal oralward current and especially lack of the small frontal cilia and so of the mode of selection of particles on the gill surface so characteristic of the Solenacea.

Gut. General form of the stomach, especially the presence of a small postero-dorsal caecum found only in the Tellinacea; deep penetration of the mid-gut and style-sac into the foot, unlike the Solenacea.

No other character mentioned is entirely confined to the one group or the other but the above list provides sure grounds for the removal of *Pharus* from the Solenacea and its inclusion in the Tellinacea.

The major difference from the more obviously typical members of the Tellinacea is the absence of a cruciform muscle (Graham, 1934*a*; Yonge, 1949). But this is also true of *Novaculina* and *Sinovacula* (subfamily Novaculininae) which, it has previously been suggested (Yonge, 1949), should be removed from the family Solenidae where they were placed by Ghosh (1920) and transferred to the Tellinacea. This was on the basis of the formation of the siphons, unquestionably a crucial character because one not liable to adaptive modification. There is much greater supporting evidence for this transfer in the case of *Pharus* where the internal structure of the siphons is also known and highly significant differences in the ctenidia and gut also observed. The absence in these three genera of the cruciform muscle,

otherwise apparently universally present in the Tellinacea, is very probably associated with the extension of pallial fusion (Type B in *Pharus* but of unknown type in the Novaculininae), along a greater part of the ventral surface; in the great majority of the Tellinacea fusion is confined to a very small region at the base of the siphons where the cruciform muscle acts as a tie for the elongate siphons (Yonge, 1949).

Elongation of the shell in a manner superficially very similar to what occurs in the Solenacea seems to have occurred several times in the Tellinacea. It has led to the appearance of *Tagelus* (with no ventral mantle fusion but with a cruciform muscle), of *Solecurtus* (with ventral mantle fusion but involving *only the inner mantle folds* i.e. Type A, and with a cruciform muscle) and of *Pharus*- and also probably of the separate group of the Novaculininae- (with more intimate ventral fusion and without a cruciform muscle). The major external difference of these elongate genera from the Solenacea is the approximately central position of the hinge and ligament, the mantle/shell having extended both anteriorly and posteriorly and not solely posteriorly. In the case of *Tagelus* the habits are also somewhat different, species of this genus occupy semi-permanent vertical burrows in mud. Species of *Solecurtus* live in deeper water and probably do not burrow deeply or perhaps even very quickly. The habits of the Novaculininae are uncertain but in *Pharus* they do resemble those of the Solenacea, living in shallow water and burrowing vertically both deeply and with great speed. An important difference may be their need, as deposit feeders, for silty conditions not present in the exposed habitat of most members of the Solenacea. In European waters *Solen* tends to occupy the environment favoured by *Tagelus* elsewhere.

SUMMARY

Pharus legumen (L.), a little-known member of the British marine fauna, is confined to the south-west and to the coasts of Wales. It inhabits a restricted zone on the margin of the lower shore and the shallow sublittoral where some silt is present.

Both in the elongation of the shell and in the habit of vertical burrowing, *P. legumen* resembles members of the Solenacea, i.e. species of *Ensis* and *Solen*.

Following description of this species with an account of the pallial structures, organs in the mantle cavity, nature of the ciliary currents on the mantle and the ctenidia, and the course of the alimentary canal, reason is given for the transference of *Pharus legumen* to the Tellinacea.

Major evidence for this decision comes from the separation of the siphons which are composed exclusively of the inner folds of the mantle margin and have the highly characteristic internal structure of those of the Tellinacea, the absence on the ctenidia of dorsal oralward currents and of the small frontal

cilia, both characteristic of the Solenacea, and finally from the nature of the stomach with its small postero-dorsal caecum and the deep penetration into the foot of the mid-gut.

The major difference from the Tellinacea generally is the absence of a cruciform muscle but this may be associated with the extensive ventral fusion of the mantle margins, not found in genera with a cruciform muscle with the exception of *Solecurtus* where it is less extensive and not so intimate (of Type A instead of Type B) as it is in *Pharus legumen*.

REFERENCES

ATKINS, D., 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. Part I. *Quart. J. micr. Sci.*, Vol. 79, pp. 181–308.

—— 1937*a*. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. *Quart. J. micr. Sci.*, Vol. 79, pp. 339–73.

—— 1937*b*. On the ciliary mechanisms and interrelationships of lamillibranchs. Part III. *Quart. J. micr. Sci.*, Vol. 79, pp. 375–421.

BLOOMER, H. H., 1903. Classification of the British species of the genus *Solen*. *J. Malacol.*, Vol. 10, pp. 41–3.

CHAPMAN, G. & NEWELL, G. E., 1956. The rôle of the body fluid in the movement of soft-bodied invertebrates. II. The extension of the siphons of *Mya arenaria* L. and *Scrobicularia plana* (da Costa). *Proc. Roy. Soc.*, B, Vol. 145, pp. 564–80.

DESHAYES, G. H. 1845–48. Histoire naturelle des Mollusques. 1. Mollusques acéphalés. *Exploration scientifique de l'Algérie*, 1840–42. Sciences Physiques, Zoologie. Paris.

FORBES, E. & HANLEY, S., 1953. *A History of British Mollusca*, Vol. 1. London: Van Voorst.

FRAENKEL, G., 1927. Die Grabbewegung der Soleniden. *Z. vergl. Physiol.*, Bd. 6, pp. 167–220.

GHOSH, E., 1920. Taxonomic studies on the soft parts of the Solenidae. *Rec. Indian Mus.*, Vol. 19, pp. 47–78.

GRAHAM, A., 1931. On the morphology, feeding mechanisms, and digestion of *Ensis siliqua* (Schumacher). *Trans. roy. Soc. Edinb.*, Vol. 56, pp. 725–51.

—— 1934*a*. The cruciform muscle of lamellibranchs. *Proc. roy. Soc. Edinb.*, Vol. 54, pp. 17–30.

—— 1934*b*. The structure and relationships of lamellibranchs possessing a cruciform muscle. *Proc. roy. Soc. Edinb.*, Vol. 54, pp. 158–87.

HOFFMANN, F., 1914. Beiträge zur Anatomie und Histologie von *Tagelus dombeyi* (Lamarck). *Jena Z. Naturw.*, Vol. 52, pp. 521–61.

JEFFREYS, G., 1865. *British Conchology*, Vol. 3. London: Van Voorst.

OWEN, G., 1958. Shell form, pallial attachment and the ligament in the Bivalvia. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 637–648.

—— 1959. Observations on the Solenacea with reasons for excluding the family Glaucomyidae. *Phil. Trans.* B, Vol. 242, pp. 59–97.

RAWITZ, B., 1892. Der Mantelrand der Acephalen. Dritter Teil. *Jena Z. Naturw.*, Bd. 27, pp. 1–232.

RIDEWOOD, W. G., 1903. On the structure of the gills of the Lamellibranchia. *Phil. Trans.* B, Vol. 195, pp. 147–284.

ROSCOFF, 1951. Inventaire de la Faune Marine de Roscoff. Mollusques. *Trav. Sta. biol. Roscoff*, Suppl. 5.

THIELE, J., 1935. *Handbuch der systematischen Weichtierkunde.* Dritter Teil. Classis Bivalvia. Jena: G. Fischer.

TRUEMAN, E. R., 1949. The ligament of *Tellina tenuis*. *Proc. zool. Soc. Lond.*, Vol. 119, pp. 719–42.

—— 1953. The structure of the ligament of the Semelidae. *Proc. malacol. Soc. Lond.*, Vol. 30, pp. 30–6.

YONGE, C. M. 1949. On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Phil. Trans.* B, Vol. 234, pp. 29–76.

—— 1952. Studies on Pacific coast Mollusks. IV. Observations on *Siliqua patula* and on evolution within the Solenidae. *Univ. Calif. Publ. Zoöl.*, Vol. 55, pp. 421–38.

—— 1957. Mantle fusion in the Lamellibranchia. *Pubbl. staz. zool. Napoli*, Vol. 29, pp. 151–71.

J. mar. biol. Ass. U.K. (1959) 38, 291–312 (*N.I.R.D. Paper No.* 2155)
Printed in Great Britain

THE FOOD OF *MEGANYCTIPHANES NORVEGICA* (M. SARS), WITH AN ASSESSMENT OF THE CONTRIBUTIONS OF ITS COMPONENTS TO THE VITAMIN A RESERVES OF THE ANIMAL

By L. R. FISHER and E. H. GOLDIE

Unit for Biochemical Research bearing on Fisheries' Problems[1] at the National Institute for Research in Dairying, Shinfield, Reading

(Text-figs. 1–10)

The importance of *Meganyctiphanes norvegica* (M. Sars) as a source of vitamin A for its predators has been demonstrated by Fisher, Kon & Thompson (1955). Basing their conclusions on the observations of Macdonald (1927) on the feeding of *M. norvegica* in Loch Fyne, Fisher, Kon & Thompson (1954) and Kon (1954) suggested a possible scheme of accumulation of vitamin A in the animal. They also made cursory examinations of the stomach contents from animals caught in the same area and their findings appeared to agree fairly well with those of Macdonald, except that from his results he concluded that phytoplankton was a major item in the diet. This conclusion has been disputed by Einarsson (1945) who called for more detailed investigation of fresh material in order to obtain a clearer picture of the feeding of north Atlantic euphausiids. Such information would be of great value in the study of the vitamin A chain in the plankton. We have, therefore, examined the stomach contents of *M. norvegica* over nearly two years to try to detect every type of food that might be eaten. In addition to our own survey, Dr J. Mauchline (1959) of the Marine Station, Millport, made a more intensive study of the food in relation to the diurnal migrations in July and November.

Earlier work on the food of *M. norvegica* in different areas records the species feeding on copepods, schizopod and decapod larvae, diatoms, dinoflagellates and detritus (Holt & Tattersall, 1905; Paulsen, 1909; Hickling, 1923–5). Macdonald (1927) concluded that larger specimens feed mainly on copepods, small specimens on diatoms and 'wet dust' or flocculent detritus, and intermediate sizes on vegetable detritus. Einarsson (1945) regarded the chief items in the diet to be detritus and crustacean fragments. He also mentioned the occurrence of a reddish-brown substance in the anterior part of the stomach. The nature of this substance has been suggested by Ponomareva (1955), who found in the stomachs of four species of Pacific euphausiids the

[1] Grant-aided by the Development Fund.

crystalline cones of compound eyes and also remains of the eyes themselves; she considered the dark reddish-brown mass to be the pigmented part of the eyes. The pigment rapidly disappeared on passage through the gut. Ponomareva examined different size-groups from the cyrtopia to the adult stages and found that the groups differed little in the composition of their food; even small euphausiids fed on copepods. She agreed with Einarsson that phytoplankton is not a basic food of euphausiids.

The local environment might account for the presence of vegetable tissues from terrigenous deposits in the Loch Fyne specimens of *M. norvegica* (Macdonald, 1927) and different material might be found in those living farther offshore. In stomachs of *Euphausia krohnii*, caught in the Bay of Biscay, we found contents similar to those of *Meganyctiphanes norvegica*, but none of the terrestrial material present in the stomachs of *M. norvegica* from Loch Fyne. Nevertheless, although *M. norvegica* may be considered as living under specialized conditions in this loch, such sea areas with deep water in close proximity to the land may be important fishing grounds. *M. norvegica* is often a component of the plankton in such areas, not only in Loch Fyne but also in, for example, the Skagerak (Poulsen, 1926), the fjords of the coast of Möre (Ruud, 1926) or the Gulf of Maine (Bigelow, 1914).

MATERIAL AND METHODS

Samples of *Meganyctiphanes norvegica* were collected with 1 m or 2 m stramin nets on one or two consecutive days during each month from September 1956, to April 1958, and in June 1958. The samples for November and December 1957 were kindly collected for us by Dr Mauchline. The area fished in Loch Fyne extended from just north of Arran in a north-westerly direction to the deep water north-east of Tarbert. Apart from one haul taken at about midnight in October 1956, all the specimens examined in 1956 and 1957 were caught during the hours of daylight between 8 a.m. and 6 p.m. A haul was taken at midnight in each monthly series during the period January to April 1958, and in June 1958.

The animals were size-grouped from the length of the carapace at 1 mm. intervals to the nearest half millimetre. Mauchline (1959) discusses the relationship of this measurement to those used by earlier workers, including ourselves. The sexes were not examined separately. Size relationships of the specimens studied are given in Table 1.

Detritus, plant material and copepods were often found in the 'basket' formed by the thoracic legs, but it seems likely that at least some of these, especially whole copepods, such as *Pareuchaeta norvegica*, became adventitiously entangled when the animals were in the net, and so we have assumed that only material that had actually entered the stomach should be considered as food.

In collaboration with Dr Mauchline, we took, with a Jenkin's mud sampler (Mortimer, 1942), kindly lent by the Freshwater Biological Association, mud samples from Loch Fyne, in the area where most of the *Meganyctiphanes norvegica* were caught. The cores, of about 3 in. diameter, were taken on 7 March 1957, at a depth of 175 m. They were cut into sections about $2\frac{1}{2}$ in. long, except the surface layer which was about $\frac{1}{2}$ in. thick and included the thin layer of water lying above it. All the cores were preserved by shaking with about twice their volume of absolute alcohol. Fat-soluble material extracted from the mud was analysed by our usual method (Fisher, Kon & Thompson, 1952).

TABLE 1. SIZE DISTRIBUTION OF SPECIMENS OF *MEGANYCTIPHANES NORVEGICA* EXAMINED

		Number of specimens of carapace length (mm)											
Date of hauls	Time	2	3	4	5	6	7	8	9	10	11	12	Total
19–20. ix. 56	D	28	22	24	17	2	0	11	22	6	9	1	142
25–26. x. 56	D	2	6	28	89	35	21	14	13	12	9	1	230
25–26. x. 56	N	0	0	0	1	14	10	0	2	3	0	1	31
22–23. xi. 56	D	0	1	9	37	21	1	35	19	4	0	0	127
19–20. xii. 56	D	0	0	4	28	8	0	32	18	6	0	0	96
9–10. i. 57	D	0	0	3	40	24	3	51	8	2	0	0	131
7–8. ii. 57	D	0	0	2	27	12	3	20	3	0	0	0	67
7–8. iii. 57	D	0	0	7	28	5	1	9	1	0	0	0	51
4–5. iv. 57	D	0	0	0	10	19	7	37	14	3	0	0	90
24. iv. 57	D	0	0	0	0	6	5	7	9	1	0	0	28
22–23. v. 57	D	0	0	0	1	6	34	27	2	2	0	0	72
19–20. vi. 57	D	0	1	1	0	6	65	48	6	1	0	0	128
10–11. vii. 57	D	0	3	42	41	3	15	15	0	0	0	0	119
14–15. viii. 57	D	0	0	2	48	86	38	116	3	2	0	0	295
11–12. ix. 57	D	0	0	0	4	16	36	97	11	0	0	0	164
9–10. x. 57	D	0	0	1	3	12	64	176	26	0	0	0	282
19. xi. 57	D	0	0	0	1	3	45	72	5	0	0	0	126
17. xii. 57	D	0	0	0	0	1	31	81	2	0	0	0	115
15–16. i. 58	D	0	0	0	0	4	42	150	9	0	0	0	205
15–16. i. 58	N	0	0	0	0	2	27	80	5	0	0	0	114
19–20. ii. 58	D	0	0	0	0	10	57	118	2	0	0	0	187
19–20. ii. 58	N	0	0	0	0	7	41	119	3	1	0	0	171
27–28. iii. 58	D	0	0	0	4	9	58	128	2	0	0	0	201
27–28. iii. 58	N	0	0	0	0	3	21	87	1	0	0	0	112
15–16. iv. 58	D	0	0	0	17	13	51	94	2	0	0	0	177
15–16. iv. 58	N	0	0	0	5	7	57	102	1	0	0	0	172
25–26. vi. 58	D	0	0	0	0	0	10	62	10	0	0	0	82
25–26. vi. 58	N	0	0	0	0	0	0	36	13	1	0	0	50
Total		30	33	123	401	334	743	1824	212	44	18	3	3765

D = Caught in daylight. N = Caught at night.

RESULTS

In considering intensity of feeding and types of food eaten we have first combined results for animals of all sizes in each monthly group. We then discuss the possible relationship between size and the kind of food eaten. So that the results should be comparable throughout the period of sampling, only day hauls are considered. Finally, the results for night and day hauls are compared.

Intensity of feeding

The intensity of feeding was determined by calculating the percentage of animals with food in the stomach in each monthly haul. However, some stomachs contained only a little detritus or other food, contrasting with others from animals that had obviously been feeding more intensively. From November 1956, a note was, therefore, made of the degree of fullness of the stomach.

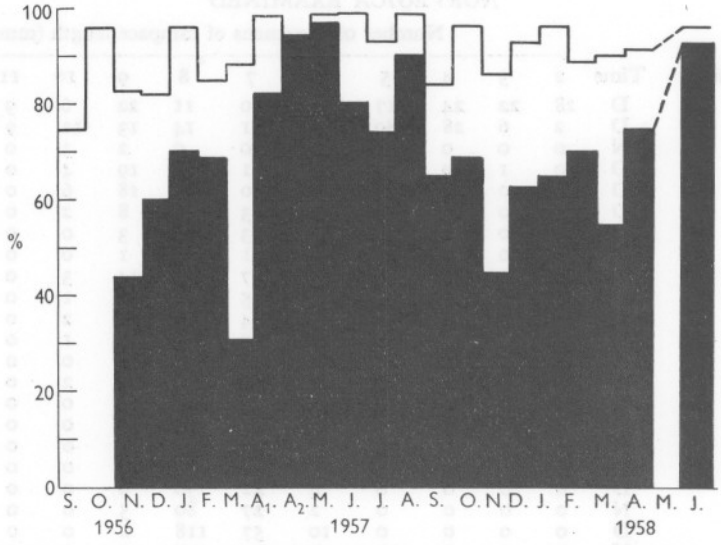


Fig. 1. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing food. Shaded portion: percentage with stomachs more than half-full.

Feeding intensity reached its peak during the spring plankton outburst in late April and May (Fig. 1). The high intensity of feeding in August was associated with the presence of numerous young euphausiids and dinoflagellates in the plankton. There was also evidence of a smaller increase corresponding to the autumn plankton surge in October 1956 and 1957. The high percentage of animals with nearly full stomachs in January and February of each year was unexpected. In January 1957, it was at first thought to be associated with an abundance of the copepod *Pareuchaeta norvegica*. In 1958, however, the species was not particularly numerous in these months. We now consider it more likely that increased feeding at this time of year is due to the greater activity associated with spermatophore transference (Mauchline, 1959).

Types of food

The main components of the diet of *Meganyctiphanes norvegica* from Loch Fyne were organic debris, mud particles and other inorganic detritus, Crus-

tacea, dinoflagellates, fern sporangia, dipteran egg membranes, diatoms, algae, and occasionally other items of terrestrial or marine origin.

Organic debris and inorganic detritus were present in many stomachs, as would be expected during daylight when these diurnally migrating animals lie on or near the bottom. Fig. 2 shows the seasonal fluctuations of these two components. The large amounts usually found indicated that this material probably forms the bulk of the diet, except possibly during the spring feeding period.

There was a marked seasonal variation in the occurrence of crustacean fragments in the stomachs (Fig. 3A). Owing to lack of time no attempt was made to identify parts of copepods separately, and all crustacean remains are considered together, save for the easily identified compound eye parts. The presence of these eyes indicates feeding on eucaridan Crustacea and their absence, when the incidence of crustacean fragments remains high, indicates that copepods were the Crustacea eaten, as during the spring increase in April and May.

In Fig. 3B the percentage of stomachs containing crustacean remains including compound eye parts is shown, and the two periods, June–November 1957 and January–April 1958, are those with the highest frequencies. From June onwards late larvae and adolescents of euphausiids are numerous in the plankton and many of the eyes in the stomachs were identified as belonging to euphausiids. The higher incidence of crustacean fragments and eyes in the early months of 1958 than in the same period in 1957 is difficult to explain, but may have been associated with the absence of sufficient alternative food in the water. It is possible that cannibalism occurs when the proportion of euphausiids to other organisms in the plankton is high either because of an abundance of euphausiids, as in the late summer, or because of a scarcity of other plankton organisms, as in the winter. On the other hand, the average size of the *M. norvegica* forming the population sampled was higher in January 1958 (carapace 8·0 mm long) than a year earlier (6·7 mm) and larger animals eat more crustaceans (see p. 299). However, in the smallest size-group (6 mm carapace) for which we have adequate values for both years there were many more animals with stomachs containing crustacean fragments in January 1958 (75%) than in January 1957 (46%), which suggests that the composition of the plankton is the determining factor.

Dinoflagellates were most frequently found in late summer and autumn with minor increases in April and January (Fig. 4). When dinoflagellates were abundant in the plankton they formed an important part of the diet of *M. norvegica*, occurring in the stomachs in enormous numbers. The species most frequently found belonged to the genera *Ceratium*, *Dinophysis*, *Phalacroma*, *Prorocentrum* and *Peridinium* (Fig. 5). *Ceratium* occurred commonly in autumn, 1956, but in the corresponding period in 1957 it was replaced by *Dinophysis* and *Prorocentrum*. A few stomachs contained *Goniaulax*, mostly in

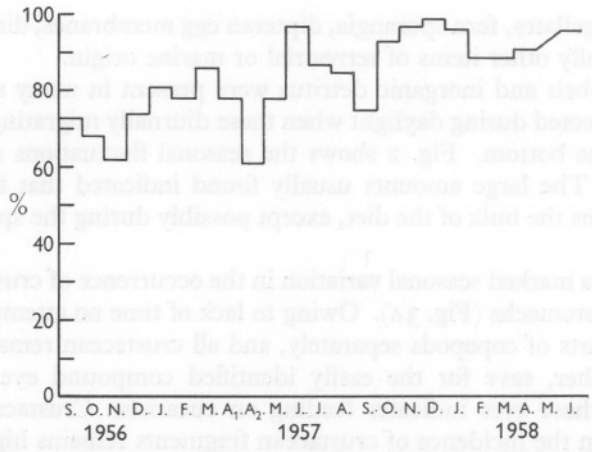


Fig. 2. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing organic debris and inorganic detritus.

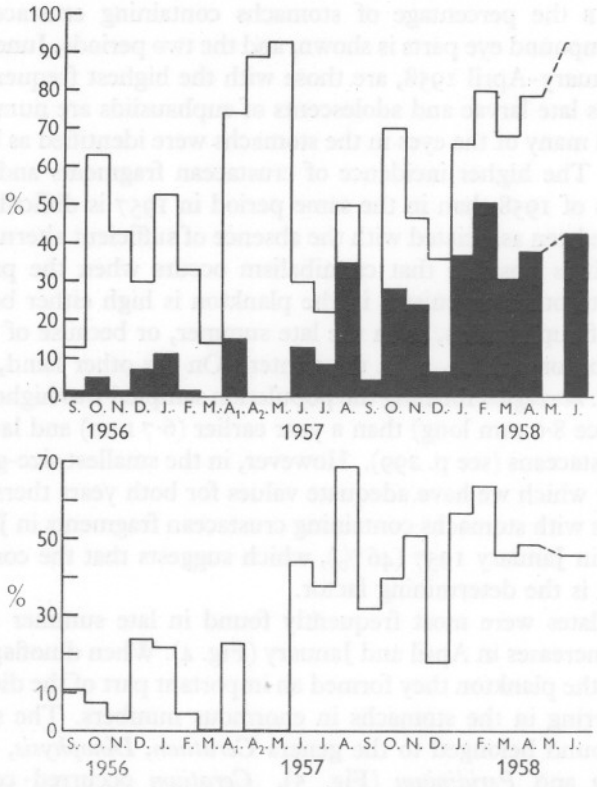


Fig. 3. A, Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing crustacean fragments. Shaded portion: percentage with stomachs containing compound eyes; B, Seasonal variation in percentage of those *M. norvegica* with stomachs containing crustacean fragments in which compound eyes were also present.

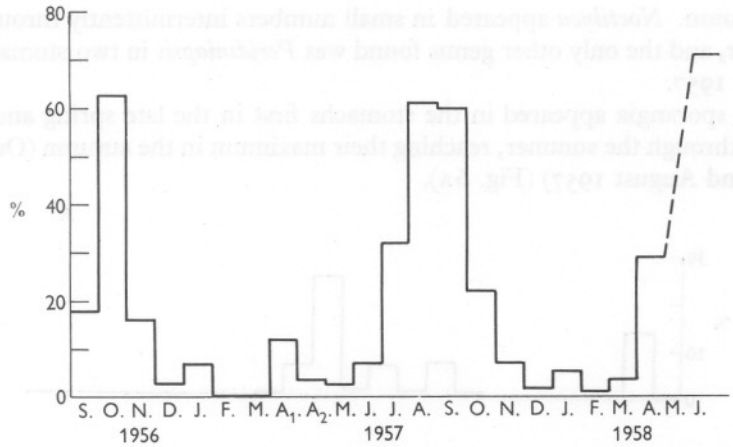


Fig. 4. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing dinoflagellates.

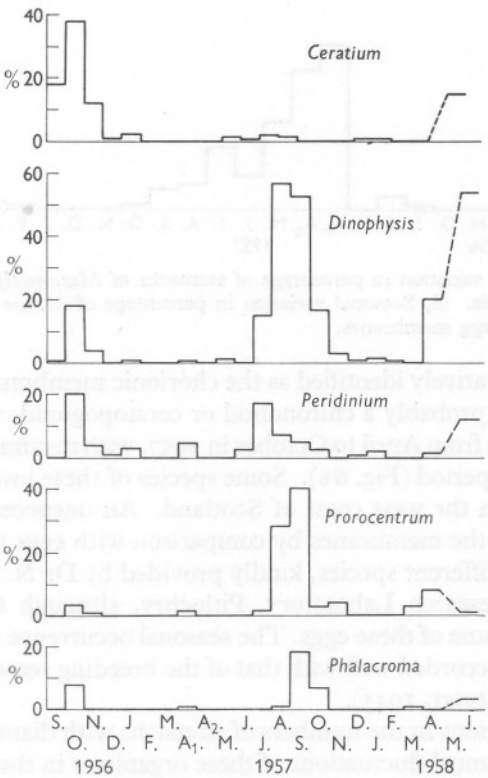


Fig. 5. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing different genera of dinoflagellates.

the autumn. *Noctiluca* appeared in small numbers intermittently throughout the year, and the only other genus found was *Peridiniopsis* in two stomachs in August 1957.

Fern sporangia appeared in the stomachs first in the late spring and continued through the summer, reaching their maximum in the autumn (October 1956, and August 1957) (Fig. 6A).

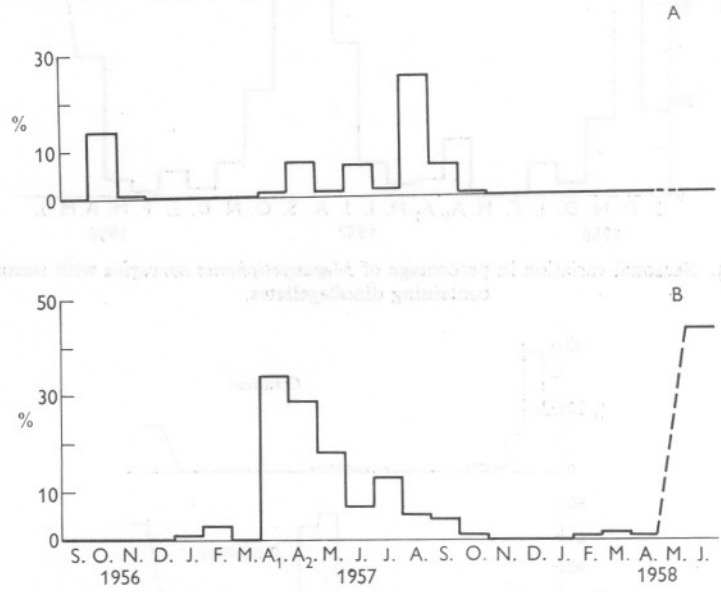


Fig. 6. A, Seasonal variation in percentage of stomachs of *Meganyctiphanes norvegica* containing fern sporangia. B, Seasonal variation in percentage of *M. norvegica* with stomachs containing dipteran egg membranes.

Structures tentatively identified as the chorionic membranes of the eggs of a dipterous insect, probably a chironomid or ceratopogonid, were found in the stomach contents from April to October in 1957, with maximal incidence at the beginning of the period (Fig. 6B). Some species of these insects are present in large numbers on the west coast of Scotland. An unsuccessful attempt was made to identify the membranes by comparison with eggs taken from female chironomids of different species, kindly provided by Dr N. C. Morgan of the Brown Trout Research Laboratory, Pitlochry, although there was a close resemblance to some of these eggs. The seasonal occurrence of the membranes in the stomachs accorded well with that of the breeding season of chironomids in the vicinity (Stuart, 1945).

Seasonal variations in the numbers of stomachs with diatoms (Fig. 7) corresponded to the annual fluctuations of these organisms in the plankton. In the first months of 1958 the numbers of stomachs with diatoms increased from January to April, more closely following the expected cycle of the plankton

than in the previous year. Diatoms were never very numerous in the individual stomachs and were not at any time predominant in the contents. The most commonly occurring were species of *Paralia* and *Thalassiosira* and more rarely we found *Coscinodiscus*, *Rhizosolenia*, *Navicula* and *Biddulphia*.

Parts of green, red and brown algae appeared in a small proportion of stomachs throughout the year; the species were not determined. Fern sporangia, insect egg membranes, diatoms, algae and such occasionally occurring items in the stomachs as chrysomonads and radiolarians, sponge spicules, *Sagitta* spp., terrestrial plant material, and various unidentifiable structures were quantitatively unimportant components of the diet.

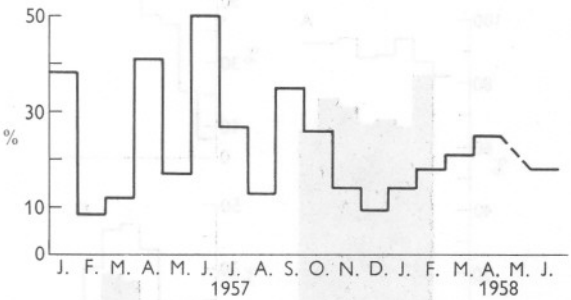


Fig. 7. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing diatoms.

Food in relation to size of animal

Stomachs were examined from animals of carapace lengths ranging from 2 to 12 mm. The smallest of these were probably late furcilia stages (Einarsson, 1945); otherwise, all the animals were post-larval. As specimens with carapace lengths of 2, 11 and 12 mm were taken only during September and October 1956, they have been disregarded partly because of their small numbers and partly because of the seasonal bias they would impart. There appeared to be no overall relationship between size and feeding intensity (Fig. 8A).

The percentage of animals with organic debris and inorganic detritus tended to increase with size (Fig. 8B). Since these components are probably mainly derived from the sea floor there is evidence that larger specimens spend a longer time there than smaller ones. By taking net hauls at different depths Mauchline (1959) has demonstrated the vertical layering of size-groups with the largest specimens lowest in the water.

The incidence of crustacean remains in the stomachs increased fairly sharply with size in the smaller animals, reaching a maximum in those of carapace length 8 mm. and then falling slightly (Fig. 8C). Our values were not so high and did not show so simple an increase to the largest size-group as those illustrated by Mauchline (1959), but his results were based on a single set of

hauls whereas our histogram is compiled from all our values. The difference may be due to seasonal variation, but no definite indication of it could be derived from an analysis of the monthly values. Compound eyes in stomach contents increased in number with the size of the animals, reaching 28% in the group with carapace length 8 mm. and then falling rather sharply (Fig. 8D). These percentages are higher than those of Mauchline (1959), undoubtedly owing to the much higher values we recorded between October 1957, and June 1958.

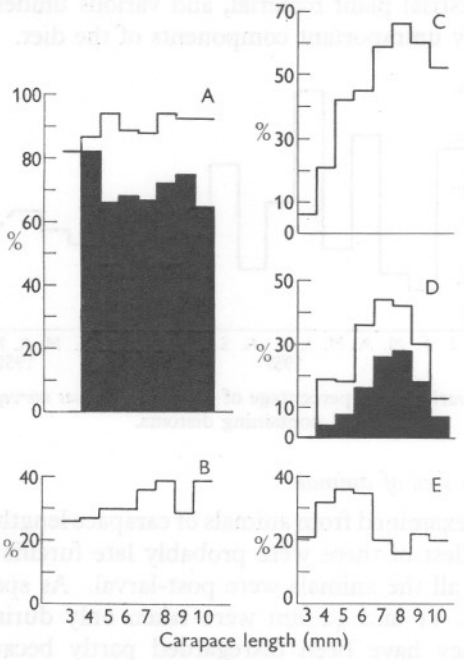


Fig. 8. A, Percentage of *Meganyctiphanes norvegica* of different size-groups with stomachs containing food. Shaded portion: percentage with stomachs more than half-full. B, Percentage of *M. norvegica* of different size groups with stomachs containing organic debris and inorganic detritus. C, Percentage of *M. norvegica* of different size-groups with stomachs containing crustacean fragments. D, Percentage of those *M. norvegica* of different size-groups with stomachs containing crustacean fragments in which compound eyes were also present. Shaded portion: percentage of all stomachs in each size-group with compound eyes present. E, Percentage of *M. norvegica* of different size-groups with stomachs containing dinoflagellates.

Dinoflagellates occurred in all size-groups but most frequently in those with carapace lengths from 4 to 6 mm (Fig. 8E). Diatoms were most frequently present (18–20%) in animals of carapace lengths 6–8 mm. Fern sporangia occurred in all size-groups, with the incidence slightly higher in those with carapace lengths from 4 to 6 mm (7–11%). There was no indication that any size of euphausiid was more likely to contain dipteran egg cases, which were

found in from 2 to 10% of animals in the size range 3–9 mm inclusive. Algae were absent from the smallest and largest animals and occurred in 6–10% of those animals with carapaces 4–9 mm long.

Seasonal variation of food in relation to size

The intensity of feeding by animals of different size-groups was calculated. Sufficient values were available to construct histograms only for the groups with carapace lengths of 6, 7 and 8 mm. In the 8 mm group (Fig. 9), which contained fairly large numbers of specimens in each month, intensive feeding occurred from December 1956 to February 1957, from April to June 1957,

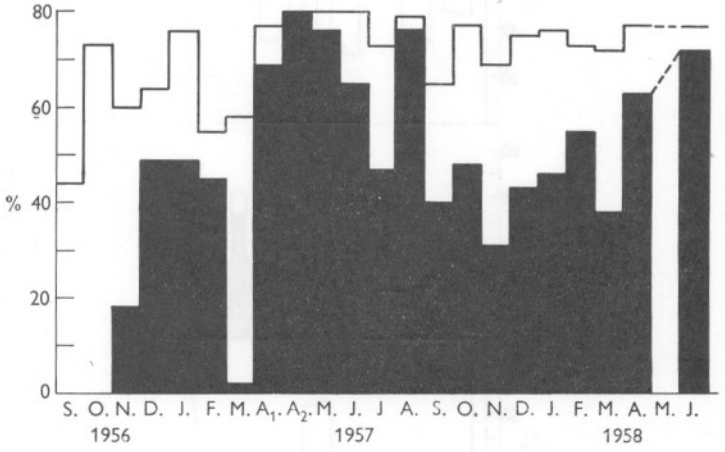


Fig. 9. Seasonal variation in percentage of *Meganyctiphanes norvegica* with a carapace length of 8 mm with stomachs containing food. Shaded portion: percentage of this size-group with stomachs more than half full.

and in August 1957. The winter feeding period occurred again from December 1957 to February 1958. Although there were some small differences, the overall picture presented by the 7 mm group was similar to that for the 8 mm specimens. Animals of this size did not appear in any numbers until January 1957, and feeding activity was at its highest in February–March, late April–June and August–October 1957. Increased spring feeding began again in April and continued through June 1958. Maximal feeding periods for the 6 mm group occurred in December 1956 to February 1957, April–June, August–October 1957, and December 1957 to January 1958. The picture presented by all three size-groups and the fragmentary information for the larger and smaller sizes leaves no doubt that there are three main feeding periods during the year, the most intensive in spring and early summer, and the other two in autumn and in winter.

As in the total population, seasonal incidence of organic debris and inorganic detritus in the various size-groups showed no consistent fluctuations.

The 8 mm group provided the most complete picture of the seasonal variations of crustacean remains in the stomachs and is representative of the other sizes. Fig. 10A shows that the main peak was, as would be expected, in April and May 1957, although, in 1958, with no information for May, the highest level did not occur until June. January 1957 and February 1958 marked the peaks of the winter feeding periods for this kind of food. August and October

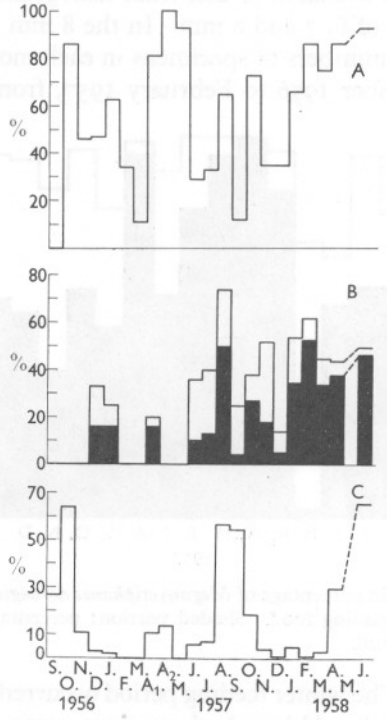


Fig. 10. A, Seasonal variation in percentage of *Meganyctiphanes norvegica* with a carapace length of 8 mm with stomachs containing crustacean fragments. B, Seasonal variation in percentage of those *M. norvegica* with a carapace length of 8 mm with stomachs containing crustacean fragments in which compound eyes were also present. Shaded portion: percentage of all stomachs in 8 mm group with compound eyes present. C, Seasonal variation in percentage of *M. norvegica* with a carapace length of 8 mm with stomachs containing dinoflagellates.

were also months in which Crustacea were present in a high proportion of stomachs, with a marked contrast in September. In the 8 mm size-group there were no animals with such food in September 1956, and those of other sizes showed a low incidence in the same month. The close similarity between the fluctuations in Fig. 10A and those in Fig. 3A is remarkable since the 8 mm size-group did not constitute the majority of the population until September 1957.

Our values for the occurrence of compound eyes in the stomachs of animals with 8 mm carapaces are again the most complete and are representative of the

other sizes. Incidence of these eyes in the winter and autumn feeding periods was high but they were almost entirely absent from the stomachs of animals caught during the main feeding period in the spring (Fig. 10B). There are probably two reasons for this absence; one is that copepods are far more numerous during the spring increase than at other times and the other is that the euphausiids themselves exhibit much less luminescence at this time of year (Mauchline, 1959). Most of the eyes in the stomachs appear to be those of euphausiids, and their predators, whether of the same or another species, are probably attracted to them by the luminescing of the photophore on the eye-stalk.

Dinoflagellates in the stomachs exhibited the same seasonal trends in all size-groups. Fig. 10C shows these for the 8 mm group, with peaks in October 1956, and August and September 1957. In 1958, a peak as high as those in previous years appeared already in June when the investigation ended. The incidence of the different genera of dinoflagellates exhibited the same seasonal patterns in each size-group as shown by the whole population irrespective of size and illustrated in Fig. 5.

Diatoms, chorionic membranes of dipteran eggs and fern sporangia showed seasonal variations in the size groups similar to those in the whole population (see Figs. 6 and 7).

Diurnal variations in stomach contents

Table 2 shows that there was little difference in the occurrence of food in the stomachs of animals collected during daylight compared with those collected at around midnight, except in October 1956, when the number of stomachs with food was lower at night than in the daytime. This finding is, similar to that of Mauchline (1959) in November for *I*-group animals, that is, those that have taken part in one breeding season. The relationship between the intensity of feeding by day and by night in different size-groups was similar to that obtaining for the whole samples regardless of size.

The incidence of those animals with stomachs more than half full was higher at night in January, March, April and June, and lower in February. The degree of fullness of the stomach was not noted for the October specimens. The occurrence of organic debris and inorganic detritus was very similar in all the day and night hauls irrespective of size of animals examined. When feeding was intensive, especially in spring, the uptake of detritus was lower and no relationship between the size of the animals, their vertical distribution, and the incidence of such material was detected. During periods of less intensive feeding a marked correlation was apparent; with increasing size of animal and, therefore, a lower position in the water, more stomachs contained organic and inorganic particles.

As fern sporangia were present more frequently at night, when the animals normally migrate upwards, we infer that the sporangia occur in the plankton

and not resting on the bottom. The incidence of dipteran egg cases did not vary so markedly between day and night, the most appreciable difference being in June when the daytime value was higher. However, since in that month feeding appeared to be at its peak, it seems likely that the egg membranes were in the plankton rather than on the bottom.

TABLE 2. INCIDENCE OF FOOD AND ITS COMPONENTS IN STOMACHS OF *MEGANYCTIPHANES NORVEGICA*

(Figures are percentages of all stomachs examined)

	Time caught	October 1956	January 1958	February 1958	March 1958	April 1958	June 1958
Food present in stomach	D	96	96	89	90	92	96
	N	81	97	85	93	94	98
Stomach more than half full	D	—	65	70	55	75	93
	N	—	70	49	92	92	98
Organic debris and inorganic detritus	D	68	96	89	89	91	96
	N	74	97	82	92	94	98
Crustacean fragments	D	63	66	79	68	79	93
	N	61	80	65	92	74	98
Compound eyes	D	4·8	37	51	31	38	43
	N	0	59	35	21	14	72
Fern sporangia	D	14	0	0	0	0	0
	N	39	0	0·6	1·8	0·6	2·0
Dipteran egg membranes	D	0	0	0·5	1·5	1·1	44
	N	0	0	1·8	1·8	1·1	34
Algal filaments	D	6·9	0·5	0·5	12	2·3	6·1
	N	32	0	0·6	5·4	5·8	4·0

D = Day. N = Night.

When dinoflagellates were abundant, as in October 1956 and June 1958, they were eaten by animals of all sizes by day and by night. In the first four months of 1958, when these organisms were found less often in the stomachs, the incidence of the three commonest genera, *Ceratium*, *Dinophysis* and *Peridinium*, tended to be higher during the day than at night and higher in smaller animals than in larger ones. *Prorocentrum* occurred in appreciable numbers in October and June and *Phalacroma* only in October and, on each occasion, they were more numerous in the animals caught at night. The difference in occurrence of these various genera by day and by night was probably associated with their vertical distribution in the plankton.

Diatoms were relatively unimportant in the diet. Usually *Thalassiosira* was found more frequently in the stomachs of specimens taken at night and *Paralia* in those taken in daylight. There was a tendency for diatoms to occur more often in the smaller animals in January, February and March, but in April and June, when they became more common in the plankton, they were found in about 20 to 30% of nearly all size-groups, with no predominance by day or night.

In October, algal filaments occurred during the day in size-groups up to and including 7 mm, and at night 9 mm, but the incidence was much higher at

night than during the day. In the other months of the diurnal study algae occurred much less often and no relationship either to size or time of catching the animals was detectable.

Analysis of mud from bottom of Loch Fyne

Mauchline (1959) has given a brief account of the organisms found on the surface of the mud samples. Living filamentous algae were present, some of littoral origin, but none was attached or growing at that depth. There were also numerous ciliates. Like Mauchline, we found protozoan remains in the stomachs of the euphausiids. Only a few diatoms, mostly *Biddulphia*, were found in the mud. Small polychaetes were present in the deposits and were occasionally also found in the stomachs. The mud contained large numbers of cumaceans and a few isopods. If these were present in the stomachs they were included with the crustacean remains and not identified separately.

TABLE 3. CAROTENOIDS IN MUD FROM THE BOTTOM OF LOCH FYNE

Depth of samples = 175 m.

Sample	Thickness (in. approx.)	Total carotenoids		β-carotene	
		μg	μg/g fat	μg	μg/g fat
A. Surface	½	18	421	Trace	—
A. 2nd layer	2½	55	1070	4·5	87
A. 3rd layer	2½	116	6250	11	574
A. Bottom layer	2½	112	3380	13	397
B. Surface	½	22	3940	Trace	—
C. Surface	½	23	1050	Trace	—

Analysis of extracts from some of the cores for vitamin A and carotenoids (Table 3) shows that vitamin A was not present in measurable amount. The carotenoid content of the organisms just mentioned as living on the surface of the mud would be included in the values given in the table for the surface layers.

DISCUSSION

Although our findings provide new information about the food and feeding habits of *Meganyctiphanes norvegica*, confirming and supplementing that presented by Macdonald (1927) and Mauchline (1959), the picture is obviously very complex. We believe we now have enough information from this study about the qualitative aspects of the diet, although the proportions of its components have been derived from subjective estimates, to try to assess the contributions of the food to the rich reserves of vitamin A present mainly in the eyes. In addition we possess a considerable amount of data, from earlier observations of other workers and ourselves, on the occurrence of vitamin A or its precursors in these components.

The most commonly occurring food substances were inorganic detritus and organic debris, particularly in the stomachs of animals caught during the day

when they were on or near the sea bottom and filter-feeding in the manner described by Mauchline (1959). Fox (1950) discussed the value of such material in the nutrition of marine animals and stressed its importance as a source of carotenoids. Marine muds have been shown to be particularly rich in carotenes (Fox, Updegraff & Novelli, 1944). Our own results (Table 3) indicate that, although β-carotene is present in the mud, a euphausiid that consumed the mud occupying a circular area of 3 in. diameter and $\frac{1}{2}$ in. thickness would not obtain enough to be measured by our technique (i.e. probably less than 1 μg). Certainly it would take in a greater amount of other carotenoids, but these would mostly be xanthophylls which, so far as we know, would not be vitamin A precursors. With allowance for greater thickness of the deeper mud samples there was some indication of an increase in carotenoid concentration and in the proportion of β-carotene with depth. Fox & Anderson (1941) found a similar increase in the ratio of carotenes to xanthophylls with the depth and, therefore, age of the mud. Our observations of living *Meganyctiphanes norvegica* in aquaria show that the animals do not penetrate far below the mud surface and so they would be unlikely to reach the layer richer in carotene.

Many stomachs contained what Mauchline (1959) has described as a 'green mush' of small particle size which he believes to be synonymous with Macdonald's (1927) 'flocculent detritus'. We do not know whether this material was derived from the bottom or from suspended matter in the sea water itself. Fox (1937) attempted to assess the importance of suspended matter and microplankton as a source of carotenoids by filtering 4000 l. of sea water. He extracted from the deposit 0·1 mg of xanthophylls and 0·02 mg of carotenes. Fox, Isaacs & Corcoran (1952) estimated that, in the sea off the Californian coast, the colloidal or otherwise finely particulate matter, which they called leptopel, included only 1·5–4% of living cells. Loch Fyne is less than 3 miles wide in the area where most of the euphausiids were caught, and the heavy rainfall (Barnes & Goodley, 1958), washes down a considerable quantity of detritus from the land, evidence of which has been provided by the occurrence of fern sporangia and dipteran egg cases in the stomachs of *Meganyctiphanes norvegica*. It is probable, therefore, that these waters contain more suspended material than those examined by Fox and his colleagues and are consequently a richer source of carotenoids than their data would suggest.

Crustacea become increasingly important in the food of *M. norvegica* as it grows. During the spring increase, copepods predominate but at other times of the year the presence of compound eyes indicated that eucaridan species are more commonly eaten. Many of these eyes were undoubtedly of euphausiid origin. In bulk of material the most important copepods in the planktonic community inhabited by *M. norvegica* are *Calanus finmarchicus* and *Pareuchaeta norvegica*. We have found occasionally small amounts of vitamin A in *P. norvegica* (Fisher *et al.* 1952) but none at all in *Calanus finmarchicus* (Fisher *et al.* 1952; cf. Euler, Hellström & Klussmann, 1934, and Lederer, 1938). We have

now analysed twenty-two other species of copepod and found the vitamin in only two species of *Gaetanus* from the Bay of Biscay (Fisher & Kon, 1959). All these results refer only to the ester and alcohol forms of vitamin A. The recent finding of vitamin A aldehyde in fish eggs (Plack, Thompson & Kon, 1958) indicated that this form might be present in other marine organisms but not detected by our normal analytical technique. Using the gradient elution method of Plack, Kon & Thompson (1959) we have found no vitamin A aldehyde in *Calanus finmarchicus*. All our evidence indicates that copepods are not a source of preformed vitamin A for euphausiids feeding on them. Earlier results show that the main carotenoid in copepods is astaxanthin possibly with small quantities of β-carotene. In the recent chromatographic analysis of extracts from *C. finmarchicus*, however, using the gradient elution method, we obtained a pigment, or pigments, which exhibited a complex absorption spectrum with three peaks all below 400 mμ. This pigment will be the subject of further study and its possible significance as a provitamin A will be investigated. Previously we demonstrated that the most striking increase in both the absolute amount and the concentration of vitamin A in *Meganyctiphanes norvegica* occurred during the spring period (Fisher *et al.*, 1954) just at the time when, as we have now confirmed, they feed mainly on copepods which contain no obvious provitamin or vitamin A, rather than at other times of year when they are eating the eyes of eucaridan Crustacea which we know to be rich in the vitamin. In laying stress on the absence of compound eyes from the stomachs during the spring feeding period (see Figs. 3 and 4), we realize that our 1958 results did not show this absence. In 1957, however, compound eyes were absent from the stomachs only in the second April and the May hauls, which were taken during the period of 74 days between the first April and the June hauls. In 1958 no hauls were taken over the 78 days that elapsed between the hauls in April and in June. Thus we may well have missed the period corresponding to that in 1957 when no compound eyes were found in the stomachs and the euphausiids were feeding on copepods. Moreover, *Calanus finmarchicus* was not caught in large numbers in the area until 13 May in 1958, which is much later for its appearance than is normal; in 1957, it became abundant between the first and second collecting trips in April.

We have suggested astaxanthin derived from copepods as a possible precursor for vitamin A in the euphausiids (Fisher *et al.*, 1954; Kon, 1954) but this pigment is present not only in copepods but in all crustaceans, especially in their eyes (Fisher *et al.*, 1952). If astaxanthin is indeed the precursor euphausiids obtain from copepods it must be in them in a more available form than in other crustaceans to account for the more rapid increase in the vitamin A reserves of euphausiids when they feed almost exclusively on copepods. In any event, the vitamin A they derive from eating other Eucarida is unlikely to account for all the reserves built up in *Meganyctiphanes norvegica*. Some additional source must exist.

After detritus and Crustacea, dinoflagellates appear to us to be the next most important component of the diet of *M. norvegica*, particularly in the younger adults. In the late summer and early autumn dinoflagellates reach their maximum incidence in the stomachs, which may be packed full of them. The only information we have about provitamins and vitamin A in dinoflagellates is for the marine species *Prorocentrum micans* (Scheer, 1940) and two species of *Peridinium*, the freshwater *P. cinctum* (Strain, Manning & Hardin, 1944) and the marine *P. trochoideum*[1]. There is no information about the vitamin itself in *Prorocentrum micans* or *Peridinium cinctum*; it was absent from *P. trochoideum*. In all three species the carotenoids included both carotenes and xanthophylls. In *Prorocentrum micans* about 10% of the carotenoids were carotenes and the rest presumably xanthophylls. The carotenes were almost entirely β-carotene in *Peridinium cinctum* and *P. trochoideum* in quantities greater than in diatoms. As in *Prorocentrum micans* most of the carotenoids in *Peridinium* were xanthophylls at a concentration higher than in most diatoms (Strain *et al.*, 1944). The three main xanthophylls separated from extracts of *P. cinctum* were peridinin (first detected in *Peridinium* spp. by Kylin (1927)), dinoxanthin and diadinoxanthin, of which the first was the most abundant (Strain *et al.*, 1944). The chemical properties of these xanthophylls are still unknown, but they are unlikely to be provitamins A. β-carotene from dinoflagellates is, however, undoubtedly an important seasonal precursor of the vitamin for *Meganyctiphanes norvegica*.

Although we agree with Einarsson (1945) and Ponomareva (1955) that diatoms do not form an important component of the diet of *M. norvegica*, those diatoms eaten will contribute to the carotenoids taken up by the euphausiid. Vitamin A itself does not occur in those species in which it has been sought, namely *Coscinodiscus concinnis*, *Skeletonema costatum* and *Thalassiosira gravida*[1]. The main carotene was β-carotene which formed up to about 5% of the total carotenoids. It was the principal carotene in several other species studied by Strain *et al.* (1944). These workers also characterized the xanthophylls. The most abundant was fucoxanthin and of the others the most important were diadinoxanthin and diatoxanthin. Goodwin (1952) has suggested that these two pigments may be *cis*-isomers of lutein and zeaxanthin respectively. If so, neither of them is a provitamin A. Zeaxanthin has the same molecular structure as β-carotene with the addition of an hydroxyl group to each of its β-ionone rings and could be an intermediate between β-carotene and astaxanthin, which has the structure of zeaxanthin with a ketone group in each β-ionone ring. It is possible, therefore, that diatoxanthin may be a connecting link between the important plant carotenoid, β-carotene, in the phytoplankton and the equally important animal carotenoid, astaxanthin, in the crustaceans feeding on it.

Filamentous algae were a constant, if only minor, constituent of the food of

[1] Fisher, L. R., London Univ. Ph.D. thesis, 1953.

Meganyctiphanes norvegica. Most of those eaten were probably green algae with carotenoids similar to those of land plants, possessing β-carotene as their principal vitamin A precursor.

Young *M. norvegica* have in their food several sources of β-carotene, namely dinoflagellates, vegetable detritus of terrestrial origin, algae and diatoms. It is probable that they convert β-carotene into vitamin A like higher animals. As they grow larger they become increasingly carnivorous, feeding more and more on Crustacea, although still taking dinoflagellates when these are abundant. The Crustacea provide them with preformed vitamin A when eucaridan species are eaten, but in the spring copepods are eaten almost exclusively and their principal carotenoid is astaxanthin. It is possible that this carotenoid may be converted into vitamin A just as it is said to be by poeciliid fish (Grangaud & Massonet, 1955). Evidence of the rapid accumulation of the vitamin at a time when the euphausiids are feeding on copepods supports this hypothesis (Fisher *et al.*, 1954).

The fern sporangia found in small numbers in the stomachs of *M. norvegica*, indicate that there may be an inkling of truth in the long-held belief that the bracken-clad hills bordering the loch nourish the Loch Fyne herring, one of the predators of *M. norvegica*, and impart to them and the kippers made from them their renowned high quality (Kerr, 1928, 1949; McCallien, 1938). Macdonald (1927) also discussed the dependence of the herring, through *M. norvegica*, on the abundance of terrigenous vegetable detritus. Essentially the reasoning is correct but we now know that the food chain is longer and more complex, involving both organic and inorganic nutrients from the land. The phytoplankton flourishes on the former and the zooplankton feeds, in turn, on it as well as on the land-derived vegetable material with its carotenoids, especially β-carotene. Such a diet produces specimens of *M. norvegica* in Loch Fyne larger than any we have seen from other waters, including the North Sea, the north Atlantic and the Mediterranean; these large animals are much richer in vitamin A than those we have analysed from the other localities mentioned (Fisher *et al.*, 1955).

Facilities for collecting the material were very kindly provided by the Director of the Marine Station at Millport and we are very grateful to him and his staff, especially the skippers and crews of M.V.'s 'Calanus' and 'Mizpah'. For much helpful discussion and criticism during the preparation of this paper we are indebted to Drs S. M. Marshall, S. K. Kon, J. Mauchline and A. P. Orr.

SUMMARY

Feeding intensity in *Meganyctiphanes norvegica* in Loch Fyne reached its peak during the spring plankton outburst, falling slightly during the summer, to be renewed in the early autumn and decreasing to a minimum in November.

There was a well-defined period of increased feeding from December to February, followed by another decrease in March.

The main food items in decreasing order of importance were organic debris and inorganic detritus, Crustacea, dinoflagellates, diatoms, algae, fern sporangia and dipteran egg membranes. Organic and inorganic material was taken at all seasons, although possibly in smaller amounts during the spring feeding period, when the euphausiids spend more time actively swimming.

Crustacean material occurred most commonly in the stomachs in the spring, when the absence of compound eyes indicated that it was probably all of copepod origin. These eyes, mostly euphausiid, occurred frequently in the stomachs from June onwards and during the late winter.

Dinoflagellates predominated in later summer when some stomachs were packed with them. Diatoms were most numerous during the spring but were never eaten to the same extent as the dinoflagellates. Fern sporangia and dipteran egg capsules appeared in the stomachs in the spring and summer months. Algae were eaten by a few animals at all seasons.

Size of the animal and feeding intensity showed no apparent relationship. The percentage of stomachs containing organic and inorganic particles increased with the size of the animal. Crustacean remains also occurred more frequently in larger animals, but less commonly in the largest specimens of all. Dinoflagellates were eaten by all sizes, with the highest incidence in those of intermediate size.

No consistent differences were observed between the intensities of feeding by day and at midnight. When feeding was intensive, especially in the spring, the relative uptake of organic and inorganic material was lower than during periods when feeding was on a reduced scale.

When the dinoflagellates were abundant in the plankton they occurred in large numbers at all times, but, when they were scarcer, the numbers of *Ceratium*, *Dinophysis* and *Peridinium* tended to be higher in stomachs by day than by night and those of *Prorocentrum* and *Phalacroma* by night.

Samples of mud taken at a depth of 175 m from the bottom of Loch Fyne contained no measurable vitamin A. The amount of carotenoid pigments and of β-carotene increased with the distance below the surface.

Preformed vitamin A is present in compound eyes and its precursor, β-carotene, in dinoflagellates, diatoms, algae and fern sporangia, all of which are eaten by *Meganyctiphanes norvegica*, and so contribute to its high vitamin A reserves. In the spring, however, when the food consists almost entirely of copepods with astaxanthin as their main, if not only, carotenoid, vitamin A concentrations increase most rapidly. It is possible that astaxanthin is a vitamin A precursor in euphausiids.

REFERENCES

BARNES, H., & GOODLEY, E. F. W., 1958. A note on rainfall in the west of Scotland. *Glasg. Nat.*, Vol. 18, pp. 45–54.

BIGELOW, H. B., 1914. Oceanography and plankton of Massachusetts Bay and adjacent waters, November 1912–May 1913. *Bull. Mus. comp. Zool. Harv.*, Vol. 58, pp. 385–419.

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

EULER, H. VON, HELLSTRÖM, H. & KLUSSMANN, E., 1934. Über den Carotenoidgehalt einiger Evertebraten. *Hoppe-Seyl. Z.*, Bd. 228, pp. 77–89.

FISHER, L. R. & KON, S. K., 1959. Vitamin A in the invertebrates. *Biol. Rev.*, Vol. 34, pp. 1–36.

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

—— —— —— 1954. Vitamin A and carotenoids in certain invertebrates. II. Studies of seasonal variations in some marine Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 589–612.

—— —— —— 1955. Vitamin A and carotenoids in certain invertebrates. III. Euphausiacea. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 81–100.

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

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

FOX, D. L. & ANDERSON, L. J., 1941. Pigments from marine muds. *Proc. nat. Acad. Sci., Wash.*, Vol. 27, pp. 333–7.

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

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

GOODWIN, T. W., 1952. *The Comparative Biochemistry of the Carotenoids*. 356 pp. London: Chapman and Hall.

GRANGAUD, R. & MASSONET, R., 1955. Recherches sur la biogénèse de la vitamine A des poissons. I. L'astaxanthine provitamine A pour *Gambusia holbrooki* Grd. *Arch. Sci. physiol.*, T. 9, pp. 245–56.

HICKLING, C. F., 1923–5. Notes on euphausiids. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 735–45.

HOLT, E. W. L. & TATTERSALL, W. M., 1905. Schizopodous Crustacea from the north-east Atlantic slope. *Rep. Sea Inl. Fish. Ire., Sci. Invest.*, Year 1902–3, App. 4, pp. 99–152.

KERR, J. G., 1928. *General Handbook of the British Association*, pp. 347–57.

—— 1949. The Scottish Marine Biological Association. *Notes roy. Soc. Lond.*, Vol. 7, pp. 81–96.

KON, S. K., 1954. Vitamine A des invertébrés marins et métabolisme des caroténoïdes du plancton. *Bull. Soc. Chim. biol., Paris*, T. 36, pp. 209–25.

KYLIN, H., 1927. Über die karotinoiden Farbstoffe der Algen. *Hoppe-Seyl. Z.*, Bd. 166, pp. 39–77.

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

MACDONALD, R., 1927. Food and habits of *Meganyctiphanes norvegica*. *J. mar. biol. Ass. U.K.*, Vol. 14, pp. 753–84.

MAUCHLINE, J., 1959. The biology of a euphausiid crustacean, *Meganyctiphanes norvegica* (M. Sars). *Proc. roy. Soc. Edinb.* (in the Press).

MCCALLIEN, W. J., 1938. *Geology of Glasgow and District.* 190 pp. London and Glasgow: Blackie.

MORTIMER, C. H., 1942. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.*, Vol. 30, pp. 147–201.

PAULSEN, O., 1909. Plankton investigations in the waters round Iceland and in the north Atlantic in 1904. *Medd. Komm. Havundersøg., Kbh.*, Ser. Plankton, Bd. 1, No. 8, 57 pp.

PLACK, P. A., KON, S. K. & THOMPSON, S. Y., 1959. Vitamin A_1 aldehyde in the eggs of the herring (*Clupea harengus* L.) and other marine teleosts. *Biochem. J.*, Vol. 71, pp. 467–76.

PLACK, P. A., THOMPSON, S. Y. & KON, S. K., 1958. Vitamin A_1 aldehyde in fish eggs. *Biochem. J.*, Vol. 68, p. 2*P*.

PONOMAREVA, L. A., 1955. The feeding and distribution of euphausiids of the Sea of Japan. *Zool. Zh.*, Vol. 34, pp. 85–97. (In Russian.)

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

RUUD, J. T., 1926. Zooplankton of the coast of Möre, 1926. *Rapp. Cons. Explor. Mer*, Vol. 41, pp. 120–4.

SCHEER, B. T., 1940. Some features of the metabolism of the carotenoid pigments in the California sea-mussel (*Mytilus californianus*). *J. biol. Chem.*, Vol. 136, pp. 275–99.

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

STUART, T. A., 1945. Chironomid larvae of the Millport shore pools. *Trans. roy. Soc. Edinb.*, Vol. 60, pp. 475–502.

J. mar. biol. Ass. U.K. (1959) 38, 313–318
Printed in Great Britain

A LINEAR PATTERN ON THE SEA FLOOR AND ITS INTERPRETATION

By A. H. STRIDE

National Institute of Oceanography, Wormley, Surrey

(Plate I and Text-fig. 1)

The redistribution of sediments by tidal streams has been demonstrated in a number of ways in the seas around Britain. For example, the numerous banks in the North Sea are elongated parallel to the streams, while the depth and position of certain channels are subject to such important changes in position that re-surveys have to be made each year or so (Robinson, 1956). In the same region the streams, reaching about a knot or more at the surface, have wrought a large area of sand floor into ridges normal to their path. Off the Dutch coast the grade of the sand (Jarke, 1956) decreases in their inferred direction of advance (Stride & Cartwright, 1958). Similar relief is found even at 90 fathoms near the edge of the continental shelf, at the western approaches to the English Channel (Cartwright & Stride, 1958). Pratje (1950) has shown that the occurrence and grade of loose sediment on the floor of the Channel as a whole is directly related to the velocity of the streams overhead.

Fresh evidence can now be given of the action of tidal streams on some flat floors of the English Channel and North Sea.

SURVEY

During April 1958 a survey of the floor (Fig. 1) was made by R.R.S. 'Discovery II' in the large open bay between Start and Dodman Points, near Plymouth, using echo-ranging equipment such as described by Chesterman, Clynick & Stride (1958). The present equipment had the following characteristics: frequency 37 kc/s; pulse length 1 ms; beam shape 1·8° (horizontal), 11° (vertical), with four side lobes in addition; tilt angle 5–7° below horizontal. A sample of the records obtained is shown in Pl. I, with the range abeam of 732 m (800 yards) as the horizontal scale.

During September 1958 five cores and photographs of the floor were taken by R.V. 'Sarsia' at positions shown near P in Fig. 1.

THE LINEAR PATTERN

The pattern has been recognized in a curved belt about 7 miles wide, roughly parallel to the Cornish coast from between Rame Head and Eddystone to Dodman Point. It does not extend as far east as the line run north-east from

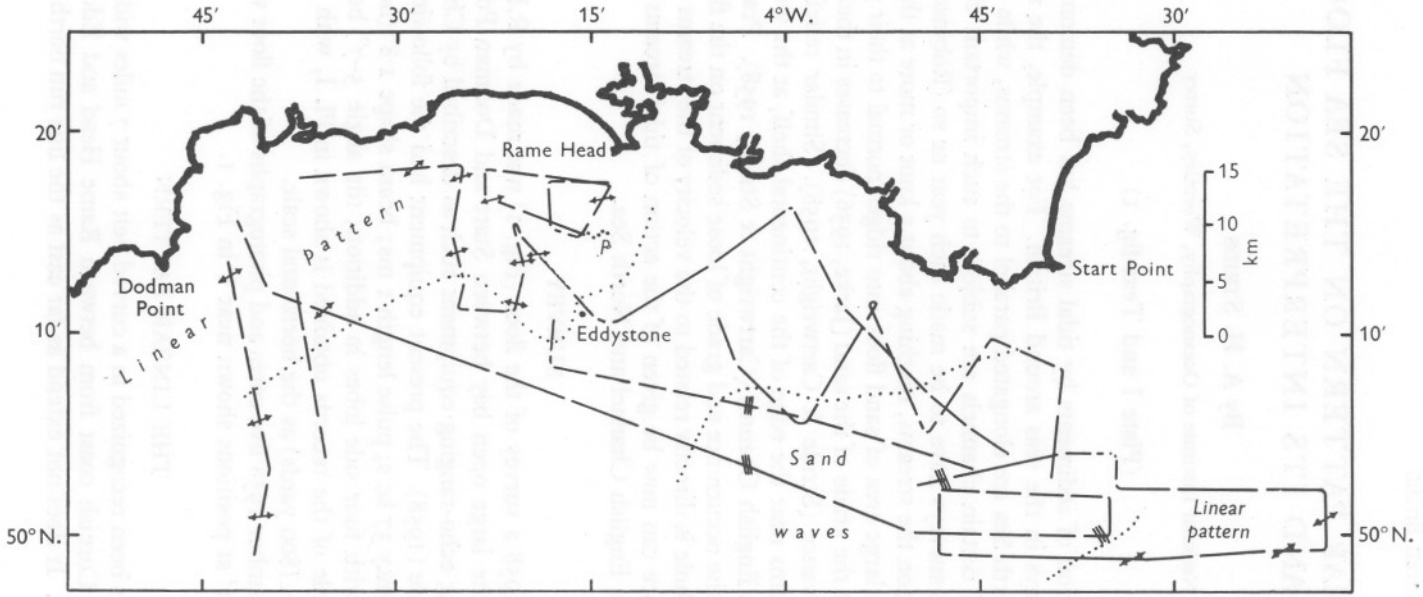


Fig. 1. Courses on which the acoustic survey was made are shown by thick lines. The patches were elongated parallel to the arrows and the crests of sand waves lay parallel to the groups of 3 lines.

Eddystone. The southern margin is gradational; the south-western limit has not yet been found. The floor is virtually devoid of relief except for the small regional depth gradient.

The acoustic pattern consists of well defined, almost parallel patches of contrasting reverberation level, such as extend across Pl. I. The low reverberation patches certainly range up to 1·8 km in length and between 50 and 200 m wide and about the same distance apart. In the middle of the region the patches are wider. Some patchiness is found farther south from here and may be present south of Start Point.

Off the East Anglian coast the patches are locally well developed, certainly reaching 1·4 km in length, yet only 90 m in width. The boundaries are generally sharp, the ends are tapered.

In each place the patches are elongated parallel to the path of the strongest tidal streams. For the Plymouth region the observed orientation of the patches is shown by the arrows in Fig. 1. The patches south of Start Point are orientated normal to the crests of sand waves occurring nearby, themselves an indicator of the direction of the streams (Chesterman *et al.*, 1958). The sand waves are up to 3 m high and mostly between 100 and 250 m apart. They are smallest and least well developed at the margins of the patch.

TABLE 1. LITHOLOGY OF CORES TAKEN NEAR EDDYSTONE

Core no.	Layer thickness (cm)	Lithology	Cumulative weight percentage of coarse fractions		
			> 12 mm	> 6 mm	> 2 mm
1	16	Fine sand with shell and rock fragments. Trace of coal	3	10	25
	10	Coarse reddish shell 'gravel' with about 30 % sand. Boundary gradational	—	—	—
2	1	Fine sand on surface	0	0	5
	13	Fine sand with shell and rock fragments. Trace of coal	—	—	—
	10	Coarse 'gravel' of shell and rock debris	—	—	—
3	13	Fine sand with shell fragments. Trace of coal	0	2	10
	4	Coarse 'gravel' of shell and rock debris with about 20 % sand	—	—	—
4	3	Fine sand with shell and rock debris	0	4	16
	12	Same as above with trace of mud	—	—	—
	8	Coarse gravel of shell and rock debris up to 2 cm diameter, with about 20 % sand	—	—	—
5	2	Fine sand	0	0	10
	16	Sand at top grading down into gravel	—	—	—
	4	Fine gravel of shell and rock debris	—	—	—

COMPOSITION OF THE FLOOR

Between Eddystone and Plymouth grab sampling has revealed that the floor may be made of almost pure sand or sand with an appreciable fraction of coarser material, greater than 2 mm in diameter (Holme, 1953).

Five cores were taken by gravity corer in this patchy floor at the positions shown in Fig. 1 and are described in Table 1 (uppermost bed first in each case).

The cores reached from 17 to 26 cm below the surface. The base of core 1 was red in colour, rather suggesting that bed rock was nearly reached, although Holme (1953) found more than 70 cm of superficial sediments nearby.

A coarse gravel, consisting mainly of fragments of large shells with subordinate sand and rock debris, was found at the base of cores 1–4, while in core 5 there was a well-graded fine gravel.

Above the basal bed there was up to 16 cm of sand carrying as much as 25% of particles between 2 and 14 mm in diameter. In cores 2 and 5 these sediments were capped by a thin layer of sand. The abundance of the coarse fractions in the surface layers is shown in Table 1, although the figures are misleading as the particles are largely platey shell fragments.

Three samples, taken by Van Veen grab, from similar patch floor near the East Anglian coast consisted of the following components:

Sample	Sand (%)	Gravel and stones (%)
1	10	90
2	60	40
3	70	30

The first sample showed that the floor was locally made of gravel and stones carrying polyzoa and hydroids. These were absent from the second sample so that the coarse fraction was probably covered by sand. In the third case the floor proved to be made largely of sand.

DISCUSSION

The acoustically patchy floor must be an expression of differences in composition since at both localities the floor was flat. The patches of low reverbation level should correspond to the patches of sand while the high reverberation level should be caused by the presence of coarser sediments. This correlation can be made with confidence for the East Anglian example because of the considerable difference in the grade of the sediments (Chesterman *et al.*, 1958). There is good reason to believe that in the surface sediments of core 1, of the Plymouth region, there is sufficient coarse, platey material to make the sediment readily distinguishable acoustically from the surface sands of cores 2

and 5. The uppermost material of the remaining cores is probably representative of the high reverberation patches also. The larger area of acoustically patchy floor, which extends to the west of the region sampled, must be due to similar types of lithological contrast.

The acoustic pattern is interpreted as representing patches of sand lying on either pebbles or sand with up to 25% shell fragments between 2 and 12 mm wide. The patches of sand are longer than broad. The ratio is largest off the east coast where the streams reach more than 2 knots.

In both localities the streams are virtually linear, flowing to-and-fro parallel to the length of the patches. There is a difference of more than 0·1 knot between the ebb and flood streams so that the sand is probably being driven into the two localities.

The growth of a similar but smaller pattern has been seen during sand storms in a desert by Bagnold (1954, p. 176) and in a flume (Casey, 1935) under an unidirectional stream. In a desert the parallel sand strips average $\frac{1}{2}$ km in length (replacing each other *en échelon*), are 1–3 m wide, 1–2 cm thick and are separated by 40–60 m of flat, pebble-covered ground. They appear to be initiated when a strong sand-laden wind blows over a uniformly rough surface (of pebbles, say) due to lateral instability of the flow, and grow so long as the drag over the sand exceeds that over the pebbles.

The author wishes to thank M. J. Tucker and A. R. Stubbs for designing their fish-detection equipment so as to make it suitable for surveying the sea floor. To these and to other colleagues the author is grateful for the help they gave in taking the records and for valuable discussion. Professor W. F. Whittard kindly made it possible to take the five cores and occupy the camera station during his cruise on R.V. 'Sarsia'.

SUMMARY

An acoustic survey has been made of two regions where there is marked geographical variation in the nature of the surface sediments. It is shown that the patches are elongated parallel to the prevailing path of the tidal streams and a mechanism of origin is suggested.

REFERENCES

BAGNOLD, R. A., 1954. *The Physics of blown Sand and Desert Dunes.* London: Methuen.

CARTWRIGHT, D. & STRIDE, A. H., 1958. Large sand waves near the edge of the continental shelf. *Nature, Lond.*, Vol. 181, p. 41.

CASEY, H. J., 1935. Über Geschiebebewegung. Dissertation der Technischen Hochschule, Berlin.

CHESTERMAN, W. D., CLYNICK, P. R., & STRIDE, A. H., 1958. An acoustic aid to sea bed survey. *Acustica*, Vol. 8, pp. 285–290.

HOLME, N. A., 1953. The biomass of the bottom fauna in the English Channel off Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 32. pp. 1–49.

JARKE, J., 1956. Der Boden der südlichen Nordsee. *Dtsch. hydrogr. Z.*, Bd. 9, pp. 1–9.

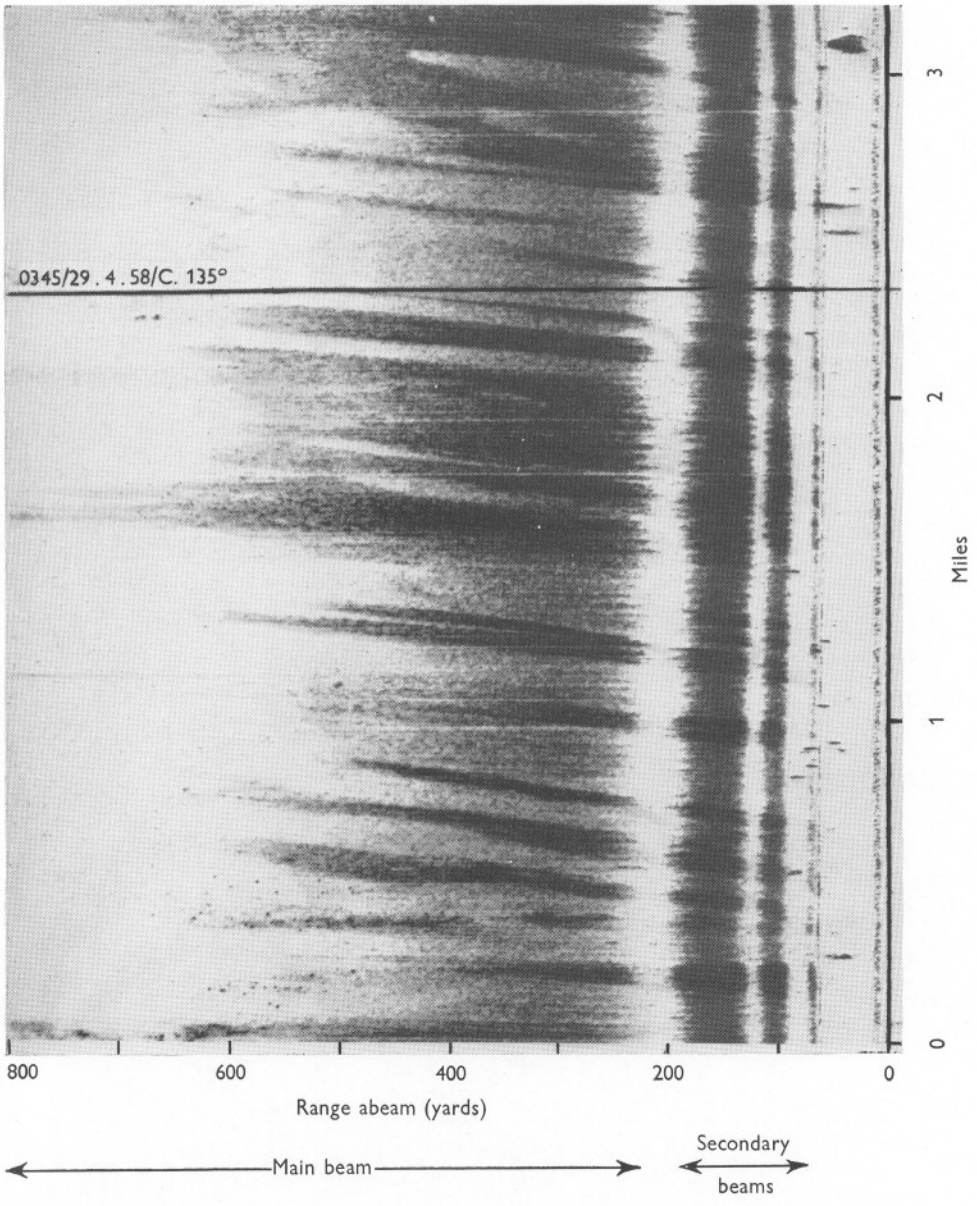
PRATJE, O., 1950. Die Bodenbedeckung des Englischen Kanals und die maximalen Gezeitenstromgeschwindigkeiten. *Dtsch. hydrogr. Z.*, Bd. 3, pp. 201–205.

ROBINSON, A. H. W., 1956. The submarine morphology of certain port approach channel systems. *J. Inst. Navig.*, Vol. 9, pp. 20–46.

STRIDE, A. H. & CARTWRIGHT, D., 1958. Sand transport at southern end of North Sea. *Dock Harb. Author.*, Vol. 38, pp. 323–324.

EXPLANATION OF PLATE I

Plate I. An acoustic map of patches on the floor near Eddystone. Range abeam of ship of 800 yards is exaggerated $\times 7$ distance scale. The light toned patches extending diagonally across the record are associated with patches of sand.


0345/29 . 4 . 58/C. 135°
3
2
1
0
Miles
800
600
400
200
0
Range abeam (yards)
Main beam
Secondary
beams

(*Facing p.* 318)

J. mar. biol. Ass. U.K. (1959) 38, 319–333
Printed in Great Britain

ELECTRON MICROSCOPICAL OBSERVATIONS ON A VERY SMALL FLAGELLATE: THE PROBLEM OF *CHROMULINA PUSILLA* BUTCHER

By IRENE MANTON

Botany Department, Leeds University

(Plates I–X)

CONTENTS

Introduction 319
Material and methods 320
External morphology 321
Internal structure 323
The flagellum 323
The nucleus 324
The plastid 325
The mitochondrion 325
Other cytoplasmic components 326
Division stages 327
Discussion 329
Summary 332
References 332

INTRODUCTION

The marine plankton flagellate hitherto known as *Chromulina pusilla* Butcher is one of the smallest of algal cells, being of bacterial dimensions (1–1·5μ, Butcher, 1952). For this reason it had seemed of interest to investigate its structure with the electron microscope as part of a general programme on ciliated cells in plants which has been in progress for some years. It soon became apparent, however, that there were more points of importance than had been expected at the outset. In particular the anatomical findings showed that the formal taxonomic description based on the light microscope was not only incomplete (an unavoidable consequence of the very small size), but that, as a further consequence of these physical limitations, it contained some rather fundamental misconceptions regarding the taxonomic position of the organism. By the kindness of correspondents and collaborators it was fortunately possible at once to follow up the anatomical evidence biochemically, and the combined data make the conclusion unavoidable that this organism is not a member of the Chrysophyceae (to which group the genus *Chromulina* properly belongs) but that its affinities lie in or near the Chlorophyceae. This naturally raises some difficult nomenclatural questions which cannot all be

immediately resolved. The anatomical facts for this particular species are not, however, in themselves dependent on nomenclature, and since this organism is not merely an electron microscopical curiosity but is also exceedingly abundant[1] in coastal waters near the British Isles, it is perhaps of some value to students of plankton that they should be made available.

I am indebted to my former technical assistant Mr B. Clarke for carrying out all the work involving shadow-cast whole mounts for external morphology, including the taking of the first four micrographs assembled on Pl. I, and also for photographic help with all the remaining Plates. As donors of material I have to thank Prof. E. W. Knight-Jones in the first instance and Dr M. Parke more recently. I am greatly indebted to the owners of the various microscopes listed below, more particularly to Dr K. R. Porter of the Rockefeller Institute whose help on this, as on many other occasions, it is a pleasure to acknowledge. Finally thanks are due to Dr G. Y. Kennedy of Sheffield for providing the chemical data quoted on p. 331 and to those members of the staff of the Marine Biological Association's Laboratory at Plymouth involved in making the arrangements and supplying him with the large quantity of material needed.

MATERIAL AND METHODS

We were first introduced to this organism in November 1951 by Dr (now Professor) E. W. Knight-Jones, who had recently isolated it into uni-algal (though not bacteria-free) culture from the Conway estuary (North Wales). We made some preliminary observations on external morphology, the only aspect of structure for which adequate electron microscopical techniques existed at that date, using shadowcast whole mounts of cells killed with osmic vapour directly on to formvar-coated carriers. This isolate had supplied the type material described taxonomically by Butcher (1952) and it was also sent to us again in 1953 from Plymouth as 'flagellate 90'. At the request of Dr M. Parke we made some comparative observations on 'flagellate 90' and a new isolation known as 'flagellate 27' obtained by her from the English Channel which proved to be indistinguishable. Finally a third isolation, made by Dr R. W. Butcher 'in June 1954 from the Thames estuary off Sheerness, Isle of Sheppey, salinity 32‰ at the surface' (Butcher personal communication), was received from Plymouth in 1956 and subsequently, as 'flagellate 90*a*'. Our information on external morphology thus relates to material of unim-

[1] Preliminary information on the relative abundance of this organism in estuarine and coastal waters on various parts of the British coasts is given by Knight-Jones (1951) and Knight-Jones & Walne (1951), but it may be of interest to quote a personal statement made by letter to the author by Professor Knight-Jones in September 1958: 'I can sum up by saying that *Chromulina pusilla* was the most abundant organism which I observed and it occurred in the majority of the samples of full salinity sea water, both from enclosed waters and the open sea.'

peachable authenticity from three different parts of the British coast, namely, Conway estuary (type culture for *Chromulina pusilla*, Plymouth no. 90), Thames estuary (Plymouth no. 90*a*) and English Channel (Plymouth no. 27).

For the anatomical investigation only flagellates 27 and 90*a* in the Plymouth collection have been used, both supplied to us as required, by Dr M. Parke. Both cultures have been embedded and sectioned several times during 1957 and 1958 but they are so similar that it has not been felt necessary to distinguish them individually in recording the results. Pls. II, III, and V–VIII are of flagellate 27 and Pl. IV, IX and X are of flagellate 90*a*.

The technical processes involved in embedding and also for whole mounts are now standard and need not be quoted in detail. The fixative was 1% osmium tetroxide buffered with acetate veronal to pH 7. All sections were cut in Leeds on a Porter Blum Sorval microtome using a glass knife and they were mounted on carbon films.

Several different microscopes have been used. For external morphology of shadow-cast material, the old Philips microscope in the Leeds Botany Department has been used and is adequate. All sections, however, have been examined on a Siemens microscope. The work was begun on the instrument in the Aeon Laboratories, near Egham, Surrey, during occasional visits in the summer of 1957. It was continued on the Siemens microscope in the Rockefeller Institute, New York, which was made available to me for two weeks at the end of December 1957. Finally it was completed on a similar instrument recently installed in the Leeds Botany Department by means of a grant from the Rockefeller Foundation.

With regard to the chemical data communicated by Dr G. Y. Kennedy of Sheffield, the methods used for the study of the pigments were a modification of those of Willstädter and Stoll 1913, the details of which are being published by Kennedy & Nicol (in the press).

EXTERNAL MORPHOLOGY

The first three figures on Pl. I show all that can easily be seen about the external morphology of the swimming cells by a study of whole mounts. Figs. 1 and 2 represent two of the strains used, at different magnifications. Both cultures are a mixture of the organism and numerous bacteria which occur with it in any field of view. In comparison with the bacteria present, the cells of the flagellate are larger (though not greatly so) and more asymmetrical. When undistorted they are more or less pear-shaped, with a laterally attached flagellum composed of two parts, namely a proximal wider portion of rather less than 1 μ in length and a much more slender distal portion of 2–3 μ long; the lengths of both parts are nevertheless somewhat variable, a matter no doubt associated with changes consequent on the growth cycle (see p. 328).

Apart from the general shape the only other features which can be detected on whole mounts are the striations which run longitudinally down the wider portion of the flagellum and which we now know to be caused by the longitudinally running fibres inside. These striations are just perceptible in the original photograph of the left hand cell in Fig. 1 and again in the more highly magnified flagellum of Fig. 3. Outside the striated portion in Fig. 3 there are faint signs of a covering membrane and the distal tip of the slender extremity ends bluntly.

Explanation of Plates I–IV

Chromulina pusilla Butcher

I

Fig. 1. Shadow-cast whole mount of flagellate 90 in the Plymouth collection, prepared in 1953 and re-examined in 1957 with the Philips microscope, showing 2 cells of the organism together with bacteria; this material was a direct descendant from the type culture used by Butcher 1952 and originally isolated from the Conway estuary by Knight-Jones. Micrograph M 575·4, magnification × 10,000.

Fig. 2. A cell and bacteria from a culture of flagellate 27 in the Plymouth collection, isolated by Dr M. Parke from the English Channel. Micrograph M 514·4, magnification × *c.* 20,000.

Fig. 3. The flagellum of a cell of flagellate 90 more highly magnified. Micrograph M 575·19 × *c.* 30,000.

Fig. 4. A section through a cell of flagellate 27 showing the two parts of the flagellum and internal cell contents which include the plastid and pyrenoid with a part of the nucleus. Micrograph MS 56 × 35,000.

II

Fig. 5. A section showing the plastid with covering membrane, lamellae and pyrenoid (*P*); part of the nucleus (*N*) and cytoplasmic vesicles. Micrograph MS 17, × *c.* 50,000.

Fig. 6. A longitudinal section to show the relative positions of the nucleus (*N*), plastid (*P*), fat body and flagellum. Micrograph MS 61, × *c.* 30,000.

Fig. 7. Longitudinal section (lower cell) to show the positions of the mitochondrion (*m*), plastid (*c*) and golgi vesicles (*xx*), relative to the flagellum. Micrograph MS 30, *c.* 55,000.

III

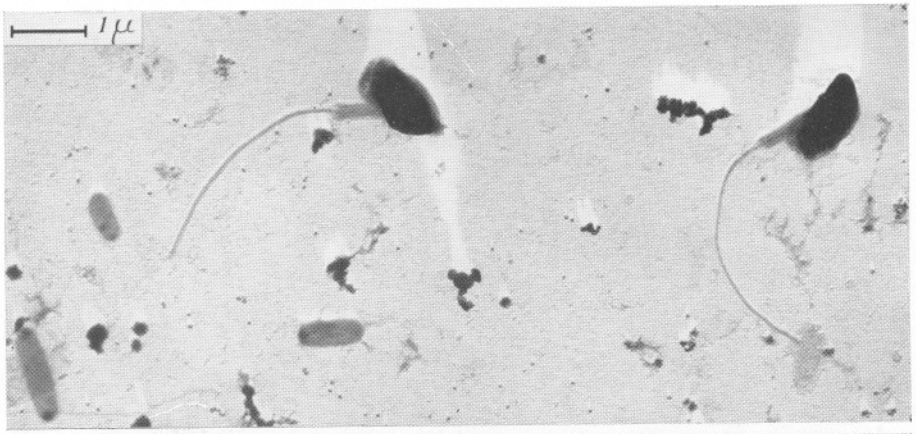
Fig. 8. Longitudinal section showing the flagellar base (*fb*) and arrangement of fibres in the flagellar axis, including the hair-point. Micrograph MS 220 × *c.* 40,000.

Fig. 9. General view of the cell shown at a higher magnification in Fig. 10. Micrograph MS 288 × 18,000.

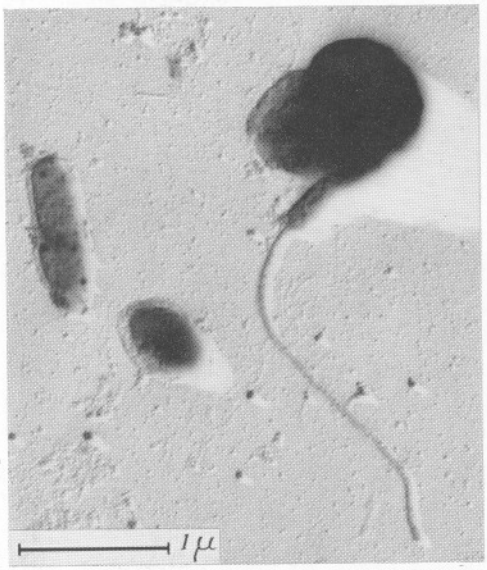
Fig. 10. Part of the cell of Fig. 9 more highly magnified to show the three-layered structure of the membrane covering the hair-point and other details of the arrangement of fibres within the flagellum; the nucleus (*N*), perinuclear space (*ps*), a bacterium in section (*B*). Micrograph MS 291, × 100,000.

IV

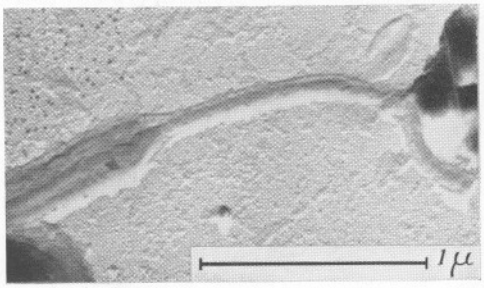
Fig. 11. A cell showing among other features the flagellum, the nucleus (*N*), the mitochondrion in TS (*m*), vesicles (*v*) not part of the peri-nuclear space (*ps*), the plastid with central pyrenoid (*P*), two peripheral small spherical bodies within the plastid and the array of parallel curved membranes near to the plastid surface and joined in pairs at intervals. Micrograph H 1237 × 90,000.



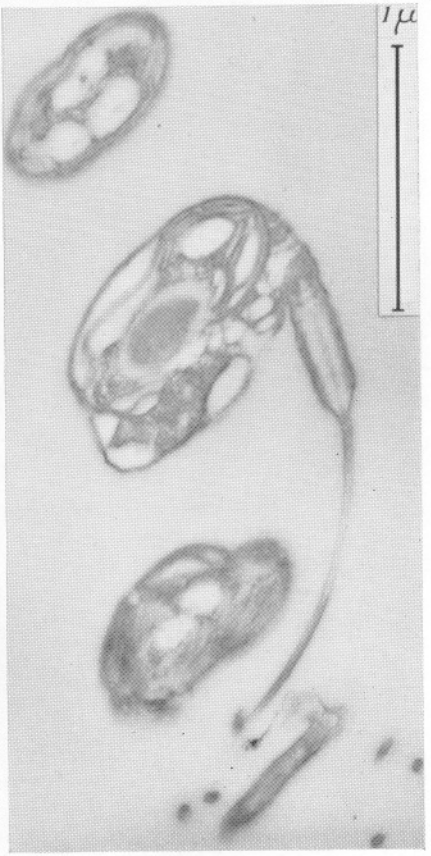
1



2

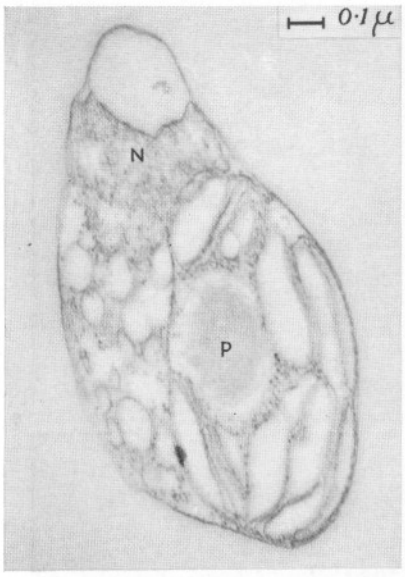


3

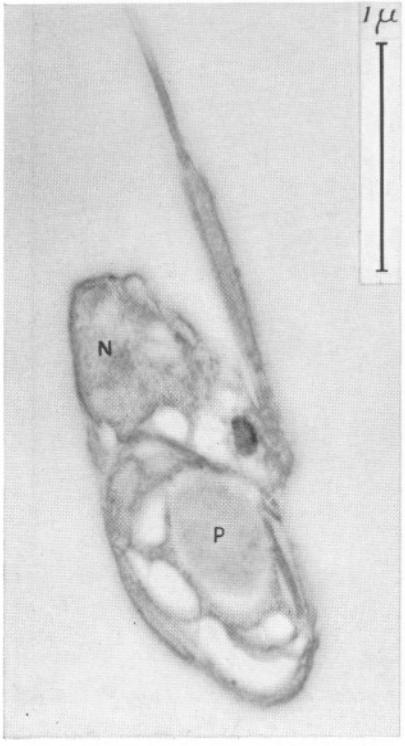


4

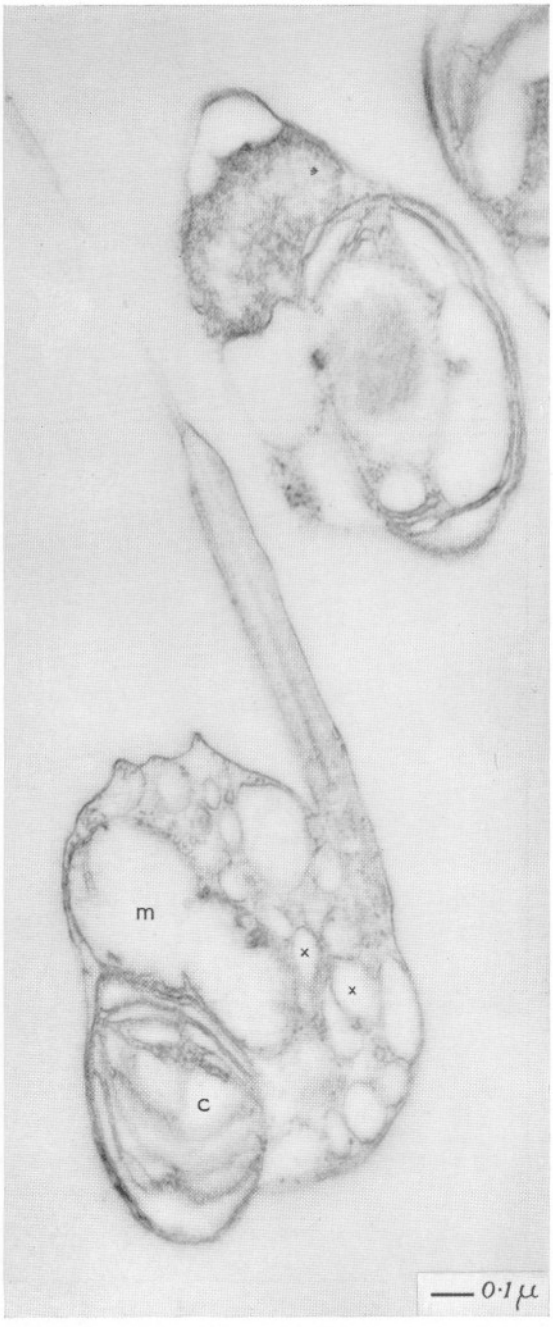
(*Facing p.* 322)



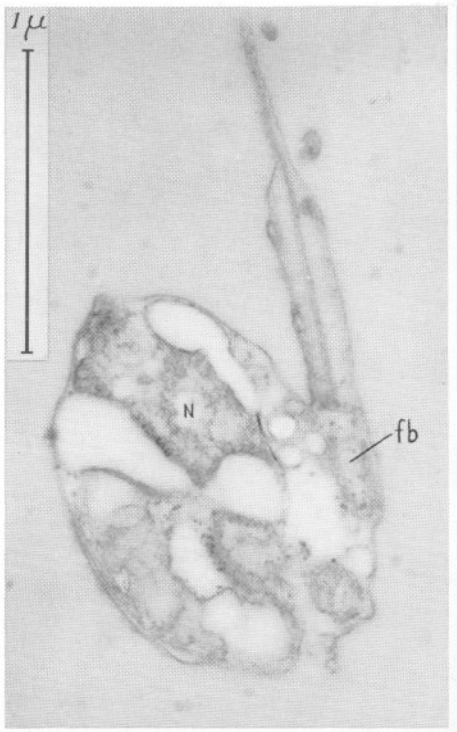
5



6

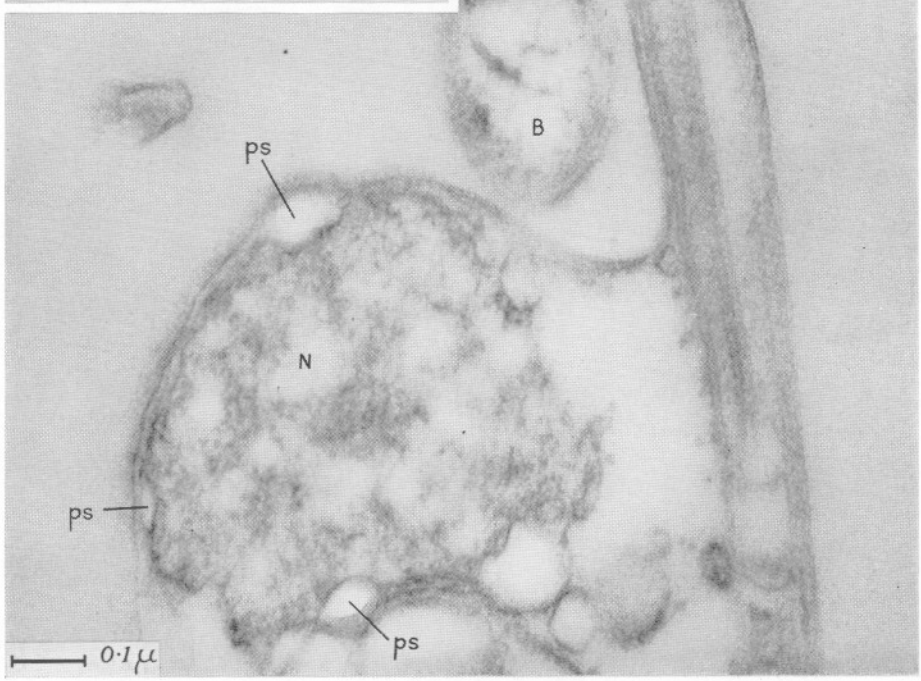


7

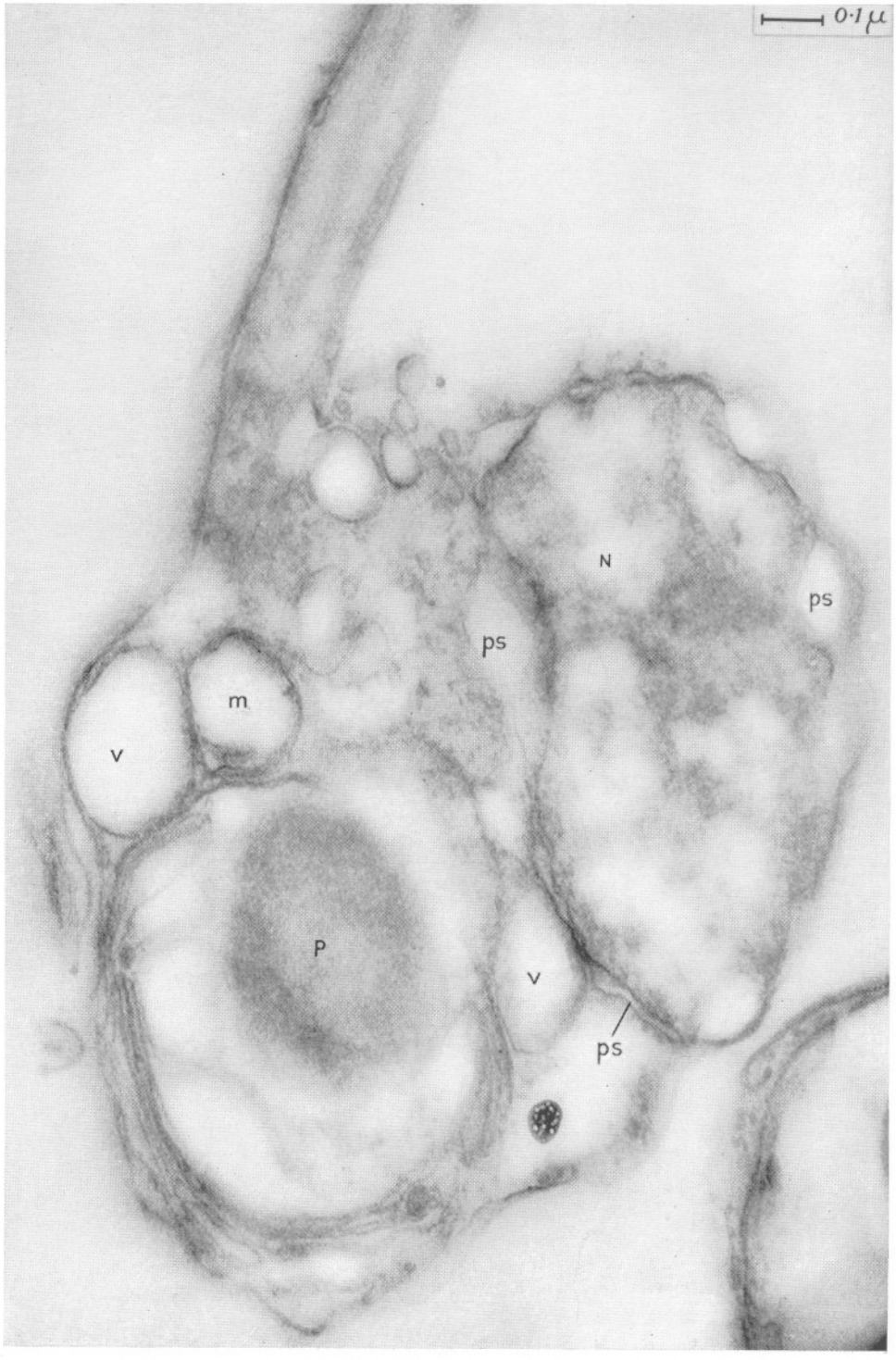


8

9



10



11

INTERNAL STRUCTURE

The flagellum

The first object of enquiry was the internal structure of the two parts of the flagellum since the distal portion is so slender that it seemed unlikely that it could contain the normal 9+2 internal fibres so characteristic of cilia and flagella in plants and animals generally, while the fatter basal part, when first seen, could have been interpreted as a sheath, and this, had it been confirmed, would have been a character of major classificatory importance.

A glance at any of the longitudinal sections included in Pls. I–III is sufficient to dispel any suggestion that the thickening of the base of the flagellum is due to a sheath. The entire surface of both parts is smoothly covered by a membrane which is continuous with the body membrane and, like it, is three-layered, being composed of two electron dense surface layers separated by a translucent central layer. This structure is most clearly demonstrated in the distal extremity of the flagellum shown at a magnification of × 100,000 in Fig. 10, Pl. III, which also shows the smooth tapering which is the only external mark of the transition region between the two parts of the flagellum.

Transverse sections of the basal part of the flagellum appear incidentally in many of the illustrations but most clearly in Plate VI. These show unmistakably that in this region the surface membrane closely covers the normal array of 9+2 strands, among which the outer 9 are double with the plane of separation of half-strands arranged radially. There is therefore nothing peculiar about this part of the flagellum except its extreme shortness.

The structure of the distal region is more difficult to investigate directly for obvious reasons. Transverse sections of it are so small that it is only by accident that one suitably orientated and of sufficient thinness to show internal structure is recorded at a magnification high enough to be of use. One such is however included in Fig. 15, Pl. VI, and if the evidence from this is amplified by comparison with longitudinal views, more especially those of Fig. 8, Pl. III, and Fig. 17, Pl. VII, the general features can be ascertained with reasonable certainty. Within the bounding triple-membrane there seems to be a core composed mainly of prolongations from the two central strands of the basal part of the flagellum, but with little or no contribution from the 9 peripheral strands. The structure is thus not that of a complete flagellum but seems to correspond more to a specialized and exceptionally elongated distal hair-point. Whether or not this hair-point is self-motile or passively dependent on motion transferred from the flagellum proper cannot be directly ascertained, but the apparent simplicity of its structure suggests the latter.

At the base of the flagellum, and separated from it by a transverse diaphragm, there is a normal type of basal body within the cell. The diaphragm is sometimes exactly level with the cell surface, e.g. Fig. 8, Pl. III, but is sometimes much more deeply seated (Fig. 9, Pl. III), a difference of position which

must also be connected with the growth cycle and which will be further discussed below (p. 329). When seen in transverse section (Fig. 14, Pl. VI, *fb*, right hand cell) the basal body appears hollow, with a wall composed of 9 peripheral fibres but with no central ones and no separate covering membrane. These are all normal features of flagellar basal bodies.

The nucleus

This is not the most conspicuous organ of the cell but is conveniently dealt with next. It can be seen at a high magnification in Figs. 10 and 11 (Pl. III and IV), after which it will be readily recognized in other parts of many of the remaining plates. The nucleus is somewhat laterally situated within the cell; there is little ascertainable internal structure except a somewhat diffuse nucleolus. (See especially Fig. 12*c* and *d*, Pl. V.)

The nuclear membrane is almost certainly of the usual type though these small cells are ill adapted for clear demonstration of the finer details owing to the strong curvature of the nuclear surface and the difficulty of detecting suitably cut cells. Signs of the perinuclear space bridged at intervals are nevertheless detectable in sections such as those of Figs. 10 and 11, though the extremely large size of certain spaces abutting on the nucleus in other sections (e.g. at one end of the nucleus in Fig. 12, Pl. V, and at both ends in Fig. 13) may be artificial distortions due to stretching of the space and local collapse of the nucleus during the processes involved in embedding.

We have been unable to trace any fibrous or other connexions between the nucleus and the flagellum.

Explanation of Plates V and VI

Chromulina pusilla Butcher

V

Fig. 12*a–e*. Series of consecutive sections through a cell passing through the nucleus (*N*), mitochondrion (*m*), plastid with central pyrenoid (*P*) and peripheral plastid lamellae somewhat distorted by the fixative, a fat body and part of the perinuclear space (*ps*) probably artificially stretched as a fixation artefact. Micrographs RS 162, 161, 160, 158, 157, ×*c.* 43,000.

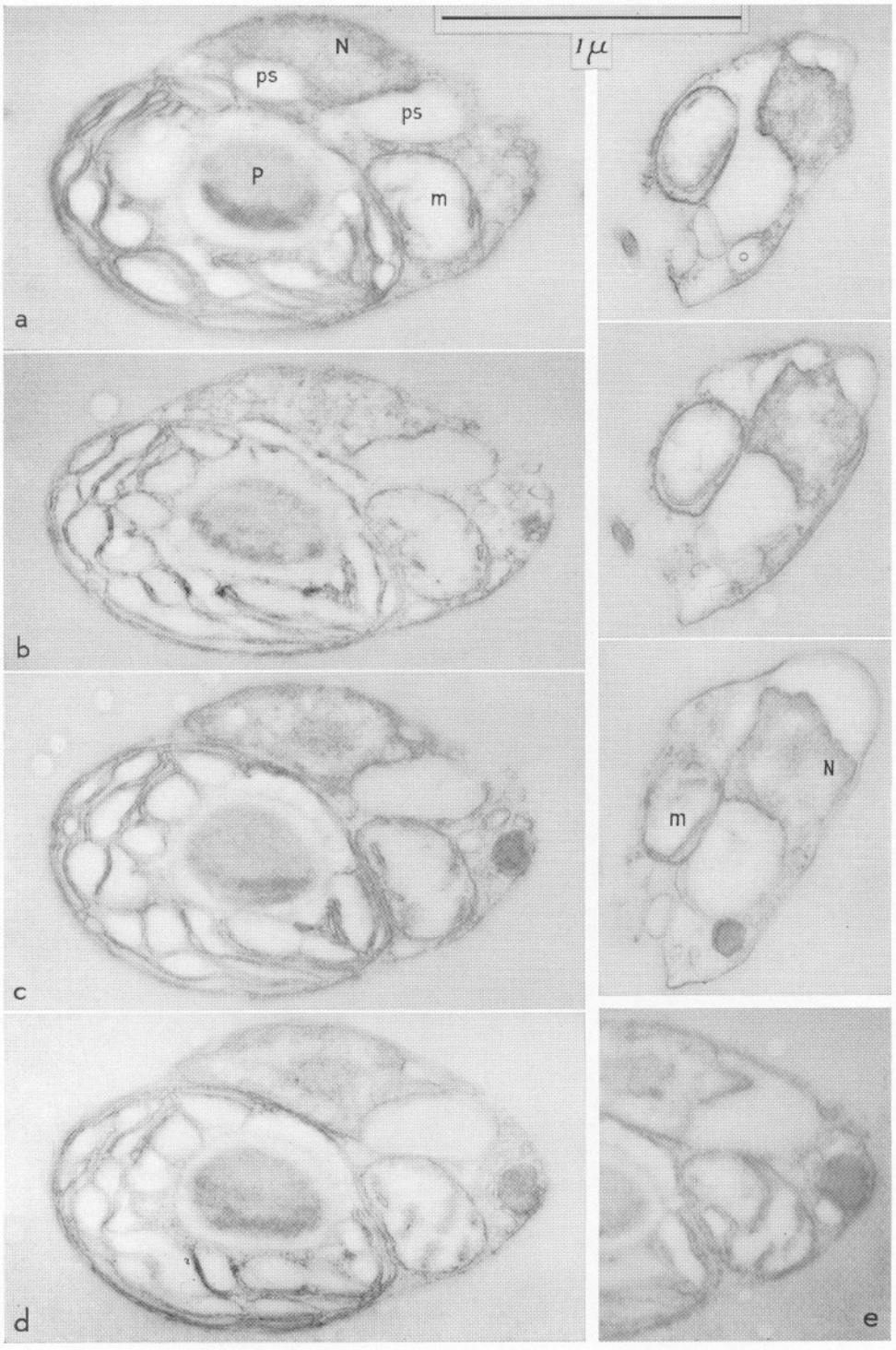
Fig. 13*a–c*. Three consecutive sections through part of another cell to show curvature of mitochondrial cristae; nucleus (*N*), mitochondrion (*m*). Micrographs RS 158, 160, 162, ×*c.* 43,000.

VI

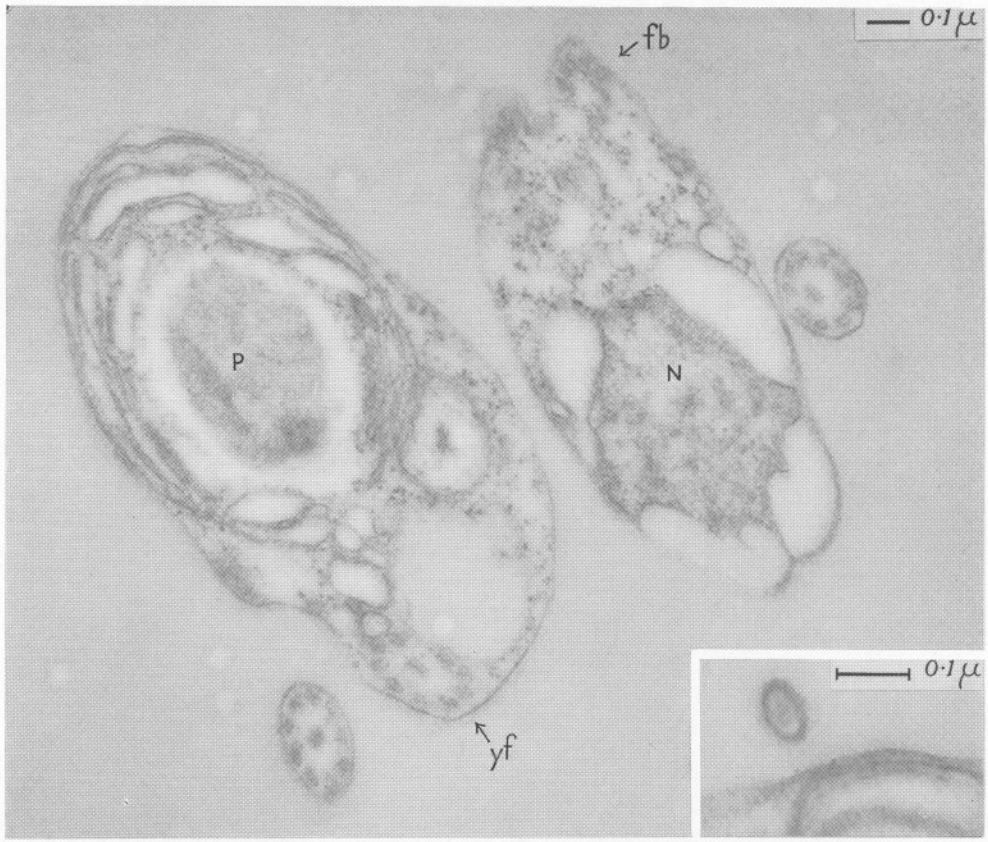
Fig. 14. Two cells in transverse section showing the different parts of the flagellum within the cytoplasm; *fb* (right-hand cell) a basal body in TS; *yf* (left-hand cell) axis of a young flagellum which has not yet emerged from the cytoplasm; nucleus (*N*), plastid with pyrenoid (*P*). For further explanation see p. 329. Micrograph RS 445 ×*c.* 55,000.

Fig. 15. TS of a flagellar tip showing contents; for further description see text p. 323. Micrograph MS 298 × 100,000.

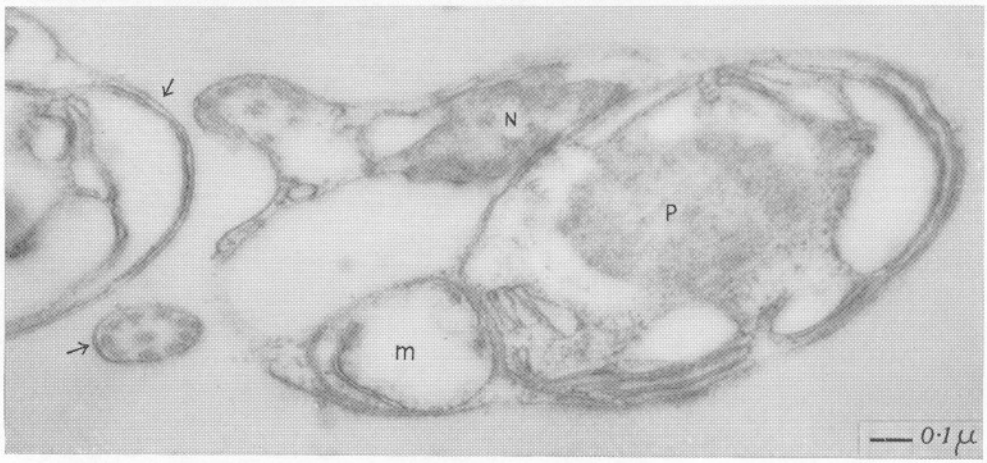
Fig. 16. TS through a cell somewhat flattened by a knife, showing details of the mitochondrion (*m*), flagellar axis, and paired lamellae in the plastid (*P*); arrows point to places where the triple structure of the surface membrane is resolved. Micrograph MS 242, × 55,000.



(*Facing p.* 324)



14 15



16

The plastid

This is by far the largest organ in the cell, and the most conspicuous part of it is the central pyrenoid which is large enough to be traversed by and to dominate almost every type of section except extremely tangential ones, as may be seen in some part of each of Pls. I–X.

There is as yet no chemical knowledge about the nature of the photosynthetic product except that tests for starch with iodine are invariably negative. No suggestions can therefore be made about the chemical nature of the dark central body forming the core of the pyrenoid and which may be expected to be the main food reserve. Outside this central dark area is a narrow lighter layer (well seen in Fig. 12*c*, Pl. V, and elsewhere), outside which is granular material which seems to be part of the normal ground substance between the outer lamellae and which, in small bands, can be encountered at all levels out to the plastid surface (see especially Fig. 5, Pl. II). The plastid surface itself is bounded by a membrane (Fig. 5 and elsewhere) and between this and the pyrenoid are a series of curved membranes joined in pairs at their edges (see especially Figs. 11, 16, 23, etc.). The enclosed spaces within each membrane pair are often distorted into lenticular blisters by the action of the fixative (e.g. Fig. 12 and elsewhere), though in more fortunately fixed specimens the array of membranes lie more evenly parallel to each other (Fig. 11, Pl. IV) in a crescentic path. They do not, however, extend without interruption over the entire circumference of the plastid, some points of interruption being related to the presence of small vesicles of unknown nature (Fig. 11); other points of interruption, which are not detectably so related are also visible in Fig. 11 and elsewhere (see for example Fig. 23, Pl. IX).

This arrangement of lamellae, which is difficult to describe verbally, but which will perhaps become sufficiently clear by further scrutiny of Pls. II, IV, V and VI, is quite unlike that of the Chrysophyceae for which preliminary reports from our own experience are available for *Synura* (see Manton, 1955) and for *Chrysochromulina* (see Parke, Manton & Clarke, 1958, 1959). It is, however, not unlike that recently described for *Chlamydomonas* by Sager & Palade (1957), allowance being made for the difference of size. This was therefore the first reason to suspect that this organism had been wrongly placed.

The mitochondrion

A second reason for suspecting a classificatory error was the fine structure of the single mitochondrion which is always to be found as a sausage-shaped organelle (round in cross-section) in close contact with both plastid and nucleus and not far removed from the base of the flagellum. It may be seen, and is labelled, in Fig. 7, Pl. II, Fig. 11, Pl. IV, both series on Pl. V, and elsewhere. The normal mitochondrial structure in all members of the Chrysophyceae, Phaeophyceae, and Xanthophyceae investigated so

far[1] shows an array of closely crowded tubular villi extending from the inside of the mitochondrial wall into the cavity and corresponding to the 'cristae' of most animal mitochondria (Palade, 1952, *et seq.*). In *Chlamydomonas*, however the mitochondria are described (Sager & Palade, 1957) as having a few, widely separated flattened cristae which scarcely interrupt the central lumen. Had this description not appeared as the present enquiry was being carried out we might have been in some doubt as to the true identity of our organelle. As it is the figures cited above, especially the two series of sections contained in Pl. V, and more highly magnified single views such as that of Fig. 16, Pl. VI, leave no doubt that this organ is not only a mitochondrion but a mitochondrion of the chlorophycean but not chrysophycean type.

Peculiar features are the facts that the organelle is single and that the cristae are often curved. The first observation will be discussed again in the section on cell division (p. 327). Curvature of the mitochondrial cristae can be seen best in Fig. 13*a–c*, Pl. V, and in Fig. 16, Pl. VI.

Other cytoplasmic components

Near to the base of the flagellum (Fig. 6, Pl. II) there is a fat body, which once seen can be picked up in other planes of section, e.g. Figs. 12*c–e*. Equally close to the base of the flagellum (Fig. 4, Pl. 1, Fig. 7, Pl. II, etc.) is a compact group of small vesicles which probably represents the equivalent of a golgi area (*x–x*, Fig. 7). There are in addition a few other vesicles of various sizes which are not of this nature. Some good examples are contained in various parts of the cytoplasm not closely associated either with the flagellum or with the nucleus (*v* in Fig. 11, Pl. IV) which seem unlikely to be either artifacts or other organelles.

Lastly there are granules. The amount of granular cytoplasm is very restricted. The large organelles (nucleus, plastid, mitochondrion) are so near to the cell surface on their outer sides that there is little except the body membrane to cover them. Only the angular spaces between these organs, and especially between them and the flagellar base, contain granular cytoplasm in an appreciable amount, together with vesicles of various sizes. This may be examined to advantage in Fig. 7, Pl. II, and Fig. 14, Pl. VI.

Finally the body membrane already discussed in a preliminary way in connexion with the flagellum can be demonstrated independently in Fig. 16, Pl. VI (arrows) as a three-layered structure identical with that of the membrane covering the surface of the flagellum.

[1] The literature on this subject is rapidly becoming too voluminous to quote in full but sample micrographs recording some of our own experience of the fine structure of mitochondria in various groups will be found in: Phaeophyceae *Fucus* (Manton & Clarke, 1956), *Scytosiphon* (Manton, 1956); Xanthophyceae *Vaucheria* (Greenwood, Manton & Clarke, 1957, Greenwood, 1959); Chrysophyceae *Chrysochromulina* (Parke, Manton & Clarke, 1958, 1959).

DIVISION STAGES

No attempt has yet been made to study nuclear division in this organism, and no stages of it other than the final stage of a binucleate cell not yet cleaved into two (Fig. 18, Pl. VII) have yet been identified. Several other manifestations of cell division are however conspicuous and, since these add very greatly to our knowledge of certain structural aspects of the other organs, it is important to include at least some of them.

Several stages in the cleavage of both plastid and mitochondrion are represented in Pls. VIII–X. The plastid seems to divide first and the cleavage passes right through the pyrenoid, starting apparently as an intucking of the plastid membrane on the side towards the nucleus. Examples of completely divided plastids are contained in Fig. 20, Pl. VIII and Fig. 26, Pl. X, an important detail of the latter being the demonstration, so far the best available for this organism, that the plastid membrane is probably compound (arrows at base). Incomplete stages are contained in Figs. 22–25, Pls. IX and X. Before scrutinizing these it will perhaps be helpful to notice that whereas Figs. 24 and 25 represent two different cells cut in planes approximately at right angles, Figs. 22*a* and 22*b* are different sections of the same cell, Fig. 22*a* being nearer to the cell surface than 22*b*. Finally Fig. 23, though at an uncertain magnification owing to an uncorrectable mistake in the record made at the time the micrograph was taken, is included for the completeness with which the very large plastid shows numerous areas of lamellar junction (marked by arrows) and an incipient cleavage furrow.

Three stages of cleavage of the mitochondrion are represented by Figs. 22, 26 and 21 (long arrow), the sequence being in the order of citation of the figures. As cleavage of the plastid is progressing the single mitochondrion seems to become partly drawn into the plastid cleavage furrow (Fig. 22*a*); it then becomes bent (Fig. 26) into a U shape, finally a depression resembling a cleavage furrow appears on the concave side (Fig. 21*b*), by which time plastid cleavage is complete and the two halves have begun to separate. Further details regarding the final separation of the mitochondrion halves have not been traced.

Observations on the mode of origin of a new flagellum are much more difficult to make and only a few stages have so far been detected, in most cases by accidental inclusion in fields recorded for other purposes, since it is almost impossible to detect them directly with the eye on the fluorescent screen of the microscope. However, a new flagellum undoubtedly forms close to an old one and it is sometimes possible, in dividing cells, to pass through the two basal bodies side by side (Fig. 19, Pl. VII). It is therefore likely that the new basal body from which the new flagellum will grow out must be formed in some structural relation to the old basal body, but what this relation is has not yet been elucidated. However, one fortunate series of sections which happened to

lie close to the field of Fig. 14 is reproduced in Figs. 17*a–f*, Pl. VII, to illustrate a very young flagellum in an early stage of growing out. It is slightly tilted and the basal body is best seen in the lowermost of the six sections (Fig. 17*f*). The central strands passing out into a very short hair point are contained in Fig. 17*d*, the tip of the short hair point itself being crossed by the path of an independent long hair point which can be seen passing obliquely through all the sections. The 9 strands of the peripheral ring are distributed among all six sections, though it should be noted that these strands are still entirely within the body membrane of the parent cell.

This observation on an undoubtedly young specimen is important for providing a clue to some of the differences in external length and in the position of the basal body relative to the surface, to which reference has been made on previous pages, especially in relation to the longitudinal sections included on Pls. II and III. It is obvious that after a new flagellum has been laid down and

Explanation of Plates VII–X

Chromulina pusilla Butcher

VII

Fig. 17*a–f*. Series of six adjacent sections passing longitudinally through a young flagellum not yet emerged from the subtending cell. For further explanation see text. Micrographs RS 440, 441, 442, 443, 444, 445, × *c.* 30,000.

Fig. 18. Sections through a dividing cell showing two nuclei. Micrograph H 2278 × 30,000.

Fig. 19. Section through a dividing cell with two ciliary bases side by side (arrows). Micrograph MS 35, × *c.* 30,000.

VIII

Fig. 20. Group of cells one of which shows a divided chloroplast. Micrograph MS 44, × *c.* 25,000.

Fig. 21*a* and *b*. Two successive sections through a field of three cells the lowest of which contains a dividing mitochondrion (21*b*) and a divided plastid (21*a* and *b*). Micrographs RS 416 and 417, × *c.* 25,000.

IX

Fig. 22*a* and *b*. Two sections (not immediately adjacent) through a dividing cell showing early stages of division of the mitochondrion (22*a*) and plastid (22*b*). Micrographs H 2113 and 2121, × 30,000.

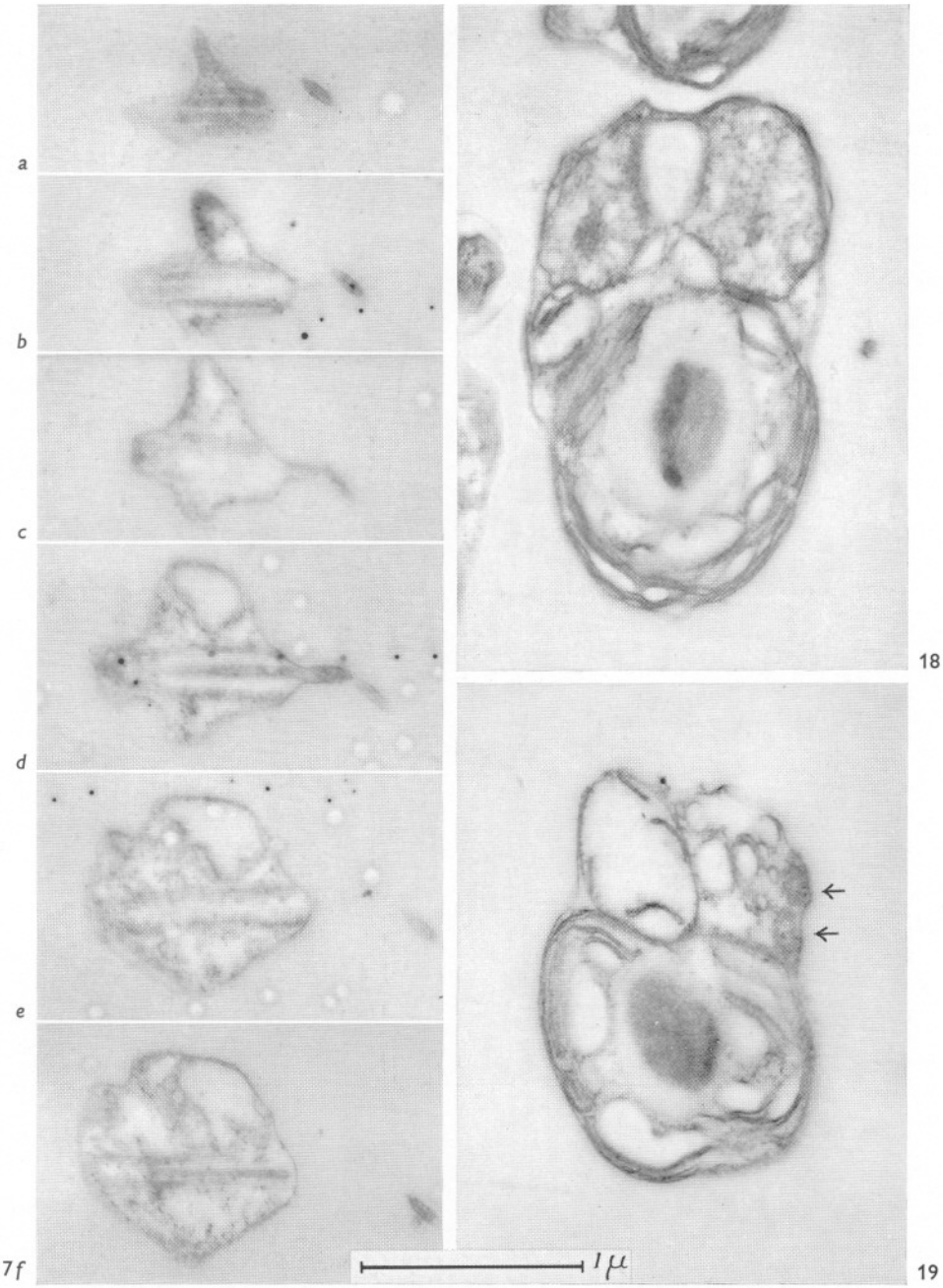
Fig. 23. A giant cell (probably a 'double-division') to show details of an early stage of fission of the plastid, arrows indicate places of fusion of lamellae. Micrograph H 2123, magnification uncertain but probably between 40,000 and 50,000.

X

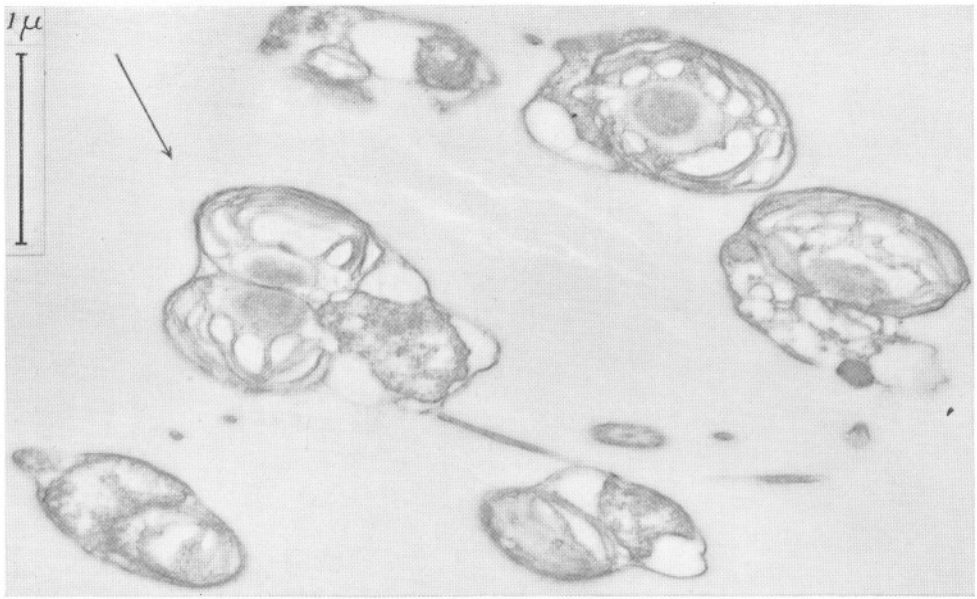
Fig. 24. A cell cut transversely to the plane of flattening showing a dividing plastid in an intermediate condition. Micrograph H 2126 × c. 30,000.

Fig. 25. A cell at approximately the same stage as that of Fig. 24 cut in a place at right angles to that of Fig. 24. This section includes a nucleus above the dividing plastid. Micrograph H 2115, × *c.* 30,000.

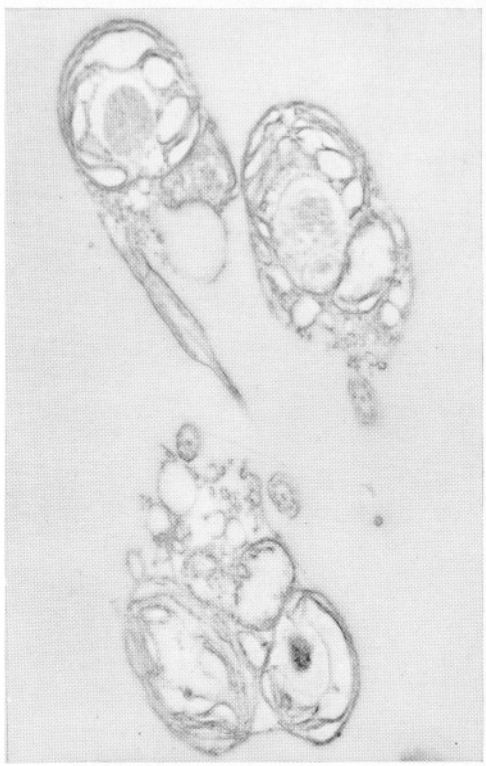
Fig. 26. A cell with a divided plastid and a dividing mitochondrion (*m*) more highly magnified to show among other details the multilayered structure of the plastid membrane (arrows). Micrograph H 2298, × 80,000.



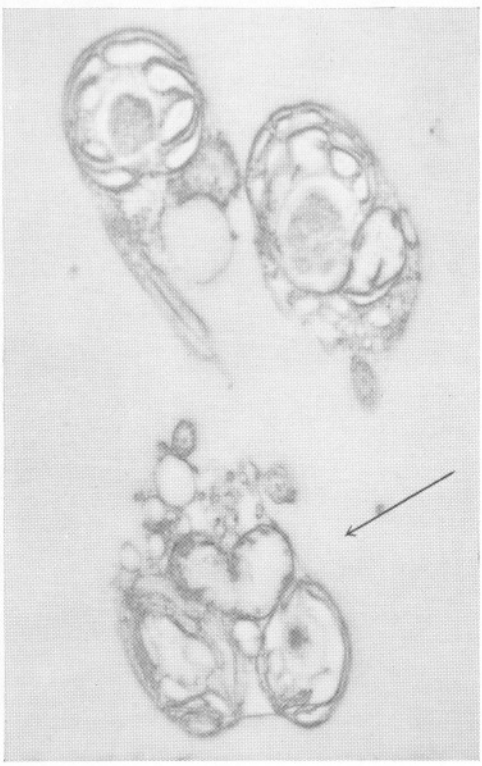
(*Facing p.* 328)



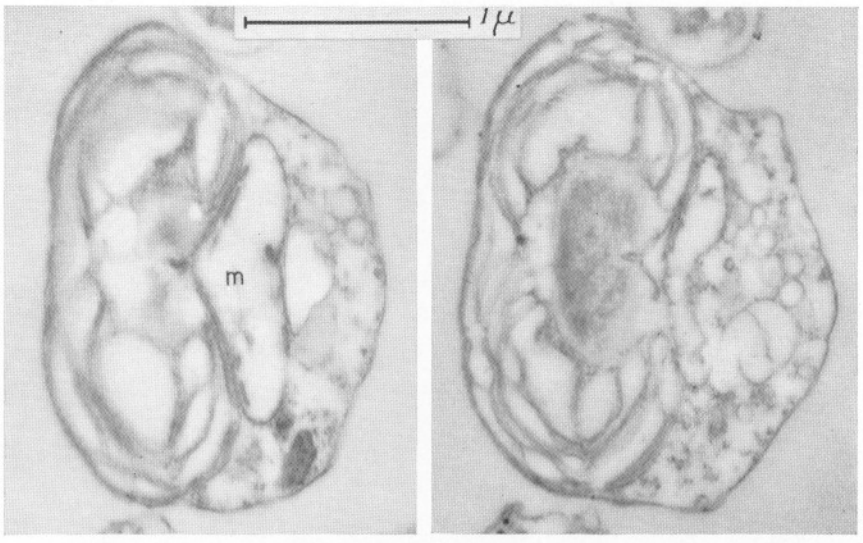
20



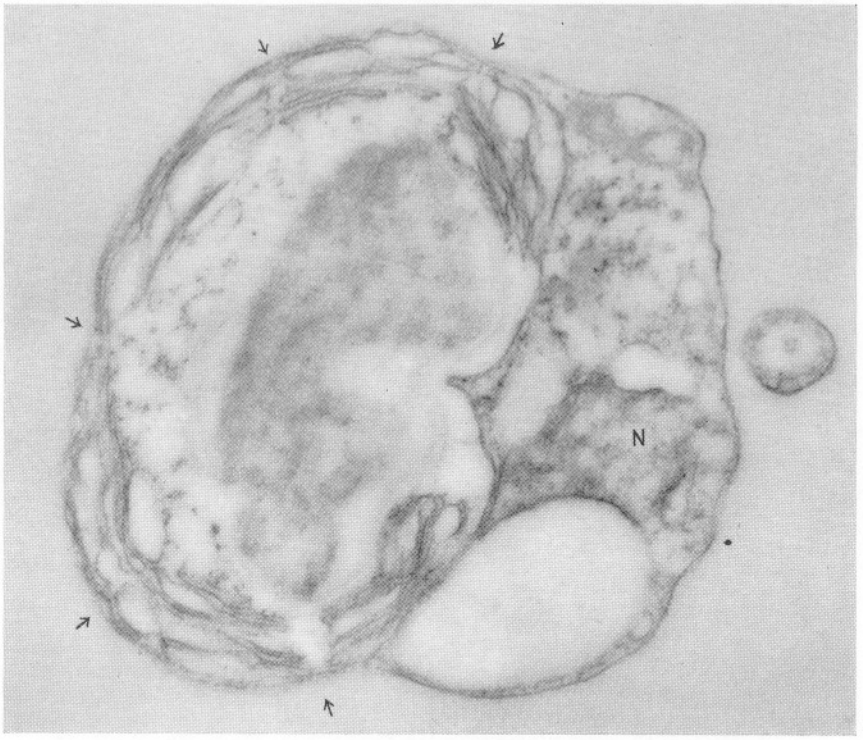
21a



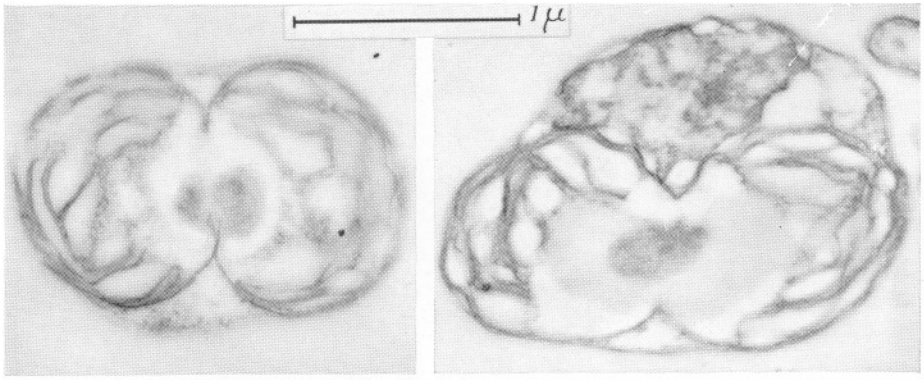
21b



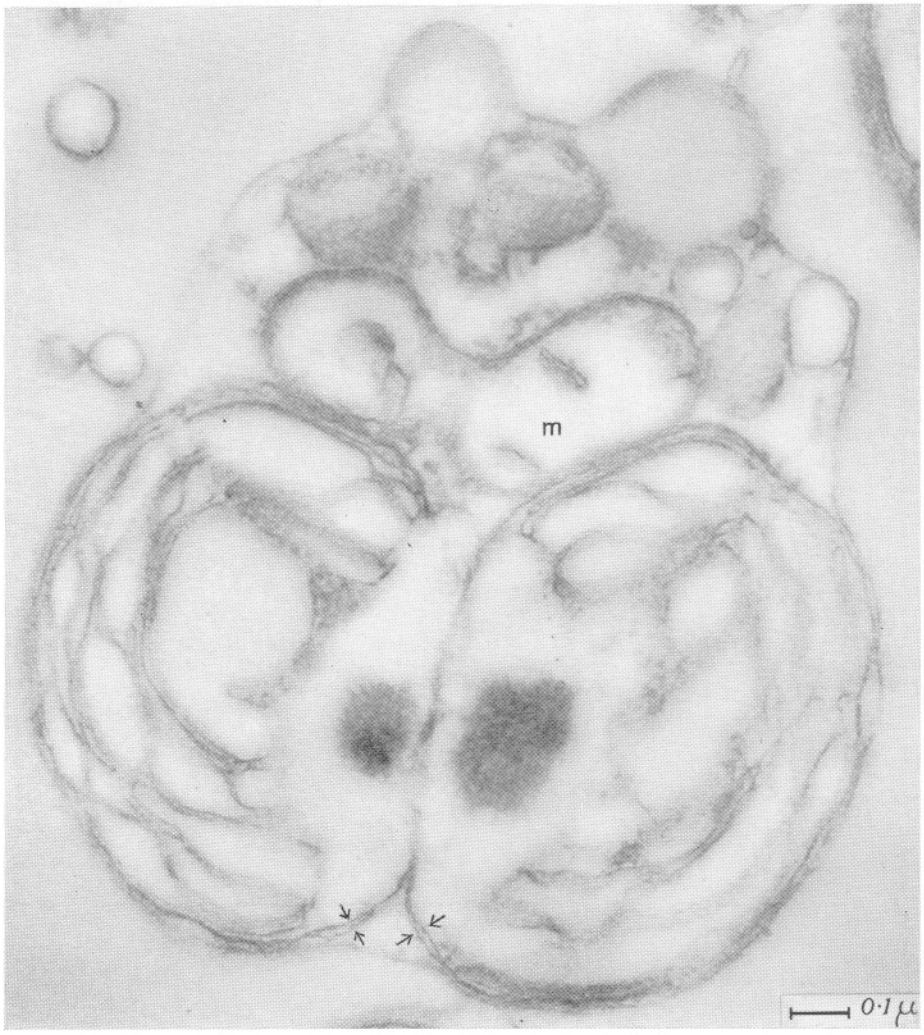
22a 22b



23



24 25



26

has elongated within the cell (not necessarily to its full size) there must at some point be an active movement of its basal body from a deep-seated position out to the surface, which is not true growth although externally it may resemble it. This movement could be very rapid, but until it has been completed a transverse section of a cell at a suitable level will show an internal flagellar axis without a separate membrane as in the left-hand cell of Fig. 14 (*yf*), Pl. VI, while a similar section taken further out can show a partially detached flagellum as in Fig. 16, Pl. VI. Comparison of both these figures with the longitudinal view of Fig. 10, Pl. III, will at once make this matter clear, and it will also be apparent by way of contrast that for any cell which had reached the condition of Fig. 8, Pl. III, a transverse section at no matter what level could at most include the flagellar basal body (*fb* in the right-hand cell of Fig. 14) within the subtending cytoplasm but no part of the flagellar axis.

DISCUSSION

Ignoring for a moment the taxonomic implications of the facts presented there are several points in the structure of this curious little organism which are of potential interest to students of fine structure. The first of these is the extreme numerical reduction of all the component parts in correlation with the greatly reduced overall dimensions of the cell. By the time that a cell is restricted to one nucleus, one plastid, one mitochondrion, one little group of golgi vesicles, one fat body, one flagellum and a very small amount of residual cytoplasm, it seems doubtful whether any further reduction would be possible without involving the fundamental structure of the organelles themselves. This has however not yet occurred in this species. Qualitatively all the organelles even the greatly abbreviated flagellum, are essentially of the same type as those of other algal cells, allowance being made for minor differences in relative dimensions of which those shown by the flagellum are the most extreme. Even here, however, the flagellum is only exceptional in the extreme disproportion between the flagellum proper (the thickened base) and its hair-point (the whiplash distal part). Hair-points are nevertheless in themselves common enough though usually short (see Manton, Clarke & Greenwood, 1955) but even long hair-points can sometimes be found, occasionally as an abnormality, as in *Fucus* (see Manton & Clarke, 1951), or even sometimes perhaps as a permanent feature (see Petersen, Caram & Hansen, 1958, on *Chordaria*). In none of these other organisms has it been shown that the hair-point contains a prolongation of the central strands, so that strict homology cannot yet be claimed. It is nevertheless at least possible that the exceptional condition about our present species might be not so much the permanent presence of a long-hair point as the fact that the flagellum proper is so short that it could be mistaken for a swollen base to the other organ. When this 1 μ long basal part is recognized for what it is the transverse dimension is not

fundamentally different from that of any other plant flagellum for which the diameter in sections is known.[1]

The reduction of the mitochondria to one only is a highly interesting feature for which we have no other examples at present. The observations made on cleavage, both of the plastid and the mitochondrion, suggest that in this organism both are self-propagating organelles which are not, and probably cannot be, reformed from any other cell component. For the mitochondria this has long been suspected, though in ordinary circumstances it is difficult to prove, since in a cell with many mitochondria it is usually impossible merely from the shapes to distinguish with certainty between division stages and coalescence. In this case the latter explanation of the shapes encountered seems excluded and we can only logically interpret the stages seen as reproduction by division.

For the plastid this is well known in many algae since green chromatophores are commonly quite large enough to be studied alive in considerable detail. In the lower algae of all colours other than blue-green it is a commonplace that mature plastids often divide without ever being resynthesized *de novo*. Nevertheless in many algae and in all or almost all higher plants the actual plastid is commonly developed not from another mature one but from a colourless primordium which alone has the capacity for division. This condition is, however, unlikely to be primitive and one may believe it not yet to have been reached by algal cells at the evolutionary level of our present flagellate.

The growth of the flagellum is a matter of considerable interest since this is a subject which, in plants, has not yet been effectively studied. Much would be learnt if it were possible to detect the first beginnings of a new flagellum. The much more limited observations made here of growth from a base inside the cell (the series of Fig. 17, Pl. VII) are nevertheless of importance in showing among other things that the flagellar membrane is not merely like the body membrane and continuous with it but is actually a part of the body surface which has been lifted up from below by growth of the flagellum itself. This could not have been deduced with certainty by observation of the mature structure, and it is a fact which will be of considerable comparative interest as knowledge of other organisms increases.

[1] Exact measurements of the diameter of cilia and flagella have not been published as such nor are they easy to obtain with any accuracy owing to the distortions inseparable from the processes involved in their study. Nevertheless a rough estimate can be obtained by comparing the published micrographs of the best fixed specimens for which, when expressed as vulgar fractions to avoid falsely suggesting greater accuracy than is justified, we obtain the following:

Sphagnum (Manton, 1957)	1/4 μ
Fucus (Manton & Clarke, 1956)	1/5 μ
Scytosiphon (Manton, 1956)	
Chrysochromulina (Parke, Manton & Clarke, 1958)	
Vaucheria (Greenwood, Manton & Clarke, 1956)	1/6 μ
'*Chromulina pusilla*'	1/7 μ

With regard to the other aspect of the investigation, namely, the taxonomic implications, it is obvious that we have here unintentionally entered a highly specialized field, of active concern to students of plankton but one which can only be effectively dealt with by an expert professional algologist, which we do not claim to be. Nevertheless the general principles are clear enough, for in addition to our own previous work on other members of the Chrysophyceae and related groups, there is a recent electron microscopical study on *Chromulina psammobia* by Fauré-Frémiet and Rouiller (1957) which proves beyond doubt that the anomalous characteristics of '*Chromulina pusilla*' are not shared by other species more properly included in that group, and that, in addition, our species lacks several characters such as the hairy appendages on a forwardly directed flagellum which are known to exist in other species of the genus.

A conclusive piece of further evidence can moreover be quoted. By co-operation between Dr M. Parke of Plymouth and Dr G. Y. Kennedy of Sheffield a large amount of material was cultured and subjected to qualitative pigment analysis. The findings (Kennedy, personal communication dated 15 November 1957) were as follows: 'present: chlorophyll *a*, chlorophyll *b*, no trace of any other chlorophyll; α and β carotene, lutein; no other pigments'. The decisive factor in this list is of course chlorophyll *b* which is entirely absent from all known representatives of the Chrysophyceae but which is virtually diagnostic of Chlorophyceae and near relatives. Its presence is the final and unequivocal proof that this organism must be removed out of the genus *Chromulina* and out of the class Chrysophyceae.

Whether, when this is done, it can properly be included within any existing genus of Chlorophyceae or whether indeed an entirely new group of non-starch-producing flagellates will be required to contain it are matters on which it would be wrong at this stage to express further opinions. Dr M. Parke is actively engaged in exploring the various alternatives and may be expected to publish her findings shortly. In the meantime it may be helpful to enumerate the taxonomically significant characters which the electron microscope has revealed and which will need to be covered by any generic diagnosis which may eventually be selected. An emended description, not including any additional facts which may still be ascertainable with the light microscope, is therefore as follows:

Unicellular pigmented flagellate, of average size *c.* $1 \times 1{\cdot}5\,\mu$, possessing chlorophylls *a* and *b*, carotenes α and β, and lutein, but not forming starch; with a single plastid possessing a large central pyrenoid covered by a shell of concentric paired lamellae resembling in a general way those of the Chlorophyceae; with a single mitochondrion possessing a sparse array of flattened cristae resembling those of certain green algae, notably *Chlamydomonas*, and with a laterally attached flagellum *c.* $1\,\mu$ long terminating in a slender hair-point *c.* $3\,\mu$ long, both parts directed backwards during swimming; the body smoothly covered by a membrane but without surface scales or cell wall; multiplication by fission involving the plastid, the mitochondrion and the nucleus with the formation of a new flagellum immediately beside the old one.

SUMMARY

The very small flagellate hitherto known as *Chromulina pusilla* Butcher has been examined morphologically and anatomically with the electron microscope. The cell is found to contain a nucleus, plastid, one mitochondrion, one small golgi area, one fat body, a small amount of granular and vesicular cytoplasm and a flagellum less than 1 μ long terminating in an extended hair-point, the whole surface being covered by a three-layered membrane. In spite of their small size all parts are structurally normal. The anatomical and biochemical evidence indicates that this flagellate has been placed in the wrong group. The details given include a few observations on division stages of the plastid and mitochondrion and on the growth of the flagellum. An emended diagnosis is inserted as a guide to the ultimate reclassing and renaming of the organism.

REFERENCES

BUTCHER, R. W., 1952. Contributions to our knowledge of the smaller marine algae. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 175–91.

FAURÉ-FRÉMIET, E. & ROUILLER, C., 1957. Le flagelle interne d'une Chrysomonadale: *Chromulina psammobia*. *C.R. Acad. Sci., Paris*, T. 244, pp. 2655–7.

GREENWOOD, A. D., MANTON, I. & CLARKE, B., 1957. Observations on the structure of the zoospores of *Vaucheria*. *J. exp. Bot.*, Vol. 8, pp. 71–86.

GREENWOOD, A. D., 1959. Observations on the structure of the zoospores of *Vaucheria*. II. *J. exp. Bot.*, Vol. 10, pp. 55–68.

KENNEDY, G. J. & NICOL, J. A. C., 1959. The pigments of *Chaetopterus*. *Proc. roy. Soc.* B (in the press).

KNIGHT-JONES, E. W. & WALNE, P. R., 1951. *Chromulina pusilla* Butcher, a dominant member of the ultraplankton. *Nature, Lond.*, Vol. 167, p. 445.

KNIGHT-JONES, E. W., 1951. Preliminary studies of nanoplankton and ultraplankton systematics and abundance by a quantitative culture method. *J. Cons. int. Explor. Mer*, Vol. 17, pp. 140–55.

MANTON, I., 1955. Observations with the electron microscope on *Synura caroliniana* Whitford. *Proc. Leeds phil. lit. Soc.* vol. 6, pp. 306–316.

—— 1956. Observations with the electron microscope on the internal structure of the zoospore of a brown alga. *J. exp. Bot.*, Vol. 8, pp. 294–303.

—— 1957. Observations with the electron microscope on the cell structure of the antheridium and spermatozoid of *Sphagnum*. *J. exp. Bot.*, Vol. 8, pp. 382–400.

MANTON, I. & CLARKE, B., 1951. An electron microscope study of the spermatozoid of *Fucus serratus*. *Ann. Bot.*, N.S., Vol. 15, pp. 461–71.

—— —— 1956. Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus*. *J. exp. Bot.*, Vol. 7, pp. 416–32.

MANTON, I., CLARKE, B. & GREENWOOD, A. D., 1955. Observations with the electron microscope on biciliate and quadriciliate zoospores in green Algae. *J. exp. Bot.*, Vol. 6, pp. 126–8.

PALADE, G., 1952. The fine structure of mitochondria. *Anat. Rec.*, Vol. 114, pp. 427–52.

PARKE, M., MANTON, I. & CLARKE, B., 1958. Studies on marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, vol. 37, pp. 209–228.

PARKE, M. MANTON, I. & CLARKE, B., 1959. Studies on marine flagellates. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp. nov. *J. mar. biol. Ass. U.K.*, Vol. 38,pp. 169–188.

PETERSEN, J. B., CARAM, B. & HANSEN, J. B., 1958. Observations sur les zoïdes du *Chordaria flagelliformis* au microscope électronique. *Bot. Tidsskr.*, Bd. 54, pp. 57–60.

SAGER, R. & PALADE, G. E., 1957. Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell. *J. biophys. biochem. Cytol.*, Vol. 3, pp. 463–87.

J. Mar. biol. Ass. U.K. (1959) 38, 335–350
Printed in Great Britain

THE EARLY GROWTH STAGES AND ADULT STRUCTURE OF THE LOPHOPHORE OF *MACANDREVIA CRANIUM* (MÜLLER) (BRACHIOPODA, DALLINIDAE)

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–11)

Although it has been stated (Thomson, 1927, p. 234; Elliott, 1953, p. 264) that the descending branches of the loop in dallinids, so far as is known, grow from the crura only, yet figures supporting this statement have not so far been published, not even by Beecher (1895) who described the very early stages of lophophore and loop in *Dallinella* (= *Terebratalia*) *obsoleta* (Dall). Tiny specimens of *Macandrevia cranium* dredged by R. V. 'Sarsia' have now made it possible for such figures to be published. It is a point of some importance as it is one of the characters given by Thomson (1927, p. 234) as distinguishing the Dallininae from the Mühlfeldtiinae (= Megerliinae Muir-Wood, 1955) and the Magellaniinae (= Terebratellinae, see Muir-Wood, 1955), for in the two latter groups the descending branches of the loop grow from both crura and septum to unite in the middle. At the time Thomson was writing, young growth stages of the loop of a member of the Laqueinae were not known. Since then, Konjoukova (1948, 1957) has studied the development of *Laqueus californicus*—which she placed in the Dallininae—and according to her the descending branches on reaching the septum join with it. She does not, however, figure any intermediate stages between absence of descending branches and complete ones. In 1941 Laqueinae was raised to family rank by Yabe and Hatai (see Muir-Wood, 1955, p. 93).

Palaeontologists have given the growth stages of the loop various names derived from different genera. Certain of the genera are fossil, and although familiar to palaeontologists, are unlikely to be so to many zoologists. Moreover, these terms are not fixed, several of them having been changed by successive authors, as may be seen from the table given by Thomson (1927, p. 232); since that date a further change has been made by Elliott (1953, p. 264), although not followed by Konjoukova in her 1957 monograph. It has therefore been decided to avoid them, relying on the figures to make the stages clear.

All figures have been drawn with the aid of a camera lucida. Mantle setae

have been omitted from the figures as they cover much space: at a shell length of 1·4 mm the anterior setae reach a length of about 0·8 mm.

Macandrevia cranium lacks the two carmine pigment spots, which have been found near the preoesophageal ganglion in certain other brachiopods.

THE EARLY GROWTH STAGES OF THE LOPHOPHORE AND LOOP

The fullest series of growth stages of the loop—without the lophophore—of *Macandrevia cranium* so far described and figured is that of Friele (1877) from a shell length of a little less than 3 mm to the adult. He remarked that at the former size 'a coherent apophysary system' first occurs. He had younger stages, but in a dried condition, and did not figure their brachial support. Elliott (1948) described and figured, from preserved material, the development of the lophophore and loop from a shell length of 5 mm to the adult.

Fine shell gravel from near La Chapelle Bank provided numbers of young *M. cranium* of shell length 1·0 mm onwards. Of two hauls, the first, that taken on 19 June 1956 at position 47° 37′ N., 7° 16′ W. and depth of 90–100 fathoms, was the richer of the two in tiny brachiopods, *Terebratulina retusa* and *Gryphus vitreus* occurring in addition to *Macandrevia cranium*. It was preserved on board and thus the lophophores were unfortunately in a contracted state. The second haul of 20 April 1958 from position 47° 35′ N., 7° 13′ W. at a depth of 105–110 fathoms was brought back under circulation and some young living *M. cranium* were found.

Stages of the lophophore at a shell length of 1·0–5·3 mm will be described and figured so as to join with and somewhat overlap Elliott's (1948) series. The stage of development of the lophophore to shell length varies somewhat in the La Chapelle Bank *Macandrevia*, so that length can only be taken as approximate for the stage under consideration; however, the La Chapelle Bank material shows earlier development of the loop than the specimens from Norway used by Friele (1877) and Elliott (1948).

The earliest stages were found in the preserved shell gravel and show the lophophore in the contracted state (Fig. 1).

The lophophore is trocholophous to the time when eleven pairs of filaments have developed—the last pair being short—with in addition a minute asymmetrical bud. The base of the lophophore, or lophophoral ridge, is a closed circle (Fig. 1 A), as described by Beecher (1895, p. 393) in *Dallinella* (= *Terebratalia*) *obsoleta* (see, however, Williams, 1956, pp. 260–1). The more recently formed filaments show a marked difference in length, indicating that the appearance of new ones is not as rapid as in later stages. The first few filaments to be formed arise from the body behind the mouth; then it would seem that the lophophoral ridge extends on to the brachial mantle. The mouth is a small opening lying in front of the bases of the first formed pair of

filaments, as described by me (Atkins, 1959) in *Platidia* and by Beecher (1895) in *Dallinella obsoleta*. In front of the mouth is a lobe which elongates laterally —running concentric to the bases of the filaments—to form the lip of the food groove or brachial fold. At the eleven pairs of filament stage the lip reaches to about the base of the fifth or sixth filament on each side. The length of the shell at this stage is approximately 1·0–1·2 mm. This mode of growth of the lip has been previously described in *Platidia* (see Atkins, 1959) and is contrary to that described by Percival (1944) and by Williams (1956, p. 260).

By the time twelve pairs of distinct filaments are present the lophophoral ridge is interrupted in the mid-line anteriorly and very slightly indented, there now being two distinct, but closely contiguous, growth regions (Fig. 1 B, C).

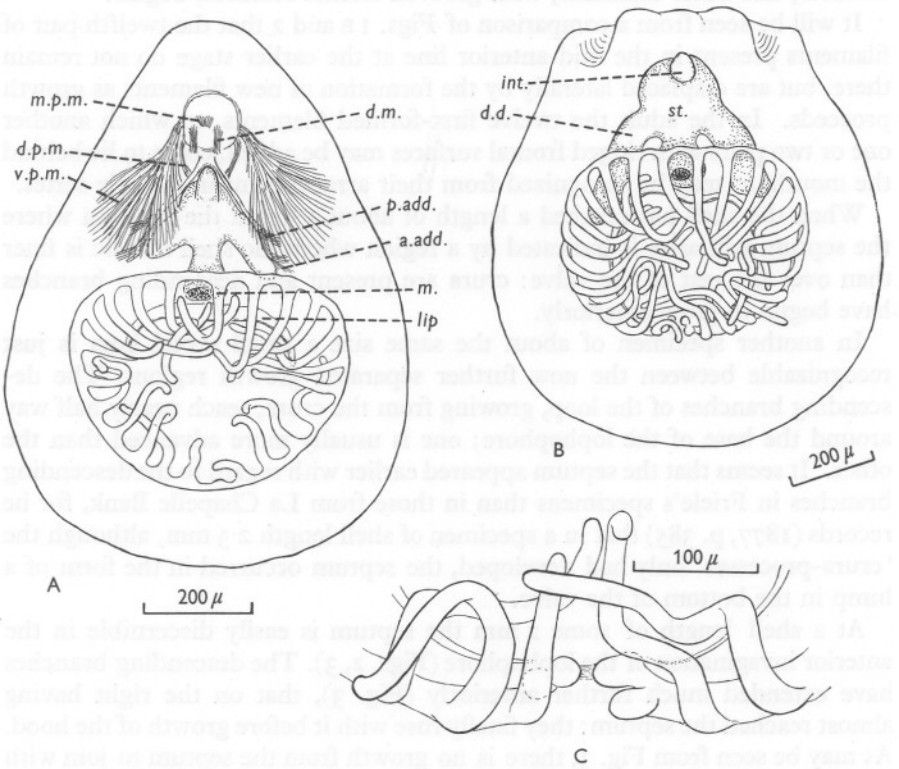


Fig. 1. *Macandrevia cranium*. A, Entire animal of shell length 1·0 mm viewed ventrally: lophophore trocholophous. B, Brachial valve of specimen of shell length 1·35 mm with very early schizolophe: muscles omitted. In both A and B the lip of the food groove (*lip*) is turned outwards exposing the mouth (*m.*). C, Growing region of the lophophore of a specimen of shell length 1·25 mm; the lip of the food groove is merely a slight ridge anteriorly. *a.add.*, anterior adductor muscle; *d.d.*, digestive diverticulum; *d.m.*, diductor muscle; *d.p.m.*, dorsal pedicle muscle; *int.*, intestine; *m.p.m.*, median pedicle muscle; *p.add.*, posterior adductor muscle; *st.*, stomach; *v.p.m.*, ventral pedicle muscle. Stained with eosin and drawn while in cedar wood oil.

The filaments are still in a single row and all have ridged frontal surfaces. In addition to the twelve pairs of recognizable filaments are two pairs of minute buds (Fig. 1 B, C) and not the one minute asymmetrical bud as in the trocholophous stage. No crura or septum can be recognized. The growth of the lip of the food groove has caught up, or nearly so, with that of the filaments, and it extends to about the base of the last formed filament on each side. Shell length is now 1·2 –1·4 mm.

From this stage onwards, as the invagination of the lophophore deepens, the rate of growth of new filaments is accelerated, the growing region bearing closely gradated buds (Fig. 2). When twelve to fourteen pairs are present, differentiation into an alternating series of inner filaments, with ridged frontal surfaces, and outer filaments, with grooved frontal surfaces, begins.

It will be seen from a comparison of Figs. 1 B and 2 that the twelfth pair of filaments present in the mid-anterior line at the earlier stage do not remain there, but are displaced laterally by the formation of new filaments as growth proceeds. In the adult the twelve first-formed filaments, to which another one or two pairs with ridged frontal surfaces may be added, come to lie behind the mouth, as may be recognized from their arrangement in a single series.

When the shell has reached a length of about 1·9 mm the position where the septum will arise is indicated by a region where the shell mosaic is finer than over the rest of the valve: crura are present and descending branches have begun to grow anteriorly.

In another specimen of about the same size a small septal boss is just recognizable between the now further separated growth regions. The descending branches of the loop, growing from the crura, reach nearly half way around the base of the lophophore; one is usually more advanced than the other. It seems that the septum appeared earlier with regard to the descending branches in Friele's specimens than in those from La Chapelle Bank, for he records (1877, p. 385) that in a specimen of shell length 2·3 mm, although the 'crura-processes' only had developed, the septum occurred in the form of a lump in the bottom of the valve.

At a shell length of some 2 mm the septum is easily discernible in the anterior invagination of the lophophore (Figs. 2, 3). The descending branches have extended much further anteriorly (Fig. 3), that on the right having almost reached the septum: they finally fuse with it before growth of the hood. As may be seen from Fig. 3, there is no growth from the septum to join with the descending branches growing from the crura. The descending branches are calcareous ribbons vertical to the valve floor, and so appear extremely narrow in dorsal and ventral view; they run slightly above the valve floor.

Fig. 2 A and B of the same lophophore stage, the one drawn living and the other preserved, show the great difference in appearance of the lophophore resulting from preservation without narcotizing (see also Atkins, 1959, p. 128). The individual shown in Fig. 2 B has the septum and descending branches

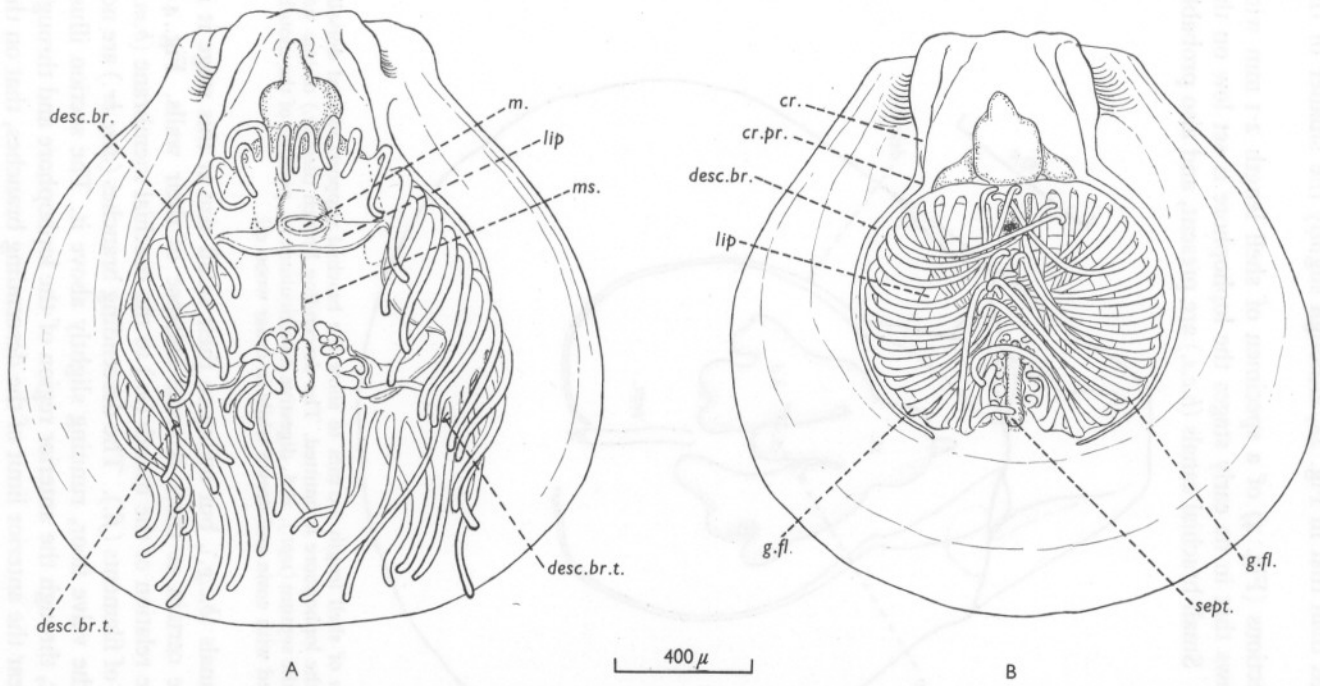


Fig. 2. *M. cranium.* A, Brachial valve, with schizolophe, of specimen of shell length of 2·25 mm, drawn living and fairly well expanded. B, Brachial valve with schizolophe in contracted state, thus exposing the well advanced descending branches of the loop. The septum as yet shows no development of a hood. Specimen of shell length 2·1 mm, drawn preserved without narcotizing. Differentiation into a double series of filaments has begun, the first outer grooved filament on each side is indicated (*g.fl.*). *cr.*, one of the crura; *cr.pr.*, crural process; *desc.br.*, descending branch of the loop; *desc.br.t.*, tip of descending branch; *lip*, lip of the food groove; *m.*, mouth; *ms.*, mesentery; *sept.*, septum.

further developed than that in Fig. 2A, although slightly the smaller of the two.

Transverse sections (Fig. 4) of a specimen of shell length 2·1 mm with schizolophe, shows that in its early stages the lophophore is set low on the brachial mantle. Small brachial canals (*b.c.s.*) are present, and also probably

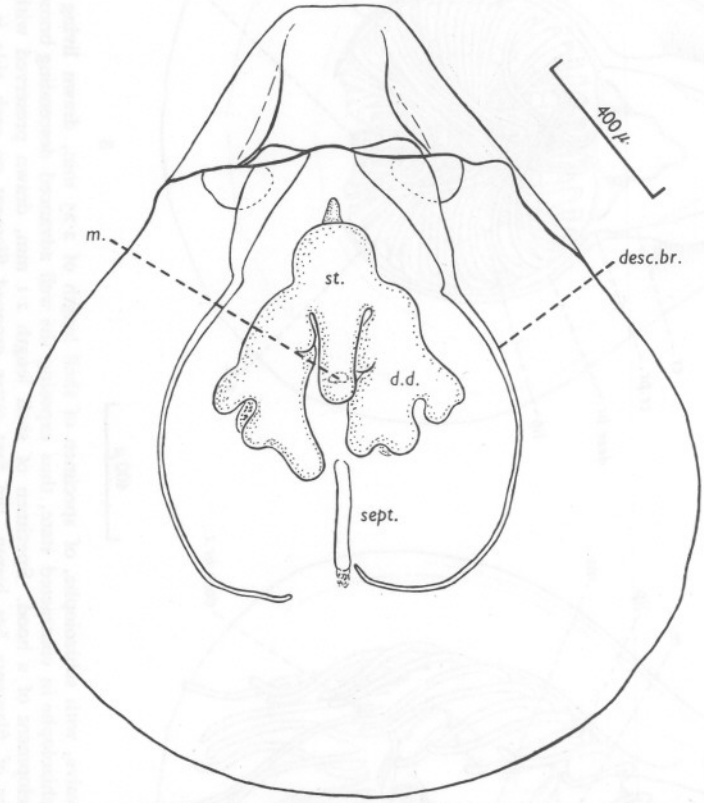


Fig. 3. *M. cranium* of shell length 2·0 mm to show the brachial support, viewed dorsally through the shell: the lophophore is omitted. The descending branch (*desc.br.*) of one side has almost reached the septum (*sept.*). *d.d.*, digestive diverticulum; *m.*, position of the mouth; *st.*, stomach. Stained with eosin and drawn while in cedar wood oil.

great brachial canals (*b.c.g.*), but owing to their small size at this stage it is impossible to be certain as there is some collapse of their walls. Fig. 4A clearly shows the relation of the mouth (*m.*) to the brachial membrane (*b.m.*) and to the circle of filaments (*fl.*). The descending branches (*desc.br.*) are not confluent with the valve floor, running slightly above it. The section illustrated in Fig. 4C, through the anterior region of the lophophore and through the septum, is near the anterior limit of the descending branches, that on the right alone being present.

As growth of the lophophore proceeds the position of the descending branches shifts inwards. A late schizolophous stage, with lateral arms deflected, at a shell length of 2·2 mm is illustrated (Fig. 5A). The, as yet widely curved, descending branches have united with the septum, which is slightly grooved ventrally—the grooving precedes formation of the hood—and is split anteriorly. Konjoukova (1957, pp. 63–4) has noted that anterior division of the septum is characteristic of the development of the brachial apparatus of the Dallininae, in which she included *Laqueus*.

In another specimen of about the same size and lophophore stage as that shown in Fig. 5A the descending branches make an acute angle with the

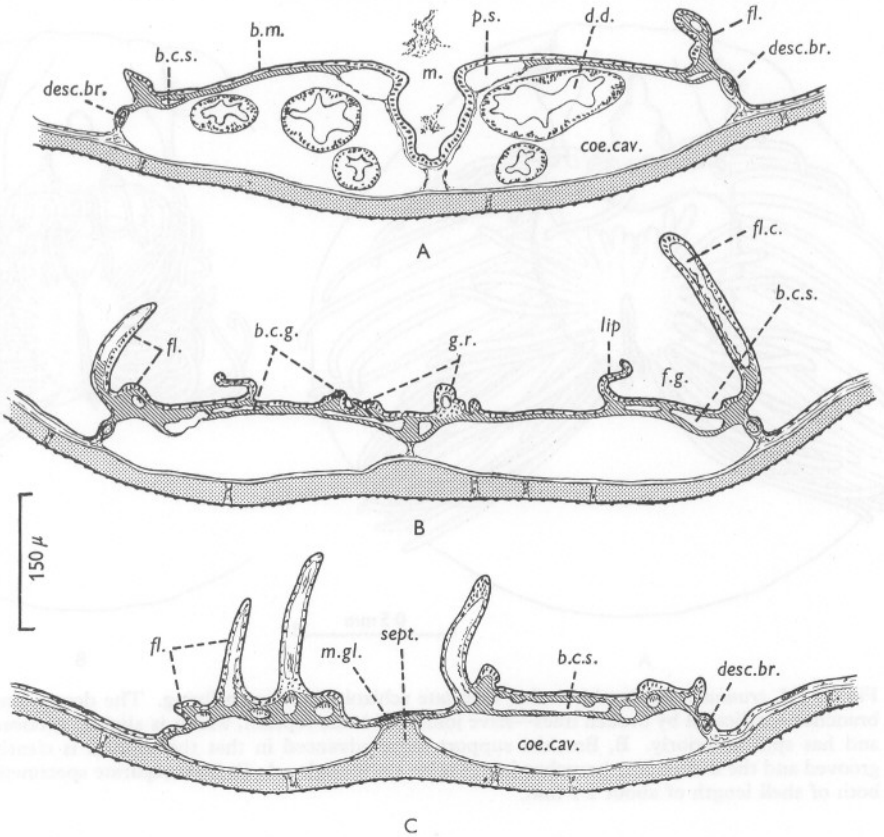


Fig. 4. *M. cranium* of shell length 2·1 mm. Transverse sections through schizolophe to show its relation to the dorsal mantle and shell, (A) through the mouth (*m.*), (B) through the twin growing regions (*g.r.*) and (C) through the anterior region of the lophophore and the septum (*sept.*). *b.c.g.*, great (?) and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *coe.cav.*, coelomic cavity; *d.d.*, tubule of digestive diverticulum; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.*, filament and filament base; *fl.c.*, filamentar canal; *lip*, lip of the food groove; *m.gl.*, mucous gland cells; *p.s.*, perioesophageal sinus. Supporting substance diagonally hatched.

septum (Fig. 5 B). This is the youngest stage of the brachial support described by Friele (1877) at a shell length of a little less than 3 mm, nearly agreeing with his fig. 1.

At a somewhat greater shell length, 2·8 mm, the lophophore is still schizolophous, but the ventral, abfrontal, surfaces of the lateral arms have widened. The hood, although narrow is clearly distinguishable. Long, spinous, processes have developed from the anterior end of the septum. This would correspond to Friele's fig. 2. Elliott (1953, p. 269) has noted the spiny nature of many dallinid loops, mature and immature, a feature almost completely lacking in the Terebratellidae.

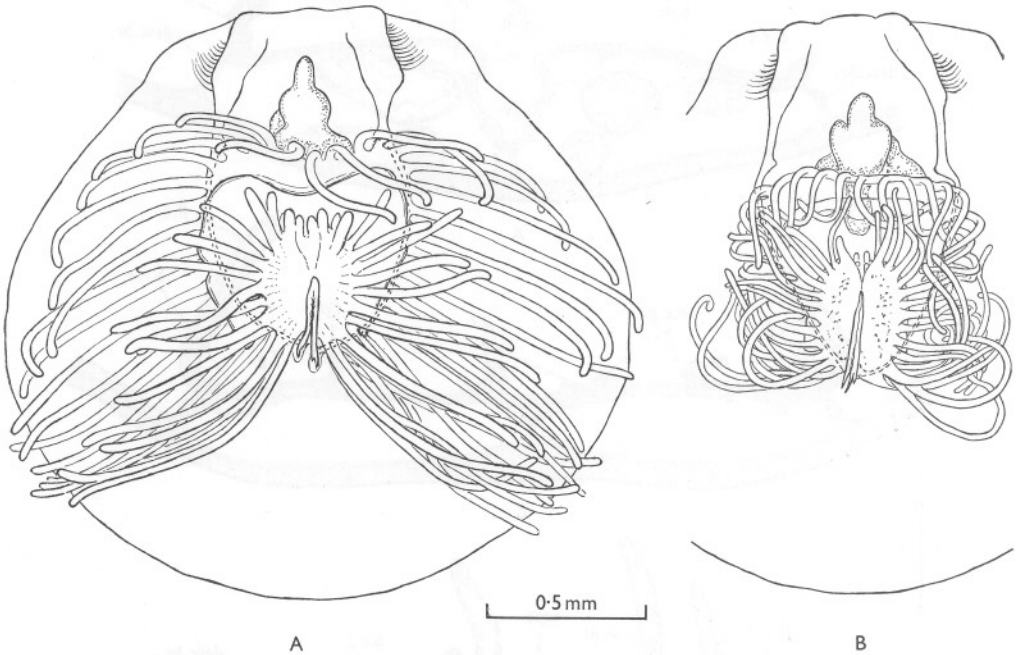


Fig. 5. *M. cranium*. A, Brachial valve with late schizolophe, drawn living. The descending branches—indicated by broken lines—have joined with the septum, which is slightly grooved and has split anteriorly. B, Brachial support more advanced in that the septum is clearly grooved and the descending branches join it at an acute angle. A, B, from separate specimens both of shell length of about 2·2 mm.

At a shell length of 3·5 mm (brachial valve 3·35 mm) the lophophore is zygolophous (Fig. 6). The hood has widened posteriorly and by resorption of its posterior closed end, the transverse band (*t.b.*) has been formed. The anterior spinous processes have increased in number. This stage was drawn from a living individual obtained from La Chapelle Bank region in September 1954. The condition of the brachial support corresponds to Friele's fig. 3.

The transverse sections of this stage, illustrated in Fig. 7, show the relation of the brachial support, and particularly of the hood, to the lateral arms. The somewhat oblique section shown in Fig. 7 A passes through the growing region (*g.r.*) of one side. At this size the great brachial canals (*b.c.g.*) are clearly distinguishable.

At a shell length of 4·2 mm (brachial valve 3·6 mm) the lophophore (Fig. 8 A) is at about the same stage of development as at the smaller shell size (Fig. 6). Growth of the hood, however, has advanced, its lower margins having diverged widely, and is now at about the stage shown in Friele's (1877) fig. 4 (brachial valve 4·5 mm long) and Elliott's (1948) fig. 2 (of shell length 5·5 mm).

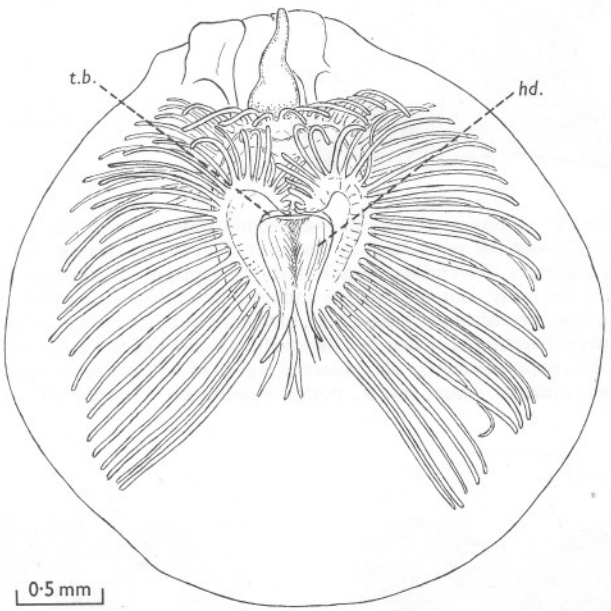


Fig. 6. *M. cranium* of shell length 3·5 mm. Brachial valve with zygolophe, drawn living. The hood (*hd.*) has widened posteriorly and the transverse band (*t.b.*) is distinct. The anterior spinous processes are long. The dorsal filaments of the lateral arms have been omitted so as to avoid confusion.

The last lophophore stage to be described here (Fig. 8 B) is at a shell length of 5·3 mm (brachial valve 4·6 mm). The lophophore is early plectolophous, with one small coil to the spiral arm. In the figure the dorsal part of the spiral arm has been omitted so that the loop should not be entirely hidden. The loop is in the stage shown in Friele's fig. 7, which according to Elliott (1948) corresponds to his figs. 4 and 5. Friele gave the length of his brachial valve as 5·6 mm; Elliott the length of his pedicle valve as 7·0 mm. The freeing of the loop from the septum, and the gradual thinning and disappearance of the

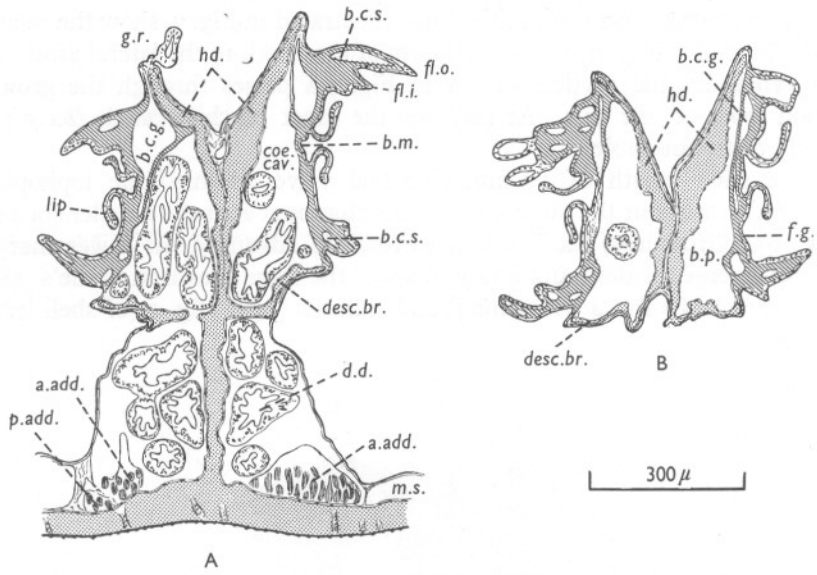


Fig. 7. *M. cranium.* Transverse sections—somewhat oblique—through the brachial valve with zygolophe of about the same stage as shown in Fig. 6, (A) through the hood (*hd.*) and the junction of the right descending branch with the septum, (B) through the lateral arms where free from the body. *a.add.*, anterior adductor muscle; *b.c.g.*, great and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *b.p.*, brachial pouch; *coe.cav.*, coelomic cavity; *d.d.*, tubule of digestive diverticulum; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.i.*, inner and *fl.o.*, outer filaments; *g.r.*, growing region of left side; *hd.*, hood; *lip*, lip of the food groove; *m.s.*, mantle sinus; *p.add.*, posterior adductor. Supporting substance shown hatched.

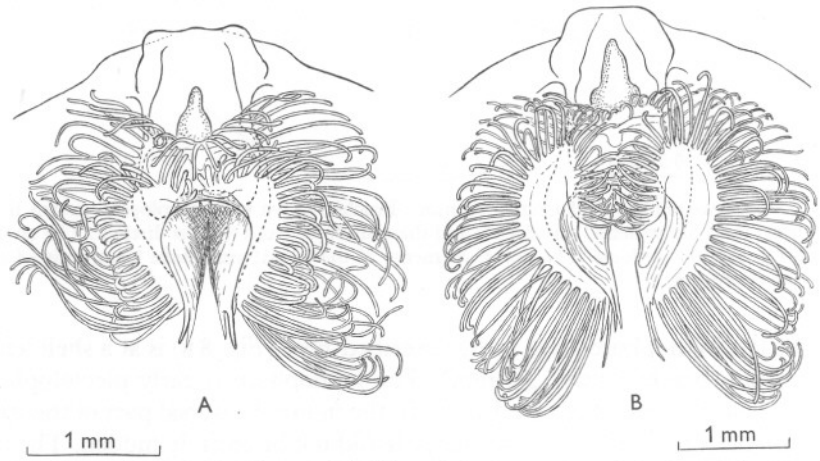


Fig. 8. *M. cranium.* A, Specimen of shell length 4·2 mm: part of the brachial valve with zygolophe, drawn preserved, unnarcotized. The hood is more advanced than that shown in Fig. 6, its lower margins having diverged. B, Specimen of shell length 5·3 mm; early plectolophe with spiral arm of one coil, the dorsal half of which has been omitted for the sake of clearness. The hood shows large lacunae. Preserved after narcotizing and drawn while in cedar wood oil.

latter takes place at this stage; it has been described by Friele (1877, pp. 381–2) and Elliott (1948, p. 301).

Although it has been found that some variation occurs in the shell length at which a certain stage of lophophore and loop is attained, it will have been seen that the loop is in a more advanced stage at a lesser shell size in the La Chapelle Bank specimens than in those of Friele and Elliott. Elliott's *Macandrevia cranium* came from the Hardangerfjord, Norway (1948, p. 316); those of Friele (1877) apparently from the same country.

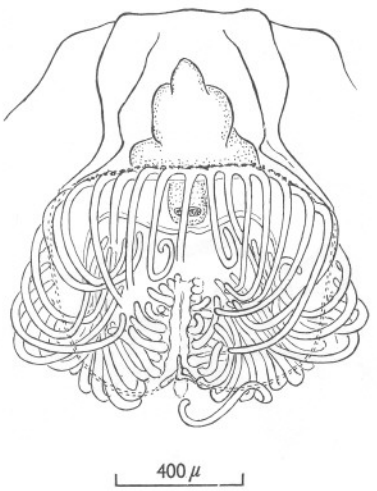


Fig. 9. *M. cranium* of shell length 2·3 mm. Schizolophe with abnormal septum, restricted to a small three-pronged structure at the junction with the descending branches (indicated by broken lines). Stained and drawn while in cedar wood oil.

The development of the brachial support of *M. cranium* is marked by exceptionally early growth of the descending branches of the loop and a late development and early disappearance of the septum.

In *Dallinella* (= *Terebratalia*) *obsoleta*, the only member of the Dallinidae in which the very early stages of the loop have been figured, a septum is present before the development of the crura, and a hood appears on the septum before growth of the descending branches begins (Beecher, 1895).

In *Laqueus californicus*, placed by Konjoukova (1957) in the Dallininae, but by Yabe and Hatai in 1941 (see Muir-Wood, 1955) in the Laqueidae, the septum also appears before the descending branches, as may be seen from her fig. 27 (p. 40).

Small individual with an abnormal septum. One individual of shell length 2·3 mm, with advanced schizolophe, had an abnormally developed septum (Fig. 9). A small calcified region, irregularly three-pronged in shape, occurred

at the junction of the descending branches with it. The remainder of the septum appeared to be represented by connective tissue only. The median dorsal mesentery was clearly visible running up its centre.

THE ADULT LOPHOPHORE

The adult lophophore is plectolophous, supported by a long calcareous loop: spicules are entirely lacking. As the shell is long the lateral and spiral arms are long. The loop extends to the extreme end of each lateral arm, and a spine projects anteriorly. The position of the twin growing regions at the apex of the spiral arm is shown in transverse section in Fig. 10A. From the sections illustrated in Fig. 10 the very largely tubular structure of the lophophore of

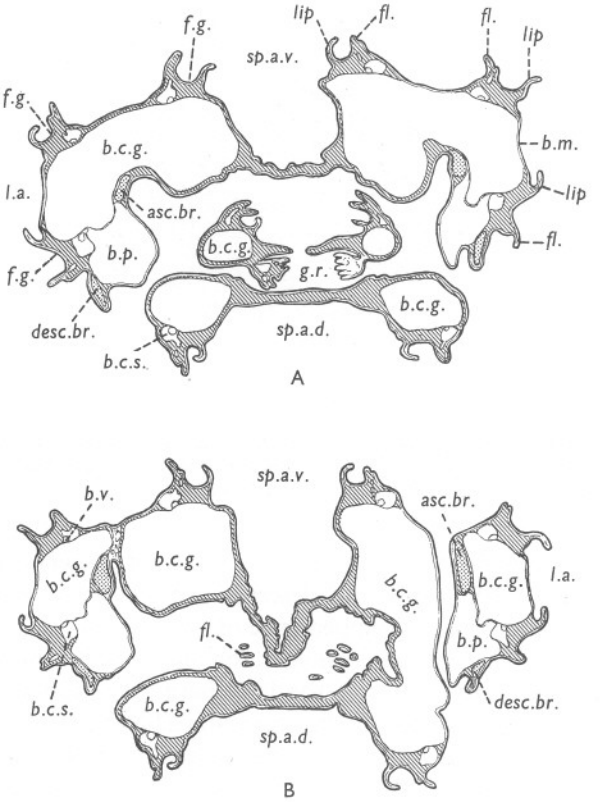


Fig. 10. *M. cranium* of shell length 17 mm. Transverse sections through adult plectolophe. A, Section through the twin growing regions of the lophophore (*g.r.*) at the apex of the spiral arm. The great brachial canals of the lateral and spiral arms are in communication. B, a section anterior to A, with the spiral and lateral arms becoming separate. *asc.br.*, ascending branch of the loop; *b.c.g.*, great and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *b.p.*, brachial pouch; *b.v.*, 'blood' vessel; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.*, filament; *l.a.*, lateral arm; *lip*, lip of the food groove; *sp.a.d.*, dorsal and *sp.a.v.*, ventral side of first coil of spiral arm. Supporting substance shown hatched.

Macandrevia cranium is apparent. There is so little tissue that on fixation a certain amount of distortion occurs, for instance the brachial membrane connecting the opposite rows of filaments of the ventral part of the first coil of the spiral arm is probably too deeply indented.

The usual canals are present in the lophophore. Small brachial canals (*b.c.s.* Figs. 10, 11), arising from the perioesophageal lacunae, run at the bases of the filaments to which they give off branches. Within the small brachial and the filamentar canals are 'blood vessels', with fine muscle fibres in their walls. The great brachial canals (*b.c.g.*) each ends in a blind sac on each side of the oesophagus (see also Hancock, 1858, p. 807). In the lateral arms they do not extend as far anteriorly as do the small brachial canals, which are continuous around the blind end of the arms. Posteriorly the great brachial

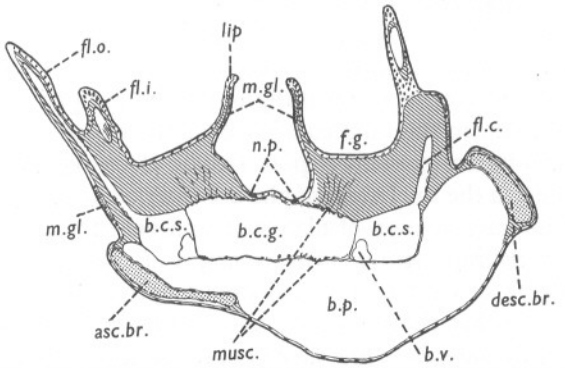


Fig. 11. *M. cranium*. Transverse sections of lateral arm of an adult. *fl.c.*, filamentar canal; *fl.i*, inner and *fl.o.*, outer filaments; *m.gl.*, mucous gland cells; *musc.*, muscle fibres; *n.p.*, principal nerve. Other lettering as in Fig. 10.

canals of the lateral and spiral arms are continuous (Fig. 10A), as noted by Hancock; anteriorly they become separate (Fig. 10B). In each lateral arm a brachial pouch, a continuation of the coelomic cavity, extends anteriorly to its extremity, reaching beyond the great and small brachial canals. In Figs. 10 and 11 it is impossible at the magnification shown to indicate the various tissues.

Muscle fibres are present in the walls of the canals, chiefly beneath the supporting substance under the food grooves and the filaments. Here they are mainly transverse in direction and on contraction compress the supporting substance, so that in sections it appears to have fibres running normal to the food groove. Contraction of these muscle fibres also causes the brachial membrane to be thrown into folds. In the lip of the food groove muscle fibres are present below the epithelium.

Nerves are difficult to distinguish in sections of the lophophore, indeed only the principal nerve (*n.p.*) could be certainly identified: its position is shown in Fig. 11.

The distribution of mucous gland cells on the lophophore is similar to that in other plectolophes examined. They occur: (1) in an abfrontal tract below the bases of the filaments; (2) on the brachial membrane of the arms; and (3) on the walls, including the lip of the food groove.

The filaments are in the usual double alternating series, the outer with grooved and the inner with ridged frontal surfaces. Behind the mouth twelve to fourteen pairs of filaments are in single series and have ridged frontal surfaces: these filaments are short. Longitudinal muscle fibres are present in the walls of the filamentar canals, chiefly in a frontal group, in which the fibres are striated.

The body of the lophophore is entirely ciliated, except possibly for mucous cells. The outer surfaces of the filaments are entirely ciliated, the cilia being in four tracts: frontal, abfrontal and paired lateral.

The ciliary feeding mechanism of *M. cranium* has been briefly described (Atkins, 1956) and it is intended to publish a full account separately.

In the various growth stages resulting in the plectolophe of the adult, new filaments are formed in two immediately adjacent regions, one on each side of the mid-line. The two growing regions remain close together in all developmental stages and in the final plectolophe are situated at the apex of the spiral arm. There is no interpolation of filaments, and yet the lateral arms increase in length commensurate with growth in length of the shell. It appears that there is a shifting around of the filaments, together with the underlying brachial canals, so that those on the ventral side of the lateral arms move around on to the dorsal side, while filaments which were originally situated on the spiral arm will move around on to the ventral side of the lateral arm. The two opposite rows of filaments of the spiral arm are distinct in that one set belongs to the left side of the body and one to the right: there are two distinct great brachial canals. In the lateral arms there is a single great brachial canal for the two rows of filaments on opposite sides of the arms: the two tubes having as it were coalesced (Hancock, 1858).

To account for the forward growth of the lateral arms in the lophophore of terebratulaceans and terebratellaceans, Williams (1956, pp. 261–3) suggested that growth in the median zone is temporarily suppressed, while two secondary growth regions arise in the antero-lateral positions of the lophophore. This suggestion seems to be unfounded. Secondary growth zones would presumably be marked by the presence of short filaments and filament buds, but such have been found only in the twin growing regions in the median indentation of the schizolophe in all brachiopods studied, including *Terebratella inconspicua* which Williams figured (1956, fig. 5 (1)). And far from growth being suppressed in the median zone, it becomes accelerated, as the presence of crowded filament buds shows (see Fig. 2). The growth of new filaments appears to be so rapid that their migration anteriorly and laterally to form the lateral arms does not keep pace with it, and the growing

regions turn either anteriorly, and finally ventro-dorsally, as in plectolophes, or laterally, as in spirolophes, encroachment on the mouth thus being avoided.

I am grateful to Dr L. H. N. Cooper for having the two dredge hauls—which contained the tiny brachiopods—taken while on hydrographical cruises. My thanks are due to Dr J. S. Alexandrowicz for translating the Russian of Konjoukova for me, and to Mr G. F. Elliott for reading the manuscript.

A grant from the Browne Research Fund of the Royal Society for the year 1956–57 and a London University table made the work possible.

SUMMARY

The early growth stages of lophophore and loop of *Macandrevia cranium* at a shell length of 1·0–5·3 mm are described. Figures are given to show that in this dallinid the descending branches of the loop grow from the crura only and fuse with the septum. The figures also show that only the two primary growth regions persist, and that no secondary growth zones arise.

The growth of the brachial support is marked by exceptionally early development of the descending branches of the loop and a late development and early disappearance of the septum.

The structure of the adult plectolophous lophophore is described.

REFERENCES

ATKINS, D., 1956. Ciliary feeding mechanisms of brachiopods. *Nature, Lond.*, Vol. 177, pp. 706–7.

—— 1959. The growth stages of the lophophore of the brachiopods *Platidia davidsoni* (Eudes Deslongchamps) and *P. anomioides* (Philippi), with notes on the feeding mechanism. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 103–32.

BEECHER, C. E., 1895. The development of *Terebratalia obsoleta* Dall. *Trans. Conn. Acad. Arts Sci.*, Vol. 9, pp. 392–9.

ELLIOTT, G. F. 1948. The evolutionary significance of brachial development in terebratelloid brachiopods. *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 1, pp. 297–317.

—— 1953. Brachial development and evolution in terebratelloid brachiopods. *Biol. Rev.*, Vol. 28, pp. 261–79.

FRIELE, H., 1877. The development of the skeleton in the Genus *Waldheimia*. *Arch. Math. Naturv.*, Vol. 2, pp. 380–6.

HANCOCK, A., 1858. On the organization of the Brachiopoda. *Phil. Trans.*, Vol. 148, pp. 791–869.

KONJOUKOVA, E. D., 1948. The post-embryonic development of the shell and of the brachial apparatus of certain Pacific brachiopods. *Bull. Acad. Sci. U.S.S.R. (Biol.)*, No. 2, p. 213. (Not seen).

—— 1957. Brachiopods of far eastern seas. *Invest. Far Eastern Seas U.S.S.R.*, Vol. 4, pp. 5–84.

MUIR-WOOD, H. M., 1955. *A History of the Classification of the Phylum Brachiopoda.* 124 pp. London: Brit. Mus. (Nat. Hist.).

PERCIVAL, E., 1944. A contribution to the life-history of the brachiopod, *Terebratella inconspicua* Sowerby. *Trans. roy. Soc. N.Z.*, Vol. 74., pp. 1–23.

THOMSON, J. A., 1927. Brachiopod morphology and genera (Recent and Tertiary). *N.Z. Bd Sci. Art*, Manual 7.

WILLIAMS, A., 1956. The calcareous shell of the Brachiopoda and its importance to their classification. *Biol. Rev.*, Vol. 31, pp. 243–87.

J. mar. biol. Ass. U.K. (1959) 38, 351–359
Printed in Great Britain

MOULTING HORMONES IN *PALAEMON* (=*LEANDER*) (CRUSTACEA DECAPODA)

II. DIFFERENCES BETWEEN POPULATIONS

By D. B. CARLISLE

The Plymouth Laboratory

(Text-figs. 1–3)

Eye-stalk removal in certain species of Brachyura and Astacura has almost invariably led to the initiation of proecdysis. In other decapod crustaceans, however, there have been significant variations in the results of this operation. Thus Travis (1951) found that eye-stalk ablation had no effect on the duration of the moult cycle in *Panulirus argus*. And in the Natantia, even in a single species, quite different results have been reported. Drach (1944) found that eye-stalk removal in *Palaemon* (= *Leander*) *serratus* (Pennant) led, as in crabs, to the initiation of proecdysis and to accelerated moulting. In ignorance of this work I repeated some of the same experiments and found that eye-stalk removal in *P. serratus* led to slower moulting (Carlisle, 1953*a*)—a result quite opposite to that of Drach. Scheer & Scheer (1954) confirm my results in the same species.

Three main types of hypothesis may be advanced to explain these contradictions: (1) that the conditions were different in the laboratories of the three groups of workers, that is to say in the laboratories of Roscoff, Plymouth and Naples; (2) that the experimental techniques adopted were different—for instance, animals might be fed or starved; (3) that the population of prawns in the three laboratories might be different and the differing results be inherent in the material.

In order to decide between these hypotheses some hundreds of *Palaemon serratus* collected at Roscoff—the laboratory where Drach performed his experiments—were brought to Plymouth and duplicate experiments were performed on these and on indigenous Plymouth specimens.

At the outset it is worthy of remark that after a little practice it was possible to distinguish between the prawns from Plymouth and from Roscoff on the basis of the colour patterns (Carlisle, 1955). Certainly about 30% of each population could be confused, but the remaining 70% were clearly different. Moreover, in colour photographs of Naples prawns, taken by Sir Francis Knowles, it was easily seen that these prawns were different again. The

differences were slight and not such as would satisfy a systematist for specific, or even subspecific, characterization, but they were nevertheless real, despite the overlap between the populations.

EXPERIMENTAL DATA AND CONCLUSIONS

The prawns were kept in running sea water in the aquarium tanks at Plymouth, either as a group of prawns in a large tank or singly in cages. The cages were made of $\frac{1}{4}$ in. mesh stainless steel, and each was 1 m square by 10 cm deep and divided into 100 cubical compartments of 10 cm side. A single prawn was placed in each compartment and the whole cage lowered into an aquarium tank. Prawns were fed daily on the flesh of *Mytilus*. For experimental purposes prawns were selected between 55 and 70 mm length measured from the tip of the rostrum to the tip of the telson. Eye-stalk ablation was performed by electrocautery; a single eye-stalk was removed on one day and the other on the succeeding day. Under these conditions the survival rate throughout the experiment was better than in earlier experiments (see Carlisle, 1953*a*).

Four groups of prawns were used: group P1—Plymouth prawns with eye-stalks removed; group P2—intact Plymouth prawns; group R1—Roscoff prawns with eye-stalks removed; group R2—intact Roscoff prawns.

In Fig. 1 is illustrated the variation of intermoult period in these four groups with alteration in temperature (small symbols). The points for the Plymouth prawns represent my own earlier experiments and those for the Roscoff prawns are plotted from the data of Drach (1944). The lines were drawn through the points by eye.

In the first experiment with these four groups of prawns a number of prawns were kept through two successive moults and the interval between these moults recorded. The temperature throughout this experiment was $13{\cdot}7 \pm 1{\cdot}1^{\circ}$ C. The results are summarized in Table 1 and diagrammatically in Fig. 2. The mean intermoult periods for the four groups are:

$$\bar{t}_{P1} = 51{\cdot}140 \pm 0.933,\ \bar{t}_{P2} = 35{\cdot}513 \pm 0{\cdot}715,$$
$$\bar{t}_{R1} = 17{\cdot}356 \pm 0{\cdot}494,\ \bar{t}_{R2} = 22{\cdot}206 \pm 0{\cdot}492.$$

where t is time in days. From this it may be calculated that the probability, P, that these four groups are drawn from the same population is less than 0·001. For all the individual comparisons, except $\bar{t}_{R1} \sim \bar{t}_{R2}$, $P < 0{\cdot}001$; for $\bar{t}_{R1} \sim \bar{t}_{R2}$, $P = 0{\cdot}001$. It is thus quite evident that all four groups are very significantly different. These four means are entered into Fig. 1 as the four large symbols in line with the temperature 13·7° C. It will be seen that they fit well with the curves drawn from the earlier data.

In the second experiment a larger number of prawns was used for a shorter period and the moulting rate followed over 15 days. The temperature during

this experiment was 12·1 ± 0·4° C. The numbers of deaths and of moults in each group on each day of the experiment are listed in Table 2 and the moult rate illustrated graphically in Fig. 3. From these data mean intermoult periods can be calculated for the four groups:

$$\bar{t}_{P1} = 103{\cdot}077, \bar{t}_{P2} = 42{\cdot}118,$$
$$\bar{t}_{R1} = 17{\cdot}092, \quad \bar{t}_{R2} = 23{\cdot}567$$

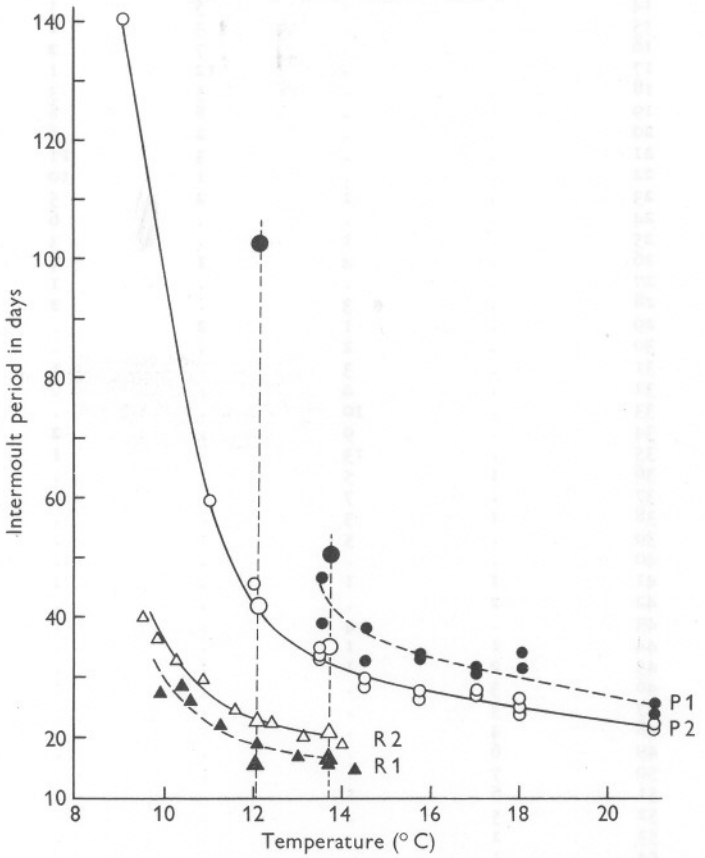


Fig. 1. Graph of the intermoult period of *Palaemon serratus* plotted against temperature. ●, P1: prawns from Plymouth whose eye-stalks had been removed; ○, P2: intact prawns from Plymouth; ▲, R1: prawns from Roscoff whose eye-stalks had been removed; △, R2: intact prawns from Roscoff. The lines are drawn through the small symbols by eye. Lines P1 and P2 represent my data; lines R1 and R2 are replotted from the data of Drach (1944). For the large symbols see the text.

These means are entered into Fig. 1 as the four large points in line with temperature 12·1° C. Once more the fit of the curves from the earlier data, extrapolated where necessary, is good. To calculate the significance of the differences in the moult rates in this experiment it is necessary to compute

TABLE 1

Days between successive moults	Number of prawns in			
	Group P1	Group P2	Group R1	Group R2
10	.	.	1	.
11	.	.	2	.
12	.	.	1	.
13	.	.	4	.
14	.	.	5	1
15	.	.	3	1
16	.	.	7	2
17	.	.	12	1
18	.	.	5	3
19	.	.	8	8
20	.	.	2	6
21	.	.	3	11
22	.	.	1	10
23	.	1	2	5
24	.	.	.	6
25	.	1	.	3
26	.	4	1	4
27	.	.	.	1
28	.	3	.	2
29	.	1	2	.
30	.	2	.	.
31	.	3	.	1
32	.	4	.	.
33	.	10	.	.
34	.	9	.	2
35	.	13	.	1
36	1	5	.	.
37	.	7	.	.
38	1	3	.	.
39	.	5	.	.
40	.	.	.	.
41	1	1	.	.
42	2	.	.	.
43	.	4	.	.
44	1	1	.	.
45	2	1	.	.
46	3	.	.	.
47	3	.	.	.
48	4	.	.	.
49	6	.	.	.
50	7	.	.	
51	6	1	.	.
52	5	.	.	
53	2	.	.	.
54	1	.	.	.
55	1	.	.	.
56	3	2	.	.
57	.	.	.	.
58	.	.	.	.
59	1	.	.	.
60	.	.	.	.
61	2	.	.	.
62	1	1	.	.
63	.	.	.	.
64	1	.	.	.
65	.	.	.	.
66	.	.	.	.
67	.	.	.	.
68	1	.	.	.
69	1	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73	.	.	.	.
74	1	.	.	.

parallel probit lines and obtain probabilities by the modified mean probit difference method outlined by Carlisle & Dohrn (1953). The various possible comparisons and the corresponding probabilities are:

$$\Delta_{(a_{P1} \sim a_{P2})} = 0{\cdot}47 \pm 0{\cdot}14;\ P < 0{\cdot}001,$$
$$\Delta_{(a_{P1} \sim a_{R1})} = 0{\cdot}97 \pm 0{\cdot}15;\ P < 0{\cdot}001,$$
$$\Delta_{(a_{P1} \sim a_{R2})} = 0{\cdot}79 \pm 0{\cdot}14;\ P < 0{\cdot}001,$$
$$\Delta_{(a_{P2} \sim a_{R1})} = 0{\cdot}50 \pm 0{\cdot}12;\ P < 0{\cdot}001,$$
$$\Delta_{(a_{P2} \sim a_{R2})} = 0{\cdot}32 \pm 0{\cdot}11;\ P < 0{\cdot}01,$$
$$\Delta_{(a_{R1} \sim a_{R2})} = 0{\cdot}18 \pm 0{\cdot}11;\ P = 0{\cdot}1,$$

where a is the mean probit and Δ the mean probit difference. All these differences except the last were highly significant.

We may conclude from these two experiments that the differences found between the four groups of prawns by independent workers in different laboratories are genuine differences inherent in the prawns, and not merely an expression of differing conditions or differing experimental techniques.

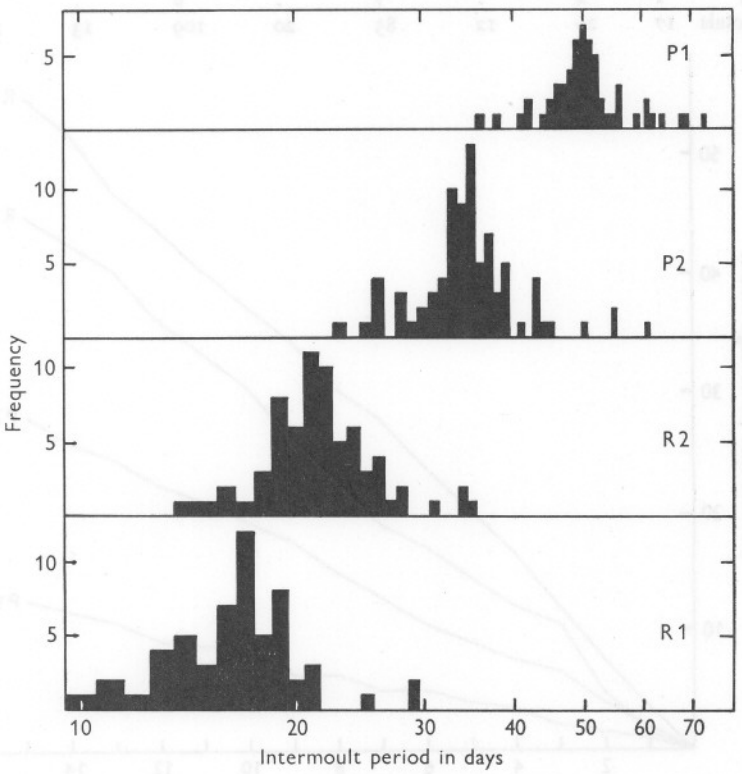


Fig. 2. Histograms drawn from the data of Expt. 1. The four groups of prawns are numbered as in Fig. 1. The time scale is logarithmic. Temperature 13·7° C.

TABLE 2. DEATHS AND MOULTS IN EXPERIMENT II

The number of animals which died without first moulting and of those which moulted on each day in each group.

Group...	P1		P2		R1		R2	
No. of animals...	205		301		198		297	
Day	Dead	Moults	Dead	Moults	Dead	Moults	Dead	Moults
1	5	2	2	6	3	7	3	4
2	3	1	5	6	4	8	5	10
3	.	1	.	8	3	9	.	17
4	3	3	.	3	2	9	.	5
5	.	1	.	4	.	7	2	7
6	.	2	1	5	.	7	.	8
7	2	.	.	4	2	8	.	6
8	.	3	.	9	.	5	.	10
9	.	.	.	8	3	8	1	11
10	2	5	3	5	.	6	.	13
11	1	.	.	5	1	6	.	8
12	.	1	.	4	.	7	1	9
13	.	3	1	7	.	6	.	12
14	.	2	.	4	2	10	1	7
15	1	2	.	7	.	6	.	7
Totals	17	26	12	85	20	109	13	134

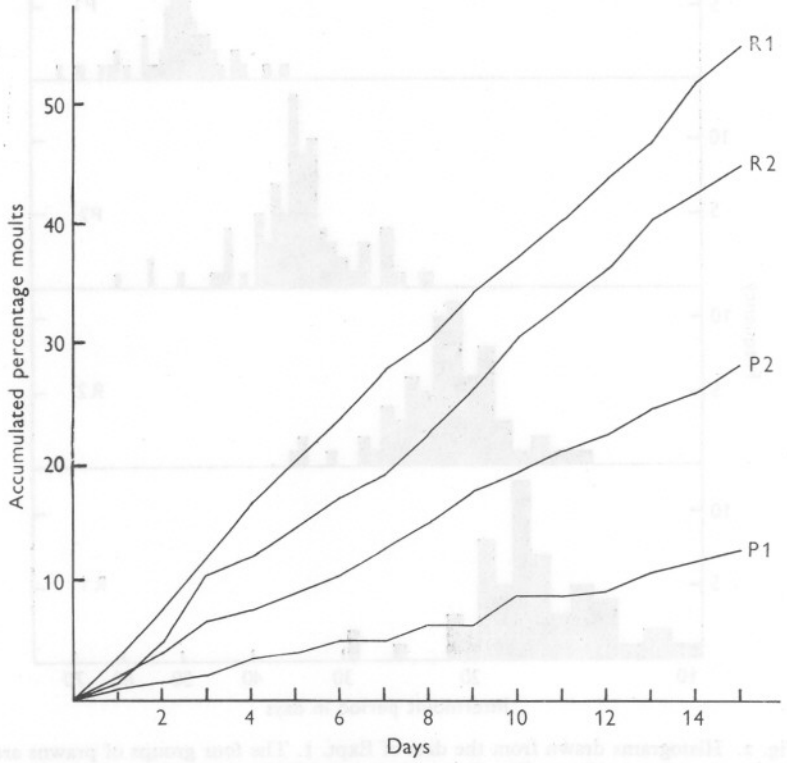


Fig. 3. Graph of the cumulative percentage of moults in Expt. 2. The four groups of prawns are numbered as in Fig. 1. Temperature 12·1° C.

There is a real difference in moult rate between intact *Palaemon serratus* from Plymouth and from Roscoff and the operation of eye-stalk ablation performed on these two populations has quite opposite effects leading to a longer intermoult period in Plymouth animals and to a shorter intermoult period in Roscoff prawns.

DISCUSSION

There can be little doubt that the results of the experiments of Drach (1944) indicate the existence of a moult-inhibiting hormone in the eye-stalks of *Palaemon serratus*. On the other hand, the results of Carlisle & Dohrn (1953) and Scheer & Scheer (1954) indicate the presence of a moult-accelerating hormone in the eye-stalks of *Palaemon*, a hormone which accelerates the processes of proecdysis. It must not be thought that these two hormones are mutually antagonistic, for the moult inhibiting hormone inhibits the onset of proecdysis, while the moult accelerating hormone accelerates the progress of proecdysis once this has begun. Both hormones, however, will affect the duration of the intermoult period; the moult inhibiting hormone by lengthening diecdysis will increase the intermoult period, while the moult-accelerating hormone by shortening proecdysis will shorten the intermoult period. Thus, in a certain measure, the length of the intermoult period will depend on the relative rates of secretion of these two hormones, and on the rates of secretion of the moult-inhibiting and moult-accelerating hormones which are known to be produced in other parts of the body (Carlisle, 1953*b*, 1954; Scudamore, 1947; Stephens, 1951). The effects of eye-stalk ablation on the duration of the intermoult period will also depend on the relative rates of secretion of these various hormones. Here then we have a possible mechanism for the different moult rates in two populations of prawns and for the opposite effects that eye-stalk ablation has on these two populations. Presumably the differences between the populations are genotypic, for besides the differences in moult rate there are also differences in colour pattern and even in the precise topography of the endocrine organs of the eye-stalk—those organs which are responsible for the secretion of the moulting hormones. This is true also for Naples prawns which are different in both these respects from Plymouth and from Roscoff prawns.

I wish to thank Prof. P. Drach for his helpful criticisms and comments and the staff and fishermen of the Station Biologique de Roscoff for their friendly collaboration.

SUMMARY

The populations of *Palaemon* (= *Leander*) *serratus* from Roscoff, Plymouth and Naples are noticeably different in the range of colour patterns, though there is some overlap between the populations from Plymouth and Roscoff at least. The mean intermoult period at any one temperature is different for these

two populations, even if kept in adjacent tanks in the Plymouth aquarium; the intermoult period is shorter for prawns from Roscoff. Eyestalk ablation leads to significant shortening of the intermoult period in prawns from Roscoff but to significant lengthening of this period in prawns from Plymouth, even when duplicate experiments are carried out in the one laboratory on collections of prawns from the two localities. An endocrinological explanation of these experiments is proposed.

REFERENCES

CARLISLE, D. B., 1953*a*. Moulting hormones in *Leander* (Crustacea Decapoda). *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 289–95.

—— 1953*b*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). IV. On the site of origin of the moult-accelerating principle—experimental evidence. *Publ. Staz. zool. Napoli*, Vol. 24, pp. 285–92.

—— 1954. On the hormonal inhibition of moulting in decapod Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 61–3.

—— 1955. Local variations in the colour pattern of the prawn *Leander serratus* (Pennant). *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 559–63.

CARLISLE, D. B. & DOHRN, P. F. R., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental evidence for a growth- and moult-accelerating factor obtainable from eyestalks. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 69–83.

DRACH, P., 1944. Étude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez *Leander serratus* (Pennant). *Bull. biol.*, Vol. 78, 40–62.

SCHEER, B. T. & SCHEER, M. A. R., 1954. The hormonal control of metabolism in crustaceans. VII. Moulting and colour change in the shrimp *Leander serratus*. *Pubbl. Staz. zool. Napoli*, Vol. 25, pp. 397–418.

SCUDAMORE, H. H., 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiol. Zoöl.*, Vol. 20, pp. 187–28.

STEPHENS, G. C., 1951. A molt-inhibiting factor in the central nervous system of the crayfish, *Cambarus* sp. *Anat. Rec.*, Vol. 111, pp. 572–73.

TRAVIS, D. F., 1951. The control of the sinus glands over certain aspects of calcium metabolism in *Panulirus argus* Latreille. *Anat. Rec.*, Vol. 111, p. 503.

APPENDIX

Some of the data from which curves P1 and P2 in Fig. 1 are drawn have been published (Carlisle, 1953*a*). Most of them, however, have not. As explained in the paper mentioned above it is possible to measure the intermoult period either directly or by calculation from the moult rate measured in a group of prawns over a period of days. By the latter method it is not possible to assign a standard error to the mean intermoult period, although it is possible to assign one to the difference between moult rates. Points on the graph (Fig. 1) have been obtained by both these methods. In Table 3 are given the mean intermoult periods corresponding to each of the points on curves P1 and P2, together with a standard error where this is appropriate.

TABLE 3. INTERMOULT PERIODS

Table of the data which generated the curves P1 and P2 in Fig. 1. Where a standard error of the mean is given the mean intermoult period was derived from a number of direct observations of individual intermoult periods. Where no standard error is given the intermoult period is calculated from the moult rate measured in a number of animals over the stated number of days.

	P1		P2	
Temperature (° C)	Intermoult period (days)	Number	Intermoult period (days)	Number
9·0	.	.	141·12 ± 11·27	93
10·0	.	.	94·63 ± 11·01	112
11·0	.	.	60·10 ± 7·33	108
12·0	.	.	45·95 ± 4·95	101
13·5	39·40 ± 2·71	5	33·78 ± 1·58	9
	35·46	(100 × 26 days)	34·62 ± 3·19	127
			47·30	(100 × 26 days)
14·5	33·10	(150 × 15 days)	29·13 ± 2·01	18
	38·74 ± 3·01	16	30·10	(150 × 15 days)
15·8	33·87	(100 × 15 days)	26·28 ± 1·99	12
	34·30 ± 2·76	9	27·00	(100 × 15 days)
17·1	31·12 ± 2·81	15	27·22	(100 × 15 days)
	32·13	(100 × 15 days)	27·84 ± 2·02	20
18·0	31·94 ± 2·11	21	24·23 ± 0·76	132
	34·50	(100 × 15 days)	25·66 ± 1·94	24
			26·47	(100 × 15 days)
21	24·11 ± 0·83	33	21·81	(100 × 15 days)
	25·97	(100 × 15 days)	22·79 ± 0·68	35

J. mar. biol. Ass. U.K. (1959) 38, 361–367
Printed in Great Britain

THE ECOLOGY OF *ECHINUS ESCULENTUS* L. QUANTITATIVE DISTRIBUTION AND RATE OF FEEDING

By G. R. FORSTER

The Plymouth Laboratory

(With Plates I and II and Text-figs. 1 and 2)

In order to provide more factual information on the effect of *Echinus* predation (Forster, 1958), further diving observations have been made during 1958. A rocky area just west of the Tinker Shoal was explored by chance and a population of *Echinus* discovered. As this area is much more readily accessible than Stoke Point rock, where most of the diving has been carried out, it was possible to establish a marker buoy and make a number of dives on exactly the same position. In the course of these dives I have attempted to measure the numerical density of the urchins and the rate of their browsing on the rock surface. Later *Echinus* 'counts' were made at many other positions near Plymouth (Text-fig. 1).

The abundance of *Echinus* has been measured by counting the number of urchins observed in a known area of sea-floor. A terylene line roughly 50 m long is laid out on a rocky part of the sea bed as is shown diagrammatically (Text-fig. 2). One end of the line is attached to the bottom of a shot rope or to the boat's anchor. The line is then drawn out to its full length with the aid of a dinghy. The diver swims along the line with a $2\frac{1}{2}$ m rod ($\frac{5}{16}$ in. galvanized wire) held at right angle to the line, counting the numbers of urchins first on one side of the line and returning along the other side; in this way a strip of rock surface 5 m wide is covered. If the line is found to be suspended between pinnacles of rock the diver can lift the 7 lb. lead at the distant end and drag it slightly until the line just touches the rocks' surface. Should part of the line be lying on sand, the actual area where urchins are being counted can be marked off by tying knots in the line.

The rate of browsing was measured by turning over a few large boulders in a position where *Echinus* were present, and placing one urchin on the newly exposed surface of each boulder. On the next dive 24 h later, if the *Echinus* was still occupying a position on the boulder, it was removed and the outline of the area which had been browsed was traced on a sheet of Ethulon. It was hoped that the undersides of these boulders, usually covered with many small barnacles (*Verruca stroemia*) would prove attractive to the *Echinus*, since *Verruca* are readily browsed on by urchins in an aquarium tank. In addition a

pair of wire frames covered with large mesh nylon netting have been used to confine a number of urchins in a small area. These frames were 1 m^2 in area and about 18 in. high. A heavy chain was laced to the edge of the netting to keep it in contact with the rock all the way round, also serving to prevent the

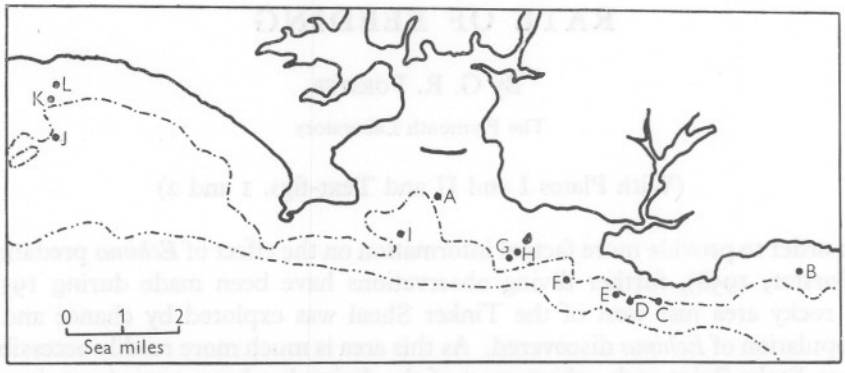


Fig. 1. Outline chart of Plymouth Sound and neighbourhood showing positions A–L, described in Table 1. The 10 and 20 fm lines are represented by lines with single and double dots respectively.

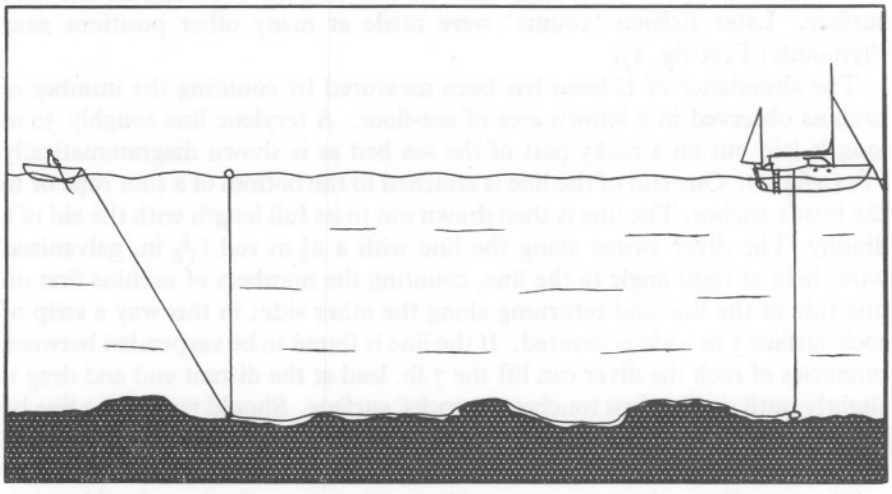


Fig. 2. Diagram showing the method of laying out the 50 m line over the rocky sea floor.

frame being moved about by wave action. After a period of several weeks the browsed area under the frame can be compared with a control area under the second frame from which *Echinus* have been excluded, or simply with the immediate surrounding rock surface if this has been kept clear of urchins during the course of the experiment.

Some tests on the browsing of *Echinus* in aquarium tanks have also been

made. The area of algal film swept clear by an urchin over a known period can be marked on the glass surface or traced directly on to Ethulon so that the area can be measured. The urchins used in these tests had all been allowed to acclimatize themselves to aquarium conditions for several weeks.

RESULTS

Table 1 shows the results of *Echinus* counts made chiefly during September and October 1958.

The average distribution of *Echinus* in the areas examined is therefore one per $4\frac{2}{3}$ m², or 868 per acre. Thus in the 4 mile strip of coast between the Mewstone and Revelstoke Point, it may be assumed from the chart that there is generally about $\frac{1}{2}$ mile separating the 7 fm from 17 fm contour; in this narrow strip alone there should be a population of nearly 1,400,000 urchins.

All the underwater observations on browsing were made in the immediate vicinity of a marker buoy situated 4 cables west of the Tinker Shoal. The marker buoy cable was attached to a projecting rock on the bottom. The following transits make it a simple matter to locate the position if the buoy should be submerged or lost. The higher anchorage beacon on Ramscliff Point is in line with the beacon on the east end of the breakwater, and the north-west corner of the Mewstone appears to be almost touching the southernmost tip of Stoke (Hilsea) Point.

The results of tests on the rate of browsing are shown in Table 2. The algal films in the last two tests were composed of *Hildenbrandia prototypus* Nardo (red) and a mixture of various green and blue-green algae. The average browsing rate for the 8 tests is 2·9 cm²/h.

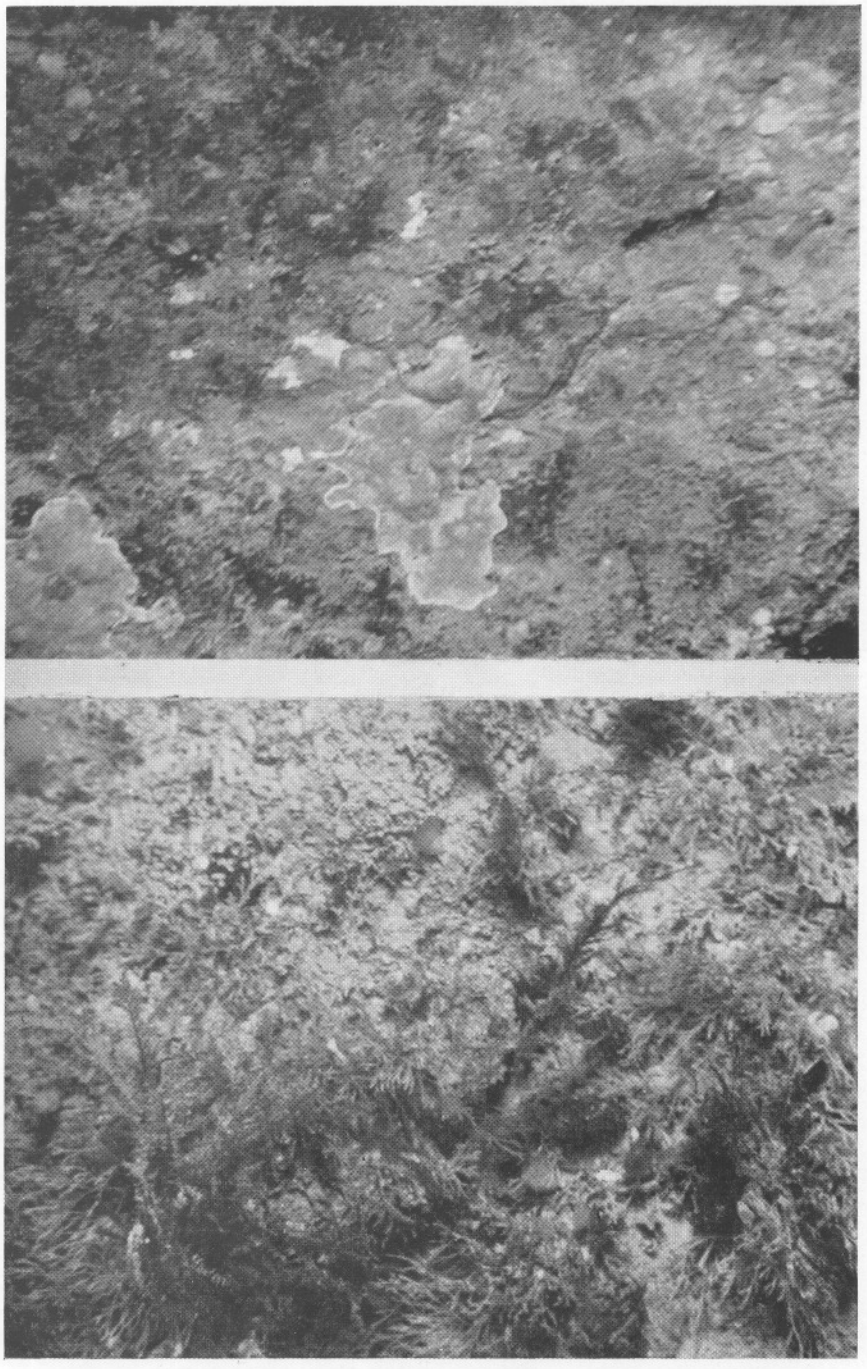
GENERAL NOTES ON BROWSING AND HABITS

Underwater observations

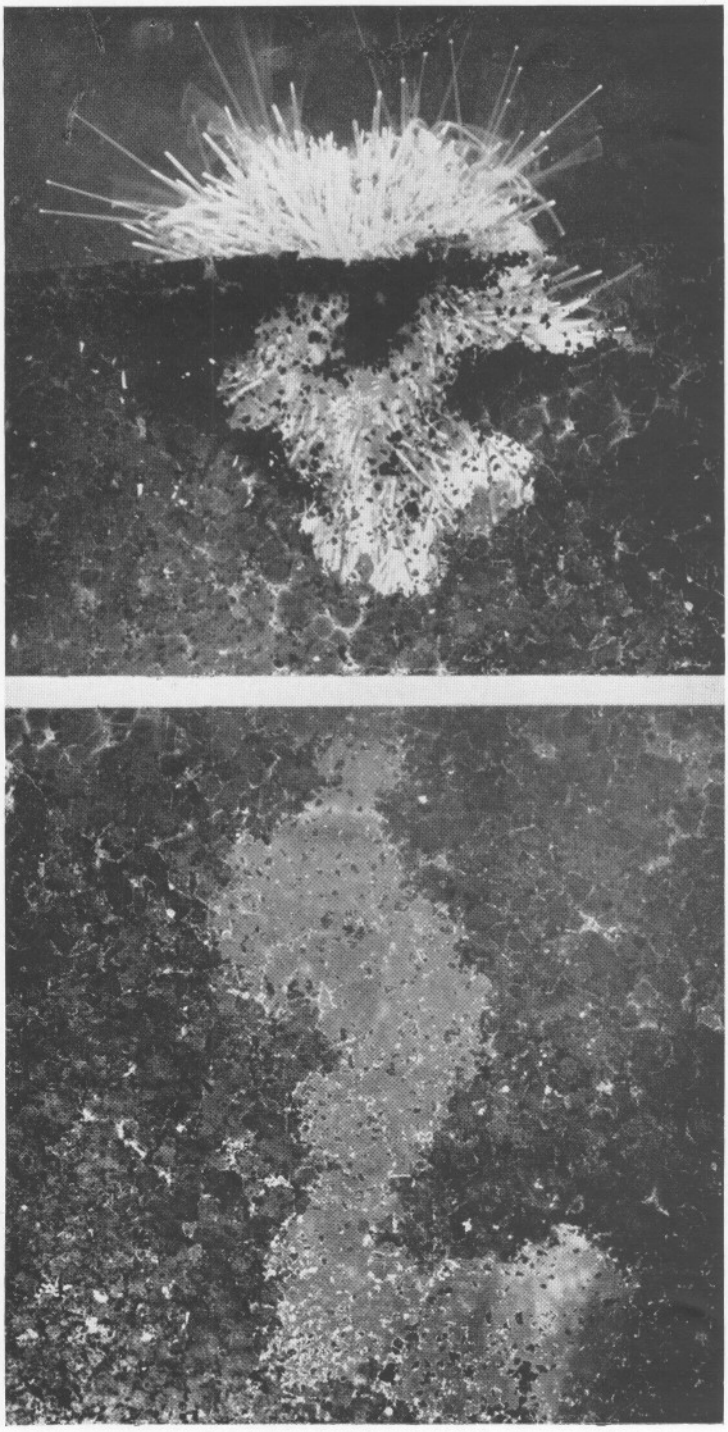
A browsed area of about $\frac{1}{4}$ to $\frac{1}{2}$ m² is usually recognizable around most of the *Echinus* examined underwater. This is not completely bare rock but is devoid of any normal brown 'fur' composed of very small filamentous algal growths encrusted by diatoms, etc., the remains of hydroid colonies similarly overgrown besides muddy tubes of amphipods and various polychaetes; it can best be described as presenting a clean 'scrubbed' appearance. In Plate I two underwater photographs show the contrast between browsed and normal rock surfaces. The browsed area formed a small part of the rock covered by a netting frame which confined six *Echinus* to 1 m² and was photographed after a month. The second photograph shows the normal appearance of the rock and was taken about 1–2 m away from the netting frame. *Lithothamnion* is usually very common in the browsed areas. Whether this browsed area is in any way similar to the territory of a limpet is not yet known. It will also be of interest to ascertain if there is any tendency for the *Echinus* to home

TABLE 1

	Position	Depth (fm)	Length measuring line (m)	Area (m^2)	No. *Echinus*	Notes
A	4 cables west from Tinker Shoa	8½–9	54	135	14	Line arranged east from marker buoy
		8½–9	54	135	11	
		8½–9	54	135	22	Line arranged north from marker buoy
		8½–9	54	135	14	
		8½–9	54	135	20	
		8½–9	54	135	4	Line arranged south from marker buoy
		8½–9	54	135	8	
		8½–9	54	135	18	Line arranged west from marker buoy
		8½–9	54	135	6	
		8½–9	50	125	17	Line running along a gully wall
B	Bigbury Bay ½ mile south of St Anchorite's rock	9	36	90	2	Line often close to or cutting across gravel patches
		9	36	90	5	
C	About 30 yards south-west from Stoke Pt (=Hilsea Pt) rock	14–15	54	135	36	Normal rock bottom
		14–15	54	135	29	
D	½ mile south-west of Stoke Pt	13	36	90	8	
		13	36	90	11	
E	*ca.* ½ mile south-west of small headland just west of Stoke Pt	—	54	135	11	Some gravel patches in vicinity
		—	54	135	8	
F	*ca.* ¾ mile south-west of Gara Pt	10–11	54	135	34	—
		10–11	54	135	29	—
		10–11	54	135	18	—
		10–11	54	135	35	—
		10–11	54	135	29	—
		10–11	54	135	23	—
G	*ca.* 4 cables south-west of Mewstone	14	36	90	30	—
		14	36	90	32	—
		12	30	75	26	—
		12	30	75	35	—
H	*ca.* 3 cables south-west of Mewstone	10–11	54	135	29	—
		10–11	54	135	19	—
		10–11	54	135	37	—
		10–11	54	135	28	—
I	1 mile south-east of Penlee Pt	9–10	54	135	41	Line running west parallel to ridges of rock
		9–10	54	135	43	
		9–10	54	135	7	Line running south cutting across ridges of rock and gullies of muddy gravel
		9–10	54	135	10	
J	Whitsand Bay *ca.* 1½ miles south of Brawn Rk (between Portwrinkle and Downderry)	10½	54	135	46	Low reefs of rock; *Echinus* slightly smaller than usual (mean diam. of 6–7·7 cm, cf. mean diam. of 8–11·3 cm from marker buoy position)
		10½	54	135	41	
		10½	54	135	75	
		10½	54	135	78	
K	Slightly closer inshore	9	54	135	63	
		9	54	135	52	
		7½–8	54	135	50	
		7½–8	54	135	33	
			Totals	5540	1187	



(*Facing p.* 364)



when they are moved from these positions. At the position in Whitsand Bay where large numbers of *Echinus* were counted, all horizontal rock surfaces were almost wholly covered by *Lithothamnion* and another encrusting red alga *Cruoria pellita* (Lyngb.) Fries. Presumably the *Lithothamnion* is either not browsed on heavily or else regenerates more quickly than other species.

At the position near the Tinker Shoal, depth 9 fm, which is near the upper limit for *Echinus* at Plymouth, almost every specimen was partly covered with algal fragments in a similar manner to *Psammechinus miliaris*, though the covering is generally rather more scanty. This form of behaviour is not usually exhibited by *Echinus* in aquaria.

TABLE 2

Type of observation	No. of *Echinus*	Area browsed	Time	Rate of browsing (cm^2/h)
Netting frame on rock, *ca.* 9 fm	6	1 m^2	25 days	2·78
Netting frame on rock, *ca.* 9 fm	3	¼ m^2	11 days	3·2
Surface of upturned boulder, *ca.* 9 fm	1	99 cm^2	22 h	4·5
	1	46 cm^2	22 h	2·1
	1	52 cm^2	18·5 h	2·9
	1	73 cm^2	18·5 h	3·9
Aquarium tank glass. Red algal film	1	161 cm^2	2 days 19 h	2·4
Aquarium tank glass. Green algal film	1	271 cm^2	8 days	1·4

Feeding in aquaria

Echinus will feed readily on the algal film growing on the glass of an aquarium tank. The *Echinus* browses steadily in one spot until after a few hours a small clear area can be observed. This area is slowly enlarged by the urchin working round its periphery leaving remarkably few remnants of the algal film (Plate II). Browsing goes on steadily both during day and night but is not generally continuous for more than a day or two. In one series of observations the rate of browsing for 24 h, apparently continuous browsing, was 5·8 cm^2/h, while for just under 3 days the rate was reduced to 2·4 cm^2/h.

EXPLANATION OF PLATES I AND II

I

Views of the rock surface near Tinker Shoal (depth 9 fm), showing (above) the effect of browsing by *Echinus*, as compared with (below) the state in the absence of *Echinus*.

II

Photographs of the glass front of an aquarium tank showing the results of browsing by *Echinus*. In the upper illustration the *Echinus* is seen in the act of feeding on the thick film of reddish-brown encrusting alga, with its mouth slightly to the left of the centre. The lower illustration shows the clear trail where the algal film has been eaten by an *Echinus*.

Sometimes a second patch on another side of the tank may be browsed for a while with the urchin subsequently feeding alternately on the two distinct areas.

DISCUSSION

The results of the *Echinus* counts show that the urchins are very abundant, particularly below 10 fm. From 10 to 15 fm they may well be considered the dominant species, for in this depth zone the average distribution is one urchin to 4·1 m^2 of rock surface. From the browsing tests (Table 2) it may be seen that 2 cm^2/h is a reasonably conservative rate to assume. Thus if an *Echinus* browses at this rate for four months of the year and at 1 cm^2/h for the remaining 8 colder months it would have covered 1·2 m^2 in the course of the year. On this basis the *Echinus* population is clearing about one-third of the rock surface every year. Naturally the browsed areas would soon be recolonized, but any feeding preferences on the part of the urchins would exercise considerable influence on the proportions of different sessile species. Further predation, possibly rather selective, must be expected from the numerous *Marthasterias glacialis*, occasional *Maia squinado* and from the various species of wrasse.

The distribution of *Echinus* in shallow water or on the shore has attracted much attention in the past. In the north of England and Scotland the species is normally present in quite shallow water—less than 5 fm and in early spring there is a small inshore migration before spawning as far as L.W.S.T. level (Elmhirst, 1922; Stott, 1931). In the south the only records of urchins found on the shore are near Mousehole (Trewavas, 1922) and in the Isles of Scilly (G. M. Spooner, unpublished notes, 1936–7). At Mousehole for the first mile of coast running southwards the 10 fm line lies only 200 yd offshore so that from low water down to 10 fm there is an average gradient of 1 in 10, a situation which is unique for South Devon and Cornwall. It therefore seems very probable that the occasional urchins taken both in this locality and at Scilly are merely strays from deeper water nearby. Reid (1935) postulates a correlation of the intertidal distribution of *Echinus* with the course of the Gulf Stream. This theory will not, however, bear any close examination, for even with the current chart as shown by Reid (for which no reference is given) *Echinus* should be present not only on the shore of north Spain but also along the French coast towards Finistère. But there are no published records of the presence of *Echinus* along the north Spanish coast, and, in a letter from the oceanographic laboratory of Santander, Dr Cuesta writes that it is present but not common. The only two French records, Roscoff and Lanvéoc near Brest (Station biologique de Roscoff, 1951; and Anthony, 1925), are also for places quite close to deeper water. Thus for the whole southern part of its range the species is basically sublittoral and it seems much more reasonable to suppose that a slightly different race is present in the northern part where there is a definite change in behaviour.

SUMMARY

The numbers of *Echinus esculentus* present in known areas have been counted at several positions near Plymouth. A total area of over 5,000 m² has been covered, and in this area the average density of *Echinus* is 868 per acre (1–4·7 m²). A few tests on the rate at which *Echinus* browse on algae and sessile animals have also been made. From these results it has been possible to estimate that where urchins are abundant they are capable of sweeping clear at least one-third of the whole rock surface in the course of a year.

The species is sublittoral for the southern part of its range.

REFERENCES

ANTHONY, R., 1925. Première note sur la faune échinologique littorale de Lanvéoc. *Bull. Soc. zool. Fr.*, T. 50, pp. 319–35.

ELMHIRST, R., 1922. Habits of *Echinus esculentus*. *Nature, Lond.*, Vol. 110, p. 667.

FORSTER, G. R., 1958. Underwater observations on shallow rocky areas in the neighbourhood of Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 473–82.

REID, D. M., 1935. The range of the sea-urchin *Echinus esculentus*. *J. Anim. Ecol.*, Vol. 4, pp. 7–16.

STATION BIOLOGIQUE DE ROSCOFF, 1951. Inventaire de la faune marine de Roscoff: Bryozoaires Echinodermes. *Trav. Sta. biol. Roscoff*, T. 2, Suppl. 4.

STOTT, F. C., 1931. The spawning of *Echinus esculentus* and some changes in gonad composition. *J. exp. Biol.*, Vol. 8, pp. 133–50.

TREWAVAS, E., 1922. Note on the occurrence of *Echinus esculentus* above low tide mark on the Cornish coast. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 833–4.

J. mar. biol. Ass. U.K. (1959) 38, 369–379
Printed in Great Britain

NOTES ON THE SPECIES OF THE PTEROMEDUSAN GENUS *TETRAPLATIA* BUSCH, 1851

By PATRICIA M. RALPH[1]
Zoology Department, Victoria University of Wellington, New Zealand

(Text-figs. 1–3)

The affinities, structure and distribution of species of the genus *Tetraplatia* Busch, 1851 have been discussed recently by Hand (1955), Beyer (1955) and Rees & White (1957*a*), but ever since these curious bipyramidal medusae (without a typical medusan bell, marginal tentacles, or gastrovascular canals) were described by Busch they have provided much interest for the systematist. There is unanimous agreement that the clearly discernible nematocyst tracts, and the body wall of ectoderm and endoderm separated by a thick, well-defined mesogloea, place them within the Cnidaria as known at present. *Tetraplatia* is usually regarded as the only member of the hydrozoan order Pteromedusae, but Komai (1939) strongly advocates scyphozoan rather than hydrozoan affinities. The purpose of this paper, however, is not to discuss the broad relationships of the Pteromedusae, but to give further evidence that *Tetraplatia chuni* Carlgren, 1909 is a good species. (The structure and affinities of *Tetraplatia* will be discussed in another paper.)

Tetraplatia possesses a body divided into apical and oral regions by an encircling horizontal groove: there are four equidistant radially situated locomotory lappets arising from the groove, two ectodermal (?) statocysts on the undersurface (adoral) of each lappet; the sexes are separate, and there are four endodermal (?) gonads—each gonad has an apical pair of finger-like processes and an oral pair, and the two pairs, are joined by a narrow isthmus in the region of the horizontal groove.

Two species, *T. volitans* Busch 1851 and *T. chuni* Carlgren 1909, are known and described. *T. chuni* lacks flying buttresses between the oral and apical regions of the body and has narrower locomotory lappets than *T. volitans*. Hand's (1955) material from off the Pacific Coast of North America (all of which he recognized as *T. volitans*) showed some specimens in which the buttresses were incomplete or fewer in number than the usual four, and others in which there were three instead of four lappets. Hand also found that, although in general there was little variation in the ratio of the width of the lappet and the space between the lappets (i.e. this space being as wide as the

[1] Nuffield Fellow, 1958.

lappet itself), in one or two specimens the space between the lappets was wider, being about twice the width of the lappet, and thus approached the condition described for *T. chuni*. Hand considered that the variation shown by his material demonstrated that *T. chuni* differs little from *T. volitans* and that it may be an aberrant form of it. Rees & White (1957*a*, *b*) working with material from the South Pacific and the North and South Atlantic Oceans recognized *T. chuni* as distinct. They regarded the absence of buttresses in *T. chuni* and the ratio of 1 : 1 of the length of the apical to oral body regions as a significant and reliable character readily separating *T. chuni* from *T. volitans*. All the material I have examined also demonstrates that *T. chuni* is a good species. There is also evidence that the juveniles of the two species can be as easily recognized as the adults although they have a similar oral to apical body length ratio of 1 : 1.

MATERIAL AND METHODS

Dr F. S. Russell very kindly made available to me 190 specimens of *Tetraplatia volitans* taken by R. V. 'Sarsia' from the Continental Shelf at the approaches to the English Channel and the Bay of Biscay, and transverse and longitudinal serial sections made by Mr A. C. G. Best. The material was collected in the summer months of May to September in the years 1955 to 1957. Ring nets were used in vertical and oblique hauls. The greatest depth fished was approximately 1000 fm but on an average the fishing depth was to about 450 fm. Hauls were made at various times of the day and night but, as collection of *Tetraplatia* was incidental to other tasks and closing nets were not used, correlation is not possible between abundance, diurnal migration and depth, etc. Fifty-seven *Tetraplatia* were taken in nine hauls in 1955, 115 from six hauls in 1956 and 18 from three hauls in 1957. The great majority of the 190 specimens were lightly stained with borax carmine in order to determine sex more readily.

In addition, Dr W. J. Rees and Mr E. White of the British Museum (Natural History) very kindly allowed me to examine all their material of *Tetraplatia* from R.R.S. 'Discovery II' (*T. volitans* and *T. chuni*); 'Carnegie VII'; H.M.S. 'Research' and F.R.V. 'Explorer' (*T. volitans*), as well as transverse sections of a single specimen of *T. volitans* from 'Discovery' collections.

The author wishes to thank Dr F. S. Russell, Director of the Plymouth Marine Laboratory, Dr W. J. Rees and Mr E. White of the British Museum (Natural History) for allowing her to examine material of *Tetraplatia* and for useful advice and encouragement, and to Miss Patricia G. Conway, Exeter, for assistance with measurements of specimens and graphs.

TETRAPLATIA VOLITANS BUSCH

Material collected by R.V. 'Sarsia'

Of the total number of 190 specimens, 173 were available for measurement, the others being damaged or too strongly contracted for accurate measurement. These range in size from 2·1 × 1·4 mm to 9·45 × 2·1 mm, the width being measured at the level of the horizontal groove across the body. The greatest number, 90 in all, were between 4·0 mm and 6·0 mm long and 1·0 mm and 2·5 mm wide. Approximately 80% of the specimens showed a dome-shaped apical tip (Fig. 1, H). The length of the oral region plotted against that of the apical region shows clearly that the length of the oral region is, in specimens above 3·0 mm long, consistently twice that of the apical region and may be more, that is, up to 5:1 (Fig. 2). Juveniles, however, usually have an oral to apical length ratio of 1:1. The ratio for both the juveniles and the adults is similar to that obtained by Hand (1955) and Rees & White (1957*b*).

Oral and apical gonad-lengths, plotted against total body length, show a similar ratio to that of the body regions; the oral lobes are usually the longer in animals above 5·0 mm long. A difference in the length of the oral and apical portions of the gonad has been noted previously. Dantan (1925, Pl. I, fig. 1) figures the oral and apical gonad pairs as approximately equal in length. Beyer's (1955) 'Brategg' specimens showed the oral pairs longer than the apical, particularly in the larger specimens. Beyer thought that the gonad in these large specimens may have been partly spent. The present material, however, indicates that a difference in length of the apical and oral portions of the gonad is probably related to the difference in length of these major body regions and that growth of the gonad in length parallels the growth in length of these regions in the animal as a whole.

Males outnumber females by approximately 5:1 in the present collection. This ratio is based on 100 animals taken over a three-year period in which collections were made by 'Sarsia', and in which the oral region of the gonad is over 0·5 mm in length. Specimens with gonads shorter than 0·5 mm in the oral region were frequently of indeterminate sex.

In the female gonad (Fig. 1, D) the width to length ratio is usually 1:2 but may be 1:3, and in the male gonad (Fig. 1, C) which is narrower and more elongated the ratio is frequently 1:3 and ranges to 1:5. Also, the range of width in the female gonad, 0·21–1·05 mm, is greater than in the male, 0·07–0·49 mm. The two extremes in male gonad width, nevertheless, occur only once, and by far the greatest number have a gonad between 0·21 and 0·28 mm in width.

All sections demonstrate that *T. volitans* has a very narrow waist, from 0·50–0·75 mm in diameter in animals whose total length ranges from 4·5 to 6·5 mm. The width of the waist was measured across the body (excluding the buttresses) in the region of the horizontal groove. One specimen sectioned

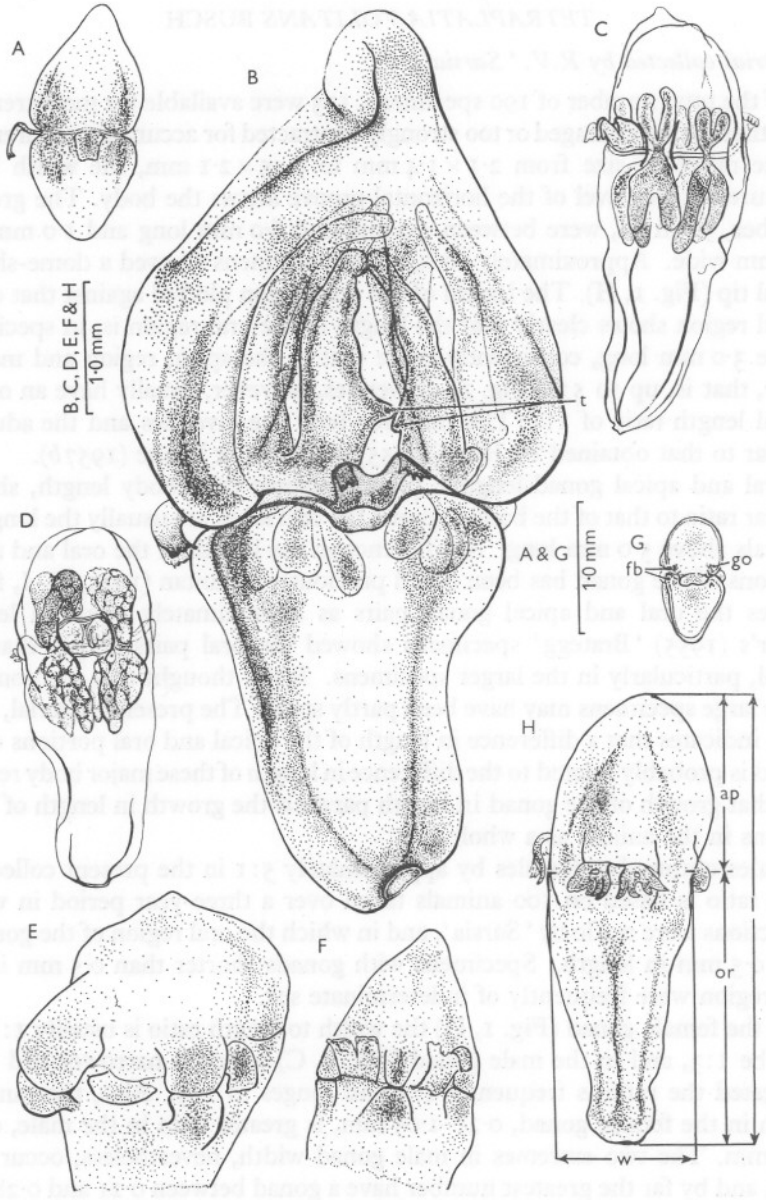


Fig. 1. A and B, *Tetraplatia chuni:* A, juvenile (from N.W. Bouvet Is.); B, adult male (from north of the Antarctic ice edge); C–H, *T. volitans:* C, male, and D, female to show oral and apical paired finger-like portions of the gonad, E–G, specimens from R.R.S. 'Discovery II' collections, E, F, to show the aberrant flying buttresses. G, juvenile to show developing flying buttresses and incipient gonad; H, diagram to show how specimens were measured.

Abbreviations: *ap*, measurement taken for length of the apical region; *fb*, flying buttress; *go*, gonad; *l*, total body length; *or*, measurement taken for length of oral region; *t*, small tubercle on male gonad of *T. chuni*; *w*, width of the body.

longitudinally shows a distinct mesogloeal septum at the equator of two buttresses.

The position of the nematocyst tracts, the nematocysts, and the general external structure of the lappet are as described by Hand (1955). Twenty-five specimens measured for width of the lappet and distance between the lappets showed the usual ratio of 1:1 as known for the species. In no other specimen examined was there any significant departure from this ratio.

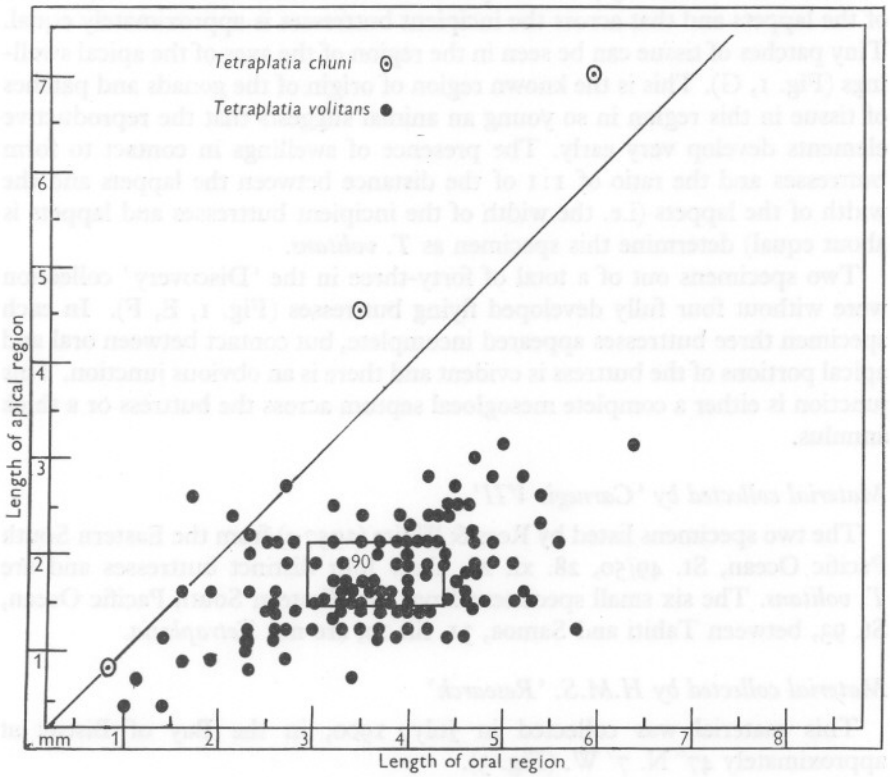


Fig. 2. Graph to show the ratio of the length of the oral region plotted against that of the apical region; line at angle of 45 degrees represents a 1:1 growth ratio of the oral/apical regions; rectangular area represents the ratio of the oral/apical growth length of the greatest number of specimens (90 in all).

Material collected by R.R.S. 'Discovery II'

Specimens examined of 'Discovery' material showed a similar oral: apical length ratio, gonad size and proportions, and ratio of males to females as did those of R.V. 'Sarsia', although gonad structure and sex were not so readily assessed as in the latter. One very young specimen 1·0 mm in total length was noted (Fig. 1, G). This has not been described before; it shows four equi-

distant swellings at the posterior margin of the oral region and four corresponding swellings opposite them at the anterior margin of the apical region. There seems little doubt that these swellings are incipient buttresses, as there is contact between three of the pairs and in the remaining pair a linking strand of tissue. It is probable that all pairs become fused at this size and that the epithelial layer which is very thin and easily damaged has been torn away. The length ratio of the oral/apical body region is approximately as one is to one, similar to that shown by juvenile specimens in the other material. The width of the lappets and that across the incipient buttresses is approximately equal. Tiny patches of tissue can be seen in the region of the axes of the apical swellings (Fig. 1, G). This is the known region of origin of the gonads and patches of tissue in this region in so young an animal suggests that the reproductive elements develop very early. The presence of swellings in contact to form buttresses and the ratio of 1:1 of the distance between the lappets and the width of the lappets (i.e. the width of the incipient buttresses and lappets is about equal) determine this specimen as *T. volitans*.

Two specimens out of a total of forty-three in the 'Discovery' collection were without four fully developed flying buttresses (Fig. 1, E, F). In each specimen three buttresses appeared incomplete, but contact between oral and apical portions of the buttress is evident and there is an obvious junction. This junction is either a complete mesogloeal septum across the buttress or a thick annulus.

Material collected by 'Carnegie VII'

The two specimens listed by Rees & White (1957*a*) from the Eastern South Pacific Ocean, St. 49/50, 28. xi. 28, show four distinct buttresses and are *T. volitans*. The six small specimens from the Western South Pacific Ocean, St. 93, between Tahiti and Samoa, 31. iii. 29, are not *Tetraplatia*.

Material collected by H.M.S. 'Research'

This material was collected in July, 1900, in the Bay of Biscay at approximately 47° N. 7° W. (Fig. 3).

The two specimens from St. 320 and the single specimen from St. 35*b* recorded by Rees & White (1957*a*) have four fully developed buttresses and are similar to other material taken by R.V. 'Sarsia' of *T. volitans* in the Bay of Biscay. The specimen from St. 33*d* is not *Tetraplatia*.

TETRAPLATIA CHUNI CARLGREN, 1909

Through the courtesy of Dr W. J. Rees and Mr E. White, of the British Museum (Natural History), I was able to examine material collected by R.R.S. 'Discovery II' from high latitudes in the South Atlantic and recognized by these authors as *T. chuni*. One specimen from north-west Bouvet Is. is a

juvenile, 1·75 × 0·52 mm, and the other from north of the Antarctic ice edge is a large mature male, 13·0 × 4·0 mm. The ratio of oral to apical body length in both these specimens is figured by Rees & White (1957*b*, fig. 1), and in the present Fig. 2. In *T. chuni*, the oral region is about the same length as, or a little shorter than, the apical. In both specimens of *T. chuni*, the distance between lappets is approximately twice that of the width of the lappet—this is more apparent in the larger one.

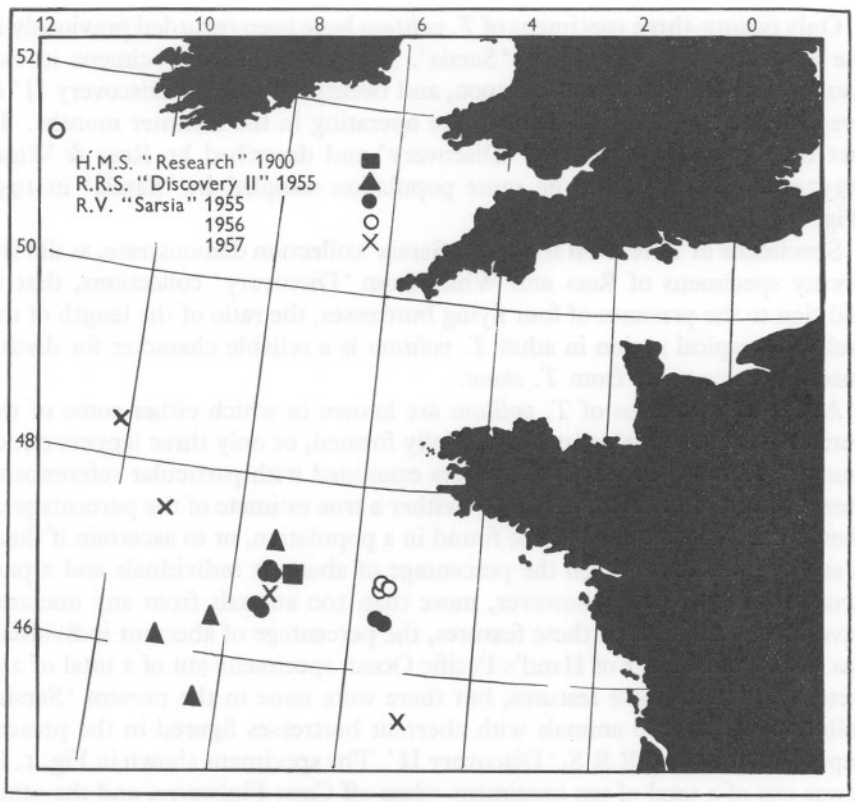


Fig. 3. Catches of *T. volitans* taken in the approaches to the English Channel.

In the large male the gonad is similar in shape (narrow and elongated) to that in *T. volitans*. There is however a distinct, small, outwardly directed tubercle on some of the apical gonad pairs (Fig. 1, B). It has not yet been determined whether these tubercles have any connexion with the exterior by means of an indentation or 'pore' as is known for some material of *T. volitans*, or whether they are in any way involved in the shedding of the gonad products; their tips come very close to the external ectodermal epithelium.

The pairs of finger-like processes of the gonads in the apical region are

greater in length (4·5 mm) than in the oral (1·5 mm). As yet, there is insufficient material described to say whether this is always so, or whether it has any relationship to the proportions shown by the major body regions. Carlgren's (1909, Pl. XI, fig. 4) original specimen also had the male gonad in the apical region of greater length than the oral.

REMARKS

Only twenty-three specimens of *T. volitans* have been recorded previously in the area investigated by R.V. 'Sarsia'. They were three specimens in two hauls by H.M.S. 'Research' in 1900, and twenty by R.R.S. 'Discovery II' in five hauls in 1955. All the ships were operating in the summer months. In fact the *Tetraplatia* taken by 'Discovery' and described by Rees & White (1957*a*) must be part of the same population sampled by 'Sarsia' in 1955 (Fig. 3).

Specimens of *T. volitans* from the 'Sarsia' collection demonstrate, as did the twenty specimens of Rees and White from 'Discovery' collections, that in addition to the presence of four flying buttresses, the ratio of the length of the oral to the apical region in adult *T. volitans* is a reliable character for distinguishing this species from *T. chuni*.

Aberrant specimens of *T. volitans* are known in which either some of the buttresses fail to develop, or are partially formed, or only three lappets out of four are present. So far, the numbers examined with particular reference to these features are inadequate to give either a true estimate of the percentage of aberrant individuals likely to be found in a population, or to ascertain if there is any correlation between the percentage of aberrant individuals and a particular locality. Where, however, more than 100 animals from any one area have been examined for these features, the percentage of aberrant individuals has been small. Four of Hand's Pacific Ocean specimens out of a total of 211 were aberrant in these features, but there were none in the present 'Sarsia' collections. The two animals with aberrant buttresses figured in the present paper were taken by R.R.S. 'Discovery II'. The specimens shown in Fig. 1, E, is one out of a total of ten specimens taken off Cape Finisterre, and the other (Fig. 1, F), is one of two specimens from the South Atlantic Ocean. Measurements of Hand's (1955) 'more robust' specimens of *T. volitans* indicate that the width of the lappet may approximate to half the width of the distance between the lappets, i.e. approaching the ratio described for *T. chuni*. However, Hand did not find any in which the lappet was as narrow as that described by Carlgren for *T. chuni* and as shown by the specimens in the present 'Discovery' collection. All the 'Sarsia' specimens had the width of the lappet approximately equal to the length of the distance between the lappets, and were typical of *T. volitans*, irrespective of contraction due to preservation.

While only three specimens of *T. chuni* are known, they all show similar

characters whether juvenile or adult. They are without flying buttresses, the oral and apical regions are almost equal in length, and they have narrow lappets, which are only half the width of the distance between the lappets. Juveniles of *T. volitans*, while possessing an oral and apical body region of equal length, have flying buttresses when approximately 1·0 mm in length and lappets in which the width is approximately equal to the distance between them.

All in all, *T. volitans* and *T. chuni* as at present known can be readily recognized and their differing characters summarized as follows. First, and probably still of greatest significance for recognizing the two species, is the presence of four flying buttresses in juvenile and adult of *T. volitans*, and their absence in *T. chuni*. Secondly, the difference in ratio between the major body regions. In *T. volitans* the length of the oral region is much greater than in the apical—a ratio of at least 2:1 and occasionally as much as 5:1 in the adult. In *T. chuni*, on the other hand, oral and apical regions are approximately equal in length, but the apical part may be slightly longer. Thirdly, the width of the lappet in relation to the width of the distance between the lappets is different in the two species. In *T. volitans* the width of the lappet and the distance between the lappets is approximately the same, while in *T. chuni* the distance between the lappets is usually twice the width of the lappet. This relationship of distance between the lappets and the width of the lappet can be seen in both juveniles and adults.

Further, in *T. volitans* the gonad pairs of the oral region are longer than those of the apical part, while in *T. chuni* they are about half the length of the apical. In the former species the difference in length of the gonad pairs in the oral and apical regions seems to be correlated with the difference in length of the major body regions. As yet, there is insufficient evidence to say whether such a relationship is a constant character in *T. chuni*. Three other features are worthy of note: the very narrow waist of *T. volitans* and the much broader waist of *T. chuni*: the dome-shaped apical tip shown by the majority of preserved specimens of *T. volitans* compared with the bluntly pointed apical tip of *T. chuni*; and the greater size of *T. chuni*. Differences in apical shape are without doubt influenced by fixation and preservation, and therefore probably unreliable as a specific character, but the difference in apical shape in *T. volitans* and *T. chuni* may indicate a difference in behaviour and prove to be characteristic of the species. Similarly differences in size may be unreliable as a specific character, particularly in *Tetraplatia* of which *T. chuni* is as yet represented by only two adult specimens. Nevertheless, both these specimens are larger than the majority of adult *T. volitans*; one of them, from north of the Antarctic ice edge, is twice the size.

It seems that *T. volitans* with its four buttresses, larger lappets in relation to body width, and narrower waist, is more highly specialized than *T. chuni*. The sequence of events that led to this higher degree of specialization may have

taken place as follows. First, there was the formation of flying buttresses by approximation of oral and apical outgrowths (of all three body-wall layers) and their fusion into a tubular, thick-walled strut between oral and apical regions of the body; the presence in the very tiny juvenile animal of the approximating outgrowths and the partial or complete septum of mesogloea in a few adult animals support this theory of the origin of the buttresses. At the moment, therefore, I cannot agree with Carlgren or Beyer (1955, p. 112) that the lack of buttresses in *T. chuni* is due to their degeneration from an originally buttressed condition. This conclusion is supported by the absence of buttresses in the juvenile *T. chuni*. Nor can I agree with Hand (1955, p. 337) that the sole function of the buttresses is to increase the digestive area, although this increase is incidental to the formation of buttresses. Their structure and shape make it reasonable to suppose that their primary function is now to afford some support for the body, even if, as indicated by the juvenile in the 'Discovery' collections, they originated as outgrowths from the gastric cavity. On the assumption that the buttresses give some support, a narrowing of the waist is possible and this would bring the lappets more closely into the groove between the buttresses, so that in effect the body becomes more streamlined. The neat fit of the lappets between the buttresses must also make for efficient swimming, as most, if not all of the locomotory beat would be effective, there being little or no lateral diversion of water. Increased growth of the oral body region may have arisen from increased swimming efficiency and the necessity for a highly mobile mouth as an efficient food catching mechanism. *Tetraplatia chuni*, on the other hand, lacking buttresses and having a long groove between narrow lappets, is probably a much less efficient swimmer than *T. volitans*. As yet, we may not have fished deeply enough to catch numbers of *T. chuni*.

SUMMARY

Collections of *Tetraplatia* made by R.V. 'Sarsia' from 1955 to 1957 are described and discussed in relation to specimens taken by R.R.S. 'Discovery II'; 'Carnegie VII'; H.M.S. 'Research' and F.R.V. 'Explorer'.

The results confirm that *T. volitans* Busch, 1951, and *T. chuni* Carlgren, 1909, are separate species which can be easily separated both in the juvenile and adult stages.

The difference in length of the gonad in the apical and oral regions in *T. volitans* is found to be correlated with the difference in length of these two major body regions. A similar gonad-length to body-length relationship can be seen in *T. chuni*, but insufficient material has been captured to ascertain whether this is a constant character in this species.

In general males occur in greater numbers than females in *T. volitans*. The known mature specimens of *T. chuni* are both males.

T. volitans is regarded as more highly specialized than *T. chuni*.

REFERENCES

BEYER, F., 1955. A new record of *Tetraplatia* (Pteromedusae). *Nytt Mag. Zool. Oslo*, Vol. 3, pp. 106–12.

BUSCH, W., 1851. *Beobachtungen über Anatomie und Entwicklung einiger wirbellosen Seetiere*. 143 pp. Berlin.

CARLGREN, O., 1909. Die Tetraplatien. *Wiss. Ergebn. 'Valdivia'*, Bd. 19, Lief. 3, pp. 75–122.

DANTAN, J. L., 1925. Contribution à l'étude du *Tetraplatia volitans*. *Ann. Inst. océanogr. Monaco*, N.S. II, Fasc. 5, pp. 429–59.

HAND, C., 1955. A study of the structure, affinities and distribution of *Tetraplatia volitans* Busch (Coelenterata: Hydrozoa: Pteromedusae). *Pacific Sci.*, Vol. 9, pp. 332–48.

KOMAI, T., 1939. On the enigmatic Coelenterate *Tetraplatia*. *Jap. J. Zool.*, Vol. 8, pp. 231–50.

REES, W. J. & WHITE, E., 1957*a*. New observations on the aberrant medusa *Tetraplatia volitans* Busch. *'Discovery' Rep.*, Vol. 29, pp. 129–38.

REES, W. J. & WHITE, E., 1957*b*. Two new records of *Tetraplatia chuni* Carlgren from the South Atlantic. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 13, Nos. 1–4, pp. 101–4.

J. mar. biol. Ass. U.K. (1959) 38, 381–394
Printed in Great Britain

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

I. HISTOLOGY OF INCRETORY ELEMENTS

By D. B. CARLISLE

The Plymouth Laboratory

(Text-figs. 1–16)

Anatomical and histological details are here given of two organ systems, which, my experiments lead me to believe, may secrete hormones concerned in the sex reversal that overtakes all male *Pandalus borealis* Krøyer. The descriptions eschew any attempt at detailed cytology, and refer exclusively to prawns taken from the population of Gullmarfjord in south-west Sweden. There is reason to believe that, as with *Palaemon* (= *Leander*) *serratus* (cf. Carlisle, 1955), different populations may vary widely in the detailed topography of the endocrine organs, as well as in appearance, life history and growth rate (cf. Horsted & Smidt, 1956).

The organ systems to be described are the X organ-sinus gland complex of the eye-stalk and the vas deferens gland system or *glande androgène*.

A note is necessary on the orientation of organs within the eye-stalk. When a prawn is at rest the eye-stalks are held outwards. In this position the eyes themselves are lateral. When a prawn is lifted out of the water, or when it is looking forward, the eye-stalks are turned forward and (in the former situation) are held in close against the head, so that the eyes hold an anterior position. This mobility of the eye-stalk, one of the characteristic features of the stalk-eyed Crustacea, makes the use of the terms anterior and posterior confusing. Following a suggestion made by Dr F. S. Russell, therefore, I propose using the terms abaxial and adaxial, with the same sense as their usage in descriptions of medusae. Under this terminology the eye will be referred to as distal (never as anterior); the side of the eye-stalk which is towards the axis of the body, i.e. the side which is medial when the eye-stalks are held in close or which lie anteriorly when the eye-stalks are turned outwards, will be called the adaxial side; the opposite side, i.e. the lateral side when the eye-stalks are held close, or the posterior side when they are turned outwards, will be called the abaxial side.

THE X ORGAN-SINUS GLAND COMPLEX

In decapods the sensory papilla has been reduced to a sensory pore, or, in many species, lost. The pore is retained in *P. borealis* and this is the only point where the X organ-sinus gland complex approaches the superficies of the eye-stalk. The gross internal anatomy of the eye-stalk differs little from that which has been reported as normal in decapods. The eyes are large as befits a deep-water animal, and the eye-stalks are relatively short and stubby, though highly mobile.

The main nervous nuclei present in the eye-stalk are the medulla terminalis (perhaps more correctly, though less usually, called the lobus terminalis) of the protocerebrum, and the medullae interna et externa. The last is succeeded by the lamina ganglionaris, the most proximal layer of the retina. The neurosecretory cells are concentrated into three major groups, one in each of the

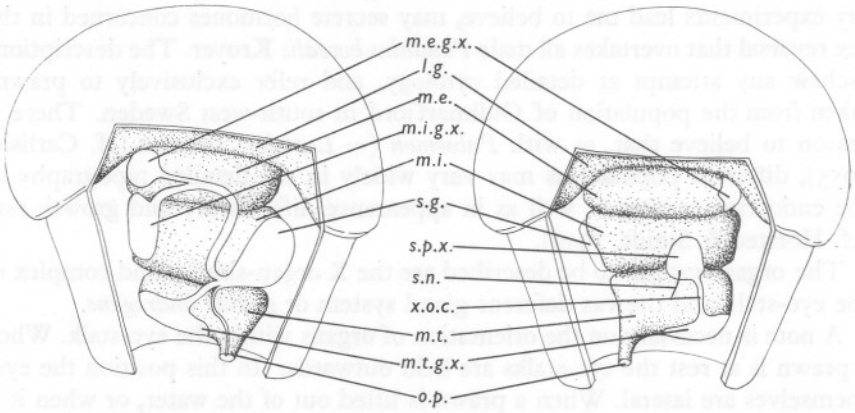


Fig. 1. Dorsal (left) and ventral (right) dissections of a left eye-stalk of *Pandalus borealis*, with all the non-nervous tissue omitted.

Lettering guide to Figs. 1–8. *d.p.s.*, dorsal pigment spot; *l.g.*, lamina ganglionaris; *m.e.*, medulla externa; *m.e.g.x.*, medulla externa ganglionic X organ; *m.i.*, medulla interna; *m.i.g.x.*, medulla interna ganglionic X organ; *m.t.*, medulla terminalis; *m.t.g.x.*, medulla terminalis ganglionic X organ; *o.p.*, optic lobe peduncle; *s.g.*, sinus gland; *s.n.*, sensory nerve of sensory pore; *s.p.*, sensory pore; *s.p.x.*, sensory papilla X organ; *x.o.c.*, X organ connective.

medullae. They may, following the terminology of Knowles & Carlisle (1956), be called the medulla terminalis ganglionic X organ (*m.t.g.x.*), the medulla interna ganglionic X organ (*m.i.g.x.*) and the medulla externa ganglionic X organ (*m.e.g.x.*), respectively (see Figs. 1–8). From these organs neurosecretory fibres run more or less directly to the sinus gland, which lies dorso-adaxially against the medulla interna. The tracts of fibres unite before entering the sinus gland and are joined by a relatively minor group of fibres coming from the brain; the four tracts thus enter together and the gland is not divided into separate lobes as in *Palaemon* (see Carlisle & Knowles, 1959). A further tract

of neurosecretory fibres, the X organ connective, runs from the *m.t.g.x.* to the sensory papilla X organ (*s.p.x.*), which is attached ventrally to the medulla terminalis and projects like a finger into the ventral blood sinus of the eye-stalk, bathed on all sides by blood (see Figs. 2, 6–7), until at its distal end it attaches to the sensory pore. This, a thinning of the cuticle, ventrally on the eye-stalk near the limit of the eye, is lined by a continuous layer of sensory nerve cells, whose axons form a nerve, distinct from the X organ connective, running to the medulla terminalis (see Figs. 1, 2).

The ganglionic X organs

Each of these groups of neurosecretory cells consists of about 30 cells compactly arranged into a discrete organ with no intermixture of normal neurones; nor are there any isolated neurosecretory cells scattered among the association areas, such as are to be found in *Lysmata* (see Carlisle, 1953*c*). The cells are about $75 \times 45\mu$, densely granulated with granules about at the limit of resolution of a $\times 45$ objective ($0{\cdot}4$–$0{\cdot}5\,\mu$). The granules have the same staining properties as in other decapods (see Carlisle & Knowles, 1959). In my preparations the cells are roughly rhomboidal in shape, with the axon forming the continuation of one end of the long diagonal (see Fig. 9). The nucleus is about 20–22 μ in diameter and in most cells contains three well-defined nucleoli.

In the *m.t.g.x.* the more adaxial cells send fibres to the sinus gland while the more abaxial ones direct their fibres to the *s.p.x.* About two thirds of the cells fall into the former and one third into the latter category. The axons of the adaxial cells leave the *m.t.g.x.* on the surface of the medulla terminalis at the adaxial corner of the X organ, and run along the surface of the medulla distally to the sinus gland, following the shortest route along the surface of the ganglionic chain. The fibres from the abaxial cells also leave on the surface of the medulla, forming the X organ connective, and run directly along the ventral surface distally to the *s.p.x.*, again following the shortest route (see Figs. 1, 2).

The axons of the *m.i.g.x.* all leave together at the distal corner of the X organ on the surface of the medulla interna. The tract runs distally on to the dorsal surface of the medulla externa, where it is joined by that from the *m.e.g.x.*, turns in a broad bend back on to the medulla interna and, first making another bend, joins with the tract from the *m.t.g.x.* which runs to the sinus gland. This tract, then, makes a broad S-bend on the dorsal surface of the medullae interna et externa.

All the tracts are on the surface of the ganglionic chain and are readily visible in the fresh dissection as opaque bluish white lines. In some few dissections there appears to be a nerve linking the *s.p.x.* with the sinus gland via the dorsal pigment spot. I am uncertain of this.

The cytology of the neurosecretory cells is in no way remarkable. Using Potter's methods, however (Potter, 1954, 1958), it was possible to distinguish

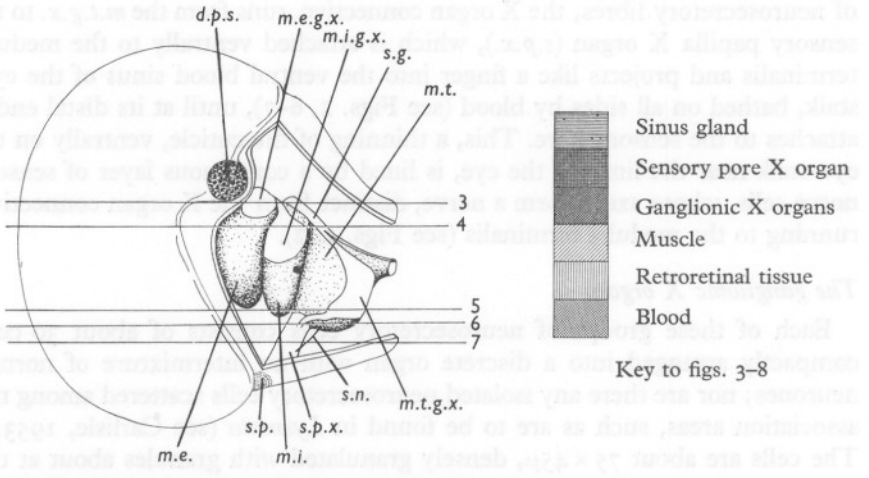


Fig. 2. Abaxial dissection of a left eye-stalk, with all the non-nervous tissue omitted. The numbered lines indicate the approximate planes of the sections illustrated in the like-numbered figures. For lettering guide see Fig. 1.

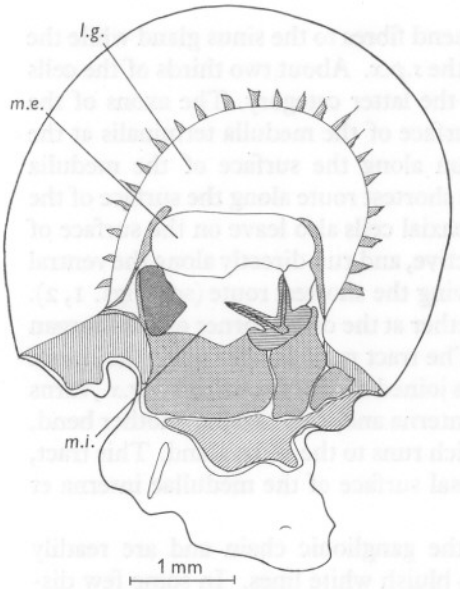


Fig. 3

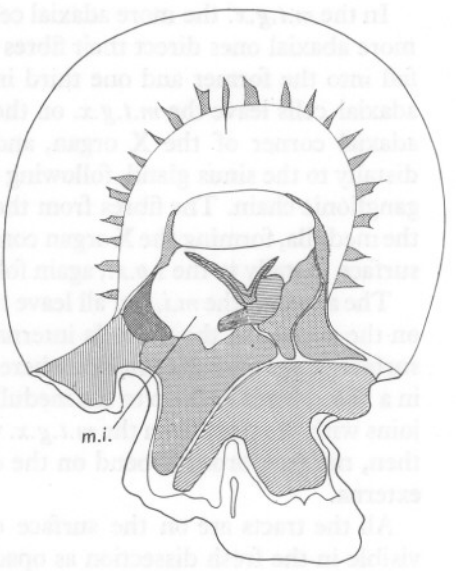


Fig. 4

Figs. 3–7. Horizontal sections through a single left eye-stalk taken in June at the levels indicated in Fig. 2. The abaxial side is to the left. The lettering guide is given below Fig. 1.

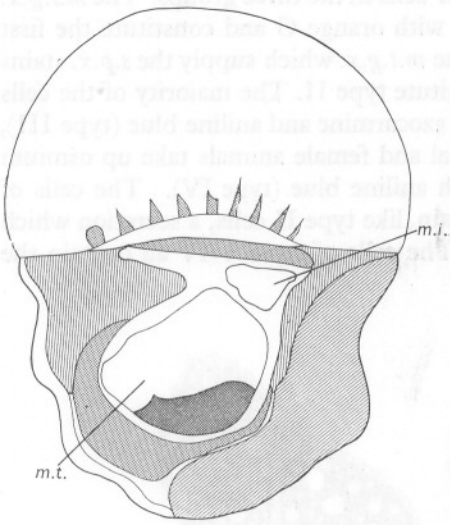


Fig. 5

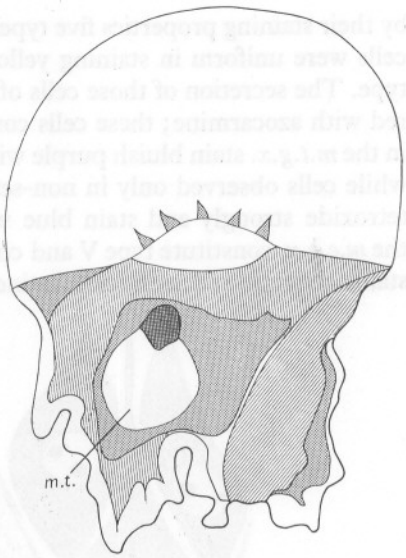


Fig. 6

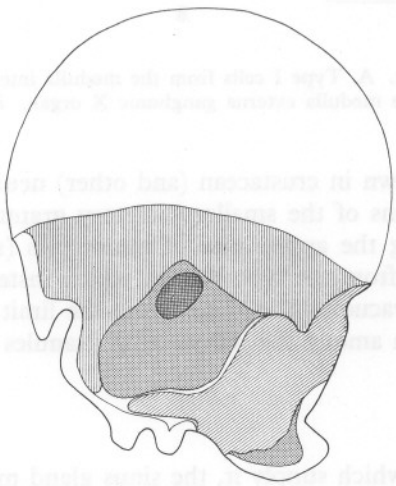


Fig. 7

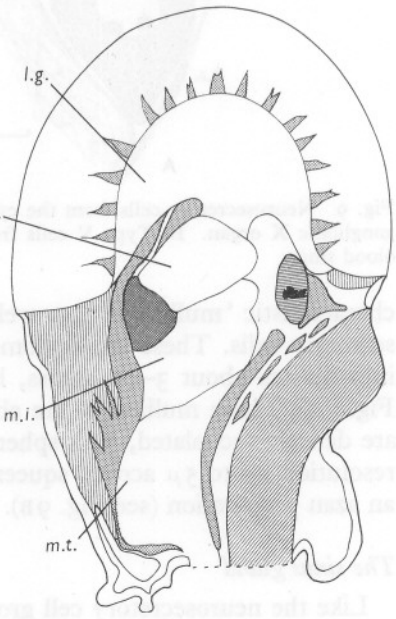


Fig. 8

Figs. 5–7. See legend to Fig. 3 opposite.

Fig. 8. An approximately horizontal, somewhat oblique section of a left eye-stalk. The adaxial side is to the left of the figure. Note particularly the position of the type IV nerve endings indicated in black in the sinus gland. The patch of retroretinal tissue to the right of the eye-stalk retains the acinar structure, although this animal was killed in June. The lettering guide is given below Fig. 1.

by their staining properties five types of cells in the three groups. The *m.i.g.x.* cells were uniform in staining yellow with orange G and constitute the first type. The secretion of those cells of the *m.t.g.x.* which supply the *s.p.x.* stains red with azocarmine; these cells constitute type II. The majority of the cells in the *m.t.g.x.* stain bluish purple with azocarmine and aniline blue (type III), while cells observed only in non-sexual and female animals take up osmium tetroxide strongly and stain blue with aniline blue (type IV). The cells of the *m.e.g.x.* constitute type V and contain, like type II cells, a secretion which stains strongly red with azocarmine. The cells of types I–IV all contain the

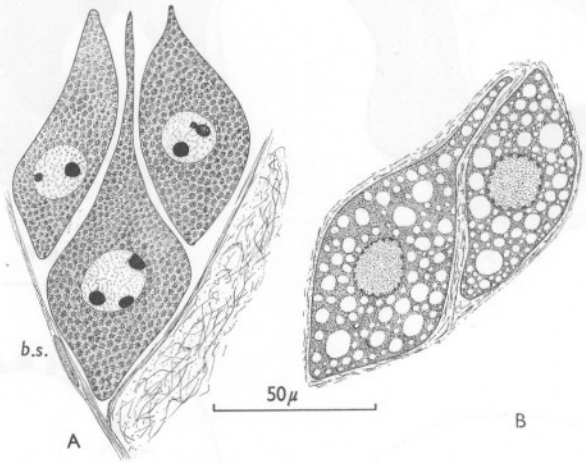


Fig. 9. Neurosecretory cells from the eye-stalk. A. Type I cells from the medulla interna ganglionic X organ. B. Type V cells from the medulla externa ganglionic X organ. *b.s.*, blood sinus.

characteristic 'mulberries', so well-known in crustacean (and other) neurosecretory cells. These are agglomerations of the smaller secretory granules into masses about 3–4 μ across, having the appearance of mulberries (see Fig. 9A). These mulberries are absent from the type V cells, which instead are densely vacuolated, with spherical vacuoles of all sizes from the limit of resolution up to 5 μ across, squeezed in among the red staining granules in an azan preparation (see Fig. 9B).

The sinus gland

Like the neurosecretory cell groups which supply it, the sinus gland may be shown to consist of at least four kinds of elements of differing tinctorial affinities. The staining properties, however, do not correspond exactly to those of the cells and it seems likely that, as in *Palaemon* (Carlisle, 1958), the secretory material undergoes a physico-chemical change as it passes down the axons. Those nerve endings which are strongly osmophilic are stained red by

azocarmine. Tracing of individual fibres shows that these endings derive from cells which are likewise osmophilic, but which stain blue with aniline blue (type IV cells). These endings are rather closely circumscribed in position (see Fig. 8). The centre of the gland is occupied by two intermingled types of endings, one staining blue with aniline blue and the other reddish mauve with azocarmine and aniline blue. The blue endings derive from type III cells, while the reddish mauve ones appear to correspond to type V cells. These latter endings are also found scattered throughout the rest of the gland among the fourth type of endings, which stain red with azocarmine and come from the type I cells of the *m.i.g.x.* The sinus gland consists almost entirely of these nerve endings of varying tinctorial affinities.

The body of the gland in a large female forms a perforated disc about 500 μ in diameter and 250 μ thick. Not more than about 25 nuclei of the connective tissue supporting cells may be seen in it and this tissue forms a minor fraction of the total tissue of the gland. As always, the gland surrounds the point where the blood vessels from the interior of the eye-stalk emerge into the outer blood sinus of the eye-stalk. It is usually stated that these blood vessels run from the inner blood sinus of the ganglionic chain, but in *Pandalus*, as in other Natantia that I have examined, there is no such inner blood sinus. The blood supply of the ganglionic chain consists of a number of small vessels which unite into two main vessels, running from the medulla interna and the medulla externa respectively to unite within the sinus gland before debouching into the outer blood sinus. As may be seen from Figs. 3 and 4, sinus gland tissue surrounds the outer part of these two blood vessels and the body of the gland is arranged around the opening into the blood sinus.

The tract of fibres from the various neurosecretory centres enters the sinus gland by the proximo-abaxial corner (see Fig. 4). The fibres immediately split up and the numerous club-shaped endings of each fibre run perpendicularly to the surface of the gland to abut on to the blood sinus or on to one of the blood vessels.

The sensory papilla X organ

Since the sensory papilla has been reduced to a sensory pore lying close against the border of the eye, this organ has come to lie internally. In *P. borealis* the cuticle is almost as thick over the sensory pore as elsewhere on the eye-stalk so that the term becomes something of a misnomer (see Fig. 12). The bulk of the organ is made up of neurosecretory axons and nerve endings, together with a rather large amount of supporting connective tissue and a groundwork of epithelium-like cells. The cytoplasm of these cells will not take up any of the common stains. About 8–10 neurosecretory fibres run into the *s.p.x.* and divide several times before terminating in the characteristic 'onion bodies' of the *s.p.x.* Each branch terminates in one of these bodies, which in section looks much like a section of an onion; the onion bodies

corresponding to all the branches of any one axon lie together and are bound into a group by a fibrous membrane. They stain with aniline blue in Heidenhain's azan technique. The whole organ is bounded on all sides, except where attached to the medulla terminalis or to the sensory pore, by a connective tissue sheath. It is cylindrical and abuts into a blood sinus (see Figs 2, 6 and 7). A few small blood vessels penetrate into its tissue. The sensory nerve cells which line the sensory pore are in no way remarkable, having scant cytoplasm and fine axons. The staining of the nuclei suggests that there may be two types, one about three times as abundant as the other (see Fig. 12).

There is a very noticeable seasonal variation in females in the histology of the *s.p.x.* (cf. that in *P. kessleri*, Aoto & Nishida, 1956). In April–June, a period between breeding seasons, when the eggs have recently hatched and the ovary has not yet begun to grow for the next breeding season, the epithelioid cells of the *s.p.x.* appear to lose the cell boundaries and to become syncytial; there is little trace of secretory material in the onion bodies (Fig. 10). In September, when the ovaries have begun vitellogenesis for the forthcoming oviposition in December, the epithelioid cells are discrete, and there is much secretory material in the onion bodies and around them. The space between the onion bodies and the surrounding fibrous sheath may be grossly distended with colloid (hyaline or loaded with secretory granules) so that the sheath surrounds a space ten times the volume of the contained group of onion bodies (Fig. 11). The younger males show a condition approximating to that of the April females; the males approaching sex reversal a condition similar to that of the September females. This is true of the males at all times of the year; there is no obvious seasonal variation in the male *s.p.x.*

THE RETRORETINAL ORGAN

The retroretinal organ of the eye-stalk lies behind the lamina ganglionaris of the retina. Like the *s.p.x.* it shows strong seasonal variation. The structure is the same in both males and females and the seasonal cycles are in step in the two sexes. It does not, therefore, vary with the state of the *s.p.x.*, which has no seasonal variation in the male. At certain seasons of the year, notably September, it has the appearance of a secretory organ, but at other times it has the appearance more of adipose tissue. It does not seem to vary with the stage of the moult cycle.

In a specimen taken in September the retroretinal organ has the appearance roughly of a portion of the exocrine tissue of a mammalian pancreas, with circular nests of cells (acini) surrounding a central lumen (Fig. 13). The cells, which are columnar, are loaded with granules. Little material is visible in the lumen of the acini. These acini fill any available space behind the retina; their distribution varies widely in different individuals and seems

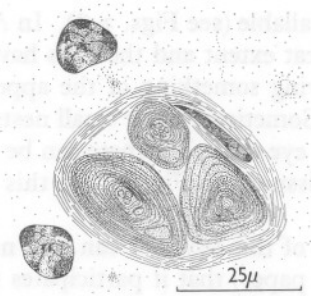


Fig. 10. A single group of onion bodies embedded in a syncytium of epithelioid tissue from the sensory papilla X organ of the eye-stalk drawn in Figs. 2–6, a specimen taken in June.

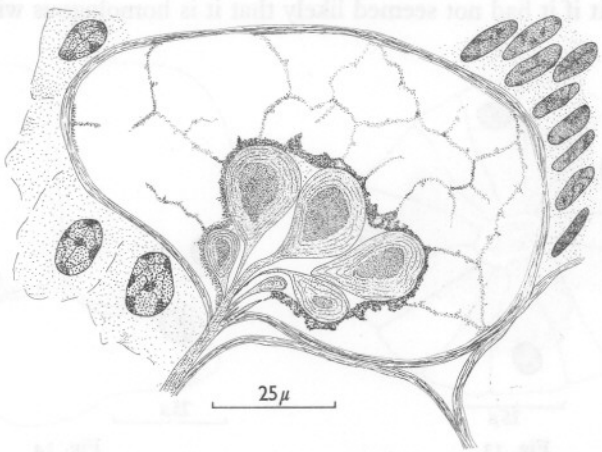


Fig. 11. A single group of onion bodies from a sensory papilla X organ of a specimen taken in September. In this section the branching of the axon into the various branches, each terminating in an onion body, is clearly seen. The onion bodies are full of secretion and the sheath is grossly distended into a cyst. Portions of other cysts are seen by the lower right corner, the discrete epithelioid cells to the left, and the nuclei of the sensory neurones of the sensory pore by the top right corner.

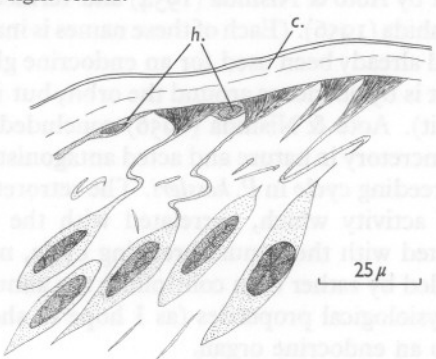


Fig. 12. Sensory cells of the sensory pore, from the same specimen as Fig. 9. Note the relative thickness of the chitinous cuticle over the pore and to one side. *c.*, chitinous cuticle; *h.*, hypodermal cells.

to depend on the space available (see Figs. 3–8). In April the acinar structure has broken down to a great extent and the cells have become much swollen with stored products, giving something of the appearance of a mammalian adipose tissue (Fig. 14). Sometimes a few small nests of acinar structure may be seen to one side of the eye-stalk, but these can be seen to grade evenly into the remaining 'storage tissue' (cf. Fig. 8). At this time of year the tissue occupies more space.

Whatever the function of this tissue, I can find no evidence (as I hope to report in detail in a later paper) that it participates in controlling the sexual cycles of *Pandalus*. Nor do I believe it to be an endocrine organ at all. Indeed, so unlike an endocrine organ is it in appearance that I should have made no mention of it if it had not seemed likely that it is homologous with the organ

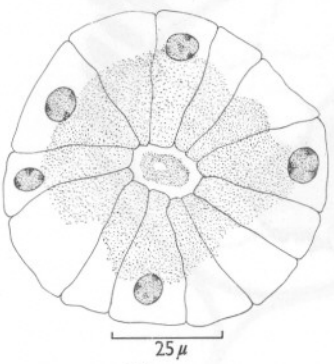


Fig. 13

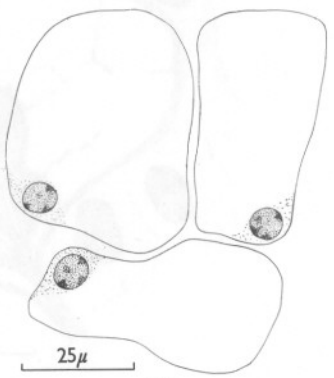


Fig. 14

Fig. 13. An acinus of the retroretinal organ from a specimen taken in September.

Fig. 14. Retroretinal tissue from the eye-stalk of a specimen taken in June.

termed the Y gland by Aoto & Nishida (1954) and termed the circum-orbital gland by Aoto & Nishida (1956). (Each of these names is inappropriate since the name 'Y organ' had already been used for an endocrine gland in Crustacea by Gabe, 1953, while it is by no means around the orbit, but in the eye-stalk, well away from the orbit). Aoto & Nishida (1956) concluded that their 'circum-orbital gland' was incretory in nature and acted antagonistically to the X organ in controlling the breeding cycle in *P. kessleri*. The retroretinal organ, although it shows cycles of activity which, correlated with the annual cycle, must perforce be correlated with the annual breeding cycle, nevertheless must be regarded as controlled by rather than controlling the annual cycle. In neither appearance nor physiological properties (as I hope to show in a later paper) can it be likened to an endocrine organ.

THE VAS DEFERENS GLAND

This gland lies in and on the wall of the vas deferens and was first described in *Orchestia* by Charniaux-Cotton (1954), who at first gave it no name. Knowles & Carlisle (1956) called it the vas deferens gland, while the original discoverer (1956) later named it *la glande androgène*. The former name seems preferable, for, by common anatomical convention, it is customary to

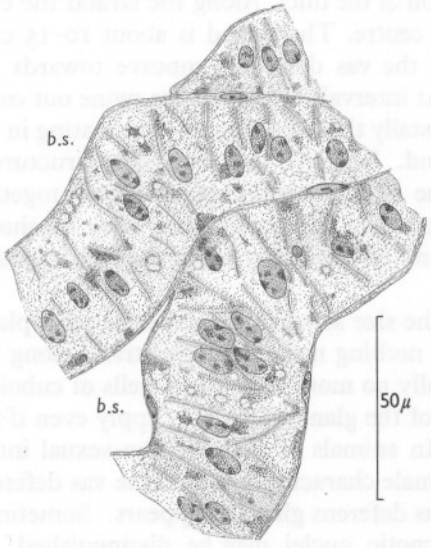


Fig. 15

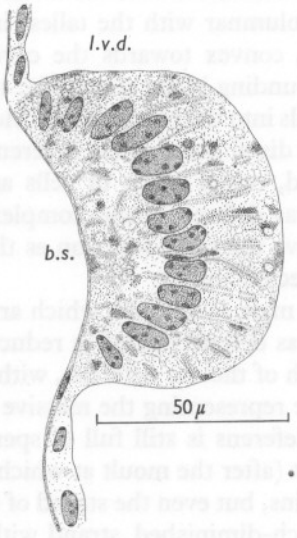


Fig. 16

Fig. 15. Part of the massive portion of the vas deferens gland. Note the fibrous sheath. *b.s.*, blood sinus.

Fig. 16. A transverse section of the strand of the vas deferens, with a portion of the squamous epithelium of the duct. Note the continuous fibrous sheath over the outer side of both duct and strand. *b.s.*, blood sinus; *l.v.d.*, lumen of the vas deferens.

name an organ from its appearance or topography and not from its supposed function; for even if the function is correctly induced this may not be the only or the main function of the organ.

In a fully functional male *P. borealis*, some few moults before sex reversal, the gland is well developed. The bulk of the organ is attached to the distal end of the vas deferens, near where it swells as a vesicula seminalis. It consists of two or more cords of cells which entwine round one another making an irregular heap (Fig. 15). The cells are columnar, about $45 \times 10\,\mu$, with nuclei about $10 \times 7\,\mu$. The cytoplasm is granulated and frequently (though not always) vacuolated. The general structure is of entwining strips of columnar epithelium, covered by a tenuous, though definite, connective tissue sheath. Close inspection shows that this massive organ (about $250 \times 100\,\mu$) is attached

to a strip of specialized epithelium of the vas deferens. In serial section this strand of cells, of the same nature as those of the bulk of the vas deferens gland, is seen to run the whole length of the vas deferens along one side. Most of my series of sections are disorientated but I believe that this strand runs along the median edge of the vas deferens. The epithelium covering the vas deferens is a single-layered pavement epithelium with much flattened nuclei covered by a thin connective tissue sheath which burgeons into a muscular sheath over the distal portion of the duct. Along the strand the cells are columnar with the tallest in the centre. The strand is about 10–15 cells wide, convex towards the cavity of the vas deferens, concave towards the surrounding blood sinus (Fig. 16). At intervals along it there twine out cords of cells into the blood sinus. More distally these are bigger, culminating in the most distal massive vas deferens gland. I believe that the whole structure—strand, minor cords of cells and the major massive group—form together the vas deferens gland complex. Certainly the massive part may be shown to have the same function as the gland of *Orchestia*, while the rest is closely connected with it.

In male *P. borealis*, which are of the size at which sex reversal takes place, the vas deferens gland is reduced to nothing more than the strand along the length of the vas deferens, with distally no more than 10–20 cells of cuboidal shape representing the massive part of the gland. This may apply even if the vas deferens is still full of sperm. In animals of the first non-sexual intermoult (after the moult at which the male characters are lost) the vas deferens remains, but even the strand of the vas deferens gland disappears. Sometimes a much-diminished strand with pycnotic nuclei may be distinguished. In the next intermoult the vas deferens itself is gone. The testis may persist for several moults after the animal has become fully functional as a female, but eventually it too degenerates leaving no trace.

DISCUSSION

The X organ-sinus gland complex of *P. borealis* is of the type which seems to be primitive in decapods and somewhat similar to that found in *Lysmata* (Carlisle, 1953*c*). It differs from that in *Palaemon* chiefly in that the latter has lost the sensory pore. One secondary feature of the system in *Pandalus* appears to be the shortening of the X organ-sinus gland tract, which seems primitively to run in an S-shaped curve (or more complicated convolutions) from the *m.t.g.x.* to the sinus gland (see Carlisle & Knowles, 1959). The complications of the course of this tract are presumably a consequence of the distortion of the topography of this region by the development and elongation of the eye-stalk. The straight route of this nerve tract in *Pandalus* is thus presumably secondary.

The vas deferens gland is not yet sufficiently well known in many species

to enable one to say whether the condition of this gland in *P. borealis* is exceptional. In Mme Charniaux-Cotton's description and figures of the gland in *Orchestia*, where it was first described (Charniaux-Cotton, 1956), the gland is seen to lie entirely outside the muscle and connective tissue sheath of the vas deferens. This I also find to be the condition in *Processa canaliculata* and *Palaemon serratus*, in both of which the sheath is continuous between the vas deferens and the gland. In four species of Pandalidae, however, the sheath runs outside the gland, which is nowhere delimited from the vas deferens, but forms indeed, in places, part of the wall of the duct (see Fig. 14). Only when sex reversal is imminent does the gland become detached from the vas deferens, and the muscular sheath pass between them. Which of these conditions is primitive and which is the more widely distributed we have as yet no means of knowing. One species which would most repay study in this connexion is *Lysmata seticaudata*, the only non-pandaloid natatian which is known to be a protandric hermaphrodite. It may be that the condition found in *Pandalus* is bound up with protandric hermaphroditism.

A cursory examination which I have made of two other species of *Pandalus* —*P. montagui* Leach and *P. bonnieri* Caullery—and of the related *Pandalina brevirostris* (Rathke), revealed but minor variations in the two incretory systems from the conditions described here for *P. borealis*. It seems likely, then, that it represents the norm for the genus. The two organ systems which I have described—the X organ-sinus gland complex and the vas deferens gland system—are known in other species of crustaceans to act as endocrine organs affecting or, in part, controlling the sexual biology. I hope to show in later papers that they have this function also in *P. borealis*. In this histological investigation it has become abundantly clear that the structure of these two systems gives one every ground for ascribing an incretory activity, and, indeed, in so far as secretion is a histological concept, these organs are, by definition, organs of internal secretion.

I should like to acknowledge the friendly assistance afforded me in this work by the former director, Dr G. Gustafson, and the staff of K. Svenska Vetenskapsakadamien Kristinebergs Zoologiska Station, and to thank Mr A. C. G. Best, who made many of the preparations upon which this account is based.

SUMMARY

The X organ-sinus gland complex of *Pandalus borealis* illustrates a condition which is relatively primitive in decapods. The complex is described and the histology and topography of the various parts figured. The only major departure from the primitive condition is the secondary shortening of the X organ-sinus gland neurosecretory tract. The annual cycle of activity in the sensory papilla X organ is briefly described and discussed. The vas deferens

gland (*glande androgène*) is described and its special features noted. The correlation between its condition and the sexual state of this protandric hermaphrodite is stressed.

REFERENCES

AOTO, T. & NISHIDA, H., 1954. (Quoted by Aoto & Nishida, 1956.)

—— 1956. Effect of removal of the eyestalks on the growth and maturation of the oocytes in a hermaphrodite prawn, *Pandalus kessleri*. *J. Fac. Sci., Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 12, pp. 412–24.

CARLISLE, D. B., 1953*a*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). IV. On the site of origin of the moult-accelerating principle—experimental evidence. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 285–92.

—— 1953*b*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex reversal. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 355–72.

—— 1953*c*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VI. Notes on the structure of the neurosecretory system of the eyestalk. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 435–47.

—— 1955. Local variations in the colour pattern of the prawn *Leander serratus* (Pennant). *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 559–63.

—— 1958. Activation of hormonal secretions: a crustacean chromactivator. *Nature, Lond.*, Vol. 182, pp. 32–4.

CARLISLE, D. B. & KNOWLES, F. G. W., 1959. *Endocrine Control in Crustaceans*. Cambridge University Press.

CHARNIAUX-COTTON, H., 1954. Découverte chez un Crustacé amphipode (*Orchestia gammarella*) d'une glande endocrine résponsable de la différenciation des caractères sexuels primaires et sécondaires mâles. *C.R. Acad Sci., Paris*, T. 239, pp. 780–2.

—— 1956. Determinisme hormonal de la différenciation sexuelle chez les Crustacés. *Ann. biol.*, T. 32, pp. 372–99.

GABE, M., 1953. Sur l'existence chez quelques Crustacés malacostracés, d'un organe comparable à la glande de la mue des Insectes. *C.R. Acad. Sci., Paris*, Vol. 237, pp. 1111–12.

HORSTED, S. A. & SMIDT, E., 1956. The deep sea prawn (*Pandalus borealis* Kr) in Greenland waters. *Medd. Komm. Havundersøg., Kbh.*, N.S., Bd. 1, No. 11, 118 pp.

KNOWLES, F. G. W. & CARLISLE, D. B., 1956. Hormonal control in the Crustacea. *Biol. Rev.*, Vol. 31, pp. 396–473.

POTTER, D. D., 1954. Histology of the neurosecretory system of the blue crab *Callinectes sapidus*. *Anat. Rec.*, Vol. 120, p. 716.

—— 1958. Observations on the neurosecretory system of portunid crabs. *Proc. 2nd int. Symp. Neurosecretion, Lund*, 1957. Berlin: Springer.

J. mar. biol. Ass. U.K. (1959) 38, 395–406
Printed in Great Britain

THE SPAWNING OF *ARENICOLA MARINA* (L).

I. THE BREEDING SEASON

By D. I. D. Howie
Zoology Department, Trinity College, Dublin

(Text-figs 1 and 2)

As a result of work by Pirlot (1933), Newell (1948), Smidt (1951) and Duncan (1953) it has become accepted that on European coasts the lugworm has a restricted breeding season occurring only in the autumn. Previously, several workers had stated that this species also spawns in the spring. Kyle (1896) reported that at St Andrews spawning took place between January and March and again between July and September. Similarly, Gamble and Ashworth (1898) and Ashworth (1904) found that on the Lancashire coast the laminarian variety spawned in the spring, although the littoral variety spawned during late summer.

Pirlot (1933), Newell (1948) and Duncan (1953) also suggest that there is some relationship between the onset of spawning and a particular phase of the tidal cycle. Thus, Pirlot (1933) observed spawning during three seasons on the Belgian coast; on each occasion there was a 2-day spawning crisis falling on either full or new moon spring tides. He suggested that the onset of spawning is influenced by the moon acting through tides. Newell (1948), in a comprehensive review of the life history, found that at Whitstable, although spawning started during springs, the peak of spawning occurred during neaps. Duncan (1953) collected data from many areas around the British Isles and, with the exception of St Andrews, again found that the peak of spawning took place during neap tides. Duncan also noted that spawning occurred later in the year on the west coast of Britain and in Ireland than on the east coast of Britain, and suggested that in any one area spawning takes place at about the same time each year.

In the present paper, the relationships between environmental conditions and the onset of the breeding season are analysed in greater detail and the possibility of a spring breeding season re-examined.

Lugworms were collected at intervals during the years 1949–53 at St Andrews, and 1953–57 (excluding 1956) at Dublin. Mr E. Latham of the Marine Laboratory, Millport, supplied worms from Fairlie Sands and Kames Bay during the spring of 1958.

The terms *laminarian* and *littoral* are used here merely to indicate whether

the worms were collected at a low level (L.W.N.T.—L.W.S.T.—laminarian) or high level (H.W.N.T.–L.W.N.T.—littoral) on the shore. Both littoral and laminarian forms were obtained at St Andrews; while near Dublin, littoral forms were collected at Booterstown, and laminarian forms at Seapoint. The worms supplied from Millport were laminarian forms.

The earlier part of this work was carried out at the Gatty Marine Laboratory, St Andrews. Grateful acknowledgement is made to Dr J. M. Dodd for help and advice, and to the Carnegie Trust for a grant in aid of research. The author is also indebted to the Meteorological Office of the Air Ministry, Edinburgh, for sea temperature records from the Bell Rock Lighthouse, off St Andrews, and air temperatures from the R.A.F. station, Leuchars near St Andrews. The Meteorological Service, Department of Industry and Commerce, Dublin, kindly supplied air temperature records from Dublin Airport (Collinstown).

THE SPRING BREEDING SEASON

The opportunity was afforded at St Andrews to re-examine, in the same area, the work of Kyle (1896) who reported the spawning of *Arenicola marina* in the spring. Lugworms were collected during February, March and April in each year from 1949 to 1953. None of the worms examined contained genital products, and there was no evidence of spawning taking place.

As regards Millport, the author is indebted to Dr J. D. Robertson of the University of Glasgow and Mr E. Latham of the Millport Marine Station for the following observations (personal communications). Robertson states that he received '*Arenicola* with near-ripe eggs and sperm' from Millport on 30 March 1956 and on 19 March 1957. Latham agreed that 'around Easter spring tides *Arenicola* collected from Fairlie Sands were ripe', but he also mentioned that specimens from Kames Bay spawned later in the year. As a result of these observations, samples were obtained from Millport at approximately monthly intervals in the spring of 1958. On 10 March, fourteen specimens were obtained from Kames Bay. None of these contained genital products. In contrast, fourteen specimens collected from Fairlie Sands on the same date all contained genital products. Of these, five females contained eggs approaching maturity (average diameter 100–180μ) while three females contained 'mature' eggs (average diameter 180–190μ). Three males contained sperm cells approaching maturity (40–80% in morulae) while three were immature (0–40% in morulae). The stage in development of the sexual products of males can be estimated by counting the relative numbers of rosettes and morulae (sperm plates) in thin films of coelomic fluid. Morulae gradually replace rosettes as spawning approaches. Newell (1948) describes the stages in the development of the germ cells in the body cavity.

In the further collection of twenty-seven worms from Fairlie Sands on 9 April, twenty-one contained genital products approaching maturity or were

mature. There was one immature specimen and five specimens which were thought to be spent. Spent females contained a few residual eggs, irregular in size; spent males contained a few morulae. In both sexes, there was an unusual amount of dark granular material in the body fluid.

There was a marked change in a sample obtained on 19 May (Table 1). Of twenty-five worms, eighteen were spent, only three contained genital products approaching maturity or 'mature' while four specimens were immature. This pattern was repeated in a sample obtained on 3 June, except for an increase in the number of immature specimens (Table 1).

TABLE 1. SAMPLES FROM FAIRLIE SANDS, MILLPORT, DURING SPRING OF 1958

Date	No. of worms	Immature[1]	Approaching maturity[2]	'Mature'[3]	Spent or no genital products
10. iii. 58	14	3	8	3	0
9. iv. 58	27	1	14	7	5
19. v. 58	25	4	1	2	18
3. vi. 58	30	12	2	0	16

[1] Gametocytes or eggs: 40–100 μ. Sperm: 0–40% morulae.
[2] Eggs 100–180 μ. Sperm: 40–80% morulae.
[3] Eggs 180–190 μ. Sperm 80–100% morulae.

TABLE 2. DEVIATIONS IN ANNULATION AND SEGMENT NUMBER AMONG SPECIMENS OF *ARENICOLA MARINA* FROM FAIRLIE SANDS, MILLPORT

	Annulation formula[1]		Chaetigerous segments		
No. of specimens	i. 2, ii. 3, iii. 4	i. 2, ii. 2 or $2\frac{1}{2}$, iii. 4	Incomplete 19	Complete 19	Incomplete 20
96	90	6	6	85	5
Percentage	93·75	6·25	6·25	88·5	5·25

[1] See Wells, 1957.

The specimens from Fairlie Sands were examined anatomically. All were large (trunk = 19 cm average length), dark worms and were collected from the laminarian zone. This suggests that they might belong to the spring-spawning 'laminarian variety' defined by Gamble & Ashworth (1898) and Ashworth (1904). However, a very large proportion of the worms from Fairlie Sands displayed the annulation of the first chaetigerous segments described for their 'littoral variety', i.e. i. 2, ii. 3, iii. 4 (formula after Wells, 1957, see also Table 2).

No evidence was thus found to support Kyle's (1896) report of a spring breeding season at St Andrews. However, there is no doubt that a large proportion of the laminarian population on Fairlie Sands, Millport, spawns in the spring (mainly between 9 April and 19 May in 1958). The immediate appearance of immature worms (containing gametocytes) after the spring breeding season at Fairlie Sands suggests that at least a proportion of this population spawns again later in the year. In view of the fact that Gamble & Ashworth (1898) and Ashworth (1904) gave accurate accounts of oviposition when

describing a spring spawning among lugworms on the Lancashire coast, it is surprising that little credence has been given to their work in recent years. Although Duncan (1953) reported autumn breeding among nearby populations (Wales, Isle of Man), this does not invalidate their observations. As shown above, the Fairlie Sands population probably breeds both in spring and autumn, while the adjacent Kames Bay population breeds only in the autumn. It may well be then, that at least on the west coast of Britain, there are isolated spring-spawning populations other than that described here. In common with worms from all other areas studied in recent years (Wells, 1957; Brewster, unpublished), the vast majority of the worms from Fairlie Sands display the characters of the 'littoral variety' as defined by Gamble & Ashworth (1898). It appears, therefore, that the occurrence of a spring spawning at Millport is unrelated to varietal differences.

Southward & Southward (1958) have recently reviewed breeding in the Arenicolidae. The belief that *A. marina* spawned only in the autumn led them to suggest that this species conforms to Orton's (1920) dictum that species towards the southern limits of their distribution should breed only in the colder months of the year. Despite the account of a spring breeding season given here, this statement is still largely true. It is significant that the spring-spawning population at Millport is laminarian in situation. It is suggested that the higher temperatures experienced by these forms, due to their greater coverage by the tide, permits gamete formation to go forward during the winter. If this is true, it might be expected that spring spawning would be common among laminarian populations. However, the larval stages of *A. marina* are found in the surface sand towards the upper reaches of the shore (Newell, 1948). It is unlikely that the larvae of a cold-water species would be able to survive the high summer temperatures experienced in this habitat, particularly towards the southern limits of its distribution. This hypothesis would account for the possibility of gamete production during the winter and at the same time the rarity of spring-spawning populations in Britain. It may be that, due to local factors, larvae of *A. marina* find an unusually sheltered habitat on Fairlie Sands.

THE AUTUMN BREEDING SEASON

The percentages of worms containing genital products in the collections made at St Andrews and Dublin during late September, October and November are given in Tables 3 and 4 and shown against minimum air temperatures and tidal conditions in Figs. 1 and 2. The air temperatures given are the lowest *at low tide* in periods of 5 days. When spawning was not observed on the shore the appearance of spent worms in the collections, i.e. a decrease in the percentage of worms containing genital products, indicated that breeding was taking place.

St Andrews

The date on which spawning commenced was most accurately determined in 1951 and 1952. A few sperm puddles were observed on the shore on 29 and 31 October 1951. It was evident that spawning had just commenced as few of the worms collected on these dates were spent (Table 3). The peak of spawning occurred on 1 and 2 November and spawning continued up to

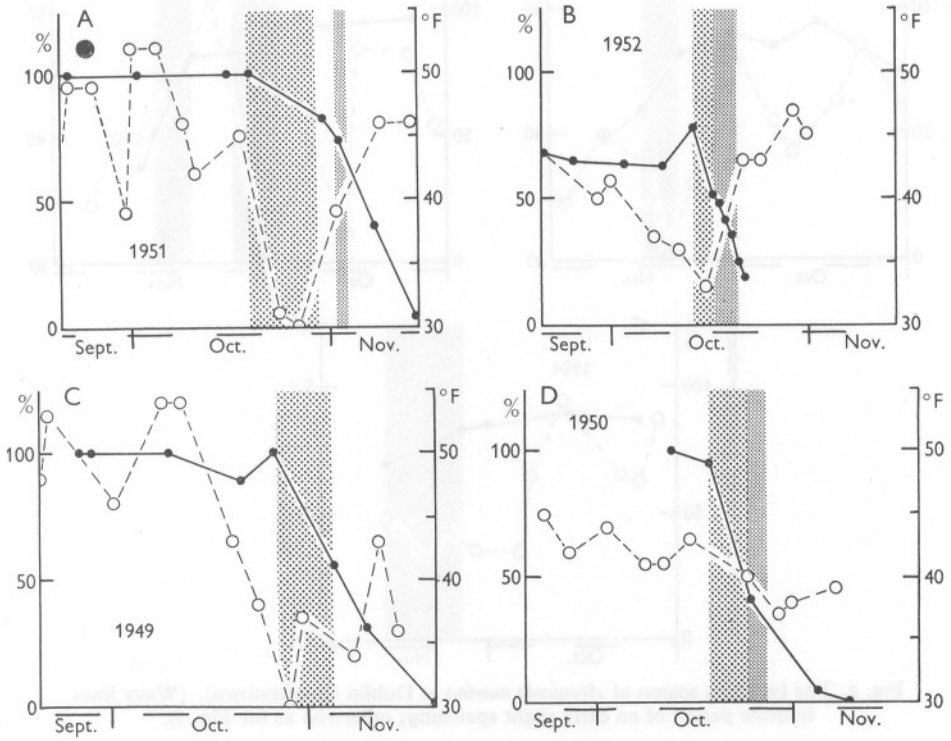


Fig. 1. The breeding season of *Arenicola marina* at St Andrews related to minimum air temperatures and tidal conditions. ○- - -○, minimum air temperatures (° F); ●——●, percentage of worms containing genital products; Large stippling, period during which spawning commenced; fine stippling, time at which peak of spawning was observed on the shore. A double line on the abscissa indicates a spring tide period.

14 November, when less than 5% contained genital products (Fig. 1A). In 1952, 527 worms were collected between 7 and 14 October; there was no evidence of a drop in the percentage containing genital products (Table 3) and no sign of spawning on the shore. On the 16th to 19th inclusive, sperm puddles appeared all over the shore (the peak) and the percentage of mature worms decreased sharply. Spawning activity had very much decreased by the 20th and 21st.

The breeding seasons in these years were similar in that (*a*) they commenced towards the end of a period of neap tides, (*b*) the peak was mainly during the succeeding full moon springs, and (*c*) the beginning of spawning coincided with a sharp fall in minimum air temperatures; this being the first drop to the seasonal minimum in each year (Fig. 1 A, B).

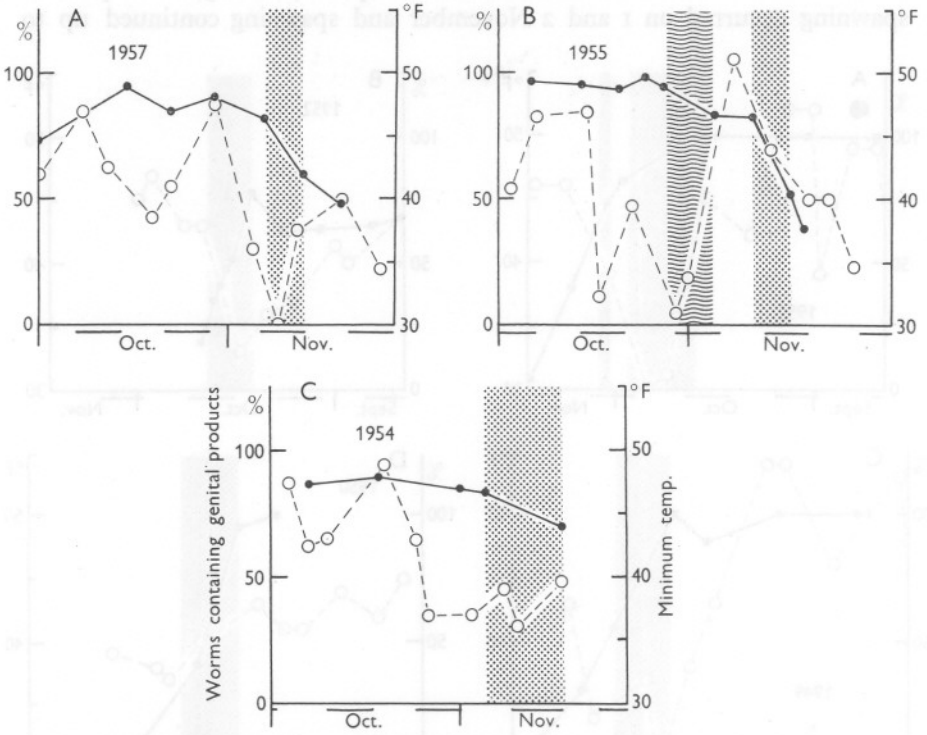


Fig. 2. The breeding season of *Arenicola marina* at Dublin (Booterstown). (Wavy lines indicate period of an early slight spawning; otherwise as for Fig. 1).

The seasons differed in relation to weather conditions. In 1951, spawning began in calm weather but storms developed during the peak. In 1952, the weather was calm throughout.

In 1949, collections were smaller and observations were made less frequently. Spawning was not observed, but collection data show that it commenced between 24 October and 3 November, i.e. probably during neap tides (Fig. 1 C). Spawning again coincided with a sharp fall in minimum temperatures. In 1950, collections were made principally from a laminarian population, and it was this population which was observed spawning on the shore. Spawning appears to have commenced between 18 and 24 October (neaps) and the peak was observed on the 24th to 26th inclusive (springs). These data

support the tidal relationships observed in 1949, 1951 and 1952. While there was a gradual fall in minimum air temperatures prior to spawning (Fig. 1 D) there was no sharp decrease similar to that observed in the other years mentioned. The spawning of laminarian forms will be discussed as a special case below.

In 1949 the breeding season was characterized by intermittent storms, while in the following year it was calm at first but stormy later in the season.

TABLE 3. RECORDS OF COLLECTIONS AT ST ANDREWS

The 1949–51 records are compounded from two populations which differ in the percentage of the total population normally containing genital products in the summer. This percentage is therefore in each case treated as 100 % and any fall in the number of worms containing genital products is percentaged accordingly. The 1952 figures are from a single population and actual percentages are given.

	Date	Total worms examined	Percentage containing genital products	Spawning observed
1949	26–28. ix.	54	100	—
	9. x.	9	100	—
	20, 21. x.	75	89	—
	24–27. x.	31	100	—
	3–5. xi.	85	56	—
	9. xi.	58	31	—
	20. xi.	20	0	—
1950	12. x.	40	100	—
	18. x.	36	94	—
	23–26. x.	52	41	+ (peak)
	4. xi.	64	5	—
	9. xi.	9	0	—
1951	20. ix.	44	100	—
	1. x.	25	100	—
	14–16. x.	25	100	—
	18. x.	21	100	—
	29–31. x.	89	82	+
	1, 2. xi.	91	73	+ (peak)
	7. xi.	34	41	+
	13–14. xi.	109	4	+
1952	19, 20. ix.	223	68	—
	23–25. ix.	328	65	—
	1–3. x.	441	64	—
	7–9. x.	320	63	—
	12–14. x.	207	78	—
	16. x.	201	52	+ (peak)
	17. x.	311	48	+ (peak)
	18. x.	164	42	+ (peak)
	19. x.	75	36	+ (peak)
	20. x.	141	25	+
	21. x.	106	18	+

Dublin (Booterstown)

Spawning in this littoral population was never observed on the shore, but the beginning of the breeding season in 1955 and 1957 can be determined within narrow limits from collection records. Taking 1957 first, the first significant drop in the percentage of worms containing genital products occurred

between 6 and 12 November (Table 4). Thus, unlike the above observations at St Andrews, spawning commenced during full moon springs (Fig. 2A). However, the beginning of spawning was again marked by a fall in minimum temperatures.

The position was more complicated in 1955. In regular collections during the summer (of approximately 60 worms/collection) the percentage of the total worm population containing genital products varied between 93% and 98%. Between 27 October and 4 November this percentage dropped to 83%.

TABLE 4. RECORDS OF COLLECTIONS FROM A SINGLE POPULATION AT BOOTERSTOWN, DUBLIN

	Date	Total worms examined	Percentage containing genital products
1957	30. ix.	41	73
	15. x.	38	95
	22. x.	49	85
	29. x.	43	91
	6. xi.	40	82
	12. xi.	50	60
	18. xi.	38	48
1955	6. x.	32	97
	14. x.	57	95
	20. x.	59	93
	24. x.	50	98
	27. x.	70	94
	4. xi.	44	83
	9, 10, 11. xi.	125	82
	16. xi.	60	52
	18. xi.	72	38
1954	6, 8. x.	45	87
	18. x.	31	90
	31. x.	72	85
	4. xi.	36	83
	15. xi.	45	69

In view of the lack of variation in sampling during the season, this drop in the percentage of worms containing genital products was interpreted as indicating the beginning of spawning. There was, however, no further increase in the number of spent worms up to 11 November (Table 4). The main spawning appears to have commenced between 11 and 16 November, during which time the percentage of worms containing genital products fell rapidly to 52%. Both the slight earlier spawning and the main spawning appear to have commenced during spring tides (Fig. 2B). Minimum air temperatures fell abruptly to 32° F on 16–18 October and there ensued a period of cold weather with the lowest temperature (31° F) occurring on 29 October. This coincided with the first slight drop in the percentage of worms containing genital products. Thereafter, air temperatures rose rapidly; the lowest recorded (at low tide) during the next 10 days was 51° F. This was followed by a second period of

falling temperatures which coincided with the beginning of the main breeding season (Fig. 2B).

Only a limited amount of data is available for the 1954 season (Table 4). The first major drop in the percentage of worms containing genital products occurred between 4 and 15 November, but this drop is of doubtful significance as no further collections were made. This period was preceded by a sharp fall in minimum air temperatures, but in this case only to 37° C. There was no record of spawning at Booterstown in 1953 (see below).

Dublin (Seapoint)

The breeding season of the laminarian population at Seapoint was never fixed with accuracy, but the following observations were made during 1953, 1954 and 1957. In 1953, 75% of the Seapoint population were spent on 4 November, although there was no indication of the Booterstown population even beginning to breed as late as 12 November, when collecting was discontinued. On 9 November 1954, 89% of the Seapoint worms were spent when breeding was just beginning at Booterstown. Finally, in 1957 there was evidence to show that breeding was under way around 9 October at Seapoint, and in this case the season did not commence at Booterstown until 6/12 November. Although in each year, spawning at Seapoint was preceded by gradually falling temperatures (as at St Andrews in 1950) the minimum recorded prior to spawning (37°–40° F) was not so low as that usually associated with spawning in littoral populations.

DISCUSSION

The evidence of Newell (1948) and Duncan (1953) that in most areas around the British Isles autumn-spawning *Arenicola* begin to breed during spring tides, while the peak of spawning occurs during neaps, cannot be disputed. Although spawning was not observed on the shore at Dublin, and in consequence little can be said about the occurrence of the peak, evidence that spawning commences during spring tides tends to support their observations. On the other hand, there is no doubt that in some areas, e.g. St Andrews, the opposite occurs. Spawning begins during neaps, and its peak occurs during springs. If Pirlot's (1933) 'spawning crisis' is equivalent to the peak of spawning his evidence supports this statement. This variation suggests that tidal or lunar conditions cannot alone control the onset of spawning in *Arenicola*. Similarly, rough and calm weather vary in relation to the breeding season. Observations made at St Andrews again agree with Pirlot in this respect.

The present paper was stimulated by the subjective observation, over many years, that spawning at St Andrews is first seen on frosty mornings. Examination of the meteorological records presented in this paper, shows that there is an apparent correlation between the first fall in air temperatures to the autumnal minimum and the onset of spawning, at least in littoral populations.

Whether this correlation has any significance depends in part on the extent to which *Arenicola* may be exposed to a sudden fall in air temperatures during the intertidal period. Southward (1958) has shown that, in winter, during daylight, air temperatures close to rocks on the shore and the body temperatures of animals are generally higher than screen air temperatures read even at a nearby site. However, on an isolated occasion when there appears to have been a sudden cold spell (18 November 1953, Table 4, p. 59) Southward shows that at 10 a.m., after 7 h exposure, screen air temperatures, shore temperatures and the average body temperatures of barnacles and top shells lay within the range 6·8° C to 7·9° C. Previously, Bruce (1928) had stated that at 9 a.m. G.M.T. air temperatures and the surface temperatures of beach sands are approximately isothermal. With a low tide at night or in the early morning, there may, therefore, be little mitigation of a sudden and severe fall in air temperature on the sand. Further, during cold weather in the autumn, the sand surface must be subjected to repeated changes in temperature between tidal and intertidal periods as even inshore waters are still relatively warm (Bruce, 1928; Southward, 1958). It might still be argued that by their burrowing habit lugworms escape these changes of surface temperature. Bruce (1928) has shown that at 20 cm the temperature of the sand is similar to that of inshore water. However, Wells (1945, 1949) has suggested that during the intertidal phase the lugworm makes periodic excursions towards the surface in the course of 'aerial respiration' or to test the temperature and salinity of the surface water. There seems little doubt that in this way the lugworm is exposed to surface conditions.

It is suggested, therefore, that the spawning of autumn-breeding *Arenicola* is stimulated, at least indirectly, by the first fall in air temperature to the seasonal minimum. The effect of this fall in temperature is emphasized by the marked difference between sea and air temperatures at that time. In view of the occurrence of a spring breeding season, reported in this paper, it may later be proved that it is merely a distinct change in temperature which is required to initiate spawning once the worms have matured. This problem is now being investigated experimentally.

Contributory evidence for the above hypothesis may be found in the fact that as the eggs develop in the coelom, they rapidly assume the size of mature eggs but are retained in the body cavity for some weeks before spawning actually takes place. A similar observation has been made by Newell (personal communication). This seems to support the idea that spawning is induced by a sudden stimulus such as temperature change rather than by rhythmic tidal influences.

The relatively early spawning of laminarian forms in response to less marked falls in temperature could be explained by the fact that these worms, covered for prolonged periods during neaps, are conditioned to relatively constant temperatures. Thus when exposed during spring tides they may respond to a

fall in temperature much less violent than that required to initiate spawning in their littoral neighbours. In this case, there would be an additional element of tidal control.

Although spawning will continue in the face of rising temperatures (Figs. 1 and 2), there may be a fixed temperature above which spawning will not take place. Thus in 1955 (Fig. 2B) slight spawning appears to have coincided with a fall in temperature and then ceased for a period of 7 days, during which the weather was unusually warm (minimum air temperature 51° F). Subsequently, there was a second fall in temperature and the main breeding season commenced. It has also been found that experiments on the artificial stimulation of spawning in *Arenicola* fail at about 57° F (14° C) but the results improve below 53° F (12° C) (unpublished work).

The above hypothesis for the environmental control of spawning in *Arenicola* might explain some of the observations in Duncan's (1953) paper. The earlier spawning among populations on the east coast of Britain than on the west coast may be due to the earlier occurrence of low temperatures on the east coast. The onset of spawning in any one area at about the same time each year may be due to the fact that the first major drop in temperature during the autumn in any given place tends to occur during the same fortnight to three weeks every year (Figs. 1 and 2).

SUMMARY

A spring spawning occurs among *Arenicola marina* on Fairlie Sands, Millport. Although laminarian in situation the vast majority of these worms belong to the 'littoral variety'. This unusual breeding season is, therefore, unrelated to varietal differences. A hypothesis is given for the possibility and at the same time the rarity of spring-spawning populations in Britain. At St Andrews the breeding season occurs during the second fortnight of October, but is unusual in that it begins during neap tides with the peak during the following springs. Breeding in the littoral population at Booterstown (Dublin) is more conventional in that it begins during spring tides; in this case during the first fortnight in November. It is shown that over several years the breeding season of littoral populations at St Andrews and Dublin is preceded by, or coincides with, the first sharp fall in air temperatures to approximately the autumnal minimum. It is suggested that this fall in temperature provides the spawning stimulus for autumn-breeding lugworms. In view of the spring breeding season reported here, it is possibly only a distinct change of temperature which is required to initiate spawning. The earlier spawning of laminarian forms in response to a lesser temperature stimulus, may be due to an element of tidal control.

REFERENCES

ASHWORTH, J. H., 1904. *Arenicola. Mem. Lpool mar. biol. Comm.*, No. 11, 188 pp., 8 pl.

BRUCE, J. R., 1928. Physical factors on the sandy beach. Pt. I. Tidal, climatic and edaphic. *J. mar. biol. Ass. U.K.*, Vol. 15, pp. 535–52.

DUNCAN, NAN, 1953. Spawning in *Arenicola marina* (L.). *Brit. J. Anim. Behav.*, Vol. 1, pp. 85–86.

GAMBLE, F. W. & ASHWORTH, J. H., 1898. The habits and structure of *Arenicola marina*. *Quart. J. micr. Sci.*, Vol. 41, pp. 1–42.

KYLE, H. M., 1896. Papers from the Gatty Marine Laboratory, St Andrews. On the nephridia, reproductive organs and post larval stages of *Arenicola marina*. *Ann. Mag. nat. Hist.*, Ser. 6, Vol. 18, pp. 295–300.

NEWELL, G. E., 1948. A contribution to our knowledge of the life history of *Arenicola marina*. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 554–80.

ORTON, J. H., 1920. Sea temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

PIRLOT, J. M., 1933. La ponte de l'*Arenicola marina*, Linné à la côte belge. *Bull. Mus. Hist. nat. Belg.*, T. 9 (No. 49), pp. 1–7.

SMIDT, E. L., 1951. Animal production in the Danish Waddensea. *Medd. Komm. Havundersøg., Kbh., Fiskeri*, Bd. 11, pp. 1–151.

SOUTHWARD, A. J., 1958. Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 49–66.

SOUTHWARD, EVE C. & SOUTHWARD, A. J., 1958. The breeding of *Arenicola ecaudata* Johnston and *A. branchialis* Aud. & Edw. at Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 267–286.

WELLS, G. P., 1945. The mode of life of *Arenicola marina* L. *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 170–207.

—— 1949. Respiratory movements of *Arenicola marina* L.: Intermittent irrigation of the tube, and intermittent aerial respiration. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 447–64.

—— 1957. Variation in *Arenicola marina* (L.) and the status of *Arenicola glacialis* Murdoch (Polychaeta). *Proc. zool. Soc. Lond.*, Vol. 129, pp. 397–419.

J. mar. biol. Ass. U.K. (1959) 38, 407–415
Printed in Great Britain

VARIATIONS IN THE ACTIVITY OF THE THYROID GLAND OF THE COD, *GADUS CALLARIAS* L., IN RELATION TO ITS MIGRATIONS IN THE BARENTS SEA

I. SEASONAL CHANGES

By A. D. WOODHEAD

Fisheries Laboratory, Lowestoft

(Text-figs 1 and 2)

Relatively little is known of the exact role of the thyroid gland in teleosts. Although the results of a few studies have suggested that the thyroid influences respiratory metabolism, as in the higher vertebrates, a greater number of experiments have failed to show that either treatment of fish with thyroid hormones or inactivation of the gland produces any significant effect upon the oxygen consumption of the fish. Thyroid activity has been linked with reproduction in some fish, although there is little evidence to show that the relationship is more than coincidental. The thyroid has also been connected with osmoregulation, but reviewing this work Pickford (1957) concludes that the relation with osmoregulation is only a secondary or collateral one. Recently Hoar (1953, 1955) has suggested that the thyroid may control the migratory response of fish. However, in the migratory species which have been studied in detail, the salmon, *Salmo salar* L. and *Onchorhynchus* spp. and the eel, *Anguilla vulgaris*, changes in the salinity of the environment during their migrations were complicating factors since salinity changes alone have been shown to stimulate thyroid activity in non-migratory fish (Olivereau, 1954).

The present paper describes the seasonal cycle of activity in the thyroid gland of the cod, *Gadus callarias* L., in the Barents Sea, and is one of a series on the physiology and behaviour of the fish. This species is of particular interest because of the great length of its monadromous spawning migration, the adults often travelling more than 800 miles before reaching the spawning grounds. The distribution and movements during the course of the life cycle have been described in detail (Trout, 1957).

The cod mature for the first time at an average age of 8 or 9 years old. Throughout the summer months, they are found on the shallow-water feeding grounds of the Bear Island–Spitsbergen Banks. In late September, at the onset of maturation, the ripening fish begin to move south and west to leave the shelf, and migrate to the spawning grounds inside the Lofoten Islands on the

north Norwegian coast. Spawning occurs each year from mid-February until mid-April. After first maturity, the cod spawns annually, the spent fish dispersing to the feeding grounds where they remain until the following autumn. The young larvae are distributed from the spawning grounds over large areas of the Barents Sea by surface currents. Lundbeck (1932) suggested that they become migratory at 3 years old. From length measurements of immature cod caught by the commercial trawlers, Maslov (1944) demonstrated a pattern of migration which has since been confirmed and extended (Trout, 1957). During the summer months, June to September, the immature fish are found in large numbers in the shallow-water feeding grounds of the Bear Island–Spitsbergen Bank. In autumn the fish begin to leave these grounds and migrate into deeper waters around the shelf where they remain throughout the winter, until the late spring, when they return to the Banks. This migration is repeated annually until the fish mature.

MATERIAL AND METHODS

The thyroid glands were collected from the cod, *Gadus callarias* L., in the Barents Sea. Mature and immature fish, from 50 to 120 cm. were selected from the catch of the M.A.F.F. Research Vessel 'Ernest Holt'. All the fish used in this investigation were caught at temperatures of between 1° and 6° C and at salinities of 34·5 to 35·2‰. The cod were killed by a sharp blow on the head; a sample of blood was taken for chemical analysis, and the thyroid was then removed. In the cod, as in the majority of teleosts, the thyroid is not encapsulated, but consists of a number of discrete follicles, closely apposed to the ventral aorta, and lying in the surrounding connective tissue. The aorta was therefore dissected out, from the heart to the level of the first gill arch;

TABLE 1. FISHING DATES, 1956–7

8–26 August	10–26 March (Spawning sample)
8–20 September	26–31 March (Norway coast)
7–23 October	23–30 April
20 November–5 December	1–10 June
20–31 January	16–31 July

this ensured that most of the gland was removed. The thyroids were fixed in Bouin's fluid for 24 h, routinely dehydrated and embedded in paraffin wax. Serial sections were cut at 4μ, and alternate slides stained with haemotoxylin and eosin, and Heidenhain's azan.

A quantitative value for the activity of the gland was obtained from measurements of the follicular cell height. Pickford & Atz (1957) have reaffirmed the reliability of this method of estimating the activity of the thyroid. In order to ensure that the same follicle was not measured twice, every twentieth section was selected for study. Four diametrically opposite cells were measured in each follicle and not less than two hundred readings were made on each fish.

Samples were obtained throughout the year, at times corresponding to the major phases of the migratory and reproductive cycles of the fish. The dates of capture are given in Table 1. Each sample consisted of ten fish, five males and five females. A statistical analysis of the results showed that there was no difference in the activity of the gland between the two sexes at any time of the year. The state of maturity was determined by examination of the gonads as the fish were dissected; in addition, the gonads were fixed, and sections studied under the microscope.

The results presented below, and in the following paper, are based on between 40,000 and 50,000 follicular cell height measurements.

RESULTS

Adult cod

The mean values for the cell height measurements of the thyroids of the mature cod are given in Fig. 1 and Table 2. The reaction of the colloid to azan staining confirmed the results obtained from cell height measurements. In resting glands the colloid stained a deep red, and had a dense laminated appearance. The surrounding follicular cells were low and stretched round the stored colloid: frequently the nucleus caused a distinct bulge in the centre of the cell. The cytoplasm of the follicular cells stained a uniform pink.

TABLE 2. SEASONAL VARIATIONS IN THE MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF THE ADULT COD

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
August	10	47·8	5·1
September	10	61·5	6·5
October	10	69·0	6·1
November–December	10	76·9	4·3
January	10	81·5	7·5
March (Spawning)	10	70·0	3·6
March (Coast)	10	67·7	5·5
April	10	54·4	3·4
June	10	50·1	2·4
July	10	54·1	4·6

75 E.P.U. = 10 μ

In active glands the colloid stained blue and appeared granular. The follicular cells were high and columnar, with a large round nucleus situated at the distal end of the cell. Cytoplasmic granules, staining a deep pink, were often present, usually surrounding the nucleus, and extending half way down the cell. The inner edge of the cell near the follicle stained much more faintly. Deep red droplets were sometimes seen at the outer edge of the colloid. In some active thyroids the follicular membrane had ruptured, and the colloid was discharged out of the follicle. Other follicles had been invaded by leucocytes.

Throughout the spring and summer months, from April to late September, the follicular cells were low, and the thyroid appeared, histologically, to be in

an inactive state. However, during this period, there was a small but significant ($p = 0{\cdot}03$) increase in follicular cell height. Marked secretory activity began in late September. The height of the follicular cells continued to increase throughout the winter, reaching a maximum in January, prior to spawning. The follicular cell height was less in fish caught in March at spawning time. The thyroid glands of cod caught some 50 miles north of the spawning grounds, and in which the gonads were ripe but not yet running freely with spawn, showed no significant difference in cell height from thyroids of 'running-ripe' cod caught on the spawning grounds, inside the Lofoten Islands. In spent fish the gland re-entered the resting condition.

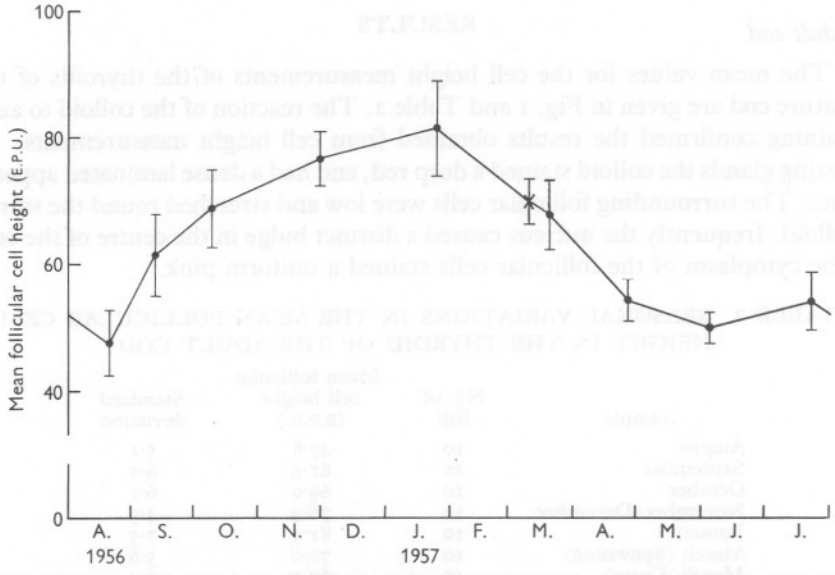


Fig. 1. The seasonal variation in the mean follicular cell height of the thyroid gland in adult cod. ×, ripe cod caught on the spawning grounds inside the Lofoten Islands. The follicular cell height is expressed in eyepiece units (E.P.U.).

A histological study of the gonads showed that throughout the summer these organs were quiescent. Maturation began in late September with the enlargement of the eggs, and the production of primary spermatocytes; at the same time the thyroid became active. Maturation continued throughout the winter migration, the gonads being fully ripe by March, as the fish reached the spawning grounds on the North Norwegian coast.

Immature cod

The cycle of activity in the thyroid of the immature cod is shown in Fig. 2 and Table 3.

The gland became active in September, as in the adult fish, but the maxi-

mum cell height was reached in December, after which the follicular cell height decreased, returning to the resting state by April. A second smaller peak of activity occurred in July, as in the adult fish. The magnitude of the thyroid cycle of the immature fish was of a lesser order than that of the adults.

The gonads of the immature fish showed no changes throughout the year. The ovaries and testes remained small, and were filled with primary germ cells.

TABLE 3. SEASONAL VARIATIONS IN THE MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF THE IMMATURE COD

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
August	10	48·4	4·5
September	10	58·3	6·2
October	10	66·5	6·5
November–December	10	67·9	7·2
January	10	64·8	8·7
March	10	55·1	3·2
April	10	49·5	4·5
June	10	48·4	4·5
July	10	53·6	4·3

75 E.P.U. = 10 μ

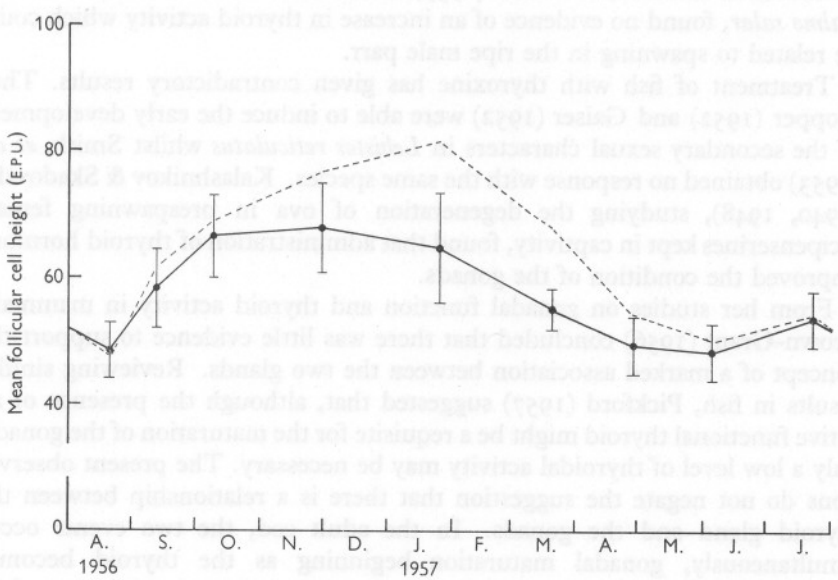


Fig. 2. The seasonal variation in the mean follicular cell height of the thyroid gland in immature cod. The broken line gives the seasonal variation in adult cod for comparison. The follicular cell height is expressed in eyepiece units (E.P.U.).

DISCUSSION

Barrington & Matty (1954) have suggested that there is a correlation between the activity of the thyroid gland and the phases of the reproductive cycle in fish. They found that in the minnow, *Phoxinus phoxinus* L., the thyroid was maximally active at spawning time, the activity decreasing as spawning ceased. Similarly, in the pike, *Esox lucius* (see Zaitzev, 1955), and in the herring, *Clupea harengus* (see Buchmann, 1940), there is increased thyroidal activity at spawning time. Lieber (1936) associated a period of thyroid activity prior to spawning, with the enlargement of the gonads in *Misgurnus fossilis*; a second period of thyroid activity occurred after spawning, but the gland was less active during the actual spawning period.

Laboratory experiments in which fish have been maintained in antithyroid drugs have suggested that these substances may have an inhibiting effect on maturation of the gonads. Chambers (1951) found that the testes of *Fundulus heteroclitus* kept in a solution of thiourea had regressed. Barrington & Matty (1952) working with the minnow, and Smith, Sladek & Kellner (1953) with *Lebistes reticulatus* achieved similar results. However, in a further series of studies on the minnow, Barrington (1954) reported that he could stimulate the development of the testes in winter, by artificial light, although the fish were immersed in thiourea, and Hoar (1939) in his studies on the Atlantic salmon, *Salmo salar*, found no evidence of an increase in thyroid activity which could be related to spawning in the ripe male parr.

Treatment of fish with thyroxine has given contradictory results. Thus Hopper (1952) and Gaiser (1952) were able to induce the early development of the secondary sexual characters in *Lebistes reticulatus* whilst Smith, *et al.* (1953) obtained no response with the same species. Kalashnikov & Skadovskii (1940, 1948), studying the degeneration of ova in prespawning female Acipenserines kept in captivity, found that administration of thyroid hormone improved the condition of the gonads.

From her studies on gonadal function and thyroid activity in mammals, Brown-Grant (1956) concluded that there was little evidence to support the concept of a marked association between the two glands. Reviewing similar results in fish, Pickford (1957) suggested that, although the presence of an active functional thyroid might be a requisite for the maturation of the gonads, only a low level of thyroidal activity may be necessary. The present observations do not negate the suggestion that there is a relationship between the thyroid gland and the gonads. In the adult cod, the two events occur simultaneously, gonadal maturation beginning as the thyroid becomes active, and the thyroid returning to a resting condition in the spent fish. But these results do not indicate whether the activity of the thyroid is necessary for gonad maturation, or whether the occurrence of the two events is coincidental. However, a similar cycle of activity occurred in the thyroid

of the immature cod, although the gonads remained quite inactive throughout the cycle.

The results of the present work suggest that the thyroid cycle in the cod may be related to the migratory activity (see Woodhead, 1959, p. 421 of this *Journal*). In both adult and immature fish, the cycle of activity in the thyroid began at the start of their southward migration over the Bear Island–Spitsbergen Bank, and declined as the migration was completed. The thyroid remained active in the adults until March, when the cod reached the Lofoten Island spawning grounds, but in the immature cod, the activity had already begun to fall by January, when most of the fish were completing their migration to the overwintering grounds.

The spawning migration of the adult cod and the overwintering migration of the immature fish are active contranatant migrations against the West Spitsbergen current. In contrast, it has been suggested that the northward return of the spent cod to the feeding grounds might be accounted for by the passive carriage of the fish within the water mass (Trout, 1957). Perhaps the simplest explanation of the active contranatant migration would be that there was a change in the reaction of the fish to water currents, probably accompanied by an increase in the general level of locomotory activity. Since fish readily orientate against a strong water current, if provided with adequate sensory clues, it seems likely that the change in the reaction of the cod to current would be in the nature of a lowered threshold for initiation of the response, rather than a completely new response. The passive denatant return of the spent fish to the feeding grounds could similarly be explained by a rise in the threshold for the reaction to current, and probably a fall in the general level of activity, the fish swimming randomly within the northward moving waters of the Norwegian current. Trout (1957) states that the cod shoals are largely pelagic at this time, which would facilitate this passive displacement.

Fontaine (1954) has emphasized the role of internal factors in the causation of migration in fish and Hoar (1955) has suggested that the secretory activity of the thyroid gland and gonads may be responsible for increased locomotory activity, sometimes involving long migrations, associated with reproduction. Hoar *et al.* (Hoar, Mackinnon & Redlich, 1952; Hoar, Keenleyside & Goodall, 1955) have shown an increased level of locomotor activity in fish maintained in thyroxine solutions, and decreased activity in thiourea; immersion in solutions of sex steroid hormones also increased the general level of activity. In the minnow, *Phoxinus phoxinus*, Woodhead (1956) has shown that during mid-April, prior to spawning, the mean swimming speed was 159% higher than in December; in April the thyroid would be maximally active (Barrington & Matty, 1954), the gonads would also be secreting hormones since the minnows had begun to assume nuptial coloration, and both gonadal and thyroid hormones may have contributed to the increased swimming speed observed in the minnow.

In the adult cod, the gonads were ripening throughout the spawning migration, when the thyroid gland was active, and the action of the thyroid and gonadal hormones may have been additive, both causing an increase in migratory activity. However, the results obtained with the immature cod, in which the gonads remained inactive, suggest that an increase in the activity of the thyroid gland alone may initiate and sustain a lengthy migration.

I would like to thank my husband for collecting the material used in this study, and for his encouragement whilst the work was in progress. Dr F. R. Harden Jones very kindly collected the sample from the cod on the Lofoten spawning grounds. Gratitude is also due to Mr Michael Graham, C.M.G., and to Mr R. S. Wimpenny, O.B.E., for their continued support of my work.

SUMMARY

A seasonal cycle of activity has been demonstrated in the thyroid gland of both adult and immature cod. The gland became active in late September and activity continued in the adults until spawning time in March; in spent cod the gland had re-entered the resting condition. In the immature fish activity had declined by March.

In the adult cod the thyroid gland was active throughout the period of maturation of the gonads, but the present results did not indicate whether these events were causally related or coincidental; a thyroid cycle occurred in the immature cod, although the gonads remained quiescent.

It has been suggested that thyroid activity may initiate and sustain the active migration of the cod.

REFERENCES

BARRINGTON, E. J. W., 1954. Hormones and control of zoological function. *Nature, Lond.*, Vol. 174, pp. 720–22.

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

—— —— 1954. Seasonal variation in the thyroid gland of the minnow, *Phoxinus phoxinus* L., with some observations on the effect of temperature. *Proc. zool. Soc. Lond.*, Vol. 124, pp. 89–95.

BROWN-GRANT, K., 1956. Gonadal function and thyroid activity. *J. Physiol.* Vol. 131, pp. 70–84.

BUCHMANN, H., 1940. Hypophyse und Thyreoidea im Individualzyklus des Herings. *Zool. Jb.*, Abt. 2, Vol. 66, pp. 191–262.

CHAMBERS, H. A., 1951. Effect of thiourea on male *Fundulus heteroclitus* (Linn.). *Anat. Rec.*, Vol. 109, p. 366.

FONTAINE, M., 1954. Du déterminisme physiologique des migrations. *Biol. Rev.*, Vol. 29, pp. 390–418.

GAISER, M. L., 1952. Effets produits par l'administration prolongée de thioureé et de thyroxine chez *Lebistes reticulatus*. *C.R. Soc. Biol., Paris*, T. 146, pp. 496–98.

HOAR, W. S., 1939. The thyroid gland of the Atlantic salmon. *J. Morph.*, Vol. 65, pp. 257–92.

—— 1953. Control and timing of fish migration. *Biol. Rev.*, Vol. 28, pp. 437–52.

—— 1955. Reproduction in teleost fishes. *Mem. Soc. Endocrin.*, No. 4, pp. 5–22.

HOAR, W. S., KEENLEYSIDE, M. H. A. & GOODALL, R. G., 1955. The effects of thyroxine and gonadal steroids on the activity of salmon and goldfish. *Canad. J. Zool.*, Vol. 33, pp. 428–39.

HOAR, W. S., MACKINNON, D. & REDLICH, A., 1952. Effects of some hormones on the behaviour of salmon fry. *Canad. J. Zool.*, Vol. 30, pp. 273–86.

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

KALASHNIKOV, G. N. & SKADOVSKII, S. N., 1940. Observations on the physiology of acipenserine fish during the period of reproduction in connection with the problem of artificial pisciculture. *Zool. Zh.*, Vol. 19, pp. 671–79 (in Russian).

—— —— 1948. Ecological and physiological study of *Sevruga* during the period of reproduction under natural and experimental conditions. *Zool. Zh.*, Vol. 27, pp. 513–24 (in Russian).

LIEBER, A., 1936. Jahrescyklus der Schilddrüse von *Misgurnus fossilis*. *Z. wiss. Zool.*, Vol. 148, pp. 364–400.

LUNDBECK, J., 1932. Biologisch-statistische üntersuchungen über die deutsche Barentsmeer—Fischerei. *Wiss. Meeresuntersuch.*, Abt. Helgoland, Bd. 18, No. 8, pp. 1–56.

MASLOV, N. A., 1944. The bottom fishes of the Barents Sea and their fisheries. *Trans. Knipovich-polyar, sci. Inst.*, Vol. 8, pp. 3–186 (in Russian).

OLIVEREAU, M., 1954. Hypophyse et gland thyroide chez les poissons. Étude histophysiologique de quelques correlations endocriniennes en particulier chez *Salmo salar* L. *Ann. Inst. océanogr. Monaco*, Vol. 29, pp. 97–296.

PICKFORD, G. E., 1957. The thyroid and thyrotrophin. In PICKFORD, G. E. & ATZ, J. W., 1957. *The Physiology of the Pituitary Gland of Fishes*. New York, Zool. Soc.

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

TROUT, G. C., 1957. The Bear Island cod: migrations and movements. *Fish. Invest., Lond.*, Ser. 2, Vol. 21, No. 6, 51 pp.

WOODHEAD, A. D., 1959. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. II. The 'dummy run' of the immature fish. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 417–22.

WOODHEAD, P. M. J., 1956. The behaviour of minnows (*Phoxinus phoxinus* L.) in a light gradient. *J. exp. Biol.*, Vol. 33, pp. 258–70.

ZAITZEV, A. V., 1955. A histological investigation of the annual changes of the thyroid gland of the pike, and the neurosecretory activity of the hypothalamic nuclei in the seasonal change of the thyrotropic function of the hypophysis. *C.R. Acad. Sci. U.R.S.S.*, Vol. 104, pp. 315–8 (in Russian).

J. mar. biol. Ass. U.K. (1959) 38, 417–422
Printed in Great Britain

VARIATIONS IN THE ACTIVITY OF THE THYROID GLAND OF THE COD, *GADUS CALLARIAS* L., IN RELATION TO ITS MIGRATIONS IN THE BARENTS SEA

II. THE 'DUMMY OF RUN' OF THE IMMATURE FISH

By A. D. WOODHEAD

Fisheries Laboratory, Lowestoft

(Text-figs. 1–3)

Describing the migrations and movements of the cod, *Gadus callarias* L., in the Barents Sea, Trout (1957) suggested that the immature fish carry out a false spawning migration. 'The pattern of migration of the immatures is basically similar to that of the matures . . . with increasing age, the immatures' southerly winter migration approached in length that of the mature cod, as if, in the years immediately prior to maturity they were making a "dummy run" towards the spawning ground.' The seasonal migrations of both the adult and immature cod have been related to changes in the activity of the thyroid gland in this fish (Woodhead, 1959). During the spring of 1956 and 1957, collections of thyroid glands from immature cod were made at stations from Bear Island to the Norwegian coast. It was hoped that a study of these glands might demonstrate further the relationship of the activity of the thyroid gland to the migration of the fish.

The thyroid glands and gonads were taken from immature cod of 50–90 cm, and preserved for histological examination by routine methods (Woodhead, 1959). Samples were taken in the four areas between Bear Island and Røst Bank, south-west of the Lofoten Islands, shown in Fig. 1. The lengths of all immature cod caught in these areas were recorded.

RESULTS

Lengths of the immature cod

The data for the lengths of the immature cod caught at Bear Island and on the Norwegian coast are summarized in Fig. 2. (An insufficient number of immature cod were caught in Areas II and IV for the construction of a length distribution histogram.) In March the length distribution of the immature fish on the Norwegian coast had a mode between 70 to 80 cm, whereas the immature cod caught at Bear Island were smaller with a mode at 55 to 65 cm. These results substantiate Trout's hypothesis of a lengthy 'dummy run' by the oldest immature cod.

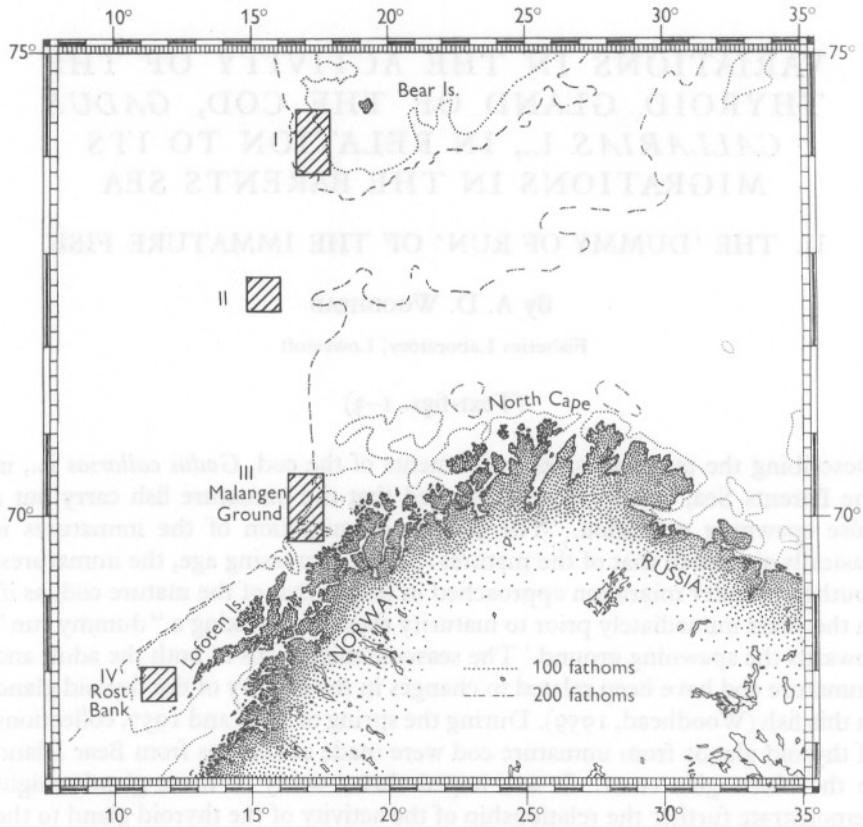


Fig. 1. Bear Island and the S.W. Barents Sea showing the four areas in which immature cod were caught in March 1957.

Thyroid activity

Thyroid glands taken from three immature fish caught on the Malangen ground (Area III) in March 1956 were found to have a significantly higher mean follicular cell height than those of a sample of ten immature cod caught at Bear Island (Area I) (Table 1).

Larger collections of material were made in the four areas shown in Fig. 1 during an extended investigation in March 1957. The activity of these thyroid glands, expressed as the mean follicular cell height for each sample of ten fish, is given in Table 2. The thyroids of the fish caught at Bear Island had the same level of activity as in the previous year, but the thyroids of the fish caught farther south were more active than those at Bear Island; thyroid activity tended to increase with the distance south of Bear Island at which the cod were caught (Fig. 3).

The thyroids of the immature cod caught in the deep water between Bear Island and the Norwegian coast (Area II) had a significantly higher level of activity ($p = 0{\cdot}02$) than those of the cod caught at Bear Island (Area I). The two groups of fish caught on the Norwegian coast also had significantly more

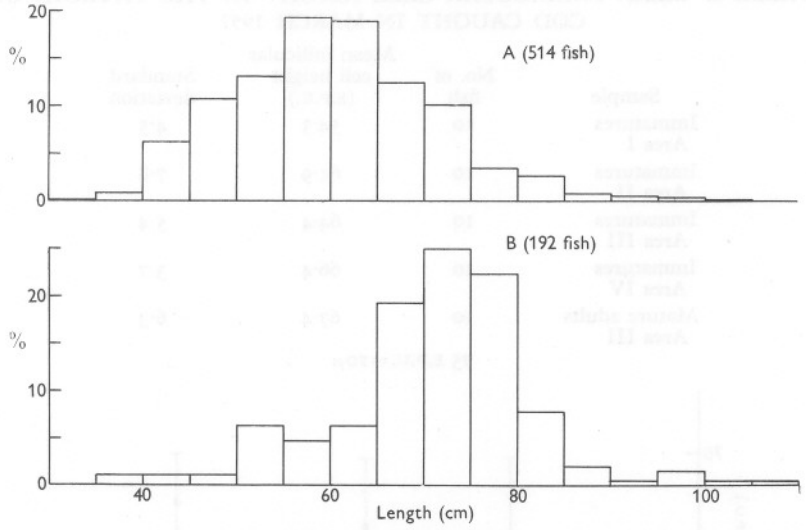


Fig. 2. The length distributions of the immature cod caught at (A) Bear Island and (B) on the Norwegian coast, in March 1957.

TABLE 1. MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF COD CAUGHT IN MARCH 1956

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
Immatures Bear Island	10	55·1	3·2
Immatures Norway coast	3	68·7	6·8
Mature adults Norway coast	10	67·7	5·5

75 E.P.U. = 10 μ

active thyroids ($p < 0{\cdot}01$) than the Bear Island fish, but although the mean follicular cell height values increased in Areas II, III and IV the means were not significantly different (Table 3).

DISCUSSION

The extent of the southerly overwintering migration of the immature cod from the feeding grounds on the Bear Island–Spitsbergen shelf increased as the fish became older, the largest immature cod migrating to the Norwegian coast, within a short distance from the spawning grounds of the adults. Examination

of the thyroids of cod which had migrated farthest south showed that they were in a more active condition than those of the immature fish caught around Bear Island. In the Bear Island fish, which had reached their overwintering grounds

TABLE 2. MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF COD CAUGHT IN MARCH 1957

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
Immatures Area I	10	54·3	4·5
Immatures Area II	10	61·9	7·8
Immatures Area III	10	64·4	5·4
Immatures Area IV	10	66·4	3·7
Mature adults Area III	10	67·4	6·3

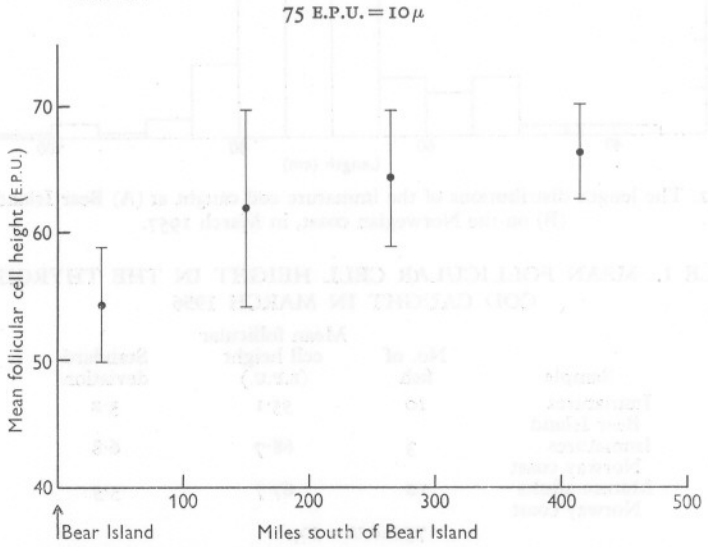


Fig. 3. The mean follicular cell height of the thyroid gland in immature cod caught in Areas I, II, III and IV, compared with the distance from Bear Island at which the fish were caught. The follicular cell height is expressed in eyepiece units (E.P.U.).

TABLE 3. COMPARISON OF THE MEAN FOLLICULAR CELL HEIGHT VALUES

Comparison between areas	Significance of difference (p)
I, II	0·02
II, III	0·4
III, IV	0·3
I, III	$<0·01$
II, IV	0·1

around the edge of the Bear Island shelf, the mean follicular cell height had declined from a January level of $64{\cdot}8 \pm 8{\cdot}7$ to a level of $54{\cdot}3 \pm 4{\cdot}5$. The fish caught on the Norwegian coast still had a follicular cell height of $64{\cdot}4 \pm 5{\cdot}4$, which was not significantly different from the January level; indeed there was no significant difference between the levels of activity in the thyroids of immature cod in the October, November–December, January and the March–Norwegian coast samples. It appears that the thyroid cycle in the immature cod does not rise to a peak as in the adults (Woodhead, 1959), but that once the gland has become active, activity continues at a steady level with a mean follicular cell height of about 66·4 E.P.U. In the large immature cod thyroid activity continues over a more prolonged period than in the smaller immatures; this coincides with the increase in the length and duration of their overwintering migration, which in the largest immatures approaches in length the spawning migration of the adult cod. It seems that in the immature cod the active contranatant migration continues as long as the thyroid gland remains active. This evidence lends further support to the suggestion that the activity of the thyroid gland in the cod may initiate and sustain active and lengthy migrations.

Although the larger immature cod carry out a winter migration which is nearly as long as that of the mature adult cod, the cycle of activity in the thyroid gland of these fish is of a lesser order, although of the same duration as that in the mature cod. Pickford has suggested (Pickford & Atz, 1957) that a minimal level of thyroid activity may be necessary for gonad maturation and it may be that in the cod this is represented by the difference between the thyroid cycle of the adults and that of the large immature fish.

SUMMARY

The average length of the immature cod caught between Bear Island and the Norwegian coast in March 1956 increased from north to south and it appeared that the length of the southerly overwintering migration increased as the fish became older. In the fish caught on the Norwegian coast the thyroid gland was still fully active, whereas in the smaller fish to the north thyroid activity had declined considerably. It is suggested that the greater length of the migration in the larger immature cod is related to the continued high level of thyroid activity.

REFERENCES

PICKFORD, G. E. & ATZ, J. W., 1957. *The Physiology of the Pituitary Gland of Fishes.* New York: Zool. Soc.

TROUT, G. C., 1957. The Bear Island cod: migrations and movements. *Fish. Invest. Lond.*, Ser. 2, Vol. 21, No. 6. 51 pp.

WOODHEAD, A. D., 1959. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. I. Seasonal changes. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 407–15.

ADDENDUM

Since this work was submitted for publication, Swift (1959) has reported that in yearling brown trout the changes in the height of the thyroid follicular epithelium are inversely related to the temperature of the environment, and he interprets this as further evidence that the basic function of the thyroid is in the control of the animal's metabolism, in this case in such a fashion as to compensate for changes in the environmental temperature. Changes in environmental temperature cannot similarly explain the seasonal cycle of activity in the thyroid of the Barents Sea cod. The cod were found at the lowest temperatures (down to $-0{\cdot}5^{\circ}$ C.) in shallow water on the Bear Island—Spitsbergen Shelf during the summer months, when thyroid activity was at a minimum. Highest water temperatures (5 to 6° C.) were encountered during mid-winter when the matures and the oldest of the immature cod were caught in warm water of Gulf Stream origin on the Norwegian coast; the thyroids of these fish were then maximally active. The comparison of thyroid glands taken from fish caught at different temperatures on the same cruise failed to show any significant differences in mean epithelial cell height, except during March when the immature cod around Bear Island at 1 to 2° C. had less active thyroids than those immatures at 5 to 6° C. on the Norwegian coast.

REFERENCE

SWIFT, D. R. (1959). Seasonal variation in the activity of the thyroid gland of yearling brown trout *Salmo trutta*. *J. exp. Biol.*, Vol. 35, pp. 120–25.

J. mar. biol. Ass. U.K. (1959) 38, 423–424
Printed in Great Britain

SEA SURFACE TEMPERATURES AT MILLPORT

By H. Barnes

The Marine Station, Millport, Scotland

Sea surface temperatures have been taken daily at Keppel Pier, Millport (55° 44′ 55″ N. lat.; 4° 54′ 20″ W. long.), for the past 10 years (1949–58), the data being included in returns to the Meteorological Office. The records are not given in their standard publication and they are, therefore, presented here to supplement those of Cooper (1958) for inshore waters at Plymouth some 400 miles farther to the south. For some years the samples were drawn in a specially constructed container fitted with a Fahrenheit thermometer which was read to the nearest whole degree; more recently the bucket method has been used, and a centigrade thermometer graduated in degrees and read to an estimated tenth of a degree. The two methods were checked against each other before the change was made and the results found to be consistent. All readings were taken at 09.00 h G.M.T., irrespective of the state of the tide. The results are shown in Table 1.

The form of the annual temperature regime is typical of boreal waters; there are, however, considerable variations from year to year, not only in the mean values but also in the temperature pattern and these variations are of ecological significance. For example, in a long cold spring the spawning of some species is delayed. In some years the temperature, after falling steadily during the autumn and winter, remains almost constant from January to April; e.g. in 1952 and 1953 when the value remained close to 7° C. during these months. In other years the temperature continues to fall during the early part of the year; in 1956, for example, this fall continued until late March, whereas in 1949 the temperature began to rise gradually in early March. In both 1954 and 1955 the temperature fell until early February, but whereas in 1954 this was followed by a slow rise, in 1955 the minimum was maintained throughout March. In 1950 the minimum was reached late in January.

Apart from day-to-day fluctuations due to local heating in shallow waters there are also, during the summer, periods of low temperature extending over 2–3 weeks; these may be the result of up-welling in the deeper basins and could be of considerable importance in the destruction of warmer water species carried into the area during the warmer months of the year.

REFERENCE

Cooper, L. H. N., 1958. Sea temperatures in Plymouth Sound. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 1–3.

TABLE 1. MEAN MONTHLY SEA SURFACE TEMPERATURES (° C.) FOR MILLPORT, 1949–58, TOGETHER WITH MAXIMA AND MINIMA ATTAINED IN EACH MONTH

	Jan.			Feb.			Mar.			Apr.			May			June		
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
1949	7·9	8·4	7·2	7·4	7·8	6·7	7·2	7·8	6·1	8·2	9·0	7·5	9·8	11·1	8·6	11·7	14·4	10·6
1950	8·1	9·1	6·4	7·1	7·7	5·5	7·6	8·4	6·9	8·2	8·8	7·6	9·2	10·2	8·1	12·0	13·4	9·7
1951	6·3	6·9	5·3	6·2	6·8	4·8	6·6	7·2	5·2	7·1	8·1	6·4	8·4	9·6	7·0	10·5	12·0	9·1
1952	7·4	8·4	6·7	7·1	8·0	5·9	7·3	7·9	6·8	8·1	8·9	7·0	9·3	10·8	7·9	10·6	12·9	9·0
1953	7·4	7·8	7·0	7·0	7·6	6·3	7·3	7·8	7·0	7·4	8·2	6·8	9·4	11·0	7·7	11·3	13·5	9·5
1954	8·7	9·9	7·2	7·0	7·6	6·4	6·5	7·0	6·0	7·5	8·2	7·0	8·7	10·1	7·5	11·0	12·3	9·8
1955	7·7	8·9	6·1	6·8	7·5	6·0	6·1	6·4	5·8	7·4	8·9	6·3	8·9	9·8	7·9	10·7	12·8	9·2
1956	7·5	8·4	6·8	6·3	6·7	5·9	6·2	6·9	5·8	7·1	7·9	6·5	9·0	11·7	7·9	10·3	11·3	9·3
1957	7·3	8·0	6·5	6·4	7·2	5·9	6·9	7·6	6·0	8·0	9·3	7·2	8·9	10·2	7·8	11·2	12·9	9·8
1958	7·2	8·0	6·4	6·4	7·0	6·0	5·9	6·5	5·2	6·3	7·3	5·5	8·1	9·8	7·1	9·7	11·4	8·4
10-year average	7·55	—	—	6·77	—	—	6·76	—	—	7·53	—	—	8·97	—	—	10·90	—	—

	July			Aug.			Sept.			Oct.			Nov.			Dec.			Annual
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
1949	12·7	14·2	11·4	13·1	15·0	13·1	14·3	15·3	13·4	13·2	13·9	11·4	11·0	12·0	10·0	9·2	10·7	8·2	10·48
1950	14·0	15·5	12·1	14·7	15·3	13·8	13·4	14·2	12·2	11·7	12·5	10·7	10·3	11·2	9·0	8·1	9·7	6·6	10·37
1951	12·5	14·0	10·9	13·1	14·8	11·1	12·9	13·5	12·2	12·0	12·9	10·8	10·7	11·5	9·7	8·9	10·1	7·4	9·59
1952	12·9	14·6	11·2	13·5	15·1	12·6	12·4	13·6	11·7	11·2	12·2	10·4	10·0	10·7	9·2	8·4	9·7	7·2	9·85
1953	13·1	14·2	11·6	14·3	15·0	13·5	13·5	14·1	13·0	12·6	13·9	11·7	10·6	11·8	9·8	9·9	10·8	9·2	10·31
1954	12·4	14·0	11·3	13·2	14·8	11·9	12·8	14·0	11·5	11·5	12·1	9·8	9·9	10·8	8·6	9·1	9·9	8·3	9·86
1955	12·8	14·7	10·5	13·6	15·1	12·3	13·5	14·5	12·9	11·8	13·0	10·8	10·6	11·0	9·9	9·0	10·0	7·8	9·91
1956	12·6	13·3	12·0	12·3	12·8	11·7	12·1	12·5	11·7	11·5	12·2	10·4	10·4	11·0	9·3	8·6	9·8	6·9	9·49
1957	12·5	13·7	11·2	12·9	13·9	12·3	12·3	13·0	11·4	11·5	12·2	10·5	10·2	10·7	9·3	8·9	9·9	7·9	9·75
1958	11·8	13·7	9·9	13·2	14·6	11·7	13·2	14·5	12·2	11·8	12·8	11·0	10·8	11·5	9·9	9·5	10·9	8·4	9·49
10-year average	12·73	—	—	13·39	—	—	13·04	—	—	11·88	—	—	10·45	—	—	8·96	—	—	9·91

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ATKINS, D., 1958. A new species and genus of Kraussinidae (Brachiopoda) with a note on feeding. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 559–81.

A new species and genus, *Pumilus antiquatus*, of kraussinid brachiopod from shallow water, Lyttelton, New Zealand, is characterized by the possession of a schizolophous lophophore in the adult, supported by both divergent lamellae and spicules: the shell is smooth, less than 5 mm long and strongly sulcate. The gonad is hermaphrodite.

Pumilus is placed in the Kraussinidae for the following reasons: (1) the brachial skeleton resembles that of immature *Kraussina rubra*; (2) the presence of spicules in the lophophore; (3) similar arrangement of muscles to that in *Megerlia*.

The ciliary feeding mechanism is briefly described. D.A.

BONE, Q., 1958. Synaptic relations in the atrial nervous system of amphioxus. *Quart. J. micr. Sci.*, Vol. 99, pp. 243–61.

The system of nerve-cells upon the gut and diverticulum of amphioxus (*Branchiostoma*) is described in detail; degeneration experiments were performed showing that these cells connect with the central nervous system by their own axons, running in the dorsal root nerves. Special attention is paid to the problems of the multi-nucleate nerve cells in the plexus, and to the possibility of asynaptic connexion between neighbouring nerve cells. No sheath cells have been observed upon the peripheral nerve fibres, either within the atrial plexus or upon the dorsal root nerve bundles.

It is suggested that the atrial system of nerve cells arises in ontogeny by emigration of cell bodies from the central nervous system; evidence is put forward supporting this suggestion.

The relation of the amphioxus visceral plexus to similar plexuses in craniates is discussed, and it is concluded that the system is not homologous with the enteric systems of nerve cells in the vertebrates. Q.B.

BRUNET, P. C. J. & CARLISLE, D. B., 1958. Chitin in Pogonophora. *Nature, Lond.*, Vol. 182, p. 1689.

The tubes of three species of *Siboglinum* and of one species of *Zenkevitchiana* consist of protein and chitin as determined by the chitosan iodine test. Acid hydrolysis of the tubes yields about 10 amino acids and D-glucosamine, while enzymic degradation yields *N*-acetyl-D-glucosamine.

No trace of glucose was found in the hydrolysates, thus discounting the suggestion that the tubes consisted of cellulose. D.B.C.

HILL, A. V. & HOWARTH, J. V., 1958. The initial heat production of stimulated nerve. *Proc. roy. Soc.*, B, Vol. 149, pp. 167–75.

The work described was an extension of research previously done at the Plymouth Laboratory (Abstract in this *Journal*, Vol. 37, p. 807). In this it was shown that a single impulse in a crab's nerve at 0° C. is accompanied by a brief heat production followed

immediately by an absorption of heat. The present paper shows records of heat production during repetitive stimulation at different frequencies at 0° C. With 5 shocks/s the diphasic character of the heat production was very clearly shown. With 10 shocks/s the instruments were too slow to show it. At all frequencies the experimental records were identical with the corresponding curves calculated from a knowledge of the deflection produced by a single impulse. Above 12° C. the instrument could not resolve the diphasic heat production at any frequency.

Similar experiments were made at University College London using medullated nerve of the frog. There was no evidence of diphasic character in the heat production, but this could be due to the instruments being too slow to detect it. Analysis of records for repetitive stimulation at higher frequency located the 'initial heat' within 0·04 s of the stimulus. J.V.H.

POTTS, W. T. W., 1958. The inorganic and amino-acid composition of some lamellibranch muscles. *J. exp. Biol.*, Vol. 35, pp. 749–64.

The sodium, potassium, and chloride content and the inulin spaces of a number of lamellibranch muscles have been measured and the intracellular concentrations of the ions calculated. The muscles examined were the large striated part and the slowest part of *Pecten* adductor, the fast and slow parts of *Mytilus* adductor and the anterior byssus retractor of *Mytilus*, the fast and slow parts of *Anodonta* adductors, and the ventricles of *Mytilus* and *Anodonta*.

The fast striated portion of *Pecten* adductor is similar in ionic composition to other striated muscles or to nerve. The intracellular concentrations of sodium and chloride are low and the potassium and chloride ions are approximately in Donnan equilibrium with the blood. The slower muscles contain much greater quantities of sodium and chloride ions and the potassium and chloride ions are not in Donnan equilibrium with the blood. The divergence from the equilibrium condition is greatest in the slowest muscles. The possible significance of these results is discussed and it is concluded that it is probable that both the potassium and the sodium ions are actively transported in the slow muscles of lamellibranch.

Analyses have also been made of the phosphate compounds and free amino acids in *Mytilus* and *Anodonta* muscles. *Anodonta* muscles contain about half the concentration of phosphate compounds that *Mytilus* muscles contain but only about 4% as much amino acids.

Mytilus muscles adapt to a 50% fall in blood concentration in part by osmotic swelling of the cells, in part by the loss of free amino acids. *Anodonta* muscles adapt to an increased concentration by the shrinkage of the muscle fibres, an increase in the sodium and chloride concentration in the cells and by an increase in the free amino acid content of the cells. In both *Mytilus* and *Anodonta* the potassium and phosphate content per cell remains relatively constant during osmotic changes. W.T.W.P.

BOOK REVIEWS

Oceanography and Marine Biology: A Book of Techniques

By H. Barnes

Published by George Allen and Unwin, Ltd., 1959. Pp. 218; illustrations 110. Price 35*s*.

The techniques described here come under four headings, viz.: Sampling the Living Organisms, The Use of Sound Waves, Some Properties of the Water itself, and Photography and Television. Those described in the first section include bacteriological sampling, plankton catching, sampling bottom fauna with trawls and dredges, and the taking of bottom samples with grabs and corers. The section on the properties of the water is concerned with temperature measurement, collection of samples, and drift and current measurements. The other two sections are up-to-date accounts of acoustic and optical methods of underwater exploration. Dr Barnes is known to be widely experienced in marine investigations and he writes with authority on underwater television. His book is readable and well arranged, and the descriptions of apparatus are accurate and lucid. The line illustrations are neat, and judiciously simplified, and the photographs help out the text. There are nearly 140 references to original sources, and an adequate index.

The book should interest the mechanically minded general reader, the student, and the newcomer to marine biology who wishes to know what can be done at sea and how it is done. Those with more experience may be disappointed that it is descriptive rather than critical of the instruments described, and that it is in no way a manual for their use. F.A.J.A.

Marine Ecology

By Hilary B. Moore

Published by John Wiley and Sons, Inc., New York (London: Chapman and Hall, Ltd.), 1958. Pp. i–xi, + 1–493, with 244 text-figures. Price 76*s*.

Dr Moore is to be congratulated on having brought together in one volume the extremely varied studies that collectively are called marine ecology. In the limited compass of a standard text-book he reviews what is known of the deep sea and the world of plankton, as well as of the specialized conditions of the intertidal zone, and pays especial attention to such perennially interesting topics as geographical distribution, vertical migration and coral reefs. The book, which is lavishly provided with tables and text-figures, is divided into three parts; the first two describe the physical environment and the various habitats available to living organisms, while the last deals with the organisms themselves. There is a large bibliography, which has been restricted, however, to references likely to be consulted by English-speaking students. This means that there are few references on the deep sea, a field in which Russian workers are now very active. A corresponding gap in the text has been filled with underwater photographs of doubtful value.

This volume provides an admirable source-book for American-style advanced courses: by judicious selection from the great detail presented, it would also serve for undergraduate studies in marine biology. A.J.S.

Molluscs

By J. E. Morton

Hutchinson and Co. Ltd., London, 1958. 232 pp. Price 10*s*. 6*d*.

This book is an introduction to the wide field of what is now known as 'functional morphology', of the molluscs. Much original work on this subject has been published in recent years by workers in this country, notably by Yonge, Graham, and Fretter, and the author has presented his subject in an eminently readable style. Chapters cover external form and habits, the main organ systems, nervous systems and behaviour, and the classification and evolution of the three main classes. This is not a text-book of anatomy, and morphology has been treated throughout in its functional and evolutionary contexts. The ecological aspects of the phylum, well covered in other works, are necessarily condensed. Published as a compact volume at a very reasonable price, this book should appeal as a source of reference to all workers concerned with the Mollusca. N.A.H.

The Underwater Guide to Marine Life

By Carleton Ray and Elgin Ciampi

First published, in 1956, by A. S. Barnes and Company Inc., New York. Published in the British Commonwealth in 1958 by Nicholas Kaye Ltd., London. Printed in the U.S.A. Pp. xiii + 338 + 16 plates and 194 text-figs. Price 30*s*.

This most attractively illustrated book was written to aid the underwater swimmer with little or no knowledge of marine life. Apart from minor errors it does on the whole succeed in giving a sound introduction to the subject, and it will enable swimmers in American waters to recognize and know something about most of the living things they are likely to meet below the surface. It must be emphasized that this is indeed an American book and it is of very limited value to divers in European seas. The species described are all from the two coasts of the United States, a large proportion of them from tropical and semi-tropical regions; few of them are to be found in Europe. In view of its purpose the larger organisms, especially the fishes, are dealt with at greatest length, the invertebrates and plant life occupying a much smaller part of the book. The sections on these could well have been expanded at the expense of the chapters on under water diving and photography, which are too short to be of real value. The numerous drawings by a Japanese artist are very pleasing; many of them are adapted from standard works. The coloured drawings are occasionally disappointing; the figure of the garibaldi, for instance, is not as brilliantly orange as it should be, and one wonders whether any octopus was ever such a grass-green as in the figure in this book. There are some excellent underwater photographs in monochrome and in colour: some European marine biologists will probably like to possess the book for these and for the general overall, though incomplete picture (heavily biased in favour of the fishes) it gives of American marine life. D. P. W.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, 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. 27 (p. 761) and Vol. 31 (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.

TERMS OF MEMBERSHIP

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officer 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.

The Commissioners of Inland Revenue have approved the Association for the purposes of Section 16, Finance Act, 1958, and that the whole of the annual subscription paid by a member who qualifies for relief under that section will be allowable as a deduction from his emoluments assessable to income tax under Schedule E.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

PAGE

J. E. MORTON. The habits and feeding organs of *Dentalium entalis* 225

T. E. THOMPSON. Feeding in nudibranch larvae 239

Z. KABATA. Ecology of the genus *Acanthochondria* Oakley (Copepoda parasitica) . 249

S. KRISHNASWAMY. Respiration in *Chondracanthus zei* de la Roche (Copepoda) . . 263

A. D. BONEY and E. D. S. CORNER. Application of toxic agents in the study of the ecological resistance of intertidal red algae 267

C. M. YONGE. On the structure, biology and systematic position of *Pharus legumen* (L.) 277

L. R. FISHER and E. H. GOLDIE. The food of *Meganyctiphanes norvegica* (M. Sars), with an assessment of the contributions of its components to the vitamin A reserves of the animal 291

A. H. STRIDE. A linear pattern on the sea floor and its interpretation 313

IRENE MANTON. Electron microscopical observations on a very small flagellate: the problem of *Chromulina pusilla* Butcher 319

D. ATKINS. The early growth stages and adult structure of the lophophore of *Macandrevia cranium* (Müller) (Brachiopoda, Dallinidae) 335

D. B. CARLISLE. Moulting hormones in *Palaemon* (=*Leander*) (Crustacea Decapoda). II. Differences between populations 351

G. R. FORSTER. The ecology of *Echinus esculentus* L. Quantitative distribution and rate of feeding 361

PATRICIA M. RALPH. Notes on the species of the pteromedusan genus *Tetraplatia* Busch, 1851 369

D. B. CARLISLE. On the sexual biology of *Pandalus borealis* (Crustacea Decapoda). I. Histology of incretory elements 381

D. I. D. HOWIE. The spawning of *Arenicola marina* (L.). I. The breeding season . . 395

A. D. WOODHEAD. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. I. Seasonal changes . . 407

A. D. WOODHEAD. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. II. The 'dummy run' of the immature fish 417

H. BARNES. Sea surface temperatures at Millport 423

ABSTRACTS OF MEMOIRS 425

BOOK REVIEWS 427

CAMBRIDGE UNIVERSITY PRESS

BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W. 1

AMERICAN BRANCH: 32 EAST 57TH STREET, NEW YORK 22, N.Y.

Printed in Great Britain at the University Press, Cambridge

(Brooke Crutchley, University Printer)