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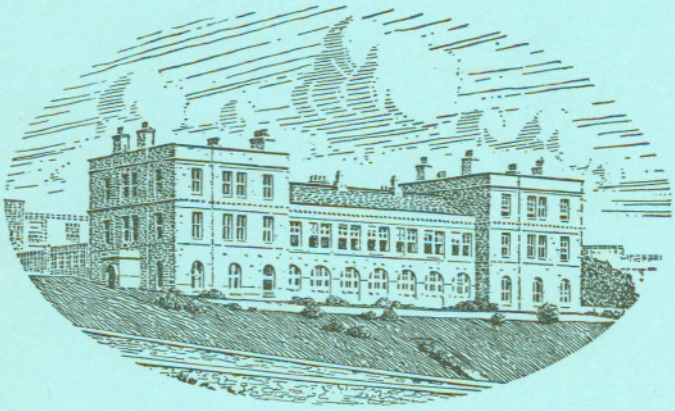

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February 1960

Love, Sexton

OBITUARY

Mrs E. W. Sexton, F.L.S. (1868–1959)

Mrs Alice Wilkins Sexton (*née* Wing) was born at Truro on 27 April 1868 and brought up by parents who, as she related, regarded natural science as no fit subject for a young lady, nor believed it proper for young ladies to study at colleges. Her later life revealed itself as a splendid reaction to this upbringing.

During her childhood some periods were spent in Banffshire and Galway, but a permanent change of surroundings occurred in 1885 when the family moved from Truro to Plymouth. Here she later married Mr L. E. Sexton, a dental surgeon with scientific interests, a keen supporter of the Plymouth Athenaeum, and a friend of the late Dr E. J. Allen. In 1900 Mr Sexton became a member of the Marine Biological Association. An outlet was now provided for his wife's interest in animals and exceptional talent for drawing and painting. As can be seen from her oldest surviving sketches, it is in this year that Mrs Sexton's work in the field of marine biology properly began.

Polychaete worms and Crustacea provided her main subject matter. The first illustrations to be published (in 1902) were twelve plates in T. V. Hodgson's report on the Crustacea of the 'Southern Cross' expedition. She then illustrated certain polychaetes for two of Dr Allen's papers published in 1904.[1] Further drawings of crustaceans and pycnogonids illustrated articles by L. H. Gough,[2] T. V. Hodgson,[3] and R. Gurney.[4] All these appeared under the signature of 'E. W. Sexton', from the circumstance that the name 'Elsie' had been adopted in place of Alice, and this name was never after to be discarded.

These earlier drawings showed an unusual ability for depicting detail accurately, a feature that was to be so conspicuous in her later work. Examination of the plates in Hodgson's 'Southern Cross' Report gives no clue that these were in fact reproduced without any reduction.

It is not surprising, then, that Prof. McIntosh accepted some of her illustrations of syllids for the current volume of his polychaete monograph (Vol II, No. 1), notwithstanding the very high standard set by Miss A. Watson, his regular artist. Some coloured illustrations of the syllid *Procerastea*, included in three plates of Allen's paper[5] published in the *Phil. Trans.* much later on,

[1] *J.M.B.A.*, Vol. 7, p. 299 and *Q.J.M.S.*, Vol. 48, p. 79 (1904).
[2] Report on the plankton of the English Channel in 1903.
[3] *Ann. Mag. nat. Hist.* (7), Vol. 14, p. 458, and *National Antarctic Exped.* 1901–4, Vol. 3.
[4] *Spolia Zeylanica*, Vol. 4, p. 126.
[5] *Phil. Trans.* B, Vol. 211, p. 131 (1921).

in 1921, can only be described as wonderful. Many, however, of her drawings and paintings of polychaetes have never been published: they survive in two albums (one started in 1901, and the other, the larger, in 1909).

But Mrs Sexton was not destined to remain simply an illustrator. In August 1906 Dr Allen went on a 5-day cruise in the S.S. 'Huxley' to the northern part of the Bay of Biscay and brought back a collection of invertebrates. He offered the Amphipoda to Mrs Sexton to examine. This task she set about with vigour and thoroughness. By the time she had dealt with the thirty-five species represented she had made contact with the leading specialists of the day, obtained loans of type material from several Museums, and had gone far in becoming a specialist herself in the study of Amphipoda. This group was, in fact, to provide the chief subject of her work for the rest of her life. Her first publications appeared in the *Proceedings of the Zoological Society* of 1908 and 1909, two first-rate taxonomic papers.[1] To a reader of these to-day it is difficult to realize that the author had had no academic zoological training.

While continuing taxonomic studies—collections from Hamburg and Königsberg Museums were next examined—Mrs Sexton now embarked on a plan to study in detail the life-history of a species that could easily be reared in the laboratory. Among Amphipoda almost nothing was then known of the number of stages passed in reaching maturity, or of the degree of change between one stage and the next. *Jassa* was first chosen, but after considerable effort the difficulty of breeding it proved too great. Its study, though renewed later, was eventually abandoned, not altogether without results of interest.[2] Attention was then turned to *Gammarus*.

It happened that both the German collections were mainly from brackish and fresh water, and contained various samples of an unnamed *Gammarus* previously confused either with *G. locusta* or with *G. duebeni*. Though particularly struck by the fact that the animals from the more saline localities were less hairy than those from fresher water, Mrs Sexton assigned them all to a single variable species, which she described as *G. zaddachi*. Various localities were then examined near Plymouth for *Gammarus*, and overlooked species were discovered (though, curiously, *zaddachi* itself remained unobserved until it was pointed out by a visitor from Australia twenty years later). Notably, a collection from the brackish ditches of Chelson Meadow, made on 4 June 1912, produced in abundance a distinct species of smallish size, to be described by Mrs Sexton as *G. chevreuxi*. For many years Chelson Meadow was its only known habitat. Two pairs of the original collection were set aside for detailed observation and for providing broods for experimental rearing in different salinities. Mrs A. Matthews assisted in this research, which was carried out in the Plymouth Laboratory: up to now Mrs Sexton

[1] *P.Z.S.* (1908): p. 370; (1909): p. 848.
[2] Sexton & Reid (1951), *J. Linn. Soc.*, Vol. 42, p. 29.

had apparently worked in her own home in Higher Compton under Dr Allen's remote control.

The results were unexpectedly fruitful, for they led to an entirely unforeseen line of research. For it was from the progeny of one of these pairs that the first mendelian recessive type was bred out—an animal with red, instead of black, eyes. Other variations affecting the eye pigments were soon observed, some behaving in a simple mendelian manner, others not. These were still relatively early days in the study of the new genetics, and hardly any invertebrates other than insects had been studied for mendelian inheritance. The outcome of the extensive breeding work then undertaken with the collaboration of Dr Allen was looked upon with more than ordinary interest, and *G. chevreuxi* began to attract attention as a useful experimental animal.

The occurrence of intersexes in one of the laboratory strains provided the occasion, in 1920, for collaboration with a rising young zoologist, J. S. Huxley, in an attempt to trace the cause of this phenomenon. While genetic work continued, the original aim of making a detailed study of the life-history of *G. chevreuxi* was eventually achieved in 1924. The successive stages of both male and female are illustrated in detail, based on the examination of successive moults of numerous individuals, and the result is a classic of its kind.

In the mean time the genetic work had received a new stimulus with the appearance of more red-eye recessive types in collections made in 1922, and another recessive concerned with the lack of body pigment. An additional incentive about this time was the belief that the 'mutant' types may have originated under the influence of heat or other abnormal conditions. As work continued through the years into the 1930's it was appreciated by degrees that the wild population carried a high proportion of recessive genes which were exposed in the laboratory by in-breeding. This in itself is a fact of much interest, its cause and significance being still imperfectly understood.

One of the mutant strains was kept going for many years in the Oxford Zoological Laboratory, where E. B. Ford used it successfully, not only in practical genetic classes but also experimentally in illustrating the essentially dynamic action of genes. Mrs Sexton had always appreciated the opportunities that *Gammarus* afforded for tracing the development of any given character during successive growth stages, a possibility not afforded by holometabolous insects like *Drosophila*.

Not long after the death of her husband Mrs Sexton was appointed to the Laboratory staff, on 1 April 1924, as the Director's Research Assistant, a post she held until 1948 when she officially retired. She had just completed a guide to the Plymouth aquarium, which L. R. Brightwell illustrated.

During the years that followed, the work involved in maintaining the diverse 'mutant' stocks of *Gammarus chevreuxi* became particularly arduous. The variety of eye-colour types had multiplied and required careful assessment.

A continuous succession of broods had to be sorted, their characters noted, and periodically re-examined; pairs had to be intelligently mated, and dead animals preserved; then histories had to be catalogued, and the final results charted. This task she fulfilled with never more than one part-time assistant—her sister Miss M. B. Wing at the beginning and then, for at least 20 years, Miss A. R. Clark.[3] The genetic work on *G. chevreuxi* was not wound up until 1936.

After this Mrs Sexton turned to accumulated arrears of *Gammarus* taxonomics, describing *G. tigrinus* from the Midlands, giving a revised account of *Marinogammarus* species (in collaboration with G. M. Spooner), and writing a fuller description of *Gammarus zaddachi*. Finally she produced a very complete review of *Jassa falcata*, including results of her earlier unpublished work, a task in which she was helped by the late D. M. Reid. She passed her 80th birthday before the latter work was completed, yet her ability to produce accurate illustrations showed no sign of deterioration. It was her failing eyesight that eventually called a halt to her remarkably sustained scientific activity. In 1957, after the death of her only daughter Mary in 1951, she left Plymouth to spend her remaining years with her son, Col. F. B. W. Sexton, in Sussex. She died on 18 February 1959 in her 91st year.

Mrs Sexton had great vitality, determination, and independence of outlook. Those who, on rare occasions, offered direct or implied criticism of her work were summarily treated, student[1] and professor[2] alike. She held firmly to any belief she had once formed.

Her great contribution was the excellence of her illustrations and descriptions. There is rarely any doubt what she is describing, and later specialists can rely on her data for forming their own interpretations. In drawing amphipods and their parts she is superior even to G. O. Sars, for paying that much more critical attention to detail. Her technique, moreover, had to be accommodated to the requirements of the line-block and dispense with shading effects, a limitation Sars never had to contend with.

Those who were privileged to know her found in her a generous and sympathetic friend. Visitors to her home at Reservoir House (to which she moved after her husband's death) were received with the kindest hospitality. Her garden was full of botanical treasures from all over the world, and these she exhibited with delight. She had special regard for young biologists whom she was always eager to help. For children also she had a particular fondness, and the photograph here reproduced shows a characteristic aspect. With her, holding a child, is her daughter Mary (Miss M. A. F. Sexton) who was Librarian at the Plymouth Laboratory when she died in 1951. G.M.S.

[1] *Nature, Lond.*, Vol. 136, p. 477. [2] *J.M.B.A.*, Vol. 21, p. 407.
[3] *J.M.B.A.*, Vol. 40, p. 459.

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THE RESPONSES AND ORIENTATION OF THE BIVALVE *LASAEA RUBRA* MONTAGU

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(Text-figs. 1–7)

Lasaea rubra, like other small Erycinacea, is unusual among eulamellibranchs in its relatively great mobility on the surface of the substrate. It can crawl about as freely as a small gastropod and lays down a mucous trail for the attachment of its progressing foot, drawing the shell and body rhythmically forward after each advance thrust of the foot. Unlike some other Erycinacea, *L. rubra* has no well-defined sole; it attaches by the sharp lower edge and part of the side of the foot, which is strongly ciliated and well supplied with mucous glands. In most burrowing eulamellibranchs, the site of communication with the surrounding water has shifted to the paired siphons at the posterior end. The Erycinacea, however, have an anterior inhalant siphon; and—as in forward moving prosobranch gastropods (Morton, 1958)—there is an obvious adaptive advantage in receiving the ingoing current from the water into which the animal is moving.

L. rubra lives normally attached by temporary byssus threads, nestling in small crevices between slate laminae, in irregularities of the rock surface, and especially in *Pygmaea* lichen tufts and inside the empty shells of the barnacle *Chthamalus stellatus* (Morton, Boney & Corner, 1957). It shows a marked thigmotaxis or preference for lateral contact. When removed from its site it appears at once very restless. Placed in a glass bowl in room light it shows three very obvious responses: it will crawl away from a source of light, it will take any opportunity to climb a sloping or vertical surface, and if enough specimens are close together they will ultimately aggregate in small clusters and attach to each other by byssus threads.

Lamellibranchs without eyes or exploratory tentacles are not usually thought of as direction-finding animals, and there appears to be as yet no account of migration by an adult eulamellibranch in response to external stimuli. This paper will present the results of some experiments to elucidate the responses of *Lasaea rubra* to the stimuli of light, gravity and lateral contact, and will attempt to show the relevance of these to its orientation and maintenance of position on the shore.

Material was obtained in the *Chthamalus stellatus* zone of the upper inter-area tidal at Tinside, Plymouth, being brought to the Laboratory in chips of rock or barnacle shells, and kept cool in the dark until used for experiments

within, at the most, 2 or 3 h of collecting. The shell being closed when out of water, the animals received no immediately previous light stimulus, and were left attached *in situ* until a few minutes before the experiment. They were then immersed in watch-glasses of sea water only for so long as was necessary to moisten the shell and allow the very rapid emergence of the foot and siphon.

All experiments were performed in a dark box, a metre each dimension, with non-reflecting blackened sides. This was closed in front by a black curtain and kept in a dark room at a cool room temperature of 15° C. The experimental substratum was a glass sheet of 13 cm square, marked out with a working field 11 cm square, gridded with centimetre squares (Fig. 1 B). The animals crawled readily on this glass, providing a record of their own trails by the secreted mucous track. After an experiment such trails could be made easily visible by gently agitating the glass sheet in a suspension of talcum powder in water, and could then be dried and varnished; in practice it was found much more convenient and equally accurate to redraw them at once at the same scale on squared graph paper.

The apparatus for light-response experiments is shown in Fig. 1 A. It consisted of a galvanized iron photographic dish (*t*) 5 cm deep painted with black marine varnish and lined with non-reflecting black 'Cobex' plastic sheeting, with vertical sides and a 2 cm. margin turned inwards horizontally at the top to absorb small amounts of light reflected from the glass. The lamp-box (*b*) held a 12 V tungsten filament lamp with a luminous intensity in the direction of the experimental plate of 9·2 candelas. The colour temperature of the lamp was 2900° K. The filament (*l*) was 8 cm behind the aperture of the box. *p*–*p* 1 represents the glass plate on the floor of the dish, and *s* the beginning of the experimental field, 7 cm inside the lighted area. *s*–*s* 1 is the length of the light gradient used in the experiments. Illumination fell off laterally along the transverse line of *s* by 1 % at 2 cm from the centre of the light path, and by 4 % at the extreme edge of the experimental field. For the conditions of the experiment, and with the steepness of the *s*–*s* 1 gradient, transverse illumination at a given level was considered as being approximately uniform. Refraction and reflexion of light at the water surface were calculated as for an average angle of incidence of 20°; and the intensity of light incident at *s*–*s* 1 was taken to be approximately 40 % of the corresponding value at the water surface. The animal having no apparatus for light concentration or image formation, the illumination *I*, received at *s*, at a distance of 20 cm from the light source may be calculated

$$I = \frac{c}{(ls)^2} \times 0{\cdot}4 = \frac{9{\cdot}2}{0{\cdot}2^2} \times 0{\cdot}4 = 9{\cdot}2 \text{ lux},$$

where *c* is the luminous intensity of the source in candelas and *ls* the distance from the source in metres. Illumination at the far edge of the experimental field, *s* 1 30 cm from the source, will be 4·1 lux.

A series of neutral filters of optical densities 0·1–4·8 were in separate experiments placed in the light path at the aperture of the lamp-box at *f*. The greatest care was taken to avoid leakage of light around the filters.

For experiments involving gravity a rectangular glass trough was used, 20 cm deep, and the experimental glass plate was raised to the angle of inclination required by varying the length of a suspending wire. In experiments at a high angle of slope animals were given 3–5 min to attach by the foot to the glass plate, while at a lower slope; after this the plate was raised, any progress already made by the animals being noted and the resultant length of trail disregarded.

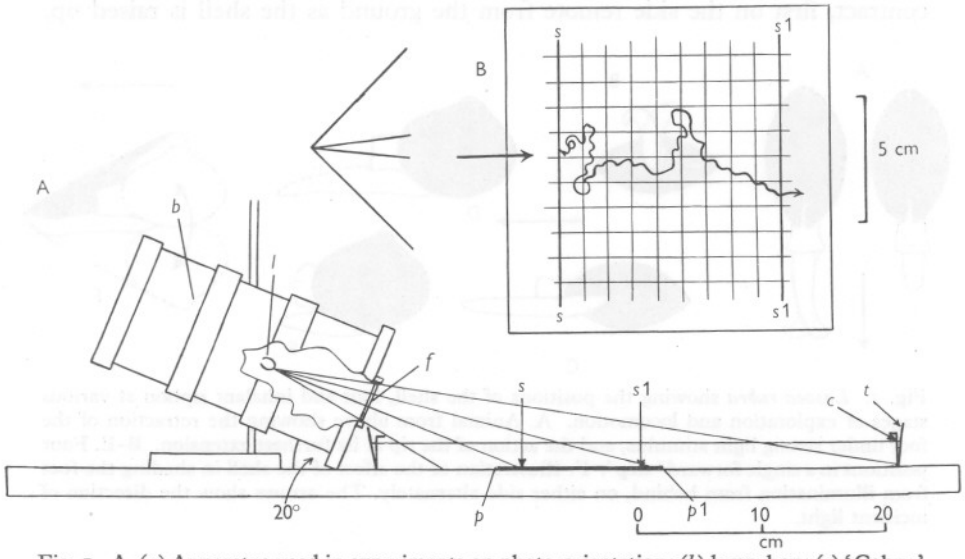

Fig. 1. A. (*a*) Apparatus used in experiments on photo-orientation; (*b*) lamp-box; (*c*) 'Cobex' dull surface lining sheet; (*f*) filter; *l*, light source of lamp; *p*–*p* 1, experimental glass plate; *s*–*s* 1, limits of experimental field; *t*, experimental trough. B. Experimental glass sheet showing the course of a trail, running across the gridded area. The arrow shows the direction of the light gradient falling off from *s* to *s* 1, and approximately uniform transversely. Also shown are the angles +45 to −45, and +5 to −5 used in the presentation of data.

MOVEMENTS

The method of locomotion is illustrated in Fig. 2. The foot forms a slender tongue, perfectly colourless and translucent, that can be thrust out a distance equal to the length of the shell. From the anterior end, above the foot, also emerges the inhalant siphon, a short stout tube, somewhat laterally compressed; its margin is plain or sometimes very slightly crenate, and the opening can be narrowed by the margin drawing in. The tip of the fully extended foot is slightly flattened against the substrate and narrowly spatulate; but this is the only suggestion of a 'sole' and when lifted free the whole

lower margin of the foot is sharp-edged and very narrow. A forward 'step' takes place in several stages (Figs. 2B–E). From the resting position with the shell lying on one side or the other, the foot is put out and freely moved about, being thrust from side to side and curved beneath the shell as its pointed tip appears to feel for purchase. It attaches to the ground by the keel-like ventral edge and the lower part of one side, which is almost opaque and possesses mucous glands and cilia (see Popham, 1940). As it becomes fully extended the main site of attachment seems to be the sharp ventral edge. With the foot so extended the heavy shell is drawn forward upon it and hauled into an upright position, ventral margin lowermost, as the retractor muscles of the foot contract, first on the side remote from the ground as the shell is raised up,

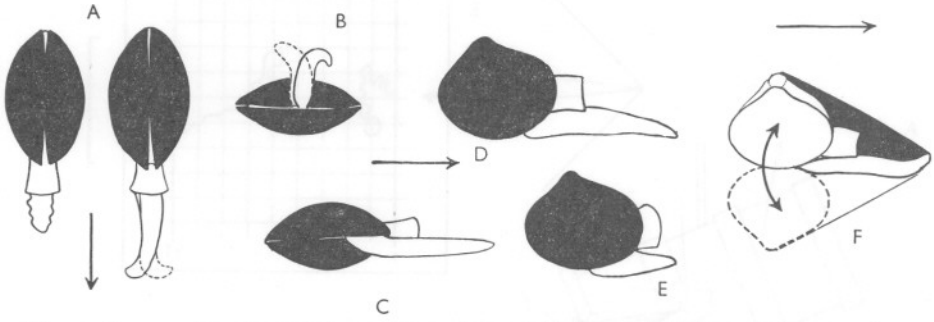

Fig. 2. *Lasaea rubra* showing the positions of the shell, foot and inhalant siphon at various stages of exploration and locomotion. A. Animal from above showing the retraction of the foot under strong light stimulus, and the action of the tip at its furthest extension. B–E. Four positions in a single forward 'step'. F. Illustration of the effect of the shell in shading the foot from illumination from behind, on either side alternately. The arrows show the direction of incident light.

and then on both sides as it is drawn sharply forward. On completion of each step the foot is very short, with an angled 'heel' behind and projects in front little more than the length of the siphon. The foot is almost immediately extended again for the next step and the shell slumps to one side or the other, being raised vertically once more at the ensuing foot contraction.

Lasaea rubra has no macroscopically differentiated light receptors, but either of the two parts of the body (foot or siphon) exposed in front could—it was suspected—be responsible for the light sense and for detecting bilateral differences in light intensity at successive points of time—that is, to serve as receptors suitable for the type of reaction called a 'klinotaxis' by Fraenkel & Gunn (1940). In *Mya* and *Cardium* the posterior siphon tips, being alone exposed at the surface, are photosensitive and bear rudimentary 'eyes'. In Pectinidae the whole mantle margin bears tentacles and eyes forming the sensory outstations of the body. In *Lasaea rubra* the siphon is short and relatively immobile. Though it is probably diffusely photosensitive, the main

exploratory role has passed to the tip of the foot, the anteriormost point of the body, which can, moreover, be freely moved about. During extension its tip can be slightly raised and curved tentatively to one side, then—after a momentary contraction—to the other. Rather more frequently after a movement to one side, the foot is partly or wholly withdrawn into the shell before emerging to make a new exploratory movement upon the other side.

By such side to side movements *L. rubra* can employ the foot as a direction receptor. The shell shades the sensitive region from light directly behind, so that relative intensity of light incidence at either side must be detected by lateral movements of the foot. With a strong light source in front of the animal, the exploratory activity of the foot is greatly intensified and on first emerging there is no attempt to grip the substrate; instead the tip of the foot is raised well clear of the ground and very freely moved about, the tip now extending narrowly, now being pulled back and contracted. Such movements may continue some time before the foot is planted and sustained forward progress is usually not attempted till the animal has swung away from the directly anterior light source.

A behavioural comparison can best be found in a very different animal, the dipteran maggot, which Fraenkel & Gunn (1940) would regard as the classic case of orientation by klinotaxis, 'a directed orientation made possible by means of regular deviations and involving comparisons of intensities at successive points in time'. The tiny pointed anterior end of the maggot has no well-defined photoreceptor. Variation in stimulation must occur as it is drawn in and covered by succeeding segments and then put out again. Thus, just as with the momentary contraction or withdrawal of the foot in *L. rubra* the 'photoreceptive region does not pass straight from one side position to the next, but goes through a series of light intensities which includes the comparative darkness of the contracted position'.

As the shell falls to one side or the other during extension of the foot, a wide shadowed zone is cast around the foot on that side (Fig. 2F). It was at first suspected that regular recumbence of the shell on one side and the other, with alternate steps, would in itself give a rough power of comparison of light intensities, making possible—as it were—a klinotaxis without lateral deviation by the foot. In Fig. 5 is shown a 20 min record of the movements of an animal away from a light source; it will be seen that there is no strictly regular alternation of the shell to right and left sides and that after slumping in one position, there is a tendency to return there for several steps. In addition, the time frequency of light comparison by the shading of the shell would be very low and much more sensitive comparison is in fact possible by the deviations of the foot. A shadowed zone on a particular side must, however, afford the animal a wider sector in which it can move transversely to the light path while still effectively shaded from direct light.

BEHAVIOUR IN DARKNESS: ORIENTATION BY LIGHT

When removed from its site and placed in the dark on a smooth flat surface, *L. rubra* is unable to orient, and crawls about to produce trails of three kinds: (i) about a third of the animals tested in the dark made a short trail of a few millimetres in length, after which they attached by the byssus thread and made no further movements; (ii) a smaller number produced trails that were difficult to follow exactly or to measure, since they were tightly convoluted with continual changes of direction; (iii) the majority produced a strongly convoluted and frequently looped trail, crawling over distances of up to 200 mm in the half-hour period of the experiment. These undirected movements have much in common with the dark behaviour described by Ullyott (1936) in the flatworm *Dendrocoelum*. They show under uniform conditions a certain basal frequency of turning, random in direction. The character of such trails can best be quantitatively represented by expressing against time the rate of change of direction (r.c.d.) in angular degrees per minute. When *Dendrocoelum* were placed in uniform non-graded light, the r.c.d. was found to increase with the intensity of light stimulation. With *Lasaea rubra* in the dark on a smooth surface, we must regard the deprivation of lateral contact as constituting in itself, as illustrated by trails of type (iii), a stimulus of intensity sufficiently high as to produce a r.c.d. equivalent to that shown by animals in light. The character of type (iii) trails and the amount of turning they show do not appreciably alter from the dark condition after the provision of uniform non-direction light (Fig. 3). Ullyott found that *Dendrocoelum* became 'adapted' to the stimulus of light, i.e. the r.c.d. at a given level of illumination fell off with time. With *Lasaea rubra* in the dark, though the stimulus of deprivation of lateral contact appears as high as that provided by light, adaptation was seldom seen: the r.c.d. was not in general lower after 30 min than at the beginning of an experiment. (The high peaks shown in Fig. 3 (1), for a trail made in directional light, fall well within the range shown by many dark trails.)

With uniform ungraded lighting, from the light source of 9·2 candelas, 20 cm vertically above, diffused by a sheet of opal glass, *L. rubra* showed few

Legend to Fig. 3.

Fig. 3. A. Rose diagrams showing the relative extent of trails produced at various angles: (*a*) aggregated results of experiments on flat surface in the dark; (*b*) results of experiments with a non-directional light gradient, illumination falling from right to left, showing klinokinesis with adaptation. B. Traced records of representative trails produced: (left) on a level surface in the dark, with examples of types (ii) and (iii) referred to above; (right) in experiments using a non-directional light gradient, illumination falling from right to left. C. Histograms showing the characteristics of various types of trail over a 30 min period: (1) in a gradient of lateral light, falling from 9·2 lux; (2) on a level surface in the dark; (3) with uniform light incident from above; (4) climbing a slope of 30° from the horizontal in the dark. Ordinate: degrees angular change of direction; abscissa: time in minutes.

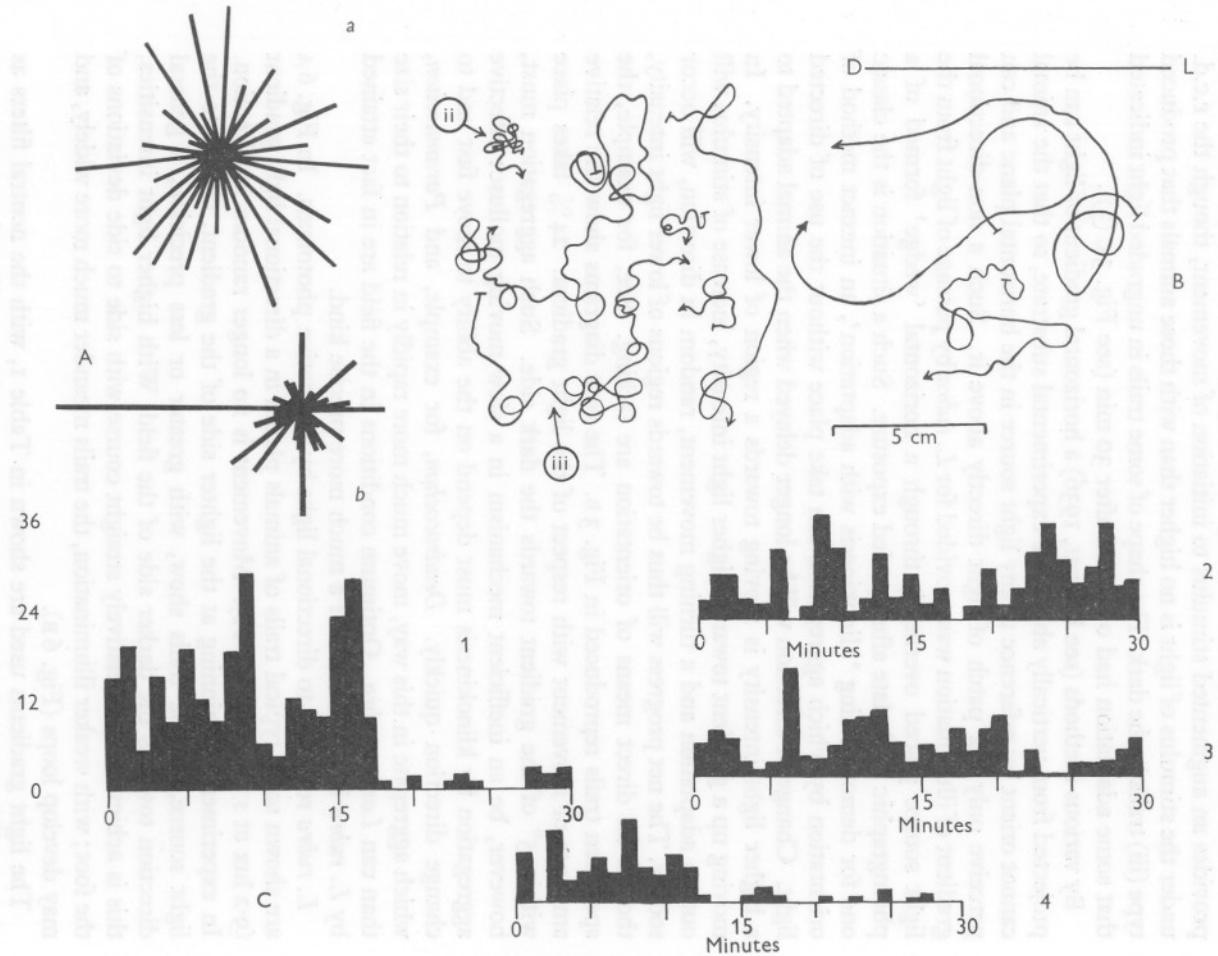

Fig. 3. For legend see opposite page.

or none of the trails of types (i) and (ii); all the animals used were highly active and made long, meandering and looping trails of type (iii). Light provides an augmented stimulus to initiation of movement, though the r.c.d. under the stimulus of light is no higher than with those animals that produced type (iii) trails in the dark. The shape of some trails in ungraded light indicated that some adaptation had occurred after 30 min (see Fig. 3 C (3)).

By various methods (see Ullyott, 1936) a horizontal gradient of light can be projected from vertically above the experimental substrate, so that the animal cannot orient by reference to any light source in the horizontal plane and can perceive only the patch of light directly above it. Such a non-directional gradient of illumination was provided for *L. rubra* by passage of light from the light source placed overhead, through a horizontal 'wedge' formed of a photographic half-plate after graded exposure. Such a situation is the classic one for demonstrating 'klinokinesis with adaptation', an inexact method of orientation by which aggregation can take place without the use of directed light. Change of direction will be longer delayed when the animal adapted to a higher light intensity is moving towards a region of lower intensity. In moving up a gradient towards higher light intensity, increase of stimulus will outrun adaptation and a turning movement, random in direction, will occur sooner. The net progress will thus be towards regions of lower light intensity, though all direct means of orientation are lacking. See, for example, the specimen trails reproduced in Fig. 3 B. The rose diagrams show the relative amounts of movement with respect of the light gradient. 24% takes place within 5° of the gradient towards the dark side. Such aggregation must, however, be an inefficient mechanism in a slow-moving mollusc: effective aggregation by klinokinesis must depend on the ability to move fast and to change direction quickly. *Dendrocoelum*, for example, and *Paramaecium*, which aggregate in this way, move much more rapidly in relation to their size than can *Lasaea rubra*. Optimum conditions in the field are in fact attained by *L. rubra* by orientation of a much more precise kind.

L. rubra responds to directional light by a negative phototaxis. In Fig. 6 A are shown some typical trails of animals placed in a directional light gradient (9·2 lux at *s* to 4·1 lux at *s*1). Movement is no longer random in direction. In experiments beginning at the lighter side of the gradient, closest to the light source, all the trails show, with greater or less precision, a general direction towards the darker side of the field. With higher light intensities, this is achieved by a relatively straight course with side to side deviations of the foot; with weaker illumination, the trails meander much more widely, and may develop loops (Fig. 6 B).

The light gradients used are shown in Table 1, with the neutral filters as shown. Efficiency of orientation was determined by totalling for each experiment the distances crawled by all the animals used, at various angles to the direction of the light gradient. The results are expressed in the rose diagrams

(Fig. 4) from which the data shown graphically could be further calculated. In the graphs, two sets of values are plotted; for each light intensity the percentage of the total linear movement represented by parts of the trails between $+5°$ and $-5°$ with the line of the light gradient, and similarly the percentage of trails formed at angles of between $+45°$ and $-45°$ with the line of the gradient. With similar rose diagrams (Fig. 3) the randomness of movement in the dark, and movement in a non-directional gradient have already been shown. In these calculations initial or intermediate parts of the trails showing small loops or continued alteration of direction, without sustained directional movement for a length of 1 cm, were disregarded.

TABLE 1. ILLUMINATION WITH THE RANGE OF NEUTRAL FILTERS USED

Density of filter*	Transmission	Illumination at *s* (lux)	Illumination at *s* 1 (lux)
0·1	0·79	7·26	3·23
0·2	0·63	5·79	2·58
0·3	0·50	4·60	2·05
0·59	0·26	2·40	1·02
0·98	0·10	0·92	0·41
2·06	0·0087	0·08004	0·03567
3·0	0·001	0·0092	0·004
4·02	0·000095	0·00087	0·00038
4·8	0·000016	0·000138	0·000060

Illumination of full moon on a clear night 0·2 lux
Illumination of a moonless night 0·0003 lux } (le Grand, 1948)

* All densities referred to here are optical densities $\left(\log_{10} \frac{\text{light incident}}{\text{light transmitted}}\right)$.

The trails made at higher light intensities undoubtedly represent klinotactic orientation; but it is not easy to mark an arbitrary distinction between these and the trails at lower intensities, some of which are better regarded as klinokinetic, with random changes of direction achieving orientation by adaptation. The loss of randomness is with increasing stimulation obviously gradual, and it is this power to make regulated deviations in relation to the direction of the gradient, followed by appropriate response, that marks the attainment of klinotaxis. A further calculation reveals what may be described as the 'inefficiency ratios' in such klinotactic and klinokinetic orientation: the total length of the trail crawled was divided by the progress achieved, i.e. the length of the straight line joining the initial and end points of the trail, regardless of its angular direction, and the result expressed logarithmically. With improving orientation at the higher light intensities this will approach zero, though such a value is never attained with photo-orientation alone.

Some measure of the amount of activity of the animals at the various light intensities was obtained by averaging the total length of all the trails obtained with each illumination level, reduced to unit time of 30 min. Too great precision should probably not be assigned to values so derived. They fall

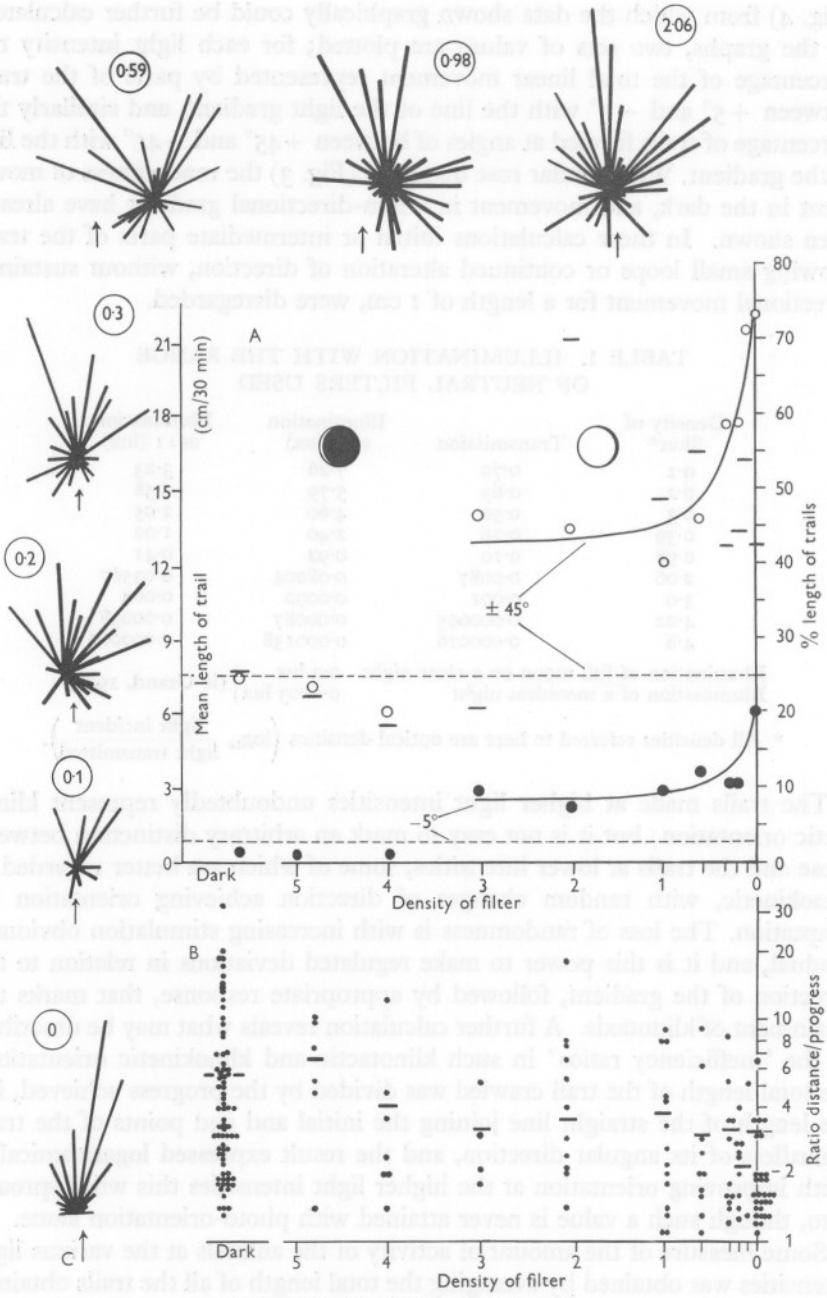

Fig 4 For legend see opposite page.

into two groups. The lower group, at light intensities below 0·01 lux, have a lower total activity, not much greater than that in the dark. Activity strongly increases with illumination from 0·1 lux upwards, with, however, a tendency to fall back at the highest levels of illumination; this last finding is an expression of the greater directness of the trail and the smaller expenditure of wide meanders and loops, at higher light intensities.

Efficiency of orientation, as assessed by the results in Fig. 4, shows three levels. First, using a 9·2 lux illumination, with transmission reduced to one ten-thousandth and below, there is no orientation. Movement at angles within 45° of the light gradients is no better than the random value to be expected with animals in the dark (in one experiment involving only a small number of trails the value falls somewhat below the theoretic random). Above this level of illumination there is a threshold where the values for orientation with the 45° and 5° angles show a strong improvement upon the random level. Orientation is now possible away from the light source and down the gradient; but as shown by their high inefficiency factor, and their form in Fig. 6 B, the trails are of the meandering and looping sort characteristic of a klinokinesis. With an illumination of above 2 lux, orientation finally achieves the character of klinotaxis; trails are much more direct or with smaller meanders (see Fig. 6 A). The curves for orientation both within angles of 45° and 5° show a steep upward trend, towards the values obtained with 9·2 lux. On the graph of Fig. 4 are inserted the values for the illumination of a moonless night and a fine night with full moon (see le Grand, 1948). Photo-orientation is clearly impossible on a moonless night; the threshold for klinokinetic orientation lies at roughly half the illumination of full moon, while the characteristics of the more precise klinotactic orientation are revealed only at illuminations of a twilight order and above.

Legend to Fig. 4

Fig. 4. Results of experiments on photo-orientation. A. Graphs showing the percentages of the total lengths of trails formed between angles of $+5°$ and $-5°$ with the line of the light gradient [●—●]; and between angles of $+45$ and $-45°$ to the gradient [○—○]. On the abscissa are represented the values for log light transmission, used in the various experiments, 0 corresponding to an illumination of 9·2 lux. The broken horizontal lines show the theoretical values for random movement as on a level surface in the dark, without orientation. The intensity of illumination on a moonless night and on a clear night with full moon is indicated by symbols on the graph (see le Grand, 1948). The short horizontal lines for each illumination represent the mean length of trail formed in 30 min in the aggregated experiments (see left ordinate), and thus give a measure of the level of activity. B. Scatter diagram showing for individual trails at various illuminations the value for the 'inefficiency factor' of distance crawled/progress achieved. Small cross-bars represent mean values. C. Rose diagrams (at the left and upper margins) showing the relative extent of the trails produced at the varying angles from the direction of the light gradient, for different levels of illumination. The illumination is represented by the values in small circles for the filter density (= minus log transmission). The light gradient falls off in the direction of the arrows.

GRAVITY RESPONSES

Efficiency of orientation greatly improves if the substrate is inclined. *Lasaea rubra* is strongly negatively geotactic, and as the angle of the experimental glass sheet was inclined more steeply from the horizontal, the trails (i) became more direct and meandered less and (ii) increasingly took the steepest path.[1]

In moving up a sloping surface, *L. rubra* attaches itself securely by two means: the viscid mucus of the foot is generally sufficient to give it a hold as effective as that of a snail on a steep surface; while at the steepest inclinations, 50° or more from the horizontal the byssal gland secretes a 'safety-line' which is fastened to the substrate at intervals of about 1·5 cm. This is thin and strong, looking like a strand of spider web, laid down parallel to the mucous trail of the foot, and crossing it occasionally if the latter should slightly meander. If the mollusc should then fall off from a steep surface, it will swing freely, still attached by the short available length of free byssus line, instead of falling the considerable distance to the bottom of the slope. By taking contact with the substrate it can at once resume crawling a centimetre or two below the point it had last reached. Such accidents are clearly recorded on the trails as short parallel lengths of double track (see Fig. 5). This safety device must be responsible for the animal's surprising freedom to wander out of crevices and to exist as a freely mobile lamellibranch on rock faces strongly exposed to wave action. Several sorts of bivalve can crawl along the surface; but few can be so apparently defenceless against wave battering and dislodgement as *L. rubra*. The safety line may also be secreted on a flat surface, though it is here much less distinct and, especially where the trails meander considerably, it is not often seen.

In experiments on inclined surfaces in the dark the experimental glass plate was sloped at 10° intervals, at angles of 10°–90° from the horizontal. The efficiency of orienting at 90° to the horizontal edge of the plate was estimated with two curves, as for the light response. The curves of Fig. 5 agree well with the curve of Hovey (1928), expressing the mean angle of upward orientation against the angle of inclination of the substrate. Even at the lowest angle of inclination, *L. rubra* has an orienting efficiency equivalent to that obtained with the highest light intensity used on the flat; and as the angle is increased this efficiency improves greatly. The trails run more directly up the slope; the meanderings are altogether eliminated, so that with slopes of 50° and above, undeviating straight trails were generally obtained, running straight up from the lower edge of the glass plate. Full efficiency is attained at 50°–60° inclination of the slope and thereafter little improvement

[1] See Fraenkel & Gunn (1940) for discussion of the view that efficiency of orientation on sloped surfaces in many animals is due to postural reflexes, with the use of the statocysts, avoiding the tendency to roll if the angle of ascent of 90° with the horizontal is too far departed from.

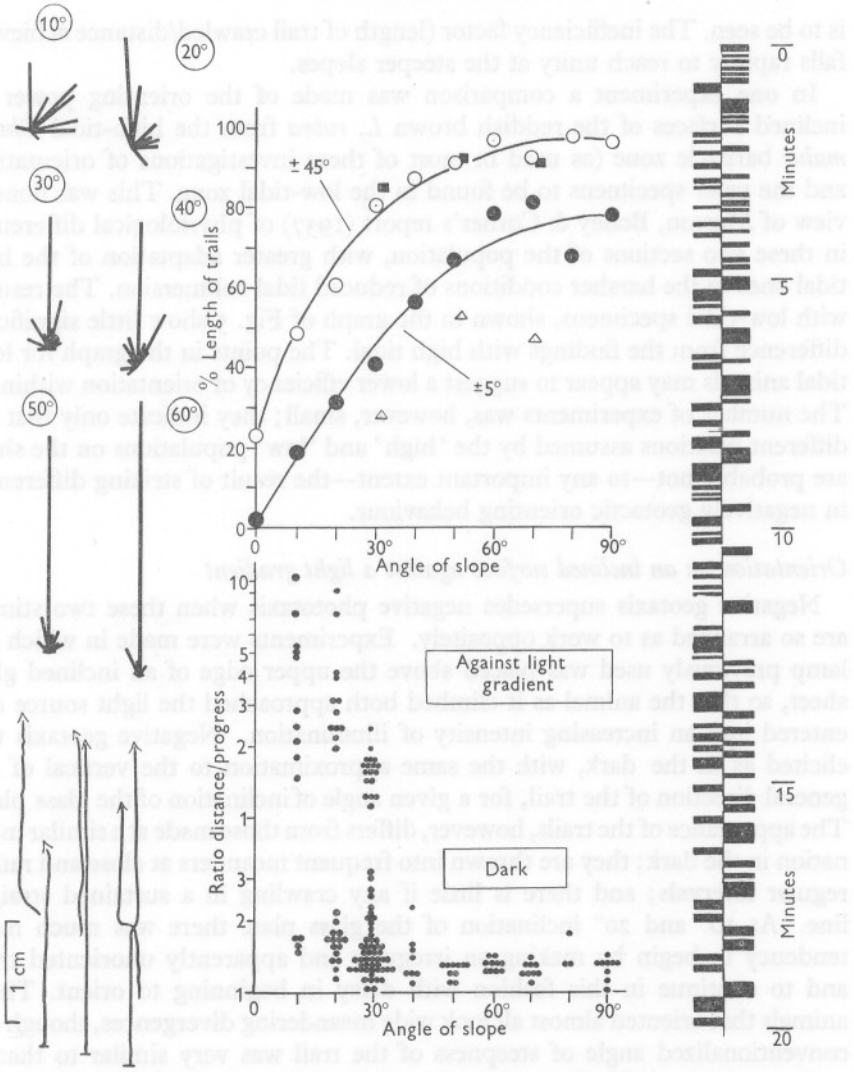

Fig. 5. Graphs showing results of experiments on orientation upon inclined surfaces. Above. Percentages of the total lengths of trails formed between angles of $+5°$ and $-5°$ to a line running directly up the slope [●—●], and between angles of $+45°$ and $-45°$ to such a line. [○—○]. The circles represent experiments with high-tidal animals which were used for most of the work; the triangles and squares show values for the few experiments with animals from low tide. Below. Scatter diagram showing for individual trails in various experiments the values for the 'inefficiency factor' of distance crawled/progress achieved. The lower points are for inclined surfaces in the dark; the upper points are for trails made on inclined surfaces by animals crawling against a light gradient. *Left.* Rose diagrams showing for various angles of inclination of the surface the relative amounts of trail produced at angles to a line running directly up the slope. *Left below.* Portions of trail produced on inclined surfaces after the animal had fallen from the glass plate and—retained by its byssal mooring line—had commenced a fresh trail. *Right.* A record of 20 min movement by a single *Lasaea rubra*, showing the periods of subsidence of the shell alternately to left and right, between 'steps' (see p. 9).

is to be seen. The inefficiency factor (length of trail crawled/distance achieved) falls rapidly to reach unity at the steeper slopes.

In one experiment a comparison was made of the orienting power on inclined surfaces of the reddish brown *L. rubra* from the high-tidal *Chthamalus* barnacle zone (as used in most of these investigations of orientation) and the paler specimens to be found in the low-tidal zone. This was done in view of Morton, Boney & Corner's report (1957) of physiological differences in these two sections of the population, with greater adaptation of the high tidal ones to the harsher conditions of reduced tidal submersion. The results, with low-tidal specimens, shown in the graph of Fig. 5 show little significant difference from the findings with high tidal. The points in the graph for low-tidal animals may appear to suggest a lower efficiency of orientation within 5°. The number of experiments was, however, small; they indicate only that the different positions assumed by the 'high' and 'low' populations on the shore are probably not—to any important extent—the result of striking differences in negatively geotactic orienting behaviour.

Orientation on an inclined surface against a light gradient

Negative geotaxis supersedes negative phototaxis when these two stimuli are so arranged as to work oppositely. Experiments were made in which the lamp previously used was placed above the upper edge of an inclined glass sheet, so that the animal as it climbed both approached the light source and entered into an increasing intensity of illumination. Negative geotaxis was elicited as in the dark, with the same approximation to the vertical of the general direction of the trail, for a given angle of inclination of the glass plate. The appearance of the trails, however, differs from those made at a similar inclination in the dark; they are thrown into frequent meanders at close and rather regular intervals; and there is little if any crawling in a sustained straight line. At 10° and 20° inclination of the glass plate there was much more tendency to begin by making an irregular and apparently unoriented trail, and to continue in this fashion with delay in beginning to orient. Those animals that oriented almost all took wide meandering divergences, though the conventionalized angle of steepness of the trail was very similar to that of trails on correspondingly steep surfaces in the dark. The inefficiency factor of length of trail crawled/distance achieved was, however, considerably increased. With animals climbing a 30° slope against the light, a few trails were relatively straight and unconvoluted; the majority meandered continuously, though with shorter and more regular divergences than at 10° and 20°. The path followed by these trails shows that the animal never crawled for long directly towards the light source, nor was either of its sides continually exposed for more than a short time to the full incidence of lateral light. The frequent changes of direction enabled the previously exposed side to be regularly shaded. The side for the time being uppermost, i.e. exposed to full lateral illumination could

not, however, be shaded from light by the shell as is possible on the flat. The heavy weight of the shell and body in climbing will always tend to slump downwards over the side already away from the light.

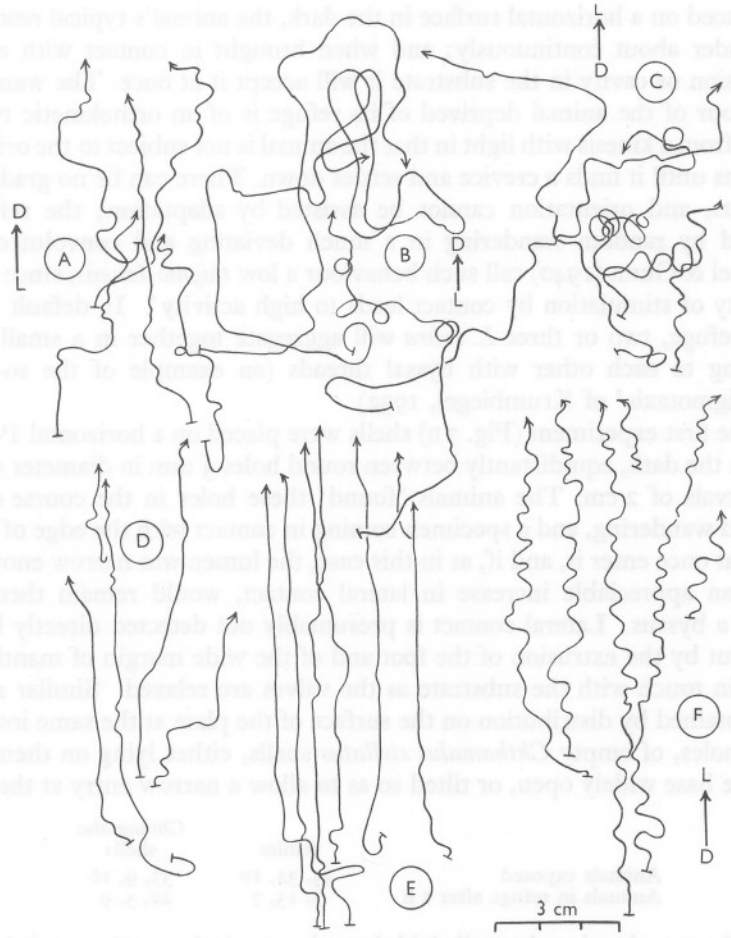

Fig. 6. Representative specimen trails with various orienting stimuli. For each group the direction of the stimulus producing orientation would be represented by a line running directly up the page. (Rather more than half natural size). A. Photoklinotactic trails in a gradient of illumination falling from 9·2 to 4·1 lux over 9 cm. B. Photoklinokinetic trails in a gradient of illumination falling from 0·080 to 0·036 lux over 9 cm. C. Trails produced in climbing a surface inclined at 30° to the horizontal, *towards* an illumination of 9·2 lux at the end-point. D. Trails produced in climbing a surface inclined at 30° to the horizontal, in the *dark*. E. Trails produced in climbing a surface at 60° to the horizontal in the *dark*. F. Trails produced in climbing a surface inclined at 30° to the horizontal, *towards* an illumination of 9·2 lux at the end-point.

RESPONSES TO LATERAL CONTACT

The normal habit of *Lasaea rubra* is to nestle in a narrow crevice or depression affording it a maximum of lateral contact. When removed from such contact, and placed on a horizontal surface in the dark, the animal's typical reaction is to wander about continuously; and when brought in contact with a small depression or cavity in the substrate it will accept it at once. The wandering behaviour of the animal deprived of its refuge is of an orthokinetic type; it differs from a kinesis with light in that the animal is not subject to the orienting stimulus until it finds a crevice and settles down. There can be no gradient of stimulus, and orientation cannot be assisted by adaptation; the refuge is attained by random wandering in a much deviating and convoluted trail. Fraenkel & Gunn (1940) call such behaviour a low *thigmo-kinesis*, since 'a low intensity of stimulation by contact leads to high activity'. In default of any other refuge, two or three *L. rubra* will aggregate together in a small heap, attaching to each other with byssal threads (an example of the so-called 'idiothigmotaxis' of Krumbiegel, 1932).

In the first experiment (Fig. 7 B) shells were placed on a horizontal Perspex plate in the dark, equidistantly between round holes 3 mm in diameter drilled at intervals of 2 cm. The animals 'found' these holes in the course of unoriented wandering, and a specimen coming in contact with the edge of a hole would at once enter it, and if, as in this case, the lumen was narrow enough to afford an appreciable increase in lateral contact, would remain there and secrete a byssus. Lateral contact is presumably not detected directly by the shell, but by the extrusion of the foot and of the wide margin of mantle that comes in touch with the substrate as the valves are relaxed. Similar results were obtained by distribution on the surface of the plate at the same intervals as the holes, of empty *Chthamalus stellatus* shells, either lying on their sides with the base widely open, or tilted so as to allow a narrow entry at the base.

	Holes	*Chthamalus* shells
Animals exposed	33, 32, 10	33, 9, 16
Animals in refuge after 1 h	8, 15, 7	22, 5, 9

From Fig. 7 B, showing the trails laid down in a typical experiment, it is clear that of those animals in refuges, all had reached their shell or hole without previously touching any other; every animal once within a refuge had stayed there, and that those remaining without refuge had never in the course of the experiment made contact with a shell or a hole.

At the point of entry into a randomly found hole, a short vertical descent is necessary from the flat surface. This would appear to be of a thigmotactic nature (as distinct from random thigmokinesis): contact with the concave interior of the hole guides the animal during its deeper entry into the refuge. Such a thigmotaxis will reverse the normal negative geotactic response.

A similar change of the general behaviour pattern in response to irregularities in the substrate has already been described by Fraenkel (1927) for the high-tidal periwinkle *Melaraphe neritoides*. This snail passes through transverse crevices on its upward climb, crawling into the bottom of a crevice and emerging by crawling out upside-down along its roof. The normally negative phototaxis is here reversed.

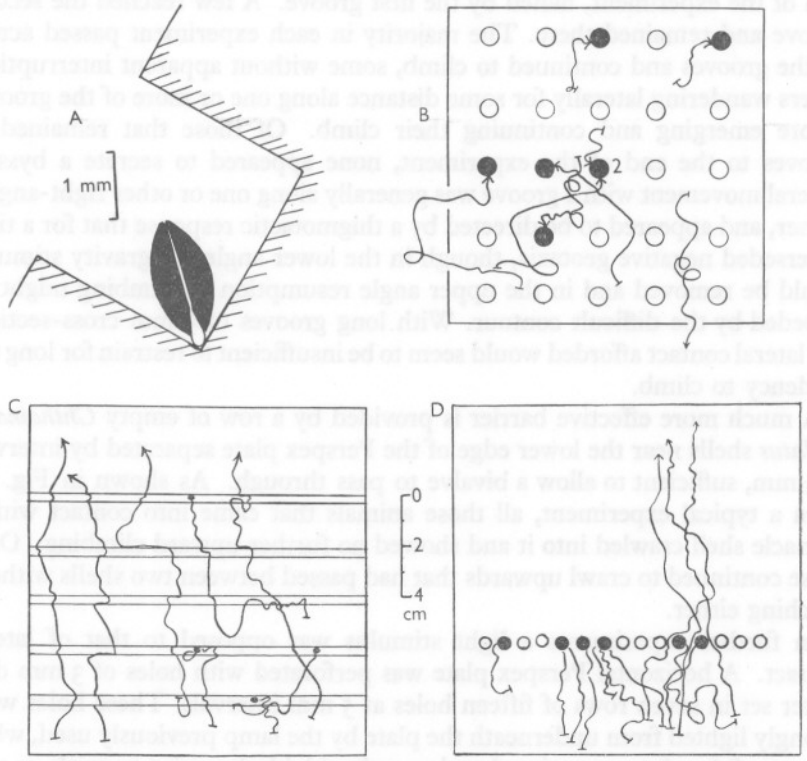

Fig. 7. A. Diagram showing typical position assumed by *Lasaea rubra* crawling along a groove cut in a Perspex sheet, with the maximum thigmotactic stimulus afforded by either of the angles. B. Diagram showing the trails of six *L. rubra* that have arrived at a refuge, crawling in the dark, with random change of direction over a smooth, level surface studded at regular intervals with holes. Black circles represent occupied holes. C. Representative trails of *L. rubra* climbing up a Perspex sheet inclined at an angle of 30° and with its surface interrupted at intervals by transverse grooves. D. Representative trails of *L. rubra* climbing a glass sheet inclined at an angle of 30°, with a barrier of a line of *Chthamalus* barnacle shells. Those animals that made contact with a barnacle were arrested and took refuge in the shells shown as black circles. Those that continued to the top of the sheet had made no contact with barnacle shells.

Thigmotaxis opposed to the stimuli of light and gravity

The lateral contact provided by a crevice or a depression will normally in *Lasaea rubra* supersede the usual responses to both light and gravity.

Experiments were made with Perspex sheets, inclined in the dark at an angle of 30° to the horizontal. The upward path of the negatively geotactic animals was interrupted at intervals of 2 cm by four transversely cut horizontal grooves, of square cross-section and measuring 3 mm along the side. Animals were placed in a row at the lower edge of the plate and allowed to climb. In each of the six experiments, a small proportion of animals were, for the 1 h duration of the experiment, halted by the first groove. A few reached the second groove and remained there. The majority in each experiment passed across all the grooves and continued to climb, some without apparent interruption, others wandering laterally for some distance along one or more of the grooves before emerging and continuing their climb. Of those that remained in grooves to the end of the experiment, none appeared to secrete a byssus. Lateral movement with a groove was generally along one or other right-angled corner, and appeared to be directed by a thigmotactic response that for a time superseded negative geotaxis, though in the lower angle the gravity stimulus would be removed and in the upper angle resumption of climbing might be impeded by the difficult contour. With long grooves of 3 mm cross-section, the lateral contact afforded would seem to be insufficient to restrain for long the tendency to climb.

A much more effective barrier is provided by a row of empty *Chthamalus stellatus* shells near the lower edge of the Perspex plate separated by intervals of 3 mm, sufficient to allow a bivalve to pass through. As shown in Fig. 7D from a typical experiment, all those animals that came into contact with a barnacle shell crawled into it and showed no further upward climbing. Only those continued to crawl upwards that had passed between two shells without touching either.

In further experiments a light stimulus was opposed to that of lateral contact. A horizontal Perspex plate was perforated with holes of 3 mm diameter set in seven rows of fifteen holes at 5 mm intervals. These holes were strongly lighted from underneath the plate by the lamp previously used, while the rest of the plate was painted underneath with black marine-varnish, so that the experimental surface on top of the plate was kept dark. A thin glass plate was placed beneath, to provide a transparent floor for the holes. Forty-five animals were randomly distributed on the lower half of the plate and a low stimulus of directional light was provided, much weaker than light in the holes. Under this stimulus the animals moved with wide meandering trails across the plate towards the area of the holes. After 8 h, out of a total of forty-five animals, thirty-four were within holes, a proportion very comparable to those that found refuge in unlighted holes or barnacle shells. Contact with the edge of a hole would appear to release a thigmotactic behaviour resulting in entry to the hole regardless of the opposing stimuli of light or gravity.

In another experiment a horizontal Perspex sheet was set up in the dark,

varnished black underneath, and pierced with forty holes 3 mm across. Two *L. rubra* were placed in each. The plate was inspected after an hour in the dark and the animals that had emerged from holes were counted and replaced. The holes were then brightly illuminated from beneath and the plate inspected again after a further hour.

Number of animals originally in holes	80
Number emerged after first hour dark and then replaced	15
Number emerged after second hour lighted	9

Of the dominance of thigmotaxis over phototaxis we have already an example in the literature, the short study of the responses of newly settled *Hiatella arctica* spat by Hunter (1949); here the animals settled in crevices provided by glass slides fixed together at an angle, even though these crevices were the most brightly lighted spots.

DISCUSSION: MAINTENANCE OF POSITION ON THE SHORE

The movements and orientation of *Lasaea rubra* from the *Chthamalus stellatus* zone, as studied here, appear to be directed to securing and maintaining the position of the population on the upper shore, and within that level to acquiring crevices, empty barnacle shells or other lodgements protecting the animals from detachment by waves and currents and from desiccation and the actions of direct light. As a dimyarian eulamellibranch of 'normal'-looking ovoid shape, *Lasaea rubra* would at first appear to be strikingly little adapted for life on wave-exposed rock faces. It has made none of the sacrifices to semi-permanent byssal attachment with alteration of symmetry undergone, for example, by the Mytilidae. Its first advantage is its minute size (see Morton, Boney & Corner, 1957). Its shell, moreover, when viewed edgewise is lentiform and well streamlined against impact of moving water.

Lasaea lacks the frail shell and obese appearance of its relative *Kellya suborbicularis*, which is much more confined to low-tidal crevices and much less peripatetic; and there is no suggestion of the wedge-shaped shell found in the burrowing Erycinacea such as *Montacuta ferruginosa*. The *Lasaea* shell is indeed of an ideal construction for lying submerged in, and filtering from the merest film of splash. There are three other important adaptive features, an extensible foot that can provide viscous attachment to the substrate, a byssal gland that can secrete an attachment line where needed, and a forward facing inhalant siphon, serving as an anteroceptor.

L. rubra may from time to time creep about on open rock surfaces and can readily alter its fixed position. In spite of the precaution of a safety line, the result of movements of disturbed water would tend to carry dislodged shells downshore. The dominant orientation reaction shown by *L. rubra* when removed from a crevice is that of negative geotaxis, by which it will climb even against a gradient of light to which at other times it would respond

negatively. Gravity must be the most continuously sustained stimulus to which *L. rubra* is exposed when out of its lodgement; and it is the stimulus most efficiently responded to, forming, so to speak, the 'coarse adjustment' mechanism, for large-scale maintenance of position. Negative phototaxis is effective only on relatively level surfaces in the absence of a strong gravity stimulus. It serves as an alternative coarse adjustment mechanism, not with the same precision as the gravity response, but nevertheless with the efficiency of a klinotaxis at the illumination of a moonlit night. Such a reaction should produce aggregation in the darkest places which will be crevices or barnacle shells or depressions suitable for nestling. The lowest threshold for photo-orientation is somewhat higher than the illumination level of a night without moonlight; and at the lower levels of detectable light, orientation is chiefly by the inefficient mechanism of klinokinesis. A low level thigmokinesis produces random wandering with very convoluted trails in the absence of a light or gravity stimulus; and once the edge of a narrow depression or crevice is randomly located, a thigmotactic response carries the animal into its narrow confines, even in opposition to the normal responses to gravity and light. Thigmotaxis is a very strong response in *L. rubra* and provides the 'fine adjustment' mechanism by which a suitable crevice is ultimately secured. Preference for lateral contact, and perhaps in part avoidance of light, may constitute a 'token stimulus' leading the animal to a place of shelter whose real advantages are, however, protection from dislodgement by waves or currents, from desiccation in a dry atmosphere and perhaps from predation.

L. rubra is seldom found singly but usually in clusters of two or three large shells with many half grown or newly born beside them. This aggregation may represent a special case of thigmotaxis; but another mechanism must be considered in the Erycinacea, that of chemotaxis. The anterior position of the inhalant siphon offers a preadaptation for direction finding by chemotaxis, and orientation towards other animals may be a rather regular faculty of the commensal Erycinidae and Galeommatidae. In *Montacuta ferruginosa* with *Echinocardium cordatum* there is a well-developed chemotaxis towards the host. I shall describe this elsewhere. In the non-commensal *Lasaea rubra* there is another possibility of chemo-orientation, an 'homoio-chemotaxis' assisting the animals to find their near neighbours at the final stage of aggregation. A hundred or so animals scattered in a Petri dish of sea water overnight will usually resolve themselves in up to a dozen heaps attached together by the byssus. In the investigation of light and gravity orientation, it was first necessary to know to what extent, if at all, the movements of *L. rubra* were influenced by the detection of others of their kind at close range. From a study of all the recorded trails, with animals distributed at 2 cm apart or more, there was no significant evidence of influence of one animal on another. Trails on a dark, a lighted, or an inclined surface, presented the same apparent randomness with respect to any other animal as they did with respect to a

given compass direction when a single animal was studied alone. In the light and gravity experiments reported here any effect of the animals upon each other appeared to be quite subdued by the stronger orienting stimulus. Yet with animals spaced as closely as 1 cm apart, there appeared instances of aggregation that seem anomalous on any theory of complete randomness. For example, in one experiment with a perforated Perspex sheet, animals were found to aggregate in one hole in a group of as many as seven, in others in fours and threes. Statistical investigation of the randomness of aggregation at very short distances must, with the general subject of chemotaxis in Erycinacea, be reserved to a later paper.

L. rubra of the low tidal zone appeared to show an essential similarity in their orienting behaviour, including that of negative geotaxis, with the high tidal ones. Low tidal *L. rubra* are less well adapted to withstand the intermittence of submersion of the upper shore, and it may be these physiological disabilities that set an upper limit to the distribution of the pale forms. Whether there may be genetical differences in the two sections of the population is not known. The lower limit of the upper shore population would tend to be maintained by their upward crawling tendency. The upper limit, about that of the upper barnacle line, must be imposed by the short duration of submersion there, with the low humidity of the atmosphere and the difficulty of crawling for any great distance on a dry substratum. *L. rubra* in the laboratory will sometimes crawl out of water on to a damp surface but never out of a saturated atmosphere. Its upper limit is quite sharply marked with no gradual falling off either in numbers or in mean size. Morton *et al.* (1957) have already mentioned the ecological advantages of a high level shore habitat to a mollusc of warm temperate distribution, reaching the northern limit of its range in Great Britain, and breeding in summer. It appears to prefer the maximum warmth of the sun on the substrate, consistent with the avoidance of desiccation; to both these requirements, the pattern of its orienting behaviour effectively contributes.

It is a pleasure to record my appreciation of the kindness and help given by the Director of the Plymouth Laboratory, Dr F. S. Russell, F.R.S., and by his staff, especially Dr E. J. Denton, for whose ready assistance with apparatus and discussion I am greatly indebted.

SUMMARY

The responses of the small bivalve *L. rubra* to light, gravity and lateral contact have been studied in relation to its orientation and maintenance of position on the shore. In the dark, on a flat surface, or in uniform light, *Lasaea* crawls freely about with meandering unoriented trails. At about half the illumination of full moon, orientation by klinokinesis with adaptation becomes

possible, rising by twilight to a negative photoklinotaxis by which darker crevices and refuges are found fairly directly. On a sloping surface *Lasaea* will crawl upwards, more directly as the slope is increased, and its negative geotactic behaviour will supersede negative phototaxis, so that it will crawl upwards even against a light gradient. As soon as chance contact is made with a small hole or crevice, an empty barnacle shell, or a groove affording lateral contact, both the light and gravity reactions are superseded: the animal will crawl downwards into a hole or enter it against a gradient of bright light from a dark surface. It is suggested that in the natural habitat negative geotaxis provides a coarse adjustment to the securing and maintenance of position, while the reactions to light and lateral contact give an increasing precision in the securing of shelter with protection from wave action, light and desiccation.

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SPECTRAL COMPOSITION OF THE LIGHT OF THE LANTERN-FISH, *MYCTOPHUM PUNCTATUM*

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(Text-figs. 1–3)

Relative spectral emission curves are available for the lights of many invertebrate animals, but none exists for fish. It seems likely that many bony fish use their photophores to signal to one another. Information about the spectral composition of fish luminescence is desirable to permit comparisons with the spectral sensitivities of fish eyes, to enable calculations to be made of luminous intensities, and to allow estimates to be made of the rate of attenuation of such lights in sea water. To further these ends, measurements were made of the spectral composition of the luminescence of the lantern-fish, *Myctophum punctatum*.

MATERIAL AND METHODS

The lantern-fish *Myctophum punctatum* Rafinesque is a pelagic species that executes diurnal migrations to the surface at dusk. Specimens were collected by R.V. 'Sarsia' at three stations: 46° 57′ N., 60° 58′ W., 4 June 1959; 46° 42′ N., 6° 09′ W., 5 June 1959; 47° 01′ N., 5° 55′ W., 7 June 1959. The fish were attracted to the side of the ship at twilight by means of a floating lamp submerged just below the surface. They were caught in hand-nets and transferred to a container of sea water on deck. Myctophids do not survive long in captivity, and the animals were used as soon as possible after capture.

Myctophids possess a series of photophores on the lower surface of the head and trunk. These light up spontaneously, and bright luminous flashes are also evoked by tactile stimulation of the fish.

The spectral composition of the light was determined by means of coloured filters and photomultiplier tubes (Fig. 1). The fish was placed in a black vessel which fitted into a black box. Above the box was a round aperture. Over this aperture was placed a thin cover-glass at 45° to the vertical axis. One photomultiplier (vertical) was placed directly over the cover-glass and the aperture; a second photomultiplier (horizontal) was placed at right angles to the vertical axis at one side of the cover-glass so as to catch reflected light from the latter. Between the cover-glass and the photomultiplier there was a disc, capable of being rotated, and containing a series of coloured filters which

could be interposed between the cover-glass and the vertical photomultiplier. The vertical photomultiplier detected the light which passed through the filters; the horizontal photomultiplier monitored the light intensity for each recording made with the other photomultiplier.

The photomultipliers were E.M.I., types nos. 6685 (vertical) and 6260 (horizontal). Both types are most sensitive in the blue region of the spectrum, and sensitivity falls off rapidly above 500 mμ. The spectral sensitivity of photomultiplier tube type no. 6685 was determined by the National Physical Laboratory; the spectral sensitivity curve is given elsewhere (Fig. 2, Nicol, 1958*a*).

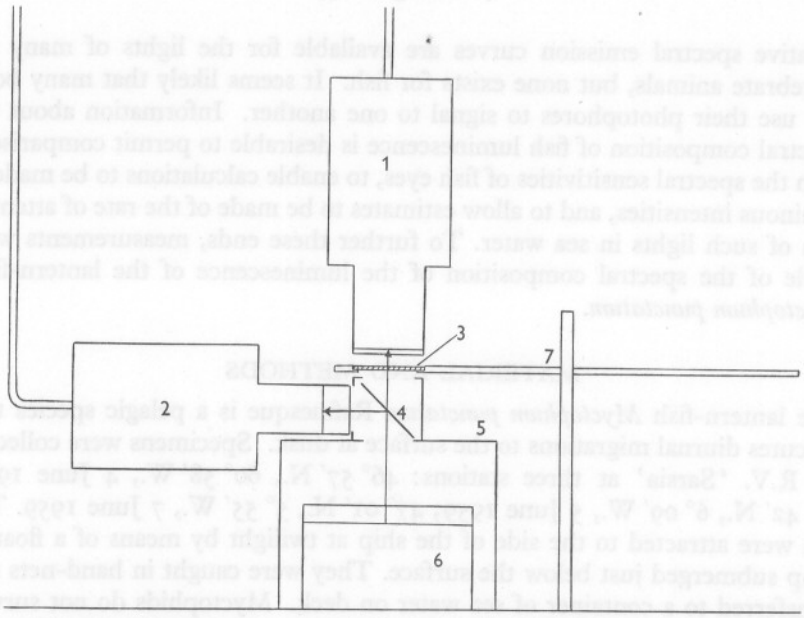

Fig. 1. Diagram of apparatus. 1, vertical photomultiplier type no. 6685. 2, horizontal photomultiplier type no. 6260. 3, filter. 4, cover-glass. 5, black box. 6, black vessel, holding sea water to contain the fish. 7, rotating disc. Arrows indicate light-paths.

Each photomultiplier was connected to a cathode-ray oscilloscope through a d.c. amplifier. Vertical deflexions of the spot on the face of the tube were photographed on moving paper. Current for the photomultipliers was provided by two stabilized power packs. Noise was filtered off by means of a 0·1 μF condenser placed across the input of each oscilloscope.

Filters were Chance OX 1 (u.v.), OV 1 (purple), and Ilford 601 (violet), 602 (blue), 603 (blue-green), 604 (green), 605 (yellow-green), 606 (yellow), 607 (orange), 608 (red), 609 (deep red). The spectral transmission of these filters was measured in a spectrophotometer. Representative transmission curves may be found in manuals of Chance Bros., Ilford Ltd., and curves

for $S_\lambda T_\lambda$ (sensitivity of photomultiplier type no. 6685 × transmission of the filters) in a previous paper (Nicol, 1957).

A lantern-fish was introduced into the box and stimulated by gently squeezing it. The response through a given filter and the monitored response were recorded, and this procedure was repeated several times with each of the filters. A fish flashed repetitively under this treatment, and each record showed a number of flashes, some of optimal size for measurement, some too bright and off screen, and some very small and difficult to measure. Representative records are shown in Fig. 2. The deflexions obtained with filters transmitting long wavelengths, viz. 606, 607, 608 and 609, were small and difficult or impossible to measure.

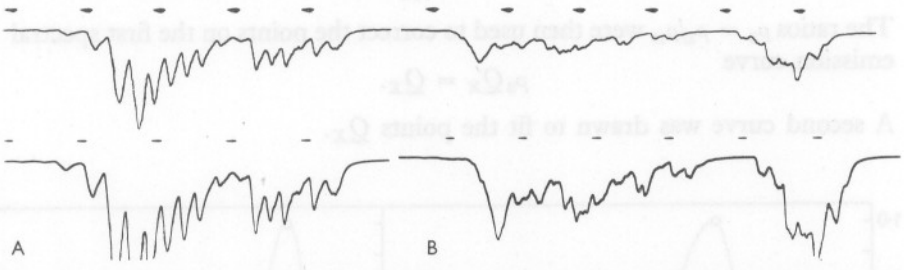

Fig. 2. Recordings of luminous responses. A, two left-hand records. Above, response recorded through filter 603, below, monitored response. B, two right-hand records. Above, through filter 602, below monitored response. Downward deflexions of traces are luminous responses. Time scale 1/sec.

The extent of the deflexions on each record, when possible, was measured. The records obtained with the monitoring photomultiplier were used to correct the records obtained with the filters and the vertical photomultiplier, so as to compensate for differences in the intensities of the response. All records obtained with filters were thus scaled to the same initial intensity. Means of the corrected records for each filter were determined. Let these values be R_X, where X refers to a given filter.

Curves were plotted for the products of the sensitivity of photomultiplier type no. 6685 (S_λ) times the transmission of the filters (T_λ), against λ. The area of each of the curves is

$$\eta_X = \int_{320}^{700} S_\lambda T_\lambda \, d\lambda.$$

The mean representative wavelength for each curve was estimated from the vertical axis bisecting the area ('centre of gravity' of the curve).

Relative spectral emission at the representative mean wavelength for each filter was calculated from

$$R_X/\eta_X = Q'_X.$$

From the values for Q'_X a spectral emission curve was constructed (first

approximation). Let the relative spectral energy levels on the curve be E'_λ. With these values, a correction was applied to compensate for the wide transmission bands of the filters. A further series of curves was constructed for $E'_\lambda S_\lambda T_\lambda$

$$\zeta_X = E'_\lambda S_\lambda T_\lambda.$$

Ratios were then obtained for each filter

$$\rho_1 = \zeta_X / \zeta_{602}.$$

Similar ratios were determined for measured responses

$$\rho_2 = \frac{R_X}{R_{602}}.$$

The ratios $\rho_3 = \rho_2/\rho_1$, were then used to correct the points on the first spectral emission curve

$$\rho_3 Q'_X = Q_X.$$

A second curve was drawn to fit the points Q_X.

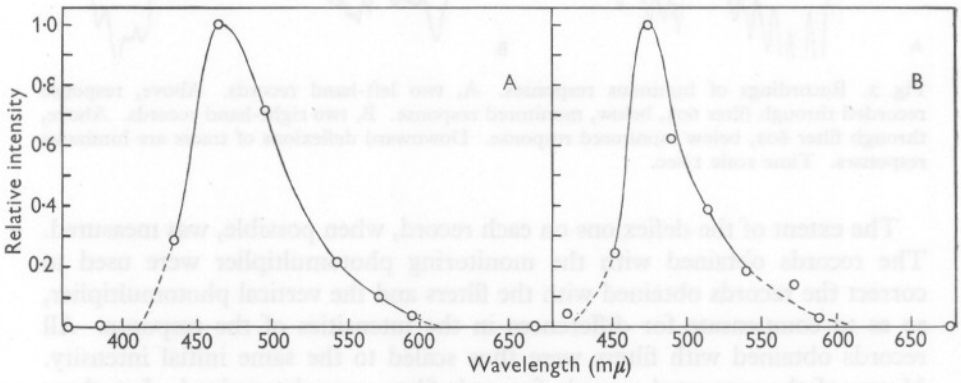

Fig. 3. Relative spectral emission curves for the light of *Myctophum punctatum*. Left (A), uncorrected curve. Right (B), corrected for wide band-width of the filters.

RESULTS

A first approximate curve (filter values Q'_X) is shown in Fig. 3A, and a corrected curve in Fig. 3B. The light is blue in colour and has a spectral range from about 410 to 600 mμ. Maximal emission lies at about 470 mμ.

These results pertain only to the light emitted by the photophores. Myctophids also possess a special light-organ, known as the 'caudal gland', on the caudal peduncle. The 'caudal gland' lights up independently of the other photophores. Its light appears blue to the eye; determination of the spectral composition was not attempted.

DISCUSSION

The lights of luminous fishes have been variously described as white, blue, blue-green, green or yellow. The majority appear to be blue or blue-green (Harvey, 1955). It is likely that several quite dissimilar chemiluminescent mechanisms are involved in the production of light by different fishes, and it is not unexpected that the lights should have different spectral characteristics. *Mycotophum* produces light intracellularly, within photophores. *Searsia*, a deep-sea species, releases a blue-green luminous secretion (Nicol, 1958*b*). *Malacocephalus laevis*, believed to owe its light to luminous bacteria, has a blue-green luminescence. Luminous bacteria, cultured from this fish, emit light having a spectral range of 430–638 mμ, and a maximum at 510 mμ.

Elsewhere I have given estimates of the intensity of the light of *Myctophum punctatum* (Nicol, 1958*b*). According to these estimates, radiant flux ranges from $0{\cdot}1 \times 10^{-9}$ μJ to 52×10^{-9} μW/cm^2 receptor surface at 1 m distance. Since a relative spectral emission curve for *Myctophum*-light was not then available, the emission curve for blue *Chaetopterus*-light was employed in the calculation. This curve is very similar to that for *Myctophum*-light, and the subterfuge now appears justified.

The photophores of myctophids have specific patterns, and it is not unlikely that they are used as intraspecific signals, during mating, schooling, or other social activities. Denton & Warren (1957) have shown that *Myctophum punctatum*, among other deep sea teleosts, possesses a golden-coloured retinal pigment (chrysopsin). The spectral characteristics of the absorption curve for this pigment are rather similar to those for the spectral emission curve of luminescence.

I wish to thank Dr E. J. Denton for helpful advice, and Mr R. G. Maddock for technical assistance.

SUMMARY

Measurements were made of the relative spectral energy in the light of the lantern-fish *Myctophum punctatum*. Light emission extends from about 410 to 600 mμ, with maximal emission at about 470 mμ.

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STUDIES ON THE GROWTH OF MARINE PHYTOPLANKTON

III. *PROROCENTRUM MICANS* EHRENBERG

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(Text-figs. 1–7)

The last of the organisms to be studied in this series was chosen as a representative of the Dinophyceae. The treatment of *Prorocentrum micans* Ehrenberg has been similar to that of *Asterionella japonica* Cleve & Müller ex Gran (Kain & Fogg, 1958*a*) and *Isochrysis galbana* Parke (Kain & Fogg, 1958*b*). The present paper includes a discussion of the results for all three organisms in the light of work by previous authors.

MATERIALS AND METHODS

The strain of *Prorocentrum micans* used originated from the Plymouth collection (No. 97). It was freed of bacteria by the method of phototaxis (Droop, 1954). Individual cells were picked out after they had traversed six 9 cm Petri dishes of sterile medium. For some experiments the original unialgal culture was used and for others the bacteria-free culture.

The methods used in culturing the dinoflagellate were similar to those used for *Asterionella* (see Kain & Fogg, 1958*a*). Pyrex glass tubes ($15 \times 2{\cdot}5$ cm) plugged with cotton wool were used as culture vessels and immersed in a constant temperature water bath at 20° C with incandescent lighting providing 5000–7000 lux.

The basic media used were Erdschreiber (Føyn, 1934), AK (Kain & Fogg, 1958*a*), AQ_N (based on natural sea water) and AQ_A (based on artificial sea water). Medium AQ was the same as AR (Kain & Fogg, 1958*a*) but without sodium silicate or tris(hydroxymethyl)aminomethane ('tris'). Soil extract, at 20 or 50 ml./l., was usually added to this medium and in all later experiments cobalamin, at 0·1 mμg/l., was included. In addition Provasoli's vitamin solution S3 (Provasoli, McLaughlin & Droop, 1957) was added on occasion. A few experiments were made in medium BD which consisted of natural sea water enriched with nitrate, phosphate and ferric chloride at the

same concentrations as in AK, with twelve vitamins (Table 1) and 50 ml./l. of soil extract. Finally medium BE was developed. It was based on natural (BE_N) or artificial (BE_A) sea water and the usual nitrate and phosphate enrichments with the addition of the trace elements and ethylenediamine tetra-acetic acid (EDTA) as in AQ but contained also glycylglycine, at 4 mM and twelve vitamins (12 V). Media were autoclaved at 15 lb./sq.in. for 1 min.

Growth was estimated solely by cell counts, as the large cell size and low population density prevented the use of optical density measurements. The same factors also precluded the use of the usual haemacytometer slides and at first the Utermohl technique (Lund, 1951) was used, all the cells in the sample

TABLE 1. THE CONCENTRATIONS IN THE MEDIA OF THE TWELVE VITAMINS (12V) USED

	μM		μM
Thiamin	0·5	p-Amino benzoic acid	0·05
Riboflavine	0·01	Inositol	1·0
Pantothenic acid	0·2	Biotin	0·002
Nicotinic acid	0·001	Folic acid	0·005
Pyriodoxine	0·01	Adenine	0·01
Cobalamin	0·0001	Guanine	0·01

being counted with an inverted microscope. Later a counting cell was constructed, suitable for use with an ordinary microscope and taking 0·5 ml. of culture. A grid of lines at 1 mm intervals on an area of 1 cm^2 was drawn on a Perspex slide by a sharp knife fixed to the tube support of a microscope. The slide was moved in relation to the knife by means of a mechanical stage, the micrometer scale of which was used to determine the position of each line. The depth of the cut was controlled by the focusing mechanism of the microscope. The grid was surrounded by Perspex walls, ground to a thickness of 0·5 cm, with four channels for the escape of excess liquid. At least 500 cells were counted to obtain an estimate of cell concentration. The distribution of cells on the grid was not quite random, there being a slightly greater concentration at the centre. Allowance was made for this if the whole grid was not scanned.

EXPERIMENTS

Growth

An example of the growth curve of *Prorocentrum* in bacteria-free culture is shown in Fig. 1. There was sometimes a lag of about 2 days. Under optimum conditions the relative growth constant k in the exponential phase was 0·3 $\log_e$units/day, corresponding to a division nearly every 2 days. Growth later became slower and the maximum population reached was usually about 50 cells/mm^3 though 80 cells/mm^3 has been recorded.

Nitrogen supply

Bacteria-free *Prorocentrum* was grown in a series of concentrations of potassium nitrate in medium BD from an inoculum washed in sterile sea

water. Cell counts were made after 21 days and the cell crop in each culture calculated by subtracting the mean cell concentration in the controls from that in the relevant culture. The cell crop in Fig. 2 is thus an expression of the response to the added nitrate, as distinct from that in the natural sea water or stored in the cells. Fig. 2 also shows the values for the added nitrate divided

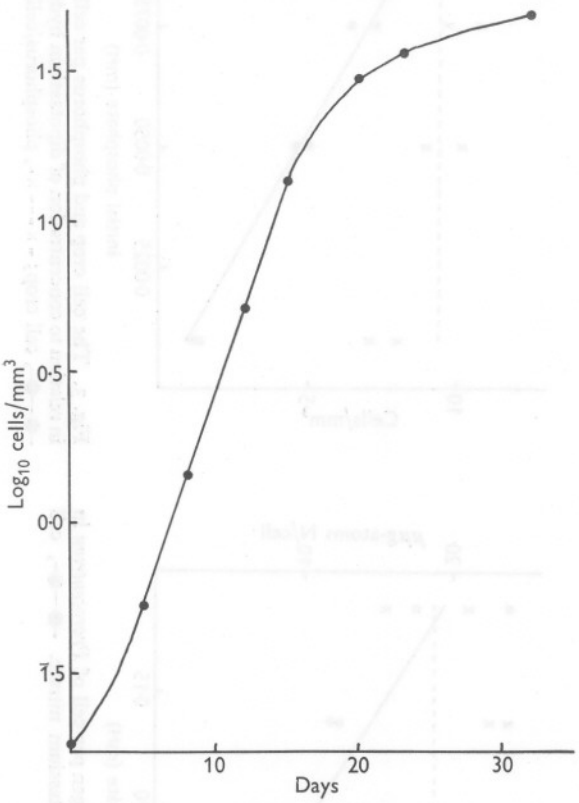

Fig. 1. The mean growth curve of three bacteria-free cultures of *Prorocentrum*.

by the cell crop obtained from it. These do not necessarily correspond to the nitrogen contents of the cells. All except the highest (2 mM) concentration gave similar values, nitrate being evidently limiting, and the mean requirement was 19 $\mu\mu$g-atoms N/cell.

Phosphorus supply

A similar experiment, also with bacteria-free cultures, was carried out in a series of concentrations of dipotassium hydrogen phosphate in medium BD and cell counts made after 29 days. The results are shown in Fig. 3.

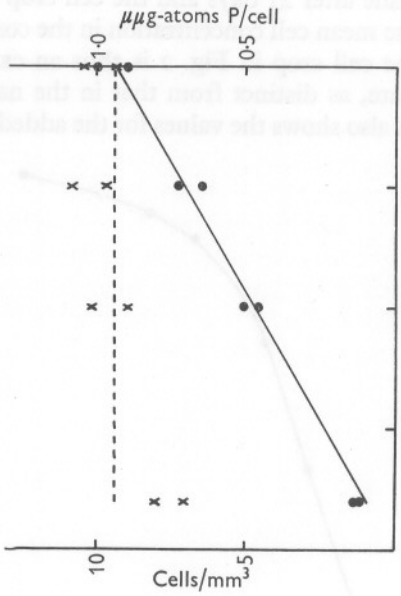

Fig. 3. The cell crop and phosphorus per cell of *Prorocentrum* in relation to concentration of dipotassium hydrogen phosphate. —●—●—, cell crop; - × - - - × -, phosphorus/cell.

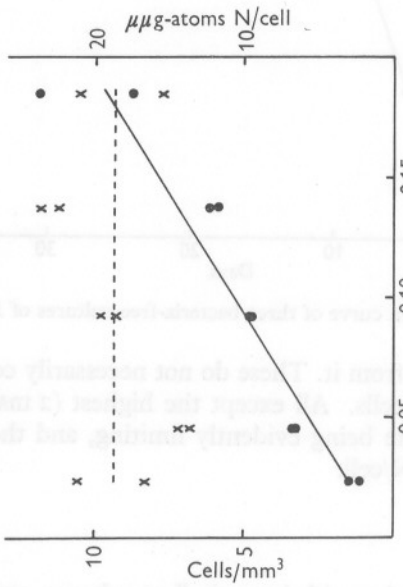

Fig. 2. The cell crop and nitrogen per cell of *Prorocentrum* in relation to concentration of potassium nitrate. —●—●—, cell crop; - × - - - × -, nitrogen/cell.

Phosphorus was evidently limiting in all but the highest concentration and the mean requirement was 0·94 $\mu\mu$g-atom P/cell.

Trace elements

In experiments carried out in Erdschreiber medium cell concentrations of the order of only 10–15 cells/mm^3 were obtained in uni-algal culture. It was found that this could be increased significantly by the addition of 0·01 mM $FeCl_3$. Later Provasoli's trace element mixture as used for *Asterionella* and *Isochrysis* (Kain & Fogg, 1958*a*, *b*) was added to natural sea water enriched with the usual nitrate, phosphate and cobalamin concentrations and 50 ml./l. of soil extract. Ethylenediamine tetra-acetic acid (EDTA) was added at 0·171 and 0·342 mM. The final cell concentrations of the uni-algal culture in

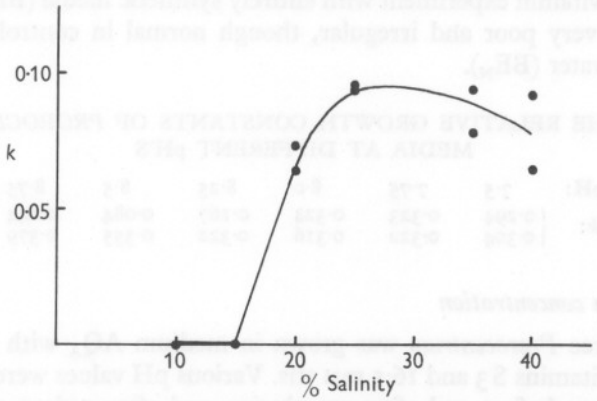

Fig. 4. The relative growth constant of *Prorocentrum* in media based on sea water of various salinities.

the latter (23·3, 23·9 and 27·8 cells/mm^3) were significantly greater than in the former (18·5, 19·4 and 14·7 cells/mm^3), with less EDTA. Medium AQ, which incorporates these concentrations of trace elements and EDTA at 0·342 mM, is therefore suitable for *Prorocentrum*.

Salinity

The uni-algal culture of *Prorocentrum* was grown in medium AK based on sea water of various salinities. All the cultures were inoculated with cells from 35‰ salinity. The relative growth constants are shown plotted against salinity in Fig. 4. A range of 20–40‰ salinity was tolerated.

Artificial sea water

Experiments with artificial sea water gave irregular results. When it was compared directly in medium AQ_A with natural sea water in AQ_N, with cobalamin and soil extract added to both, growth took place in all the cultures,

but after 46 days the cell concentrations were significantly lower in artificial sea water (2·54, 2·24 and 2·08 cells/mm^3) than in natural sea water (3·06, 2·70 and 3·24 cells/mm^3). On the other hand in the experiments on hydrogen-ion concentration, in which artificial sea water was used with soil extract, the relative growth constant was as high as has been observed (k = 0·38). Also the experiment to determine which of the twelve vitamins was necessary (see page 43) was based on artificial sea water without soil extract and the relative growth constant was normal, from a washed inoculum. Thus there is nothing unsuitable about the substances present in the artificial sea water though a medium made up from it may lack some stimulatory factor present in variable amounts in natural sea water. That this latter is the case with *Prorocentrum* as well as with *Asterionella* (see Kain & Fogg, 1958*a*) was strongly indicated by a further vitamin experiment with entirely synthetic media (BE_A) in which growth was very poor and irregular, though normal in control cultures in natural sea water (BE_N).

TABLE 2. THE RELATIVE GROWTH CONSTANTS OF *PROROCENTRUM* IN MEDIA AT DIFFERENT pH'S

pH:	7·5	7·75	8·0	8·25	8·5	8·75
k:	0·294	0·323	0·324	0·167	0·084	0·244
	0·304	0·322	0·316	0·322	0·355	0·379

Hydrogen-ion concentration

Bacteria-free *Prorocentrum* was grown in medium AQ_A with soil extract, cobalamin, vitamins S3 and 16·5 mM tris. Various pH values were maintained by adjustments before and after autoclaving and after 5 days growth. The relative growth constants, calculated from cell counts made after 7 days, are shown in Table 2. The variable results above pH 8 were probably due to differences in precipitation during autoclaving. Apart from this there was no apparent effect between pH 7·5 and 8·75.

Light

In the course of an experiment on *Prorocentrum* it was noted that identical cultures in separate tanks at the same temperature illuminated by fluorescent and incandescent lamps, respectively, showed markedly different growths. Those in incandescent light flourished while those in fluorescent light ceased growth after two divisions. In investigating the optimum light intensity for this organism therefore, both types of lamp were used. As the spectral composition of light from the two types is different, the energy output per unit of illumination (e.g. lux) is not the same from each. It is therefore necessary to use energy units in direct comparisons.

In two experiments at relatively low light intensities the lag time was

measured by extrapolating the exponential growth line back to the inoculum level. The results of this are shown in Fig. 5*a* (in incandescent light) and Fig. 5*b* (in fluorescent light, not inhibitory on this occasion). In both there was a reduction of lag with increasing light intensity, at least up to the saturation level.

Four experiments were made with incandescent light, using lamps of different wattages, with cultures at various distances from the source. The results are combined in Fig. 6*a*. The saturation level (at 20° C) was at about

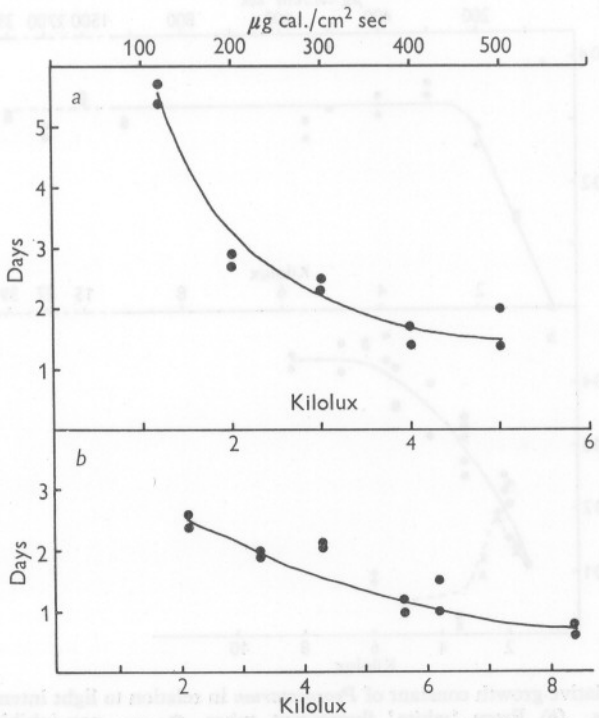

Fig. 5. The lag time of cultures of *Prorocentrum* in relation to light intensity. (*a*) From tungsten lamps. (*b*) From 'white' fluorescent tubes. The energy scale at the top applies to both (*a*) and (*b*).

300 μg-cal/cm² sec (or 3000 lux as indicated by a selenium photocell light metre). There was no inhibition at 3900 μg-cal/cm² sec (or 39,000 lux), an intensity which was obtained at about 10 cm from a 1000 W bulb.

The fluorescent tubes used were 'white'. The inhibition of growth by this type of light was observed in four separate experiments, involving two different makes of lamp, with identical control cultures in incandescent illumination in which growth was normal. The relative growth constants of some of these are shown as crosses in Fig. 6*b*. Below 150 μg-cal/cm² sec the growth constant increased with light energy but above this value it declined markedly.

In one experiment (shown as circles in Fig. 6*b*) over the same range of energy no inhibition was observed at all, and the relative growth constant increased with light energy to saturation at about 400 μg-cal/cm^2 sec. That the saturation energy was higher than that for incandescent light may be attributed to the less suitable spectral distribution of energy in fluorescent light or, since the relative growth rate achieved was higher in the latter, to the removal of some other limiting factor. It is not known what factors cause the inhibition by this

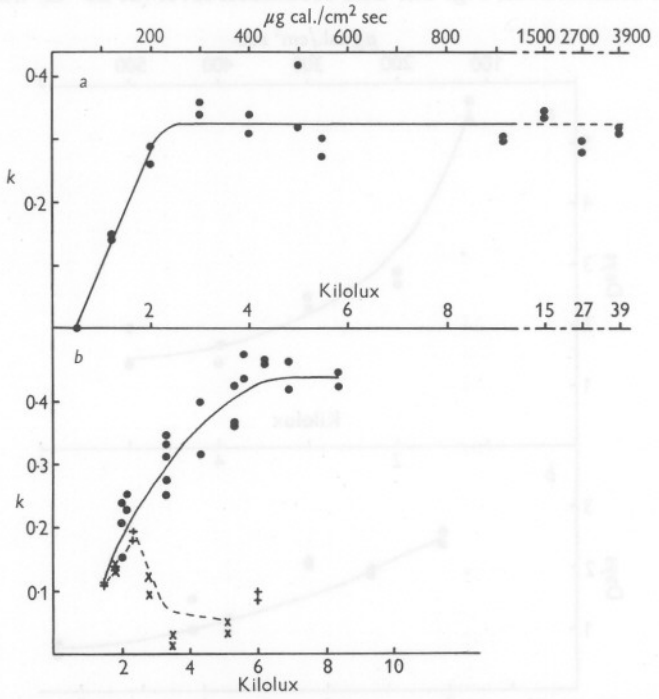

Fig. 6. The relative growth constant of *Prorocentrum* in relation to light intensity. (*a*) From tungsten lamps. (*b*) From 'white' fluorescent tubes. ●——, non-inhibitory; × +---, inhibitory.

type of light. The possibilities include the medium, the physiological state of the cells and the age of the fluorescent tubes.

In investigating the cause of the inhibition it is necessary to compare the emission spectrum of this type of fluorescent lamp with that of an incandescent tungsten filament bulb. These are shown in Fig. 7*a*, where the total relative energy, represented by the area below the curves, has been made the same between 380 and 720 mμ. The position of the principal peak in emission in the region of least absorption by *Prorocentrum* (Fig. 7*b*) indicates the unsuitability of this type of light for photosynthesis. It is possible that the wave-band 425–445 mμ could contain sufficiently more energy in fluorescent

than incandescent light to account for the inhibition. The other possibility is that the ratio of wavelengths is in some way responsible. In this connexion it is worthy of note that the action spectrum of phototaxis of *P. micans* was found by Halldal (1958) to have its peak at about 575 mμ (see Fig. 7*b*), very near to the peak in emission of 'white' fluorescent light.

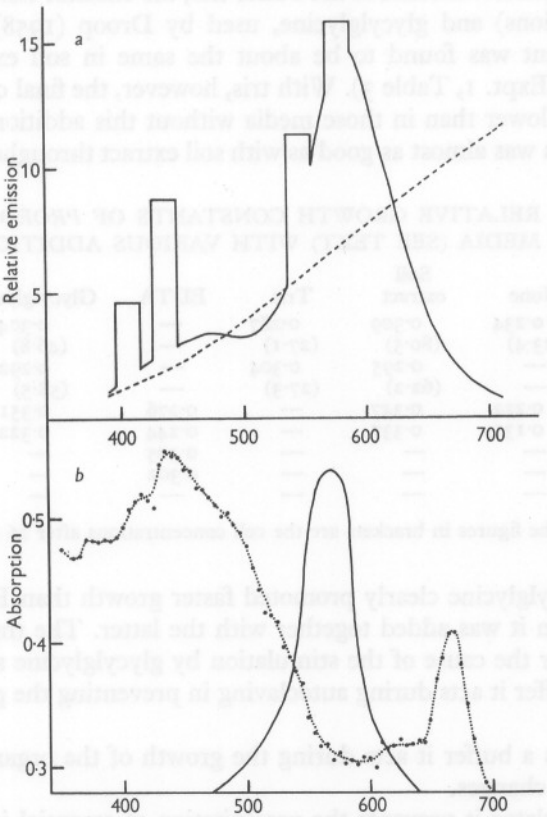

Fig. 7. (*a*) The emission spectra of lamps giving equal energy between 380 and 720 mμ. —, Ekco 'white' fluorescent tubes (by permission of Ekco-Ensign Electric Ltd.); - - -, tungsten filament lamp. (*b*) —, the phototaxis action spectrum of *Prorocentrum micans* (from Halldal, 1958); ···●···, the absorption spectrum of *P. micans* suspended in glycerol.

Stimulatory substances

In our early experiments on *Prorocentrum* in Erdschreiber medium it was found that soil extract was essential for good growth, especially when the culture was bacteria-free. In attempts to characterize the stimulatory factor a number of substitutes were tried. In all these experiments a mixture of twelve vitamins (see Table 1) in addition to nitrate, phosphate and iron was included in the basal sea-water medium. The results, expressed in terms of

relative growth constants and in one case as the cell concentration at the end of the experiment, are shown in Table 3. It is clear that even in the presence of the vitamins soil extract was still stimulatory. Its action cannot be attributed to its providing any of these particular substances and the likely possibilities are that it was acting as a buffer or a chelator. The effect of its addition was therefore compared with that of the buffer tris, the chelator EDTA (with trace element additions) and glycylglycine, used by Droop (1958). The relative growth constant was found to be about the same in soil extract, tris and glycylglycine (Expt. 1, Table 3). With tris, however, the final cell populations attained were lower than in those media without this addition. With glycylglycine growth was almost as good as with soil extract throughout. In Expt. 2

TABLE 3. RELATIVE GROWTH CONSTANTS OF *PROROCENTRUM* IN MEDIA (SEE TEXT) WITH VARIOUS ADDITIONS

Expt.	None	Soil extract	Tris	EDTA	Glycylglycine	Glycylglycine and EDTA
1*	0·234	0·309	0·287	—	0·304	—
	(23·4)	(80·5)	(27·1)	—	(46·8)	—
	—	0·295	0·304	—	0·292	—
	—	(62·2)	(27·3)	—	(58·5)	—
2	0·222	0·347	—	0·276	0·351	0·391
	0·135	0·337	—	0·244	0·322	0·369
3	—	—	—	0·305	—	0·402
	—	—	—	0·302	—	0·365
	—	—	—	—	—	0·350

* The figures in brackets are the cell concentrations after 26 days.

(Table 3) glycylglycine clearly promoted faster growth than EDTA both by itself and when it was added together with the latter. The three most likely possibilities for the cause of the stimulation by glycylglycine are:

(1) As a buffer it acts during autoclaving in preventing the precipitation of essential ions.

(2) Again as a buffer it acts during the growth of the organism, reducing inhibitory pH changes.

(3) As a chelator it prevents the precipitation of essential ions or reduces the concentration of toxic ions.

If the first were the case then its superiority over EDTA would disappear if the autoclaving process were omitted. However, in Seitz-filtered media which were not autoclaved, the stimulation was still significant. In Expt. 3 (Table 3) the initial pH of the medium in both cases was about 6·4 and the final pH with EDTA alone was 8·2 while with glycylglycine it was 7·7 As the initial pH was the same and the final values both within the optimum range for the organism (Table 2) it is unlikely that the buffering action of glycylglycine is important in the stimulatory effect. It seems probable that it acts as a chelator, being in some way more suitable than either tris or EDTA.

Vitamins

Droop (1957) has already reported the requirement for cobalamin of the organism. In order to determine which of the twelve vitamins used in the current media were necessary, thirteen duplicate cultures were grown in medium BE_A, in each one of twelve of which a different vitamin was omitted. The inoculum was washed and the concentration of the previous medium was 0·06%. Cotton-wool plugs were not used. The cell concentrations after 11 and 18 days are shown in Table 4. The requirement for cobalamin was confirmed by the low and stationary (after 11 days) cell concentration in its absence. Growth was also less in the absence of biotin. None of the other vitamins

TABLE 4. THE CELL CONCENTRATIONS IN CULTURES WITH VITAMIN OMISSIONS

(Cells/mm³)

	11 days		18 days	
No omission (i.e. with twelve vitamins)	13·8	13·4	32·9	34·2
No thiamin	16·1	13·5	33·3	27·6
No riboflavine	15·9	14·0	31·2	24·9
No pantothenic acid	14·3	6·46	24·0	15·9
No nicotinic acid	12·8	9·44	25·7	23·8
No pyridoxine	11·6	12·7	23·6	30·7
No cobalamin	2·68	3·08	3·14	3·00
No *p*-amino benzoic acid	14·7	12·4	28·9	29·7
No inositol	14·3	12·8	27·8	32·3
No biotin	7·95	7·69	12·0	9·67
No folic acid	15·9	12·6	31·4	28·2
No adenine	17·2	11·4	30·8	31·0
No guanine	11·7	15·5	27·8	27·4

appear to have been necessary, though it is doubtful whether a single experiment, even using a washed inoculum, is sufficient to establish the independence of the organism from external sources of these substances.

DISCUSSION

These results obtained with *Prorocentrum micans* will now be considered, together with those presented in previous papers of the series (Kain & Fogg, 1958*a*, *b*) for *Asterionella japonica* and *Isochrysis galbana*.

Nitrogen supply

Nitrate has been the only source of nitrogen used in our work but appears to be the most generally suitable source for phytoplankton. In common with workers studying various other kinds of algae (see Fogg, 1959) we have been unable to demonstrate the limitation of exponential growth rate by nitrate concentration. Barker (1935) had already shown that the exponential growth

of *Prorocentrum micans* is unaffected by variation in nitrate concentration between 0·016 and 16 mM. At limiting concentrations, the final yield of cell material is proportional to the amount of nitrate originally supplied (Spencer, 1954; Miller & Fogg, 1957) and a minimum nitrogen content per cell is approached. From our results it is possible to determine the minimum nitrogen requirement per cell which is not necessarily equivalent to minimum nitrogen content per cell, since a proportion of the nitrogen assimilated may be liberated in extracellular form. Some values for the minimum nitrogen content or requirement per cell are given in Table 5. From approximate cell volumes determined from models, values for the mean nitrogen content per unit volume of protoplast have been calculated and are seen to be of the same order for the various organisms considered. Above the minimum value the

TABLE 5. MINIMUM NITROGEN CONTENT OR REQUIREMENT PER CELL OF VARIOUS ALGAE

Species	Cell volume μ^3	$\mu\mu$g-atoms N/cell	μg-atoms N/mm^3	Author
Asterionella japonica	920	0·25	0·27	Kain & Fogg, 1958*a*
A. formosa	1,800	0·4	0·4	Lund, 1950
Isochrysis galbana	31	0·051	1·6	Kain & Fogg, 1958*b*
Prorocentrum micans	17,000	19	1·1	This paper
Peridinium I	3,200	1·5	0·47	Barker, 1935
Monodus subterraneus	120	0·0228	0·19	Miller & Fogg, 1957

nitrogen content per cell may vary greatly and it is to be expected that such variations are accompanied by considerable changes in metabolic activity and pattern (Fogg, 1959).

Phosphorus supply

Orthophosphate has been the only source of phosphorus used in our experiments and, as for nitrate, it is difficult to determine the concentrations which would limit the exponential growth rate. Ketchum (1939*b*) reported that variation in phosphate concentration between 0·00053 and 0·0016 mM had no effect on the relative growth constant of *Nitzschia closterium*. The growth of some algae is inhibited by high phosphate concentrations of the order of 0·5 mM (Chu, 1943; Provasoli & Howell, 1952). For *Asterionella japonica* we have found that the relative growth rate remains constant within the range 0·01 to 0·31 mM phosphate. The phosphorus content of algal cells may vary within wide limits according to conditions, but for a given species the minimum content is fairly constant (Ketchum, 1939*a*; Goldberg, Walker & Whisenand, 1951; Mackereth, 1953). Values for the minimum phosphorus requirement of various algae, determined as for minimum nitrogen requirements, are given in Table 6, from which it seems that, while the mean phosphorus requirement or content per unit volume of protoplasm may be fairly constant for the

marine species, those for freshwater species may be an order of magnitude lower.

Salinity

Of the three organisms studied, *A. japonica* is the least tolerant of variations in salinity, having a well-defined optimum in relative growth rate between 30 and 35‰ salinity. In contrast, the relative growth rate of *Isochrysis galbana* is little affected by variation between 15 and 40‰ salinity. The results obtained for *Prorocentrum micans* (optimum between 25 and 40‰ salinity) are in reasonable agreement with those of Braarud & Rossavik (1951).

TABLE 6. MINIMUM PHOSPHORUS CONTENT OR REQUIREMENT PER CELL OF VARIOUS ALGAE

Species	Cell volume (μ^3)	$\mu\mu$g-atoms P/cell	μg-atoms P/mm^3	Author
Asterionella japonica	920	0·05	0·05	Goldberg *et al.*, 1951
Nitzschia closterium	580	0·0066	0·011	Ketchum, 1939*a*
Asterionella formosa	1,800	0·0018	0·001	Rodhe, 1948
	—	0·0006	0·0003	Lund, 1950
	—	0·002	0·001	Mackereth, 1953
Isochrysis galbana	31	0·00097	0·03	Kain & Fogg, 1958*b*
Prorocentrum micans	17,000	0·94	0·055	This paper
Peridinium I	3,200	0·159	0·05	Barker, 1935
Monodus subterraneus	120	0·00089	0·007	Miller & Fogg, 1957

Temperature

Previous determinations of the effects of temperature on phytoplankton organisms (Barker, 1935; Ryther, 1954; Spencer, 1954; Nordli, 1957) have shown the lower and upper limits for growth to be about 5° and 30° C and the optima to be usually between 15° and 27° C. The precise values obtained in a given experiment will vary according to conditions, e.g. light intensity and the presence of bacterial contaminants may have important effects. Our results are in general agreement with those of previous workers and it seems that the optimum temperatures for the growth of the three species studied are higher than any they are likely to encounter in nature.

Light intensity

The comparison of results obtained by different workers on the effect of light intensity on algal growth is difficult. Two sets of units (of illuminance and of irradiance), which are not readily interconvertible, are in use; the geometry of the apparatus used and the density of the suspension may have effects making the actual light intensity reaching the cells considerably different from that recorded by a photometer; and the calibration of commercial photometers cannot be relied upon (Myers, 1946). Determinations of effects on photosynthesis cannot be taken as being equivalent to the effects

on growth since saturating intensities for the former may be much higher than for the latter (Myers, 1951). Our determinations of the effect on growth gave values which are of the same order as those found in other studies on algae. The saturating intensity for *Asterionella japonica*, *c.* 4000 lux from a tungsten lamp (400 μg-cal/cm^2 sec) is about the same as those reported for various fresh-water *Chlorella* species, a *Scenedesmus* and a *Chlamydomonas* (Sorokin & Krauss, 1958). That for *Isochrysis galbana* is lower, *c.* 1500 lux (150 μg-cal/cm^2 sec), but similar values have been recorded for *Chlorella pyrenoidosa* (Myers, 1953). The relative growth rate of *Prorocentrum micans* reaches its maximum at 300–400 μg-cal/cm^2 sec. We have not observed light inhibition of the growth of either *Asterionella japonica* or *Isochrysis galbana*; the highest light intensities used being well below 20,000 lux, at which intensity inhibitory effects on the growth of Chlorophyceae become apparent (Sorokin & Krauss, 1958). No inhibition of the growth of *Prorocentrum micans* was found by us in light of an intensity of 39,000 lux (3900 μg-cal/cm^2 sec) from tungsten lamps. On a number of occasions, though not invariably, we have observed inhibition at 3000 lux (200 μg-cal/cm^2 sec) from 'white' fluorescent tubes. Nordli (1957) has also recorded inhibition of the growth of dinoflagellates by relatively low intensities of light from fluorescent lamps but Haxo & Sweeney (1955), who used light from 'white' fluorescent tubes, found 5000–8000 lux to be optimal for *Gonyaulax polyedra* and made no mention of inhibitory effects. Nordli (1957) attributed the more marked inhibition of *Ceratium furca* by continuous light from fluorescent lamps to the overstimulation of phototaxis, but further work is needed to establish this point.

Hydrogen-ion concentration

Until buffers such as tris(hydroxymethyl)aminomethane were used (Provasoli, McLaughlin & Pintner, 1954) there was difficulty in controlling the pH of sea-water media and information on the effect of pH on the growth of marine phytoplankton is correspondingly scanty. Our results support the conclusion of Bachrach & Lucciardi (1932) that the pH optimum for marine diatoms is near to that of natural sea water. The lower limit of the optimum range is less than pH 7·5 for the three organisms studied by us. *Asterionella japonica* seems to be the most sensitive to alkaline conditions, an appreciable decrease in relative growth constant occurring above pH 8·25, while *Prorocentrum micans* is the most tolerant, the maximum relative growth constant being maintained to at least pH 8·75. Our results for *P. micans* are in good agreement with those obtained previously for this organism by Barker (1935).

Other factors affecting growth

Requirements for organic substances have now been established for many marine phytoplanktonic organisms (see the reviews by Droop, 1957 and by

Provasoli, 1957, 1958*a*, *b*). Our failure to grow *Asterionella japonica* in the absence of bacteria and other pieces of evidence suggest that this organism has a requirement for one or more organic growth factors and there are also indications that it produces an autoinhibitor in the course of growth. The chemical nature of these substances is as yet entirely unknown. *Prorocentrum micans*, isolated by us into bacteria-free culture, appears to require cobalamin and biotin in addition to some other possible substance. Since our work was completed it has been reported that *Isochrysis galbana* has requirements for cobalamin and thiamin (Provasoli, 1958*b*).

THE LARGE-SCALE CULTURE OF MARINE PHYTOPLANKTON

The mass culture of microscopic algae is a possible means of producing feedingstuffs or organic materials for industry. While the use of sea water rather than fresh water as a medium for this has no intrinsic value it might be

TABLE 7. GROWTH CHARACTERISTICS OF SOME UNICELLULAR MARINE ALGAE AND OF *CHLORELLA PYRENOIDOSA*

Species	Author	Conditions	Relative growth constant ($\log_e$ units/day)	Final cells/ml
Phaeodactylum tricornutum	Spencer, 1954	Laboratory culture	1·7 (25° C)	—
	Raymont & Adams, 1958	Large-scale tanks	0·69 (13·5°—18° C)	$3·2 \times 10^7$
Chlorella sp. (marine)	Loosanoff, 1951	Large-scale tanks	—	$3·5 \times 10^7$
Dunaliella bioculata	Eddy, 1956	Laboratory culture	1·1 (25° C) 1·8 (29° C) 2·9 (33·5° C)	$2·5 \times 10^7$ $1·2 \times 10^7$ $7·2 \times 10^6$
Asterionella japonica	Kain & Fogg, 1958*a*	Laboratory culture	1·2 (20–25° C)	$4·0 \times 10^6$
Isochrysis galbana	Kain & Fogg, 1958*b*	Laboratory culture	0·55 (20° C)	$2·4 \times 10^7$
Prorocentrum micans	This paper	Laboratory culture	0·3 (20° C)	$5·0 \times 10^4$
Chlorella pyrenoidosa	Thacker & Babcock, 1957	Large-scale continuous aseptic culture	2·0 (25° C)	$3·7 \times 10^8$

more economic in certain circumstances. The general principles underlying the large-scale culture of algae have been considered in several publications (e.g. Tamiya, 1957; Thacker & Babcock, 1957; Fogg, 1957) and here it is our intention only to comment briefly on such features of the organisms studied by us as seem important for their growth in large-scale cultures.

The maximum relative growth rates of *Asterionella japonica*, *Isochrysis galbana* and *Prorocentrum micans* are lower than those of other organisms which have been considered from this point of view (Table 7), but this is not necessarily of great importance since in mass culture the aim should be to achieve a linear phase of growth in which yield per unit time is proportional

to the amount of light received. Here, there may be an advantage in using organisms such as *Asterionella japonica* and *Prorocentrum micans* which require relatively high intensities for saturation and which may thus give greater yields for a given amount of light. The maximum yield per unit volume of medium is of considerable importance for successful large-scale culture, since dense suspensions are necessary for economic handling. The maximum final cell concentration achieved in cultures of marine algae have so far been only about one-tenth of those achieved in ordinary large-scale cultures of *Chlorella* (Table 7) and even when differences in cell size are allowed for the yield per unit volume of the former remains poor by comparison. In our densest cultures nitrate, phosphate, salinity, hydrogen-ion concentration and carbon dioxide were not limiting and the nature of the factors determining the final population density is unknown; natural sea water with simple additions of nitrate and phosphate is not necessarily the medium giving maximum yields of these organisms and it may be that adjustment of ionic ratios, as has proved successful with the freshwater alga *Monodus* (Miller & Fogg, 1957), or addition or removal of organic factors will be necessary to obtain worthwhile improvements in yield. Finally, it may be pointed out that harvesting is one of the most expensive processes in the mass culture of algae and that organisms such as *Asterionella japonica* and *Prorocentrum micans*, being considerably larger, are more readily separated from the culture medium than is *Chlorella*.

Part of this work was carried out under extra-mural contract with the Institute of Seaweed Research. We are grateful to the Institute for a maintenance grant made to one of us (J.M.K.). We are also very grateful for the technical assistance of Miss W. B. Chapman.

SUMMARY

The dinoflagellate *Prorocentrum micans* has been grown in bacteria-free, as well as uni-algal, culture under controlled conditions and its growth measured by means of cell counts.

The relative growth constant was about 0·3 $\log_e$ units/day. Ranges of salinity of 20–40‰ and of pH of 7·5–8·75 were tolerated. The optimum incandescent light intensity was 3000–39,000 lux (300–3900 μg-cal/cm^2 sec). Fluorescent light of more than 2000 lux (150 μg-cal/cm^2 sec) was usually inhibitory, a phenomenon which may have been associated with phototaxis. The lag time decreased with increasing light intensity.

The provision of a suitable chelator seemed very important for this organism. Ethylenediamine tetra-acetic acid was fairly satifactory but glycylglycine was better. Its requirement for cobalamin was confirmed and it was also found to need biotin. Prolonged growth in a completely synthetic medium was not achieved.

A final cell concentration of 50–80 cells/mm^3 was obtained. The minimum nitrogen requirement was 19 $\mu\mu$g-atoms N/cell and the minimum phosphorus requirement 0·94 $\mu\mu$g-atoms P/cell.

The results from the three organisms studied in this series are compared with those obtained by other workers.

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STEROID SULPHATASE, ARYLSULPHATASE AND β-GLUCURONIDASE IN MARINE INVERTEBRATES

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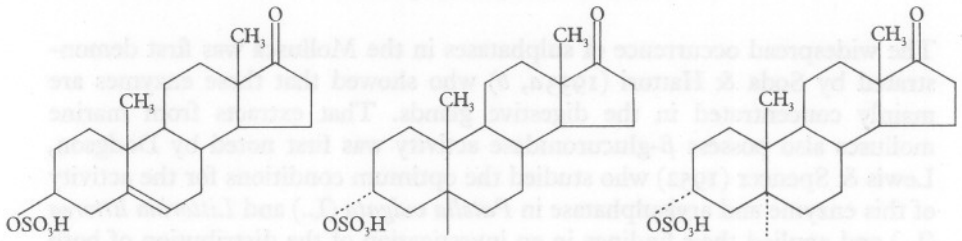
The widespread occurrence of sulphatases in the Mollusca was first demonstrated by Soda & Hattori (1933*a*, *b*) who showed that these enzymes are mainly concentrated in the digestive glands. That extracts from marine molluscs also possess β-glucuronidase activity was first noted by Dodgson, Lewis & Spencer (1952) who studied the optimum conditions for the activity of this enzyme and arylsulphatase in *Patella vulgata* (L.) and *Littorina littorea* (L.) and applied their findings in an investigation of the distribution of both enzymes among various other marine molluscs (Dodgson, Lewis & Spencer, 1953).

Molluscan tissues have also been investigated as a possible source of a sulphatase capable of hydrolysing the steroid conjugates normally encountered in human urine (Henry & Thevenet, 1952; Stitch & Halkerston, 1953*a*, *b*; 1956; Jayle & Baulieu, 1954; Savard, Bagnoli & Dorfman, 1954; Roy, 1954, 1956*a*; Leon, Bulbrook & Corner, 1960). The main reason for such studies has been the need to find an alternative to acid hydrolysis of the steroid conjugates, a method which causes much destruction and alteration of some of the liberated steroids. However, although many of the gastropods studied possess an enzyme capable of hydrolysing dehydro*epi*androsterone sulphate (I), and some species belonging to this class possess an enzyme which will also hydrolyse aetiocholanolone sulphate (II), it has not been possible to detect an enzyme which would effect the hydrolysis of androsterone sulphate (III). This means that complete enzymic hydrolysis of all the steroid conjugates present in human urine cannot be achieved with any of the molluscan enzymes studied so far. Moreover, sulphatases from other sources, such as mammalian liver (Gibian & Bratfisch, 1956; Roy, 1957), fungi (Cohen & Bates, 1949; Stitch & Halkerston, 1953*b*), and bacteria (Buehler, Katzman & Doisy, 1950) have also proved to be unsuitable for this purpose.

The present work is an extension of an earlier study (Leon *et al.*, 1960) in which a search was made for a convenient source of a 'steroid sulphatase'

that would effect the hydrolysis of androsterone sulphate. In this paper the term 'steroid sulphatase' is used to describe any enzyme which will effect the hydrolysis of a steroid sulphate. However, since the role of these enzymes in mammalian and molluscan tissues is not known, the use of this term is not intended to imply physiological function. In the earlier investigation all the animals used belonged to the mollusca, but in the present study species representative of eight additional marine invertebrate phyla have been examined, and the activities of arylsulphatase and β-glucuronidase in these animals have been examined in relation to their diets, habitats and methods of feeding. No steroid sulphatase has been detected in any marine animal tested other than certain molluscs of the class gastropoda, and there appears to be no correlation between the activity of this enzyme and the diet of the



animal in which it is present. Moreover, even the most active preparations of steroid sulphatase so far studied have no measurable influence on the hydrolysis of androsterone sulphate, and an enzyme capable of effecting this change has therefore still to be found.

MATERIALS AND METHODS

Animals. Test species were collected from the intertidal zone or from the off-shore waters in the vicinity of Plymouth, with the exception of *Helix pomatia* L., specimens of which were gathered in a Sussex garden. This terrestrial species was included for comparison because enzyme preparations from *Helix* have been used for the hydrolysis of steroid conjugates. Full names of all the test species are given in Tables 1 and 2, but are not hereafter used in any part of the text other than the summary.

Extraction of enzymes. Acetone-dried powders were prepared from the tissues of approximately twenty animals of each test species. Exceptions to this were *Marinogammarus*, *Ligia* and *Procerodes* where much larger numbers of test animals were used; and *Maia*, *Cancer*, *Clionacelata*, *Ciona* and *Sepia* where the number of test animals was only two or three. Whenever possible the digestive gland was the tissue used in preparing the enzymes, but in many instances dissection of this organ was prohibited by the smallness of the test species, and the whole animal was then used.

Estimations of enzyme activity. Arylsulphatase was assayed using dipotassium 2-hydroxy-5-nitrophenylsulphate (NCS) and potassium nitrophenylsulphate (NPS) as substrates; the method employed was that described by Dodgson *et al.* (1953).

Androsterone, dehydro*epi*androsterone and aetiocholanolone sulphates (AS, DHAS and AeS respectively), were used in estimations of steroid sulphatase activity by the method of Roy (1956*a*, *b*). β-Glucuronidase was assayed by the method of Dodgson *et al.* (1953), using *p*-chlorophenylglucuronide monohydrate as the substrate.

RESULTS

β-Glucuronidase and arylsulphatase

The experimental findings, summarized in Tables 1 (Mollusca) and 2 (other phyla), demonstrate the widespread occurrence of β-glucuronidase and arylsulphatase among marine invertebrates. Thus, each of the twenty-three species tested was found to possess some arylsulphatase activity, although two species with low activity towards NCS (*Ligia* and *Antedon*) were inactive when NPS was used as substrate. β-Glucuronidase activity was found in eighteen of the species tested, but was not detected in *Turritella* (Mollusca), *Marinogammarus* and *Ligia* (Arthropoda), *Clionacelata* (Porifera) and *Amphioxus* (Cephalochorda).

The number of species used in experiments with molluscs was sufficient to allow examination of the possibility of a correlation between enzyme activity on the one hand and diet and habitat on the other (Table 3). The figures in the first row of the table include all the species of molluscs tested and show that β-glucuronidase activity is much greater in the herbivorous than in the carnivorous or detritus-eating species. There is a similar but less marked difference in the mean values for arylsulphatase activity. These figures include the results obtained using acetone-dried powders prepared from two herbivorous and two detritus-eating species where the whole animal was used. If the enzymes are mainly concentrated in the digestive gland then only the results of experiments in which these glands were used are strictly comparable. The figures in the second row of the table are derived from the results on species where it was possible to dissect out the digestive glands, and they emphasize the difference in β-glucuronidase levels between the herbivorous, the carnivorous, and the detritus-eating species. The figures show again the raised arylsulphatase levels in the herbivores compared with the carnivores but the mean value for the arylsulphatase of the detritus-eating species is not significantly different from that of the herbivores. It is worth mentioning that the differences between the enzyme activities of herbivorous and carnivorous molluscs would be even greater were it not for the anomalous result obtained using *Nassarius*, which possesses a very high arylsulphatase and β-glucuronidase activity, but is described as carnivorous (see Table 1). However, although the animal is reported to feed largely on carrion, there is a possibility that it may inadvertently ingest a certain amount of plant material as well, and so, to some extent, be omnivorous (A. Graham, private communication).

TABLE 1. β-GLUCURONIDASE AND ARYLSULPHATASE ACTIVITY IN MOLLUSCS

Species	Test material: Habitat	Test material: Feeding and diet	Source of enzyme	Enzyme activities*: Arylsulphatase NCS	Enzyme activities*: Arylsulphatase NPS	Enzyme activities*: β-Glucuronidase
Patella vulgata (L.)	Intertidal	Browses on algal sporelings and diatoms (1)	Visceral hump	652,335	157,450	155,900
Littorina littorea (L.)	Intertidal	Browses on algal sporelings and diatoms (1) (3)	Whole animal without shell	585,860	168,920	69,070
Lepidochitona cinerea (L.)	Intertidal	Diatoms and algal detritus (1)	Whole animal	41,200	25,220	10,890
Patina pellucida (L.)	Intertidal	*Laminaria* and attached sporelings and diatoms (1) (2)	Whole animal	25,620	7,450	23,790
Aplysia punctata (Cuvier)	Intertidal	*Ulva*, *Fucus* (1) (4)	Digestive gland	287,390	55,030	28,520
Nucella lapillus (L.)	Intertidal	Carnivorous (barnacles and mussels) (1)	Digestive gland	96,260	39,550	15,480
Nassarius reticulatus (L.)	Offshore; mud deposits	Carnivorous (barnacles and mussels (1)	Digestive gland	435,930	235,610	75,800
Buccinum undatum L.	Offshore; gravel and mud deposits	Carnivorous (crabs, worms, etc.) (1)	Digestive gland	227,830	81,400	14,620
Turritella communis (Risso)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (7)	Whole animal	181,440	37,070	0
Crepidula fornicata (L.)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (1) (5)	Digestive gland	74,100	9,360	8,310
Pecten maximus (L.)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (6)	Digestive gland	941,110	449,050	16,910
Sepia officinalis (L.)	Pelagic	Carnivorous (*Crangon*, etc.)	Digestive gland	58,860	19,490	9,740
Helix pomatia (L.)	Terrestrial	Herbivorous (green plants)	Digestive gland	759,670	367,260	230,700
			Crop fluid	2,551,170	823,190	58,460

Arylsulphatase activity tested at pH 5·5; β-glucuronidase activity at pH 4·0; Activities are expressed as μg. phenol liberated/g powder/h.
References: (1) Graham, 1955; (2) Graham & Fretter, 1947; (3) Graham (unpublished observations); (4) Eales, 1921; (5) Orton, 1912; (6) Hunt, 1925; (7) Yonge, 1947.

* These figures for enzymic activity have been previously reported by Leon *et al.* (1960) and are reproduced here to show the correlation between enzyme activity and ecological data.

TABLE 2. β-GLUCURONIDASE AND ARYLSULPHATASE ACTIVITY IN ANIMALS OF PHYLA OTHER THAN MOLLUSCA

Phylum	Test material: Class	Test material: Species	Habitat	Feeding and diet	Source of enzyme	Enzyme activities: Arylsulphatase NCS	Enzyme activities: Arylsulphatase NPS	Enzyme activities: β-Glucuronidase
Arthropoda	Malacostraca	*Cancer pagurus* L.	Intertidal	Carnivorous	Digestive gland	157,200	45,860	15,480
Arthropoda	Malacostraca	*Maia squinado* (Herbst)	Offshore; sandy or rocky localities	Carnivorous; also browses on algal tufts (1)	Digestive gland	280,460	77,580	7,450
Arthropoda	Malacostraca	*Marinogammarus marinus* (Leach)	Intertidal	Browses; chiefly vegetable detritus	Whole animal	38,780	9,940	0
Arthropoda	Malacostraca	*Ligia oceanica* (L.)	Intertidal	Browses; *Fucus* and small epiphytic algae on large sea weeds (3)	Whole animal	7,270	0	0
Coelenterata	Anthozoa	*Calliactis parasitica* (Couch)	Offshore; attached to shells inhabited by *Eupagurus*	Carnivorous	Whole animal	21,470	7,640	5,450
Echinodermata	Crinoidea	*Antedon bifida* (Pennant)	Offshore; mud deposits	Ciliary feeder; detritus and small living organisms	Whole animal	10,040	0	7,740
Porifera	Demospongiaria	*Clionacelata* (Grant)	Offshore; boring in rocks	Ciliary feeder; detritus and small living organisms	Whole animal	88,290	28,850	0
Platyhelminthes	Turbellaria	*Procerodes ulvae* (Oersted)	Intertidal	Browses; unicellular algae (2)	Whole animal	9,700	3,630	9,310
Tunicata*	Ascidiacea	*Ciona intestinalis* (L.)	Offshore; attached to submerged structures	Ciliary feeder; detritus (4)	Whole animal	—	—	—
Cephalochorda*	—	*Amphioxus lanceolatus* (Pallas)	Offshore; shell gravel	Ciliary feeder; detritus (5)	Digestive system	109,420	22,550	0
Annelida	Polychaeta	*Chaetopterus variopedatus* (Renier)	Offshore; muddy gravel	Ciliary feeder; detritus (6)	Whole animal	36,010	14,140	24,500

Experimental conditions as in Table 1.
References: (1) Carlisle (1957); (2) Spooner (unpublished observations); (3) Nicholls (1931); (4) MacGinitie (1939*b*); (5) Hunt (1925); (6) MacGinitie (1939*a*).
* Sub-phyla of the Chordata.

Although Dodgson *et al.* (1953) state that enzymic activity cannot be correlated with feeding habits, re-examination of their results shows the same trend as that in Table 3—namely, that the herbivorous molluscs have greater levels of β-glucuronidase and arylsulphatase activity than the carnivorous species.

Reference to both Tables 1 and 2 shows that of the eight species examined which are thought to feed on detritus, four were without β-glucuronidase-activity; whereas, of a further fourteen species which appear to be definitely carnivorous or herbivorous, only one (*Ligia*) was found to lack the enzyme. Correlation between habitat and enzyme activity merely reflects the fact that the herbivorous species are intertidal, whereas the carnivorous animals may, in addition, be pelagic or live off-shore in gravel or muddy deposits. Tables 1 and 2 show that many of the species that contain sulphatase and β-glucuronidase dwell on a substratum of muddy composition and the question arises of whether their enzymes are of bacterial origin, not being extracted from the tissues of the animals but from the bacterial flora that inhabit them. However, this does not seem likely, for Dodgson, Melville, Spencer & Williams (1954) have shown that the bacteria present in the digestive organs of certain marine molluscs have only a very low arylsulphatase activity; and it has been found in the present work that acetone-dried powders, prepared from samples of mud collected from the habitats of the various test animals, possessed only a slight enzymic activity, far less than would account for the activities of the preparations obtained using the tissues of the animals concerned.

'*Steroid sulphatase*'

'Steroid sulphatase' activity appears to be confined to the phylum mollusca; it was not detected in any of the other invertebrate phyla. It was only found in seven of the mollusca tested, each of these belonging to the subclass Prosobranchia of the class Gastropoda. Moreover, the enzyme was not found in all members of the Prosobranchia; and it was absent from the only species representing a different subclass of the gastropods, *Aplysia* from the Opisthobranchia (Table 4). The gastropods can be divided into two groups, according to the specificity of their steroid sulphatase. Thus, enzymes from four of the species tested would only effect the hydrolysis of DHAS, whereas enzymes from three species were active when both DHAS and AeS were used as substrates. In Table 4 the species are listed in order of maximum DHAS sulphatase activity, and it is of interest to note that the species with the highest activity are those which will also hydrolyse AeS. Two of these species belong to the carnivorous order, Stenoglossa; the third is the herbivorous land snail, *Helix*. It was not possible to detect an enzyme that would effect the hydrolysis of AS in any of the species tested. There is no apparent correlation between steroid sulphatase activity and diet and habitat because, of the marine molluscs that possess this enzyme, three are carnivores and three are herbivores.

TABLE 3. THE RELATIONSHIP BETWEEN ENZYMIC ACTIVITY AND FEEDING HABITS IN THE MOLLUSCA

The enzyme levels found for the digestive gland of *Helix pomatia* are included but not those found for the crop fluid.
The figures in the table are mean levels, calculated from the data in Table 1.
The figures in brackets in the columns headed NCS refer to the number of results from which the mean was derived. Units of enzyme activity are those defined under Table 1.

	NCS			NPS			β-Glucuronidase		
	Herbivores	Carnivores	Detritus	Herbivores	Carnivores	Detritus	Herbivores	Carnivores	Detritus
All preparations	462,175 (5)	204,720 (4)	309,463 (4)	151,222	94,013	130,175	101,596	29,810	9,027
Digestive gland preparations only	566,465 (3)	204,720 (4)	507,600 (2)	193,247	94,013	229,205	138,373	28,910	12,610

TABLE 4. STEROID SULPHATASE ACTIVITY OF VARIOUS MOLLUSCS

Activities are expressed as μg steroid liberated/g Powder A

Species	Order	Subclass	Class	Steroid sulphatase AeS	Steroid sulphatase DHAS
Nassarius reticulatus	Stenoglossa	Prosobranchia	Gastropoda	10,409	10,650
Helix pomatia	—	—	Gastropoda	8,138	8,147
Buccinum undatum	Stenoglossa	Prosobranchia	Gastropoda	12,056	3,592
Nucella lapillus	Stenoglossa	Prosobranchia	Gastropoda	0	2,900
Patella vulgata	Archaeogastropoda	Prosobranchia	Gastropoda	0	2,012
Patina pellucida	Archaeogastropoda	Prosobranchia	Gastropoda	0	900
Littorina littorea	Mesogastropoda	Prosobranchia	Gastropoda	0	891
Turritella communis	Mesogastropoda	Prosobranchia	Gastropoda	0	0
Crepidula fornicata	Mesogastropoda	Prosobranchia	Gastropoda	0	0
Aplysia punctata	Aplysiomorpha	Opisthobranchia	Gastropoda	0	0
Pecten maximus	Pseudo-lamellibranchia	—	Lamellibranchia	0	0
Lepidochitona cinerea	—	—	Polyplacophora	0	0
Sepia officinalis	Decacera	—	Cephalopoda	0	0

The very active preparation of steroid sulphatase obtained from the digestive glands of *Nassarius* has been used in studies of the enzyme under different environmental conditions. The findings have shown that when DHAS is used as the substrate enzymic activity at 37° C. is four times as great as that at 12° C., and that when the test medium consists of 50% sea water, all enzymic activity disappears.

DISCUSSION

β-Glucuronidase and arylsulphatase

Vertebrates possess enzyme systems that effect the conjugation of phenolic substrates with sulphuric and glucuronic acids. Many phenolic substances, both of endogenous and exogenous origin, are excreted in the urine after conjugation as sulphates or glucuronides. In contrast, the physiological significance of β-glucuronidase, and various sulphatases which are concerned in the hydrolysis of glucuronic and sulphuric acid conjugates and are of widespread occurrence, has not yet been established. The possible function of these enzymes in the marine invertebrates is the hydrolysis of glucuronides and sulphates present in the diet. Thus, the finding that so many species of marine invertebrates possess arylsulphatase and a β-glucuronidase implies that these animals, although they show marked differences of phyla, habitat and method of feeding, must all live on diets containing sulphuric acid and glucuronic acids in conjugated form. These substances occur as structural units in certain polysaccharides and mucopolysaccharides. Thus, the hemicelluloses of plant origin contain glucuronic acid, and other polysaccharides such as hyaluronic acid and chondroitin sulphate, found in animal tissues, contain sulphuric acid as well. While the polysaccharide commonly found in marine plants (alginic acid) and animals (chitin) possesses uronic acids other than glucuronic as the structural unit, it is possible that polysaccharides other than alginic acid and chitin occur in the diets of marine animals. However, the exact substrates on which the invertebrate enzymes act are still unknown (Roy, 1956*a*).

The view that β-glucuronidase and arylsulphatase are involved in digestion is supported to some extent by the finding that, compared with carnivorous molluscs, herbivores possess a much higher enzyme activity; for it seems likely that this is because the latter species have to deal with a diet consisting of plants with thick cell walls.

'Steroid sulphatase'

Unlike arylsulphatase and β-glucuronidase, steroid sulphatase appears to have no obvious role as a digestive enzyme. The animals that possess it have very varied diets and feeding habits, which also closely resemble those of certain members of other classes and phyla from which the enzyme is absent.

The distribution of steroid sulphatase is interesting because the enzyme has been detected in only two classes in the ten phyla so far examined. These are the mammalia (Gibian & Bratfisch, 1956; Roy, 1957); and certain molluscs of the class Gastropoda, subclass Prosobranchia. Moreover, among marine gastropods, only those of the order Stenoglossa were found to possess an enzyme capable of hydrolysing AeS. However, the significance of the distribution of steroid sulphatase remains difficult to assess until a more comprehensive study has been made using a larger number of specimens from each phylum.

Preliminary results which show that a steroid sulphatase of use in the hydrolysis of AeS is exclusively confined to the order Stenoglossa (among marine invertebrates) are sufficiently interesting to make further investigation worthwhile. In addition, the discovery that *Nassarius* provides a rich source of sulphatase for use in steroid analysis warrants a more detailed investigation of the nature of the enzyme found in this animal. However, the specificity of the steroid sulphatase, present in the few available species that have been found to possess it, is such that the enzyme cannot be used to achieve complete hydrolysis of all the urinary steroid conjugates; the sulphates of 3α:5α steroids can still only be hydrolysed by chemical methods.

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SUMMARY

A study has been made of the distribution of β-glucuronidase, arylsulphatase and 'steroid sulphatase' in nine marine invertebrate phyla.

Species representative of all the phyla examined possess β-glucuronidase and arylsulphatase, but only certain gastropod molluscs, subclass Prosobranchia have a 'steroid sulphatase'. Two marine species, *Buccinum undatum* and *Nassarius reticulatus*, possess an enzyme of specificity similar to that of the sulphatase obtained from the land snail, *Helix pomatia*, in that it assists the hydrolysis of both aetiocholanolone and dehydro*epi*androsterone sulphates. However, the 'steroid sulphatase' prepared from three other marine species, *Patella vulgata*, *Patina pellucida* and *Littorina littorea*, has a higher specificity, assisting the hydrolysis of only the latter substrate. An enzyme that will effect the hydrolysis of androsterone sulphate has yet to be found.

Experiments with molluscs have shown that the β-glucuronidase and arylsulphatase activities of herbivorous species are, in general, greater than those of carnivores. Further experiments, using members of all the phyla examined, have shown that, compared with herbivorous and carnivorous species, animals

which feed on detritus are more often lacking in β-glucuronidase activity. However, this correlation between diets and enzymic activity is not found in experiments with 'steroid sulphatase' and there is evidence consistent with the view that this enzyme may not perform any physiological function related to the hydrolysis of steroid sulphates in the few species in which it has been found.

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A CHECK-LIST OF BRITISH MARINE HALACARIDAE (ACARI), WITH NOTES ON TWO SPECIES OF THE SUB-FAMILY RHOMBOGNATHINAE

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(Text-figs. 1–4)

Mites of the family Halacaridae are abundant on the sea shore, and in the sea down to considerable depths. Their identification is not an easy matter; there is no one account which can be used to identify all the British species, and the use of any one of the general accounts of European species, such as André (1946) or Viets (1936), by itself might easily lead to misidentification.

The purpose of the present list is to record the species which have so far been found around the British Isles, and to indicate sources of reliable descriptions of each species. A list of the British species of this family was given in a general list of British Acari by Turk (1953), and this has provided the starting point for the present list; no reference to occurrence in Britain before Turk's list is given, apart from one by Halbert (1920) which was left out by Turk. Twelve of the thirty-six species in the present list were not recorded by Turk. One species, *Halacarus zosterae* (Fab.), recorded by Turk has been omitted because the original description is quite inadequate to decide even which genus the creature should belong to. Eight species are known from British fresh water; these have not been included in the present list.

Only full species are given in this list, infra specific categories have been omitted. When the specific name is followed only by the author and date it indicates that the species was described from British material, and has not appeared in a general account of the group. The references in square brackets following the date indicate the source of a reliable description, while the succeeding references in rounded brackets give the authority for including the species as British.

The names in this list generally follow those given by Viets (1956) except that I have adopted Newell's (1947) classification of the Rhombognathinae, and the genus *Copidognathus* has not been divided into subgenera.

FAMILY HALACARIDAE Murray 1876

Subfamily Halacarinae Viets 1927

Gen. *Halacarus* Gosse, 1855

Subgen. *Halacarus* Gosse, 1855 s.str.

ctenopus Gosse, 1855 [Newell, 1947] (Turk, 1953)
actenos Trouessart, 1889 [Newell, 1947] (Turk, 1953)
bisulcus Viets, 1927 [Viets, 1936] (Spooner, 1959)

Subgen. *Thalassarachna* Packard, 1871 (= *Halacarellus* Viets, 1927)

basteri (Johnston, 1836) [Newell, 1947] (Turk, 1953)
southerni (Halbert, 1915)
areolatus (Halbert, 1915)
subterraneus (Schulz, 1933) [Newell, 1947][1]

Gen. *Copidognathus* Trouessart, 1888

granulatus (Hodge, 1863) [André, 1946, as *C. glyptoderma* Trs.] (Turk, 1953)
rhodostigma (Gosse, 1855) [André, 1946] (Turk, 1953)
loricifer André 1946 [André, 1946] (Turk, 1953)
fabricii (Lohmann, 1889) [André, 1946] (Turk, 1953)
tabellio (Trouessart, 1894) [André, 1946] (Turk, 1953)
lamellosus (Lohmann, 1893) [André, 1946] (Turk, 1953)
oculatus (Hodge, 1863) [André, 1946] (Turk, 1953)
gracilipes (Trouessart, 1889) [André, 1946] (Turk, 1953)
gibbus (Trouessart, 1889) [André, 1946] (Turk, 1953)

Gen. *Agauopsis* Viets, 1927

brevipalpus (Trouessart, 1889) [André, 1946] (Halbert, 1920)

Subfamily Porohalacarinae

Gen. *Caspihalacarus* Viets, 1928

hyrcanus Viets, 1928 [Viets, 1928*a*] (Green, 1956)

Subfamily Rhombognathinae

Gen. *Rhombognathus* Trouessart, 1888

notops (Gosse, 1855) [Newell, 1956] (Turk, 1953)
magnirostris Trouessart, 1889 [Newell & André, 1959][2]
lionyx Trouessart, 1900 [Newell & André, 1959][2]

[1] This species has not previously been recorded from Britain. I have identified a single specimen from the Essex coast, near the mouth of the Thames.

[2] I have British specimens which agree with Newell and Andrés' recent redescription of these species.

Gen. *Isobactrus* Newell, 1947

setosus (Lohmann, 1889) [Newell, 1947] (Turk, 1953)
levis (Viets, 1927) [Newell, 1947] (Green, 1956*a*)
uniscutatus (Viets, 1939) [Viets, 1939] (Green, 1956*a*)

Gen. *Rhombognathides* Viets, 1927, emnd. Newell, 1947

pascens (Lohmann, 1889) [Newell, 1947] (Turk, 1953)
spinipes (Viets, 1933) [Willmann, 1952] (Green, 1956*a*)
seahami (Hodge, 1860) [Newell, 1947] (Turk, 1953)
trionyx (Trouessart, 1899) [André, 1946] (Turk, 1953)
merrimani Newell, 1947 [Newell, 1947] (Green, 1956*b*)
mucronatus (Viets, 1927) [Newell, 1947][1]

Gen. *Metarhombognathus* Newell, 1947

armatus (Lohmann, 1893) [Newell, 1947] (Green, 1956*b*)
nudus (Viets, 1928*b*) [Sokolov, 1952] (see below)

Subfamily Simognathinae

Gen. *Simognathus*

minutus (Hodge, 1863) [André, 1946 as *S. sculptus*] (Turk, 1953)

Subfamily Lohmanellinae

Gen. *Lohmanella* Trouessart, 1901

falcata (Hodge, 1863) [André, 1946] (Turk, 1953)

Gen. *Scaptognathus* Trouessart, 1889

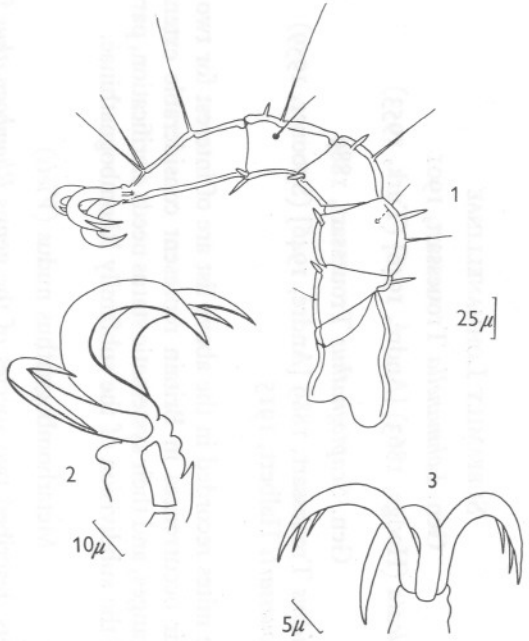
tridens Trouessart, 1889 [André, 1946] (Spooner, 1959)
trouessarti Halbert, 1915

Two of the mites recorded in the above list are of interest for two distinct reasons. Their occurrences in Britain represent considerable extensions of their known ranges, and their systematic status needs clarification, particularly in relation to the subdivision of the subfamily Rhombognathinae.

Metarhombognathus nudus (*Viets*)

In 1928*b* Viets described two species of the genus *Rhombognathus* from the Murmansk coast. Both species were found on the same alga, and one, *R. nudus*, was found only as the adult, while the other, *R. contectus*, was found

[1] Not previously recorded from Britain. I have a specimen from the Tees estuary, collected by Miss E. Clay.



Figs. 1–3. *Metarhombognathus nudus*. 1, Leg IV of adult. 2, terminal claws of leg I of adult. 3, terminal claws of leg I of larva.

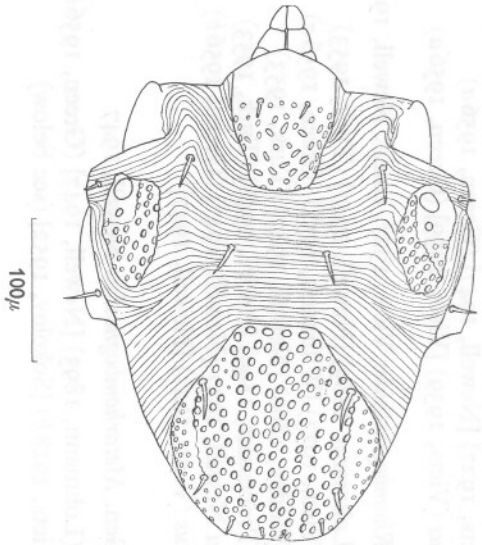


Fig. 4. Deutonymph of *Metarhombognathus nudus*, dorsal view of body. This specimen has a squarer posterior border to the anterior dorsal plate than most of the other specimens examined.

only as the deutonymph. Newell (1947), on the basis of his experience with a similar pair of forms, suggested that the two were in fact only one species. As Newell puts it 'It is highly improbable that two extremely closely allied forms (as shown by the unique structure of the lateral claws) could occupy the same habitat, both forms being numerous, and yet one be known only as the adult and the other only as the deutonymph'.

The two species have not apparently been recorded in the literature since the original descriptions and a repetition of these by Sokolov (1952), who still regards them as separate species. In Viet's (1956) register of the Halacaridae the two forms are maintained as separate species.

In March 1959 specimens agreeing with the description of *R. nudus* were found in small splash pools at the bottom of the cliffs at Cullercoats, Northumberland. The mites were very numerous in these pools, and all stages from larvae to adults were found.

The principal difference between *R. nudus* and *R. contectus* is that the dorsal plates of the former are small while those of the latter are large. When a random sample of twenty-five specimens from Cullercoats was examined in detail after clearing in lactic acid three specimens were found to be larvae. These had very small dorsal plates; the body length, from the front of the anterior dorsal plate to the posterior tip of the body, ranged from 216 to 223 μ. Five of the series were protonymphs, with small dorsal plates, and body lengths ranging from 223 to 295 μ. Seven deutonymphs, ranging in length from 350 to 380 μ, were found; these all had large dorsal plates and would be identifiable as *R. contectus*. Ten adults, with small dorsal plates, and having body lengths of 320–500 μ, completed the series.

This result of examining a random sample clearly indicates that *R. contectus* is the deutonymph of *R. nudus*. The latter name takes precedence owing to page priority. The finding that the protonymph has small dorsal plates like the adult is in agreement with Newell's description of the protonymph of *Metarhombognathus armatus* Lohmann, which has deutonymphs with large dorsal plates and adults with small dorsal plates. Newell has produced conclusive evidence that the deutonymphs of *M. armatus* with large dorsal plates are followed by adults with small dorsal plates by dissecting an adult from a quiescent deutonymph.

Further evidence that *R. contectus* is the deutonymph of *R. nudus* can be taken from the structure of the lateral claws. In the larvae, protonymphs and deutonymphs the lateral claws each carry two teeth on their inner borders (Fig. 3). This agrees with Viets figure of the claws of *contectus*. Nine of the adults had lateral claws with a single tooth on the inner border (Fig. 2), while one had two of its lateral claws with two teeth on their inner borders; it is noteworthy that this was the smallest of the adults. The loss of one of the teeth on the lateral claws appears to be normal when the adult stage is reached.

A further question is raised by the generic, or subgeneric, position of this

species. The difficulty is that two different systems of classification have been proposed. Viets (1936, 1952, 1956) regards the subfamily Rhombognathinae as consisting of a single genus *Rhombognathus* Trouessart, which he subdivides into three subgenera. Newell (1947, 1953) regards the subfamily as comprising four genera. Using Viet's system the present species would be called *Rhombognathus* (*Rhombognathopsis*) *nudus*. I have, however, adopted Newell's system for reasons which will become apparent in the discussion of the next species.

Rhombognathides merrimani *Newell*

This species has been recorded in Britain from the following localities: Isle of Man (Green, 1956*a*); Skokholm Island, Pembrokeshire (Green, 1956*a*); Gwendraeth Estuary, Carmarthenshire, on *Cladophora*, and in the gut of *Gobius minutus* (Green, unpublished records). Previous to these records it was known only from North America.

Newell (1947) has given an admirably detailed description of this species, so that no further description is necessary here. The most important feature of this species, from a systematic point of view, is its occurrence in two varieties: *merrimani* having two claws on all its legs, and *needleri* having three claws on legs I and II, and two claws on legs III and IV. Apart from this difference in claw number the two varieties are identical. My British specimens include both varieties, and I am unable to find any other difference between them, so that I am convinced with Newell that they belong to the same species. Viets (1950, 1952, 1956) does not accept this; he regards the two forms as not only separate species, but belonging to different subgenera. This is necessary if Viet's classification of the Rhombognathinae is followed, since the subgenera are separated entirely on claw characters, as follows:

Leg	I	II	III	IV
Rhombognathus	2	2	2	2
Rhombognathides	3	3	2	2
Rhombognathopsis	3	3	3	3

If the two forms of *R. merrimanni* are conspecific, as Newell and I believe, then Viet's classification must fall, and a substitute be found. I have adopted Newell's system because it is based upon an analysis of a considerable number of characters and it groups together species which are obviously closely allied although they may differ in claw number. The alternative to Newell's system would be to keep the single genus *Rhombognathus* and not divide it further, this would be a negative attitude, resulting in a genus of some thirty species with no expression of affinities within the group.

The specimens of *Metarhombognathus nudus* were collected while running a marine biology course for students at the Dove Marine Laboratory, Cullercoats. It is a pleasure to thank Dr H. O. Bull and his staff for the facilities which they made available to us.

SUMMARY

A check list of British marine mites of the family Halacaridae is given.

Metarhombognathus nudus (Viets), hitherto known only from the Barents Sea, is recorded from Cullercoats, Northumberland. Evidence is presented which indicates that the form described as *Rhombognathus contectus* Viets is the deutonymph of *Metarhombognathus nudus*.

The known distribution of *Rhombognathides merrimani* Newell in Britain is summarized, and its systematic status in relation to the classification of the Rhombognathinae is discussed. It is recommended that Newell's classification of this subfamily be adopted.

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A NEW SPECIES AND GENUS OF BRACHIOPODA FROM THE WESTERN APPROACHES, AND THE GROWTH STAGES OF THE LOPHOPHORE

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

(Plate I and Text-figs. 1–14)

A new species and genus of brachiopod has been dredged by R.V. 'Sarsia' on three cruises to the Western Approaches of the English Channel and two to the La Chapelle Bank region. In general appearance it closely resembles *Dallina septigera* (Lovén) with which it appears to have been confused by Fischer & Oehlert (1891).

In 1956 it was obtained on 13 June at position 48° 33′ N., 10° 05′ W. at a depth of 570–770 fathoms, four specimens of shell length 17–25 mm were taken together with seven *D. septigera*.

On 3 May 1957 at position 48° 33′ N., 10° 01′ W., depth 580–680 fathoms, a specimen of shell length 22 mm and an entire shell, 19 mm long, were taken accompanied by one *D. septigera*. The bottom in both positions was complex, mostly stone, shell and coral gravel, with some mud and boulders. The brachiopods were attached to worn fragments of coral, shell or stone.

During a cruise in the winter of 1958 the new species and *D. septigera* were dredged in some numbers and on this occasion there was little mixing of the two species. On 28 November at position 48° 24′–26′ N., 10° 12′–08′ W., depth 540–650 fathoms, twelve specimens (shell length 3·6–24 mm) of the new species were taken: no *Dallina* occurred in this haul. On the 29 November at position 48° 32′–33′ N., 10° 10′–09′ W., depth 375–490 fathoms, amongst twenty-seven *D. septigera* was a single specimen (shell length 15 mm) of the new species and also six *Macandrevia cranium* (Müller). On 30 November at position 48° 38′ N., 9° 47′–48′ W., depth 510–550 fathoms, eighteen individuals (shell length 2.7–22 mm) of the new species were dredged together with a single *Dallina septigera*. On the same date in position 48° 39′–38′ N., 9° 45′–50′ W., 580–510 fathoms, one perfect specimen (shell length 19 mm), parts of a further three and one entire shell of the new species were obtained, together with one *D. septigera*, and the posterior ends of another two. On this 1958 cruise the new species was taken mainly attached to the dead region of growing coral, *Lophelia prolifera* (L.) Probably because the coral afforded shelter, a number of small specimens were found, the smallest being of shell length 2·7 mm and width 2·3 mm.

In the La Chapelle Bank region (47° 11′–14′ N., 6° 13′–11′ W., 625 fathoms) on 2 July 1959 one specimen (smashed) of shell length 7–8 mm was dredged, together with two *Dallina*.

From a second cruise in the La Chapelle Bank region (47° 37′ N., 7° 27′ W., 395 fathoms) on 11 July 1959, five of the new species (shell length 15–25 mm) were obtained in the same dredge haul as sixty-two *Dallina*. These brachiopods were from a chalky bottom and were attached to minute pieces of calcareous rock, and a few to living *Limopsis* (about 8 mm high) and *Limopsis* valves. In both species the pedicles were short. The specimens of the new species were of a broad and deep type; the four large ones had the following proportions: (1) 25 mm long, 24 mm wide, 19 mm deep (Pl. I, fig. 4); (2) and (3) 24 mm long, 22 mm wide, 17 mm deep; (4) 24 mm long, 22 mm wide, 16·5 mm deep. The new species is almost as variable in general shape as is *Dallina septigera*.

Altogether some forty-six specimens of the new species, shell length 2·7–25 mm, have been dredged.

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

Fallax gen.nov.

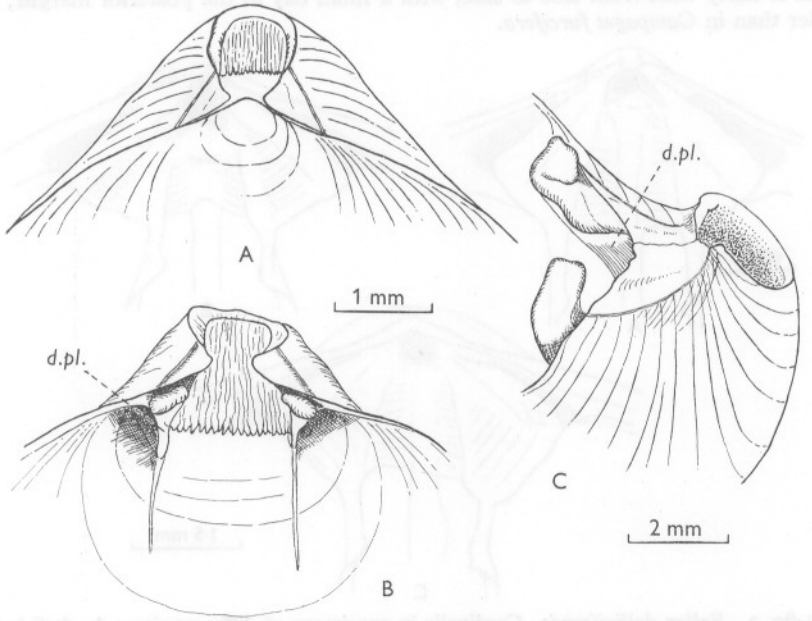
Dallinid brachiopod with hinge teeth supported at all sizes by dental plates: deep, sessile pedicle collar. Loop passing through the growth stages characteristic of dallinids to reach the adult form, which is campagiform: ascending branches very broad, joined with the descending as far posterior as the junction with the septum, the two forming a gutter. Adult lophophore plectolophous. Spicules abundant, but not coarse, occurring in the lophophore, including the outer filaments, the body wall and over the mantle sinuses.

Fallax dalliniformis sp.nov.

The shell is variable in shape, elongate ovate to subpentagonal—rarely almost as wide as long—and broadest anteriorly (Pl. I). The two largest specimens were 25 mm long, 22·5 mm wide, 16·5 mm deep (Pl. I, fig. 5 and Text-fig. 5) and 25 mm long, 24 mm wide, 19 mm deep (Pl. I, fig. 4). The hinge line is curved; the shell is broadly sulcate to intraplicate; the test fairly thin and smooth; growth lines little marked; punctations somewhat finer and denser than in *Dallina septigera*; colour in the young creamy white, in adults fulvous or brownish owing to some deposit, possibly of manganese oxide. The beak is erect, the beak-ridges rounded. The deltidial plates are disjunct in the young (Text-fig. 1A); fusion occurs at a shell length of some 10 mm and the pedicle opening is then entire; the line of fusion is generally apparent even in adults (Text-fig. 1C). The otherwise circular foramen runs down in a small 'v' in front, the base of the 'v' being produced inwards as a small projection on each side (Text-fig. 1C), into which runs a beak ridge; in individuals with short pedicles the projections are sometimes absent owing to abrasion. Dental plates support the strong hinge teeth at all sizes, vertical in the young (Text-fig. 1B) they become curved in adults. (Dental plates are absent in *D. septigera* brought in by R.V. 'Sarsia', at least down to a shell length of 11·5 mm.) From the anterior corner of each dental plate a narrow ridge runs

forward for a short distance. The deep sessile pedicle collar has a striated appearance owing to the longitudinal direction of the pits (Text-fig. 1B).

Cardinalia characterized by a platform which in adults tends to jut ledge-like over the rather thick septum (Text-fig. 2B, C). Even in the young the anterior edge of the platform is approximately at right angles to the septum, making a T-shape with it (Text-fig. 2A). (In *D. septigera* the cardinalia are less heavy, the hinge plates are generally markedly excavate, and in both young and adults the inner hinge plates curve gently to the thin-edged septum, giving a V-shaped outline.)



Text-fig. 1. *Fallax dalliniformis*. A, umbonal region of specimen of shell length 7·3 mm and width 6·8 mm. Deltidial plates are disjunct at this stage. B, beak of same specimen tilted somewhat on to its tip so as to reveal the dental plates, with ridges running forward from their anterior corners, and the sessile pedicle collar of striated appearance. C, beak of adult specimen of shell length 24 mm and width 20 mm. Oblique side view to show the shape of the unworn foramen and the conjunct deltidial plates. *d.pl.*, dental plate.

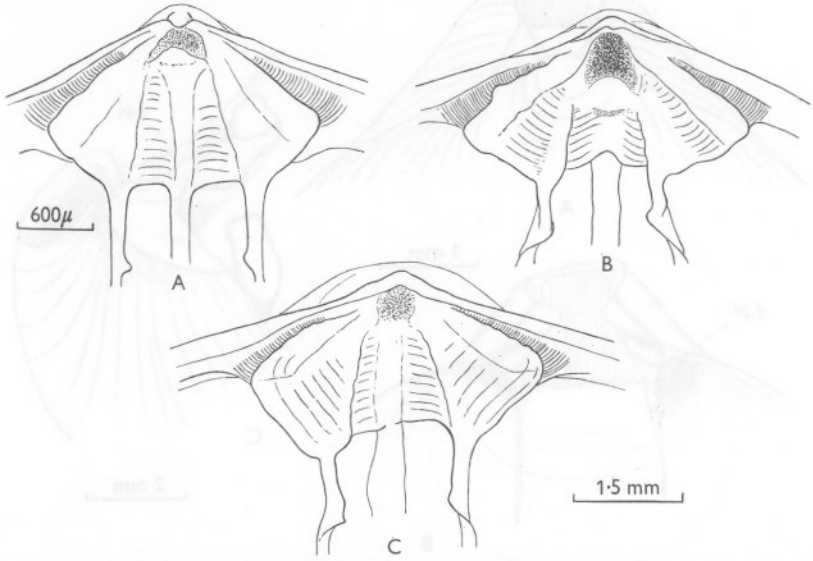
In young *Fallax dalliniformis* the crura arise from the inner socket ridges (Text-fig. 8): in adults their position shifts nearer the mid-line. No obvious crural bases, separating outer and inner hinge plates, as in *Dallina septigera*, can be distinguished, possibly because of the heavier cardinalia.

A cardinal process is absent; the diductor muscles are inserted on the floor of a small depression in front of the dorsal umbo (Text-fig. 2C), as in *Macandrevia cranium* (see Thomson, 1927, p. 240); in some individuals a small boss (Text-fig. 2B), in others a triangular elevation (Text-fig. 2A) is present in front of the depression.

In the brachial valve the median septum, rather broad posteriorly, extends forward for about three-quarters of its length.

The loop does not reach as far forward as the septum (Text-fig. 3). The crura are short (Text-fig. 2) and the crural processes small. The adult loop is campagiform, rather than terebrataliform, resembling that of *Campages furcifera* Hedley (Hedley,

1905). The descending branches are connected with the septum in all the large specimens obtained, and the very broad ascending branches are joined with the descending as far posteriorly as the junction with the septum, the two forming a gutter (Text-fig. 3). Some specimens, not always the largest, show a greater degree of resorption of the ascending branches than do others (Text-fig. 3C, D), but even so, the ascending form a gutter with the descending as far backwards as the junction of the latter with the septum. The loop illustrated is very similar to that of a specimen of '*Dallina septigera*', 25 mm long figured by Fischer & Oehlert (1891, pl. v, fig. 9, *ac*). The transverse band is fairly wide from side to side, with a small bay in the posterior margin; it is wider than in *Campages furcifera*.



Text-fig. 2. *Fallax dalliniformis*. Cardinalia in specimens of different sizes: A, shell length 11·5 mm and width 11·0 mm; B, shell length 17 mm and width 14 mm; C, shell length 21 mm and width 17 mm.

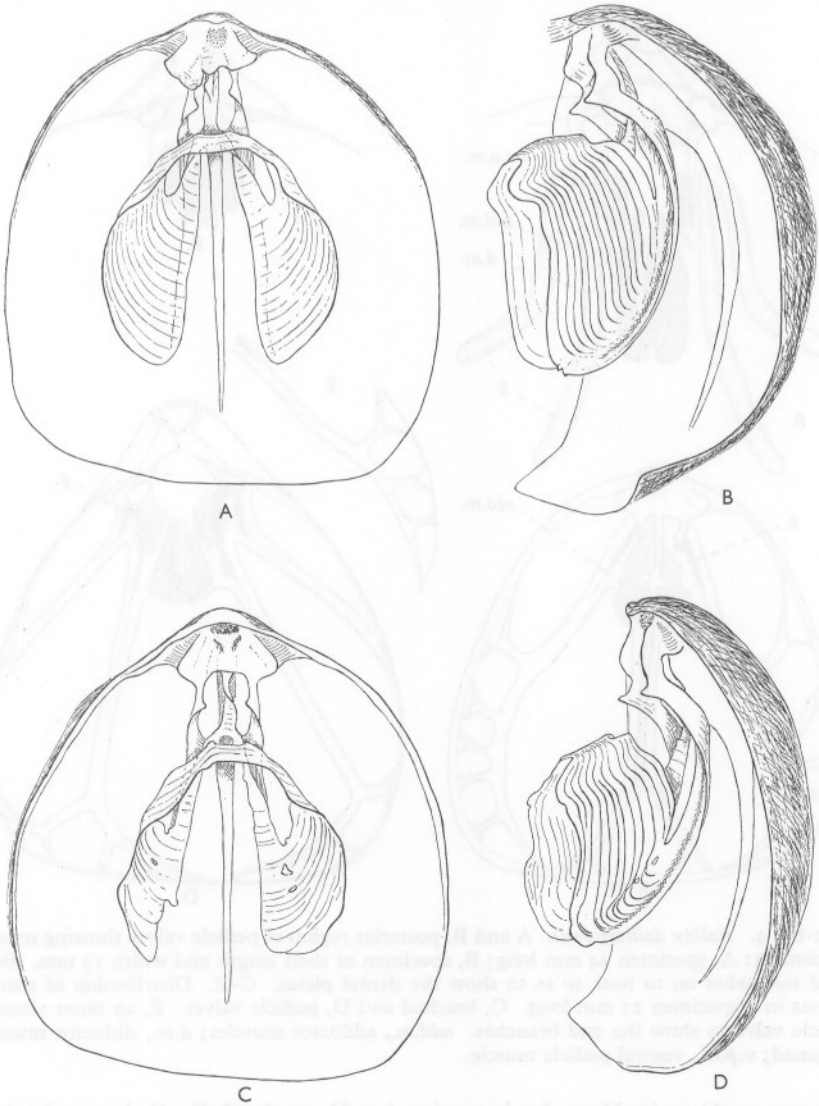
The slender pedicle, very short in the young, reached a length of 11 mm in adults attached to large pieces of coral, but was short in those attached to rock and small objects.

The ventral pedicle muscle impressions are strongly marked and bounded laterally by ridges running from the anterior corners of the dental plates (Text-fig. 4A, B). A median elongated depression bounded laterally by raised ridges, marks the position of attachment of the diductor and adductor muscles; the impressions of the two are not clearly separated. The position of the ventral pedicle muscles relative to that of the adductor and diductor muscles changes with age, as does the relative size (Text-fig. 4A, B); at a shell length and width of 15 mm the surface of attachment of the ventral pedicle muscles is large (Text-fig. 4B). In the brachial valve the anterior and posterior adductor muscles are inserted separately on each side of the septum, the attachment of the two being almost in line with one another. The dorsal pedicle muscles are inserted on the hinge plate.

Two pairs of mantle sinuses are present in each valve.* In the brachial valve

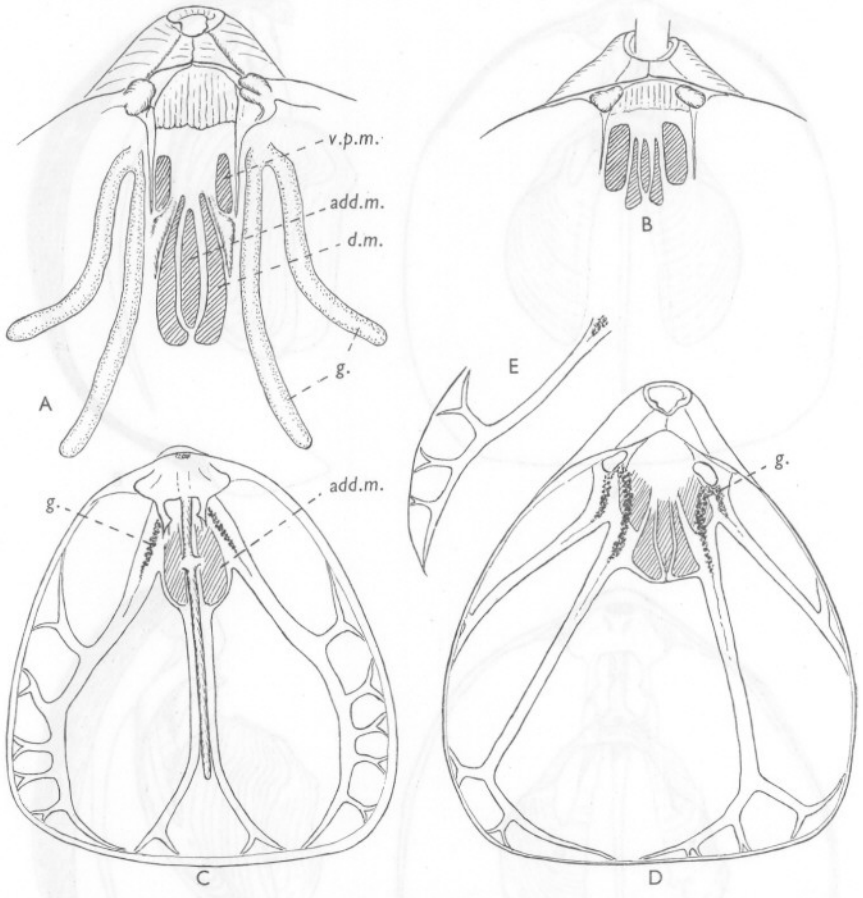
* The mantle sinuses were demonstrated by soaking the valves in an aqueous solution of Aniline blue for a few days.

(Text-fig. 4C) the inner pair run alongside the median septum to its anterior end and then diverge, each sinus branching near the mantle margin. The outer pair after their origin from the coelomic cavity run almost parallel with the valve edge giving off short exterior branches. This distribution is similar to that in *Dallina septigera* (see Fischer &



Text-fig. 3. *Fallax dalliniformis*. Interior of brachial valve of two specimens. A and B, of shell length 24 mm and width 20 mm; the left inner socket ridge is abnormally large. C and D, of shell length 21 mm and width 17 mm. The ascending branches show uneven development. The right descending branch had an abnormal growth anterior to the crural process, near an injury and mend to the valve: these abnormalities have been omitted.

Oehlert, 1891) except that the lateral sinuses appear to diverge more widely and in the only *Fallax* examined the first branch arose at some considerable distance from the coelomic cavity. In the pedicle valve (Text-fig. 4D, E) the inner pair diverge widely, and the inner branch of each is long, the two almost meeting on the mid-anterior margin. In *Dallina septigera* these sinuses are nearly parallel (Fischer & Oehlert, 1891,



Text-fig. 4. *Fallax dalliniformis*. A and B, posterior region of pedicle valves showing muscle attachment: A, specimen 24 mm long; B, specimen of shell length and width 15 mm. Both tilted somewhat on to beak so as to show the dental plates. C–E. Distribution of mantle sinuses in a specimen 21 mm long. C, brachial and D, pedicle valves. E, an outer sinus of pedicle valve to show the end branches. *add.m.*, adductor muscles; *d.m.*, diductor muscle; *g.*, gonad; *v.p.m.*, ventral pedicle muscle.

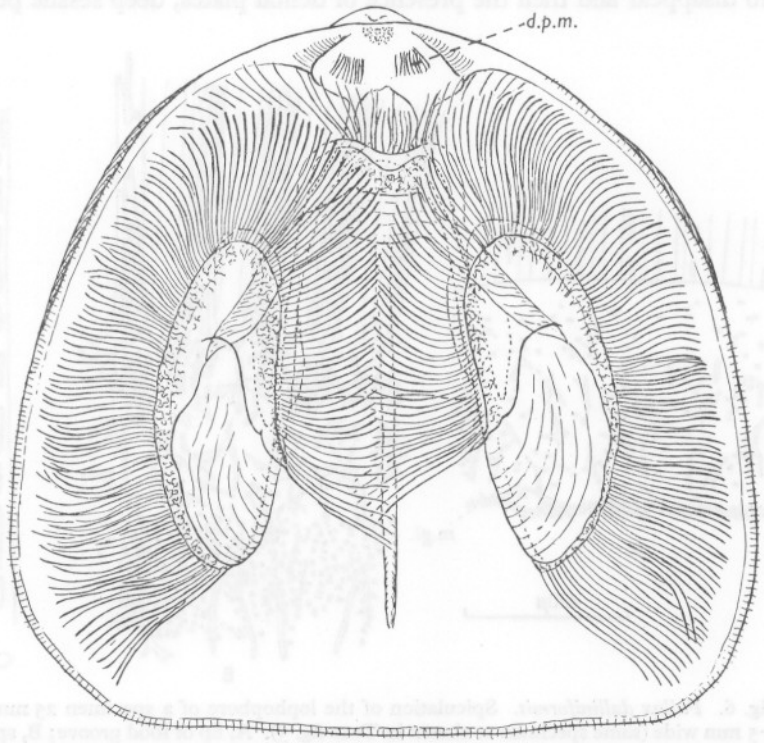
and own work), as in *Macandrevia cranium* (see Hancock, 1858). Owing to the deep concavity of the ventral valve the outer branches of the lateral sinuses are not visible in ventral view, only when the valve is tilted on to its side (Text-fig. 4E).

The distribution of the gonad is as in *M. cranium* (see Hancock, 1858) and *Dallina septigera* (see Fischer & Oehlert, 1891). The sexes appear to be separate, but sectioning

has not been carried out, and it is possible that alternation in the production of sex cells may occur.

The mantle setae are short and closely set in the adult.

The adult lophophore is plectolophous, with an alternating series of inner and outer filaments, except behind the mouth where some thirty-six are in single series. Spicules are present in the lophophore, including the outer, grooved filaments to about half of



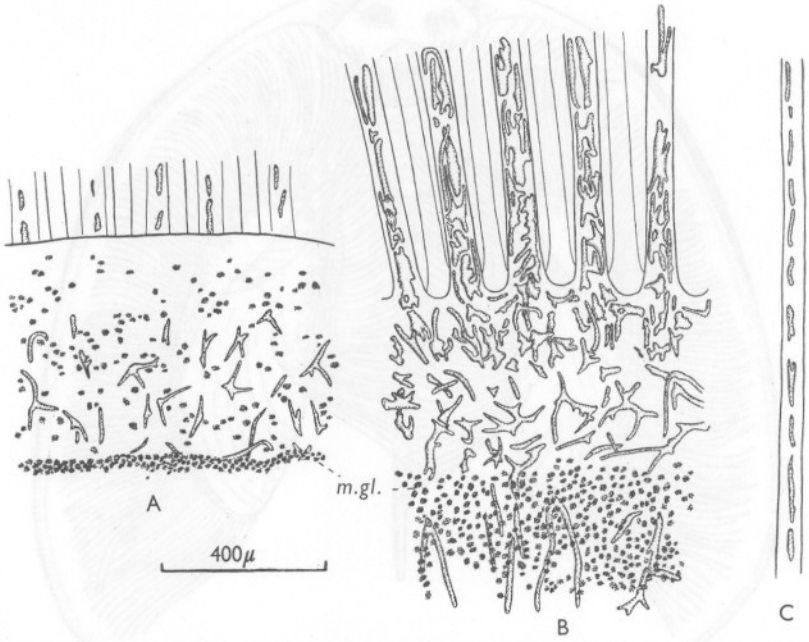
Text-fig. 5. *Fallax dalliniformis* of shell length 25 mm and width 22·5 mm. Brachial valve with plectolophe drawn living: alimentary canal omitted. Loop added after clearing in cedar wood oil. *d.p.m.*, dorsal pedicle muscle.

their length, in the body wall and over the mantle sinuses (Text-figs. 6, 7), in this differing strikingly from *D. septigera*. The spicules are fairly fine, of irregular spidery branching, especially those in the body wall and over the mantle sinuses (Text-fig. 7). The deep band of mucous cells at the base of the filaments when full of spherules hides the spicules in that position (see Text-fig. 14).

Fallax dalliniformis lacks the two carmine pigment spots found in connexion with the preoesophageal ganglion in certain brachiopods, as does also *Dallina septigera* and *Macandrevia cranium*.

The ciliary feeding mechanism is as described for *M. cranium* by Atkins, (1956). In large living specimens the valves may gape anteriorly as much as 6 mm.

With practice *Fallax dalliniformis* can be distinguished externally from *Dallina septigera* by the difference in the shape of the pedicle opening. The presence of abundant spiculation is the character by which living *Fallax dalliniformis* is most quickly distinguished from *Dallina septigera*, but in specimens long preserved in alcohol or in formalin, unless neutralized, these tend to disappear and then the presence of dental plates, deep sessile pedicle



Text-fig. 6. *Fallax dalliniformis*. Spiculation of the lophophore of a specimen 25 mm long and 22·5 mm wide (same specimen as shown in Text-fig. 5). A, lip of food groove; B, spicules in outer grooved filaments and between their bases and the loop, seen from the abfrontal surface, C, outer grooved filament at extreme distal region of spiculation. *m.gl.*, mucous gland cells.

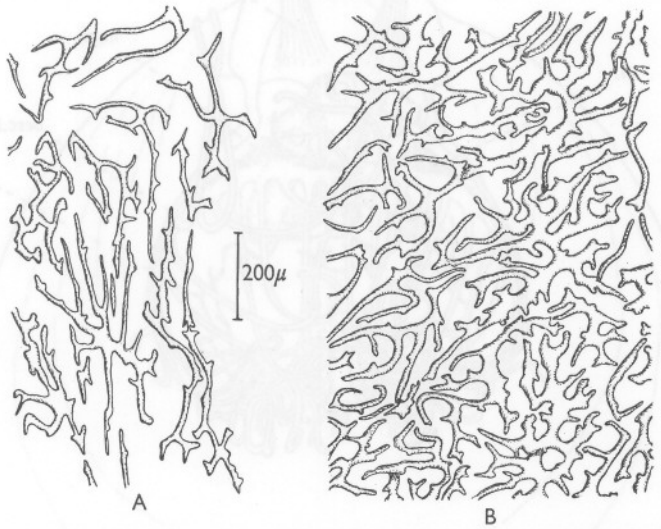
collar and the differences in cardinalia and loop clearly separate the new species from *D. septigera*. Although *D. septigera* can be described as lacking spicules, in one individual a few minute, widely scattered spicules were found in the lophophore after a careful search. Dall (1871, p. 16) has recorded the presence of a very few exceedingly delicate spicules in the floor of the greater mantle sinuses in *D. floridana*.

Type locality

Western Approaches to the English Channel in an area 48° 24′–39′ N., 9° 45′–10° 12′ W., at a depth of 375–770 fathoms.

Type specimens

As the first specimens obtained in 1956 have been damaged in the post, the single *Fallax dalliniformis* of 3 May 1957, 48° 33′ N., 10° 01′ W., 580–680 fathoms has been chosen as the holotype (Pl. I, fig. 1). Two specimens of 28 November 1958, 48° 24′–26′ N., 10° 12′–08′ W., 540–650 fathoms (Pl. I, figs. 2, 3) and one of 11 July 1959, 47° 37′ N., 7° 27′ W., 395 fathoms (Pl. I, fig. 4) have been chosen as paratypes. These specimens will be deposited in the British Museum (Natural History) when they can be conveyed there safely.



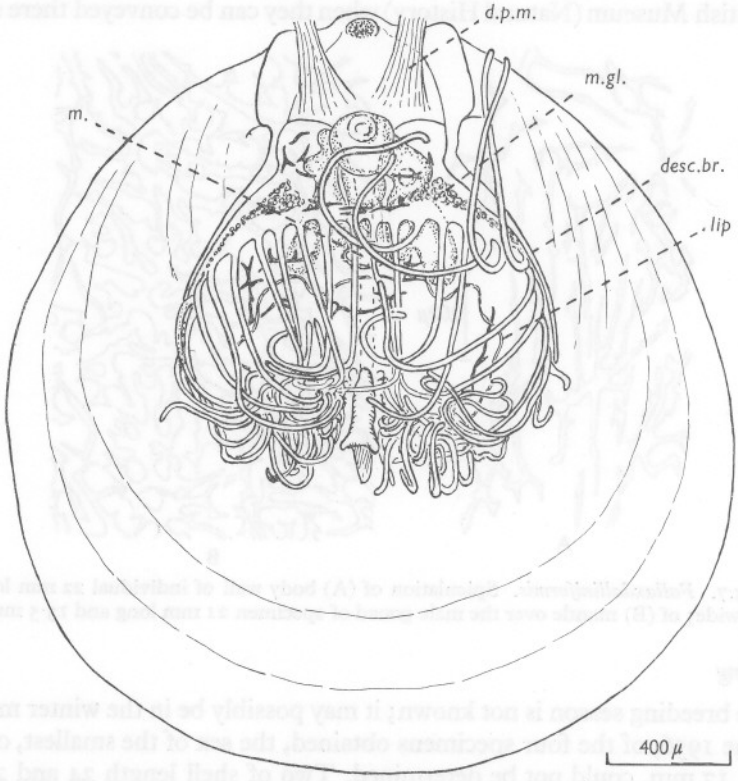
Text-fig.7. *Fallaxdalliniformis*. Spiculation of (A) body wall of individual 22 mm long and 16 mm wide; of (B) mantle over the male gonad of specimen 21 mm long and 15·5 mm wide.

Breeding

The breeding season is not known; it may possibly be in the winter months. In June 1956, of the four specimens obtained, the sex of the smallest, of shell length 17 mm, could not be determined. Two of shell length 24 and 25 mm were males with tailed sperm in the gonad, and the fourth, of shell length 21 mm, was a female. In both sexes the gonad was small. In November 1958 seven males (shell length 16–24 mm) had tailed sperm in their gonads. Two specimens (shell length 19 and 24 mm) had gonads of fair size; in the other five they were small. In five females of shell length 18–22 mm although the ova were large and round, the gonads were mostly small. In six of shell length 9–20 mm no gonad was discernible, and in a further two of shell length 20 and 22 mm sex could not be determined without sectioning. The four large *F. dalliniformis* of July 1959 had the gonads visible through the shells which were not opened.

GROWTH STAGES OF THE LOPHOPHORE AND LOOP

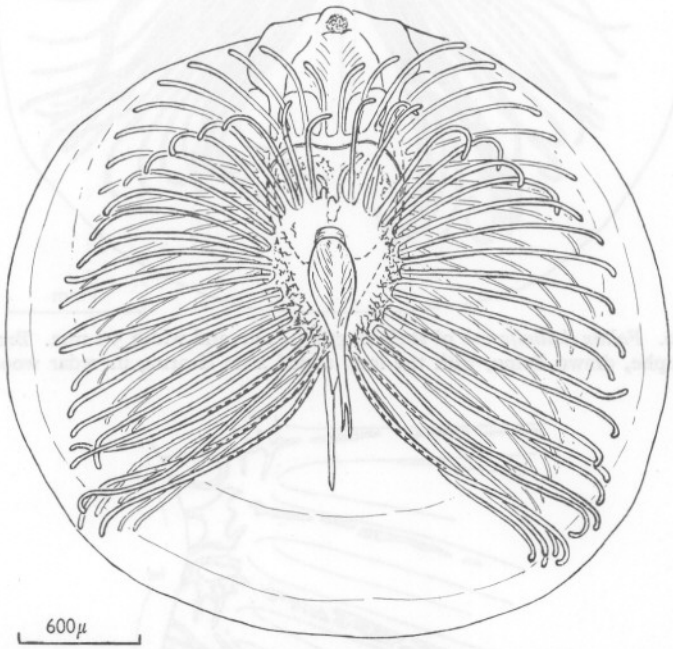
Some few immature individuals have been found allowing of certain of the growth stages to be described. The smallest, of shell length 2·7 mm and width 2·3 mm already showed spicules at the bases of the filaments, on the brachial membrane of the schizolophous lophophore and in the body wall. In this individual it appeared as though the widely curved descending branches were



Text-fig. 8. *Fallax dalliniformis* of shell length 2·9 mm and width 2·4 mm. Brachial valve with schizolophe; the descending branches (*desc.br.*) are incomplete. Preserved specimen. *d.p.m.*, dorsal pedicle muscle; *lip*, edge of lip of food groove; *m.*, mouth; *m.gl.*, mucous gland cells. Spicules are indicated.

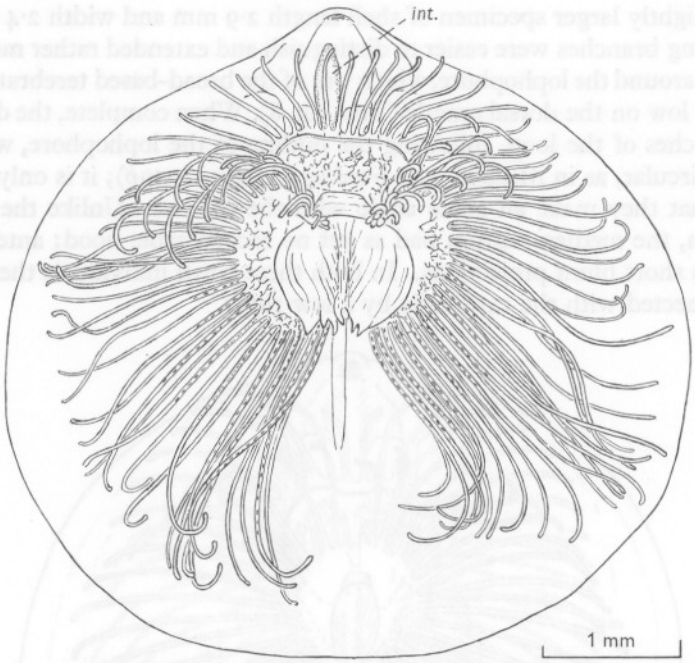
continued by long spicules; it was, however, impossible to be certain that these were not fractured ends of the branches. If they were, then the descending branches extended about half way around the lophophore. (It is not intended to imply that the loop is formed by fusion of spicules, but possibly they support the lophophore until the descending branches are fully formed.) The septum posteriorly bore a small hood of somewhat irregular shape.

In a slightly larger specimen of shell length 2·9 mm and width 2·4 mm the descending branches were easier to distinguish and extended rather more than half way around the lophophore, which was of the broad-based terebratellacean type, set low on the dorsal mantle (Text-fig. 8). When complete, the descending branches of the loop, following the outline of the lophophore, would be almost circular, as in *Macandrevia cranium* (Atkins, 1959*b*); it is only in later stages that they make an acute angle with the septum. Unlike the smaller specimen, the median septum had as yet no recognizable hood; anteriorly it bore two short blunt projections. In both these small individuals the septum was connected with the cardinalia by a low ridge.

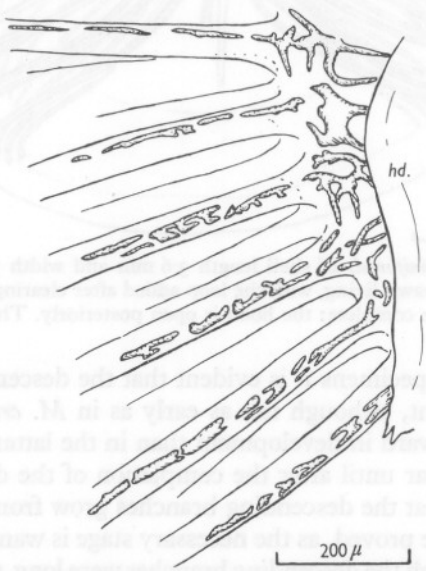


Text-fig. 9. *Fallax dalliniformis* of shell length 3·6 mm and width 3·3 mm. Brachial valve with late schizolophe, drawn living, with the loop added after clearing in cedar wood oil. The descending branches are complete: the hood is open posteriorly. The gut is omitted.

From these two specimens it is evident that the descending branches arise early in development, although not as early as in *M. cranium*, and that the septum is more forward in development than in the latter species, in which a hood does not appear until after the completion of the descending branches (Atkins, 1959*b*). That the descending branches grow from the crura only can unfortunately not be proved, as the necessary stage is wanting. It is, however, probable, for although the descending branches were long, no lateral projections were present on the septum.



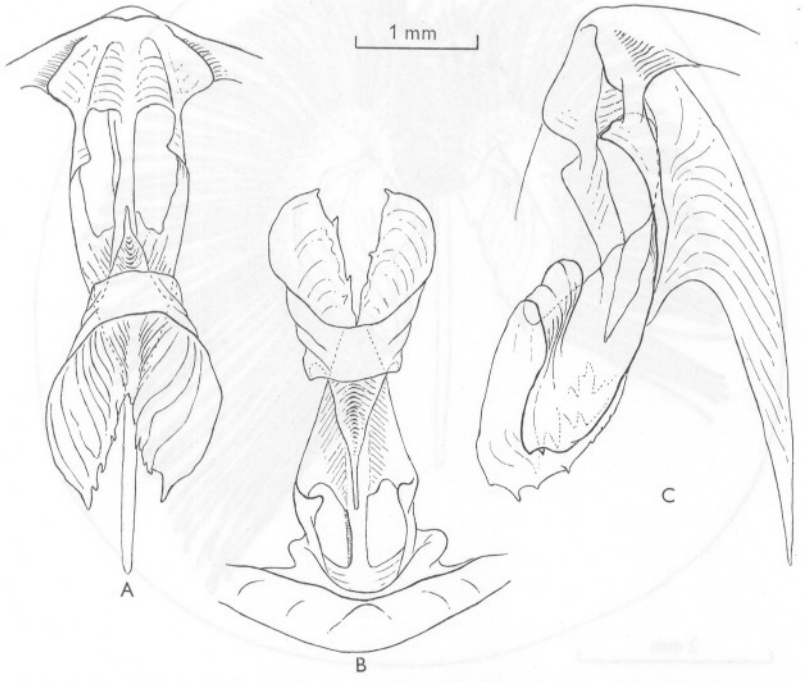
Text-fig. 10. *Fallax dalliniformis* of shell length 5·4 mm and width 5·1 mm. Brachial valve with zygolophe, drawn living, with the loop added after clearing in cedar wood oil. *int.*, intestine.



Text-fig. 11. *Fallax dalliniformis*. Part of the lophophore shown in Text-fig 10, enlarged to show the spicules. *hd.*, hood.

At a shell length of 3·0 mm and width of 2·7 mm the lophophore was late schizolophous and the lateral arms were already deflected. The descending branches joined the septum at an acute angle: the hood was long, narrow and entire; the septum was produced into a long spine anteriorly. Spicules were now present in a few of the outer filaments situated at the anterior ends of the lateral arms.

Three specimens of nearly the same size and of about the same stage of development of the lophophore—late schizolophous to very early zygolophous —were obtained. In one of shell length 3·6 mm and width 3·2 mm, the angular

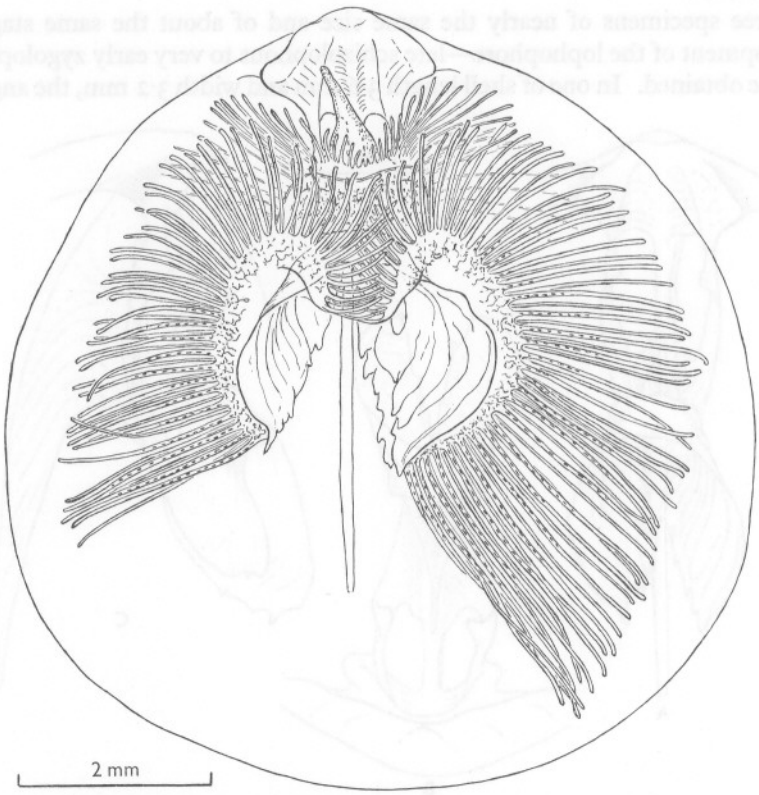


Text-fig. 12. *Fallax dalliniformis*. Loop of specimen 7·3 mm long and 6·8 mm wide. A, ventral view; B, as seen when the valve is standing on its anterior edge; C, side view.

hood was the least developed of the three, the posterior end being entire. Anteriorly the septum had spinous projections. The hood of a slightly smaller individual of shell length 3·6 mm and width 2·7 mm, was fairly broad and somewhat asymmetrical; the posterior end had undergone resorption. Anterior spinous projections were short and from their appearance it is possible that the hood had been damaged in life. The hood of the third individual of shell length 3·6 mm and width 3·3 mm was long, fairly narrow with high sides and was open posteriorly (Text-fig. 9). Anteriorly the septum was produced into long spines. It is evidently at about the size of these three

specimens, when the lophophore is late schizolophous, that resorption of the posterior end of the hood occurs.

At a shell length of 4·8 mm and width of 4·4 mm the lophophore was early zygolophous; the hood, somewhat asymmetrical in shape, had widened; the transverse band was distinct. Long anterior spines still remained.

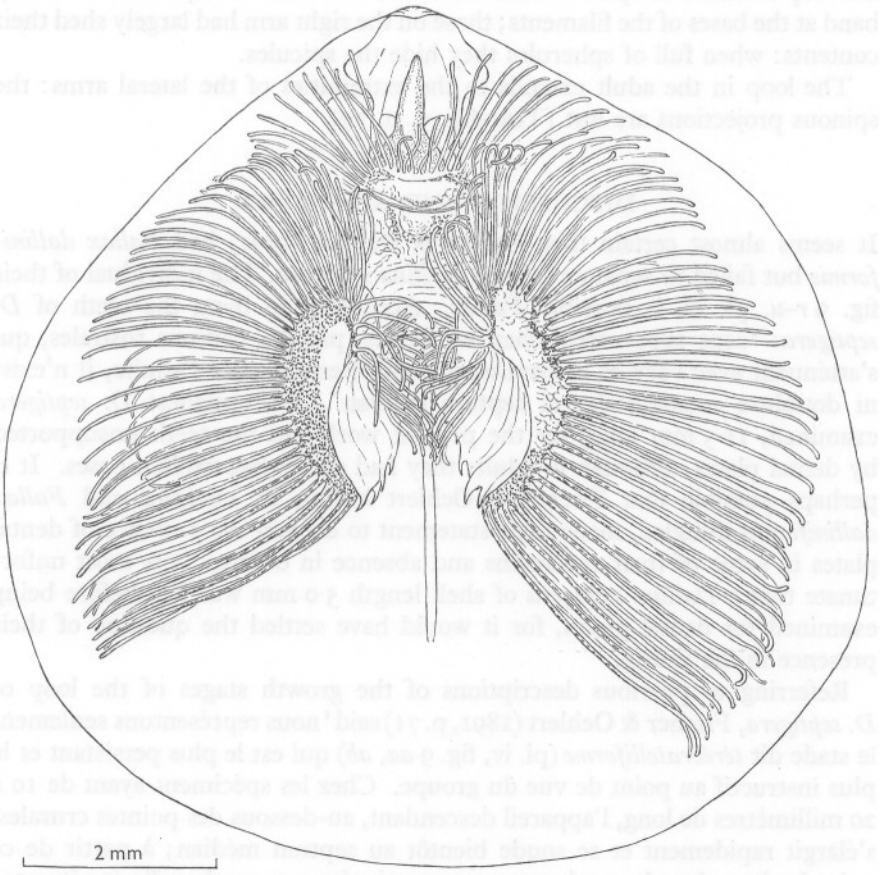


Text-fig. 13. *Fallax dalliniformis* of shell length 8·7 mm and width 7·8 mm. Brachial valve with early plectolophe; the lateral arms most unevenly developed. Spicules are indicated.

The lophophore was zygolophous at a shell length of 5·4 mm and width of 5·1 mm. The hood, or ascending branches of the loop, had widened greatly: slight anterior bifurcation was evident with short spines bordering it (Text-fig. 10). Spicules in part of the lophophore are shown enlarged in Text-figure 11. The loop extended to the middle of the valve.

A specimen of shell length 7·3 mm and width 6·8 mm was without flesh, so that three aspects of the loop could be drawn (Text-fig. 12). The hood was deeply divided anteriorly, the sides of the bifurcation being spinous. The sides of the hood, or broad ascending branches, were attached to the ventral surface

of the septum as far backward as the posterior attachment of the descending branches, the two sides forming a ventrally facing gutter, extending beyond the transverse band of the loop. This is a slightly younger stage than that shown by Friele (1877, pl. iv, fig. 13) of *Dallina septigera* at a shell length of about 6·0 mm and is very similar.



Text-fig. 14. *Fallax dalliniformis* of shell length 9·2 mm and width 8·9 mm. Brachial valve with early, almost symmetrical, plectolophe. The position of the mucous cells on the lophophore is indicated where these are full of spherules, as on the left lateral arm and certain regions of the right arm. Spicules are only visible where the mucous cells have shed their contents.

At a shell length of 8·7 mm and width of 7·8 mm the lophophore was early plectolophous, with some three-quarters turn to the spiral arm (Text-fig. 13). In this specimen the lateral arms were most unequally developed. Spines were present along the inner, dorsal edges of the ascending branches. Resorption had begun posteriorly in the larger, right ascending branch. Spicules

were present over the oesophagus, contrary to the condition in *Platidia* (Atkins, 1959*a*).

In an individual of shell length 9·2 mm and width 8·9 mm with lateral arms almost symmetrically developed (Text-fig. 14), the early plectolophe had rather more than a complete turn to the median arm. The anterior region of the loop retained its spinous character. Mucous cells were present in a deep band at the bases of the filaments; those on the right arm had largely shed their contents: when full of spherules they hide the spicules.

The loop in the adult extends to the extremities of the lateral arms: the spinous projections are lost (Text-fig. 5, p. 77).

DISCUSSION AND AFFINITIES

It seems almost certain that Fischer & Oehlert (1891) had *Fallax dalliniformis* but failed to separate it from *Dallina septigera*. The individual of their fig. 9 *r–u*, pl. iv, is possibly *Fallax*. According to them the teeth of *D. septigera*: 'sont supportées, chez les jeunes, par des cloisons rostrales, qui s'atténuent avec l'âge et disparaissent complètement chez l'adulte, il n'exist ni doublure sous-apicale, ni septum médian'. The smallest *D. septigera* examined, 11·5 mm long, for the present work had the teeth unsupported by dental plates, although in adults they had somewhat swollen bases. It is perhaps possible that Fischer & Oehlert having *D. septigera* and *Fallax dalliniformis* mingled, made their statement to explain the presence of dental plates in some of their specimens and absence in others. It is most unfortunate that a *Dallina septigera* of shell length 5·0 mm was lost before being examined for dental plates, for it would have settled the question of their presence in the young.

Referring to previous descriptions of the growth stages of the loop of *D. septigera*, Fischer & Oehlert (1891, p. 71) said 'nous représentons seulement le stade dit *térébratelliforme* (pl. iv, fig. 9 *aa*, *ab*) qui est le plus persistant et le plus instructif au point de vue du groupe. Chez les spécimens ayant de 10 à 20 millimètres de long, l'appareil descendant, au-dessous des pointes crurales, s'élargit rapidement et se soude bientôt au septum médian; à partir de ce point les branches descendantes sont constituées par une lamelle étroitement repliée sur elle-même en forme de gouttière et dont la partie externe persiste seule dans les appareils arrivés à leur complet état de développement.' Thus according to them the gutter is formed entirely by the descending branch. It has been shown (p. 73–4) that this 'térébratelliforme' stage of Fischer & Oehlert's '*D. septigera*' is the adult loop of *Fallax dalliniformis*—indeed their fig. 9 *aa* and *ab*, pl. iv represent a somewhat idealized loop of *F. dalliniformis* with the cardinalia of that species—and the gutter is formed not by the descending branch alone, but by the fused descending and ascending branches, as in *Campages furcifera* Hedley. Thomson (1927, fig. 74*f*) reproduced

their figure as a terebrataliform stage. In *Dallina septigera* dredged by R.V. 'Sarsia' the loop is entirely free from the septum from a shell length of about 13 mm., and the ascending and descending branches are fused for a variable, but generally short, distance anteriorly (Atkins, 1960).

Fallax dalliniformis obtained in November 1958 from coral exhibited abnormalities of shell and internal structure, including asymmetry of shell valves (Pl. I, fig. 2), loop (see Text-fig. 13) and cardinalia (Text-fig. 3A). Asymmetry of the shell resulting from injury was probably caused by the crowded branches and polyps of the coral; that of the loop and cardinalia may follow, or possibly be caused by predatory animals. As mentioned previously these *Fallax* from coral tended to have pedicles of up to 11 mm long.

It is impossible to place this new species in *Dallina* because of the presence at all sizes of dental plates, deep sessile pedicle collar, the difference in cardinalia and the presence of abundant spiculation; the presence of dental plates precludes it being placed in *Japanithyris*, a genus of which little is known; a new genus, *Fallax*, has therefore been created.

Fallax dalliniformis no doubt should be placed in the Dallinidae and most probably in the Dallininae, as now constituted because of its loop form, presence of dental plates, early development of the descending branches of the loop, probably growing from the crura only, and anterior bifurcation of the septum in the early stages, with spines on the loop. But while it is said (Thomson, 1927, p. 231) of the Dallininae that spicules are occasionally present, but never abundant, they are abundant, although not coarse, in *F. dalliniformis*, which in this differs from any genus of Dallininae so far described. It is possible that spiculation is present in other genera of the subfamily and has escaped notice, as apparently by Fischer & Oehlert (1891) in certain of their specimens of '*Dallina septigera*'. Mr G. F. Elliott tells me that among specimens labelled *D. septigera* in the British Museum (Natural History) one dried specimen with T-shaped cardinalia shows glistening membranes suggesting strong spiculation.

In the presence of spicules *Fallax dalliniformis* approaches *Laqueus*, but the adult loops are entirely different, as is the folding. In addition to spicules it agrees with *Laqueus* in the presence of dental plates, sessile pedicle collar and absence of cardinal process, all characters in which it also agrees with certain of the Dallininae. Thomson (1927, p. 259) records of *Laqueus* 'small spicules present over the pallial sinuses, but not extending to the body-wall or lophophore'. In the few *L. californianus* (Küster) from Puget Sound, North America, that I examined, long delicate spidery spicules were present not only over the pallial sinuses, but also in the body wall, at the bases of the filaments behind the mouth, and between the two carmine pigment spots and the transverse band; I was unable to find them in the filaments or between their bases and the loop. Incidentally this species of *Laqueus* possesses two carmine pigment spots near the preoesophageal ganglion.

Although the adult loop of *Fallax dalliniformis* resembles that of *Campages*, and especially that of *C. furcifera* Hedley, *Fallax* differs from *Campages* in possessing dental plates at all ages, lacking a cardinal process and possessing abundant spiculation.

My thanks are due to the Captain and crew of R.V. 'Sarsia' who dredged the brachiopods, and especially to Dr A. J. Southward and Mr G. R. Foster who were in charge of the scientific work, and who took great care of these delicate brachiopods during the journeys back to the laboratory. Those from the second cruise to the La Chapelle Bank region were very kindly picked out and cared for by Mr H. Gill (Cambridge University). I am indebted to Mr A. C. G. Best for the photographs in the plate: faults in blacking out the background are my responsibility. Mr G. F. Elliott most kindly read the manuscript. The work was done while occupying a London University table.

SUMMARY

A new species and genus, *Fallax dalliniformis*, of dallinid brachiopod is described from the Western Approaches to the English Channel, in the area 48° 24′–39′ N., 9° 45′–10° 12′ W., depth 375–770 fathoms, and from the La Chapelle Bank region 47° 11′–37′ N., 6° 11′–7° 27′ W., 395–625 fathoms. It is homoeomorphic with *Dallina septigera* (Lovén), with which it occurred in some dredge hauls. It is characterized by the possession of dental plates to the hinge teeth, deep sessile pedicle collar, a campagiform loop in the adult, spiculation in the lophophore, the body wall and over the mantle sinuses: in all these characters and in the cardinalia it differs from *D. septigera*. Shell length 25 mm.

Growth stages of the lophophore from schizolophous to plectolophous are described. The loop passes through the growth stages characteristic of dallinids as far as the campagiform stage, which in this species is the adult loop.

EXPLANATION OF PLATE I

Fallax dalliniformis sp. et gen.nov.

Fig. 1A–D. Dorsal, ventral, lateral and frontal views of the holotype, 22 mm long, 19 mm wide and 15·5 mm deep. May 1957, 48° 33′ N., 10° 01′ W.; 580–680 fathoms.

Figs. 2–4. Paratypes to show variation in shape.

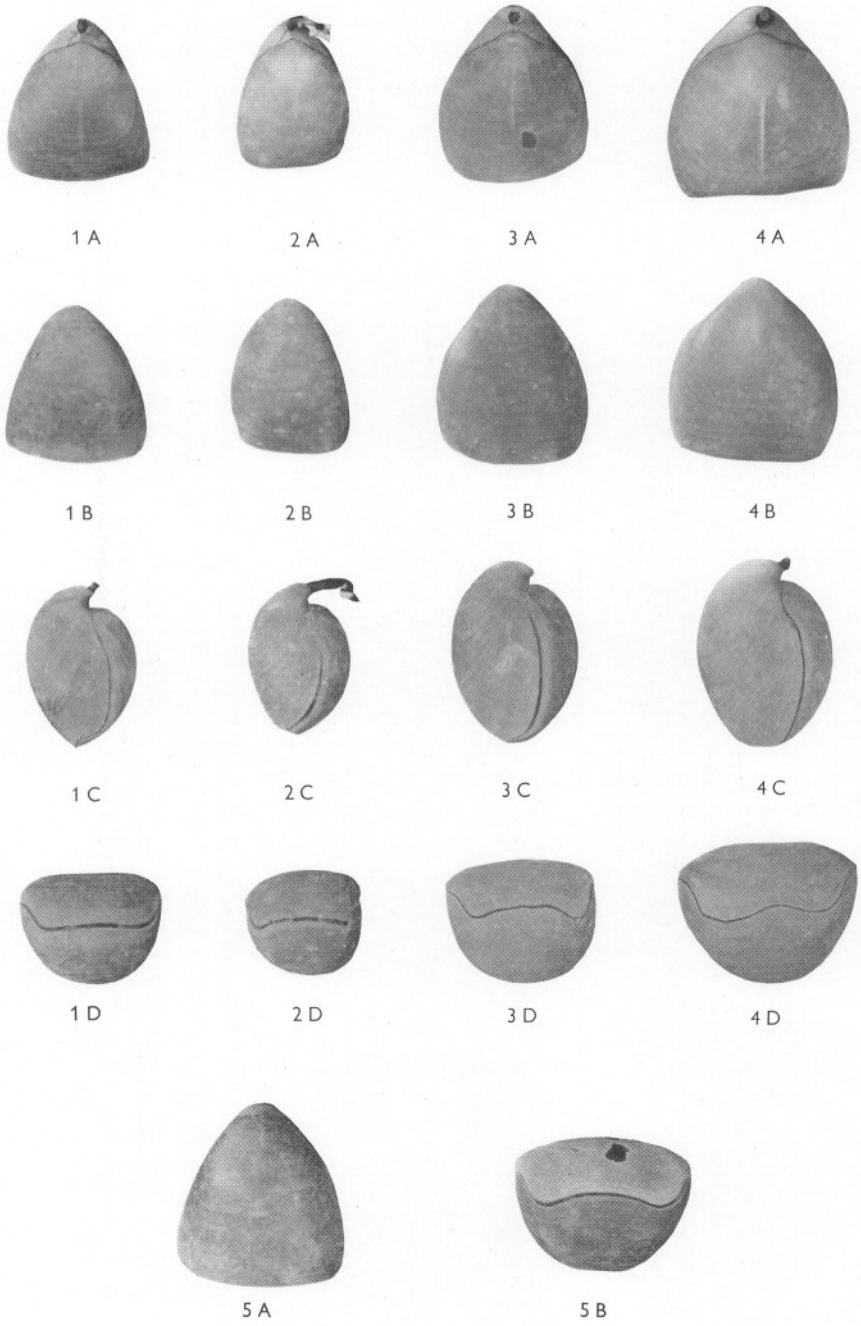
Fig. 2A–D. Specimen 21 mm long, 15·5 mm wide and 15 mm deep. November 1958, 48° 24′–26′ N., 10° 12′–08′ W.; 540–650 fathoms, on coral. Foraminifera attached to pedicle.

Fig. 3A–D. Specimen 24 mm long, 20 mm wide and 17 mm deep. Obtained on same date and from same position as previous specimen.

Fig. 4A–D. Specimen 25 mm long, 24 mm wide and 19 mm deep. July 1959, from chalk bottom, 47° 37′ N., 7° 27′ W.; 395 fathoms.

Fig. 5A, B. Ventral and frontal views of specimen 25 mm long, 22·5 mm wide and 16·5 mm deep. (The same specimen of which the brachial valve with plectolophe is shown in Text-fig. 5; the loop is now smashed.) June 1956, 48° 33′ N., 10° 05′ W.; 570–770 fathoms.

(All photographed while in water. Approximately natural size.)



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A NOTE ON *DALLINA SEPTIGERA* (LOVÉN), (BRACHIOPODA, DALLINIDAE)

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

(Plate I and Text-figs. 1–5)

The discovery of a homoeomorph, *Fallax dalliniformis*, of *Dallina septigera* (Lovén) dredged with that species (Atkins, 1960) and, it is believed, sometimes confused with it, certainly by Fischer & Oehlert (1891), has made a brief redescription desirable. During the present work more than a hundred *D. septigera* of shell length 5–31 mm have been obtained, and most of them examined internally.

The original description in Latin—without figures—of *Dallina septigera* (= *Terebratula septigera*) was by Lovén in 1846 (p. 183). Unfortunately his description does not now clearly separate his species from *Fallax dalliniformis*, except perhaps his failure to mention the presence of dental plates, pedicle collar and spiculation. It is doubtful how much weight can be attached to this, for on the same page he failed to mention the presence of dental plates in *Macandrevia cranium*. He gave the length as 28 mm, width as 21·5 mm and depth as 17 mm.

The Stockholm Natural History Museum, the most likely Museum to possess Lovén's type specimen, unfortunately does not have it, and it is probable that he did not designate one. The Museum, however, does possess old collections of dried shells of this species from Norway and the North Sea, which Lovén probably studied. Through the kindness of Dr H. Mutvei a large specimen from these was loaned to me: he stated that it is not known with certainty whether it was collected before or after 1846—the date of publication of Lovén's paper. This specimen (Pl. I, fig. 2A–D), of length 29 mm, width 22 mm and depth 18 mm, has no dental plates; the pedicle collar is a narrow rim, slightly more marked than in some of the *Dallina septigera* dredged by R.V. 'Sarsia'. The loop is attached to the crura only and is somewhat stouter than in my specimens of about the same size. The ascending branches are wide, narrowing abruptly to the transverse band, which is deeply embayed anteriorly, and less so posteriorly. The band is narrower (antero-posteriorly) than in the majority of 'Sarsia' specimens, in which it generally resembles that figured by Sars (1878, Tab. 1, fig. 2*g*): the depth of the band, however, is variable. The inner hinge plates form a V with the septum. Dr Mutvei mentioned that the shells comprising these old collections have similar external shape.

Gray (1853, p. 59) was the next to describe *D. septigera* under the name *Waldheimia septata*. He seems to have given an abstract of Lovén's description with the same shell measurements: he also failed to provide figures.

In 1855 (p. 441) Davidson described *D. septigera* from a single specimen originally in the possession of S. Hanley, and gave figures of the dorsal, lateral and frontal views of the entire shell. These figures show the typical shape of the beak and pedicle opening of the species, and his failure to mention dental plates makes it almost certain that he had it. His statements that the deltidium was in two pieces and the beak ridges well defined would seem to be errors.

Dall in 1871 was apparently the first to give a figure of the loop, and from this it is obvious that he was dealing with *D. septigera*. His figure clearly shows that in the adult the loop was attached to the crura only, it also shows the V-shaped junction of the inner hinge plates with the septum. He stated: 'hinge plate longer than wide, anterior point passing forward between the crura'. He gave the length as 1·20 in., width as 1·10 in. and depth as 0·80 in., and mentioned that this large specimen originated from Jeffreys. Although he does not refer to the absence of dental plates in *D. septigera* he (1921, p. 359) remarked of *D. floridana* 'there are no props to the dental plates', apparently meaning no props to the teeth.

Friele (1877) described certain of the growth stages of the loop of *D. septigera*. That he had this species appears certain, for he figured the inner hinge plates running in a V to the septum and showed a young stage (size not given) in which the branches of the loop were free from the septum, thus distinguishing it from *Fallax dalliniformis*. The crura in his specimens—drawn with camera lucida—have the appearance of being somewhat shorter than in the 'Sarsia' *Dallina septigera*.

Jeffreys (1878, pp. 407–9) described *D. septigera* under the name *Terebratula septata* Philippi. He noted that in the full grown state the loop is attached only to the hinge plate: from this it is clear that he had *D. septigera*. His specimens, the largest of which was an inch and three-tenths long, were obtained by the 'Porcupine' expeditions. The dredgings in the Western Approaches to the English Channel from which Jeffreys obtained some of his specimens were near the positions where R.V. 'Sarsia' dredged, it therefore seems possible that he may have obtained *Fallax dalliniformis* as well as *Dallina septigera*.

Sars (1878, p. 11 and pl. I, fig. 2), under the name *Waldheimia septata*, gave good figures of the entire shell in dorsal, lateral and frontal view and also the brachial valve in ventral and side view. From the two latter views in particular it is evident that Sars was dealing with *Dallina septigera*: the hinge plates run in a V to the septum and the loop is attached to the crura only, while the descending and ascending branches are joined for but a short distance anteriorly. The crural processes end in characteristic slender curved points.

He gave the length as 36 mm: the adult he figured was somewhat longer than broad.

Davidson in his Linnean Society Monograph (1886) again described *D. septigera* (= *Waldheimia septigera*). He repeated that the deltidium was in two pieces, but omitted his earlier statement concerning well-defined beak ridges. He reproduced some of Sars's figures, but gave a new one of the ventral view of the adult loop (pl. 11, fig. 4) and added an anterior view (pl. 11, fig. 6). The figures show that the adult loop is attached to the crura only and is therefore not that of *Fallax dalliniformis*. Moreover, he stated (p. 57): 'the connection between the process of the lamellae and the septum is severed in a specimen 6 lines in length, and in a specimen 8 lines in length the lamellae are separated and the character of the loop is that of adult *Waldheimia*'. He gave the length as 1 in. 8 lines, that is approximately 42 mm.

Davidson (1886), in discussing Jeffreys' mistaken naming of *Dallina septigera* as *Terebratula septata* Philippi, mentioned that Seguenza had examined the perfect loop in several adult examples of Philippi's *T. septata* from the Pliocene and found it to be three times attached. Thomson (1927, p. 251) placed *septata* in his new genus *Japanithyris*.

Mr G. F. Elliott most kindly examined all the specimens of '*Dallina septigera*' in the British Museum (Natural History) and from his description there is evidently a mixture of *D. septigera* and *Fallax dalliniformis*, but the specimens of the latter may have been added since Davidson's time.

As already mentioned (Atkins, 1960) it seems clear that Fischer & Oehlert (1891) had both *Dallina septigera* (called by them *Magellania septigera*) and its accompanying homoeomorph, and that their description is compounded of characters of the two. Of their figures on pl. iv, fig. 9*aa*, *ab* of the brachial valve of a specimen of shell length 14 mm is almost certainly *Fallax dalliniformis*, and it is possible that fig. 9*r–u*, exterior views of a shell, is also. Fig. 9*x–z* is the loop of *Dallina septigera*, but although it would seem by the dotted line connecting fig. 9*x* and 9*y* that *y* is the loop of *x* enlarged, yet although 9*x* shows the V-shaped meeting of hinge plates with septum, fig. 9*y* shows the meeting T-shaped; this is perhaps the artist's error; in this figure there is also some appearance of transverse connecting bands between the descending branches and the septum, almost obliterated, whether intentionally or not, by shading. On pl. v, fig. 9*ac* of the loop of a specimen 25 mm long is almost certainly of *Fallax dalliniformis*.

Certain of the statements of Fischer & Oehlert apply to *Dallina septigera* only, namely: (1) 'il n'existe ni doublure sous-apicale...' in the ventral valve; (2) 'plateau cardinal...acuminé en avant'; (3) 'mince septum médian'; and (4) loop connected to crura only as in their pl. iv, fig. 9*x* and *z*.

Their statement that the 'dents sont supportées, chez les jeunes, par des cloisons rostrales, qui s'atténuent avec l'âge et disparaissent complètement

chez l'adulte' needs confirmation. In *D. septigera* dredged by R.V. 'Sarsia' dental plates are absent from a shell length of at least 11·5 mm, whereas in *Fallax dalliniformis* they are present at all the sizes obtained.

Fischer & Oehlert (1891, p. 69) mentioned that they had several times found the 'terebratelliforme' stage in individuals of large size appearing fully adult. These were almost certainly *F. dalliniformis* Atkins, 1960.

Thomson (1927, pp. 252–3) evidently based his description of *Dallina septigera* at least partly on that of Fischer & Oehlert (1891, pp. 64–71) and therefore mentioned certain characters absent in *D. septigera* but present in its homoeomorph *Fallax dalliniformis*. The description of *Dallina septigera* given here is based on Thomson, omitting those characters which belong to *Fallax dalliniformis* and emphasizing those which distinguish it from the latter species.

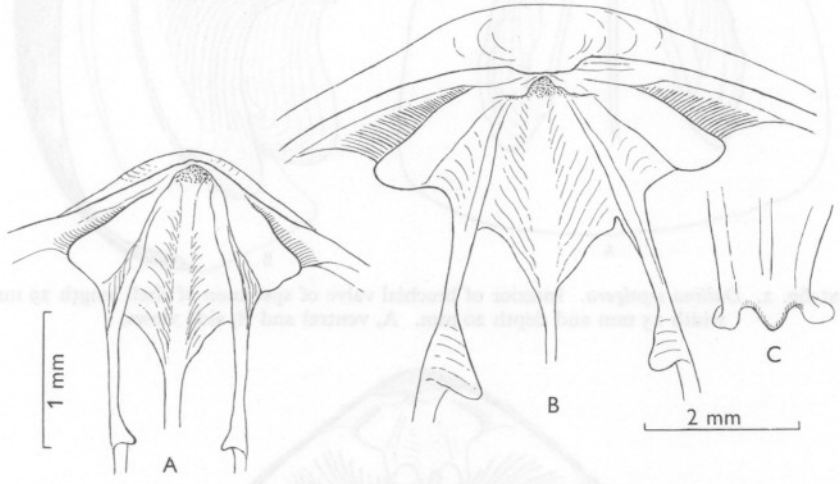
The presence of dental plates is said to be characteristic of the Dallininae, but as Thomson (1927, p. 178) remarked in a footnote *Dallina* itself is an apparent exception, although—evidently following Fischer & Oehlert (1891)—he stated that they were present in the young stages of *D. septigera*, but absent in adolescence (p. 238). Hatai (1940, pp. 316–18, 319, 320–21, 322) noted the absence of dental plates in *D. raphaelis* (Dall), *D. raphaelis albida* (Dall), *D. obessa* Yabe and Hatai and *D. elongata* Hatai. He (p. 320) omitted to mention their absence in *D. triangularis* Yabe and Hatai, but inferred it by saying 'interior features as in *D. raphaelis*'. Dall (1921, p. 359) had previously remarked of *D. floridana* 'there are no props to the dental plates nor septum in the pedicle valve'. This is difficult to follow unless he intended to write there are no props to the teeth. Hatai (1940) in his description of Japanese species of *Dallina* noted the large complete circular foramen, although in *D. raphaelis albidus* it was said to be somewhat transverse. In *D. raphaelis*, *D. raphaelis albidus* and *D. elongata* he described a short sessile striate pedicle collar, but omitted mention of this in *D. obessa* and *D. triangularis*, although it is implied in the latter. All the Japanese species have a symphytium with or without a raised median ridge.

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

Dallina septigera (Lovén)

Shell large, very variable in shape, generally subpentagonal, broadest anteriorly (Pl. 1, fig. 2A–D), but varying from elongate triangular (Pl. 1, fig. 1A–D) to equilateral triangular, in which the width may slightly exceed the length (Pl. 1, fig. 3A–D). Hinge-line curved, shell broadly sulcate to intraplicate, test thin, smooth. Beak prominent, erect, without beak ridges, foramen large, *circular*, complete, ?mesothyrid, attrite. Symphytium with raised median ridge: it is complete at a shell length of 11·5 mm and possibly earlier. *Hinge teeth unsupported by dental plates*, at least from a shell length of 11·5 mm, the smallest examined, that of 5 mm shell length having been lost; in adults the bases of the teeth somewhat swollen. *Pedicle collar represented merely*

by a narrow inner rim about 1 mm deep. Cardinalia characterized by excavate hinge-plates supported by the median septum, and separable into inner and outer hinge plates by the crural bases, which are not united laterally with the socket ridges (Text-fig. 1A, B and Thomson, 1927, fig. 26). In a large specimen of shell length 27 mm, width 28 mm and depth 20 mm (Text-fig. 3) the hinge-plates were only slightly excavate. *The anterior edges of the inner hinge-plates form a long* V *with the septum.* Cardinal process absent in the young, tendons of diductor muscles attached in a rounded depression (Text-fig. 1A): in adults the area of attachment consists of a median depression with on each side a small elevation (Text-fig. 1B), the end of the tendon being tripartite (Text-fig. 1C). Median septum thin, fairly high posteriorly, gradually lessening in height anteriorly, and extending forward to three-quarters the length of the valve. *Adult loop dalliniform*, attached to the crura only, extending

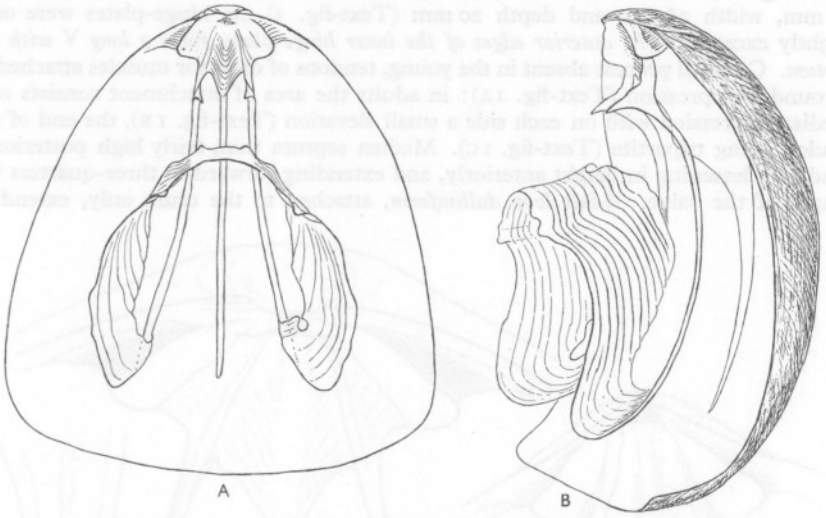


Text-fig. 1. *Dallina septigera.* Cardinalia of two specimens: A, of shell length 11·5 mm, width 10 mm; B, of shell length 24 mm, width 21·5 mm; C, dorsal end of tendons of diductor muscles removed from specimen shown in B.

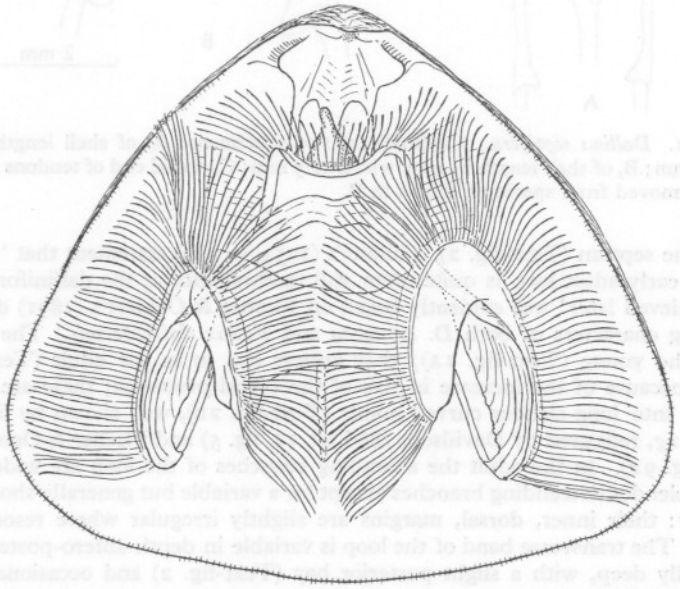
beyond the septum (Text-fig. 2). (Elliott's (1953, p. 269) statement that 'in *Dallina* itself the early adult loop is quite often still terebrataliform, the dalliniform pattern being achieved later' was evidently based on Fischer & Oehlert's (1891) description combining characters of both *D. septigera* and *Fallax dalliniformis*.) The crura are long in the young (Text-fig. 1A); they appear less so in the adult (Text-fig. 1B) possibly because of the increase in size of the crural processes; these are large and produced into long slender curved points (Text-fig. 2B), well shown by Sars (1878, pl. 1, fig. 2*g*, refigured by Davidson, 1886, pl. 11, fig. 5) and Fischer & Oehlert (1891, pl. IV, fig. 9*z*). In the adult the ascending branches of the loop are wide, but free from the slender descending branches except for a variable but generally short distance anteriorly: their inner, dorsal, margins are slightly irregular where resorption has occurred. The transverse band of the loop is variable in depth antero-posteriorly, but is generally deep, with a slight posterior bay (Text-fig. 2) and occasionally a pronounced anterior one.

Muscular impressions not strongly marked. Four pallial sinuses present in each valve. Adult lophophore plectolophous (Text-fig. 3), with nineteen to twenty-four

filaments in single series behind the mouth. The specimen figured is one of two in which the width slightly exceeded the length. Spicules mostly absent, when present are minute and widely scattered (Atkins, 1960).



Text-fig. 2. *Dallina septigera*. Interior of brachial valve of specimen of shell length 29 mm, width 25 mm and depth 20 mm. A, ventral and B, side views.

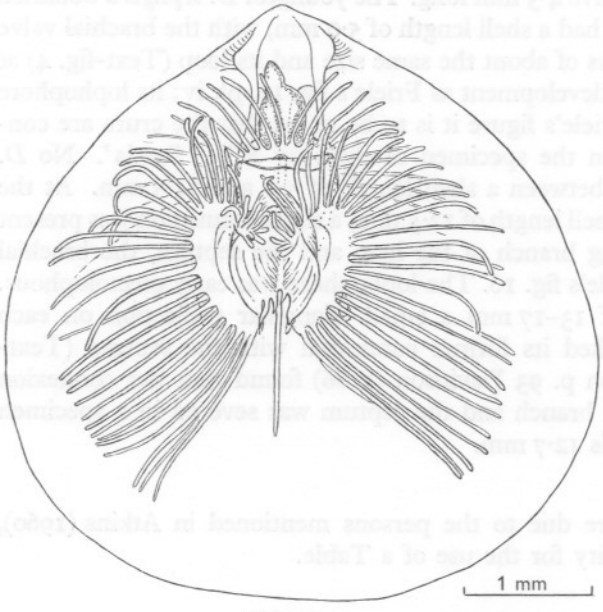


Text-fig. 3. *Dallina septigera*. Brachial valve with plectolophous lophophore of male individual of shell length 27 mm, width 28 mm and depth 20 mm: one of two specimens obtained in which the width exceeded the length.

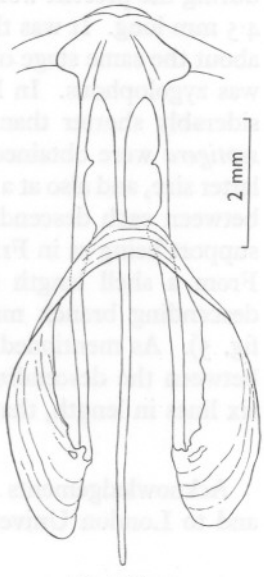
Dallina septigera agrees with *Macandrevia cranium* (Müller) and *Fallax dalliniformis* in lacking the two carmine pigment spots found in certain other brachiopods in connexion with the preoesophageal ganglion.

The ciliary feeding mechanism is as described for *Macandrevia cranium* by Atkins (1956).

The largest specimen obtained was 31 mm long, 25 mm wide and 20 mm deep: it came from position 47° 37′ N., 7° 27′ W. at a depth of 395 fathoms.



Text-fig. 4



Text-fig. 5

Text-fig. 4. *Dallina septigera*. Brachial valve with zygolophous lophophore of specimen of shell length 5 mm (brachial valve 4·5 mm long). The lip is turned forwards exposing the mouth.

Text-fig. 5. *Dallina septigera*. Ventral view of loop of specimen of shell length 13 mm and width 11·5 mm.

Two specimens in which the width slightly exceeded the length had the following dimensions: (1) 27 mm long, 28 mm wide, 20 mm deep (Pl. 1, fig. 3); (2) 23 mm long, 24 mm wide, 15 mm deep.

Breeding. In the seven *Dallina septigera* (shell length 12·5–26 mm) obtained in June 1956 and the single specimen of May 1957 (shell length 24 mm) the gonads were too immature for the sex to be determined without sectioning. Of those obtained in November 1958, twenty-four were examined for sex: ten (shell length 18–27 mm) were males, tailed sperm being present in all; three (shell length, 23, 24, 25 mm) had the gonad well developed. Four (shell length 16–24 mm) were females; two of these (shell length 16 and

24 mm) had large rounded eggs. In ten individuals (length 11·5–22 mm) no gonad could be discerned. Most of the *D. septigera* of July 1959 had the gonad visible through the shell, but in those examined sex products were immature.

GROWTH STAGES OF THE LOPHOPHORE

A number of growth stages of the loop were figured by Friele (1877), the smallest with brachial valve 4·5 mm long. The youngest *D. septigera* obtained during the present work had a shell length of 5·0 mm, with the brachial valve 4·5 mm long. It was thus of about the same size and its loop (Text-fig. 4) at about the same stage of development as Friele's fig. 12, pl. iv; its lophophore was zygolophous. In Friele's figure it is to be noted that the crura are considerably shorter than in the specimen dredged by R.V. 'Sarsia'. No *D. septigera* were obtained between a shell length of 5·0 and 11·5 mm. At the latter size, and also at a shell length of 12·5 mm, a slight connexion was present between each descending branch of the loop and the septum, the brachial support being as in Friele's fig. 16. The lophophore was early plectolophous. From a shell length of 13–17 mm a small triangular projection on each descending branch marked its former connexion with the septum (Text-fig. 5). As mentioned on p. 93 Davidson (1886) found that the connexion between the descending branch and the septum was severed in a specimen six lines in length, that is 12·7 mm.

Acknowledgements are due to the persons mentioned in Atkins (1960), and to London University for the use of a Table.

SUMMARY

Following the discovery of a homoeomorph, *Fallax dalliniformis* Atkins 1960, of *Dallina septigera* (Lovén) occurring with that species and confused with it by Fischer & Oehlert, *D. septigera* has been redescribed.

EXPLANATION OF PLATE I

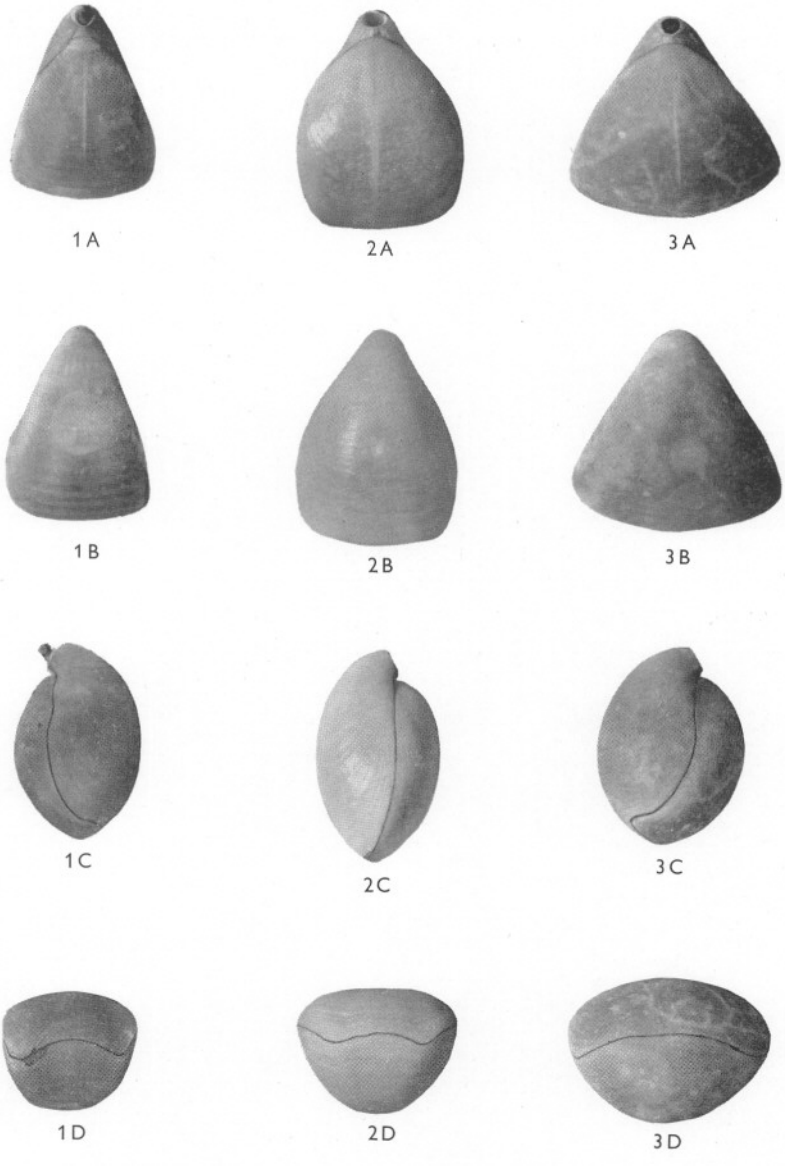
Dallina septigera (Lovén), to show variation in shape.

Fig. 1, A–D. Dorsal, ventral, lateral and frontal views of specimen 26 mm long, 18 mm wide and 17 mm deep, from position 48° 39′–38′ N., 9° 45′–50′ W.; depth 580–510 fathoms. The light circular area on the pedicle valve marks the former position of a *Verruca*.

Fig. 2, A–D. Dorsal, ventral, lateral and frontal views of a specimen 29 mm long, 22 mm wide and 18 mm deep from the Stockholm Natural History Museum. Locality Norway and North Sea. The symphytium had evidently been injured and later repaired by the animal.

Fig. 3, A–D. Dorsal, ventral, lateral and frontal views of specimen 27 mm long, 28 mm wide and 20 mm deep, from position 48° 32′–33′ N., 10° 10′–09′ W.; depth 375–490 fathoms.

Figs. 1 and 3 photographed while in water; Fig. 2 photographed dry. Approximately natural size.



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ENTIONELLA MONENSIS SP.NOV., AN ENTONISCID PARASITE OF THE SPIDER CRAB *EURYNOME ASPERA* (PENNANT)

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(Text-figs. 1–8)

The family Entoniscidae (Isopoda-Epicaridea) is included in the superfamily Bopyrina, which comprises the families Bopyridae, Dajidae and Entoniscidae. The Bopyridae are parasitic in the branchial cavity or on the abdomen of decapods, mainly Macrura and Anomura. The Dajidae occur in the incubatory chamber or on the dorsal surface of mysids and euphausiids, and rarely on Brachyura. The Entoniscidae are found in the visceral cavity of Anomura and Brachyura (an exception is *Synalpheion*, in a macruran, Coutière 1908), surrounded by a membrane of host origin, and communicating with the branchial cavity of the host by a pore. Together with this internal habitat the family has extreme morphological specialization. Females of the Bopyridae have distinct thoracic segmentation and well-developed peraeopods; in the Dajidae the peraeopods are distinct, but segmentation is less evident; in adult females of the Entoniscidae, however, the peraeopods consist of only the enlarged oostegites, with rudimentary endopodites in some species, and the thorax has only traces of segmentation.

Giard & Bonnier (1887) considered that entoniscids were morphologically and physiologically external parasites, that the sheath was formed by invagination of the body wall of the host, and that the external medium circulated within it. However, Miyashita (1941) considers that there is little, if any, circulation, and shows that in *Entionella fluviatilis* Miyashita the fluid within the sheath is indistinguishable from the haemolymph of the host. Veillet (1945) has shown that in *Portunion maenadis* (Giard) the younger stages are totally internal; the host secretes a sheath, which only later develops an opening to the exterior. The family may therefore be considered truly endoparasitic.

The Entoniscidae have been studied in only a few areas, and it is probable that only a small proportion of the total species have been recorded. The most recent survey of the family is by Shiino (1942). There are thirty-one known species, which are distributed as follows.

Europe, 12 spp.; South America—Atlantic coast, 5 spp.; North America—Atlantic coast, 3 spp.; North America—Pacific coast, 1 sp.; Japan, 10 spp.

Two species have been recorded previously from British waters, *Pinnotherion vermiforme* from *Pinnotheres pisum* and *Portunion maenadis* from *Carcinus maenas* (Marine Biological Association, 1957, pp. 206–7).

Material examined

102 specimens of *Eurynome aspera* were collected offshore around the south of the Isle of Man, and two were infected by the entoniscid described below. A male crab, carapace length 17·5 mm, dredged on 7 November 1958 in 40 m approximately 6 km west of Fleshwick contained two ripening female parasites. A second male, carapace length 16·5 mm, dredged on 24 February 1959 in 65 m approximately 9 km west of Calf Sound contained an ovigerous female and a male.

Genus **Entionella** Miyashita 1941

Female. First oostegite with well-developed ascendant, lateral and recurrent lobes. Thorax without ovarian processes. Heart on third abdominal segment. Convoluted pleural lamellae on first and second abdominal segments only.

Male. Peraeopods jointed. Thoracic terga expanded laterally as flat plates. Abdominal segments 1–4 with hyposphenians ('crochets ventraux médians', Giard & Bonnier, 1887).

Entionella monensis sp.nov.

Description

Female. The following description is of the ovigerous specimen (Fig. 2). The cephalogaster is bilobed and swollen; ventrally it bears the first and second antennae, which are rounded lobes flanking the mouth (Fig. 3, *an* 1, 2). Posterior to these are the maxillipedes (Fig. 3, *mxp*), each having a bilobed prominence, which bears a free flattened third lobe dorsally. The respiratory folds (*rf*) or 'corps spongieux' of Giard & Bonnier (1887) are small and simple, and ventral to the maxillipedes.

The thorax is dull orange in colour, of even width and without ovarian processes. The peraeopods are represented only by five pairs of oostegites which arise dorsally, the reduced dorsal surface of the thorax lying between their bases (Fig. 4, *dst*). Oostegites 2–5 form the brood chamber, but this is open and eggs lie between the oostegites and the host membrane. Oostegite 1 is large and has three lobes; the ascendant and lateral lobes are both folded and hood-like, while the recurrent lobe is large and flat, and lies along the lateral and ventral surfaces of the thorax enfolded by oostegites 2–5 (Fig. 1). According to Giard & Bonnier (1887) the function of the first pair of oostegites is to agitate the contents of the brood chamber, and to prevent the eggs and

embryos being crushed. However as the oostegites are more or less immobile, the latter is probably their principal use. There is a blood vessel, the marginal sinus (*ms*), which emerges from the base of the oostegite and passes around the free borders of all three lobes. On the ascendant lobe there is a median vessel (*mv*) which joins the marginal sinus distally. There are two branching blood vessels on the lateral lobe, and one median vessel on the recurrent lobe which sends branches towards the marginal sinus (Fig. 2). The ascendant lobe is stiffened by a clear firm rod which runs alongside the median vessel (Fig. 2, *sr*). The other oostegites are without prominent blood vessels or stiffening supports.

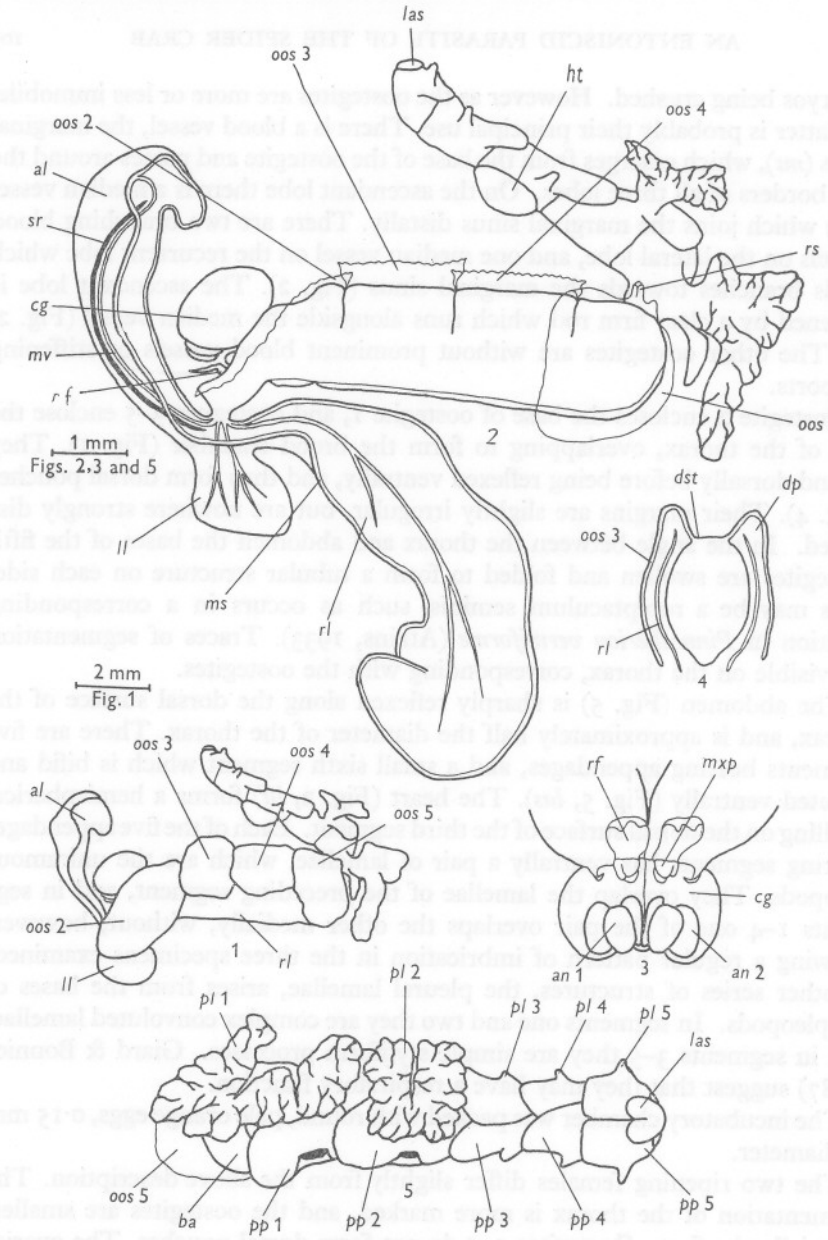
Oostegite 2 encloses the base of oostegite 1, and oostegites 3–5 enclose the rest of the thorax, overlapping to form the brood chamber (Fig. 1). They extend dorsally before being reflexed ventrally, and thus form dorsal pouches (Fig. 4). Their margins are slightly irregular, but are nowhere strongly dissected. In the angle between the thorax and abdomen the bases of the fifth oostegites are swollen and folded to form a tubular structure on each side. This may be a receptaculum seminis, such as occurs in a corresponding position in *Pinnotherion vermiforme* (Atkins, 1933). Traces of segmentation are visible on the thorax, corresponding with the oostegites.

The abdomen (Fig. 5) is sharply reflexed along the dorsal surface of the thorax, and is approximately half the diameter of the thorax. There are five segments bearing appendages, and a small sixth segment which is bifid and situated ventrally (Fig. 5, *las*). The heart (Fig. 2, *ht*) forms a hemispherical swelling on the dorsal surface of the third segment. Each of the five appendage-bearing segments has ventrally a pair of lamellae, which are the uniramous pleopods. They overlap the lamellae of the preceding segment, and in segments 1–4 one of the pair overlaps the other medially, without, however, showing a regular pattern of imbrication in the three specimens examined. Another series of structures, the pleural lamellae, arises from the bases of the pleopods. In segments one and two they are complex convoluted lamellae, and in segments 3–5 they are simple styliform processes. Giard & Bonnier (1887) suggest that they may have a respiratory function.

The incubatory chamber was packed with round, pale orange eggs, 0·15 mm in diameter.

The two ripening females differ slightly from the above description. The segmentation of the thorax is more marked, and the oostegites are smaller, especially the first. Oostegites 3–5 do not form dorsal pouches. The ovaries contain tightly packed polygonal eggs, orange in colour, diameter 0·1 mm approximately.

The distance from the anterior end of the cephalogaster to the rear of the thorax, and the length of the abdomen, respectively, for the three female specimens were: ovigerous female—7·0, 5·0 mm; first ripening female —6·4, 4·8 mm; second ripening female —7·2, 5·4 mm.



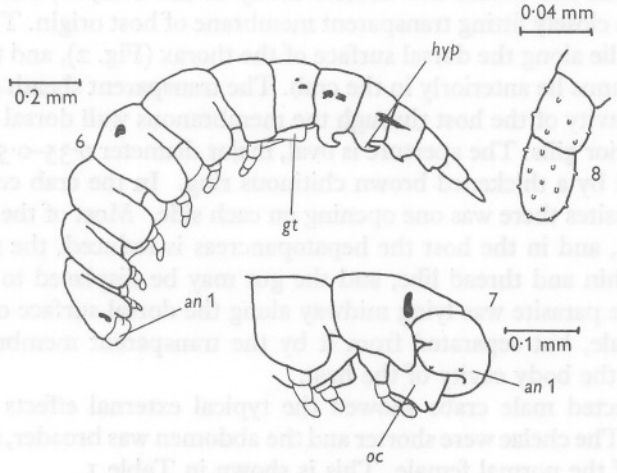
Figs. 1–5. *Entionella monensis*, female.

Fig. 1. Oostegites *in situ*.
Fig. 2. Oostegites 2, 3 and 4 cut off at base.
Fig. 3. Cephalogaster ventral view.
Fig. 4. Diagrammatic transverse section at region of third oostegite.
Fig. 5. Abdomen, ventral view.

Abbreviations: *al*, ascendant lobe of first oostegite; *an* 1–2, antennae 1–2; *ba*, base of abdomen; *cg*, cephalogaster; *dp*, dorsal pouch; *dst*, dorsal surface of thorax; *ht*, heart; *las*, last abdominal segment; *ll*, lateral lobe of first oostegite; *ms*, marginal sinus; *mv*, median vessel; *mxp*, maxillipede; *oos* 1–5, oostegites 1–5; *pl* 1–5, pleural lamellae 1–5; *pp* 1–5, pleopods 1–5; *rf*, respiratory folds; *rl*, recurrent lobe of first oostegite; *rs*, receptaculum seminis; *sr*, stiffening rod.

Male (Fig. 6). Only one specimen has been found, which occurred in the visceral cavity of the crab, separated from the female by the host membrane. This may be the normal position, for Atkins (1933) found that in *Pinnotherion vermiforme* all the males discovered occurred free in the body cavity of the host. In other species of entoniscid the male occurs within the incubatory chamber or on the surface of the female.

The total length is 1·65 mm. The general colour is pale, but the hepatopancreas gives an orange colour to the posterior thoracic and anterior abdominal segments, and there are several scattered brown chromatophores.



Figs. 6–8. *Entionella monensis*, male.
Fig. 6. Lateral view.
Fig. 7. Head.
Fig. 8. Fourth peraeopod.

Abbreviations: *an* 1, first antenna; *gt*, genital tubercle; *hyp*, hyposphenians; *oc*, oral cone.

The head is blunt, and has a curved boss around its anterior margin. This is formed of the enlarged first antennae (*an* 1), which in most other species are swellings on either side of the anterior of the head. The second antennae are absent, as in *Entionella okayamaensis* Shiino. Ventrally there is a suctorial oral cone which encloses the mandibles; it has no scale-like projections on the lower lip. No maxillae or maxillipedes could be found.

The thorax has six free segments, the third to the eighth, the first and second being fused with the head. The terga of segments 2–7 are produced laterally as broad flat processes. There are six pairs of peraeopods, on segments 2–7, segment 8 lacking appendages but bearing a pair of lateral genital tubercles. The posterior peraeopods are slightly larger than the anterior ones, and all are of three segments, the terminal one rounded. The two distal segments bear a number of small blunt tubercles (Fig. 8).

The first four abdominal segments have mid-ventral projections or hyposphenians ('crochets ventraux médians', Giard & Bonnier, 1887), those on segments one and two being strongly hooked (*hyp*). The last segment, the sixth plus the telson, is pointed and unforked.

Type specimens have been deposited at the British Museum (Nat. Hist.). The ovigerous female has been selected as the holotype (register no. 1959.vi.22.1) and the male as an allotype (register no. 1959.vi.22.2).

Relation with host

The female lies within the visceral cavity of the host, separated from the viscera by a closely fitting transparent membrane of host origin. The abdomen is flexed to lie along the dorsal surface of the thorax (Fig. 2), and the cephalogaster and anus lie anteriorly in the crab. The transparent sheath opens to the branchial cavity of the host through the membranous wall dorsal to the bases of the anterior gills. The aperture is oval, major diameter 0·35–0·5 mm, and is surrounded by a thickened brown chitinous ring. In the crab cotaining two female parasites there was one opening on each side. Most of the body cavity is occupied, and in the host the hepatopancreas is reduced, the male genital ducts are thin and thread like, and the gut may be displaced to one side.

The male parasite was lying midway along the dorsal surface of the thorax of the female, but separated from it by the transparent membrane, and so actually in the body cavity of the host.

The infected male crabs showed the typical external effects of parasitic castration. The chelae were shorter and the abdomen was broader, approaching the form of the normal female. This is shown in Table 1.

TABLE 1. A COMPARISON OF INFECTED MALE CRABS WITH NORMAL MALES AND FEMALES

Sex and condition of crab	Carapace length (mm)	Chelar propodus length (mm)	Ratio: carapace L. / propodus L.	Width of A.S. 4 (mm)	Ratio: carapace L. / width of A.S. 4
Parasitized male	17·5	6·6	2·65	2·55	6·86
Parasitized male	16·5	6·4	2·56	2·20	7·50
Normal male	16·5	10·4	1·59	1·55	10·64
Normal male	18·0	12·2	1·47	1·65	10·90
Normal female	16·0	5·4	2·96	3·85	4·16
Normal female	17·0	6·0	2·83	4·10	4·15

DISCUSSION

Entionella monensis sp.nov. is included in the genus *Entionella* because the female has no ovarian processes, and there are folded pleural lamellae only on the first two abdominal segments. The three species of the genus may be distinguished by means of Table 2 (partly based on that of Shiino, 1954).

E. fluviatilis and *E. okayamaensis* both occur in Japan, in hosts spending part of their life in fresh and brackish water. The discovery of *E. monensis*

extends the range of the genus to European waters. There are four other genera which also occur in both of these areas: *Entoniscus* Müller, *Pinnotherion* Giard & Bonnier, *Portunion* Giard & Bonnier and *Cancrion* Giard & Bonnier.

TABLE 2. CHARACTERS SEPARATING THE SPECIES OF THE GENUS *ENTIONELLA*

		E. fluviatilis	*E. okayamaensis*	*E. monensis*
Female	Oostegite 1	All three lobes folded	Recurrent lobe not folded; many secondary pockets	Recurrent lobe not folded; no secondary pockets
	Pleural lamella 1	No stalk	Long stalk	No stalk
	Pleural lamellae 3–5	Distinct	Indistinct	Distinct
Male	Last abdominal segment	Deeply bifid	Shallowly bifid	Undivided
	Thoracic segment 8	No expansion	Lateral expansions	Lateral expansions
	Thoracic segment 2	Not fused with cephalon	Fused with cephalon	Fused with cephalon

SUMMARY

The relationships of the Entoniscidae to the other families of the Bopyrinae are briefly discussed. The nature of the parasitism of the family is considered. The characters of the genus *Entionella* are given, and a new species, *E. monensis* is described (mature female and mature male). Its relations with the host, the spider crab *Eurynome aspera*, are described. A table is provided to separate the species of the genus *Entionella*.

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HISTOLOGY OF THE LIGHT ORGANS OF *PHOLAS DACTYLUS* (LAMELLIBRANCHIA)

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(Plate 1 and Text-fig. 1)

The piddock, *Pholas dactylus* L., gives off a luminous secretion when irritated. The luminous glands which produce the secretion are two longitudinal stripes in the exhalant siphon, a pair of triangular organs in the mantle cavity near the base of the siphon, and a stripe around the ventral rim of the mantle (Panceri, 1872).

The histology of the light-organs has been described several times. A light-organ is covered by a simple columnar ciliated epithelium, below which are many glandular cells, which discharge through the surface epithelium. The outer part of the glandular layer consists of a mass of large mucus cells. Deeper lies a second glandular region containing large cells with long necks that extend to the external epithelial surface. Dubois (1892, 1914, 1928) believed that the photogenic tissue was made up of two kinds of secretory cells; these were the superficial ciliated cells, which possessed glandular bases (fixed secretory cells); and deeper lying glandular cells derived from clasmatocytes (migratory secretory cells). Rawitz (1891) clearly distinguished a mucous from an underlying photogenic layer. The latter, according to Förster (1914), contains pyriform cells with long necks. He believed that he could distinguish a secretory cycle in the photogenic cells. Exhausted cells at the beginning of the cycle possessed an alveolar cytoplasm; granules began to appear in the cytoplasm; the granules increased in number and stained intensely with iron haematoxylin. Those photogenic cells which were filled with granules were in the active secretory state. Transitional stages between the inactive (or depleted) cells and the active (granular) cells were rare. An account by Dahlgren (1916) is based on the work of Förster (1914).

While examining some sections of the light-organs of *Pholas*, I made certain observations which differed from the published accounts. Moreover, the latter were difficult to reconcile with one another. Therefore, I undertook the following study of the light-organs of *Pholas dactylus*.

METHODS

The triangular organs and the siphonal cords were excised together with a little contiguous tissue. The material was fixed in Zenker's fluid, and cut in

polyester wax (Steedman, 1957). Stains used were: thionin; Alcian blue and neutral red; mucicarmine; Giemsa; Masson's trichrome stain; van Gieson's stain; a modification of Masson's, consisting of Weigert's haematoxylin, aniline blue and xylidine ponceau; iron haematoxylin; Ehrlich's haematoxylin and eosin.

OBSERVATIONS

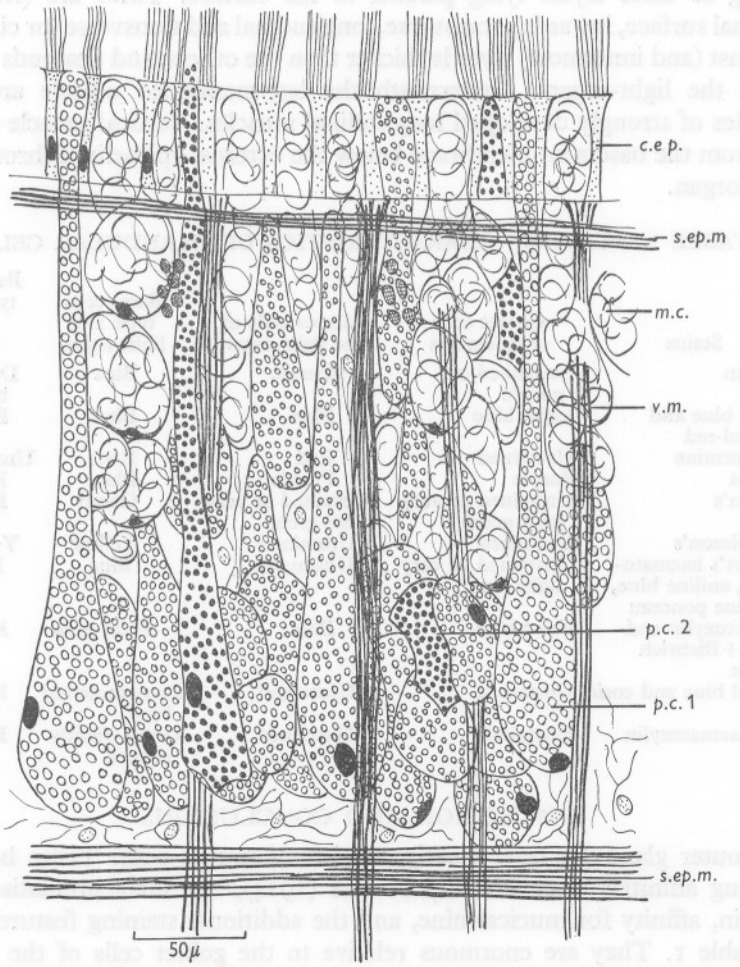
The general histological picture described by earlier workers is confirmed (Text-fig. 1; Pl. I). The epithelium overlying the light-organ, as elsewhere, contains ciliated columnar cells. Between them open the distal extremities of the underlying glandular cells. In vertical section, the ciliated epithelial cells expand externally into cones, which arch over the necks of the glandular cells. Underneath the epithelium is a thick and dense layer of mucus cells, some 180 μ deep, which extend down to and into the outer part of the photogenic layer (Text-fig. 1; Pl. I). These mucus cells are large, up to 180 μ long and about 13 μ wide at the base. They open by fairly wide necks, 6–7 μ across, between the epithelial cells. Except for their very large size, these cells are typical mucus cells. Their staining affinities are listed in Table 1. With some stains they are coloured much more intensely than the ordinary mucus goblet cells occurring elsewhere in the epithelium.

Below the mucus cells there is another dense glandular layer. This, the photogenic tissue, consists of closely packed secretory cells. They occupy a layer approximately 120 μ thick, beginning about 160 μ from the surface. Thus, the mucus layer and the photogenic layer overlap to some extent.

Two kinds of cells can be distinguished in the photogenic layer by their staining affinities (see Table 1). Let these cells be known as types 1 and 2 (Text-fig. 1). Both types of cells have granular contents. Photogenic cells of type 1 are more abundant (Text-fig. 1; Pl. I). The granules are basophilic to some plasma stains: they stain weakly with iron haematoxylin and eosin, red with mucicarmine, green with light green, etc. Cells of type 2 are infrequent, relative to type 1. Their coarse granules are acidophilic; they stain red with eosin, neutral red and xylidine ponceau, and possess stronger affinity for iron haematoxylin.

Photogenic cells, type 1 (Text-fig. 1). These are packed closely together, up to 270 μ long and 15 μ wide at the base. They have the shape of long pyriform sacs with long necks, 4–5 μ wide, extending to the epithelial surface. The small nucleus lies basally, either in the lateral wall or at the bottom of the cell. The interior of the cell is packed with coarse granules, having an average diameter of about 1·3 μ. The cell is invested by a thin cytoplasmic sheath staining with xylidine ponceau. The secretory granules extend through the necks of the cells to the external surface; discharged to the exterior, they lose their identity. A homogeneous flocculent precipitate lies over the external surface of the epithelium.

Photogenic cells, type 2 (Text-fig. 1). These have about the same dimensions as cell type 1. They extend well down into the photogenic layer. At the base they are about 11 μ wide; the necks are 4·5 μ wide where they pass through the epithelial layer. A small nucleus lies against the wall of the cell in the basal



Text-fig. 1. Semi-diagrammatic representation of the triangular light-organ of *Pholas dactylus*, from vertical sections. Legend: *c.ep.*, ciliated epithelium; *m.c.*, mucus cell; *p.c.* 1., photogenic cell type 1; *p.c.* 2, photogenic cell type 2; *s.ep.m.*, subepithelial muscle; *v.m.*, vertical muscle.

region. The interior of the cell is packed with coarse spherical granules, having an average diameter of about 1·1 μ. They extend up to the external epithelium, where they can be seen emerging from the mouths of the cells. The cytoplasmic wall is difficult to distinguish.

Previous workers (Dubois, 1892, 1914; Förster, 1914) have laid much emphasis on the muscle system in the vicinity of the light-organ. The following description is based on the siphonal cord (Text-fig. 1; Pl. I), but the arrangement is similar in the triangular organ. There is a subepithelial muscle consisting of three layers lying parallel to the surface. These are (from the external surface, inwards), transverse, longitudinal and transverse (or circular). The last (and innermost) layer is thicker than the others, and descends underneath the light-organ. Underneath the last-mentioned muscle are thick bundles of strongly developed longitudinal muscles. Vertical muscle strands run from the basement membrane below the external epithelium through the light-organ.

TABLE 1. STAINING CHARACTERISTICS OF GLANDULAR CELLS

Stains	Goblet cells of epidermis	Mucus cells of the light-organ	Basal cells type 1 of light-organ	Basal cells type 2 of light-organ
Thionin	Unstained or purple	Purple	Blue	Darker blue
Alcian blue and neutral red	Light blue	Blue	Blue	Red
Mucicarmine	Unstained	Red	Red	Unstained
Giemsa	Purple	Purple	Blue	Blue
Masson's	Unstained or very faint green	Unstained or very faint green	Green	Red
Van Gieson's	Unstained	Unstained	Yellow	Yellow
Weigert's haematoxylin, aniline blue, xylidine ponceau	Unstained or very light blue	Unstained	Blue	Red
Haematoxylin and eosin + Biebrich scarlet	Faint blue	Blue	Faint pink	Red
Methyl blue and eosin	Unstained	Unstained	Predominantly blue	Red
Iron haematoxylin	Unstained	Unstained	Grey—faintly stained	Black

DISCUSSION AND CONCLUSIONS

The outer glandular layer clearly consists of mucus cells. These have the staining affinities discovered by Förster (1914), viz. metachromatism with thionin, affinity for mucicarmine, and the additional staining features listed in Table 1. They are enormous relative to the goblet cells of the surface epithelium elsewhere. Moreover, there are differences in the staining affinities of these two types of mucus cells, since the mucinogen of the ordinary goblet cells stains poorly or not at all with thionin and mucicarmine. Possibly, different types of mucins are produced by goblet cells of the epithelium and mucus cells of the light-organ. Certainly, the large size and numerical abundance of these cells in the light-organs indicate that they are concerned with photogeny, and their functional role may be to produce a mucin-carrier for the photogenic secretion.

There are two kinds of secretory cells in the lower glandular or photogenic layer. Both types of cells are filled with granular inclusions which differ in staining affinities. As a generalization, the granules of cell type 1 have rather poor staining affinities; those of cell type 2 are acidophilic and have strong staining affinities. The staining characteristics allow the two kinds of cells to be distinguished clearly from each other. Cells of both types can be seen discharging through the external epithelium.

Förster's interpretation of a transformation in the photogenic cells from an alveolar to a granular condition seems to be based on failure to distinguish two kinds of secretory cells in the inner glandular or photogenic layer. Dahlgren (1916) accepted Förster's account, and his artist clearly illustrated two kinds of cells in the photogenic layer, viz. alveolar cells with a coarse meshwork and cells with darkly staining small granules. The latter, presumably, were stained with iron haematoxylin (cf. elsewhere in Dahlgren's work). The alveoli appear to be unstained granules of cell type 1, and are grossly exaggerated in size. A most interesting feature of Dahlgren's illustration is that the alveolar cells (photogenic cells, type 1) are shown discharging at the surface; granules of the other kind of cell (photogenic cells, type 2) are shown as extending along the neck of the cell to the external surface.

Förster (1914) considered that the outer glandular layer (of mucus cells) was concerned solely with the production of mucus. Dubois (1892, 1914) demonstrated the basic luciferin-luciferase reaction in the secretion of *Pholas* (cf. Harvey, 1957). Dahlgren (1916), without evidence, assigned luciferin to granular cells of the inner glandular (i.e. photogenic) layer, luciferase to the outer glandular (i.e. mucus) layer. Since there are two kinds of secretory cells in the photogenic layer, it is logical to link them with the production of luciferin and luciferase; possibly, the luciferase corresponds to the eosinophilic granules of less abundant cell type 2. This is mere speculation, however.

Previous authors (Dubois, 1914; Förster, 1914) have assumed that the luminous secretion of *Pholas* is discharged by muscular contraction. The luminous gland cells of *Pholas* much resemble those of *Chaetopterus*. The latter discharges a luminous secretion from unicellular glands, and its photogenic tissue lacks muscle fibres. In both animals the luminescence is under nervous control; the gland cells are filled with granules, and these emerge through an open pore at the apex of the cell. Instead of invoking a muscular mechanism, I would suggest that the walls of these cells consist of oriented contractile protein capable of expressing the cell contents. A model may be found in the contractile behaviour of monolayers of actomyosin (Bennett, 1956; Giese, 1957).

I am grateful to Mr A. C. G. Best for the excellent histological preparations and for the photography.

SUMMARY

Earlier work dealing with the histology of the light-organs of *Pholas dactylus* is reviewed.

Beneath the surface epithelium of the light-organ, two glandular layers can be distinguished. One, an outer glandular layer, contains mucus cells. The other, an inner layer, contains two kinds of granular cells, which can be separated by their staining characteristics. The latter are listed in Table 1. Both kinds of granular cells of the inner layer discharge their contents upon the external surface. The photogenic secretions are produced, most probably, by the glandular cells of the inner layer.

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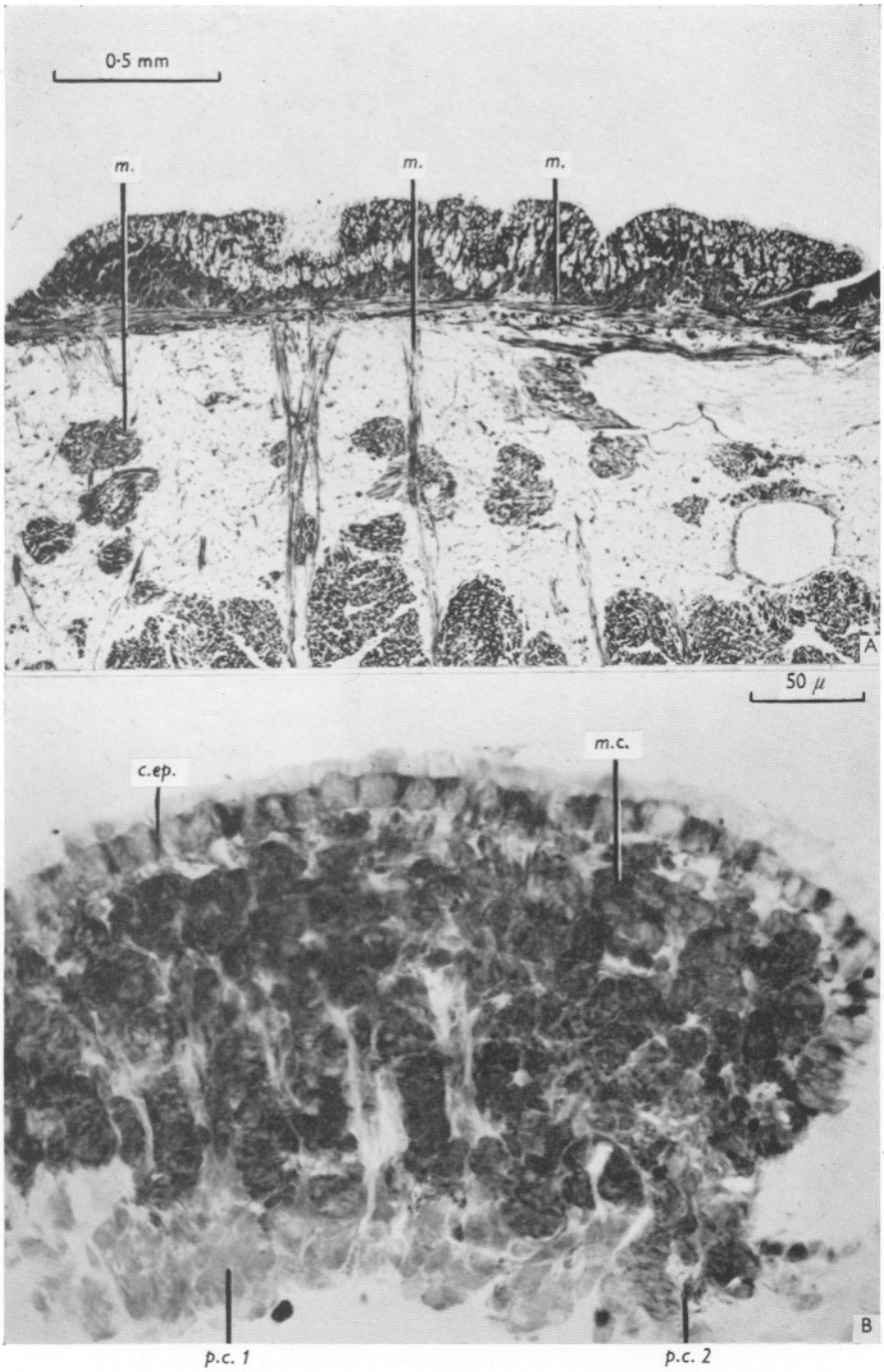
EXPLANATION OF PLATE I

PLATE I. A. General view of a triangular light organ of *Pholas dactylus*. Iron haematoxylin, acid fuchsin-ponceau 2R, aniline blue. Mucus cells unstained; photogenic cells dark. B. View of siphonal light-organ, showing mucus cells. Giemsa. Legend: *l.o.*, light organ. *m.*, muscle. *c.ep.*, ciliated epithelium. *m.c.*, mucus cells. *p.c.* 1, *p.c.* 2, photogenic cells, types 1 and 2, respectively. *p.l.*, photogenic layer. Mucus cells dark; photogenic cells, type 1, light; photogenic cells, type 2, with dark granules.



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DEFENSIVE ACID-SECRETION IN MARINE GASTROPODS

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(Text-fig. 1)

An important function of the gastropod shell is to protect the body against attack by predators. If the shell of a whelk is broken away and the soft animal is then offered to a hungry cod, it is eaten readily. Many successful gastropods, however, lack an external shell as adults (although it is certain that all have evolved from shelled ancestors) and might be expected to be eaten by fish as readily as is the naked body of a broken whelk. The purpose of this paper is to describe part of an investigation into whether this is in fact true.

Some authors have considered that many of these naked gastropods (the majority of which belong to the Opisthobranchia) escape being eaten by their cryptic coloration and their habit of 'skulking under stones or in crannies' (Crossland, 1911, p. 1063). Some have considered that many opisthobranchs are distasteful in various ways and believed the behaviour and coloration of such forms to be 'warning' in nature. Thus it might be expected that, under experimental conditions, cryptically coloured opisthobranchs would be accepted as food by fish while the more vividly coloured ones would be rejected, for the usual line of reasoning indicates that aposematic or warning coloration and behaviour accompany the possession of some defensive mechanism, while cryptic coloration and habits usually characterize animals which would be quickly killed and eaten if plainly visible to their enemies. Few experiments have been done with opisthobranchs. Herdman & Clubb (1892) describe some interesting experiments with marine aquarium fish and nudibranchs, but these were inconclusive since only a very few species of the latter were used. Crossland (1911) established that fish refused live chromodorid nudibranchs thrown to them from a boat in shallow water, while readily accepting other food and even taking formalin-soaked oysters. Many workers tended to be over-subjective and were handicapped by disagreements, often resulting from the description of animals removed from their natural surroundings. For instance, Crossland (1911, p. 1064) states that 'Tritonids ...are fairly conspicuously coloured', while most other authorities would agree that tritoniids resemble closely the alcyonarians on which they live and feed. Similarly, Cooke (1895, p. 73) states that *Pleurobranchus membranaceus*

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is 'conspicuously marked with red-brown and yellowish "warning" colours. *Haminoea* and *Philine*, on the other hand, are good to eat, and consequently possess "protective" coloration'; these statements are based on faulty knowledge of these opisthobranchs.

It is clear that a full re-examination of the subject is necessary. To this end, I have made observations on as wide a variety of naked gastropods as possible, attempting to relate the results obtained to the facts of their natural history and anatomical and histological structure. The present paper describes the results of a part of this work, dealing with some gastropods which proved to rely on the production of an acid fluid for the deterrence of would-be predators. That *Pleurobranchus membranaceus* was able to secrete an acid substance has been known for many years (Garstang, 1890). Other species of gastropods now found to do this are: *Berthella plumula* (Opisthobranchia:Pleurobranchidae); *Philine quadripartita* (Opisthobranchia:Philinidae); *Lamellaria perspicua* (Prosobranchia:Lamellariidae); *Velutina velutina* (Prosobranchia: Lamellariidae). *Velutina* is not truly naked but observations on it are included here for it raises some interesting points. Some observations on *Pleurobranchus* are given by Thompson & Slinn (1959).

The classification and nomenclature employed herein are those advocated by the Port Erin Fauna list (to be published).

METHODS

In the feeding-acceptability tests the fish used were in glass-fronted tanks in the Port Erin Aquarium, illuminated artificially, and usually fed twice per week with chopped herring or boiled mussels. Experiments were always carried out when the fish were to be expected to be hungry and this was confirmed by giving them some of their usual food before and after the experiments. The gastropod to be tested was dropped into the tank from above and allowed to sink to the bottom; the responses of the various fish were noted. Particular care was taken to ensure that the gastropods to be used in the tests were not damaged or in any way feeble, for previous experience had shown that, when vitality is low, resistance to predators may fall. It is admitted that the results of such tests must be interpreted with caution for a variety of reasons; the reliability of the results obtained will be discussed below.

Material for sectioning was fixed in Zenker's fluid (with or without acetic acid) or Lewitsky's fluid with sodium chloride added, cleared with amyl acetate and embedded in Hance's rubber wax (Gurr). Sections were cut at 4 and 6 μ. The stains employed were either the azan or the alum haematoxylin of Heidenhain, with, as counterstains for the latter, eosin and alcian blue 8GS (Steedman, 1950). In addition, preparations stained intravitally with neutral red were useful.

RESULTS OF ACCEPTABILITY TESTS

Tests with fish

Some tests carried out using *Pleurobranchus membranaceus* have been described by Thompson & Slinn (1959). In the present investigation further experiments with this species have yielded confirmatory data.

With *Philine quadripartita*, *Lamellaria perspicua* and *Berthella plumula*, essentially similar results were obtained in the present study. No tests were carried out using *Velutina velutina*, for only one live specimen was available and this was required for study of the histology of the foot.

Fish used in the tests were *Blennius pholis*, *Acanthocottus bubalis*, *Pholis gunnellus*, *Pleuronectes platessa*, *Spinachia spinachia*, *Gadus morrhua*, *Pollachius pollachius* and *P. virens*. (Tests with *Agonus cataphractus* and *Ciliata mustelas* were unsatisfactory for these fish invariably ignored test organisms.) The usual reaction to the test animal was for the fish to inspect it closely; the height in the tank at which the fish became aware of the mollusc and the means of inspection (olfactory or optical) varied with the different species of fish. Occasionally a fish would take one of the test molluscs into its mouth, but invariably rejected it violently and immediately. In a tank containing several pollack (*Pollachius pollachius*) a single test mollusc may be inspected, taken into the mouth and rejected by every fish present before it reaches the tank bottom. No evidence that any of the fish 'learned' to ignore these molluscs was obtained. There is little point in describing in detail the reactions of the various species of fish employed, for the behaviour of fish with regard to test foods has been described particularly fully by Bateson (1890).

To summarize: none of the four species of gastropod used was ever seen to be ingested by any of the fish employed; occasionally the fish would 'taste' the mollusc but always rejected it. The following observations are relevant to the contention that the tests yielded reliable data, and reflect the probable behaviour of these fish when encountering these molluscs in nature: (*a*) in nature, large numbers of swimming *Pleurobranchus* were ignored by shoals of coalfish (*P. virens*) (Thompson & Slinn, 1959); (*b*) a detailed study of the gut contents of gadoid fish from the seas around the south of the Isle of Man (personal communication from A. K. Nagabhushanam) revealed none of the molluscs under consideration, except for a specimen of *Velutina velutina* from the stomach of a coalfish, *P. virens*, in December 1956).

Tests with anemones

Thompson & Slinn (1959) state that *Tealia felina* rejected live *Pleurobranchus* in laboratory tests. This has been confirmed and found to apply also to *Berthella plumula*, but with this important proviso: rejection only occurs predictably the first time the test is performed with any individual gastropod; if a rejected pleurobranchid is immediately placed in the tentacles of a fresh

Tealia, the mollusc is often ingested readily. The significance of this is difficult to estimate; escape from the first anemone may have temporarily exhausted the deterrent reserves of the pleurobranchid.

ACID SECRETION

If the molluscs under consideration are roughly stimulated with a glass rod and a pH paper is applied, it can be seen that a strongly acid secretion has been produced. The pH estimations were made with B.D.H. wide-range papers; the colour change indicated the acidity of the secretion to be approximately pH 1. The secretion is not produced continuously but only after a disturbance; pH papers resting lightly upon the animal while it is creeping exhibit no colour change.

Thompson & Slinn (1959) found that the nature of this acid secretion in *Pleurobranchus* was relatively simple; it may be considered to be a mixture of sulphuric and hydrochloric acids, the former predominating. No organic acid radicals were identified nor were proteins present in appreciable amounts. The proportion of sulphate to chloride was much higher in the secretion than in either sea water or molluscan blood (Robertson, 1949).

In *Pleurobranchus*, *Berthella*, *Philine* and *Lamellaria* the acid secretion was produced at any point on the body surface; even the gills of the two pleurobranchid species are able to secrete this acid if disturbed. In *Velutina* the foot certainly was able to produce acid, but it is not certain whether in addition the mantle has this capacity.

ACID-SECRETING TISSUES

Pleurobranchus membranaceus

Thompson & Slinn (1959) describe the histology of the mantle and foot epidermis. The 'empty' appearance of the epidermal cells is characteristic of acid-secreting tissues in the gastropods investigated. Scattered unicellular mucus glands are also present, especially in the pedal epidermis.

Sections through the gill show epidermal acid cells to be abundant. The metapodial gland (which develops as maturity is reached) has a totally different structure, packed with large compound multicellular glands of complex cytological aspect; its functions are presumably connected with adhesion to the substratum.

In sections through the mantle, cilia are never detectable (although plainly evident in life) but they are clearly demonstrable in sections through the foot.

Berthella plumula

The epidermis of *Berthella* shows only minor differences from that of *Pleurobranchus*. The contents of the acid cells in life stain red with neutral

red; it is well known that this stain (for which the name 'neutral' red is misleading) can act as an indicator, red being the colour it adopts in acid media. Squashing of an excised piece of the mantle beneath a cover-glass causes the acid fluid to be released by rupture of some of the cells. The secretion at first takes the form of large spherules, one from each epidermal cell, but they soon merge and disperse through the surrounding medium.

Simple calcareous spicules are present in the subepidermal layer of the mantle. That these can exist in such close proximity to cells containing a strongly acid fluid is rather surprising; when a small piece of the mantle is compressed under a cover-glass the acid is freed and the spicules rapidly dissolve.

As in *Pleurobranchus*, cilia are not detectable in the mantle after fixation, and the metapodial gland, which develops late in life, is histologically complex and does not seem to be concerned with acid secretion.

Philine quadripartita

The whole integument is able to secrete the acid fluid. The epidermis differs markedly from that of the pleurobranchids; the acid glands are large, flask-shaped, subepidermal sacs, appearing as minute white specks in life and empty in sections. Between the sacs is a network of muscle fibres; discharge of the acid is probably brought about by the contraction of these fibres. Unicellular subepidermal mucous glands are also present, discharging to the exterior through separate ducts perforating the simple ciliated epidermis.

Lamellaria perspicua *and* Velutina velutina

As in *Philine*, the epidermis is simple and ciliated, while the acid glands take the form of subepidermal sacs communicating with the exterior by separate ducts perforating the epidermal layer. Muscle fibres are present around the sacs. The shell of *Velutina* is covered externally by a peculiar horny sheath; it seems probable that one of its functions is to protect the calcareous shell against the acid secretion of the animal's own foot. Fig. 1 illustrates diagrammatically the two types of epithelium found in acid-secreting gastropods.

DISCUSSION

All the gastropods described in this paper were found to be distasteful to fish; all are able to secrete a strongly acid fluid; all show similar glands in the skin. Strong circumstantial grounds exist for believing the glands to be in all cases the producers of the acid, and the acid to be the deterrent to the fish.

Bateson (1890) discovered that even dilute acids are repellent to a variety of fish. He states (p. 247): 'Conger are equally willing to eat a piece of squid or pilchard if it is covered or smeared with spirit, cheese of various sorts,

anchovy extract, or *Balanoglossus*, as if it had been unpolluted. On the other hand, they will refuse cooked or tainted food and food which has been soaked for a few moments in dilute acids. The same remarks apply generally to the other fishes'. There can thus be little doubt that it was the acid secretion which brought about the rejection of the gastropods used in my experiments. This is further supported by the fact that dead or dying *Berthella* were eaten readily;

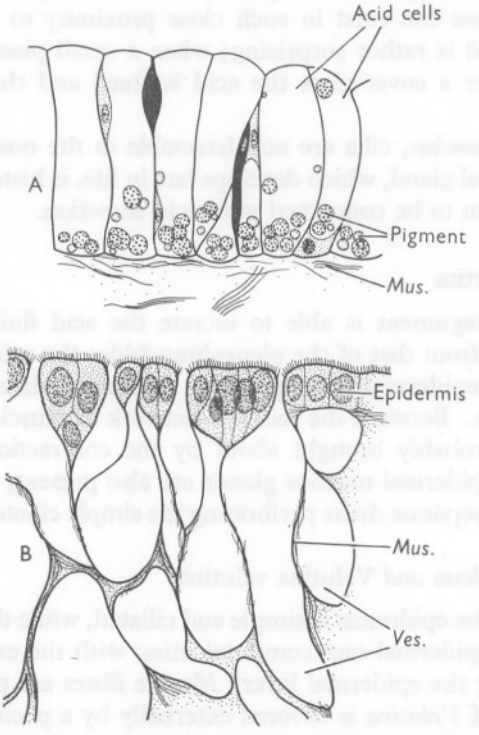

Fig. 1. Diagrams of sections through the two types of acid-secreting epithelia found in gastropods. A, characteristic of *Berthella plumula* and *Pleurobranchus membranaceus*; B, characteristic of *Philine quadripartita*, *Lamellaria perspicua* and *Velutina velutina*. Mucous glands are shown black. *Mus.*, muscle fibres. *Ves.*, empty acid vesicles.

the deterrence is essentially a living process and cannot be explained simply on the grounds that the test foods were unfamiliar to the fish.

That the acid is produced by the glands described in this paper is difficult to prove, but is supported by much indirect evidence: (1) the histological structure of the median buccal gland (acid gland) of *Pleurobranchus* (Thompson & Slinn, 1959) is remarkable for the 'empty' appearance of the cells in stained sections. Because these cells undoubtedly secrete acid, and because all the glands described in the present paper resemble them so closely, there are grounds for believing the function of all to be the same. (2) Excised pieces of

the mantle skirt of *Berthella* can secrete acid; this rules out any possibility that the fluid might reach the exterior by seepage from the median buccal gland of the pleurobranchids. (3) Preparations of small excised pieces of the mantle of *Berthella* showed that rupture of the epidermal cells and liberation of the contained fluid (which neutral red staining showed to be acidic) was followed closely by the solution of the subepidermal calcareous spicules. This is proof of the acid-secreting function of the 'empty' epidermal cells of *Berthella*, and provides further grounds for believing, by analogy, the glands of the other species to have the same function.

Thus we may suppose that: (*a*) large distinctive glands are present in the skin of the five species dealt with; (*b*) when the animal is disturbed acid is discharged by these glands; (*c*) this acid secretion will usually cause the rejection of the mollusc by any fish which attempts to ingest it.

None of the five species is vividly coloured, notwithstanding Cooke's (1895) opinion that *Pleurobranchus* shows warning coloration. All, from *Philine* which lives submerged beneath sand or mud (Brown, 1934) to *Lamellaria*, which may bury itself in the ascidians on which it feeds (Herdman, 1893), are fairly effectively camouflaged. None shows any warning coloration or behaviour.

Acid secretion must have arisen independently in the Pleurobranchidae, the Philinidae and the Lamellariidae, which are distinct, well-defined taxonomic groups. This independent evolution of a similar defence mechanism in three groups of gastropods makes it all the more interesting that, while two of the groups (Philinidae and Lamellariidae) have evolved subepidermal acid glands (Fig. 1 B), the third (Pleurobranchidae) makes use of the epidermal cells for the purpose (Fig. 1 A). Perhaps the former condition was derived from an epithelium similar to that of modern pleurobranchids.

Although a considerable number of British gastropods have been tested during the present investigation, many more species undoubtedly would prove to have acid glands, if more naturalists were aware of the possibility of their existence. It is to be hoped that workers with the necessary skill will interest themselves in the chemical nature of these acid secretions; it would be of particular interest to know whether essentially the same acids are produced by the various groups. Furthermore, the functioning of the epidermal cilia in animals whose external pH may vary from 1 to 7 within a few seconds deserves further attention.

The author is indebted to Mr J. S. Colman for critical reading of the manuscript and for the provision of laboratory facilities. In addition it is a pleasure to acknowledge the assistance given by Mr R. G. Hartnoll, Mr D. Eggleston and Mr P. J. Miller in collecting the material on which this paper is based. The work was done while the author was holder of a Leverhulme Fellowship in the University of Liverpool.

SUMMARY

The ability, on being abruptly disturbed, to secrete through the skin strongly acid fluids (approximately pH 1) is recorded for five species of British gastropod molluscs. These are the prosobranchs *Lamellaria perspicua* and *Velutina velutina* and the opisthobranchs *Philine quadripartita*, *Berthella plumula* and *Pleurobranchus membranaceus*. All but *Velutina* lack an external shell as adults.

The tissue responsible for the acid secretions presents a characteristically 'empty' appearance in histological preparations. In *Lamellaria*, *Velutina* and *Philine* the acid glands take the form of subepidermal sacs communicating with the exterior through separate ducts perforating the simple ciliated epidermal layer. In *Berthella* and *Pleurobranchus* the secretion is elaborated within the ciliated epidermal cells themselves.

The ability to secrete acid fluids through the skin appears to have arisen independently at least three times, for the three families concerned in this study (Pleurobranchidae, Philinidae and Lamellariidae) are not closely related.

Tests in large aquaria led to the conclusion that the above-mentioned gastropods are invariably rejected as food by a variety of species of fish. After a consideration of the reliability of such tests, it is concluded that it is in fact the acid-secretion mechanism of the gastropod which is responsible for the deterrence of predatorial fish.

It is suggested that the ability to secrete acids through the skin may be a widespread phenomenon among gastropods.

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DEFENSIVE ADAPTATIONS IN OPISTHOBRANCHS

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(Text-fig. 1)

This is the third and concluding part of an investigation into defensive mechanisms in some British gastropods. The first part (Thompson & Slinn, 1959) dealt with a single species of opisthobranch, *Pleurobranchus membranaceus*, which proved able to secrete a strong acid if disturbed. The second part (Thompson, 1960*a*) recorded further instances of acid secretion. The present paper describes the remainder of my work on opisthobranchs.

The two earlier studies were rendered more easy to interpret by the relatively simple nature of the defensive fluid; in the forms to be dealt with herein it has not been possible to attempt any biochemical investigations in view of the complexity and diversity of the materials involved.

Observations on defensive adaptations in opisthobranchs were made by Garstang (1889, 1890*a*), by Herdman & Clubb (1892), by Crossland (1911) and by Crozier (1917). Cott (1940), in *Adaptive Coloration in Animals*, summarizes the work of Garstang and Crossland, but omits any reference in the text to that of Crozier or of Herdman & Clubb. It is unfortunate that Cott's account is marred by some misconceptions regarding opisthobranchs; for instance, after mentioning the batteries of nematocysts possessed by some nudibranchs, he states (p. 254) in a section entitled 'Poison in defence': 'So effective are these batteries as a deterrent, that fishes have been known to eat shelled molluscs such as *Margaritifera* which had been long pickled in formalin, in preference to fresh specimens of the Nudibranch *Chromodoris*.' Chromodorid nudibranchs do not possess nematocysts. Similarly, in a section entitled 'Warning coloration in other groups of animals', Cott mentions Garstang's (1890*a*) work as follows: 'Certain species in the suborder [Tectibranchia], such as *Scaphander lignarius*, *Haminoea hydatis*, and *Philine aperta*, are largely preyed upon by fishes and are inconspicuously coloured. On the other hand, the related *Oscanius membranaceus*, a form rendered highly distasteful by defensive acid secretions, "is not eaten by fishes, and is handsomely coloured with red-brown and yellowish markings"' (Cott, 1940, p. 270). It has already been pointed out (Thompson & Slinn, 1959) that *Pleurobranchus* (= *Oscanius*) *membranaceus* does not exhibit warning coloration; that *Philine*

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can secrete a fluid of the same acidity (approximately pH 1—see Thompson, 1960*a*) as that of *Pleurobranchus*; and, furthermore, I know of no evidence that *Scaphander*, *Philine* and *Haminoea* are 'largely preyed upon' by fish (see the discussion to the present paper). Cott's arguments here are based on faulty knowledge of the opisthobranchs concerned.

It was clear that a re-investigation of the subject of defence in opisthobranchs was necessary; to this end, I have made observations on as wide a variety of forms as possible, attempting to relate the morphology and natural history of the mollusc to data resulting from aquarium experiments with fish.

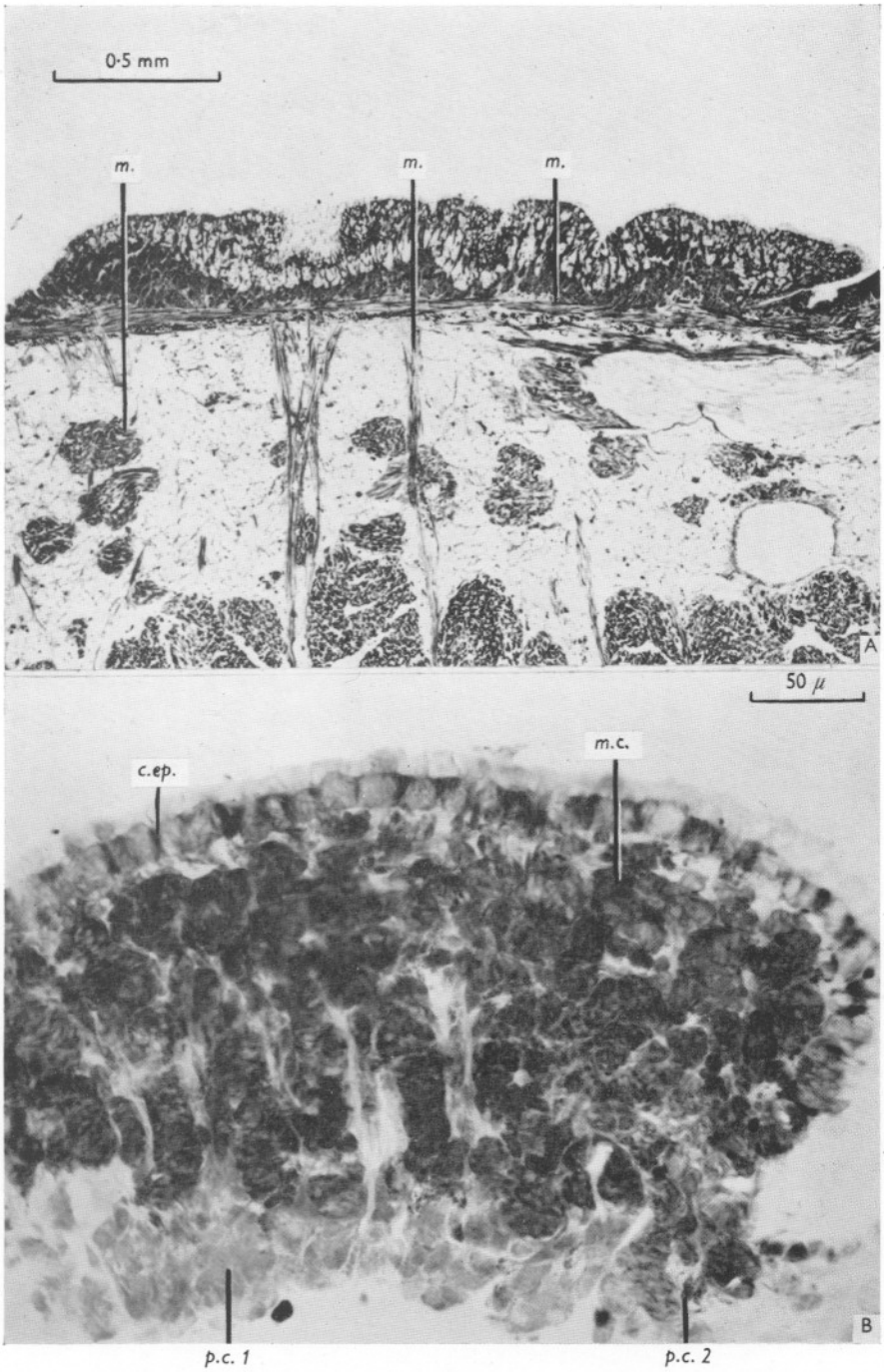
The nomenclature and classification of British animals mentioned herein are those advocated by the Port Erin Fauna list (to be published). In the case of foreign species, the name employed is that used by the author cited.

METHODS

Material for sectioning was fixed in the fluids of Zenker (with or without acetic acid), Helly, Perényi, Bouin, Lewitsky (with added sodium chloride) or Ciaccio. Of these, the last two gave by far the best results. The embedding medium used was Hance's rubber wax (Gurr) and sections were cut at from 4 to 10 μ. Stains employed included the azan and the alum haematoxylin of Heidenhain, safranin and light green, Mayer's haemalum, eosin and alcian blue 8GS (Steedman, 1950). Preparations stained intravitally with neutral red were useful.

In the feeding-acceptability experiments, the procedure and the species of fish used were exactly as described in an earlier paper (Thompson, 1960*a*). These experiments were carried out in the Port Erin Aquarium. Each species of opisthobranch was tested at least twelve times; with the more readily obtainable species of nudibranch, the number of tests made was nearer thirty. Because of the difference in size between the various species of opisthobranchs, the fish to which any individual mollusc was offered was selected partly on the grounds of its size; there would have been little point, for instance, in offering the tiny *Elysia* or *Doto* to a large cod, for such fish often ignore very small objects. Particular care was taken always to ensure that the opisthobranchs tested were not damaged or otherwise enfeebled, for experience showed that resistance to enemies falls if vitality is low.

To test the taste of various opisthobranchs to the human palate, the specimens were first rubbed between the fingers in order to initiate any defensive reaction, and then placed in the mouth; the reactions of the observer were recorded. The results of these tests were not uniformly reliable for difficulty was experienced in separating the saline taste of the medium from the taste of any defensive secretion; it is probable that the human tongue fails to detect some of these secretions, especially in the case of the smallest molluscs. The tests were carried out by the author and Mr D. Eggleston.



(*Facing p.* 114)

The acidity of the skin secretions was estimated by means of B.D.H. pH papers.

RESULTS

Feeding-acceptability tests

The results of the aquarium tests were rather uniform. No healthy opisthobranch (with a solitary exception) was ever seen to be ingested by any of the fish used. The exception was a single *Hero formosa* which was eaten by an *Acanthocottus*, while other individuals were invariably rejected. Dead or severely damaged opisthobranchs were, however, usually taken readily. The fish usually inspected the test organism closely (the manner of inspection varying with the species of fish); on some occasions a healthy opisthobranch was taken into the mouth but rejection almost invariably followed. In a large tank containing a number of excited hungry fish, a mollusc may be 'tasted' by every individual present before it reaches the bottom. The test organisms were seldom damaged in the process of tasting and rejection. There is no point in giving here any details concerning the behaviour of the fish used in these experiments, since Bateson (1890) describes particularly fully the reactions of various species of fish to test foods.

Further observations

In Table 1, data concerning the histology of the skin, natural history and defensive behaviour are listed for the species of opisthobranchs investigated. These data are derived mainly from the present investigation. For each species reference is made to a work in which appears a good illustration; in this way lengthy descriptions of the external features have been avoided. Epidermal mucous glands have been ignored; wherever the term gland is used alone, it may be taken to mean non-mucous gland.

DISCUSSION OF LITERATURE

Records of the predation of fish on bottom-living opisthobranchs are few. Of the species listed in Table 1, only *Tritonia hombergi*, *Scaphander lignarius* and *Philine quadripartita* have been found to be eaten by fish in nature. The jaws of *Tritonia hombergi* have been found occasionally by the author in the stomachs of dogfish (*Scyliorhinus canicula*) dissected by students at University College, Bangor; Ford (1921) found *Scaphander lignarius* in the stomachs of two dogfish of this same species; Todd (1903) found *Philine quadripartita* in the stomachs of a very few fish. *Aeolidia papillosa* (a British nudibranch not dealt with in the present study) was recorded by Homans & Needler (1944) from the stomachs of young haddock (*Melanogrammus aeglefinus*). *Akera bullata* has been found (Lemche, 1929) in the stomach of *Limanda limanda*.

TABLE 1. SUMMARY OF WORK ON DEFENSIVE

(1) Species	(2) Source of data	(3) Reference to good illustration	(4) pH of skin secretion	(5) Taste to tongue	(6) Tests on fish	(7) Cnido-sacs
Aplysia punctata	P	Eales, 1921; Wilson, 1937	N	Bitter†	X	Abs.
Scaphander lignarius	P	P.-F., p. 57	N	Bitter	X	Abs.
Philine quadripartita	T(2)	P.-F., p. 65	*ca.* 1	Bitter-Sour	X	Abs.
Berthella plumula	T(2)	B.Y., Pl. 18	*ca.* 1	Bitter-Sour	X	Abs.
Pleurobranchus membranaceus	T.S.	T.S.	*ca.* 1	Bitter-Sour	X	Abs.
Hermea dendritica	P, G	A.H., 3, pl. 40	—	—	—	Abs.
Elysia viridis	P	Eliot, pl. VII	N	O	X	Abs.
Duvaucelia plebeia	P	A.H., 2, pl. 3	N	—	—	Abs.
Tritonia hombergi	P	A.H., 2, pl. 2	N	Bitter‡	X	Abs.
Dendronotus frondosus	P	A.H., 3, pl. 3	N	O	X	Abs.
Doto coronata	P	A.H., 3, pl. 6	N	O	—	Abs.
Hero formosa	P	Eliot, pl. IV	N	O	*	—
Eubranchus tricolor	P	A.H., 3, pl. 34	N	O	X	Pres.
Facelina auriculata	P	A.H., 3, pl. 12, 13	N	O	X	Pres.
Archidoris pseudoargus	P	A.H., 1, pl. 3	N	O	X	Abs.
Jorunna tomentosa	P	A.H., 1, pl. 5	N	O	X	Abs.
Acanthodoris pilosa	P	A.H., 1, pl. 15	N	O	X	Abs.
Onchidoris pusilla	P	A.H., 1, pl. 13	N	O	—	Abs.
O. fusca	P	A.H., 1, pl. 11	N	O	X	Abs.
O. muricata	P	A.H., 1, pl. 9	N	O	X	Abs.
Adalaria proxima	T(1)	A.H., 1, pl. 9	N	O	—	Abs.
Goniodoris nodosa	P	A.H., 1, pl. 18	N	O	X	Abs.
Polycera quadrilineata	P	A.H., 1, pl. 22	N	O	X	Abs.
Ancula cristata	H.C.	A.H., 1, pl. 25	—	O	X	Abs.

Notes

Column (1), name of species as in *Port Erin Marine Fauna* MS.

Column (2) refers to the following sources: P, present article; T(1), Thompson, 1958; T(2), Thompson, 1960*a*; T.S., Thompson & Slinn, 1959; G, Garstang, 1890*a*; H.C., Herdman & Clubb, 1892.

Column (3) references include: B.Y., Barrett & Yonge, 1958; A.H., Alder & Hancock, 1845–55, Families 1, 2, or 3, as indicated; P.-F., Pruvot-Fol, 1954; Eliot, 1910 (supplement to Alder & Hancock); T.S., Thompson & Slinn, 1959.

Column (4): N, neutral.

Column (5), taste to human tongue: O, None.

Column (6), acceptability to fish in tests: X, always refused; * one eaten by *Acanthocottus bubalis*, others rejected.

Column (7), cnidosacs present (Pres.) or absent (Abs.).

† Both purple secretion and body.

‡ Byne (1893) states that this secretion may blister human hands.

ADAPTATIONS IN OPISTHOBRANCHS

Species	(8) Non-mucous skin glands: Epid.	(8) Non-mucous skin glands: Subepid.	(9) Regions of body poor in non-mucous skin glands	(10) Behaviour if abruptly disturbed	(11) Appearance in natural surroundings
Aplysia punctata	Ab.	Ab.	Pedal sole and parts of mantle	Epipodial flaps brought together, pedal sole contracted	Inconspicuous in colour and attitude
Scaphander lignarius	O	Ab.	None	General contraction	Half-buried in sand
Philine quadripartita	O	Ab.	,,	,, ,,	Buried beneath sand
Berthella plumula	Ab.	O	,,	,, ,,	Not strikingly coloured but usually easy to see
Pleurobranchus membranaceus	Ab.	O	,,	General contraction, often followed by swimming motions	When swimming: conspicuous but not strikingly coloured. When creeping: camouflaged
Hermea dendritica	O	Ab. in d.p.	Whole body excluding dorsal papillae	Body contracted, dorsal papillae held erect	Difficult to see on *Bryopsis* and other Algae
Elysia viridis	O	Ab. (Fig. 1H)	Pedal sole and dorsal epidermis	Epipodial flaps brought together, pedal sole contracted	Well camouflaged among littoral and sublittoral Algae
Duvaucelia plebeia	Ab.§	O	Rhinophores and pedal sole	Rhinophores retracted, pedal sole contracted	Well camouflaged on *Alcyonium digitatum*
Tritonia hombergi	Ab.§ (Fig. 1F)	O	,, ,, ,,	,, ,, ,,	,, ,, ,,
Dendronotus frondosus	Ab.	Ab.‖	,, ,, ,,	,, ,, ,,	Well camouflaged on hydroids
Doto coronata	Ab.	Ab. in d.p.	Whole body excluding dorsal papillae	Body contracted, dorsal papillae held erect	Difficult to see on hydroids
Hero formosa	—	—	—	,, ,, ,,	Well camouflaged on hydroids
Eubranchus tricolor	O	O	—	,, ,, ,,	Never inconspicuous
Facelina auriculata	O	O	—	,, ,, ,,	,, ,,
Archidoris pseudoargus	Ab. in d.m.	Ab. in d.m.	Ventral parts of body, rhinophores and branchiae	Branchiae and rhinophores retracted; if detached from substratum foot contracted	Well camouflaged in colour, attitude, and texture
Jorunna tomentosa	Ab. in d.m.	O	,, ,, ,,	,, ,, ,,	,, ,, ,,
Acanthodoris pilosa	Ab. in d.m. (Fig. 1B)	O	,, ,, ,,	,, ,, ,,	,, ,, ,,
Onchidoris pusilla	O	Ab. in d.m. (Fig. 1D)	,, ,, ,,	,, ,, ,,	,, ,, ,,
O. fusca	Ab. in d.m.	O	,, ,, ,,	,, ,, ,,	,, ,, ,,
O. muricata	O	Ab. in d.m.	,, ,, ,,	,, ,, ,,	,, ,, ,,
Adalaria proxima	O	,,	,, ,, ,,	,, ,, ,,	,, ,, ,,
Goniodoris nodosa	O	,,	,, ,, ,,	,, ,, ,,	,, ,, ,,
Polycera quadrilineata	O	Ab. espec. in d.p.	,, ,, ,,	Body contracted, dorsal papillae held erect	Never inconspicuous
Ancula cristata	O	,,	,, ,, ,,	,, ,, ,,	,, ,, ,,

Column (8), non-mucous skin glands may be epidermal (epid.) or subepidermal (subepid.): Ab., abundant; O, not present; d.p., dorsal papillae; d.m., dorsal mantle; espec., especially.

§ Glands of two types in these two species.

‖ Subepidermal glands occur in adults, especially in the dorsal papillae (see Thompson, 1960*b*).

In addition, predation by various carnivores on pelagic gymnosomes and thecosomes is known to be widespread (personal communication from J. E. Morton). On the other hand, in a recent detailed study of the gut contents of gadoid fish from the seas around the south of the Isle of Man, no opisthobranchs were found (personal communication from A. K. Nagabhushanam).

There are instances where certain species of opisthobranchs have been wrongly said to be widely eaten by fish. A particularly glaring example of this is provided by Cott (1940), who stated that *Scaphander lignarius* was widely preyed upon by fish, giving Garstang (1890*a*) as the source of this information. While Garstang did state that *Haminoea* and *Philine* were 'largely eaten by fishes', he clearly did not claim that *Scaphander* also was. It is now necessary to inquire more deeply into Garstang's claim regarding *Haminoea* and *Philine*. He gives as his source for this Verrill (1873), but perusal of Verrill's paper shows that no mention occurs of *Haminoea hydatis* or of *Philine*. The only hard fact that emerges is that there appears to be no evidence that *Scaphander lignarius*, *Haminoea hydatis* or *Philine quadripartita* are widely eaten by fish.

DISCUSSION OF RESULTS

Feeding experiments with fish

The feeding experiments carried out in this study gave uniform results which seem to show clearly that all the opisthobranchs tested were distasteful to the fish used. It is well known that the results of such aquarium experiments must be interpreted with caution and the possibility that the fish employed might be behaving abnormally was always borne in mind. However, the obvious health of the fish, their vigorous appetites and, above all, their eagerness to take and swallow *dead* or *damaged* opisthobranchs, showed beyond reasonable doubt that some deterrent attribute possessed by healthy opisthobranchs rendered them distasteful to fish. This is not to suggest that these experiments show that fish *never* eat these opisthobranchs in nature.

Predators other than fish

Mention may be made here of some experiments with shore-crabs (*Carcinus maenas*) and with the anemones *Tealia felina* and *Anemonia sulcata*. While the anemones would often ingest opisthobranchs placed within reach of their tentacles, consistent results were impossible to obtain and their interpretation is difficult. It has already been recorded (Thompson, 1960*a*) that *Berthella* and *Pleurobranchus* were usually refused by *Tealia*; it has now been found that the eolidaceans *Facelina* and *Eubranchus* also enjoy some kind of immunity from this anemone and others. McMillan (1941) records similar results. All the other opisthobranchs tested were, however, seen to be ingested by anemones on various occasions, but never *predictably*. The significance of

these results hinges on whether this predation occurs in nature; unfortunately, I can offer only the negative information that I have never seen it happen.

Tests with shore-crabs were also unsatisfactory, but more uniform; the crabs always ignored the test organisms.

Defensive attributes

Nematocysts

That eolidacean nudibranchs possess cnidosacs at the tips of their dorsal papillae has been known for many years. There can be little doubt that these nematocysts discourage fish from ingesting these molluses, for Cott (1940) mentions that cnidarian tissues are highly distasteful to a variety of fish; my experiments confirm this. That some have been known to be devoured by fish (Homans & Needler, 1944) is not a convincing argument against the theory that one of the functions of the eolidacean cnidosacs is to deter predatory fish.

Acid secretions

Three species of opisthobranch (and two of prosobranch) gastropods are known to possess the ability to secrete a strong acid through the skin if they are roughly disturbed (Thompson, 1960 *a*). Because Bateson (1890) found fish to refuse any food which had been soaked in dilute acids it is believed that these acid-secreting gastropods are generally refused as food by fish in nature. This is supported by aquarium and other observations.

Other secretions (see Fig. 1)

In all the other opisthobranchs which have been studied in this investigation (see Table 1), skin glands have been found whose position and function can only be explained satisfactorily as defensive. They are always present in addition to the usual mucous glands associated with ciliated epithelia. They are always present in greatest abundance in the areas of the skin which would be first encountered by an inquisitive fish in nature. In species which possess dorsal papillae projecting some way out from the body, these glands are always concentrated in the papillae. Papillae of this kind are usually non-retractile, and may be rapidly regenerated if damaged. On the other hand, those dorsal processes which do not contain these glands (such as the rhinophoreal tentacles or the anal branchiae of some nudibranchs) are always retractile and are quickly concealed if the animal is disturbed. The behaviour of the opisthobranch if molested roughly is always such as to bring about the concealment of those regions of the body which are not endowed with these glands.

Histologically these defensive glands exhibit considerable diversity; they may be epidermal or subepidermal, unicellular or multicellular; their secretion may consist of fluids alone or may contain hyaline concretions; the secretion

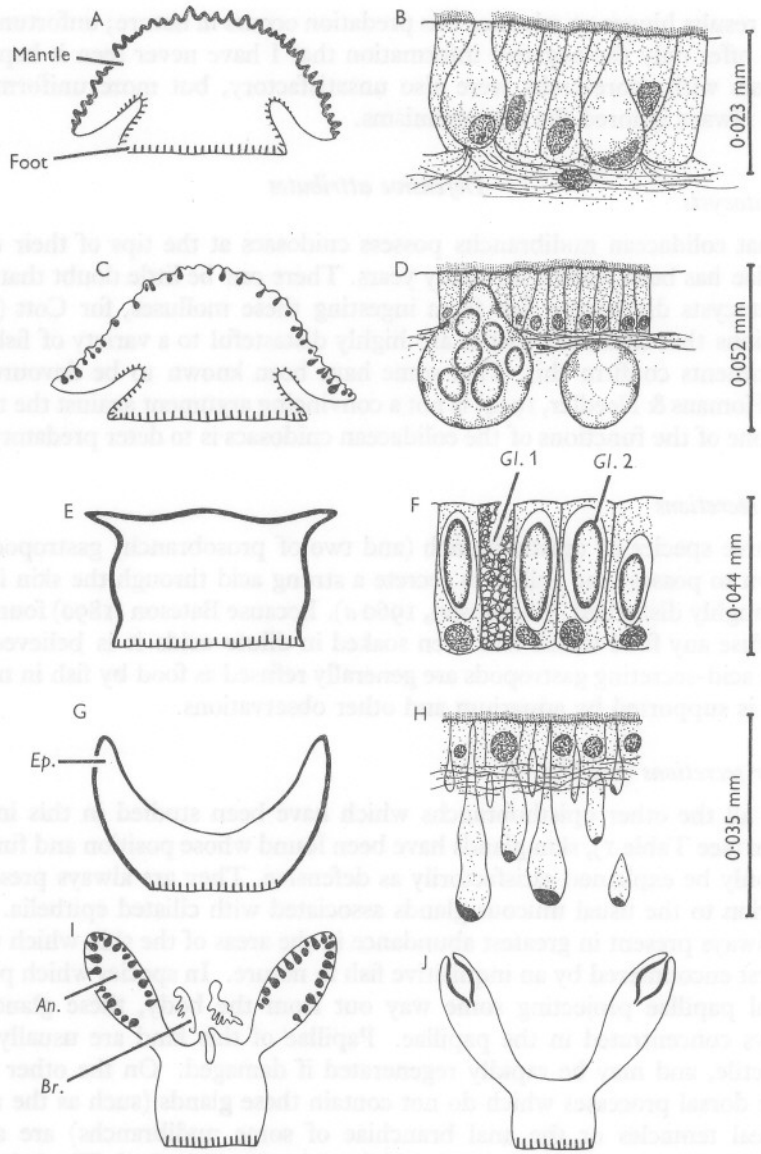


Fig. 1. Illustrating the structure and distribution of defensive glands in some representative opisthobranchs. In the diagrammatic transverse sections areas provided with defensive attributes are shown black while areas rich only in mucous glands are hatched. A, diagrammatic transverse section through *Acanthodoris pilosa*; B, portion of the dorsal integument of *A. pilosa* at greater magnification; C, diagrammatic transverse section through *Onchidoris pusilla*; D, portion of the dorsal integument of *O. pusilla* at greater magnification; E, diagrammatic transverse section through *Tritonia hombergi*; F, portion of the dorsal integument of *T. hombergi* at greater magnification; G, diagrammatic transverse section through *Elysia viridis*; H, portion of the epithelium of the epipodial flaps of *E. viridis* at greater magnification; I, diagrammatic transverse section through *Polycera quadrilineata*, passing through the level of the anus; J, diagrammatic transverse section through *Facelina auriculata*, passing through a pair of cerata and showing the position of the cnidosacs. *An.*, anal tubercle; *Br.*, anal branchia; *Ep.*, epipodial flap; *Gl.* 1, gland of type 1; *Gl.* 2, gland of type 2.

may or may not taste bitter to the human tongue. More than one type of gland may be present in a single species.

The mucus which is secreted profusely if an opisthobranch is roughly handled may decrease the animal's attractiveness as food; this mucus may even aid the effectiveness of the defensive secretions by hindering their too-rapid dispersal.

Spicules

All the dorid nudibranchs have abundant small papillae and calcareous spicules in the skin and it is probable that the spiculose texture of the mantle, while perhaps not of positive deterrent value, would diminish their attractiveness as food to a fish. It is interesting to note that sponges may be refused as food by fish in experiments (Garstang, 1890*b*).

Behaviour

The opisthobranchs studied may be classed as follows, on the basis of their behaviour both in undisturbed conditions and when abruptly disturbed:

(i) Opisthobranchs which, for most of their lives at least, behave in a way which indicates an apparent desire for concealment; this concealment may be achieved by means of coloration, attitude, texture, habitat selection, or a combination of any or all of these.

(*a*) Forms which, if abruptly disturbed, respond merely by a general contraction of the body, e.g. *Scaphander*, *Berthella*, *Philine*, *Pleurobranchus* (which may subsequently attempt to swim away). In all these forms the defensive glands are distributed over the whole naked surface of the body.

(*b*) Forms which, if abruptly disturbed, retract or otherwise conceal parts of the body which are not liberally endowed with skin glands, thus presenting a would-be predator only with those parts which are provided with defensive attributes, e.g. *Dendronotus*, *Doto*, *Aplysia*, *Elysia*, *Hermaea*, dorid and tritoniid nudibranchs.

(ii) Opisthobranchs whose behaviour is always characterized by an apparent disregard for concealment. They invariably possess well-developed dorsal papillae and, if an individual is abruptly disturbed, these papillae are held erect while the rest of the body, which is always provided to a lesser extent with any defensive attributes, is contracted, e.g. *Ancula*, *Polycera*, *Eubranchus*, *Facelina*.

Coloration

Opisthobranchs which would, on the grounds of their behaviour, fall into class (i) above are always difficult to detect (at least to the human eye) in their natural surroundings. This resemblance or camouflaging is usually achieved by a combination of the coloration of the body and of such dorsal processes as

may be present, the slow-moving habits, the texture of the body and the selection of a suitable habitat (many nudibranchs are found in narrow crevices, under stones, etc., while *Scaphander* and *Philine* lie partly or wholly covered by sand).

Those opisthobranchs which fall into class (ii) above were always brightly coloured, the pigmented areas often being confined to the dorsal papillae. Since I have no evidence that any of the fish employed in my tests 'learned' to avoid any species of opisthobranch, it is best that the terms 'warning' or 'aposematic' coloration be avoided here; nonetheless there is no doubt that the colours, behaviour and form of *Ancula*, *Polycera* and the eolids investigated render these animals readily visible to the human eye both in aquaria and in the field. The inference is that they are similarly obvious to fish.

An important point which emerges from a consideration of opisthobranchs in their natural surroundings is that the usual dividing line between brightly coloured, distasteful forms on the one hand, and cryptically marked, readily edible forms on the other, does not hold. In my experiments, no facts emerged which might support a theory that the conspicuous forms (such as *Polycera* or *Eubranchus*) were any more or any less acceptable to fish than the cryptically coloured species (such as *Aplysia* or *Archidoris*). All the opisthobranchs studied were distasteful to fish. The phenomenon is not new; Cott (1940) mentions that there are a number of species of poisonous snakes which are cryptically coloured. Cott dismisses the idea that this apparent anomaly points to some deep-rooted weakness in the theory of adaptive coloration in animals in the following words (with which I am in complete agreement): '...there is nothing irreconcilable in the fact that some well-defended forms rely upon concealment as a first line of defence...while others gain an advantage by being recognized through their conspicuousness as easily as possible' (Cott, 1940, p. 155). On the other hand, it is now necessary to disagree with Cott when he states (1954, pp. 55–6): '...a broad correlation has been established between cryptic coloration and relative palatability on the one hand, and between conspicuousness and deterrent attributes on the other. Such a relationship, supported by a great body of experimental and observational evidence...is found among many groups of animals, including tectibranch molluscs (Garstang, 1889–90).'

Anomalous opisthobranchs

The species investigated in the present work were selected solely on the grounds of availability; the conclusions arrived at are thus based on a number of opisthobranchs chosen more or less at random from the British fauna. Unfortunately, several species of nudibranchs, which appear to provide exceptions to my conclusions, were not available for study. The orange-red dorid *Archidoris pseudoargus flammea* and the supposedly cryptically coloured

eolid *Aeolidia papillosa* are cases in point; going farther afield, the brilliantly marked chromodorid nudibranchs of warmer seas would clearly repay further study.

This work was done while I was the holder of a Leverhulme Fellowship in the University of Liverpool. I am indebted to Mr J. S. Colman for critical reading of the manuscript and for the provision of laboratory facilities. In addition, it is a pleasure to record my indebtedness to Mr D. Eggleston, Mr R. G. Hartnoll, Mr P. J. Miller and Mr K. Reddiah, for their help in collecting the material on which this paper is based. Mr D. Eggleston also kindly gave assistance in the gustatory tests. Dr A. K. Nagabhushanam gave guidance in the literature concerning the gut contents of fish.

SUMMARY

Feeding-acceptability tests with a variety of opisthobranchiate gastropods and marine aquarium fish gave rather uniform results; in such experiments, opisthobranchs were almost invariably refused as food by fish.

Descriptions are given of the glands which, placed often in the most vulnerable yet least vital regions of the body of the opisthobranch, are believed to produce deterrent secretions.

The results of these investigations are related to facts concerning the natural history of the animals concerned, and the conclusions reached are considered in the light of modern theories concerning the adaptive coloration of animals.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

XI. OBSERVATIONS ON VERTICAL MIGRATION ESPECIALLY IN FEMALE *CALANUS*

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(Text-figs. 1–5)

A number of different factors have been shown to affect the vertical migration of the zooplankton, among them changes in light intensity, temperature, salinity and the presence of food (see Russell, 1927; Hardy & Paton, 1947; Cushing, 1951; Bainbridge, 1953; Harris, 1953; Moore, 1958; Banse, 1959). The object of the present study was to find out whether in female *Calanus* there was any relation between vertical migration and the degree of ripeness.

When *Calanus* were taken by tow-nettings near the laboratory it was noticed that the percentage of adult females in the ripe condition was generally greater in hauls made close to the surface than in those from deep water. For example in the spring of 1953 a series of hauls was made near the surface in Fairlie Channel where the maximum depth is about 40 m, and at 60–80 m off Garroch Head a few miles away where the depth is 115 m. Throughout the period of examination there was a much higher percentage of ripe females in the shallow than in the deep hauls (Table 1, Fig. 1). The deep hauls show the progressive ripening of the females during the spring reaching a peak in percentage ripe which coincides with the spring diatom increase in the middle of March. It also coincides with the maximum number of eggs produced by laying females. In shallow water the sequence is not so obvious because of the high percentage of ripe females at the surface most of the time.

A tendency for ripe female *Calanus* to keep in the surface waters might be expected since the eggs, which are denser than sea water (Salzen, 1956), are usually found in the upper 30 m (Nicholls, 1933). In the Clyde area adults which moult from the overwintering stock of Stage V in January and February show a marked migration, and if migration to the surface for egg-laying takes place one might expect to find a difference in behaviour of females in different stages of ripeness during the spring. It is only during early spring that a good proportion of immature and medium, as well as ripe, females is certain to be found and this is therefore the best time to study their migrations.

Vertical distribution stations were worked in the spring of three years,

1953 (2 February), 1954 (16 March) and 1959 (9 February) and on each occasion hauls were taken every 3 h for 27 h. On all three occasions the weather was calm with only light airs. There was sunshine during the day, but in 1959 the sun shone only through a haze. There was bright moonlight in 1953 and 1954 but in 1959 the night was overcast and raining. Times of sunrise and sunset are shown in the figures.

The position chosen for the observations was the deep water between the

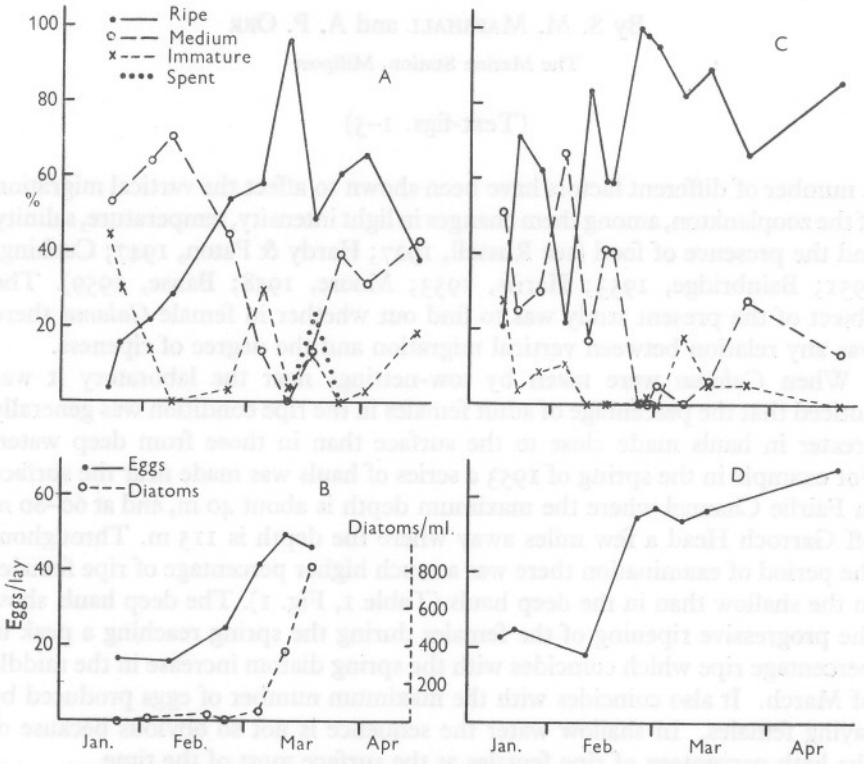

Fig. 1. Maturity of female *Calanus* in deep and shallow hauls in 1953. A and B, deep water, Garroch Head; C and D, near surface, Fairlie Channel.

south-east of the island of Bute and the Little Cumbrae. Because of the small numbers of *Calanus* present at the end of the winter, vertical hauls with a closing net would not have given sufficient numbers for studies of ripeness. Thirty-minute hauls with 50 cm diameter coarse tow-nets (26 meshes to the inch) were therefore taken simultaneously at four depths from a trawl warp to the end of which was attached a depressor (Barnes, 1951). Tows were made at a constant speed and the depth of the nets during tows checked by an Admiralty pattern depth recorder. The top net fished just below the surface, the next at 20–25 m, the third at about 45 m and the lowest net at

60–70 m. Because of the time required for hauling and untying, the deep nets were towed for a few minutes longer than the shallow on each run. There is no simple correction which can be applied for this, but the differences are unlikely to have interfered seriously with the results.

An objection to the use of horizontal tows at a small number of depths is that important differences in the concentration of organisms might be missed. If so one would expect to find changes in the total numbers from

TABLE 1. MATURITY IN SPRING, 1953

Date	No. examined	% Females: Immature	Medium	Semi-ripe	Ripe	Spent	Eggs per lay	Diatoms (cells/ml.)
Garroch Head, 60–80 m.								
2 Jan.	47	45	43	9	4	0	—	—
26 Jan.	51	31	28	26	16	0	17	0
2 Feb.	536	14	20	44	22	0	—	10
9 Feb.	51	0	22	49	29	0	16	—
19 Feb.	—	—	—	—	—	—	—	35
24 Feb.	39	3	3	41	54	0	25	0
5 Mar.	40	30	10	3	58	0	42	50
12 Mar.	25	4	0	0	96	0	50	370
19 Mar.	45	13	4	9	49	24	46	818
26 Mar.	31	0	7	32	61	0	—	—
2 Apr.	32	3	13	19	66	0	—	—
16 Apr.	32	19	34	9	38	0	—	—
Fairlie Channel, near surface								
19 Jan.	43	28	23	28	21	0	23	
23 Jan.	56	4	9	16	71	0	25	
29 Jan.	71	9	17	13	62	0	—	
5 Feb.	45	11	38	29	22	0	—	
11 Feb.	23	0	4	13	83	0	18	
16 Feb.	87	0	2	39	59	0	—	
17 Feb.	27	0	0	41	59	0	—	
25 Feb.	20	0	0	0	100	0	55	
27 Feb.	50	2	0	0	98	0	—	
2 Mar.	21	0	0	5	95	0	58	
9 Mar.	28	18	0	0	82	0	54	
16 Mar.	18	6	6	0	89	0	57	
26 Mar.	35	6	14	14	66	0	—	
20 Apr.	14	0	7	7	86	0	68	

top to bottom during the 27 h. There was in fact a rise in total numbers in the early evening in two of the years which may have been because of an influx from below the depth of the bottom net. Otherwise total numbers remained reasonably constant. The percentage of the total catch in different stages and states of maturity also remained fairly constant throughout.

The tow-nettings were fixed in 5% formalin and afterwards the numbers of males, females and Stage V counted in aliquot samples from each haul. When numbers allowed it at least 200 of the females were taken for staining and clearing to determine ripeness.

Three different methods of staining were tried. In the first year (1953)

the females were stained in methylene blue, dehydrated in dioxan and cleared and examined in creosote. This sometimes gave a very clear picture but the results were not consistent because the largest and ripest eggs hardly stained at all and were easy to miss. Eventually samples of this series were stained with celestine blue B (see below).

In 1954 the stain used was borax carmine and the examination was made in xylol. This method proved fairly satisfactory and the results were later checked against the celestine blue method. Staining with celestine blue B (Gray, 1958) proved the best method and was adopted for the 1959 hauls. We should like to thank Dr H. F. Steedman of the Zoology Department of the University of Glasgow for suggesting the method and for his help.

The specimens were put in a glass tube closed at the lower end by coarse bolting silk, and were washed and then stained in celestine blue B (14 ml. to 86 ml. 1% acetic acid). They were then taken through a series of alcohols and cleared in diethyl phthalate.

Unfortunately the living females especially when ripe look rather different from the fixed, stained and cleared specimens and the categories chosen, immature, medium and ripe, do not necessarily correspond exactly to those used for living material (Marshall & Orr, 1952). The eggs appear smaller and the ripe female less full of eggs. In February and March, however, the number of large eggs in a ripe female is very much smaller than it is later in the year.

The ripest eggs do not as a rule stain so heavily as the unripe, and the arbitrary criterion we have used in distinguishing the ripe from the medium is that there should be a row of these larger and paler eggs in the lower part of the oviduct (Fig. 2 C). There is no such definite criterion to distinguish the immature from the medium, but in the immature the ovary is small, the oviducal diverticula are represented by two short single rows of small eggs not extending to the front of the head and the oviducts have a single row, often discontinuous, of small eggs (Fig. 2 A). In the medium, the oviducal diverticula extend almost to the front of the head and may have more than one row of eggs each; the eggs in the oviduct are larger and pressed more closely together forming a single or a double row (Fig. 2 B). As a rule, in distinguishing the different stages, most weight was given to the appearance of the oviducts.

MIGRATIONS

A study of the vertical distribution of *Calanus* in spring has already been made for a deep station in this area (Nicholls, 1933). The four distributions, all of the same generation, within 8 weeks (although in different years), and within the same area, might be expected to resemble one another closely, but in fact they do not.

The earliest, that of Nicholls on 25 January 1932, although based on rather small numbers, shows the clearest results. Apart from this it differs from the

rest in having been done in much deeper water and with a closing net. It shows the usual pattern for the overwintering generation in which the females migrate most, in close agreement with light changes, the males migrate less, and the Stage V hardly at all.

In 1959 (9–10 February) the behaviour of females and males followed this pattern (Fig. 3 and Table 2), but the Stage V also showed a distinct migration slightly more marked than that of the males. From 9.00 to 15.00 h all stages

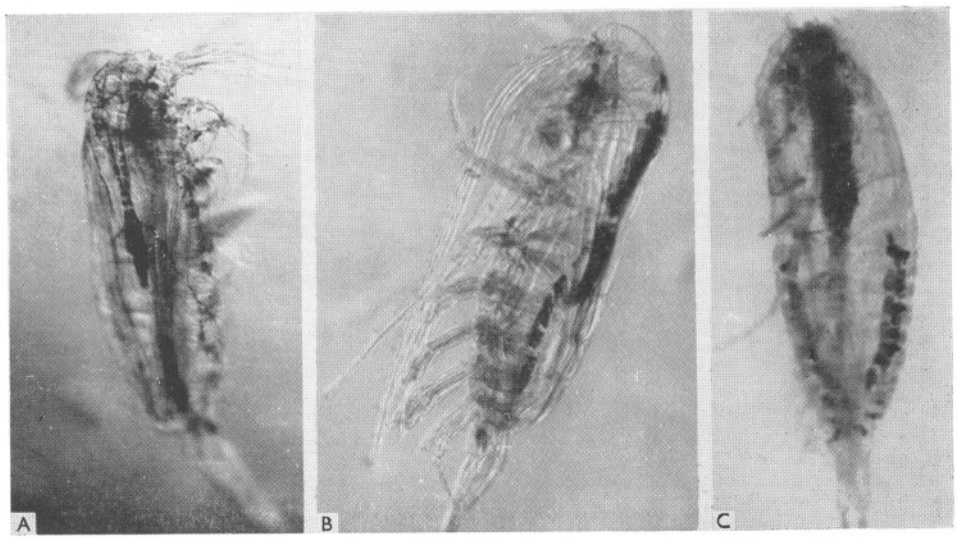

Fig. 2. Female *Calanus* stained with celestine blue. A, immature: the ovary, oviducal diverticula and right oviduct are visible. B, medium: oviducal diverticula extend farther forward and eggs are larger. C, ripe: note the two sizes of eggs in the oviduct.

were concentrated in the bottom net (62 m), but between sunset and sunrise the distribution was much more even throughout the water column with a tendency for the females to be concentrated in the upper two nets and the males and Stage V in the lower two.

In 1953 (2–3 February) the main difference was that the *Calanus* were higher in the water (Fig. 3), the highest numbers being usually in the net at 45 m in spite of clearer sunshine than in 1959. Apart from this the females showed the usual migration upwards between sunset and sunrise. This movement was less marked in the males and Stage V. All, however, behaved less regularly than in 1933 or in 1959.

In 1954 (17–18 March) the behaviour was surprisingly different. A considerable proportion of all the *Calanus* remained in the two top nets throughout the 27 h and at 9.00 and 12.00 h, the brightest hours, were even more concentrated there, a distribution quite opposite to those found earlier in

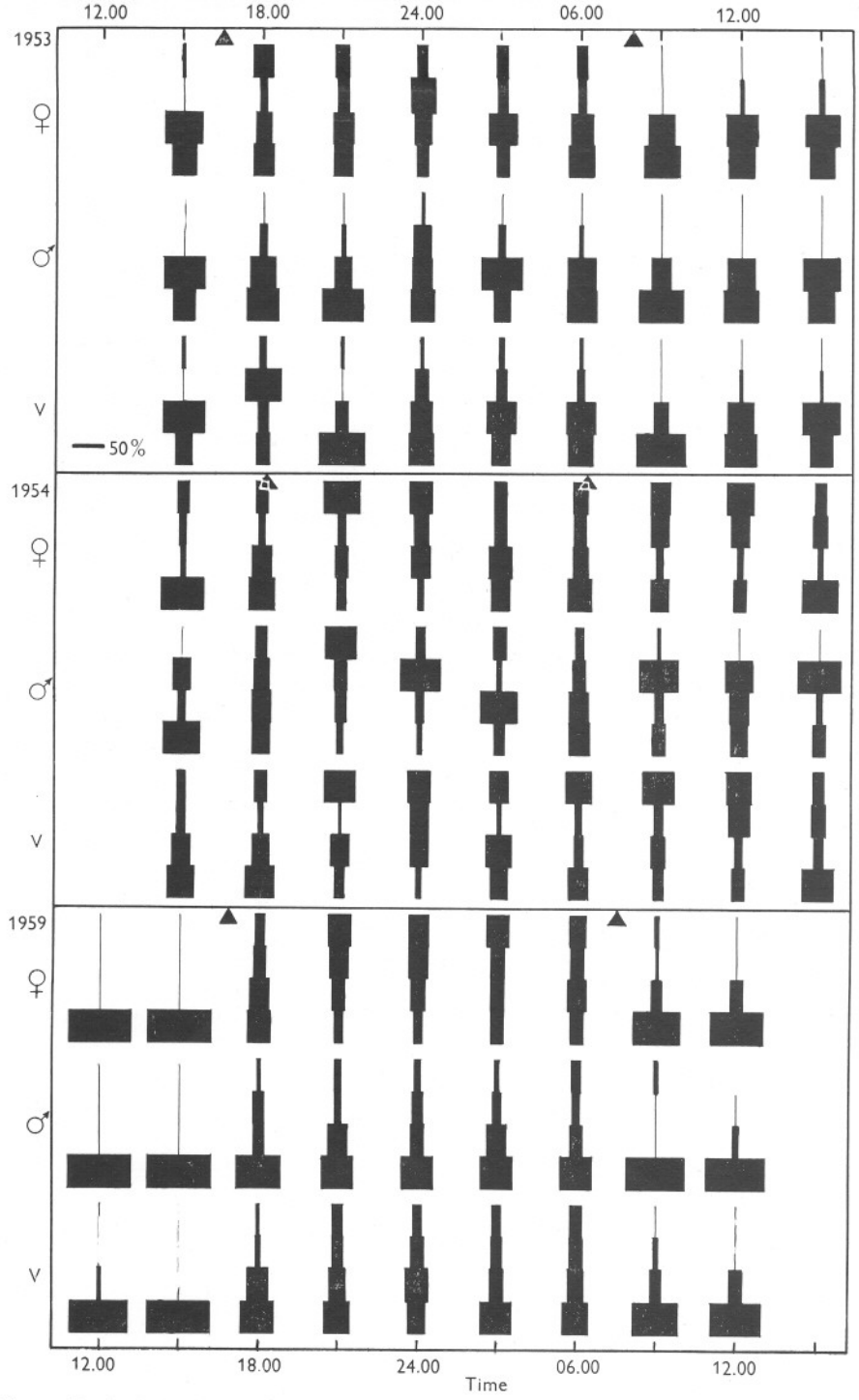

Fig. 3. Vertical distribution of male, female, and Stage V *Calanus* at four depths. The arrows show times of sunset and sunrise.

TABLE 2. VERTICAL DISTRIBUTION OF FEMALE, MALE AND STAGE V *CALANUS*

		2–3 February 1953							16–17 March 1954							9–10 February 1959						
		♀		♂		V			♀		♂		V			♀		♂		V		
Haul	Time	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
I. 1	15.00	77	1·3	9	0·4	5	1·3	15.00	1013	23·9	2	1·4	61	13·4	12.00	13	0·3	2	0·6	0	0	
2		355	5·9	38	1·6	27	7·0		603	14·2	42	28·4	65	14·3		32	0·8	1	0·3	5	2·3	
3		3380	56·7	1480	64·4	250	65·4		359	8·5	19	12·8	133	29·3		119	2·9	3	1·0	14	6·4	
4		2150	36·1	770	33·5	100	26·2		2260	53·4	85	57·4	195	42·9		3928	96·0	302	98·1	200	91·3	
II. 1	18.00	3750	32·5	80	2·2	110	10·4	18.00	547	17·9	16	18·2	46	19·2	15.00	10	0·1	6	1·0	1	0·2	
2		1400	12·1	440	12·0	600	56·5		344	11·3	21	23·9	18	7·5		55	0·6	2	0·3	4	0·6	
3		2670	23·1	1410	38·4	160	15·1		983	32·2	26	29·6	66	27·5		21	0·2	1	0·2	5	0·8	
4		3730	32·3	1740	47·4	190	17·9		1175	38·5	25	28·4	110	45·8		8990	99·1	580	98·5	650	98·5	
III. 1	21.00	1690	20·7	80	2·8	20	4·0	21.00	2410	45·8	95	48·5	250	48·3	18.00	995	15·8	11	5·0	18	6·7	
2		1600	19·6	170	6·0	10	2·0		795	15·1	40	20·4	24	4·6		1150	18·2	18	8·3	23	8·5	
3		2590	31·7	780	27·4	110	22·0		1126	21·4	37	18·9	159	30·7		2024	32·1	35	16·0	89	33·1	
4		2280	27·9	1810	63·7	360	72·0		936	17·8	24	12·2	85	16·4		2134	33·9	154	70·6	139	51·6	
IV. 1	24.00	602	16·6	40	3·3	14	5·7	24.00	1395	20·9	20	13·5	155	35·4	21.00	2357	36·8	61	12·6	67	18·3	
2		1385	38·1	345	28·9	55	22·5		2045	30·7	95	64·2	125	28·5		1882	29·6	57	11·8	60	16·3	
3		975	26·8	370	31·0	85	34·8		2220	33·3	20	13·5	120	27·4		1281	20·1	138	28·5	95	25·9	
4		670	18·4	440	36·8	90	36·9		1010	15·1	13	8·8	38	8·7		863	13·5	228	47·1	145	39·5	
V. 1	03.00	531	17·7	26	2·3	14	8·1	03.00	925	13·1	30	20·3	75	28·0	24.00	1808	31·8	54	10·9	66	13·7	
2		468	15·6	105	9·2	29	16·8		1315	18·7	8	5·4	18	6·7		1731	30·5	83	16·7	107	22·3	
3		1390	46·4	725	63·4	80	46·2		2795	39·7	85	57·4	105	39·2		1274	22·4	109	21·9	170	35·4	
4		607	20·3	287	25·1	50	28·9		2005	28·5	25	16·9	70	26·3		869	15·3	252	50·6	137	28·5	
VI. 1	06.00	673	16·4	40	2·2	13	5·3	06.00	931	22·7	18	13·3	83	40·1	03.00	2049	35·9	29	5·7	65	13·3	
2		467	11·4	110	6·0	23	9·4		951	23·2	30	22·2	26	12·6		1304	22·9	68	13·3	80	16·4	
3		1430	34·7	850	46·2	110	44·7		997	24·3	42	31·1	30	14·5		1086	19·2	152	29·9	108	22·2	
4		1545	37·5	840	45·7	100	40·6		1219	29·8	45	33·3	68	32·9		1264	22·1	260	51·0	234	48·0	
VII. 1	09.00	46	1·1	13	0·7	3	1·1	09.00	1983	25·5	7	4·1	171	47·4	06.00	1491	26·9	44	15·3	59	17·3	
2		83	2·0	15	0·8	6	2·1		3167	40·7	107	62·9	52	14·4		1196	21·6	32	11·1	66	19·3	
3		1690	40·3	560	30·1	60	21·5		940	12·1	24	14·1	77	21·3		1651	29·7	62	21·6	84	24·6	
4		2370	56·5	1270	68·3	210	75·3		1694	21·8	32	18·8	61	16·9		1210	21·8	149	51·9	133	38·9	
VIII. 1	12.00	132	2·2	8	0·5	7	2·3	12.00	1930	40·8	2	2·0	86	35·3	09.00	247	5·4	15	5·1	9	3·3	
2		477	7·9	60	3·6	20	6·5		1703	36·0	45	44·0	82	33·6		224	4·9	5	1·7	18	6·5	
3		3040	50·5	680	41·3	130	42·3		351	7·4	28	27·4	32	13·1		750	16·4	7	2·4	50	18·0	
4		2370	39·3	900	54·6	150	48·8		745	15·8	27	26·5	44	18·0		3356	73·3	268	90·8	200	72·2	
IX. 1	15.00	150	2·6	26	2·2	4	1·9	15.00	660	15·3	5	3·9	37	16·8	12.00	13	0·3	0	0·0	3	1·0	
2		359	6·3	34	2·9	13	6·3		1348	31·2	86	66·2	47	21·4		33	0·8	4	1·0	2	0·7	
3		2960	52·1	650	55·1	120	58·0		422	9·8	12	9·2	31	14·1		697	17·6	29	7·2	63	20·5	
4		2210	38·9	470	39·8	70	33·8		1893	43·8	27	20·8	105	47·7		3220	81·3	368	91·7	240	77·9	

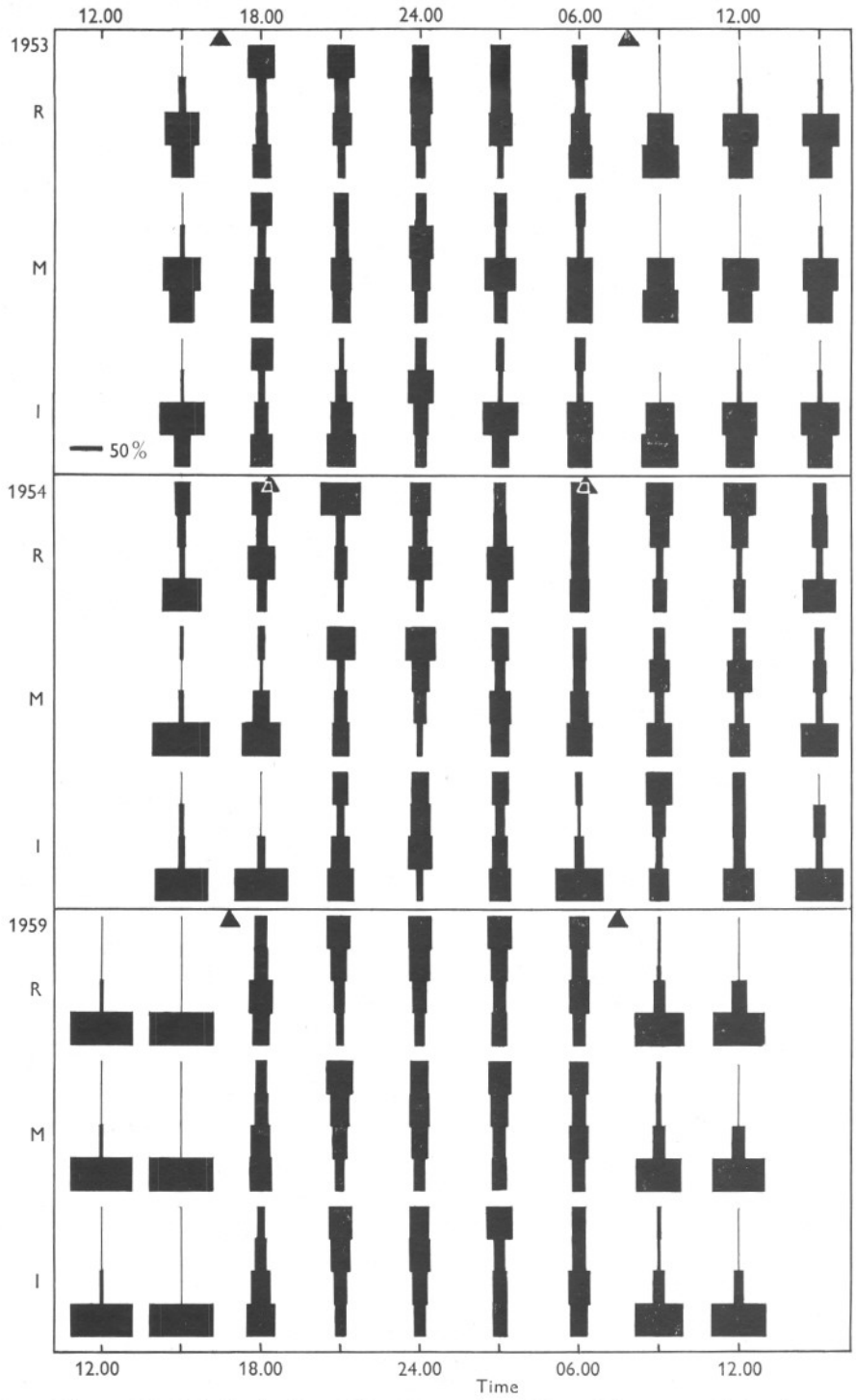

Fig. 4. Vertical distribution of ripe (R), medium (M), and immature (I) female *Calanus* at four depths.

the year. There was, however, a rise from the bottom from 18.00 to 24.00 h followed by a descent till 6.00 h and then the curious secondary rise at 9.00 and 12.00 h. At 15.00 h the distribution on the first day differed considerably from that on the second. The weather was hazy instead of clear

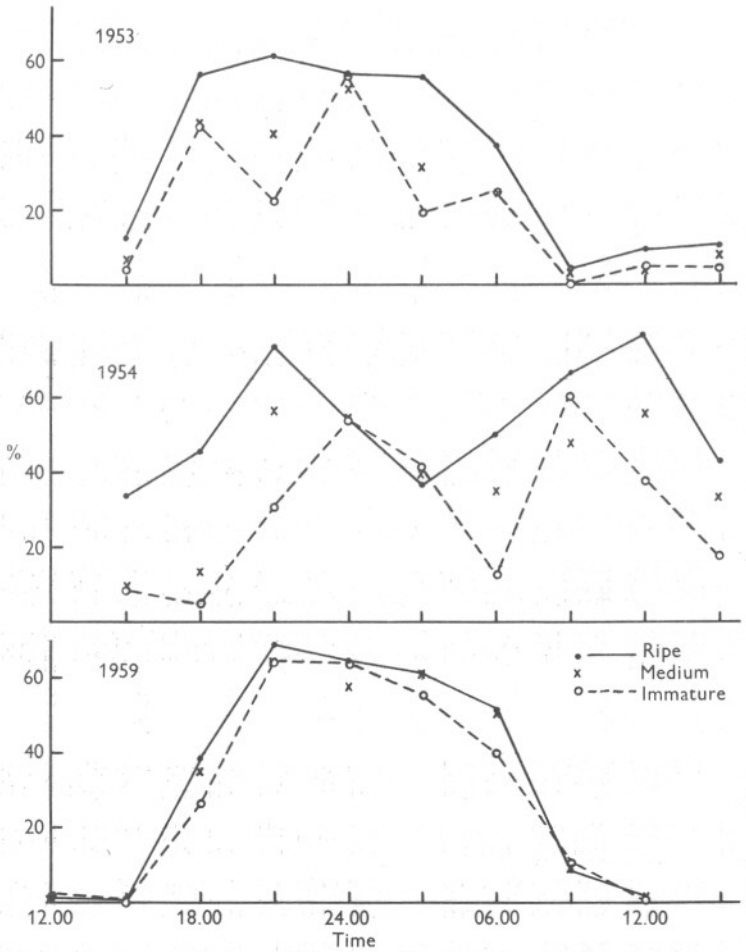

Fig. 5. Percentage of ripe and immature female *Calanus* in the two top nets.

sunshine. More of all stages were higher in the water on the second day and the males were actually richest in the second net.

Figs. 4 and 5 and Table 3 show the vertical distribution of the females when separated into three states of maturity—ripe, medium and immature. In 1953 and 1959 all resemble one another closely, but the immature show a slightly later and less marked rise and an earlier descent than the ripe. This is perhaps more clearly seen in Fig. 5, where the percentage of ripe and

TABLE 3. VERTICAL DISTRIBUTION OF RIPE, MEDIUM AND IMMATURE ♀ *CALANUS*

		2–3 February 1953						16–17 March 1954						9–10 February 1959					
		Ripe		Medium		Immature		Ripe		Medium		Immature		Ripe		Medium		Immature	
Haul	Time	No.	%	No.	%	No.	%	Time	No.	%	No.	%	No.	%	Time	No.	%	No.	%	No.	%
I. 1	15.00	9	0·9	23	0·9	6	0·4	15.00	522	21·9	31	6·6	4	1·1	12.00	5	0·3	5	0·4	3	0·3
2		114	11·7	160	6·0	59	3·6		282	11·9	10	2·1	28	7·6		9	0·5	12	0·9	12	1·4
3		524	53·9	1476	55·1	1143	69·9		166	7·0	17	3·6	30	8·1		57	3·0	40	3·0	25	2·9
4		322	33·1	1018	38·0	426	26·0		1408	59·2	411	87·6	308	83·2		1837	96·3	1277	95·7	823	95·3
II. 1	18.00	818	40·1	1459	32·1	1330	33·6	18.00	435	30·1	47	9·7	16	2·7	15.00	5	0·2	3	0·1	3	0·1
2		331	16·2	506	11·1	356	9·0		221	15·3	17	3·5	17	2·9		19	0·7	22	0·6	16	0·7
3		339	16·6	1108	24·4	905	22·9		600	41·5	122	25·1	66	11·2		7	0·2	7	0·2	7	0·3
4		552	27·1	1477	32·5	1363	34·5		189	13·1	300	61·7	489	83·2		2884	98·9	3734	99·2	2364	98·9
III. 1	21.00	145	6·9	773	21·6	628	39·4	21.00	1754	61·4	219	44·1	73	23·3	18.00	423	17·5	370	16·7	185	11·2
2		321	15·4	677	18·9	355	22·3		350	12·3	61	12·3	24	7·7		506	20·9	407	18·3	253	15·3
3		691	33·0	1144	31·9	432	27·1		531	18·6	90	18·1	86	27·4		862	35·6	680	30·6	498	30·2
4		932	44·6	989	27·6	179	11·2		221	7·7	127	25·6	131	41·7		627	25·9	765	34·4	715	43·3
IV. 1	24.00	181	23·9	254	16·3	159	15·7	24.00	805	32·0	400	45·6	45	25·0	21.00	945	39·5	782	38·7	653	33·4
2		250	33·1	568	36·5	412	40·8		565	22·5	234	26·6	53	29·4		702	29·4	524	25·9	603	30·9
3		218	28·8	433	27·8	248	24·6		883	35·2	166	18·9	66	36·7		482	20·1	442	21·9	370	18·9
4		107	14·2	301	19·3	191	18·9		259	10·3	78	8·9	16	8·9		262	11·0	272	13·5	329	16·8
V. 1	03.00	196	28·8	228	17·8	95	12·4	03.00	396	17·6	235	25·2	64	26·9	24.00	716	33·0	528	28·7	562	32·7
2		184	27·0	173	13·5	53	6·9		430	19·1	136	14·6	34	14·3		683	31·5	528	28·7	528	30·7
3		239	35·1	615	47·9	418	54·5		882	39·2	305	32·7	57	23·9		461	21·2	475	25·8	367	21·3
4		62	9·1	267	20·8	201	26·2		541	24·0	256	27·5	83	34·9		310	14·3	310	16·8	261	15·2
VI. 1	06.00	195	22·7	273	14·0	161	15·0	06.00	359	25·4	133	19·2	27	8·6	03.00	791	35·6	693	33·1	576	40·3
2		125	14·5	205	10·5	114	10·6		348	24·6	110	15·9	13	4·2		572	25·8	559	26·7	214	15·0
3		250	29·1	735	37·6	403	37·5		343	24·2	177	25·6	43	13·7		360	16·2	398	19·0	322	22·5
4		290	33·7	738	37·8	397	36·9		365	25·8	271	39·2	230	73·5		495	22·3	446	21·3	318	22·2
VII. 1	09.00	17	2·2	21	1·0	0	0·0	09.00	1126	39·4	168	16·5	100	40·7	06.00	557	28·4	625	29·0	317	22·0
2		19	2·5	44	2·2	2	0·2		784	27·4	320	31·4	48	19·5		452	23·1	483	22·4	260	18·1
3		296	38·8	867	42·4	477	44·2		298	10·4	143	14·0	25	10·2		570	29·1	607	28·1	474	33·0
4		430	56·4	1110	54·3	600	55·6		648	22·7	387	38·0	73	29·7		381	19·4	442	20·5	387	26·9
VIII. 1	12.00	45	3·0	63	2·2	10	0·8	12.00	1140	50·3	90	17·7	34	17·9	09.00	88	4·3	88	6·1	70	6·2
2		99	6·6	192	6·8	51	4·3		597	26·3	195	38·3	38	20·0		87	4·3	85	5·8	52	4·6
3		802	53·2	1565	58·6	608	51·4		152	6·7	61	12·0	29	15·2		354	17·3	256	17·6	179	16·0
4		562	37·3	994	35·3	512	43·4		379	16·7	162	31·9	89	48·6		1510	74·1	1025	70·5	820	73·2
IX. 1	15.00	62	4·1	49	1·8	7	0·7	15.00	326	19·9	109	13·2	2	1·2	12.00	5	0·4	3	0·2	5	0·4
2		98	6·5	176	6·5	38	3·8		382	23·2	166	20·2	29	16·7		13	0·9	5	0·4	15	1·2
3		836	55·5	1436	53·1	599	60·2		121	7·4	75	9·1	16	9·2		286	20·8	230	17·2	181	14·5
4		509	33·8	1044	38·6	351	35·3		811	49·4	473	57·4	127	73·0		1074	78·0	1101	82·2	1046	83·8

immature in the two top nets is shown. In general the medium state females are intermediate between ripe and immature.

In 1954 the immature were concentrated in the bottom net from 12.00 to 18.00 h and showed an irregular rise in the dark with a curious dip at 6.00 h. The ripe, on the other hand, tended to concentrate at the surface even in the daylight hours and to be more evenly distributed in the dark. As before the distribution of the medium state was intermediate.

Calanus finmarchicus is the dominant form in the Clyde sea area, but during the winter an appreciable proportion of the *helgolandicus* form may be present. However their numbers were too small for a study of their diurnal migration except in the spring of 1954. The females were then much more mature than those of the *finmarchicus* form, about 90% ripe as against 70%.

The pattern of vertical migration differed from that for the *finmarchicus* in that the tendency to concentrate in the upper nets during the day was more marked. During the night, on the other hand, they were deeper than the *finmarchicus*. When the females were separated into ripe, medium and immature, it was seen that, as with the *finmarchicus* form, the ripe tended to be higher in the water for most of the time. The immature were too scarce to give reliable results.

DISCUSSION

The state of the gonad can now be added to the other factors which influence vertical migration. It is most easily distinguished in females, and it is the female which might be expected to come to the surface at night. Apart from the necessity for feeding, eggs are at this time of year laid mainly between midnight and 2.00 h (Harding, Marshall & Orr, 1951). In the early spring months before the diatoms have begun to increase in numbers these factors may not be operative. Phytoplankton is scarce and evenly distributed and, although there are often some eggs laid in February, the majority seem to be laid in March or even April. However, the hauls taken in deep water and near the surface in 1953 (Fig. 1) show that the percentage of ripe females is higher near the surface even in February.

A surprising feature of the results is the difference in migration in different years. The most unexpected difference is the behaviour of the Stage V. During the winter they are normally found in deep water, and Nicholls (1933) found that they did not migrate. We, on the other hand, have found that in all three years they migrated almost as much as the adults. Another point of interest is that the *Calanus* were deeper in 1959 than in the two other years although their behaviour was similar in 1953 and 1959.

On many occasions in 1950 and 1951 Gauld examined catches from three different depths throughout 24 h in the Clyde sea area and he says (1953): 'that only on seven of the thirteen occasions on which hauls were taken, were

the catches of the top net distinctly greater in darkness than in daylight'. This also shows how variable vertical migration may be from year to year and perhaps even from month to month.

The tendency of the *Calanus* in 1954 to remain near the surface even at noon on the second day was surprising. It almost looks as though two populations were present, a surface living and a deep living. The first generation of the year, which does live mainly at the surface, has appeared as adults in these waters as early as the middle of March and it seemed possible that there was here a mixture of the overwintering and the first generation. Had two generations been present, however, the immature should have belonged to the later surface-living rather than to the overwintering one and actually the immature were found concentrated in the bottom net from 12.00 to 18.00 h. In addition, the clearest indication of the presence of the adults of the first generation came nearly a month later, on 16 April, when the proportion of males and of unripe females rose considerably, and 30% of the latter were carrying spermatophores. An examination too of nearly 6000 measurements of the lengths of the females in the vertical distribution hauls showed that the size curve was symmetrical with no suggestion of two modes as would have been expected had the two generations been mixed.

The chief difference between the 1954 observations and those for other years is that the station was worked a month later. In 1953 and 1959 the ratio of ripe:medium:immature was about 20:50:30 while in 1954 it was 70:20:10. It is possible that the behaviour of females may show a gradual change with advancing ripeness rather than an abrupt change with the generation. The anomalous results for the females might thus be explained by the fact that the majority were ripe and were staying near the surface to lay their eggs; in this case one would have expected the Stage V and males to have behaved normally. Their distributions, however, are also anomalous although less markedly so.

Banse (1959) has stressed the importance of temperature and salinity gradients for the vertical distribution of the zooplankton. It seems improbable that here the gradients in either temperature or salinity had a measurable effect on the migrations recorded. Water samples were taken at the stations and the vertical gradient in temperature in 1959 was less than 1° C (7·1–7·8° C) and in salinity less than one part per thousand (33·15–32·57‰). In 1959, however, the water was warmer than in the other two years (1953, 6·3–7·3° C; 1954, 6·2–6·9° C).

SUMMARY

A comparison of vertical distribution stations worked in several different years shows that the diurnal vertical migration varies considerably from one to another. The state of maturity of the females influences it in that ripe females migrate more and remain higher in the water than immature.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ALEXANDER, R. McN., 1959. The physical properties of the swimbladders of fish other than Cypriniformes. *J. exp. Biol.*, Vol. 36, pp. 347–55.

The properties of the swimbladders of seventeen species of teleost from the orders Clupeiformes, Anguilliformes, Gasterosteiformes, Gadiformes and Perciformes were examined. Determinations of sinking factor and swimbladder volume are reported. In no case could excess internal pressure be detected, and the new swimbladder wall was found to exercise no appreciable constraint on changes of buoyancy of the fish with depth. These fish are contrasted with the Cypriniformes, where excess internal pressure has been found in all species examined, and where changes of swimbladder volume and so of buoyancy with depth are reduced by the constraint of the swimbladder wall.

The marine species examined were studied at the Plymouth Laboratory. R. MCN. A.

COOPER, L. H. N., 1958. Océanographie expérimentale en Méditerranée. *Trav. Cent. Rech. Etud. océangr.*, T. 3, Fasc. 2, pp. 17–25.

The Ligurian and Balearic Seas provide a small-scale model for the study of the formation of bottom water in the North Atlantic. It is suggested that (*a*) nowhere in the deep ocean does truly vertical mixing between surface and bottom occur but that sinking occurs helically and waters may be re-cycled several times during the process; (*b*) the abyssal water of the Western Mediterranean is composite and derived firstly from water from the Eastern Mediterranean by way of the Sicily–Tunis and Capraia Channels at 300–400 m depth and secondly from water over the narrow shelf of the Cote d'Azur, Riviera di Ponente and coast of Tuscany cooled by the mistral and tramontana winds; (*c*) when the strong winds drop, the heavy water cascades over the edge of the shelf and at a depth of 300–400 m mixes with the water from the Capraia Channel to produce a very heavy water which sinks not vertically but along an inclined plane. Whilst sinking it may incorporate enveloping water, poor in oxygen, which may be recycled in the abyss.

L. H. N. C.

COOPER, L. H. N., 1959. Calm patch. *Yachting World*, Vol. 3, p. 250.

An explanation is offered of a calm patch about 30 miles long and a cable in width observed by a yachtsman in a rough sea off the coast of Portugal. The phenomena may have been a convergence over the continental edge between conflicting currents. In this an oil film of biological origin had become concentrated.

L. H. N. C.

DAY, ALAN A., 1959. The continental margin between Brittany and Ireland. *Deep-Sea Res.*, Vol. 5, pp. 249–65.

The continental slope between Brittany and Ireland is steep and cut by many canyons, with the exception of the central region which forms a broad smooth spur. Scarps up to 110 miles in length are associated with the continental margin, and these, together

with the steep areas of the slope proper, are considered very probably to have been developed by faulting. Cores and bottom samples from the area are shown mainly to represent Recent and Pleistocene deposits, with the exception of three which represent Tertiary and possibly Cretaceous strata.

New names applied to topographic features in the area are: Meriadzek Terrace, Goban Spur and Pendragon Scarp.

A. A. D.

HAWES, F. B., 1958. Preliminary observations on the settlement of the actinula larva of the *Tubularia* larynx (Ellis & Solander). *Ann. Mag. nat. Hist.*, Ser. 13, Vol. 1, pp. 147–55.

This paper records the results of an uncompleted study of the actinula. The latter are discharged by movements of the gonophore at differing stages of development. Attachment to a substrate is effected initially by nematocysts at the tips of the aboral tentacles. The nematocysts are frequently not fully developed when the actinula is discharged with the result that the free-living stage is prolonged.

Movements described by previous authors as walking, swimming and substrate testing appear to be parts of an inherent rhythmic cycle seen more clearly in the adult.

F. B. H.

DAY, A. A., HILL, M. N., LAUGHTON, A. S. & SWALLOW, J. C., 1956. Seismic prospecting in the western approaches of the English Channel. *Quart. J. Geol. Soc. London*, Vol. 112, p. 15.

On the basis of the measured seismic velocities, the layering found at 25 seismic stations in the area of the western approaches of the English Channel is divided into four classes which are respectively correlated with semi-consolidated Cretaceo-Tertiary sediments, the New Red system, the palaeozoic system, and a metamorphic basement. The last of these appears to form a long deep trough. The palaeozoic floor is depressed in a trough of somewhat variable depth, bounded on the north by an outcrop of the metamorphic basement which is probably a westward extension of the upthrust Lizard-Start metamorphic belt. Contour maps of the layering are produced.

A. A. D.

KAMPA, E. M., BODEN, B. P. & ABBOTT, B. C., 1959. Electrical response to illumination of the Euphausiid Crustacean eye. *Nature, Lond.*, Vol. 183, pp. 1820–1.

The electrical potentials developed in the eyes of three species of euphausiid crustaceans, *Meganyctiphanes norvegica*, *Euphausia pacifica* and *Nematoscelis difficilis*, during stimulation by light have been measured. Silver-silver chloride gauze served as the indifferent electrode. The recording microelectrode, which penetrated the eye, was made of tungsten wire. Signals from the microelectrode were amplified and recorded with an oscilloscope or an ink-writer. Intensity, duration and colour of the stimulating light flash were varied. Response to a 100 msec flash is rapid with a small *a*-wave lasting about 20 msec, a main response which reaches a peak in about 40 msec, and a final overshoot in the other direction. Over a defined range of light intensities, the height of the main response varies directly with the logarithm of the intensity. At the upper end of this range the signal reaches a limiting value of 200–300 μV. Flashes of longer duration induce response at both on and off. The euphausiid eye is most sensitive to blue-green light.

E. M. K.

WICKSTEAD, J. H. 1959. A predatory copepod. *J. Anim. Ecol.*, Vol. 28, pp. 69–72.

During analysis of some Indo-West-Pacific plankton it was noted that the copepod *Candacia bradyi* A. Scott very frequently had another animal grasped in its 1st maxillipedes. This animal was usually a chaetognath—*Sagitta enflata* Grassi. A detailed analysis was made of some samples and, after due consideration was given to the fact that it was preserved material which was being dealt with, the evidence is that this species of copepod is wholly carnivorous. Some selectivity is shown in its feeding, the prey normally being a chaetognath, particularly *S. enflata*. It is shown that the 1st maxillipedes are not suitable for filter feeding but do have all the essentials of a raptorial organ.

The suggestion is also made that *Tortanus gracilis* (Brady) is mainly, probably wholly, carnivorous, feeding mainly on other copepods.

J. H. W.

BOOK REVIEW

SEA SHELLS OF TROPICAL WEST AMERICA. MARINE MOLLUSKS FROM LOWER CALIFORNIA TO COLOMBIA

By A. MYRA KEEN

Stanford University Press. London: Oxford University Press. £5.

This book is the first attempt to catalogue and describe as a whole the marine shells of the Panamanian province, covering the west coast of Central America between the Gulf of California and Colombia. The marine fauna of this region is perhaps remarkable in showing greater resemblances to that of the Caribbean than to the Indo-Pacific regions, due to a continuity between the two areas through a natural Panama canal or seaway during former geological periods. The scope of this work lies between that of a monograph and a collector's handbook, but descriptions are included of all species found within the 100 fathom line, save the smaller gastropods which are cited only by genus. Dichotomous keys for identification are included where necessary, each species being briefly described, and in most instances illustrated. The reader will be gratified to find that the illustrations lie invariably on the same or facing page to that of the descriptions to which they apply. The illustrations vary in quality—there are some good colour plates, but a number of the black and white photographs, some necessarily reproductions of lithographs rather than actual specimens, are not as clear as they might have been.

This handbook will no doubt prove as valuable to the research worker as to the amateur conchologist, for the text, which is written as far as possible in non-technical language, loses nothing thereby in accuracy. Essentially a monograph of shells, one must not expect to find descriptions of the soft parts of the animals, and by the same token the section on cephalopods is restricted to a description of the paper nautiloids.

N.A.H.

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

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Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

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 the 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.

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