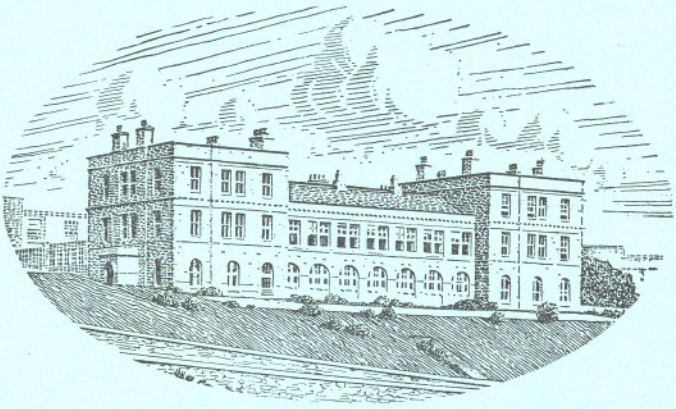
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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GREGARIOUSNESS AND SOME OTHER ASPECTS OF THE SETTING BEHAVIOUR OF *SPIRORBIS*

By E. W. Knight-Jones

Marine Biological Station, Bangor

(Text-figs. 1, 2)

CONTENTS

INTRODUCTION

Further study of the phenomenon of gregariousness during settlement, demonstrated in the oyster *Ostrea edulis* (Cole & Knight-Jones, 1949; Knight-Jones, 1951) and the barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950), seemed to require laboratory experiments, for which a viviparous animal with a brief, lecithotrophic, pelagic stage appeared to be needed.

In the Bangor area tube-worms of the genus *Spirorbis* are amongst the most abundant and easily obtained of such animals. They have proved to be excellent material for investigations on setting behaviour. This account deals mainly with *S. borealis*, the species which settles abundantly on *Fucus*, but some field observations and a single series of experiments on *Spirorbis pagenstecheri* are included.

Previous accounts of the behaviour of *Spirorbis* larvae are very incomplete and occasionally misleading. Shively (1897) observed attachment, but gave few details, Neu (1933) counted the numbers setting on plates of various colours, and Garbarini (1936*b*) made some observations which are referred to later (p. 214). Thorson (1946) gives further references.

This work was carried out in the Department of Zoology at the University College of North Wales, where Prof. F. W. Rogers Brambell, F.R.S., has

kindly given us rooms until our Station is built. I am very grateful to him and to his Staff for their hospitality and help. Dr H. A. Cole, of the Fisheries Experiment Station, Conway, encouraged me at an early stage with the information that his records of sessile organisms competing with oyster spat had given evidence of gregariousness in *Spirorbis*.

Breeding Periodicity and Liberation of Larvae

Fucus serratus covered with *Spirorbis borealis* was collected frequently between late May and early October from near Bangor Pier at about low-water mark. Breeding was in progress throughout this period. Adults on pieces of *Fucus* were put into dishes containing about 200 ml. of sea water, into which the larvae were liberated.

Breeding periodicity in *S. borealis* was first demonstrated by Garbarini (1933), who found that liberations of larvae at Concarneau and Roscoff occurred only during a few days at each quarter of the moon. Later (1936*a*) he found that growth of oocytes to ripeness lasted about 14 days, that the incubation period was of similar duration, and that breeding was synchronized so that at each quarter a batch of larvae was liberated, a batch of eggs was spawned and a batch of oocytes began to grow. Liberations of larvae at Bangor showed similar periodicity. During July and August mass liberations occurred regularly during the few days before and after the moon's quarters. Very few larvae were obtained in the intervening periods. During June (when the weather was hot) breeding was less regular and less well synchronized. Many tubes were opened at various times and practically all contained embryos, which tends to confirm that a fresh spawning follows closely upon liberation.

Synchronization of breeding appeared to be less perfect than in Garbarini's material, for larvae were rarely unobtainable, but it proved to be a waste of time to attempt experiments except during the week in which each liberation peak occurred.

No larvae appeared for the first few minutes after adults were put into a dish. It seemed that a short period was needed to recover from disturbance, even if the adults were over-ripe for liberation. In freshly collected material only occasional individuals liberated their larvae, even during peak periods, but in material which had been in the laboratory for a few hours mass liberations of larvae could be obtained by (i) exposing to light dishes which had been kept for a few hours in the dark, (ii) changing the water of dishes, whether in the light or in the dark, (iii) immersing adults which had been kept out of water for a few hours or overnight. Such changes were various in their effects. The oxygen tension and pH were usually raised, but when the change was to fresh sea water from sea water which had contained brightly illuminated *Fucus*, both were lowered. Probably over-ripe adults will react by liberation to any improvement in their surroundings. Liberation appeared to be

a voluntary act on the part of the adults because it was accompanied by marked protrusion of the body and by rocking movements, and because the larvae appeared helpless when they first emerged from the parent tube. They often remained motionless for about a quarter of a minute, entangled in the parent's tentacles. Then they gradually freed themselves and swam away towards the light. Usually twenty or thirty larvae, probably the whole brood, were liberated at the same time by each adult. Occasionally only two or three larvae made their appearance in a dish, suggesting that a few may be liberated before or after the majority of the brood.

The Pelagic and Searching Phases

After liberation the larvae swam straight towards the light, each following in the path of the one in front. Swimming was relatively rapid, about 3 mm./sec. (the larvae being about 0·4 mm. long). It was largely or entirely effected by the large cilia of the prototroch. These showed metachronal waves which moved anticlockwise, seen from the apical end. The body rotated in the opposite direction.

The types of behaviour shown by larvae are summarized diagrammatically in Fig. 1. On reaching the lighted side of the dish the larvae swam backwards and forwards against the glass, usually near the surface of the water. They generally kept a straight course except when they hit an obstruction or found themselves going away from the light, when they turned and swam towards the light again, orientating directly towards it if free to do so. The length of this purely pelagic phase varied in different broods, but was usually between 15 min. and 2 hr.

After this period larvae frequently left the lighted side of the dish. Their swimming appeared rather random, with a photo-negative tendency. They generally swam straight, but if their course took them near a piece of *Fucus* they often turned and swam towards it as though able to perceive its dark surface. When kept for some hours in a clean glass vessel containing nothing suitable for attachment the majority collected near the bottom on the side remote from the light.

On making contact with a suitable surface a larva started to crawl on its ventral side at a speed of about 1 mm./sec., keeping a straight course unless it met an obstruction. On reaching the edge of a *Fucus* frond it generally crawled round on to the other side. Such a period of crawling rarely lasted more than a few minutes, and was sometimes very brief. The larva then swam off again. It often became positive to light for a further short period, before seeking another surface to crawl upon. Brief periods of swimming and crawling followed one another thus for 1 or 2 hr. During this time the larvae explored the dish thoroughly. They were often seen to encounter previously settled individuals, turn, crawl onwards, and eventually swim off again. They seemed to hurry

about, with their entire output of energy given up to visiting as many different surfaces as possible.

The pelagic and searching phases were often much shorter in larvae liberated by parents which had been kept in crowded dishes or out of water for 1 or 2 days. Such larvae sometimes settled immediately they reached the glass side of the dish, if this bore a bacterial film. This behaviour appeared to be the result of long overdue liberation.

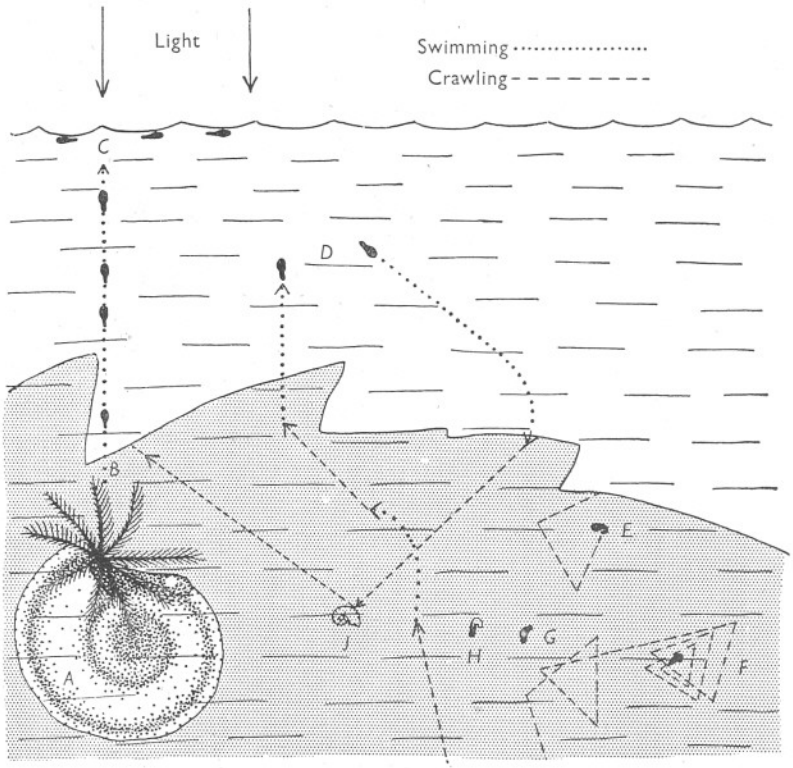

Fig. 1. Successive phases in behaviour of larvae of *Spirorbis borealis* illustrated diagrammatically. Stippled area represents frond of *Fucus serratus*. *A*, incubation within parent's tube; *B*, liberation and positive phototaxis; *C*, pelagic phase at surface; *D*, searching phase, with larvae swimming at random, or alternately negative and positive to light, interspersed with periods of crawling in straight lines; *E* and *F*, larvae about to settle and just settled, to show frequent changes of course during crawling; *G* and *H*, metamorphosis and formation of calcareous tube; *J*, individual 2 weeks after settlement.

Settlement

A larva which was about to settle was recognizable by the fact that it frequently changed direction whilst crawling. During the straight crawling of the searching phase the narrow, tail-like abdomen remained projecting

longitudinally, or occasionally twitched from side to side. When about to settle the posterior end of the abdomen was frequently pressed against the substratum, to which it appeared to adhere, and the abdomen was strongly flexed so that the trunk turned sideways, usually through an angle of about 120°. The larva crawled a short distance in the new direction before again using its abdomen to steer itself through another change of course.

A larva which had begun to change direction rarely swam off again, but often continued to crawl for many minutes and sometimes covered a few centimetres in its wanderings. Eventually it began to cross backwards and forwards many times over the area within 2 or 3 mm. radius of the spot upon which it was finally to settle. Crawling became gradually slower and turning more frequent until progress ceased and attachment took place. Very similar behaviour is shown by oyster larvae which are preparing to settle (Prytherch, 1934; Cole & Knight-Jones, 1939).

In the free-swimming larva of *Spirorbis borealis* there is a superficial white spot in the dorsal mid-line at the posterior end of the thorax, sufficiently large to be seen easily with a hand-lens. Under a low-power microscope this was seen to alter in shape when the body was flexed violently, as though it was a sac full of fluid. It may conveniently be termed the attachment gland from the part it plays during setting.

Immediately before setting movement in each direction was no more than about a millimetre before a flexure of the abdomen turned the body round. As crawling ceased the abdomen continued to wave from side to side and the whole body wriggled and alternately stretched and contracted, the muscles apparently taut and very active, but the movements slight. After a few seconds a cloud of milky liquid issued from the attachment gland, apparently through a narrow pore at its posterior end. The gland rapidly emptied and the fluid spread over the surface of the trunk and abdomen, losing its opacity during dispersion, and apparently forming the initial tube. The body continued to wriggle and rock about, as though to aid the dispersion of the fluid and to ensure adhesion to the substratum. Anteriorly, the folds of the collar, which had been pressed inconspicuously against the sides of the trunk in the free-swimming larva, became erected almost perpendicularly (see figures in Fewkes, 1885) and then bent backwards again. They appeared to be beginning to enclose and mould the anterior lip of the tube which was being formed. This is probably their function, for glands secreting the calcareous tube have been demonstrated under the collar of serpulids (Swan, 1950). Posteriorly, the body became shorter and broader, so that the previously abrupt demarcation between the broad trunk and narrow abdomen disappeared. The worm, with unceasing small movements, rolled first one way and then the other, and finally completely over ventral side upwards. The large, ventro-lateral eyes, which had been next to the substratum in the crawling larva, became very conspicuous as they faced upwards. The rudiment of the operculum, which

was difficult to distinguish in the larva, now appeared on the left side of the head and grew with almost visible speed, whilst the head shrank so that the eyes, formerly widely separated, approached one another closely.

Fixation, shortening of the abdomen, and rolling ventral side upwards took place within 1 or 2 min. after crawling had ceased. Very striking growth of the operculum at the expense of the larval head took a further 5 or 10 min. The change was sufficiently rapid to be termed a cataclysmic metamorphosis. The trunk and abdomen were then enclosed in an almost transparent, straight tube, which, being only about 0·25 mm. long, could not accommodate the anterior end. This initial length of tube, derived wholly or in part from the secretion of the attachment gland, remained straight and semi-transparent, but a white, opaque, calcareous extension was added to it anteriorly. This gradually elongated and immediately started to bend to the occupant's left as the inner whorl of the future tube. About 5 hr. after settlement growth at the mouth of the tube had carried its axis through 90°. The occupant then had a large operculum and rudimentary tentacles, which it could withdraw completely into the tube.

Need for Bacterial or Algal Filming of the Substratum

Surfaces immersed in sea water become covered with a film of micro-organisms after periods varying from a few hours to a few days (see ZoBell, 1946). There is evidence that larvae of a variety of sessile animals settle more readily on filmed surfaces than on clean surfaces (ZoBell & Allen, 1935; ZoBell, 1938, Scheer, 1945; Miller, Rapean & Whedon, 1948; Pyefinch & Downing, 1949; Cole & Knight-Jones, 1949).

Although previous accounts had described *S. borealis* as setting on glass, it was soon found that larvae in clean glass beakers metamorphosed only after a considerable delay. A series of experiments was carried out in which batches of larvae were divided between pairs of 50 ml. beakers. Each pair had contained sea water, with or without the addition of an algal culture (*Chlamydomonas* or *Synechococcus*) during the previous few days. One beaker of each was wiped clean with cotton-wool before use, the other was left with a visible film, which always included bacteria and was often coloured green with algal cells. The *Spirorbis* were examined after they had been in the beakers 24 hr. (Table I). In the aggregate 91 % had metamorphosed in the filmed beakers, only 15 % in the beakers which had been wiped. The results were consistent throughout with films of various origins.

A high proportion of larvae kept in clean beakers eventually became abnormal, showing some of the changes of metamorphosis, but a few retained the larval form for 2 or 3 days. The majority of these aged larvae lay motionless but some swam when disturbed. Five such larvae were placed, 48 hr. after liberation, in a flask which had contained a culture of the alga *Prasinocladus*

and which bore a green film on the glass inside. After a further 24 hr. two of the five had metamorphosed and secreted tubes, showing that a greatly prolonged planktonic life may sometimes be followed by successful metamorphosis.

It was then found that the great majority of larvae would seek out and settle upon a filmed cover-slip in a clean beaker, and would not settle upon a clean cover-slip in a filmed beaker. Larvae encountering a filmed glass surface behaved differently from those encountering a clean one. On striking clean glass larvae either swam off again with no more than momentary hesitation,

TABLE I. EARLIER METAMORPHOSIS OF *SPIRORBIS BOREALIS* IN FILMED BEAKERS THAN IN CLEAN BEAKERS

(In each experiment a batch of larvae was divided between a pair of 50 ml. beakers, one with the inside surface filmed, the other wiped clean. After 24 hr. the numbers metamorphosed and unmetamorphosed were counted. In all, 91 % had metamorphosed in the filmed beakers, 15 % in the clean ones.)

Exp. no.	Origin of film	Nos. metamorphosed/unmetamorphosed in Filmed beakers	Clean beakers
1	Beakers had contained sea water, to which a culture of *Chlamydomonas* had occasionally been added, for 14 days	14/4	0/20
2		16/2	4/15
3		16/1	0/16
4		21/5	1/21
5		11/0	3/7
6		30/0	6/24
7	A culture of *Synechococcus* for 4 days	12/1	0/17
8	Sea water in dim light for 14 days	21/0	9/10
	Totals	141/13	23/130

or swam over the surface with scarcely diminished speed. On striking a filmed surface they stuck to the film as soon as they touched it and then started crawling upon it at less than half the swimming speed. They appeared to have some difficulty in leaving a filmed surface. A larva about to swim away was often seen with its anterior end clear, its prototroch vigorously active, and its posterior end apparently attached to the surface by an invisible, gradually lengthening thread. Suddenly the larva would accelerate to normal swimming speed.

It seemed possible that clean glass might be too smooth for the larvae to crawl upon, so paired pieces of slate and granite, carefully matched, were selected as offering surfaces which were mechanically rough. One of each pair was immersed for 48 hr. in an algal culture (*Synechococcus* or *Prasinocladus*), the other was left dry. Then each pair was exposed to larvae in a 250 ml. dish. Many larvae encountered the freshly immersed stones, but none were seen to crawl upon them. Larvae that encountered the other stones, which were tinted green from the cultures in which they had been lying, immediately started to crawl. Eighty larvae settled on these stones, only two on the freshly immersed ones. (Table II).

It seems highly probable that *Spirorbis* settles on filmed surfaces rather than clean surfaces because it can crawl on them more easily, and that crawling is due to cilia sticking to bacterial films or algal surfaces. There is practically no interval between contact with a filmed surface and adhesion to it, so there is scarcely time for deliberate choice, but mechanical roughness is unlikely to be one of the factors involved.

TABLE II. LARVAE OF *SPIRORBIS BOREALIS* SOUGHT OUT FILMED SURFACES IN PREFERENCE TO FRESHLY IMMERSED ONES

(In each experiment larvae in a clean 250 ml. dish were presented with a pair of stones, one of which had been immersed in an algal culture for 48 hr., the other left dry for the same period immediately prior to the experiment.)

Exp. no.	Stone	Culture	Nos. settled on stones which were: Filmed	Freshly immersed
1	Granite	*Synechococcus*	36	0
2	Granite	*Prasinocladus*	18	0
3	Granite	*Prasinocladus*	4	2
4	Slate	*Synechococcus*	22	0
Totals			80	2

EFFECT OF PREVIOUSLY SETTLED *SPIRORBIS* ON CHOICE OF SUBSTRATUM

Fucus was found to be suitable for settlement whether its surface had been wiped or not. When larvae were put into thoroughly wiped 250 ml. bowls with pieces of *F. serratus*, all the larvae settled on the *Fucus*, unless the bowls were placed in the dark, when many settled on the surface film. Similar behaviour during darkness has been recorded in *Bugula* (Grave, 1930; Lynch, 1949*a*). To avoid it most experiments were carried out in the light.

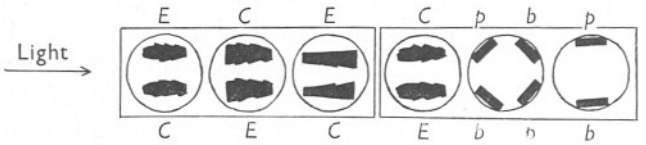

Fig. 2. In laboratory tests for gregariousness similar pieces of *Fucus*, some (*E*) bearing previously settled *Spirorbis borealis*, others (*C*) bare, were exposed side by side to larvae in 250 ml. dishes. These were contained in boxes which were white inside, both sides being equally illuminated. The diagram shows two boxes, each with three dishes. Results are given in Table III. Two dishes are shown with slate blocks, some bearing *S. borealis* (*b*), others *S. pagenstecheri* (*p*), which were used to test whether larvae could distinguish between the two species. Double pairs of blocks were used in Exps. 1–10 (see Table V), single pairs in Exps. 11–20.

As the larvae were photosensitive the dishes were placed in narrow boxes, white inside, the long axes of which were directed towards a window so that both sides appeared equally illuminated. For each experiment two similar pieces of *Fucus* were obtained from immediately above a dichotomy and placed on opposite sides of a dish, one bearing previously settled *Spirorbis*, the other

a bare control. Experimental and control pieces were placed on alternate sides in adjacent dishes to guard against there being an undetected difference in

TABLE III. SETTLEMENT OF *SPIRORBIS BOREALIS* ON TWO SEPARATE SIMILAR PIECES OF *FUCUS*, ONE WITH PREVIOUSLY SETTLED *SPIRORBIS*, THE OTHER BARE

(In every experiment, except those marked with asterisks (in which the larvae showed little discrimination or appeared to have been influenced by light), at least twice as many settled amongst the previously settled *Spirorbis* as on the bare *Fucus*.)

Exp. no.	No. of previously settled *Spirorbis*	No. of larvae settling on	
		Experimental *Fucus*	Bare control
1	20	18	1
2	10	14	0
3	4	4	1
4	16	31	2
5	25	22	2
6	15	50	5
7	10	25	3
8	9	52	2
9	10	6	9*
10	10	44	0
11	8	51	1
12	12	12	16*
13	20	11	2
14	19	17	8
15	19	22	0
16	12	29	2
17	43	25	0
18	16	44	4
19	70	27	2
20	49	34	23*
21	84	7	0
22	46	15	7
23	29	4	2
24	31	13	1
25	35	43	11
26	22	24	59*
27	35	85	2
28	15	7	1
29	143	61	27
30	151	64	25
31	123	113	23
32	115	83	25
33	102	74	38*
34	111	112	49
35	46	29	5
36	56	10	21*
37	28	48	7
38	47	15	23*
	Totals	1345	409

illumination between the two sides (Table III). Freshly liberated larvae were then pipetted into the dishes in varying numbers.

During this series of experiments the following conditions were varied, but the changes did not affect the results, which are given in Table III. At first,

previously settled *Spirorbis* were removed from the controls. This often involved damage to the *Fucus* so similar damage was done to the surface of the other piece of each pair, either by removing tubes or by picking pieces away with the point of a needle. A few tests with bare pieces of *Fucus* indicated that damage discouraged settlement, so some experiments (nos. 8–11) were carried out in which the experimental pieces were more damaged than the controls. Later (nos. 17–24 and 29–34) the *Fucus* used was entirely undamaged, apart from the scissor cuts by which the pieces were obtained, and *Spirorbis* was allowed to settle on the experimental pieces shortly before the experiments, whilst the controls remained in neighbouring vessels which contained no larvae. In some experiments (nos. 12–16 and 29–34) the water in the dishes was kept circulating by air bubbles, in the remainder it was left unstirred.

In thirty-one out of the thirty-eight experiments the results were consistent, with a ratio of at least 2:1 in favour of the *Fucus* which bore previously settled *Spirorbis*. It is probable that some of the remaining seven experiments involved larvae which were in poor condition, for they were from adults which had been kept for 1 or 2 days in the laboratory. In the three experiments of these (nos. 26, 36 and 38) which showed a clear majority settling on the controls, it appeared that the dishes had not been equally illuminated, and that the larvae had been collecting towards their darker sides.

Altogether 1345 larvae settled on the *Fucus* which bore previously settled *Spirorbis*, 409 on the bare control pieces. In the earlier experiments (nos. 1–24) when the numbers of larvae in each dish varied between 5 and 57, over six times as many settled on the experimental pieces as on the controls. In the later ones, in most of which between 50 and 100 larvae were used, the ratio was only about 2·5:1. This recalls results obtained with *Ostrea edulis* (Cole & Knight-Jones, 1949), which showed that gregariousness tended to be masked when settlement was heavy, probably because a few less discriminating larvae settled early upon the control surfaces, thereby encouraging further settlement upon them.

Prolongation of the Searching Phase in Isolated Individuals

The movements of larvae did not appear to be directed towards previously settled individuals, so it seemed probable that gregariousness might be accounted for by random swimming with settlement taking place more readily on *Fucus* which already bore *Spirorbis* than on bare *Fucus*. The possibility that metamorphosis might be delayed in isolated larvae was therefore investigated.

In each of three preliminary experiments 10 larvae were isolated in 50 ml. beakers, and 20 were put together into another similar beaker. To ensure, as far as possible, that the isolated and crowded larvae in each batch were comparable genetically, they were pipetted from a small liberation and put out

alternately, one into isolation and two into the crowded beaker. All thirty may have come from the same parent. A piece of *Fucus* which bore five previously settled individuals was presented to the crowded larvae, pieces of bare *Fucus* to the isolated ones. The beakers were left under similar conditions of illumination and examined at intervals of about 20 min. When it was seen that

TABLE IV. *SPIRORBIS BOREALIS*. PERCENTAGES METAMORPHOSED IN EXPERIMENTAL BATCHES OF LARVAE WHICH WERE CROWDED AND/OR WITH *FUCUS* BEARING PREVIOUSLY SETTLED INDIVIDUALS AND IN CONTROL BATCHES OF LARVAE WHICH WERE ISOLATED WITH BARE *FUCUS*

(Differences of less than 20 % are marked by asterisks. The other, more considerable, differences indicate consistently earlier metamorphosis in the experimental batches.)

Exp. no.	Period (hr.)	No. of crowded larvae	Percentages metamorphosed: Experimental	Control
	Crowded with previously settled *Spirorbis*			Isolated
1	3	20	90	60
2	1	20	95	70
3	1	20	85	40
		Mean	90	57
	Crowded with initially bare *Fucus*			Isolated
1	2½	25	60	50*
2	2	20	90	50
3	2	50	100	33
4	1½	100	95	75
5	1½	100	85	80*
6	1½	45	98	90*
7	1½	50	30	30*
8	2	50	90	44
9	2	48	87	60
10	6	49	58	20
		Mean	79	53
	Single larvae with previously settled *Spirorbis*			Isolated
1	2	—	44	10
2	1	—	78	70*
3	1	—	89	20
4	1	—	90	60
5	1	—	100	70
6	1	—	50	60*
7	3	—	100	60
8	3	—	70	30
9	3	—	80	56
10	1½	—	56	50*
		Mean	76	49

most of the larvae in the crowded beaker had settled, the numbers which had metamorphosed and which were still moving were recorded, first in the crowded beaker and then in the other beakers. Because they were examined later the isolated larvae had a few minutes longer in which to metamorphose than the others. Nevertheless, it was found in each experiment that 85–95 %

of the crowded larvae had metamorphosed, but only 40–70 % of the isolated ones (Table IV).

Using similar methods, a series of ten experiments was then carried out, in which the crowded larvae, between 20 and 100 in number, were presented with bare *Fucus*. In the aggregate 79 % of the crowded larvae metamorphosed, but only 51 out of the 96 isolated ones (the 4 remaining isolated larvae could not be found, having probably adhered to the inside of the pipette). Consistently in six out of the ten experiments 20–67 % more of the crowded larvae metamorphosed than of the others. In the remaining four experiments the differences were 10 % or less. This does not prove that the presence of unmetamorphosed larvae promotes settlement. The earlier settlement in the crowded beakers may possibly have been due to a few less discriminating larvae attaching themselves and metamorphosing, thus encouraging further settlement.

In each of another series of ten experiments twenty isolated larvae were divided into two batches, one of which was presented with pieces of *Fucus* bearing from 5 to 10 previously settled *Spirorbis*, the other with similar bare pieces. A total of 73 out of 96 larvae in the former batch metamorphosed, 48 out of 99 in the latter (the remaining larvae were lost). In seven out of the ten experiments 25–70 % more larvae metamorphosed in the former than in the latter. In the remaining three experiments the differences were 10 % or less.

The length of the period of larval life appeared to be rather similar in larvae from the same parent, but it varied widely in different batches. As Table IV shows, 100 % of one batch metamorphosed within an hour, but only 60 % of another in 6 hr. The results show that the searching phase was prolonged by isolation, but do not show by how much it was prolonged. As a mere conjecture, the prolongation often appeared to be about an hour. This is probably sufficient to account for the gregariousness shown in the laboratory experiments.

Ability of Larvae to Distinguish their own Species from an Allied Species

S. pagenstecheri is abundant on stones and shells in the lower half of the tidal zone on most shores near Bangor. Its tube coils so that the mouth faces anticlockwise from an observer's viewpoint, whereas that of *S. borealis* is clockwise. Its larvae are colourless (apart from their red eyes) and very small, whereas those of *S. borealis* are much larger and reddish brown in colour.

S. borealis appeared to be rather more common on stones than *S. pagenstecheri* was on *Fucus*, so smooth slate was chosen as providing an experimental surface suitable for both species. Eight similar blocks measuring 24 × 24 × 4 mm. were prepared. These were immersed in sea water for a day, to allow a bacterial film to develop, and were then divided into two batches, with each block in

a separate, freshly wiped beaker. Larvae of *Spirorbis borealis* were added to one batch, and larvae of *S. pagenstecheri* to the other. These soon settled on the blocks. The blocks were then exposed in pairs (Fig. 2), one of each pair bearing *S. borealis*, the other *S. pagenstecheri*, in 250 ml. dishes to which larvae of both species were added. After 24 hr. the numbers of both species which had settled on each block were recorded. The few *S. borealis* which had settled on the *S. pagenstecheri* blocks were then removed, whilst the *S. pagenstecheri* were removed from the other blocks. This was done with the point of

TABLE V. *SPIRORBIS* LARVAE SETTLED AMONGST THEIR OWN SPECIES IN PREFERENCE TO AN ALLIED SPECIES

(In a series of experiments larvae of *S. borealis* and *S. pagenstecheri* were put together in a 250 ml. dish containing paired blocks of smooth slate, one of each pair bearing previously settled *S. borealis*, the other *S. pagenstecheri*. Inconsistent results are marked by asterisks.)

	Nos. of previously settled		Nos. of larvae of: *S. borealis* setting on blocks bearing		*S. pagenstecheri* setting on blocks bearing	
Exp. no.	*S. borealis*	*S. pagenstecheri*	*S. borealis*	*S. pagenstecheri*	*S. pagenstecheri*	*S. borealis*
1	18	58	20	6	26	10
2	34	65	6	3	29	14
3	40	90	21	3	43	5
4	58	132	30	10	16	7
5	92	70	12	7	7	5*
6	4	24	38	14	24	29*
7	32	35	56	6	40	13
8	83	71	15	4	3	14*
9	93	72	18	33*	—	—
10	30	49	1	0	16	0
			Blocks changed round			
11	4	7	9	0	7	1
12	13	14	12	0	7	1
13	25	21	17	1	—	—
14	5	12	8	1	7	1
15	13	19	5	2	12	1
16	12	4	7	0	9	1
17	19	13	9	0	17	3
18	5	1	6	0	1	0
19	11	2	6	3	10	3
20	14	12	11	1	8	0
	Totals		307	94	282	108

a needle to avoid disturbing the bacterial films, which were probably similar on all the blocks since they had all been exposed under similar conditions for the same period. The experiments were then repeated.

After ten experiments the results seemed conclusive (Table V). In the aggregate, and in seven or eight out of ten separate experiments, more than twice as many larvae of each species settled on the blocks which bore their own species than on the other blocks. To guard against the possibility of there being some intrinsic difference between the blocks of each pair, the blocks were all wiped

clean and changed round, so that those which had previously borne *S. borealis* bore *S. pagenstecheri* and vice versa. Another similar series of ten experiments was then carried out, using small numbers of larvae and taking care to obtain these only from freshly collected adults. The results which followed were still more conclusive. About 90 % of the larvae of each species sought out their own species in preference to the other.

It is scarcely conceivable that the larvae could have distinguished mechanically between their own species and the allied one. The tubes of the previously settled individuals grew quickly, so that the size ranges of the two species overlapped one another considerably. Those searching larvae which were seen to encounter tubes never paused to explore their shape or size, but always moved away immediately. It seems highly probable that some chemical sense played the main part in recognition of their own species.

Further Aspects of the Behaviour during Settlement

Garbarini (1936*b*) carried out experiments on larvae from *S. borealis*, which he kept in an aquarium on a clump of *Fucus*. With circulating sea water he was able to keep them thus in good condition for more than 2 months. During the initial photopositive phase larvae attached themselves to every object which they could not go round, whatever its nature. Later they appeared to be attracted or repelled by neighbouring objects. Then, if a larva passed near *Fucus*, it went straight to the surface, crawled upon it and attached itself. Few larvae settled on *Laminaria* and still fewer on *Himanthalia*, *Ascophyllum*, *Rhodymenia* and stones and other inanimate objects. None attached to ascidians. *Botryllus* seemed repellant. He visualized the contrasting behaviour towards *Fucus* and *Botryllus* as due to substances secreted into the water by these organisms.

Similarly rapid settlement, without a searching phase, was observed at Bangor in larvae liberated by adults which had been kept in the laboratory for more than 1 or 2 days (p. 204). The apparent attraction towards *Fucus* may be a response of photonegative larvae towards the dark fronds (p. 203). A few experiments tended to confirm that *Fucus* is much more favourable for settlement than *Ascophyllum*. It has already been shown that freshly immersed inanimate surfaces are very unfavourable (p. 207).

Fucus is not overwhelmingly favourable for settlement. When larvae were put into a dish, the glass of which bore a bacterial film and some previously settled *Spirorbis* on its lighted side, they all settled on the glass amongst the *Spirorbis* though there was *Fucus* in the dish. Settlement on glass cover-slips bearing a bacterial film and 10 to 50 *Spirorbis*, which had settled a short time previously, was compared with settlement on a bare piece of *Fucus* of similar size. In a single experiment in the light 25 larvae settled on the *Fucus*, 5 on the cover-slip. It seemed likely that the searching larvae found the dark

surface of the *Fucus* visually during their repeated reconnaissances, so four other experiments were carried out in the dark. In these 76 larvae settled on the cover-slip, none on the *Fucus*. Light and photosynthesis are complicating factors, but in the dark gregariousness was clearly stronger than a possible predilection for *Fucus*.

Fewkes (1885) noted that *Spirorbis* larvae in aquaria sometimes settled, metamorphosed and floated on the surface film 'until the increasing specific gravity of their bodies sinks them to their future homes', but it is very unlikely that re-attachment to another surface can occur after metamorphosis. At Bangor settlement on the surface film took place particularly readily in the dark and when there was a bacterial scum there.

TABLE VI. PROVIDED THE SURFACE WAS NOT CROWDED (< 10/CM.^{2}) MORE LARVAE OF *SPIRORBIS BOREALIS* SETTLED ON *FUCUS* WHICH BORE MANY *SPIRORBIS* THAN ON SIMILAR PIECES WHICH BORE FEWER

(In each experiment larvae were presented with two similar pieces of *Fucus* of surface area 4–8 cm.^{2}, one bearing many more recently settled *Spirorbis* than the other.)

	Fucus with many *Spirorbis*		*Fucus* with few *Spirorbis*	
Exp. no.	(Nos. previously settled)	Larvae setting	(Nos. previously settled)	Larvae setting
1	(80)	49	(20)	29
2	(83)	11	(18)	2
3	(54)	15	(21)	1
4	(120)	16	(18)	0
5	(32)	13	(7)	3
6	(26)	14	(5)	12
7	(120)	8	(10)	0
8	(38)	14	(5)	1
Totals		140		48

Settlement took place very frequently in concavities, such as the slight grooves alongside the midrib of *Fucus* or the angle at the periphery of a beaker's base. This was probably a response to contact stimuli. It could not be sheltering from currents or light when it occurred in still water and with a transparent substratum.

In eight experiments (Table VI) larvae attached in far greater numbers to *Fucus* which bore many recently settled *Spirorbis* (5–10/cm.^{2}) than to pieces which bore fewer (1–2/cm.^{2}), but the crawling larvae left areas which were crowded with 10–20 recently settled *Spirorbis* per cm.^{2} and spaced themselves out very evenly on adjoining less crowded areas. In a further five experiments (Table VII), pieces bearing 1–4/cm.^{2} proved to be more favourable for settlement than similar pieces crowded with 20–50/cm.^{2}. Apparently the gregarious tendency operates until it has led to a crowding of about 10/cm.^{2}, but larvae arriving thereafter try to find less crowded areas nearby.

This spacing out tendency is not due to physical lack of space because larvae proved to be capable of settling at over 40/cm.^{2} when they were given

no other choice. It must involve discrimination, which is probably exercised during crawling. The behaviour immediately prior to settlement is well suited to ensuring, as far as possible, that there is a clear area of 1 or 2 mm. radius round the place of attachment (p. 205, and Fig. 1).

A spacing-out tendency on very crowded surfaces has also been recorded in the barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950).

TABLE VII. MORE LARVAE SETTLED ON *FUCUS* WHICH BORE FEW *SPIRORBIS* THAN ON SIMILAR SMALL PIECES WHICH WERE VERY CROWDED (>20/CM.^{2})

(In each experiment larvae were presented with two similar pieces of *Fucus* of surface area 1–2 cm.^{2}, one with few or no recently settled *Spirorbis*, the other crowded to an artificially high degree (the previously settling larvae having been given no other surface for attachment).)

	Fucus with crowded *Spirorbis*		*Fucus* with few *Spirorbis*	
Exp. no.	(Nos. previously settled)	Larvae setting	(Nos. previously settled)	Larvae setting
1	(53)	10	(4)	37
2	(62)	9	(2)	16
3	(59)	11	(3)	11
4	(50)	14	(0)	20
5	(63)	11	(5)	30
Totals		55		114

SOME FIELD OBSERVATIONS

Sample patches of *Fucus serratus* at different levels on the shore were examined during one of the periods in which *Spirorbis borealis* was liberating larvae. Recently settled individuals were numerous about low-water mark, particularly on the new growth bordering on old stems which were thickly covered with old *Spirorbis*. Very few were found on the abundant *Fucus* at about half-tide, which bore no older stages of *Spirorbis*, though a few laboratory experiments had indicated that *Fucus* collected from above half-tide was as favourable for settlement as some from low-water mark. One may speculate that liberations occurred during the ebb tide or at low water, so that larvae, settling within a few hours, got little opportunity of reaching the higher levels of the shore, but so far there is no evidence for such behaviour. It is probable that the heavy settlement at low-water mark, where adults were numerous, was largely due to the gregarious tendency, and that this usually prevents large numbers of larvae from settling at an unfavourably high level on the beach.

Very few *Spirorbis* were found on *Ascophyllum*, even in areas where this was intermingled with *Fucus* bearing abundant *Spirorbis* (see p. 214).

S. pagenstecheri was comparatively rare on *Fucus*, but when one individual was noticed on a frond a search generally revealed several others nearby. It was common on stones and shells in damp places in the lower half of the tidal zone. It is probably short-lived, for in many places the stones bore only tubes which were empty or occupied by *Polydora*. When the collection of

adults for laboratory experiments was begun, much time was wasted in the difficult task of examining the tubes to see whether or not they contained live *Spirorbis*. Then it was noticed that amongst live tubes there were usually many very small but conspicuously white tubes of recently settled individuals, whilst no such concentrations of young individuals were to be found round dead tubes. This might suggest that the larvae had settled immediately after liberation, but laboratory observations showed that larvae from freshly collected adults were positively phototactic for at least a quarter of an hour after liberation, and usually for longer than this. Probably they are so in the field, which would lead to their being widely dispersed.

Shells of *Nucella lapillus* were often covered with *Spirorbis*, either with the dextral tubes of *S. pagenstecheri* or the sinistral tubes of *S. granulatus* or *S. borealis*. Whilst some *Nucella* bore tubes which were all dextral, others from the same area bore only sinistral tubes. Mixtures of two species on such a small area as a *Nucella* shell appeared to be comparatively uncommon. *Spirorbis* on rocks and stones occurred in clumps, often of a single species. Isolated individuals were comparatively rare.

These observations left a strong impression that the specifically gregarious tendency demonstrated in the laboratory was of great importance in the field.

Gregariousness as Probably a General Feature of the Settlement Behaviour of Marine Larvae

Thorson (1950) found that about 70–80 % of bottom-living invertebrates have planktonic larvae, and reviewed the evidence that many of these larvae are able to postpone metamorphosis for days or even weeks, and to search actively for a suitable substratum. 'In a water area with a bottom current of only half a knot the larvae forced towards the bottom by their photonegativity and testing the substratum at intervals may be carried over a distance of 24 km. in 24 hr., i.e. 170 km. per week, and their chance of finding a suitable substratum for settling and metamorphosis seems to be great.' It is usually possible to relate the patchiness of the benthos to differences in the substratum, but sometimes it is difficult. *Pecten maximus*, for example, lives on a sandy bottom which may extend for vast areas, yet Priol (1930) described this species as essentially gregarious. During dredging near Bangor, in the wide sandy area of Red Wharf Bay, it was found commonly in some places but not at all in hauls from neighbouring, apparently similar, ground.

The distribution of marine animals is probably greatly influenced by the settlement behaviour of their planktonic larvae. In general, little is known about this behaviour. No evidence for gregariousness during settlement had been revealed when Allee (1931), having described crowded populations of certain littoral species, which appeared to be far below the level of organization at which a 'social appetite' was likely to occur, concluded that aggregations of

primitive animals form frequently as the result of forced movements. This must be true. Berrill (1950) may well have been justified in describing the aggregations, which are usually typical of the ascidian *Styelopsis grossularia*, as due to the viviparous development and insensitivity of the tadpoles to light, factors tending to 'keep the children at home', though he gave the free-swimming period as of many hours.

In laboratory experiments on ascidian larvae (Grave, 1935, 1941, 1944; Grave & Nicoll, 1940) metamorphosis was hastened by crowding or by aqueous extracts of the adults or larvae.[1] This is suggestive. The distribution of ascidians often suggests specific gregariousness. In 1948 *Ascidiella aspersa* was abundant and *Ascidia conchilega* was comparatively sparse on certain oyster grounds worked from the Fisheries Laboratory at Burnham-on-Crouch, Essex. *Ascidia conchilega* occurred in clumps, with few isolated individuals, as though the larvae were gregarious and could distinguish their own species from the other.

So far tests for gregariousness have been carried out only on *Ostrea edulis*, *Elminius modestus* and the two species of *Spirorbis* dealt with here. These animals were not chosen for research because they were obviously more gregarious than other marine invertebrates, but because of their economic importance or convenience. The fact that the tests gave positive results in each species, representing three main phyla, suggests that gregariousness will prove to be a rather general feature of settlement behaviour. Though it has been demonstrated only in sessile species, in which it is likely to be particularly important, it will probably occur in others also, since most marine invertebrates have limited powers of locomotion as adults.

Andrews (1949), having observed that Folliculinidae constantly occurred in clumps, suggested that the behaviour could hardly serve for protection or feeding, but that breeding might be aided by clumping, if a process of conjugation occurred in the imperfectly known life cycle of the group. Breeding is likely to be the main advantage to be derived from gregariousness, but it is not the only one. Another is that larvae will probably find, in places where the species is already established, suitable conditions for their own survival.

[1] Glaser & Anslow (1949) have suggested that this may have been due to copper, which they found to be present in ascidians and which has been shown to accelerate their metamorphosis. The grounds for this idea are weakened by the facts that (i) Grave (1935) found that tissue extracts which rapidly and consistently induced metamorphosis in larvae of the same species had at most only a slight effect on another species, and (ii) a variety of unfavourable conditions and dilute solutions of other poisons besides copper have been shown to accelerate metamorphosis, not only in ascidians (Zinkin, 1938), but in *Bugula* (Lynch, 1949*b*) and *Tubularia* (Pyefinch & Downing, 1949). The same criticism may now be made of Prytherch's conclusion that copper is necessary for metamorphosis in larvae of the American oyster. Prytherch (1934) put forward, in support of this, a controversial idea regarding the function of the pigment spots in the larval mantle (Cole, 1938) and an interpretation of some results, showing peak settlement at low water, which has also been questioned (Korringa, 1940). It should not yet be accepted that copper has a peculiar or necessary effect on metamorphosis in either oysters or ascidians.

This will be particularly important in species with closely restricted habitats. Often the factors which make places unsuitable for a species are complex or do not operate all the time. The presence of adults is a simple test of suitability. Without gregariousness many larvae of *S. borealis* would probably settle, during high water, on the *Fucus* in the upper half of the tidal zone, where they would be doomed to desiccation during subsequent ebbs (see p. 216).

Gregariousness in oysters proved to be most marked when settlement was light and to be masked when settlement was very heavy, probably because a few less discriminating larvae soon settled on bare surfaces, stimulating others to follow (Cole & Knight-Jones, 1949; see also p. 210). This phenomenon seems bound to accompany gregariousness in general and to have the following results, beneficial to the species. In poor breeding years settlement of larvae will be concentrated round the parent stocks, replenishing these as far as possible. This happened during the poor oyster spatfall of 1947 in the River Crouch, Essex (Knight-Jones, 1951). In normal breeding years old stocks will be built up and the range of the species may be extended to new areas, where pioneer larvae will be followed by others. In exceptionally good breeding years larvae will settle abundantly in every area to which they drift in sufficient numbers. Great increases in the range of the species will occur and many larvae will settle in unusual and often unsuitable places. Oyster larvae are said to have settled abundantly (and most unusually) on reeds in flooded ditches during the exceptionally good spatfall of 1935 in the River Crouch, Essex. Settlement in such places is mass suicide, but population pressure on the old beds is thereby reduced. This pressure may become too great in exceptionally good breeding years. At Conway, in 1940, practically all *Mytilus edulis* of marketable size were smothered and killed by a continuous covering of young mussels (Ministry of Agriculture and Fisheries, 1946, p. 57). In *Elminius* and *Spirorbis* the spacing-out tendency on crowded surfaces (p. 216) and the normally short span of life guards against such a disaster.

Summary

Spirorbis borealis and *S. pagenstecheri* were chosen for laboratory experiments on settlement behaviour because they are viviparous, with larvae which soon settle.

S. borealis liberated larvae mainly at about the moon's quarters.

After liberation larvae were positively phototactic for a period (15 min. to 2 hr.), then swam more at random. For a further period of 1 or 2 hr. they visited a large number of different surfaces, crawling upon them and then swimming off again.

When about to settle the crawling larvae changed direction with gradually increasing frequency by flexing the abdomen. Eventually they attached themselves, with wriggling movements, by the milky secretion of a gland on the

dorsal surface, which formed the initial, semi-transparent tube. Within 1 or 2 min. they rolled over on to the dorsal side and embarked upon a cataclysmic metamorphosis.

Larvae settled on surfaces of glass or stone only if the surfaces bore bacterial or algal films. In clean vessels larvae did not metamorphose, except abnormally or after a considerable delay, unless a filmed surface or a piece of *Fucus* was provided for attachment. Searching larvae did not stick to wiped or freshly immersed surfaces and appeared to be unable to crawl upon them, but immediately they touched filmed surfaces they stuck to them and started to crawl.

Many more larvae settled on pieces of *Fucus* which bore previously settled *Spirorbis* than on bare controls.

Settlement occurred earlier in larvae which were crowded, or accompanied by previously settled individuals, than in isolated larvae.

Paired slate blocks, one bearing previously settled *S. borealis*, the other *S. pagenstecheri*, were exposed to larvae of these two species. The great majority of the larvae settled on the blocks which bore their own species rather than on those which bore the other.

Fucus was very favourable for settlement of *S. borealis*, but in the dark bare *Fucus* proved less favourable than filmed glass surfaces which bore previously settled *Spirorbis*. Larvae often settled in concavities, probably in response to contact stimuli. Gregariousness soon led to a crowding of from about 10 to 20 recently settled individuals per cm.^{2}, but larvae arriving thereafter sought less crowded areas nearby, on which they spaced themselves out.

In the field settlement of *S. borealis* occurred mainly about low-water mark, on *Fucus* which already bore *Spirorbis*. Stones which bore live adult *S. pagenstecheri* could easily be distinguished from others which bore dead tubes, because many small, white tubes of recently settled individuals occurred amongst the adults.

Gregariousness during settlement has been demonstrated in each of the only four species so far investigated (*Ostrea edulis*, *Elminius modestus* and two species of *Spirorbis*), representing three main phyla. It is probably a general feature of the settlement behaviour of planktonic larvae, helping them to find suitable habitats, to maintain old breeding stocks and to form new ones.

REFERENCES

ALLEE, W. C., 1931. *Animal Aggregations*. Chicago.

ANDREWS, E. A., 1949. Folliculinid life-cycle. *Journ. Morph.*, Vol. 84, pp. 401–9.

BERRILL, N. J., 1950. *The Tunicata*. Ray Society, No. 133. London.

COLE, H. A., 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. *Journ. Mar. Biol. Assoc.*, Vol. 22, pp. 469–84.

COLE, H. A. & KNIGHT-JONES, E. W., 1939. Some observations and experiments on the setting behaviour of larvae of *Ostrea edulis*. *Journ. Cons. Int. Explor. Mer*, Vol. 14, pp. 86–105.

COLE, H. A. & KNIGHT-JONES, E. W., 1949. The setting behaviour of larvae of the European flat oyster, *Ostrea edulis* L., and its influence on methods of cultivation and spat collection. *Fish. Invest.*, Ser. II, Vol. 17, No. 3.

FEWKES, W., 1885. On the larval forms of *Spirorbis borealis*. *Amer. Nat.*, Vol. 19, pp. 247–57.

GARBARINI, P., 1933. Rhythme d'émission des larves chez *Spirorbis borealis* Daudin. *C.R. Soc. Biol., Paris*, T. 112, pp. 1204–5.

—— 1936*a*. Rhythmes de croissance des oocytes et d'incubation des larves chez *Spirorbis borealis* Daudin. *C.R. Soc. Biol., Paris*, T. 122, pp. 157–8.

—— 1936*b*. Le choix du support pour les larves de *Spirorbis borealis* Daudin. *C.R. Soc. Biol., Paris*, T. 122, pp. 158–60.

GLASER, O. & ANSLOW, G. A., 1949. Copper and ascidian metamorphosis. *Journ. Exp. Zool.* Vol. 111, pp. 117–39.

GRAVE, B. H., 1930. The natural history of *Bugula flabellata* at Woods Hole, Massachusetts, including the behaviour and attachment of the larva. *Journ. Morph.*, Vol. 49, pp. 355–83.

GRAVE, C., 1935. Metamorphosis of ascidian larvae. *Publ. Carnegie Instn. Wash.*, No. 452, pp. 209–92.

—— 1941. Further studies on the metamorphosis of ascidian larvae. *Biol. Bull. Woods Hole*, Vol. 81, pp. 286–7.

—— 1944. The larva of *Styela* (*Cynthia*) *partita*; structure, activities and duration of life. *Jour. Morph.*, Vol. 75, pp. 173–91.

GRAVE, C. & NICOLL, P. A., 1940. Studies of larval life and metamorphosis in *Ascidia nigra* and species of *Polyandrocarpa*. *Pap. Tortugas Lab. Wash.*, Vol. 32, pp. 1–46.

KNIGHT-JONES, E. W., 1951. Aspects of the setting behaviour of larvae of *Ostrea edulis* on Essex oyster beds. *Cons. Int. Explor. Mer, Rapp.-Proc. Verb.*, Vol. 128, pp. 30–4.

KNIGHT-JONES, E. W. & STEPHENSON, J. P., 1950. Gregariousness during settlement in the barnacle *Elminius modestus* Darwin. *Journ. Mar. Biol. Assoc.*, Vol. 29, pp. 281–97.

KORRINGA, P., 1940. Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *O. edulis*. *Arch. Néerl. Zool.*, Vol. 5, pp. 1–249.

LYNCH, W. F., 1949*a*. Modification of the responses of two species of *Bugula* larvae from Woods Hole to light and gravity: ecological aspects of the behaviour of *Bugula* larvae. *Biol. Bull. Woods Hole*, Vol. 97, pp. 302–10.

—— 1949*b*. Acceleration and retardation of the onset of metamorphosis in two species of *Bugula* from the Woods Hole region. *Journ. Exp. Zool.*, Vol. 111, pp. 27–51.

MILLER, M. A., RAPEAN, J. C. & WHEDON, W. F., 1948. The role of slime film in the attachment of fouling organisms. *Biol. Bull. Woods Hole*, Vol. 94, pp. 143–57.

Ministry of Agriculture and Fisheries, 1946. *Fisheries in War Time. Report on the Sea Fisheries of England and Wales by the M.A.F. for the years 1939–44.* London: H.M.S.O.

NEU, W., 1933. Der Einfluss des Farbtons der Unterlage auf die Besiedlung mit *Balanus* und *Spirorbis*. *Internat. Rev. Ges. Hydrobiol. Hydrograph.*, Bd. 28, pp. 228–46.

PRIOL, E., 1930. La Coquille Saint-Jacques (*Pecten maximus*). *Rev. Trav. Pêches Marit.*, T. 3, pp. 143–73.

PRYTHERCH, H. F., 1934. The role of copper in the setting, metamorphosis and distribution of the American oyster, *Ostrea virginica*. *Ecol. Monogr.*, Vol. 4, pp. 49–107.

PYEFINCH, K. A. & DOWNING, F. S., 1949. Notes on the general biology of *Tubularia larynx* Ellis & Solander. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 21–43.

SCHEER, B. T., 1945. The development of marine fouling communities. *Biol. Bull. Woods Hole*, Vol. 89, p. 103.

SHIVELY, M. A., 1897. The anatomy and development of *Spirorbis borealis*. *Proc. Acad. Nat. Sci. Philadelphia*, Vol. 49, pp. 153–60.

SWAN, E. F., 1950. The calcareous tube secreting glands of the serpulid polychaetes. *Journ. Morph.*, Vol. 86, pp. 285–314.

THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Komm. Danmarks Fisk. Havund., Ser. Plankton*, Bd. 4, Nr. 1, 523 pp.

—— 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, Vol. 25, pp. 1–45.

ZINKIN, L. N., 1938. Stimulation of metamorphosis in ascidian larvae. *C.R. Acad. Sci. U.S.S.R.*, Vol. 18 (3), pp. 213–16.

ZOBELL, C. E., 1938. The sequence of events in the fouling of submerged surfaces. *Contr. Scripps Inst. Oceanogr.*, No. 35.

—— 1946. *Marine Microbiology*. Waltham, Mass.

ZOBELL, C. E. & ALLEN, E. C., 1935. The significance of marine bacteria in the fouling of submerged surfaces. *Journ. Bact.*, Vol. 29, pp. 239–51.

ON THE BIOLOGY OF *MYTILICOLA INTESTINALIS* (STEUER)

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(Text-figs 1–3)

Introduction

The copepod parasite *Mytilicola intestinalis* was first described by Steuer (1902) from the gut of *Mytilus galloprovincialis* (Lam.) in the Gulf of Trieste. Monod & Dollfus in 1932 recorded the same species from *M. edulis* from Marseilles. In 1939 the parasite was first recorded on the German North Sea coast by Caspers near Cuxhaven, and in 1947 by Ellenby from Blyth, Northumberland. It is now widespread along the English south coast in *M. edulis*, but the distribution still shows some irregularities that are discussed in this paper.

I am grateful to Prof. J. E. G. Raymont for facilities at University College, Southampton, and for the use of a research table at Plymouth; to Mr F. S. Russell, F.R.S., and the staff of the Plymouth Laboratory of the Marine Biological Association for their assistance; and to Dr D. P. Wilson for facilities to collect at Exmouth. Dr H. A. Cole and Mr J. N. R. Grainger have kindly given me information from their papers not yet published. Dr D. J. Crisp and Dr H. G. Stubbings have assisted by sending me several samples of mussels, and I am grateful also for information received from several other friends named in the text.

Description

Steuer's description includes the following points. Length: male about 4 mm., female about 8 mm. Body elongated and worm-like. Thoracic segments with paired dorsal processes. Segmentation of abdomen incomplete. Genital openings paired, female carrying two long, narrow egg-sacs, in which eggs are arranged with some regularity. These features are shown in Fig. 1. The head carries a median eye, first antennae of four joints, and second antennae of three joints, the last forming a hook. Lateral to the maxillary base Steuer notes the presence of a soft pocket in the body wall receiving the tip of the antenna. I am unable to recognize this as a permanent structure.

The nomenclature of the mouthparts given by Steuer (1902) was revised by him (1905), when he recognized the opening of the shell gland (excretory

gland), but he continued to follow Claus's older work and named the appendage with the excretory gland as 1st maxilliped. Claus (1895) had agreed with Giesbrecht and Hansen to call this appendage the maxilla, preceded by a maxillule and followed by maxillipeds. Wilson (1910) confirmed this arrangement for the parasitic types.

Dollfus (1932) followed Wilson in the main terminology, but criticized the identification of the mandibles by Steuer. It is clear that *Mytilicola* lacks one

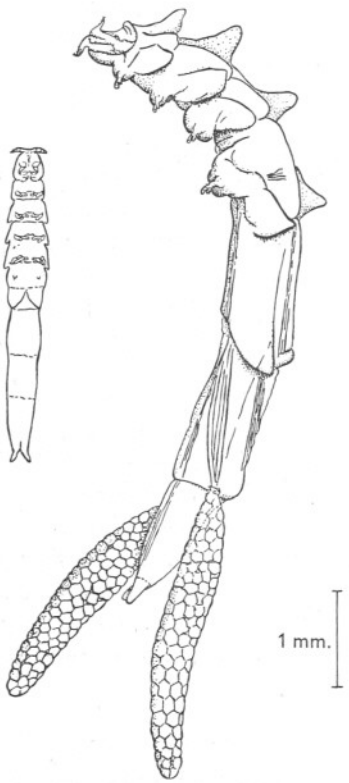

Fig. 1. *Mytilicola intestinalis*, adult male (left) from the ventral aspect, and female (right) with egg-sacs in left dorso-lateral view. The dorso-lateral processes are shown, by which the animal presses the ventral surface with appendages against the opposite wall of the host's intestine.

of the characteristic pairs of appendages, and Steuer thought this to be the post-mandibular pair. Dollfus, by a comparison with *Lichomolgus*, *Ergasilus*, *Panaietis* and *Trochicola*, has shown that in all probability the mandibles are lost and the first mouthparts present are the maxillules. My examination of the larval stages supports Dollfus's conclusion (see below, and Fig. 3).

My interpretation of the head of the female is shown in Fig. 2A. Whole specimens have been mounted direct in polyvinyl lactophenol and lignin pink,

and also dissections have been made with the Labgear Harding micromanipulator. The base of the maxillule is deeply embedded in the head, and the projecting part fits closely in a depression of the anterior margin of the maxillary base. The maxillules are wide apart, and their movements contribute only slightly to the feeding process. They may assist in movement through the gut of the host, or in maintaining a hold while the antennae are released and moved forward.

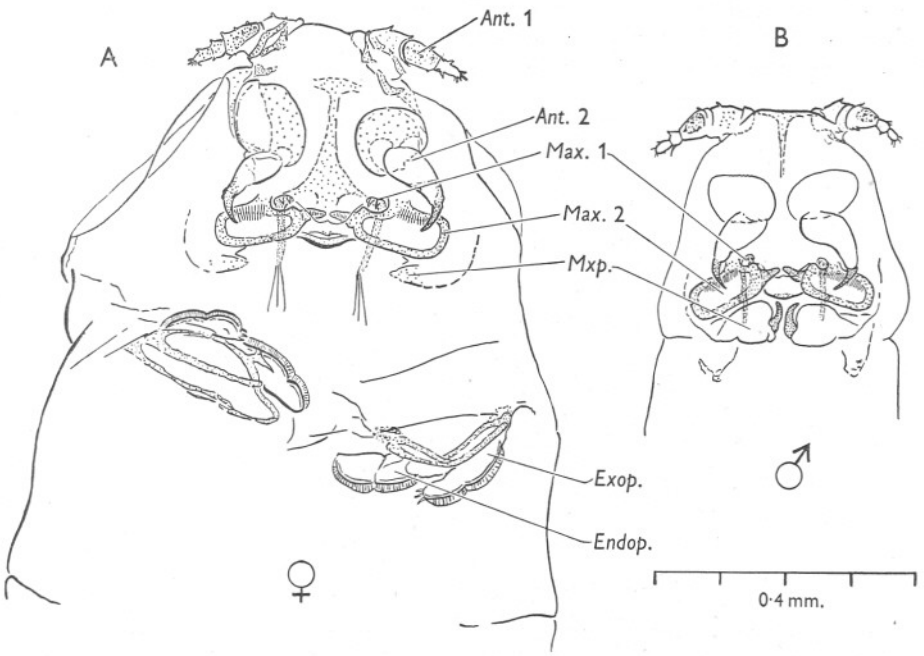

Fig. 2. *Mytilicola intestinalis*. A, head and first free thoracic segment of a mature female. B, the head of a male, in ventral view. *Ant.* 1, antennule; *Ant.* 2, antenna; *Max.* 1, maxillule; *Max.* 2, maxilla; *Mxp.*, maxilliped, vestigial in the female. In both sexes the upper and lower lips are shown, the former covering the inner ends of the maxillae. *Exop.*, exopodite, and *Endop.*, endopodite of the typical thoracic limb.

The base of the maxilla is thickly sclerotized. On the posterior border it carries the aperture of the excretory gland. The inner side of the base carries a pointed projection bearing minute serrations. This part of the maxilla meets its counterpart of the opposite side and is used to push food into the mouth. As noted above, the anterior margin of the maxilla is hollowed around the maxillule, and lateral to this it bears a row of ridges which lie adjacent to the hook of the antenna. Each ridge projects at its posterior end, forming a small tooth. It appears that these structures also assist in maintaining position in the host. Behind the maxillae is a pair of quite vestigial maxillipeds.

The upper lip is a well-defined structure, overlapping the inner processes of

the maxillae. The lower lip is less prominent, but it forms a firmly sclerotized line between the maxillary bases.

In the male, Fig. 2B, the head appendages follow closely the structure described for the female. The maxillipeds are, however, well developed. Each has a large swollen base, and a strong hook curved in a forward direction.

The first four pairs of thoracic feet are similar in both sexes. The base is a double chitinous ring, carrying exopodite and endopodite each of two joints. Their outer edges carry a ridge of thin cuticle with some vertical striation which may easily be mistaken for fine hairs. A few small spines and bristles occur on each foot. The fifth pair of feet are reduced to short bristled pegs.

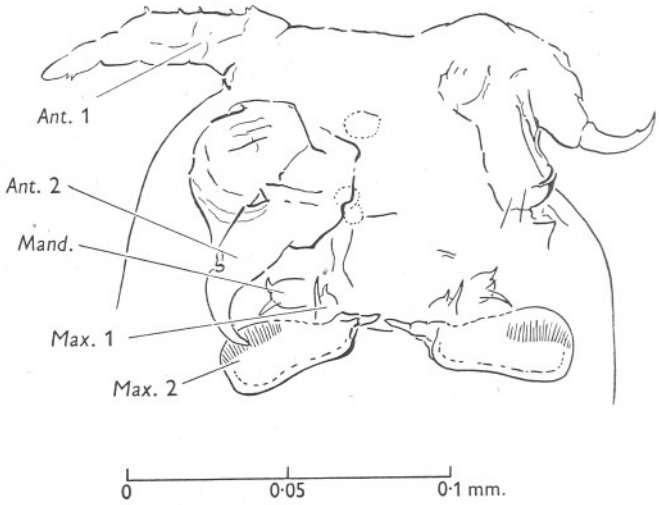

Fig. 3. *Mytilicola intestinalis*, head of a larva of the second parasitic instar, showing the mandible (*Mand.*) which is subsequently lost. The relationship between the maxillule (*Max.* 1) and maxilla (*Max.* 2) is very similar to that of the adult.

Habitat and Life History

The worm-like parasites lie in the recurrent intestine or rectum of *Mytilus edulis*, but not in the direct intestine or style sac. Generally they have a bright red colour and are easily seen, but occasionally quite active individuals are found with no colour. The female parasite occupies most of the lumen of the gut and presses the ventral surface close against the wall with the aid of the paired dorsal thoracic processes. Between these processes the main stream of the host's food is allowed to pass. In the intact gut I have seen little activity, but as soon as contact with the gut wall is lost the parasite begins vigorous peristaltic contractions. If only the posterior half is exposed the parasite can quickly crawl farther into the gut, but when fully exposed its power of locomotion is greatly reduced. At such times a frequent upward flick of the

head is noticed, and it is probable that such a movement in the normal environment would ensure that some of the food mass passed to the oral surface of the parasite. Adult females appear to be always orientated with the head towards the oncoming food, but the smaller males seem to move about more freely. Probably the male maxilliped is used in pairing. Despite the large numbers of fresh specimens examined, pairing has never been observed, and it is safe to assume that the association is quite temporary.

When a group of mussels is kept in the laboratory for 2 or 3 days the parasites may move down the gut until the egg-sacs protrude from the anus, but no adult parasite has been seen to leave the host. Females may be found with the eggs in various stages of development, but they are seldom found with eggs ready to hatch. Parasites removed from the host may retain the egg-sacs even after all the eggs have hatched, but they are easily detached at any stage. It seems probable that only mechanical forces in the host gut cause the shedding of egg-sacs, and this not long before hatching.

The youngest egg-sacs, with an opaque pink colour, were removed and cultured in beakers containing 150 c.c. Plymouth 'outside' sea water. No special filtration or sterilization of the water was found necessary. Slow aeration was maintained and the larvae were fed with cultured *Chlamydomonas*. The average temperature was 18° C.

Under these conditions the embryos develop a translucent brown tint, with a prominent red eye in each. Hatching occurs in about 7 days, and the nauplius and metanauplius instars are passed on the eighth day. These two larvae show a strong positive phototropism, which was seen by all previous workers, who also noted that the reaction is lost at the third instar (1st copepodid). The copepodid is an active swimming stage with two pairs of biramous thoracic limbs. Its movement in culture vessels is occasional or spasmodic, and since it is markedly more dense than the water, it tends to swim in the lower part of the vessel. This may be important among factors limiting the distribution.

The first copepodid is the infective stage, and although Pesta (1907) failed in his efforts to infect mussels, Caspers (1939) and Grainger (private communication) found no difficulty. The present author has reared many larvae, and carried out the infection of a mussel measuring 11 mm. long. From this host twenty-two fourth- and fifth-instar parasites were recovered after 3 weeks. This probably does not reflect a normal growth rate, for such a heavy infection of a small host is unusual, and the copepodids may have had only a minimum of food.

Caspers figured three parasitic instars before the adult form, and no attempt has been made here to check his observations. Dollfus has noted that previous figures of the larvae do not show the detail needed to decide the fate of the mandible and maxillule. I have examined all the earlier larvae, and Fig. 3 shows the head of a fifth-instar (second parasitic) larva. Although their cuticle is but

slightly thickened, it is possible to discover at this stage both pairs of appendages, and therefore to confirm that the mandible is the appendage missing from the adult head.

Distribution

Altogether over a thousand mussels have been examined, by opening the whole intestine of each specimen, from sites extending from Kent to Cornwall. In the neighbourhood of Southampton mussels have been examined at all times of the years 1948–50, and no significant seasonal variation was detected. On each occasion females with egg-sacs were present. Males were always the more numerous, a typical sample yielding 182 males:53 females. Larval stages were seldom found. Although some may have been overlooked because of their small size, I believe it is safe to infer that the time occupied by the three larval parasitic instars is short when compared with the average span of adult life.

In many places it was difficult to secure an adequate sample of mussels, but Table I shows that the absence of the parasite cannot be assumed at any of these sites.

Further reports have been received, and are gratefully acknowledged as follows:

Whitstable, Kent, the parasite is common (Dr G. E. Newell)
Littlehampton, Sussex, mussels are not numerous but the parasite is present (Mr E. W. Baxter)
Poole Harbour, Dorset, very abundant 1950 (Dr H. A. Cole)
Falmouth Harbour, Cornwall, present 1949 (Dr H. A. Cole)
Teignmouth, Devon, absent 1949 (Dr H. A. Cole)
Fowey, Cornwall, absent 1949 and 1950 (Dr H. A. Cole)
Conway, Caernarvonshire, absent (Dr H. A. Cole)
Co. Cork, Eire, the parasite is common (Mr J. N. R. Grainger).

Discussion

Estuarine and Marine Environments

Caspers has suggested that the parasite may be indicative of polluted water, being particularly prevalent in the shallow waters of estuaries. He does not give a detailed description of his sites, but Neuharlingersiel and Karolinensiel would appear from the map to be protected shallows and not true estuaries, and these areas he found to be heavily infested. Other areas such as Norddeich and Busum appear to be similar, yet the parasite was absent. From all the more exposed situations and deeper waters he obtained no *Mytilicola*. The density of the population in the infected areas led Caspers to suppose that the parasite was not a recent immigrant, but had been previously overlooked.

Ellenby, reporting a heavy infestation at Blyth in 1946, was convinced that the parasite was newly arrived, for mussels had been regularly examined

from that area over a period of some years. The same experience obtains at Southampton, where the Woolston mussels were at least relatively free until 1948. Seventy parasites were then recovered from twenty-four hosts in a sample of thirty-nine mussels. This density of population shows that the parasite is able to establish itself rapidly in the muddy and often shallow waters of

TABLE I. SOUTH COAST DISTRIBUTION OF *MYTILICOLA INTESTINALIS*

Locality	No. of *Mytilus* opened	No. infected	Type of site and remarks
Birchington, Kent	18	9	Mussel bed offshore
Bognor, Sussex	16	10	Open shore
Langstone Bridge, Hayling Island	5	4	Attached to stones in mud; protected
Horsey Island, Portsmouth Harbour	5	1	Attached to stones in wall; protected
Sandown, Isle of Wight	19	12	Low down on pier. Flat shore.
Titchfield, Hants	78	60	Mussel bed offshore, protected by island
Hook Bungalow, Hants	9	9	Stones on flat shore
Woolston, Southampton (River Itchen)	39	24	On pier and from the ground; estuarine
Eling, Southampton (River Test)	50	19	Wreck on muddy bottom near L.W.
Milford-on-sea, Hants	8	6	On groynes; exposed
Barton-on-sea, Hants	5	0	On groynes; exposed
Mudeford, Christchurch (River Avon)	45	16	Concrete blocks on shore
Sandbanks, Poole Harbour	3	2	Protected
Studland Bay, Dorset	13	13	On low rocks
Exmouth, Devon:			
(*a*) On quay wall	50	0	Vertical surface, strong current
(*b*) Bull Hill Bank	100	1	Extensive mussel bed in estuary
Goodrington Sands, Paignton	36	2	From flat rocks among sand
Brixham, Devon:			
(*a*) On floating pontoon	134	13	From sides and under
(*b*) On sea bed	18	1	Few mussels only under rocks
Steer Point, River Yealm, Devon	50	46	Very protected bed in estuary
Cattewater, Plymouth	113	4	In cracks of quay wall. Protected and estuarine
Plymouth Sound:			
(*a*) Rum Bay	23	1	On rocks near H.W.
(*b*) Pier piles	107	5	All on nearly vertical surfaces
(*c*) Drake's Island	50	1	
(*d*) Promenade wall	15	1	
(*e*) West Hoe	20	1	
Neal Point, River Tamar	77	70	Specimens dredged in estuary
Anthony Quay, River Lynher	53	49	From stones in mud. Protected estuary
Cawsand Bay, Cornwall	20	0	Small specimens on rocks near H.W.

harbours and estuaries. It is unlikely that such an obvious parasite would have been overlooked for long at Plymouth, yet the mussels now in the Tamar and Lynher estuaries show an average of eleven parasites per host.

Ellenby noted that the mussels of the only bed of purely marine character available in his area, at Holy Island, contained no *Mytilicola*. This agreed with

Caspers's observations. The records given here show that *Mytilicola* is quite capable of parasitizing mussels of a purely marine environment, either on open shores (Bognor, Sandown, Studland), or in more protected places (Titchfield). In fact from all the marine areas examined negative results were obtained only at Barton-on-Sea, Hants, and Cawsand Bay, Cornwall. At the former site the sample was obviously inadequate, and *Mytilus* was itself barely established on the shore. At Cawsand the mussels were better established, but they were not numerous and individuals were somewhat stunted in growth.

Whatever factors may limit the distribution of *Mytilicola intestinalis*, the species is not restricted to estuarine environments, although these may provide optimum conditions for its growth. In view of the power of rapid colonization here shown, it seems likely that those few estuaries that remain free will not for long maintain their immunity.

The Invasion of New Areas

Established beds of *Mytilus edulis* may become parasitized in either of two ways. First, by the larvae of *Mytilicola* being distributed by tidal and other currents. Secondly, by the introduction of adult or post-metamorphic *Mytilus* which are already parasitized.

The range over which the first method may operate will be limited by the period of free larval life, which appears to be short. If the observations made here are reliable as an indication of natural development, the truly planktonic phase lasts only 3 or 4 days and the copepodid larvae then begin to move downward in the water. The total free life is 10–14 days. If during this time the larvae are dispersed to areas with a low incidence of *Mytilus* there may be no effective spreading of the parasite.

All the facts known at present show that only sexual reproduction can occur, and it involves the presence of the two sexes in one host. It may be significant that in the following samples showing a low incidence of infection only a single parasite was recorded in any one host.

Site	Infected hosts	Total sample
Cattewater, Plymouth	4	113
Plymouth Sound	9	215
Brixham	14	152
Paignton	2	36
Exmouth	1	150

A slightly higher number was found at Mudeford, at the mouth of the Hampshire River Avon where, from a total of forty-five *Mytilus*, sixteen were found to harbour twenty-three parasites. Mudeford is not far away from either Poole Harbour or Southampton Water, either of which could act as a source of infection. No such sources are known in the neighbourhood of either Exmouth, Paignton or Brixham, but the survey of this area is not yet completed and no mussels have been examined from the Dart estuary.

The mussel colonies of the Tamar and Lynher estuaries, Plymouth Sound and the Cattewater show striking differences of infection which call for explanation. In each area quite dense colonies are to be found, though rather less in the Cattewater. Ten mussels taken at random at Anthony Quay (River Lynher) contained 115 parasites of which thirty-nine were females carrying egg-sacs. We might expect the production of larvae from this area to be sufficient to infect rapidly all mussels in the Sound, but this has not yet occurred. In the Lynher and Tamar the mussels are on flat beds of mud and stones; in the Sound all are on steep or vertical surfaces free of mud, and in the Cattewater most of the mussels were taken from crevices in the quay walls and none were found by dredging.

It is suggested that because of the sinking effect in the infective copepodid stage mussels on the bottom are much more susceptible to infection than those raised on steep surfaces. Experiments are now being started at Southampton to test the relative rates of infection of buoyed and bottom-living groups of mussels. The evidence afforded by the material from Brixham is inconclusive, but not encouraging to this hypothesis. From a floating pontoon 134 mussels were examined, and thirteen were infected, each with a single parasite. Very few mussels were to be found on the sea-bed in this area, and only one out of eighteen was infected.

The spreading of *Mytilicola* by introduction of infected *Mytilus* may occur accidentally on driftwood or on shipping, or by the deliberate importation of mussels where they are used for bait. It appears most likely that shipping across the North Sea has introduced the parasite to harbours and estuaries along our eastern and southern coasts, and into Eire. Yet some have remained relatively free (Exmouth and Fowey) which are visited by a moderate amount of coastal traffic, while others (Yealm River) have a heavy infection and little or no shipping. Where *Mytilus* is used either for food or as bait the introduction of specimens from another port would be rash unless it were accompanied by a very careful examination for *Mytilicola*.

Effect upon the Host

Caspers could not find any direct evidence of a harmful effect upon the host, but he records that the rate of filtration of water was reduced. It would be reasonable to suppose that an individual harbouring eight or ten parasites, which are common numbers, must suffer some deprivation of food. Taken as a whole, I have the impression that the parasitized stocks are less well nourished, but I have seen a specimen, apparently in normal condition, that contained a female *Pinnotheres* and twenty-seven *Mytilicola*.

The subject is being investigated at the Fisheries Experiment Station, Conway, and Dr Cole informs me in a personal communication, that where parasites are abundant severe loss of condition followed by death may result.

The numbers of *Mytilus* have been greatly reduced in the Tamar off Neal Point during recent years, but until my observations in 1948 the parasite had not been recorded there. I detect a slight reduction in the *Mytilus* population at Woolston, Southampton, over the last 7 years, but could not attribute this to the parasite. Sections of the infected intestine do not reveal any damage to the tissues.

Summary

Mytilicola intestinalis, a copepod parasite of *Mytilus edulis*, is now widespread along the English south coast.

The mouthparts are redescribed, and the loss of mandibles suggested by Dollfus is confirmed by the discovery of both mandibles and maxillules in the second parasitic larval stage.

Mytilicola is a comparatively recent immigrant. It has achieved a very high density of infection in harbours and estuaries, but also occurs in exposed and fully marine situations.

Factors influencing the distribution are discussed, and the infection of further estuarine populations of mussels is considered likely.

Owing to the behaviour of the infective copepodid larvae it is suggested that the parasite may be slow to colonize mussels that are raised above the sea-bed. This hypothesis is to be tested by experiment.

REFERENCES

Caspers, H., 1939. Über Vorkommen und Metamorphose von *Mytilicola intestinalis* Steuer (Copepoda paras.) in der südlichen Nordsee. *Zool. Anz.*, Bd. 126, pp. 161–71.

Claus, C., 1895. Über die Maxillarfüsse der Copepoden. *Arb. Zool. Inst. Wien.*, Bd. 11, pp. 49–64.

Ellenby, C., 1947. A copepod parasite of the mussel new to the British fauna. *Nature*, Vol. 159, pp. 645–6.

Monod, T. & Dollfus, R. P., 1932. Les Copépodes parasites de mollusques. *Ann. Paras. Hum. Comp.*, Vol. 10, pp. 129–204, 295–9.

Pesta, O., 1907. Die Metamorphose von *Mytilicola intestinalis* Steuer. *Zeitschr. f. Wiss. Zool.*, Bd. 88, pp. 78–98.

Steuer, A., 1902. *Mytilicola intestinalis* n.gen. n.sp. aus dem Darme von *Mytilus galloprovincialis* Lam. (Vorläufige Mitteilung). *Zool. Anz.*, Bd. 25, pp. 635–7.

—— 1905. *Mytilicola intestinalis* n.gen. n.sp. *Arb. Zool. Inst. Wien*, Bd. 15, pp. 1–46.

Wilson, C. B., 1910. The classification of the copepods. *Zool. Anz.*, Bd. 35, pp. 609–20.

ON THE VARIABILITY OF REPLICATE PLANKTON SAMPLES AND SOME APPLICATIONS OF 'CONTAGIOUS' SERIES TO THE STATISTICAL DISTRIBUTION OF CATCHES OVER RESTRICTED PERIODS

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(Text-figs. 1–4)

The variability to be expected in replicate plankton hauls, using several methods of hauling and a number of different nets, has been examined by Winsor & Clarke (1940). Some of the variability is due to true sampling variations, inevitable when discrete entities in suspension are sampled, and some is due to inadequacies of sampling technique. Winsor & Walford (1936) found that the variability of their vertical net hauls could be explained on the basis of a random distribution of the population, the variations being ascribed to variations in the volume of water filtered in successive hauls. They fully realized that the alternative, namely a non-random distribution of the population, was not disproved; but they considered that since widely different organisms showed little difference in variability the evidence was strongly in favour of a random distribution. They did note, however, that agreement between these different organisms was less marked when the numbers caught were large.

Analysis of net data by Barnes (1949*a*) was not at variance with the above hypothesis, agreement with Winsor & Walford's results being quite satisfactory. However, when the volume of water filtered was carefully controlled by the use of a plankton pump the variability was of a similar order and the distribution of χ^2 as well as its relation to sample size was similar (Barnes, 1949*b*). This cast doubt upon the validity of the assumption of a random distribution of population.

Ricker (1937) has examined the variability of catches of fresh-water plankton, and has pointed out that the variance is often greater than the mean and that this is evidence of aggregation of the organisms. This work has been extended by Langford (1938), who compared the mean and variance of a number of hauls taken at one station as well as over an area. He found evidence that some of the organisms were aggregated while others could be considered as randomly distributed. However, only a small number of hauls were examined. Similar work by Southern & Gardiner (1926), Ruttner (1930)

and Baldi, Cavalli & Pirocchi (1945) has been done on various fresh-water lakes. The problem is in many ways similar to that of the distribution of individual plants, and there has been considerable progress in this work (see reviews by Ashby, 1936, 1948).

The detailed form of the distribution[1] can be determined if a number of samples are available for the construction of frequency diagrams. In the present paper a modified method of collecting small samples in large numbers is described and this has, for the first time, enabled frequency distributions to be set up for plankton.

One of us (H. B.) would like to take this opportunity of thanking Dr R. A. Robb for many valuable and friendly discussions on statistical matters, and we both wish to thank him and Dr F. J. Anscombe for criticism of this paper.

We also wish to thank Captain Stewart and the crew of the *Calanus* for their assistance in the collection of the samples.

The Collection and Counting of the Samples

In order to obtain a large number of small samples, in which the volume was accurately controlled, the following procedure was adopted. A series of fourteen matched aspirators was set up in a rack, and underneath the stopcock of each a copper tube 1 in. diameter was fastened to a bar running across the front of the whole series. A piece of fine bolting silk (200 meshes/in.) was held over the base of the tube by means of a 'jubilee' clip. The use of aspirators with a narrow neck enabled an accurate volume to be rapidly taken. The mean volume of the aspirators was 5867 ml. with a standard deviation of 84 ml., and therefore a coefficient of variation of only 1·4 %. In all the experiments water was pumped continuously, one person filling up the aspirators until they overflowed. The tap was then opened and the water filtered through the silk on the copper tube. After rinsing the aspirator with filtered sea water and passing the washings through the copper tube the silk was carefully removed, the plankton washed off into a small bottle or specimen tube and a little formalin added. By employing ten people the whole process was kept more or less continuous during the sampling period. Welch (1948) has recently criticized pump-sampling technique, but his objection, namely that water is drawn from depths other than that nominally sampled, does not apply to these experiments, where only small volumes were taken (for example 720 l. *total* in series I) and where some relative movement of ship and water must have taken place.

At first it appeared probable that a reversed-microscope technique would be most suitable for the examination and counting of the plankton. In the

[1] 'Distribution' is used throughout in both the statistical and general sense; it is clear from the context which meaning is intended.

earlier samples, therefore, after allowing the plankton to sediment in the specimen tube and removing the excess water, it was transferred to a small Perspex vessel holding about 40 ml. and allowed to settle for at least 2 hr. before examining with a reversed microscope. When the sample was clean and the animals were undamaged this was satisfactory, but some of the samples had a great deal of detritus and this, concentrated on the restricted area of the bottom, hid the smaller animals. In addition, it was impossible to re-orientate one animal without disturbing a large area of the sample, and if many animals had to be thus moved the whole sample was disarranged.

With the later samples therefore this reversed microscope technique was abandoned. It was found best to let the plankton settle in the specimen tube and then draw off the overlying water by suction through a tube whose mouth was covered with fine silk, taking care not to disturb the settled plankton, and to wash this out into a small Bogorov-type tray made of Perspex and holding about 8 ml. The plankton was then examined with a dissecting microscope. A number of samples were counted by both methods; when the samples were clean the numbers were the same but when there was much detritus the reversed-microscope count gave a smaller number. Although passage through the pump, on the whole, did not damage the animals appreciably, some of the nauplii had lost their long tail spines (e.g. *Temora* and *Centropages*) and a good many of the *Oithona* copepodites had lost the abdomen. It was then sometimes impossible to assign them to the correct copepodite stage.

Three series of samples have been taken. In the first two series (10 and 11 February 1949) 120 and 300 samples respectively were taken from a single constant depth of 10 m. In the third (3 May 1949) four depths, 1, 5, 8 and 10 m. were sampled (see pp. 252–5). In all the experiments the boat was allowed to drift.

Series I and II were taken at a depth of 10 m. in 60 m. of water, in the channel between the Islands of Cumbrae and Bute (about 4° 55′ W., 55° 47′ N.), there being a distinct tendency during the experiment to drift to the south-east. For several days previous to this the wind speed had averaged 19 m.p.h. and during series I it varied from 10 to 14 m.p.h. from a direction of 300° backing to 290°. There was rather more wind on the following day, 20–25 m.p.h., from direction 290° to 270°. The weather was bright on the 10th, overcast on the 11th. Both these series were collected between 10.30 and 14.00 hr. High tide was 9.28 and 10.38 hr. at Greenock on the 2 days, so that the samples were collected on the ebb tide. During the first two series no observations were made of temperature or salinity. However, the sea-surface temperatures were in the neighbourhood of the winter minimum, between 7·0 and 7·5° C., while the salinity was about 31·5 $^{\circ}/_{\circ\circ}$, these figures being obtained in routine observations at Keppel Pier.

In series III, 400 samples were collected at four depths, 1, 5, 8 and 10 m., the depth at the station being 60 m. The position was somewhat different,

slightly to the east of south of Little Cumbrae Island, about 4° 57′ W., 55° 41′ N., the ship again being allowed to drift. The wind had been very light for the preceding days (mean 7 m.p.h.), but during the morning of the 3rd it rose and remained at 15–20 m.p.h. during the experiment, the direction being 200–215°. The sky was overcast throughout. During this series temperature and salinity observations were made, the observations being taken as nearly as possible to correspond with the middle of the period during which any part of the collection was being made. The observations are summarized below (Table I), further reference to them being made later.

TABLE I. TEMPERATURES AND SALINITIES OF POSITIONS SAMPLED IN SERIES III

	Period									
	i		ii		iii		iv		v	
Depth (m.)	Temp. (° C.)	Salinity (‰)	Temp. (° C.)	Salinity (‰)	Temp. (° C.)	Salinity (‰)	Temp. (° C.)	Salinity (‰)	Temp. (° C.)	Salinity (‰)
1	9·64	31·85	9·61	31·78	9·70	31·67	9·78	31·65	9·90	31·73
5	9·23	32·00	9·46	31·89	9·64	31·78	9·56	31·74	9·70	31·82
8	9·00	32·09	9·21	32·03	9·60	31·80	8·88	32·03	8·90	31·92
10	8·76	32·07	9·18	32·09	9·58	31·65	8·81	32·09	8·79	31·96
Time B.S.T.	10.15 to 11.03		11.09 to 11.49		11.59 to 13.05		13.13 to 14.01		14.09 to 15.03	

A PRELIMINARY COMPARISON WITH PREVIOUS INVESTIGATIONS

It will facilitate subsequent discussion if the results are considered using the methods of previous workers, and for this purpose only those from the simpler series (I and II) will be used. In previous work (Winsor & Walford, 1936; Barnes, 1949*a*, *b*) comparison has been almost entirely limited to paired hauls (see, however, Winsor & Clarke, 1940). χ^2 has been calculated from such paired catches, and the resultant distribution of χ^2 compared with that expected on a Poisson distribution. Using this method the results from two former sets of data (Barnes, 1949*a*, *b*) and from the present series are compared. For the present series counts of all organisms were used when $n_1+n_2>9$, paired samples being obtained by grouping sets of ten small samples in consecutive pairs.

The results are shown as the distribution of χ^2 and as the relationship of χ^2 to sample size (Tables II and III). In spite of the very great differences in volume of water filtered, several cubic metres with the nets and several hundred litres in the present samples, the results are very similar. The most noticeable feature, as was originally pointed out by Winsor & Walford for their own data, is the very large number of values showing a high χ^2 ($>3{\cdot}841$) (Table II), and the fact that the larger values of n_1+n_2 contribute largely to this value of χ^2; both features are clearly common to all the collections.

A further point can be considered at this stage. It was emphasized by Winsor & Clarke that in their results the standard deviation of the catches was roughly proportional to the mean, and they therefore use logarithmic values to stabilize the variance in their analyses. In the present series, although not directly proportional, the standard deviation increases roughly linearly with the mean, and strictly a transformation of the type $1/\beta \sinh^{-1}\beta\sqrt{x}$ is more appropriate. However, in view of the arbitrary character of some of the

TABLE II. THE DISTRIBUTION OF χ^2 FOR POISSON (EXPECTED ON A RANDOM DISTRIBUTION) AND NET, PUMP AND PRESENT SAMPLES FOR ALL ORGANISMS

	Poisson	Nets	Pump	Series I and II, present observations
0–0·0039	5	4·5	5·5	5·7
–0·0158	5	1·5	3·6	1·6
–0·0642	10	3·7	3·6	6·6
–0·148	10	5·2	9·1	9·1
–0·455	20	10·4	9·1	10·1
–1·074	20	11·2	5·5	14·2
–1·642	10	9·0	12·7	11·3
–2·706	10	11·9	7·3	10·4
–3·841	5	11·2	3·6	7·6
>3·841	5	31·3	40·0	23·4

TABLE III. SUM OF χ^2 BY SAMPLE SIZE (POPULATION DENSITY)

(Numbers in brackets indicate number of samples in each group.)

n_1+n_2	Net	Pump	n_1+n_2	Series I and II, present observations
5–40	128·9 (50)	8·6 (12)	10–50	197·9 (133)
40–170	100·5 (44)	65·4 (19)	50–100	156·7 (72)
170–400	69·1 (19)	51·7 (7)	100–150	154·1 (35)
400–1000	161·5 (9)	128·7 (11)	150–200	99·5 (23)
>1000	1726·2 (12)	227·7 (6)	200–250	64·8 (12)
			250–350	64·4 (20)
			350–(3029)	343·2 (23)

divisions made in considering the results, the more complex transformation was not used, the logarithmic transformation being considered adequate. When both transformations were used on selected parts of the data there was no difference in the conclusions to be drawn.

THE STATISTICAL CONCEPTS USED

The study of variation leads naturally to the concept of frequency distributions in which the frequency of the variable quantity under consideration takes each of its possible values. In plankton sampling the number of organisms is discrete, and therefore the frequency distribution is essentially discontinuous. When there is a relatively small number of organisms compared with elemental

units of medium, then with the organisms randomly distributed the sampling distribution is described by Poisson's limit to the Binomial Expansion. The relative frequencies with which the samples will contain 0, 1, 2, ..., organisms is given by the series, $e^{-m}, me^{-m}, \frac{m^2}{2!}e^{-m}, \ldots, e^{-m}\frac{m^k}{k!}, \ldots$, where m is the mean number per sample.

Alternatively one may write, $P(x=k)=\frac{m^k e^{-m}}{k!}$, where $P(x=k)$ denotes the probability that a sample will contain k organisms. Agreement between a theoretical and an experimentally determined distribution may be tested by means of the χ^2 test, and $P(\chi^2)=0{\cdot}05$ will be considered the acceptable limit of significance (the classes are grouped when the expected value is less than 5; the degrees of freedom are $n-2$ for testing Poisson). A supplementary test is to calculate the variance and its ratio to the mean. With a Poisson distribution this ratio is unity and the departure from unity is a measure of dispersion. This ratio has been termed the relative variance by Clapham (1936), but Fisher's coefficient of dispersion (Blackman, 1942) appears a more suitable term. The coefficient of dispersion itself has a distribution, and in order to be significantly different from unity it must be greater (or less) by $2\sqrt{[2n/(n-1)^2]}$, where n is the number of samples. The coefficient of dispersion is sensitive as regards aggregation but it will not detect certain types of skew distribution.

It has been found, particularly by botanists studying the distribution of individual plants, that when a Poisson distribution does not fit their data, there are often too many samples containing no individuals and too few containing one individual when compared with the appropriate Poisson series, and as a result the observed frequency curves sometimes show bimodality. New series comparable with Poisson series have been developed which show this same tendency, such series being originally termed 'contagious' by Pólya (1931). Those developed by Neyman (1939) and Thomas (1949) seem to be particularly suitable for biological work (Archibald, 1948, 1950; Beall, 1940; Barnes & Stanbury, 1951).[1] Both are based on the same fundamental assumption, namely, a random distribution of the groups and a random number per group. The presence of a particular individual in a given region increases the probability of there being other individuals present (hence the term contagious series). Beall, working on insects, found that Neyman's Type A (which is the most easily calculated) is the most generally useful of the three Neyman series, and only Type A will be considered here. When the three types are different Beall showed that passing from Type A to B to C the expectation for the 0-frequency class falls, and for classes immediately after 0 tends to rise and then to fall for all subsequent classes. The Type A series, which was developed with

[1] For a further discussion of such series see Anscombe (1949, 1950).

particular reference to the emergence of insect larvae from egg masses, when put into the recurrent form of Beall is[1]:

$$P(x=0)=e^{-m_1(1-e^{-m_2})},$$

$$P(x=k+1)=\frac{m_1m_2e^{-m_2}}{k+1}\sum_{t=0}^{t=k}\frac{m_2^t}{t!}P(x=k-t).$$

The distribution is determined by two parameters m_1 and m_2 in contrast to the Poisson series which is completely determined by one parameter, the mean. These parameters can be taken to be proportional to the mean number of groups per sample (m_1) and to the mean number of individuals per group (m_2). They can be estimated from the first and second moments, since

$$m_2=(\mu_2-\mu_1')/\mu_1', \quad m_1=\mu_1'/m_2.$$

Since

$$\mu_2/\mu_1'=m_1m_2/m_1m_2(1+m_2),$$

it is clear that where m_2 becomes very small and m_1m_2 is finite the distribution approaches Poisson, where

$$\mu_2/\mu_1'=1.$$

Thomas's series, with similar fundamental assumptions, also expresses a contagious distribution. It is given by the following:

$$P(x=0)=e^{-m},$$

$$P(x=k)=\sum_{r=1}^{k}\frac{m^re^{-m}}{r!}\left[e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!}\right].$$

The parameter m is the mean number of groups per sample, whilst $1+\lambda$ is the mean number of individuals per group. As before, the parameters are obtained from the first and second moments:

$$\mu_1'=m(1+\lambda), \quad \mu_2=m(1+3\lambda+\lambda^2).$$

Clearly, as λ becomes very small the distribution approaches Poisson. Since this series is the sum of the products of two simple Poisson series

$$\frac{e^{-m}m^r}{r!} \quad \text{and} \quad e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!},$$

the terms can be obtained by interpolation from tables (Pearson, 1948).

The frequency distributions given by both Neyman's and Thomas's series are very similar, as might be expected, since they are based on similar fundamental assumptions regarding the population (Barnes & Stanbury, 1951).

[1] $P(x)$ denotes the probability that the number of individuals in any one sample is x. In applying the χ^2 test, 3 degrees of freedom should be subtracted from the total when dealing with the Neyman and Thomas distributions.

However, from the point of view of interpreting the aggregation mentioned, Thomas's parameters are more useful, since the mean number of groups per sample and the mean number of individuals per group are at once evident.

The Hauls of Series I

This was the first series using the new technique and 120 samples were collected in $2\frac{1}{2}$ hr.; improved organization enabled more samples to be taken in the same time in later series.

In this series the dominant organisms were: *Pseudocalanus minutus* (Krøyer), *Microcalanus pygmaeus* G. O. Sars, *Temora longicornis* (Müller), *Oithona similis* Claus (all as nauplii), lamellibranch larvae and small eggs (not identified). A small number of nauplii of *Acartia clausi* Giesbrecht were found.

Since the samples were taken over a considerable period of time it is first necessary to examine the results for possible changes in the population during the period of sampling. For this purpose the samples have been grouped in consecutive sets of fifteen, and the results are shown in Table IV and Fig. 1. It is clear from the figure that the organisms are behaving differently. The lamellibranch larvae and the egg population appear to be constant throughout, but *Pseudocalanus* and *Microcalanus* show a sharp, and *Temora* a less sharp, change at about sample 60, whilst *Oithona* nauplii show a tendency to change somewhat later in the series. The change in population is presumably a change either with time or with distance. There is little evidence to suggest that the change is dependent upon time *per se*, as for example in vertical migration. It is rather to be ascribed to a change in space consequent upon the relative changes between boat and water mass with time.

The above suggestion can be tested by considering the first four sets as replicates belonging to period 1 and the second four to period 2 (corresponding to the change indicated by Fig. 1 at sample 60) and an analysis for *Oithona* nauplii, lamellibranch larvae and eggs is given in Table V.

The mean squares for P and P × S are not significant[1] when tested against the appropriate residual, and one can assume that the same population was being sampled throughout. By contrast a similar analysis of *Pseudocalanus*, *Microcalanus* and *Temora* nauplii is given in Table VI.

With these species, although the P × S interaction is not significant, that is the species were caught in the same proportion in the two periods, the value for P is highly significant.

It seems therefore that two distinct populations of *Pseudocalanus*, *Microcalanus* and *Temora* were sampled during the experiment, the boundary between these two populations being sharply defined. Furthermore, there co-existed constant populations of lamellibranch larvae, eggs and to a large

[1] It will be assumed throughout that the results and the tests of significance are to apply to these experiments only.

TABLE IV. COUNTS OF ORGANISMS IN SERIES I

Sample nos. ...	1–15	16–30	31–45	46–60	61–75	76–90	91–105	106–120
Pseudocalanus nauplii	7	8	13	17	38	41	33	26
Microcalanus nauplii	38	41	47	46	101	94	109	70
Temora nauplii	8	25	16	29	33	55	36	67
Oithona nauplii	29	23	20	26	30	28	33	43
Lamellibranch larvae	47	51	35	34	55	43	55	39
Eggs	9	7	11	1	8	9	6	12

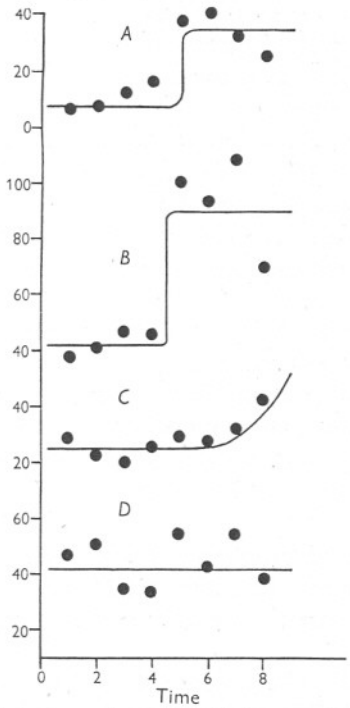

Fig. 1. Counts for repeated hauls, series I, for (A) *Pseudocalanus* nauplii, (B) *Microcalanus* nauplii, (C) *Oithona* nauplii, (D) lamellibranch larvae.

TABLE V

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	1	0·1149	0·1149
Residual 1	6	0·2812	0·0469
Species (S)	2	2·9845	1·4928
P × S	2	0·0244	0·0122
Residual 2	12	0·5329	0·0444

TABLE VI

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	1	1·0583	1·0583
Residual 1	6	0·1939	0·0323
Species (S)	2	1·1263	0·5632
P × S	2	0·0307	0·0154
Residual 2	12	0·2070	0·0173

extent *Oithona*. The biological relationship between these populations can be a matter for much speculation. If they have a common origin in a much denser concentration it may mean that one portion of the water mass in which this dense concentration was originally present has maintained its identity to a greater extent or for a longer time than the rest of the water mass. If this were so it is perhaps rather surprising to find extremely sharp boundaries. On the other hand, the two populations may have entirely different origins and may have come together as a result of water movements.

These possibilities might have been tested by a closer examination of the composition of the two populations, for example by size measurements or by finding the proportions of the different nauplius stages, but unfortunately the samples were thrown out before this was realized. The foregoing is based on the idea that the movement of copepod nauplii is dependent on the movement or mixing of water masses. Alternatively, such a mixing of populations may be due to the active movement of swarms of copepods away from denser centres of distribution.

The mean square for $P \times S$ is not significant in the analysis for *Pseudocalanus*, *Microcalanus* or *Temora* nauplii, although the value for P is highly significant, that is to say the proportions of the different species did not change significantly although the total numbers changed in the two halves of the experiment. This perhaps adds weight to the suggestion that the populations have a common origin and have become separated by a greater dilution of one portion.

In forming frequency distributions, the populations uniform throughout, namely *Oithona similis* nauplii, lamellibranch larvae and eggs, will be considered first. The frequency distributions and the various parameters are shown in Table VII.

Oithona similis nauplii. The coefficient of dispersion is not significantly different from unity and $P(\chi^2)$ for Poisson is 0·2–0·1, indicating an adequate fit to a random distribution. However, there is a clear indication of an excess of the 0-frequency class suggestive of aggregation or clumping, and it is clear from Table VII that both Neyman's and Thomas's series give a better fit to the observed results. From the Thomas parameters the estimated mean number of nauplii per clump $(1+\lambda)$ is 1·1.

Lamellibranch larvae. The results are very similar to those for *Oithona*. The coefficient of dispersion and the χ^2 test indicate an adequate fit to a Poisson series. Again the 0-frequency class is high, suggesting aggregation. The Neyman and Thomas series, again similar as would be expected, give a better fit and the mean number per clump as derived from $(1+\lambda)$ is 1·4. It should be noted that the mean is higher than with the previous species, and the increase in the mean is accompanied by an increase in the mean size of the aggregates.

Eggs. These small eggs were not identified and belonged to several different organisms and perhaps because of this none of the series gives an adequate fit.

There are an insufficient number of degrees of freedom to test the series but the 0-frequency class, although high, does not appear to be as high as required by contagion.

TABLE VII. SERIES I. ANALYSIS OF FREQUENCY DISTRIBUTIONS OF THE 'UNIFORMLY' DISPERSED FORMS. (120 SAMPLES)

(Pn., Poisson; Ny., Neyman; Th., Thomas; C.D., coefficient of dispersion.)

	Oithona similis nauplii	Lamellibranch larvae	Eggs
Mean	1·933	2·992	0·517
Variance	2·349	4·849	0·975
C.D.	1·215	1·621	1·885
$2\sqrt{[2n/(n-1)^2]}$	0·260	0·260	0·260
Ny., m_1	9·000	4·819	0·583
Ny., m_2	0·215	0·621	0·886
Th., m	1·736	2·204	0·336
Th., λ	0·114	0·358	0·537
$P(\chi^2)$			
Pn.	0·2–0·1	0·1–0·05	<0·05
Ny.	0·5–0·3	0·7–0·5	<0·05
Th.	0·5–0·3	0·8–0·7	<0·05

Frequency class	Found	Expected Pn.	Ny.	Th.	Found	Expected Pn.	Ny.	Th.	Found	Expected Pn.	Ny.	Th.
0	**23**	17·4	21·1	21·1	**11**	6·0	12·9	13·3	**80**	71·6	85·2	85·8
1	**28**	33·6	33·0	32·8	**24**	18·0	20·8	20·4	**26**	37·0	18·2	16·8
2	**34**	32·5	29·3	29·1	**20**	27·0	23·2	23·0	**12**	9·6	9·9	10·6
3	**17**	20·9	19·2	19·1	**23**	26·9	20·7	19·9	**0**	1·7	4·2	4·3
4	**8**	10·1	10·2	9·9	**17**	20·1	15·9	15·9	**0**	0·1	1·6	1·6
5	**7**	3·9	4·7	4·2	**11**	12·0	11·0	11·4	**1**	—	0·5	0·6
6	**3**	1·6[1]	2·5[1]	3·8[1]	**5**	6·0	6·9	7·3	**0**	—	0·4	0·2
7	—	—	—	—	**4**	2·6	4·1	4·2	**1**	—	—	0·1
8	—	—	—	—	**3**	1·0	2·3	2·3	—	—	—	—
9	—	—	—	—	**1**		1·2	1·2	—	—	—	—
10	—	—	—	—	**0**	0·3[1]	0·6	0·6	—	—	—	—
11	—	—	—	—	**0**		0·3	0·3	—	—	—	—
12	—	—	—	—	**1**		0·1[1]	0·1[1]	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—

[1] In this and all subsequent tables of this kind the last class shown of the expected values is obtained by subtraction and includes any higher frequency classes.

The two remaining species are dealt with in the two periods each with uniform population, the results of the analyses being given in Table VIII.

Pseudocalanus minutus nauplii (*samples* 1–60). An excellent fit to a random distribution is obtained and no further comments on this series are necessary.

Microcalanus pygmaeus nauplii (*samples* 1–60). The results for this copepod are in excellent agreement with a random distribution, $P(\chi^2)$ for Poisson being 0·9 and the coefficient of dispersion very little different from unity, although the population density is moderately high.

Pseudocalanus minutus nauplii (*samples* 61–120). $P(\chi^2)$ with Poisson is 0·1–0·05, but for Neyman and Thomas series it is much higher (0·9), showing a closer agreement and again indicating some aggregation.

Microcalanus pygmaeus nauplii (*samples* 61–120). In the second part of this series the population density increased very considerably, and since there is

TABLE VIII. SERIES I. ANALYSIS OF FREQUENCY DISTRIBUTIONS OF *PSEUDOCALANUS* AND *MICROCALANUS*

(Pn., Poisson; Ny., Neyman; Th., Thomas; C.D., coefficient of dispersion.)

	Samples 1–60		Samples 61–120	
	Pseudocalanus minutus nauplii	*Microcalanus pygmaeus* nauplii	*Pseudocalanus minutus* nauplii	*Microcalanus pygmaeus* nauplii
Mean	0·750	2·867	2·300	6·233
Variance	0·801	3·304	3·671	14·419
C.D.	1·068	1·152	1·596	2·314
$2\sqrt{[2n/(n-1)^2]}$	0·371	0·371	0·371	0·371
Ny., m_1	—	—	3·858	4·745
Ny., m_2	—	—	0·596	1·313
Th., m	—	—	1·715	3·364
Th., λ	—	—	0·341	0·853
$P(\chi^2)$	Pn., 0·7–0·5	Pn., 0·9	Pn., 0·1–0·05 Ny., 0·95–0·90 Th., 0·98–0·95	Pn., <0·05 Ny., 0·2–0·1 Th., 0·2–0·1

						Expected				Expected		
Frequency class	Found	Expected Pn.	Found	Expected Pn.	Found	Pn.	Ny.	Th.	Found	Pn.	Ny.	Th.
0	**28**	28·3	**2**	3·4	**11**	6·0	10·6	10·8	**2**	0·1	1·9	2·1
1	**23**	21·3	**12**	9·8	**13**	13·8	13·5	13·1	**3**	0·7	3·1	3·0
2	**6**	8·0	**14**	14·0	**13**	15·9	12·5	12·5	**6**	2·2	4·7	4·7
3	**2**	2·0	**15**	13·4	**8**	12·2	9·5	9·4	**5**	4·7	5·8	5·7
4	**1**	0·4	**9**	9·6	**7**	7·0	6·3	6·3	**6**	7·3	6·4	6·4
5	—	—	**3**	5·5	**4**	3·2	3·7	3·7	**7**	9·2	6·5	6·5
6	—	—	**2**	2·6	**2**	1·2	2·1	2·0	**5**	9·6	6·2	6·2
7	—	—	**1**	1·1	**1**	0·4	1·1	1·0	**8**	8·6	5·6	5·7
8	—	—	**1**	0·4	**1**	0·3	0·7	1·2	**1**	6·7	4·7	4·8
9	—	—	**1**	0·2	—	—	—	—	**2**	4·6	3·9	3·9
10	—	—	—	—	—	—	—	—	**6**	2·9	3·1	3·1
11	—	—	—	—	—	—	—	—	**4**	1·6	2·3	2·4
12	—	—	—	—	—	—	—	—	**0**	0·8	1·7	1·8
13	—	—	—	—	—	—	—	—	**3**	0·4	1·3	1·3
14	—	—	—	—	—	—	—	—	**0**	0·1	0·9	0·9
15	—	—	—	—	—	—	—	—	**2**	0·5	1·9	1·5

a large number of classes the number in any one class is small. Poisson is not an adequate fit, whilst Neyman and Thomas give $P(\chi^2) > 0{\cdot}05$. The high population density is accompanied by a higher degree of aggregation, the Thomas estimate of the mean number of nauplii per clump being 1·9.

THE HAULS OF SERIES II

This series was taken on the day following series I, at the same depth, and the abundant species were the same. The total time of sampling was $3\frac{1}{2}$ hr.,

somewhat longer than in series I, but a total of 300 samples was taken. The values for *Pseudocalanus*, *Temora* and *Oithona* are shown in Table IX, in which, since the numbers in any one sample were very small, they have been grouped into ten periods with paired sets of fifteen samples in each period. The analysis of Table IX is given in Table X.

TABLE IX. SERIES II. RESULTS FOR THREE ABUNDANT SPECIES

Period ...	1		2		3		4		5		6		7		8		9		10	
Pseudocalanus nauplii	17	25	14	22	32	40	28	27	24	28	29	23	14	17	21	22	8	13	21	14
Temora nauplii	22	41	28	32	51	49	44	62	63	45	62	48	45	60	72	56	40	65	95	72
Oithona nauplii	31	33	22	26	15	50	26	38	16	25	21	24	27	26	19	22	19	26	29	33

TABLE X

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	9	0·2877	0·0320
Residual 1	10	0·2081	0·0208
Species (S)	2	1·6100	0·8050
P × S	18	0·5670	0·0315
Residual 2	20	0·1755	0·0088

The P × S mean square is significant and one cannot assume that the whole population was constant throughout the sampling period. The population of *Oithona* nauplii appears, however, to be uniform and the analysis confirms this suggestion. Table IX suggests that the populations of both *Pseudocalanus* nauplii and *Temora* also are uniform between periods 3–8 inclusive, and this is confirmed by analysis.

The population with respect to the three species appears to be uniform between 1 and 2 as indicated by Table XI.

TABLE XI

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	1	0·0130	0·0130
Residual 1	2	0·0538	0·0269
Species (S)	2	0·0890	0·0445
P × S	2	0·0078	0·0039
Residual 2	4	0·0207	0·0052

The results for *Microcalanus* (Table XII) include nauplii and copepodite stages, and inspection suggests that three populations were sampled during the experiment. The numbers were moderately large, and for analysis they may be grouped in paired sets of ten for fifteen separate periods. When all the developmental stages of this species are considered the analysis of variance is shown in Table XIII.

The value for P × St is significant, indicating that the proportion of the stages changed during the experiment. Further, the highly significant value for periods indicates significant changes in the total *Microcalanus* population.

The indication that three populations were sampled has been tested by analysis of the three sections. Analysis of the first section of nauplii and copepodite stages I and II (periods i–v) is shown in Table XIV.

TABLE XII. SERIES II. RESULTS FOR *MICROCALANUS* NAUPLII, AND STAGES I AND II COPEPODITES

Initial section of hauls

	i		ii		iii		iv		v	
Nauplii	76	153	167	145	171	154	126	165	213	146
Stage I	27	18	19	23	30	16	11	14	29	25
Stage II	29	29	34	37	24	10	17	20	29	34

Central section of hauls

	vi		vii		viii		ix		x		xi		xii	
Nauplii	168	115	106	84	86	84	114	80	88	83	98	117	111	83
Stage I	9	5	5	1	2	4	4	2	11	5	2	2	1	11
Stage II	10	11	4	7	10	5	3	3	5	1	4	6	4	9

Late section of hauls

	xiii		xiv		xv	
Nauplii	85	112	141	169	130	99
Stage I	10	19	13	4	2	5
Stage II	18	36	24	23	5	7

TABLE XIII

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	14	6·5243	0·4660
Residual 1	15	0·8270	0·0551
Stages (St)	2	25·9737	12·9869
P × St	28	2·6901	0·0961
Residual 2	30	1·1141	0·0371

TABLE XIV

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	4	0·1802	0·0451
Residual 1	5	0·1031	0·0206
Stages (St)	2	4·5074	2·2537
P × St	8	0·1953	0·0244
Residual 2	10	0·1078	0·0108

The value for neither P × St nor the periods is significant, and it can therefore be assumed that the population as regards these stages was uniform in the first five periods.

The analysis for periods vi–xi, grouping into three sets since stages I and II are few in numbers, is shown in Table XV.

Again a uniform population can be assumed.

It will be remembered that P × St was significant for three developmental stages. However, if only copepodite stages I and II are considered this distinction disappears as seen in Table XVI, although the value for P still remains significant.

The proportions of stage I to stage II therefore remained the same although the totals changed.

The other organisms present in quantity were lamellibranch larvae, and inspection of the results suggests considerable variability at the beginning and towards the end of the experiment. However, analysis indicates the central section to be uniform (Table XVII).

TABLE XV

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	2	0·0971	0·0486
Residual 1	3	0·1450	0·0483
Stages (St)	2	7·3855	3·6928
P × St	4	0·0408	0·0102
Residual 2	6	0·2686	0·0448

TABLE XVI

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	14	7·9771	0·5698
Residual 1	15	1·2045	0·0803
Stages (St)	1	0·4527	0·4527
P × St	14	0·9044	0·0646
Residual 2	15	0·6082	0·0405

TABLE XVII

Source of variation	Degrees of freedom	Sum of squares	Mean square
Periods (P)	11	0·2203	0·0200
Residual	12	0·1663	0·0138

The situation is obviously more complex than in series I and the division by inspection in the first instance arbitrary. It will be as well therefore to recapitulate the results of the variance analyses in order to get a clear picture of the component populations.

(i) *Oithona* was uniform throughout. (ii) *Pseudocalanus* and *Temora* were uniform between 1 and 2 and between 3 and 8 at different population densities. (iii) *Microcalanus* nauplii and copepodite stages I and II were uniform from periods i to v and also from vi to xi but at a different population density. (iv) If only *Microcalanus* stages I and II are considered, they were caught in the same proportions throughout although the population density changed. (v) Lamellibranch larvae were uniform in the central section. (vi) There was considerable variation towards the end of the experiment.

The existence of a number of different populations sampled during the experiment is clear and confirms the situation evident in series I. However, there is a distinct tendency towards irregularity at some of the boundaries, which have to some extent been selected in an arbitrary manner. On the hypothesis, suggested in the discussion on series I, that populations may be associated with water masses, one might infer that this variability at the population boundaries was due to the breaking up and mixing of these water masses. Apart from these boundary variations, a tendency towards a division into three populations for all the species considered (except *Oithona*) is reasonable. This suggests that all three have some common origin and, in view of their different biological characters, further strengthens a physical interpretation. There is only limited information on the length of time taken by *Microcalanus* to develop from egg to stage II, but it is probably about a fortnight (see Marshall, 1949). The constancy in the proportions of three developmental stages at two different population densities and of copepodite stages I and II throughout suggests a common biological origin, unless egg production had taken place at the same time and environmental conditions had remained the same in the two different regions sampled.

Using only those populations shown to be uniform the frequency distributions can again be constructed (Tables XVIII, XIX, XX).

Oithona nauplii—all samples. The results are shown in Table XVIII. Poisson is a very good fit but even so the 0-frequency class is high and both the contagious series give a higher $P(\chi^2)$ indicating a tendency to aggregation.

The less abundant species. An excellent fit [$P(\chi^2) = 0{\cdot}9$–$0{\cdot}8$] to Poisson is obtained, and although the population density is higher than in the other populations where Poisson was a less adequate fit, it should be remembered that this is the distribution of a grouped set of animals, each one of which is at a much lower population density.

Pseudocalanus nauplii (*central section*, 3–8). The numbers in the first section are small, and only the central section has been analysed. The results are shown in Table XVIII and Poisson is a very satisfactory fit.

Temora nauplii (*central section*). Again only the central section is analysed, and the results are shown in Table XVIII. As with *Pseudocalanus*, a satisfactory fit to Poisson is obtained even though the population density is double.

Lamellibranch larvae (*central section*). Again only the central section is used with similar results to those from the two preceding copepod nauplii. No account was taken of different species or size groups, but the larvae were mostly at an early stage of development and comparitively uniform in size.

Microcalanus nauplii (*initial and central sections*) The results are shown in Table XIX. The population density is high in both sections, but it is to be noted that the higher population density in the first section is due to a greater number of clumps per sample (8·8 against 5·1) rather than to an increase in the

mean number of nauplii per clump (1·852 and 1·889). The Neyman and Thomas series both give a $P(\chi^2) > 0{\cdot}05$.

Microcalanus copepodite stage I. This was the only copepod of which stages other than nauplii were taken in numbers sufficient for analysis in the small volume of water selected for these experiments. The results are shown in Table XX. Poisson is a very good fit for the initial section of stage I. For the

TABLE XVIII. SERIES II. ANALYSIS OF FREQUENCY DISTRIBUTIONS (1)

(Pn., Poisson; Ny., Neyman; Th., Thomas; C.D., coefficient of dispersion.)

	Oithona nauplii (all samples)	Rare species (all together)	*Pseudocalanus* nauplii (central section)	*Temora* nauplii (central section)	Lamellibranch larvae (central section)
Mean	1·758	0·930	1·690	3·640	2·808
Variance	2·377	1·029	1·789	4·250	3·249
C.D.	1·352	1·106	1·059	1·168	1·157
$2\sqrt{[2n/(n-1)^2]}$	0·163	0·164	0·212	0·212	0·260
Ny., m_1	4·999	—	—	—	—
Ny., m_2	0·352	—	—	—	—
Th., m	1·474	—	—	—	—
Th., λ	0·192	—	—	—	—
$P(\chi^2)$					
Pn.	0·3–0·2	0·9–0·8	0·5–0·3	0·5–0·3	0·7–0·5
Ny.	0·5–0·3	—	—	—	—
Th.	0·5–0·3	—	—	—	—

Frequency class	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Ex-pected Pn.	Found	Ex-pected Pn.	Found	Ex-pected Pn.	Found	Ex-pected Pn.
0	**62**	52·0	68·5	69·2	**121**	118·4	**36**	33·2	**7**	4·7	**6**	7·2
1	**92**	91·4	84·7	84·1	**109**	110·1	**57**	56·1	**17**	17·1	**24**	20·3
2	**68**	80·5	67·3	67·3	**48**	51·2	**37**	47·4	**33**	31·2	**32**	28·5
3	**45**	47·2	41·7	41·9	**18**	15·9	**32**	26·7	**39**	37·9	**21**	26·7
4	**22**	20·8	22·0	22·1	**2**	3·7	**13**	11·3	**27**	34·6	**17**	18·8
5	**6**	7·3	10·3	10·3	**0**	0·7	**4**	3·8	**26**	25·3	**9**	10·5
6	**4**	2·1	4·4	4·4	**2**	—	**1**	1·5	**11**	15·4	**7**	4·9
7	**1**	0·5	1·7	1·7	—	—	—	—	**10**	8·0	**2**	2·0
8	**1**	0·1	0·6	0·6	—	—	—	—	**7**	3·6	**1**	0·7
9	**0**	0·1	0·2	0·2	—	—	—	—	**3**	2·2	**1**	0·4
10	**0**	—	0·1	0·1	—	—	—	—	—	—	—	—
11	**1**	—	0·5	0·1	—	—	—	—	—	—	—	—

central section the numbers of stage I and the frequency classes are small, and the χ^2 test is therefore very insensitive, but a tendency to aggregation is clear.

Microcalanus copepodite stage II (*central section only*). Here again the numbers and the frequency classes are small but the same tendency as seen in stage I is apparent.

Microcalanus copepodite stage III (*initial section only*). Again with the low population density the numbers and frequency classes are low. However, Poisson gives a fair fit.

TABLE XIX. SERIES II. ANALYSIS OF FREQUENCY DISTRIBUTIONS (2)

(Pn., Poisson; Ny., Neyman; Th., Thomas; C.D., coefficient of dispersion.)

Microcalanus pygmaeus nauplii

	Early section	Central section
Mean	16·330	9·600
Variance	37·759	22·664
C.D.	2·312	2·360
$2\sqrt{[2n/(n-1)^2]}$	0·286	0·233
Ny., m_1	12·450	7·060
Ny., m_2	1·312	1·360
Th., m	8·816	5·082
Th., λ	0·852	0·889
$P(\chi^2)$		
Pn.	<0·05	<0·05
Ny.	0·7–0·5	0·7–0·5
Th.*	0·5–0·3	0·8–0·7

Frequency class	Early section Found	Early section Expected Pn.	Early section Expected Ny.	Early section Expected Th.	Central section Found	Central section Expected Pn.	Central section Expected Ny.	Central section Expected Th.
0	**0**	0	0	0	**0**	0	0·8	0·9
1	**0**	0	0·1	0·1	**2**	0·1	1·9	2·0
2	**0**	0	0·1	0·2	**4**	0·5	3·7	3·7
3	**0**	0	0·3	0·3	**3**	1·5	5·8	5·8
4	**2**	0	0·6	0·6	**5**	3·6	8·0	8·0
5	**0**	0·1	1·0	1·0	**8**	6·9	10·0	9·9
6	**2**	0·2	1·6	1·6	**16**	11·1	11·6	11·5
7	**0**	0·5	2·2	2·2	**13**	15·2	12·5	12·5
8	**6**	1·0	3·0	3·0	**12**	18·2	12·9	12·9
9	**3**	1·8	3·8	3·8	**13**	19·4	12·7	12·7
10	**2**	3·0	4·5	4·5	**15**	18·6	11·9	12·0
11	**7**	4·5	5·2	5·2	**15**	16·3	10·9	10·9
12	**8**	6·1	5·8	5·8	**9**	13·0	9·6	9·6
13	**4**	7·6	6·2	6·3	**9**	9·6	8·2	8·2
14	**4**	8·9	6·4	6·5	**7**	6·6	6·8	6·9
15	**11**	9·7	6·4	6·5	**4**	4·2	5·5	5·6
16	**6**	9·9	6·3	6·5	**4**	2·5	4·4	4·4
17	**8**	9·5	6·0	6·2	**6**	1·4	3·4	3·4
18	**6**	8·6	5·6	5·8	**2**	0·8	2·6	2·6
19	**4**	7·4	5·1	5·4	**0**	0·4	1·9	2·0
20	**4**	6·1	4·6	4·8	**2**	0·1	1·4	1·4
21	**4**	4·7	4·0		**1**	—	3·5	3·1
22	**3**	3·5	3·5		—	—	—	—
23	**3**	2·5	2·9		—	—	—	—
24	**2**	1·7	2·5		—	—	—	—
25	**3**	1·0	2·0		—	—	—	—
26	**1**	0·7	1·6		—	—	—	—
27	**0**	0·4	1·3	23·7	—	—	—	—
28	**2**	0·2	1·0		—	—	—	—
29	**1**	0·1	0·8		—	—	—	—
30	**3**		0·6		—	—	—	—
31	**0**	0·3	0·5		—	—	—	—
32	**0**		0·4		—	—	—	—
33	**1**		4·1		—	—	—	—

* Calculated with later frequency classes grouped as shown.

Table XX. Series II. Analysis of Frequency Distributions

(Pn., Poisson; Ny., Neyman; Th., Thomas; c.d., coefficient of dispersion.)

Microcalanus pygmaeus copepodites

	Stage I (initial section)	Stage I (central section)	Stage II (central section)	Stage III (initial section)
Mean	2·217	0·353	0·529	0·560
Variance	2·783	0·468	0·675	0·675
c.d.	1·255	1·325	1·276	1·205
$2\sqrt{[2n/(n-1)^2]}$	0·371	0·261	0·261	0·274
Ny., m_1	8·686	1·087	1·925	2·727
Ny., m_2	0·255	0·325	0·275	0·205
Th., m	1·952	0·292	0·461	0·507
Th., λ	0·136	0·205	0·148	0·105
$P(\chi^2)$				
Pn.	0·7–0·5	<0·05	0·5–0·3	0·1–0·05
Ny.	0·8–0·7	—	—	—
Th.	0·9–0·8	—	—	—

Frequency class	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Expected Pn.	Expected Ny.	Expected Th.
0	**8**	6·5	8·5	8·5	**89**	83·6	88·0	88·9	**74**	70·1	74·8	75·1	**67**	62·3	65·7	65·7
1	**16**	14·5	14·6	14·4	**20**	29·5	22·4	21·2	**32**	37·1	30·1	30·0	**27**	34·9	30·0	30·0
2	**14**	16·1	14·4	14·3	**8**	5·2	6·5	6·9	**10**	9·8	10·1	10·3	**11**	9·8	9·9	10·0
3	**9**	11·9	10·5	10·5	**2**	0·7	2·3	2·0	**1**	1·7	2·8	2·9	**4**	2·0	3·4	3·3
4	**7**	6·6	6·3	6·1	—	—	—	—	**2**	0·3	1·2	0·7	—	—	—	—
5	**2**	2·9	3·3	2·8	—	—	—	—	—	—	—	—	—	—	—	—
6	**4**	1·5	2·4	3·4	—	—	—	—	—	—	—	—	—	—	—	—

The Hauls of Series III

In this series it was hoped to obtain further information both on the frequency distributions of populations and on their vertical relations. The set up was as follows.

Samples were pumped continuously for a period of about 5 hr., during which time the boat was allowed to drift. With the hose at a depth of 1 m., twenty samples were taken as in the previous series. The hose was then lowered to 5 m., and, after washing out with water from that depth, a further twenty samples were collected. This was repeated at 8 and 10 m. The hose was then brought back to 1 m. and the sequence of samples and depths repeated five times. There are therefore four series of depths, twenty samples at each depth, and what may be termed five periods, each period corresponding to a complete vertical set of four depths, giving a total of 400 samples. Each set of twenty samples took about 10–15 min., each period, therefore, 40–60 min. and five periods 200–300 min.; in point of fact the whole series took 5 hr. (less 12 min.). Salinities and temperatures were determined for each depth corresponding to the sets of twenty samples from that depth, (see p. 236). Such a division into periods has, of course, an arbitrary character since changes *may* have been taking place at different depths at different rates. However, it is the most natural grouping that can be adopted.

On this occasion the more numerous species were: *Calanus finmarchicus* (Gunnerus), *Centropages hamatus* (Lilljeborg), *Temora longicornis* (Müller), *Acartia clausi* Giesbrecht, *Oithona similis* Claus, all as nauplii, *Calanus* eggs and lamellibranch larvae. Attention will first be confined to copepod nauplii and eggs.

For the analysis of variance the twenty samples of any set have been grouped into four sets of five which are considered as replicates for the given depth and period. The analysis is shown in Table XXI.

Table XXI

Source of variation	Degrees of freedom	Mean square
Periods (P)	4	0·4866
Depths (D)	3	0·5532
P × D	12	0·1768
Residual 1	60	0·0312
Species (S)	5	27·1112
P × S	20	0·1889
D × S	15	0·5616
P × D × S	60	0·0890
Residual 2	300	0·0147

The values for the mean squares indicate a complex situation. If the results are again to be interpreted as applicable to this experiment only, then, when the mean squares are tested against the appropriate residual, they are found to be significant. It is perhaps simpler, under these circumstances, to

consider the organisms separately. The mean squares are given in Table XXII, the degrees of freedom of each source of variation being the same throughout. Again for this particular experiment all the mean squares, with few exceptions, are significant. Thus had the catches for, say, *Calanus* nauplii been obtained by drawing a net vertically through the water column from 10 to 0 m. at each period, then a significant difference would have been found between the catches, equivalent to a significant value of P, the summing over depths being done by the technique. Similarly, the significance of D would have been found had horizontal hauls been taken at the appropriate levels over the given periods, the summation again being done by the sampling technique. The

TABLE XXII. SERIES III. THE MEAN SQUARES

Source of variation	Degrees of freedom	*Calanus* nauplii	*Calanus* eggs	*Centropages*	*Temora*	*Acartia*	*Oithona*
Period (P)	4	0·3183	0·0466	0·2978	0·4128	0·2333	0·0975
Depth (D)	3	0·3211	0·0499	0·2880	1·7440	0·6520	0·3060
P × D	12	0·1129	0·0372	0·0829	0·2525	0·0627	0·0741
Residual	60	0·0134	0·0053	0·0155	0·0225	0·0199	0·0286

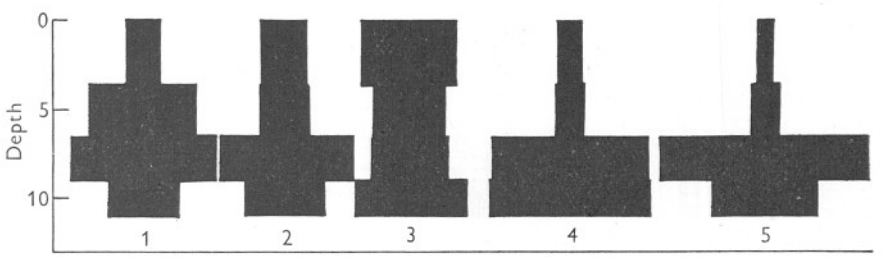

Fig. 2. Data for series III, for *Temora longicornis* nauplii, showing percentage occurrence of total at various depths.

significance of the P × D interaction can be illustrated by using the results for *Temora* in a more conventional way. In Fig. 2 the proportions at the various depths are shown as a percentage of the total, the value at each depth being taken to represent a column reaching half-way between that depth and the depth sampled above and below. The change in that proportion as one passes from period to period is clearly evident. In view of the time of day and the shortness of the interval between two consecutive periods it is hardly possible that these changes represent regular vertical migrations of the *Temora* population between 1 and 10 m. throughout the area of which these catches are samples. However, it must be pointed out that similar changes, based on catches expressed as percentages, and taken at intervals not longer than the beginning and the end of this series, have been held to imply vertical migration, although in such work the regularity of the change over longer periods and its correlation with factors such as light intensity strengthen the argument.

Fig. 3 represents the populations sampled in series III, and salinities are shown in the same type of figure. The salinity samples were taken as far as possible at the mid-point in time of the equivalent set of plankton samples, and the large range of values emphasizes that conditions in this area are not necessarily typical of the open sea.

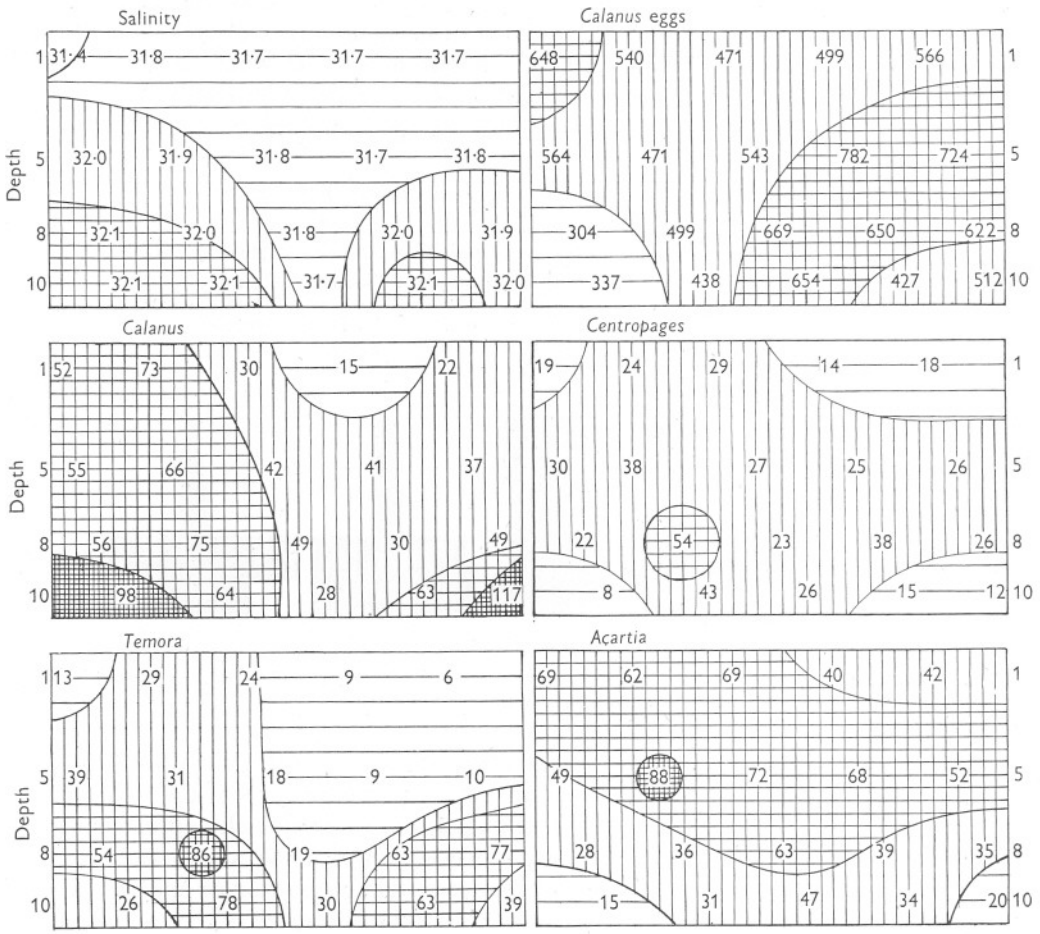

Fig. 3. Population density distributions compared with salinity (top left), for all hauls of series III. Vertical, depth in metres. Horizontal, time (assumed to represent some sort of spatial scale owing to drift). Each diagram shows up to four grades of shading indicating numerically: salinity (‰), <31·6, 31·6–31·8, 31·8–32·0, >32·0; *Calanus* eggs, <400, 400–600, >600; all nauplii, <20, 20–50, 50–80, >80.

The boundaries of the copepod nauplius populations are again selected somewhat arbitrarily, but they are in the main substantiated by variance analyses which need not be quoted. The arbitrary horizontal axis is a time-scale, but as

has already been noted (p. 236) it presumably corresponds with spatial changes in the water.

There is considerable resemblance between all these diagrams, including that for salinity. The regions of high salinity at 8 and 10 m., at the beginning and end of the series, are marked by distinct populations in most of the species, although the relative population density varies according to the species. Thus with *Calanus* nauplii these are regions of high, but with *Centropages* and *Acartia* of low, population density. The population density of these two areas is, however, similar for any one species and had greater depths been sampled in the central period this area might have extended across the figure.

In the third period the low salinity water extends to 10 m., and with *Calanus*, *Temora* and *Centropages* nauplii the populations at all depths during that period are fairly uniform. At the surface, or the surface and 5 m., there is in most species an area of low numbers within the low salinity area.

In general, although the population boundaries are not defined by salinity boundaries, they are most often contained within them; only occasionally does a uniform population spread over a wide range of salinities as it does, for example, for *Acartia* nauplii at 1 and 5 m. and for *Calanus* nauplii during the first two periods.

In several cases the population boundaries are sharp (e.g. *Temora*), but in others there is a suggestion that the nauplii have spread out from one or two centres of high population density. Thus for *Calanus* nauplii there are two apparent centres of distribution in deep water corresponding with high salinity water, and for *Acartia* nauplii one centre at 5 m. from which, although it is situated in low salinity water, the high numbers are spreading out across the boundaries into higher salinity water.

If in the early developmental stages regions of high population density exist, resulting from the production of a large number of eggs, then as time goes on there will be a tendency for these to become dispersed through physical forces, and such information would be essential for a complete interpretation of the figures.

The physical background has been emphasized in suggesting how these populations may be developed, maintained and eventually disseminated, but it must be remembered that the work has dealt mainly with nauplius stages. Clearly with later stages which perform vertical migrations a population could not in this way be restricted to a small water mass. We do not wish to maintain that a given population is always associated with a given mass of water as characterized by its salinity (see above) and it is possible too that a small body of water, apparently homogeneous, may contain separate plankton populations. A large body of water, homogeneous physically by the usually accepted standards, may certainly contain many populations (cf. Baldi *et al.* 1945; Rae & Rees, 1947). However, coincidence of physical conditions and nauplius populations have also been demonstrated above, and it is possible

that quite small physical differences which make up what might be termed the 'microclimate' would, on detailed investigation, be found to exist in the regions where those observations were made. It would be interesting to extend this work by trying to follow the history of some of these small zooplankton patches and to apply a similar technique to later developmental stages.

The Populations of Series III

Some of the uniform populations were sufficiently extensive to enable frequency distributions to be formed. Since, however, the results were of very

Table XXIII. Series III. Analysis of Frequency Distributions

(Pn., Poisson; Ny., Neyman; Th., Thomas; c.d., coefficient of dispersion.)

	Oithona	*Temora*	*Centropages* 1	*Centropages* 2
Mean	1·825	1·690	5·810	5·160
Variance	2·316	1·690	9·550	5·518
c.d.	1·269	1·000	1·644	1·069
$2\sqrt{[2n/(n-1)^2]}$	0·201	0·320	0·286	0·320
Ny., m_1	6·784	—	9·026	—
Ny., m_2	0·269	—	0·644	—
Th., m	1·597	—	4·232	—
Th., λ	0·143	—	0·373	—
$P(\chi^2)$				
Pn.	<0·05	0·5–0·3	0·1–0·05	0·3–0·2
Ny.	0·3–0·2	—	0·7–0·5	—
Th.	0·5–0·3	—	0·5–0·3	—

Frequency class	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Expected Pn.	Found	Expected Pn.	Expected Ny.	Expected Th.	Found	Expected Pn.
0	**42**	32·2	40·4	40·5	**17**	14·8	**3**	0·3	1·4	1·5	**0**	0·5
1	**59**	58·8	56·3	56·6	**20**	25·0	**3**	1·7	4·2	4·2	**5**	2·4
2	**36**	53·7	46·8	46·8	**22**	21·1	**6**	5·1	7·8	7·8	**6**	6·1
3	**34**	32·7	29·5	29·6	**16**	11·9	**13**	9·8	10·9	10·9	**15**	10·5
4	**18**	14·9	15·4	14·7	**5**	7·3	**10**	14·2	12·9	12·8	**10**	13·6
5	**6**	5·4	7·0	5·8	—	—	**14**	16·5	13·3	13·2	**10**	14·0
6	**5**	2·2	4·6	6·0	—	—	**16**	16·0	12·3	12·3	**8**	12·0
7	—	—	—	—	—	—	**6**	13·3	10·5	10·5	**9**	8·9
8	—	—	—	—	—	—	**10**	9·7	8·3	8·4	**9**	5·7
9	—	—	—	—	—	—	**9**	6·2	6·0	6·3	**8**	6·3
10	—	—	—	—	—	—	**4**	3·6	4·3	4·4	—	—
11	—	—	—	—	—	—	**2**	1·9	2·9	3·0	—	—
12	—	—	—	—	—	—	**0**	0·9	1·9	1·9	—	—
13	—	—	—	—	—	—	**1**	0·4	1·2	1·2	—	—
14	—	—	—	—	—	—	**2**	0·2	0·7	0·7	—	—
15	—	—	—	—	—	—	**1**	0·1	1·4	0·9	—	—

much the same type as those already given, it is not necessary to give them for each population. A selection is given as follows:

Oithona. Here Poisson is not an adequate fit but Neyman and Thomas are a good fit to the data.

Temora. Here Poisson is an adequate fit, indicated by a $P(\chi^2)$ value of 0·5–0·3, although again the 0-frequency class is in excess.

Centropages 1. The population density is high (5·81), yet Poisson gives an adequate fit. However, the agreement in earlier classes is to some extent the result of grouping.

Centropages 2. Here, as with the previous distribution, Poisson is an adequate fit, even though again the population density is high (see p. 261).

The Sampling Variations

The results from series I and II are shown in Tables VII, VIII, XVIII, XIX and XX, and the numerical data in the top halves of these tables should be compared. (The number per m.^{3}, if required, is readily obtained by multiplying

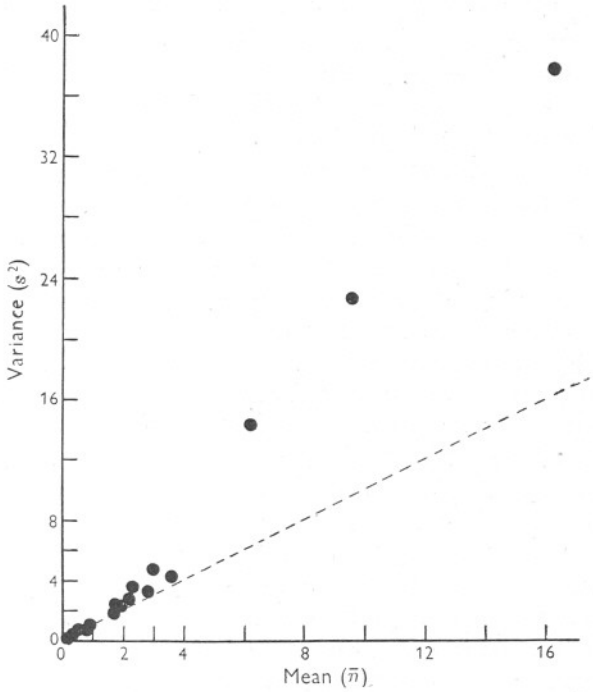

Fig. 4. The relation between the variance (s^2) and the mean ($\bar{n}$) of the populations from series I (Tables VII and VIII) and series II (Tables XVIII, XIX and XX).

the mean by 170.) In Fig. 4 the mean population density is plotted against the variance using the values from series I and II. It is clear that as the population density increases the sampling variance increases. If all the populations were randomly distributed then points would be scattered about a line corresponding to mean population density = variance (as indicated in Fig. 4). Only at low population densities is there a close approach to a random

distribution. As the population density increases there is clear evidence of aggregation, i.e. the chance of an organism being present is increased by the presence of an organism already there, so that the frequency distributions fit those of a contagious series.

It is now possible to explain the observed distribution of χ^2 in previous work both with nets and pumps. The variation, particularly noted at high population densities, is to be ascribed largely to the greatly increased true sampling variation with increasing population density. As will be discussed later, this change depends to some extent on the biological character and history of the population, but inspection of the above tables shows that in general the Poisson distribution begins to be an inadequate fit when the population density exceeds a value of 1000 organisms per m.^{3}. Now in the early work (Tables II and III) the great change in the mean value of χ^2 begins in the 170–400 n_1+n_2 group, increases considerably at the 400–1000 group, becoming very great at the >1000 group. The change can be taken at somewhere in the region of a population density of 200–300 organisms per sample. In the pump samples about 200 l. of water were taken, so this corresponds to a population density of 1000–1500 organisms per m.^{3}, agreeing with the above calculation. The change can therefore be ascribed largely to sampling variations dependent upon the population sampled, and not to inadequacies in sampling technique. With the net samples the increase in χ^2 began at catches of the same order, and nominally a much larger volume, about 11 m.^{3}, was filtered. The change is therefore taking place at a lower population density than expected. This may in part still be ascribed to errors of technique, such as variations in the volume filtered, but in contrast to a pump haul taken at a single depth it should also be remembered that in a net haul a whole series of populations even of any one organism can be sampled at different levels of the haul, so that the organisms may be concentrated in a relatively small proportion of the volume filtered. Under these circumstances minimal population densities are obtained by the use of 11 m.^{3} volume.

The preliminary examination of the data indicated that the standard deviation was roughly proportional to the mean. In Fig. 4 the estimated variance is plotted against the mean, and a shallow curve is obtained. The form of the curve and general consideration of heterogeneous distributions (see Anscombe, 1949) suggest that an equation of the following type expresses the relation between variance and mean

$$\sigma^2=\bar{n}+c\bar{n}^2, \tag{1}$$

or in a more convenient form as

$$\frac{\sigma^2}{\bar{n}}=1+\frac{1}{k}\bar{n}. \tag{2}$$

There are a number of sets of estimates of $1/k$ (one from each distribution) and

Anscombe has shown that an unbiased estimate of the value for any set, $1/\hat{k}_i$, is given by

$$\frac{1}{\hat{k}_i}=\frac{s_i^2-\bar{n}_i}{\bar{n}_i^2}\left(1+\frac{s_i^2}{N_i\bar{n}_i^2}\right), \tag{3}$$

where s_i^2, $\bar{n}_i$ are the estimated variance and mean for the ith set, and N_i is the number of samples in that set. The values of $1/\hat{k}_i$ are shown in Table XXIV. There is some variation but no apparent correlation of this value with the mean. (Four high values, one from Table VII and three from Table XX, stand out as somewhat different from the rest, and it is of interest to note that in contrast to the other data, which refer to nauplii, these values are derived from eggs and copepodite stages.)

TABLE XXIV

Table	Population	Estimated $1/\hat{k}_i$	Table	Population	Estimated $1/\hat{k}_i$
VII	*Oithona* nauplii	0·112	XIX	*Microcalanus* nauplii (initial)	0·080
VII	Lamellibranch larvae	0·208			
VII	Eggs	1·767	XIX	*Microcalanus* nauplii (central)	0·142
VIII	*Pseudocalanus* (1–60)	0·053			
VIII	*Microcalanus* (1–60)	0·093	XX	*Microcalanus* copepodites, stage I (initial)	0·116
VIII	*Pseudocalanus* (61–120)	0·262			
VIII	*Microcalanus* (61–120)	0·212	XX	*Microcalanus* copepodites, stage I (central)	0·949
XVIII	*Oithona* nauplii	0·201			
XVIII	Rare species	0·115	XX	*Microcalanus* copepodites, stage II (central)	0·532
XVIII	*Pseudocalanus* nauplii	0·035			
XVIII	*Temora* nauplii	0·046	XX	*Microcalanus* copepodites, stage III (initial)	0·373
XVIII	Lamellibranch larvae	0·056			

Now although an attempt has been made to fit distributions to these populations, an efficient estimate of a common value for the k's can, none the less, be obtained. The method used is equivalent to taking an appropriately weighted average of the separate estimates of $1/\hat{k}$, and $\hat{k}$ is chosen so that it satisfies the equation

$$\Sigma T_i(\hat{k})=0,$$

where

$$T_i(\hat{k})=\frac{(N_i-1)s_i^2-(N_i-1-\hat{k}^{-1})(\bar{n}_i+\bar{n}_i^2/\hat{k})}{(\bar{n}_i+\hat{k})^2}. \tag{4}$$

If the value for eggs (Table VII) is ignored, $1/\hat{k}=0{\cdot}121$; if the high values from Table XX (copepodites) are also ignored the values of $1/\hat{k}=0{\cdot}118$. It is sufficient to take a value of 0·12.

It will be remembered that the equation originally derived from paired observations on the assumption of a random distribution of organisms and variability due to variable volume of water filtered was

$$\chi^2=1+K^2n, \tag{5}$$

where K^2 lay between 0·04 and 0·05. For paired observations $\chi^2=s^2/\bar{n}$, and

clearly the equation is of the same form as that given above where $K^2 = 1/k$ with an estimated value of 0·12. Now K is not to be regarded as constant, as Winsor & Walford pointed out (they were, for example, not dealing with nauplius stages of copepods, but with a wide variety of organisms), and indeed Silliman (1946) in pilchard egg investigations obtained a value of $K^2 = 0{\cdot}12$, and here the agreement is striking. Now equation (1) was derived from experiment in which the volume of the samples was carefully controlled; the variability can therefore be ascribed to non-random distribution of the organisms (perhaps superimposed on some sampling variation), and it would seem an adequate explanation for Winsor & Walford's results.

Discussion

In the previous sections some of the features of the animal populations sampled by pump during periods of from 2 to 5 hr. have been considered. The results, however, have a bearing upon plankton work in general, which may now be considered.

Unlike most net catches the volume of water taken in a pump sample is accurately known, being unaffected by many of the factors causing errors in net hauls. Correct estimates of the population are therefore obtained, and these values are readily converted to organisms per m.^{3}, in the present instance, by multiplying by 170. The numbers obtained tend to be larger than those usually reported at this time of the year in this area (Marshall, 1949), which is to be expected since previous estimates have been based on net hauls.

In the present experiments there is no information on the relative movements of ship and water, but the rapid changes in population suggest very strongly the presence of numerous three-dimensional 'swarms' which may be quite small in volume, smaller than those which have been so clearly shown by Hardy & Gunther (1935) for Antarctic plankton, and by several workers for the North Sea, using the continuous plankton recorder (Rae & Rees, 1947). The evidence is in favour of their being restricted laterally and, since the samples of series III were taken at several depths, their vertical limits also are sometimes found to be quite small. If the patterns of Fig. 3 were extended into three dimensions they would give a picture of the situation.

Hitherto most of the work on 'swarms' has been confined to discontinuities in a horizontal direction. Variations in population density in a vertical direction for a given organism are well known, and a movement of these populations is implicit in results which indicate vertical migration. The evidence from the present work relates largely to nauplius stages and these are not known to show diurnal migration. Stress has been laid on the possibility that the 'swarms' are confined in a particular water mass, but if such small 'swarms' exist in the late developmental stages which do show diurnal migration then a similar relation to the water mass would be impossible. If such adult

swarms exist and if they preserve their identity during migration some other force must be responsible. This may be a 'positive' biological force such as causes swarming in other animal groups. Since, however, vertical migration is usually considered to be a response to a change in light intensity, and since this acts only in a vertical direction, the movement may occur much more freely in a vertical than a horizontal direction; with a consequent tendency to reduce horizontal dispersion. A repetition of this type of work extended vertically and horizontally and over a longer period is proving of considerable interest.

This evidence, derived largely from a consideration of nauplii, suggests that the distinct populations maintain their identity for some time, and are gradually broken down by physical forces and reduced by mortality as the organisms go through their development. It might be expected, therefore, that copepods which retain their eggs until the nauplii hatch (*Pseudocalanus* and *Oithona* of those here discussed) would, at a given time from hatching, be less widely distributed than those laid singly in the water (the other copepods concerned). There is no evidence about the age from hatching of the nauplii caught, and strict comparisons between the two types cannot therefore be made. It is interesting to observe, however, that in series I the *Pseudocalanus* at a mean population density of 2·300 has a coefficient of dispersion of 1·596, whereas *Microcalanus* with a higher mean population density (2·867) has a coefficient of dispersion of only 1·152. Further, *Temora* in series II with a mean population density of 3·640 and *Centropages* in series III (population density 5·160) have coefficients of dispersion of 1·168 and 1·069, respectively. The results from *Oithona* are in general agreement.

Summary

A brief review of previous work on the sampling variation encountered in the course of plankton work is given, and leads to the suggestion that the observed variability is not entirely accounted for by technical errors. A non-random distribution (statistical) of the population is suggested.

If the distribution is not random (Poisson) then the application of contagious distributions should be considered. A short account of two such distributions, Neyman's and Thomas's, is given; the parameters of the latter seem to be more readily interpreted in plankton sampling.

An account of the method of collection and counting of numerous small samples taken continuously over several hours is given. In the first two series the collections were made at a constant depth. The results suggest the existence of comparatively well-defined populations of a number of organisms, chiefly copepod nauplii. Series III, in which samples were taken at several depths, confirms this suggestion and indicates a similar state of affairs in a vertical direction. The population changes, it is suggested, might be

associated with different masses of water which have maintained their identity over a period during which the populations have been developed, and the association of a number of species maintained.

Frequency distributions are set up, and in general when the population density is low the distribution closely approaches that of Poisson. At higher population densities the Neyman and Thomas series give a better fit, indicating clumping of the organisms. An estimate of the mean number per clump is obtained.

The results are considered in relation to Winsor & Walford's work and it is shown that the sampling variation can be explained as dependent upon the non-random distribution of the organisms sampled. This explanation is adequate for earlier data.

REFERENCES

ANSCOMBE, F. J., 1949. The statistical analysis of insect counts based on the negative binomial distribution. *Biometrics Amer. Stat. Assoc.*, Vol. 5, pp. 165–73.

—— 1950. Sampling theory of the negative binomial and logarithmic series distributions. *Biometrika*, Vol. 37, pp. 358–82.

ARCHIBALD, E. E. A., 1948. Plant populations. I. A new application of Neyman's contagious distribution. *Ann. Bot.*, N.S., Vol. 12, pp. 221–35.

—— 1950. Plant populations. II. The estimation of number of individuals per unit area of species in heterogeneous plant populations. *Ann. Bot.*, N.S., Vol. 14, pp. 7–21.

ASHBY, E., 1936. Statistical ecology. *Bot. Rev.*, Vol. 2, pp. 221–35.

—— 1948. Statistical ecology. II. A reassessment. *Bot. Rev.*, Vol. 14, pp. 222–45.

BALDI, E., CAVALLI, L. L. & PIROCCHI, L., 1945. Condizioni della distribuzione del mesoplancton nel pelago di un grande lago oligotrofo (Lago Maggiore). *Mems. Istituto Ital. Idrobiol. Milano*, Vol. 2, pp. 253–90.

BARNES, H., 1949*a*. Statistical study of the variation in vertical plankton hauls, with special reference to the loss of the catch with divided hauls. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 429–46.

—— 1949*b*. On the volume measurement of water filtered by a plankton pump, with some observations on the distribution of planktonic animals. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 651–62.

BARNES, H. & STANBURY, F. A., 1951. A statistical study of plant distribution during the colonization and early development of vegetation on china clay residues. *Journ. Ecol.*, Vol. 39, pp. 171–81.

BEALL, G., 1940 The fit and significance of contagious distributions when applied to observations on larval insects. *Ecology*, Vol. 21, pp. 460–73.

BLACKMAN, G. E., 1942. Statistical and ecological studies in the distribution of species in plant communities. I. Dispersion as a factor in the study of changes in plant populations. *Ann. Bot.*, N.S., Vol. 6, pp. 351–70.

CLAPHAM, A. R., 1936. Over-dispersion in grassland communities and the use of statistical methods in plant ecology. *Journ. Ecol.*, Vol. 24, pp. 232–51.

HARDY, A. C. & GUNTHER, E. R., 1935. The plankton of the South Georgia whaling grounds and adjacent waters, 1926–7. *Disc. Rep.*, Vol. 11, pp. 1–456.

LANGFORD, R. R., 1938. Diurnal and seasonal changes in the distribution of the limnetic crustacea of Lake Nipissing, Ontario. *Publ. Ontario Fish. Res. Lab.*, No. 56, pp. 1–142.

MARSHALL, S. M., 1949. On the biology of the small copepods in Loch Striven. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 45–122.

NEYMAN, J., 1939. On a new class of 'contagious' distributions applicable in entomology and bacteriology. *Ann. Math. Stat.*, Vol. 10, pp. 35–57.

PEARSON, K., 1948. *Tables for Statisticians and Biometricians*. Cambridge University Press.

PÓLYA, G., 1931. Sur quelques points de la théorie des probabilités. *Ann. Inst. Henri Poincaré*, T. 1, pp. 117–62.

RAE, K. M. & REES, C. B., 1947. Continuous plankton records: The Copepoda in the North Sea, 1938–1939. *Hull Bull. Mar. Ecol.*, Vol. 2, pp. 95–132.

RICKER, W. E., 1937. Statistical treatment of sampling processes useful in enumeration of plankton organisms. *Arch. Hydrobiol.*, Vol. 31, pp. 68–84.

RUTTNER, F., 1930. Das Plankton des Lunzer Untersees, seine Verteilung in Raum und Zeit während der Jahre 1908–1913. *Int. Rev. Hydrobiol.*, Vol. 23, pp. 1–149, 161–287.

SILLIMAN, R. P., 1946. A study of variability in plankton townet catches of Pacific pilchard *Sardinops caerulea* eggs. *Journ. Mar. Res.*, Vol. 6, pp. 74–83.

SOUTHERN, R. & GARDINER, A. C., 1926. Seasonal distribution of Crustacea of the plankton of Lough Derg and River Shannon. *Sci. Investig. Fish. Ireland*, Vol. 1, pp. 1–170.

THOMAS, M., 1949. A generalization of Poisson's binomial limit for use in ecology. *Biometrika*, Vol. 36, pp. 18–25.

WELCH, P. S., 1948. *Limnological Methods*. Philadelphia.

WINSOR, C. P. & CLARKE, G. L., 1940. A statistical study of variation in the catch of plankton nets. *Journ. Mar. Res.*, Vol. 3, pp. 1–34.

WINSOR, C. P. & WALFORD, L. A., 1936. Sampling variations in the use of plankton nets. *Journ. Cons. Int. Explor. Mer*, Vol. 11, pp. 190–204.

AN APPARATUS FOR THE COLLECTION OF PLANKTON IN THE IMMEDIATE VICINITY OF THE SEA-BOTTOM

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(Text-figs. 1–3)

In connexion with a study of plankton-benthos interrelationships off the coast of Northumberland, an apparatus was designed for the collection of plankton in the immediate vicinity of the sea-bottom, a layer usually missed by ordinary plankton sampling gear. The present account is a brief description of this apparatus, together with some notes on its handling at sea and the work carried out with it on board the Dove Marine Laboratory's boat, the *Pandalus II*.

I was fortunate in having the willing co-operation of numerous people who helped in various ways, and to whom I am very greatly indebted. These included my father, Mr E. Bossanyi; Mr T. E. F. Sanderson of Eastcote, Middlesex; Prof. A. C. Hardy, Oxford University; Dr C. E. Lucas, Fishery Laboratory, Aberdeen; Prof. A. D. Hobson, Director of the Dove Marine Laboratory; Dr H. O. Bull, Assistant Director; Messrs Ellis and Co., Engineering Works, Swalwell, County Durham; the Department of Mechanical Engineering of King's College, Newcastle upon Tyne; and the entire scientific, boat and technical staff as well as research students at the Dove Marine Laboratory. Without them, the design, construction and employment of the apparatus would not have been possible.

Description of the Apparatus

The present apparatus differs from previous designs of bottom-plankton nets (e.g. Russell, 1928; Hardy & Lucas, personal communication), chiefly in being provided with a closing device which operates only by contact with the sea-bottom. It thus eliminates the disadvantage of the earlier designs which necessarily fished both on the way down to and up from the bottom. At the same time, the nature of the closing mechanism avoids the necessity of any extra ropes or cables for messengers etc., and allows the apparatus to be shot rather like a beam trawl from a boat that is slowly moving as well as from a stationary one.

The apparatus is essentially a sledge constructed of tubular steel ($1\frac{1}{4}$ in. diameter) on runners, on which a removable plankton net with bucket is mounted, and bearing in addition two closing doors which fit over the mouth of the net (Figs. 1–3).

The frame consists of two parts, each made in one piece with all joints firmly welded so as to be both strong and waterproof. These are the sledge frame (Fig. 3A), which bears the runners (*u*) and to which the ropes are shackled by means of eyes, and the net frame (Fig. 3B), which is mounted on its vertical bars. This frame can thus move vertically on the sledge frame with a total range of about a foot and can be clamped at any level within this range by wing nuts at (*v*). The actual net with its bucket fits into the net frame, and is fastened at the front end by hooks fitting into perforated steel laths (*w*) on the net frame, whilst at the rear end the bottom of the bucket is fixed to a steel plate (shown in black) by a wing nut. The net frame bears a coarse steel gauze on its lower aspect to protect the net from below, and also the doors, each of which is welded to it by two strong hinges at (x, x'; y, y'). All cross-bars of both frames are above the level of the runners.

The net (Fig. 1) is a cone of four sections sewn together, and is constrained into a rectangular shape at its mouth. As in ordinary plankton nets, it has a canvas rim at each end for attachment, achieved, in this net, by means of the hooks already mentioned at the front, and the bucket at the rear.

The bucket (Fig. 1) is constructed on the lines of Hensen's quantitative bucket (Hensen, 1895, pp. 69–71), with a non-filtering lower portion from which there is an outflow (corked during a tow), and a filtering upper portion consisting of the finest bolting silk (200 meshes/in.) which enables the bucket to be used with nets of that or coarser mesh. It is fastened to the net by a threaded rim which screws with a few turns into a ring clamped to the rear canvas of the net. When several nets of different mesh but of the same size and shape are in use therefore, one and the same bucket can easily be fixed to any one net, provided each of these has its own threaded ring clamped to it.

The doors (Fig. 3C) each consist of two steel plates movably bolted to one another. The door proper is permanently welded to the net frame by the hinges already referred to, and closes half of the net mouth-opening. The arm projects out at right angles to the whole apparatus when the door is shut, and its lower end projects 2–3 in. or more below the level of the runners. Because the arm is adjustable relative to the door proper, it can always be kept at this level whatever the vertical position of the net frame in the sledge frame may be. When the apparatus is lowered to the sea-bed and dragged forwards, the lower end of each arm thus digs into the ground and is swept back so as to lie parallel with the frame, thereby opening the doors forwards. The adjustable springs which are attached to the upper margin of the door proper and the upper net frame bar on each side (z, z') are extended and put under tension by this action, so that when the apparatus and hence the arms are lifted clear of the bottom, they pull the doors shut. Care was taken in the design to ensure that the areas of door and arm on each side of the points of attachment to the hinges are equal, thus cancelling out to all intents and purposes the effect of water resistance. The only effective forces on the 'open-

shut' mechanism therefore are the resistance of the sea-bottom to the arms and the pull of the springs.

The dimensions of the whole apparatus (maximum length 7, maximum width 3, and maximum height 2 ft. approximately), are determined by those of the net it contains. These in turn are determined by two factors: the net must be large enough to obtain a reasonable catch in a short time, and its filtering area must be large enough relative to its mouth area to allow efficient filtering to take place at ordinary plankton net towing speeds (about 1 knot). A consideration of the dimensions of various commonly used nets such as described by Ostenfeld & Jespersen (1924) and Künne (1929, 1933) led to the conclusion that a ratio of 6:1 would in practice meet this requirement, and the present net was made accordingly.

Handling the Apparatus at Sea

Owing to its considerable weight (about $1\frac{1}{2}$ cwt.) the apparatus cannot be handled without either a derrick or a davit with a winch. Given these requirements, together with a minimum of three people who have learnt the necessary drill for shooting and hauling, the apparatus can be quite comfortably used even in a moderately choppy sea, though in the latter case the steersman must take care to keep the vessel stern into the waves to reduce swaying to a minimum. Two fairly heavy ropes are necessary ($2\frac{1}{4}$ in. circumference), one of which, the lifting rope, is used solely for lowering the apparatus to the bottom and later raising it from just beneath the surface on to the deck, whilst the other, the tow rope, is employed for towing the apparatus along the bottom and hauling in to just below the surface afterwards. With a davit of sufficient height, the apparatus could be hauled in completely by the tow rope and swung in over the deck, hanging in a vertical position. The disposition of the ropes is shown in Fig. 2.

A good deal of information as regards the working of the apparatus when being towed along the bottom could be gleaned from the presence and position of scratch marks on the runners and lower ends of the arms. These parts were therefore often coated with paint sometime before a haul, and scratch marks observed afterwards (see Fig. 2).

Work done with the Apparatus, 1949–50

The apparatus described here is of course not suitable for use on rocky bottoms. It is doubtful whether any type of sledge arrangement can be used effectively on such grounds. The work carried out with it has been confined to sandy and hard muddy bottoms of which there are great stretches in shallow and deep water off the coast of Northumberland. On these bottoms the apparatus has been found to work very well and a considerable amount of material has been collected with it. It is also planned to use the apparatus on softer muddy

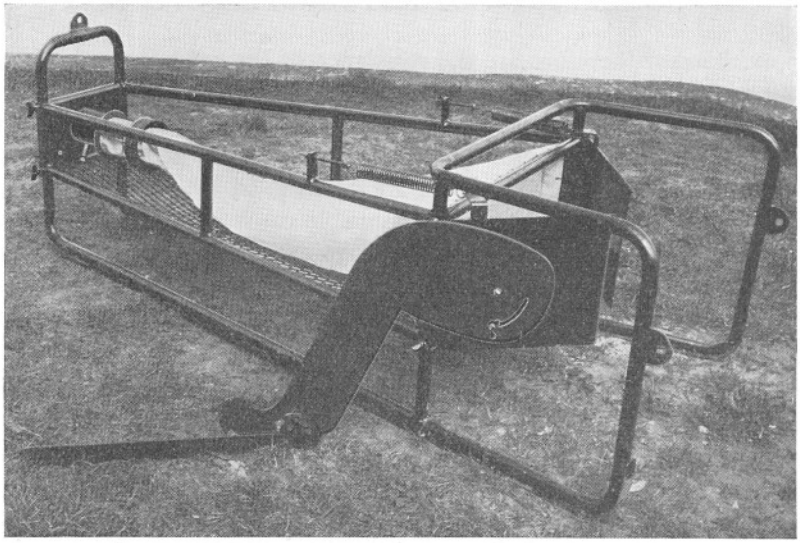

Fig. 1. The whole apparatus assembled with the doors partially opened. The extension on the arm of the door was an experimental addition which proved to be unnecessary.

Fig. 2. The apparatus hanging on the davit and about to descend into the sea, showing the davit swung out overboard, and the lifting and tow ropes. Note also the areas scratched clean on the lower ends of the arms during a previous tow.

bottoms, which may present some difficulties owing to its considerable weight, and on shingle and gravelly areas. Collections of plankton above rocky bottoms will have to be tackled by different means, e.g. using a pump and hose, for the smaller species especially. Apart from various trial runs at different places in the initial stages of the work, the apparatus was used fairly regularly with a net

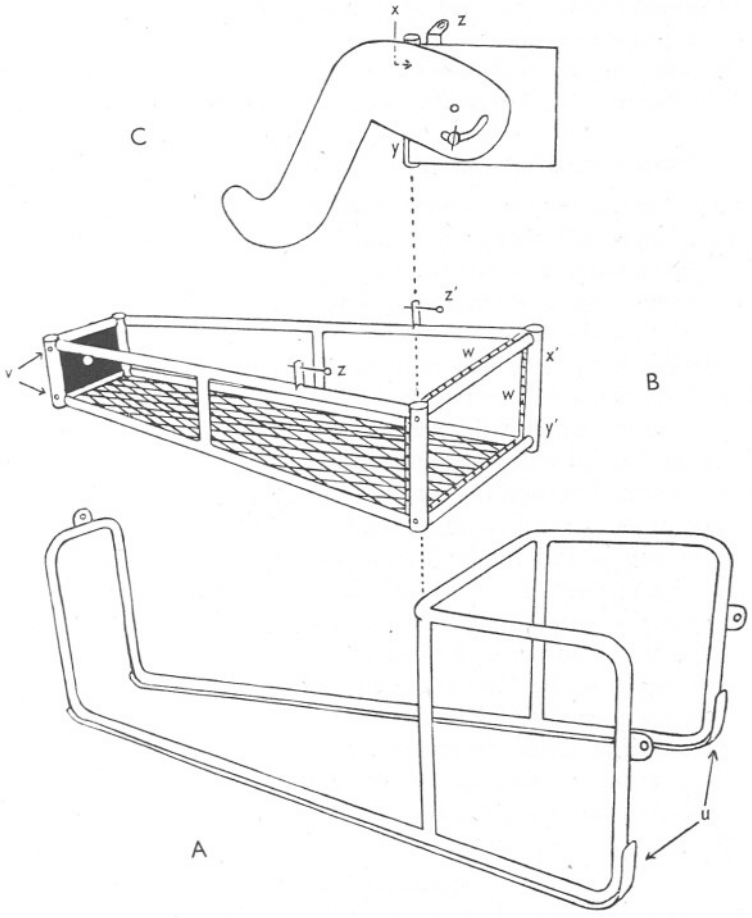

Fig. 3. Portions of the apparatus: A, the sledge frame; B, the net frame; C, one of the doors complete with arm. *u*, the runners; *v*, threaded holes for wing nuts for clamping net frame on sledge frame, also present at the front end; *w*, steel laths with holes 3 in. apart for hooks of net; *x*, *x'*, *y*, *y'*, position of hinges on door and net frame; *z*, *z'*, points of attachment of springs on door and net frame.

of 40 meshes/in. at two stations in the vicinity of Blyth harbour, Northumberland. One of these was in Blyth Bay a few hundred yards offshore in 5–6 fathoms on a sandy bottom; the other in Cambois Bay (north-east of Blyth) in 13–15 fathoms on a bottom of sandy mud. Collections were made at different states of the tide, at different times of the day, and also at night. The

duration of tow varied somewhat according to circumstances, but was usually 20 min. to ensure an adequate catch, though this was often much more than sufficient.

The catches obtained were not appreciably contaminated with sand or mud if the apparatus had been working properly, even with the net frame fixed at its lowest possible position on the sledge frame. Small coal particles, extremely abundant in the sea in this area, however, often contaminated the catches and had to be separated off in the laboratory later.

A detailed analysis of the catches will be discussed in a subsequent paper.

Possible Improvements in Design of the Apparatus

The model employed so far is an experimental one, and a number of improvements have suggested themselves during the course of the work with it. Minor ones include, amongst others, better streamlining of the frame, perhaps a greater range of vertical movement of the net frame on the sledge frame, and the attachment of the bucket to the net by means of a bayonet clip. Total weight cannot be much reduced in future models, especially if the apparatus is to be used in any appreciable depth of water, unless a paravane system is mounted which will carry it to and keep it at the bottom without disturbing the flow of water into and through the net. Such paravanes would have to be mounted beyond the range of the current system through the net.

The door system should be improved by eliminating the arms which project out at right angles and render the apparatus rather more clumsy than is necessary, and placing others *beneath* the frame. In addition, it is possible that the doors may tend to flap whilst the apparatus is being towed and thus influence the water flow into the net and thereby the catch obtained. The doors could therefore be substituted by a vertically moving, flexible shutter operated by such arms acting against the pull of springs.

However, during visual observation of the present apparatus at work in very shallow water, no flapping of the doors was observed and the motion along the bottom was quite satisfactory.

REFERENCES

Hensen, V., 1895. Methodik der Untersuchungen. *Ergebnisse der Plankton-Expedition der Humboldt-Stiftung*, Bd. 1, B, 200 pp.

Künne, C., 1929. Vergleich der Fangfähigkeit verschiedener Modelle von Plankton-Netzen. *Cons. Perm. Int. Explor. Mer, Rapp. Proc. Verb.*, Vol. 59, 37 pp.

Künne, C., 1933. Weitere Untersuchungen zum Vergleich der Fangfähigkeit verschiedener Modelle von vertikal-fischenden Plankton-Netzen. *Cons. Perm. Int. Explor. Mer, Rapp. Proc. Verb.*, Vol. 83, 36 pp.

Ostenfeld, C. H. & Jespersen, P., 1924. Standard net for plankton collections. *Cons. Perm. Int. Explor. Mer, Publ. Circ.* No. 84, 16 pp.

Russell, F. S., 1928. A net for catching plankton near the bottom. *Journ. Mar. Biol. Assoc.*, Vol. 15, pp. 105–8.

THE SEASONAL ABUNDANCE OF YOUNG FISH XI. THE YEAR 1949

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(Text-figs. 1, 2)

The 1949 production of young fish and plankton in Plymouth off-shore waters continued to remain at the low level characteristic of the last few years. Collections were made by half-hour oblique hauls of the 2 m. stramin ring-trawl; the dates are given in Table I. The fortnightly averages of all young fish, excluding clupeids, are shown in Table II with comparative figures for 1948 and for the period 1930–34. The highest value, in the second half of June, may have been unduly weighted, since only one collection was made in the fortnight. The monthly total catches of young fish are given in Table III. The sum of the monthly averages for all young fish (459) was slightly higher than the 1948 value (311[1]), due to larger catches of clupeids; young fish other than clupeids continued to decrease. A few post-larval herring were present in January, and for the third successive year young *Mugil* spp. occurred; two in October and one in December. No young plaice were caught, although a very small number of plaice eggs were present in January and February. Plankton, other than young fish, was very sparse throughout the year, *Calanus* being even scarcer than in 1948. *Sagitta setosa* was the dominant *Sagitta* species, although never abundant, and as in recent years the numbers were particularly low from February to June. A few *S. elegans* occurred in November and December (maximum, 66 specimens). The abundance and percentages of the *Sagitta* species are shown in Fig. 1.

In the account of conditions in 1948, it was noted that small immature *S. setosa* appeared to predominate in the catches, indicating perhaps a further step in the progressive decline of the macroplankton of the area. In order to examine this question, the maturity stages of all the 1949 catches of *setosa* were determined. But the low numbers caught preclude any reliable information concerning trends in the proportions of the stages, and make it difficult to interpret the data regarding the numbers of mature populations and of spawnings during the year. It would seem, however, that there were not more than five mature populations and probably only five spawnings. This, in contrast to at least six breeding populations and eight spawnings in the period September 1930—August 1931 (Russell, 1932), points to a considerably lowered productivity.

[1] Misprinted as 331 in the 1948 report.

Three specimens of *Muggiaea atlantica*, which was present throughout 1948, occurred in January and February. During the summer no *Muggiaea* were caught, until small numbers of *M. kochi* appeared in October–December (maximum, 33 specimens). Three small *Aglantha* were present in the catch of 14 November. *Liriope*, which occured in considerable quantity at the end of 1948, continued to be present in 1949 until 1 February. A single salp was taken on 5 January and one *Themisto* on 20 January.

The numbers of pilchard eggs in the catches are given in Table IV. The monthly averages have again fallen very considerably since the low averages of 1948.

TABLE I. DATES ON WHICH COLLECTIONS WERE MADE, 1949

All 2 miles east of Eddystone, unless otherwise stated

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
3	1*	7	11	2†	8	1	3	1	4	7	8
5*	7	14	13*	4	9*	11	8*	5	6*	8*	12
10	14	21	14	9*	14	22	8	8*	8	14	19
14‡	21	29	25	10	24	26	26	12	10	28	22*
14§	28		27‡N	16				19	17		28
14			29N	24				26	27		
17			29N	30							
20											
24											
31											

* Station E1. † Station L5. N, night collection.
‡ Station L4. § Station L6.

TABLE II. FORTNIGHTLY AVERAGE CATCHES OF ALL YOUNG FISH EXCLUDING CLUPEIDS, 1930–34, 1948 AND 1949

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1930–34 (average):												
1st fortnight	5	10	27	110	635	299	100	152	48	11	7	2
2nd fortnight	8	15	89	374	573	78	85	48	9	6	3	2
1948:												
1st fortnight	+	3	15	12	nr	13	9	9	+	4	0	1
2nd fortnight	0	16	15	nr	65	32	17	5	0	+	+	0
1949:												
1st fortnight	+	1	0	1	10	22	20	22	+	+	1	2
2nd fortnight	+	+	2	11	18	77	26	1	1	0	1	+

nr, no records. + average of less than 1.

It is necessary that a note should be included in these records concerning the quality of the stramin used in the 2 m. ring-trawl. Pre-war stramin has an average of sixteen threads in both warp and weft to the inch when dry and unused. It is evenly woven and there is only slight variation in the thickness of the threads, so that the actual holes between the meshes have a very fair uniformity of size; the threads are also free of projecting tufts. Stramin of this quality became unobtainable after the war. The post-war stramin has an average of fourteen threads in the warp and thirteen in the weft to the inch. It is very unevenly woven, the thickness of the strands is extremely variable and

TABLE III. MONTHLY TOTAL CATCHES OF POST-LARVAE IN HALF-HOUR OBLIQUE HAULS WITH 2 M. STRAMIN RING-TRAWL, 1949

The number of hauls per month is shown by the small figure against each month at the head of the column. A + is used in the 2nd, 4th and 6th lines to denote monthly average of less than 0·5.

	Jan.[10]	Feb.[5]	Mar.[4]	Apr.[7]	May[7]	June[4]	July[4]	Aug.[4]	Sept.[6]	Oct.[6]	Nov.[4]	Dec.[5]	Total	Sum of monthly averages
Total young fish	85	11	3	69	314	304	692	272	27	119	189	15	2100	..
Monthly average, T.Y.F.	9	2	1	10	45	76	173	68	5	20	47	13	..	459
T.Y.F., less clupeids	5	5	1	49	93	144	94	66	4	3	4	5	473	..
Monthly average, ditto	1	1	+	7	13	36	24	17	1	1	1	1	..	103
All clupeid spp.	80	6	2	20	221	160	598	206	23	116	185	10	1627	..
Monthly average, ditto	8	1	1	3	32	40	149	51	4	19	46	2	..	356
Clupea harengus	31	..	..	..	..	..	..	..	..	..	..	..	31	3·1
Gadus pollachius	..	..	..	..	6	1	..	..	..	..	..	..	7	1·11
Gadus merlangus	..	..	..	..	3	5	..	..	..	..	..	..	8	1·68
Gadus minutus	..	..	..	7	..	..	..	..	11	..	..	..	7	1·0
Gadus luscus	3	2	..	..	..	..	..	..	..	..	2	3	10	1·8
Gadus callarias	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Onos spp.	..	..	..	3	26	11	3	..	..	..	..	..	43	7·88
Phycis blennoides	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Molva molva	..	..	..	..	1	..	..	..	..	..	..	..	1	0·14
Merluccius merluccius	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Raniceps raninus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Capros aper	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Zeus faber	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Arnoglossus spp.	..	..	..	..	..	1	8	9	1	..	..	..	19	4·67
Rhombus spp.	..	..	..	..	3	2	..	..	1	..	..	..	6	1·1
Scopthalmus norvegicus	..	..	..	3	1	} 1 {	..	..	..	..	..	..	4 } 9	0·57 } 1·39
Zeugopterus punctatus	..	..	..	2	2		..	..	..	..	..	..		0·57
Zeugopterus unimaculatus	..	..	..	..	..		..	..	..	..	..	..	..	..
Pleuronectes platessa	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Pleuronectes limanda	..	..	..	1	..	..	..	..	..	..	..	..	1	0·14
Pleuronectes flesus	..	..	..	..	4	..	..	..	..	..	..	..	4	0·6
Pleuronectes microcephalus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Solea vulgaris	..	..	..	..	1	10	..	..	..	..	..	..	11	2·64
Solea variegata	..	..	..	2	1	14	..	..	..	..	..	..	17	3·94
Solea lascaris	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Solea lutea	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Serannus cabrilla	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Caranx trachurus	..	..	..	..	..	..	8	20	..	1	..	..	29	7·17
Mullus surmuletus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Morone labrax	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ammodytes lanceolatus	..	..	..	4	20	..	1	..	..	..	..	..	25	3·85
Ammodytes tobianus	..	..	..	} 1 {	..	..	1	..	..	..	..	..	1 } 8	0·25 } 1·49
Ammodytes marinus	..	..	..		..	..	..	..	..	..	..	..	..	..
Ammodytes immaculatus	2	2	1		..	..	..	..	..	..	1	..	6	1·1
Cepola rubescens	..	..	..	..	..	..	..	2	..	..	..	..	2	0·39
Callionymus spp.	..	..	..	2	13	51	6	10	..	..	..	..	82	19·3
Labrus bergylta	..	..	..	..	..	1	..	..	..	..	..	..	1	0·25
Labrus mixtus	..	..	..	..	1	1	..	..	..	..	..	..	2	0·39
Ctenolabrus rupestris	..	..	..	..	5	19	18	2	..	..	..	..	44	10·71
Crenilabrus exoletus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trachinus vipera	..	..	..	..	..	..	3	2	..	..	..	..	5	1·25
Scomber scombrus	..	..	..	..	..	..	16	9	..	..	..	..	25	6·25
Gobius spp.	..	..	..	19	..	..	6	4	..	..	1	..	30	5·46
Lebetus scorpioides	..	..	..	1	1	..	2	1	..	..	..	..	5	1·03
Blennius ocellaris	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Blennius pholis	..	..	..	1	3	3	..	..	..	..	..	..	7	1·32
Blennius gattorugine	..	..	..	..	1	23	21	5	..	..	..	..	50	12·64
Chirolophis galerita	..	1	..	..	..	..	..	..	..	..	..	..	1	0·2
Mugil spp.	..	..	..	..	..	..	..	..	..	2	..	1	3	0·53
Agonus cataphractus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trigla spp.	..	..	..	..	..	1	2	2	..	..	..	..	5	1·25
Cottus spp.	..	..	..	1	1	..	..	..	..	..	..	..	2	0·28
Cyclopterus lumpus	..	..	..	..	..	..	..	..	1	..	..	..	1	0·34
Liparis montagui	..	..	..	1	..	..	..	..	..	..	..	..	1	0·14
Lepadogaster bimaculatus	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Lophius piscatorius	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Pipe fish	..	..	..	..	..	..	..	..	1	..	..	..	1	0·17
Unidentified	..	..	..	..	..	..	..	..	1	..	..	..	1	0·17

there are noticeably more thinner threads than in the pre-war material. In consequence the mesh holes are considerably larger and very much less uniform than in the pre-war stramin. The threads are also very tufted and fuzzy.

Since it was important that this series of observations should continue,

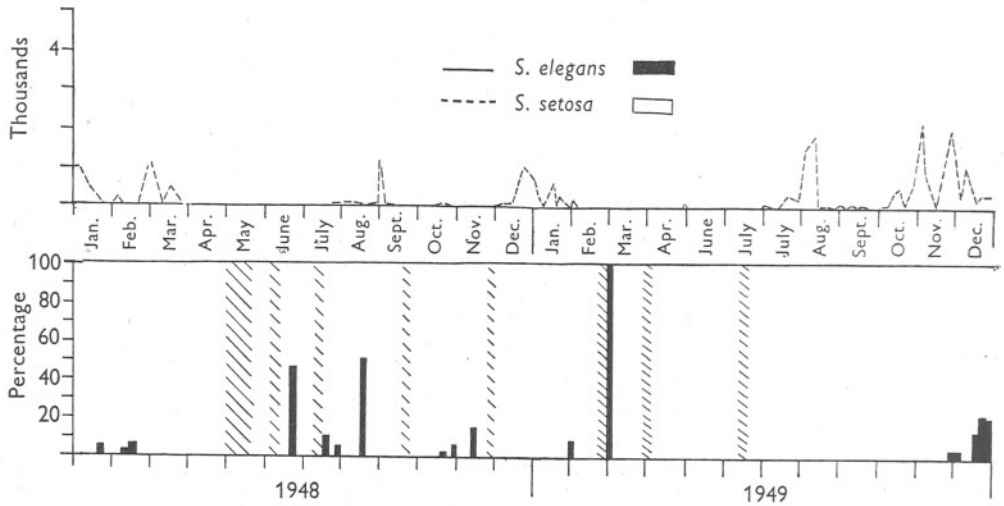

Fig. 1. Above, curves showing the abundance of *Sagitta elegans* (——) and *S. setosa* (– – – – –) in half-hour oblique hauls with the 2 m. stramin ring-trawl in 1948 and 1949. Below, percentage composition of the *Sagitta* populations during the same period: *S. elegans*, black; *S. setosa*, white; no *Sagitta*, hatched. (Continued from Corbin, 1949, p. 710, fig. 1.)

Table IV. Pilchard Egg Catches, 1949

See Table I for dates of hauls

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	0*	0	0*	6	630	420	13	0	42	107	0	4
		3		36	590	716	6	0	20	448	0	3
		63		49	412	1,610	9	0	96	1	1	1
		0		56	184	568	1	24	2	61	40	0
		0		31	1,328				9	1		18
				157	1,160				0	0		
				228	836							
Monthly average, 1949	0	13	0	80	734	828	+	+	28	103	10	+
Monthly average, 1937–39 and 1946–47	+	0	+	478	5,868	14,093	6,196	385	415	305	398	+

\+ Average of less than 10.
* None taken in any haul in these months.

the 2 m. ring-trawls had to be made up in the inferior material despite the discrepancy of mesh size. The post-war stramin has been in use since mid-September 1947.

The larger mesh of the post-war stramin will undoubtedly have resulted in a loss of some of the catch, particularly the smaller organisms. There has certainly been a very marked poverty of copepods in the catches of the last 2 years. On the other hand, considerable numbers of small *Sagitta* and clupeid larvae have at times been caught, and it is possible that the fuzziness of the threads may have had a slight effect in reducing the actual mesh size.

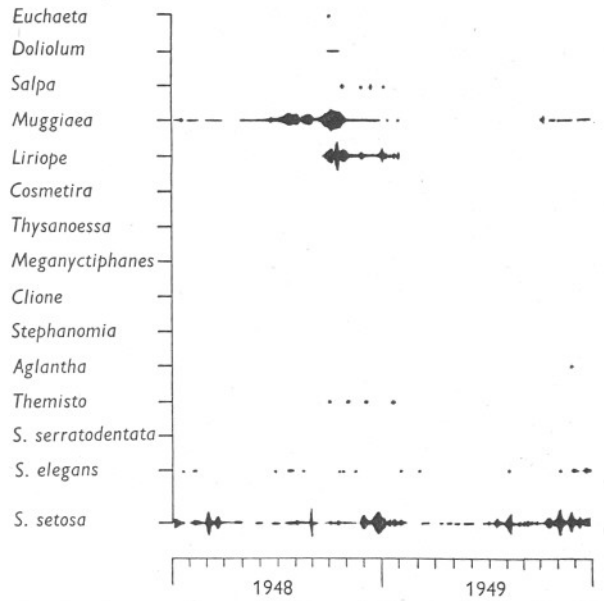

Fig. 2. Diagram showing the occurrence of plankton indicators in the collections off Plymouth in 1948 and 1949. *Note.* The *Muggiaea* present in October–December 1949 were all *M. kochi*. (Continued from Corbin, 1949, p. 711, fig. 2.)

A measure of the loss of catch will be possible by comparative hauls, when stramin of the pre-war quality becomes available.

There is thus some doubt as to the reliability of comparisons between data obtained with the pre- and post-war stramin. Nevertheless, it would seem improbable that major fluctuations would have been obscured, and this appears to be upheld by the continued decline in the sum of the monthly averages of young fish excluding clupeids and in the monthly average catches of pilchard eggs in 1948 and 1949, during both of which years the poorer stramin was in use.

REFERENCES

CORBIN, P. G., 1949. The seasonal abundance of young fish. X. The year 1948. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 707–12.

RUSSELL, F. S., 1932. On the biology of *Sagitta*. II. The breeding and growth of *Sagitta setosa* J. Müller in the Plymouth area, 1930–31, with a comparison with that of *S. elegans* Verrill. *Journ. Mar. Biol. Assoc.*, Vol. 18, pp. 147–60.

NOTE ON A METHOD FOR DETERMINATION OF AMMONIA IN SEA WATER

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Since the estimation of ammonia in sea water by existing methods either presents difficulties or requires much time, an alternative based on a different principle has been explored.

This method depends upon the action of ammonia on sodium hypobromite in an alkaline medium, when

$$2NH_3 + 3NaBrO = N_2 + 3NaBr + 3H_2O.$$

If the sea water is acidified before the hypobromite is added, the ammonia present will not react. The effect therefore may be measured of adding the hypobromite, in equal quantities, to two samples of sea water, one untreated and one acidified. The difference represents an equivalent of ammonia. Excess hypobromite is estimated colorimetrically by adding Bordeaux B dye solution, which is decolorized by the hypobromite in acid solution.

I have the pleasure to thank very gratefully Dr H. W. Harvey, F.R.S., to whom I am much indebted for suggesting the problem, for his criticism and contribution to the work. The discussions with Dr L. H. N. Cooper and Mr F. A. J. Armstrong were also very helpful and I thank them cordially.

This work was done at the Plymouth Laboratory, and I am deeply indebted to the Director, Mr F. S. Russell, F.R.S., for his kindness in allowing me unrestricted facilities in the Laboratory.

The Method

Solutions Required

(i) Hydrobromic acid 0·23N, containing 1·9 % HBr. (ii) Sodium hypobromite 0·001N, made by diluting 0·1N stock solution, which is prepared by dissolving 2·5 g. NaOH and 1·25 ml. fluid bromine in 500 ml. of distilled water. This 0·001N-NaBrO is made alkaline by adding 1 % of 2N-Na_2CO_3. (iii) Bordeaux B, 0·014 g. of dye per litre. (iv) Ammonium sulphate: stock solution; 0·4716 g. $(NH_4)_2SO_4$ per litre; working solution is obtained by diluting 4 ml. of stock solution to 500 ml. 0·25 ml. of this solution contains 2 μg. ammonia-N.

Procedure

Exactly 50 ml. of the sea-water sample is transferred to each of three 100–150 ml. flasks by pipette. Using an all-glass syringe (Krogh's pipette) there is then added to

flask A: 1 ml. of (i) solution, followed immediately by
2 ml. of (ii) solution, followed immediately by
5 ml. of (iii);

flask B: 2 ml. of (ii) and then after 1 min.
1 ml. of (i) followed immediately by
5 ml. of (iii);

flask C: 0·25 ml. of (iv) (which is equivalent to the addition of 40 mg. ammonia-N/m.^{3}) and thereafter as for flask B.

The flasks are allowed to stand covered for 2 hr. or more (overnight) and the absorption of blue green light is then measured. Colorimetric measurements are conveniently made in a 4 cm. cuvette in the instrument described by Harvey (1948), the light passing through two Ilford filters, no. 404R + no. 403R.

If α, β and γ is the increase in optical density over that of distilled water, of the contents of the three flasks in the same cuvette, then $\beta - \alpha = K \times$ hypobromite destroyed by ammonia present in the water sample, and $\gamma - \beta = K \times$ hypobromite destroyed by the added 40 mg. ammonium-N/m.^{3}.

The concentration of ammonia in the sample of sea water is therefore $\frac{40(\beta - \alpha)}{\gamma - \beta}$ mg. ammonia-N/m.^{3}.

Example

The following shows a typical analysis carried out in triplicate on a sample of sea water collected from offshore:

Difference in Optical Density in 4 cm. Cuvette, Compared with Distilled Water

Exp.	α	β	γ	$\frac{40(\beta-\alpha)}{\gamma-\beta}$ of mg. ammonia-N/m.^{3}
I	0·305	0·338	0·381	30·7
II	0·302	0·335	0·376	32·2
III	0·298	0·332	0·380	28·3
IV	0·306	0·339	0·374	37·7

The values of α in this experiment indicated that 64 % of the added dye had been decolorized.

The α value varies with the concentration of hypobromite solution, which

decreases with age; it also varies with different sea-water samples, being greater for harbour waters.

The value of $\gamma - \beta$ bears a strict linear relation to the quantity of ammonia nitrogen added up to a total of about 140 mg. N-NH_3/m.^{3} present. The estimated value of ammonia nitrogen is not affected by the addition of 10 mg./l. of glucose and is not significantly affected by the addition of 40 mg. nitrite nitrogen per m.^{3}. When amino-acetic acid is added, about 30 % of the amino-nitrogen behaves as ammonia. During storage the ammonia content of unfiltered sea waters was found to decrease, as had been observed by Redfield & Keys (1938).

Comparison with Determinations Made by Distilling Sea Water in a Current of Air under Reduced Pressure

The method of Krogh (1934) was followed, 30 ml. of sea water (at pH 10–11 obtained by adding 0·25 N-NaOH, using cresol phthalein as indicator) being distilled in a current of air at a pressure of 70–80 mm. mercury.

The use of a 0·006 % solution of potassium indigo disulphonate, instead of napthyl red, gave a sharper end-point and obviated the necessity of titrating each sample at the same speed and of thoroughly cleaning the titration vessels in order to rid them of all traces of oxidation products of napthyl red. Traces of these products interfere with the titration (Buljan, 1951).

It was found that if amino-acetic acid was added to the sea water, 16–20 % of the amino-nitrogen distilled over as ammonia. Hence in both methods, the estimated values for ammonia nitrogen include a part of any amino-nitrogen present. Determinations carried out by the distillation method, in duplicate from the same sample of unfiltered sea water, did not show close agreement, sometimes differing by as much as 11 mg. ammonia-N/m.^{3}.

The following table gives results (in mg. ammonia-N/m.^{3}) obtained by the two methods:

	By distillation	By photometric method
Raw unfiltered sea water collected offshore	29·9	37·7
	35·6	30·7
	25·3	32·2
	21·8	28·3
Mean	28·15	32·2
Another unfiltered sea water collected offshore	18·1*	25·5
Stored water	16·8*	7·5
Water from Plymouth Sound	27·8*	30·8
Polluted sea water (a mixture of water from densely populated aquarium and open sea water)	40·6*	48·0
Plymouth Sound sea water with artificially destroyed ammonia	3·9	0·0
Total of means	135·35	144·0

* Mean of duplicate determinations.

The totals of the mean values, obtained by each method, are similar. Differences between replicate values appear to be due to similar experimental errors inherent in both methods.

The photometric method is more simple and rapid than distillation.

The time available for this investigation did not allow further study of discrepancies between the two methods.

REFERENCES

BULJAN, M., 1951. *Arhiv, Za Kemiju, Zagreb* (in the Press).

HARVEY, H. W., 1948. The estimation of phosphate and of total phosphorus in sea-waters. *Journ. Mar. Biol. Assoc.*, Vol. 27, pp. 337–59.

KROGH, A., 1934. A method for the determination of ammonia in water and air. *Biol. Bull. Woods Hole*, Vol. 67, pp. 126–31.

REDFIELD, A. C. & KEYS, A. B., 1938. The distribution of ammonia in the waters of the Gulf of Maine. *Biol. Bull. Woods Hole*, Vol. 74, pp. 83–92.

AGE AND GROWTH OF *CALLIONYMUS LYRA* L.

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(Plate I and Text-figs. 1–4)

CONTENTS

The Common Dragonet, *Callionymus lyra* L., is one of the commonest fishes in the Plymouth area, and is widely distributed in European seas. Recent reports indicate that it occurs also off the coast of West Africa (Fowler, 1936; Poll, 1949). This fish, like others of the genus, attracts attention because, although it has very little economic importance, it is strikingly coloured and the sexes are markedly different. Work has been done on the breeding by Holt (1897, 1898), and by Holt & Scott (1898); on ova and larvae by M'Intosh (1885), M'Intosh & Prince (1889), Cunningham (1891), Holt (1897), Ehrenbaum (1905–9), Fage (1918), Mielck (1925), Duncker, Ehrenbaum, Kyle, Mohr & Schnakenbeck (1929); on seasonal abundance and distribution of post-larvae off Plymouth by Russell (1930–47) and Corbin (1948); and on the skeleton by Günther (1861) and Ford (1937). The mature males are provided with remarkable secondary sexual characters both in coloration and in relative lengths of snout and of median fins, which render them so different from the females that they were originally regarded as different species and known as the Gemmeous Dragonet (male *C. lyra* L.) and the Sordid Dragonet (female *C. lyra* L. = *C. dracunculus* L.) respectively (Donovan, 1808; Yarrell, 1859; Couch, 1863). The sexual dimorphism and seasonal variation of this species has been much studied by Holt (1898), Smitt (1892–95), Gallien (1934), Letaconnoux (1949) and Desbrosses (1949). Very little information has so far been provided about its age and growth, with which the present paper deals.

I am greatly indebted to Mr F. S. Russell, F.R.S., for suggesting this investigation to me; and to Mr G. A. Steven for advice and assistance while

it was being carried out and for much help in the preparation of the manuscript; to Mr G. M. Spooner for statistical advice and assistance; to Dr T. J. Hart and Mr M. D. Menon for help in the use of the radial bone for age determination; and to Dr J. S. Alexandrowicz who took the photographs. I have also the greatest pleasure in recording my indebtedness to the British Council and to the Trustees of the Ray Lankester fund for financial assistance without which this work could not have been done.

Collection of the Material

All the specimens for this study were trawled off Plymouth by the research vessels *Sula* and *Sabella* and the research launch *Gammarus* during a period of 18 months, from December 1948 till May 1950. Some young fish were obtained also in the autumn and winter of 1950. During the first few months of the investigation the specimens were caught mainly by ordinary otter-trawl and partly by small beam-trawl (shrimp-netting), and very few fish below 50 mm. in length were collected. In order to obtain more young fish an Agassiz trawl with fine mesh and an otter-trawl with small mesh cover to the cod-end were also used later on. Nevertheless, the difficulty of obtaining sufficient numbers of young fish had not been solved. The total number of fish thus collected for this work amounts to over 4000 specimens varying from 20 to 240 mm. in standard length, including both sexes.

Monthly samples were taken and treated separately. Each fish was labelled and measured to the nearest millimetre, from the tip of the snout to the tip of the distal end of the longest caudal ray (total length) and from the tip of the snout to the distal end of the caudal hypural (standard length or body length). Then they were weighed to the nearest gram in a Salter spring balance and sexed. The large males above 80 mm. can easily be sorted out by means of the male secondary sexual characters, but those below that length were all confirmed by examining the gonads. Before preservation in formalin for other purposes, an otolith or the complete right pectoral girdle, or both of them, were removed and kept in small numbered envelopes for later study.

Determination of Age and Rate of Growth

There are no published data on the age and growth of this fish. Dr P. Desbrosses told me, in October 1949, that he had successfully used the otoliths to determine the age of *C. lyra*, but his results have not yet been published.

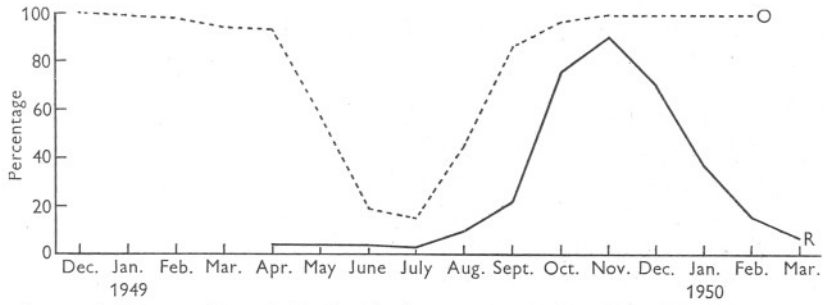
Age Reading from the Otolith

As *C. lyra* is devoid of scales, the otoliths were at first used for the age determination. The otolith belongs to the labrid type; its outer side is flat, inner side convex, dorsal rim domed and regular, and ventral rim curved (Frost, 1928). It is quite thin, and the number of rings on the fresh otoliths

can easily be read under a binocular dissecting microscope against a dark background. If the otolith has been dried it must be immersed in water before examination, otherwise the rings cannot be distinguished easily. The central area of the otolith is opaque, with alternating rings of transparent (slow growth zone) and opaque (rapid growth zone) material. Altogether, 2407 otoliths taken

TABLE I. PERCENTAGE OF FISH WITH SLOW OR RAPID GROWTH ZONES AT THE MARGIN OF THE OTOLITH AND OF THE SECOND RADIAL BONE

		Percentage of otoliths with			Percentage of radial bones with	
Month	No. examined	slow growth zone at the margin	rapid growth zone at the margin	No. examined	slow growth zone at the margin	rapid growth zone at the margin
Dec. 1948	72	100	0	—	—	—
Jan. 1949	200	99	1	—	—	—
Feb. 1949	119	98	2	—	—	—
Mar. 1949	64	94	6	—	—	—
Apr. 1949	88	93	7	45	4	96
May 1949	101	57	43	126	4	96
June 1949	206	19	81	134	4	96
July 1949	390	15	85	97	3	97
Aug. 1949	230	43	57	162	10	90
Sept. 1949	146	86	14	95	22	78
Oct. 1949	82	96	4	75	75	25
Nov. 1949	124	99	1	274	90	10
Dec. 1949	164	99	1	311	70	30
Jan. 1950	241	99	1	236	37	63
Feb. 1950	180	99	1	177	18	82
Mar. 1950	—	—	—	132	7	93



Text-fig. 1. *Callionymus lyra*, *O* (broken line), percentages of otoliths with transparent (slow growth) zone at margin, from December 1948 till February 1950. *R* (continuous line), percentages of radial bones with slow growth zone at margins, from April 1949 till March 1950.

from specimens of various ages of both sexes have been examined, over a period of 15 months, from December 1948 to February 1950 inclusive. The results confirm that the rings on the otoliths are formed annually, as there is a complete turning over of one sort of margin to the other during a certain period of the year (Table I; Text-fig. 1). The opaque rings are laid down cover-

ing the outer margin of the transparent zone during the summer months. It is a gradual increase in intensity of calcification rather than an increase in width during a short period. The transparent zone begins to appear after July, and the percentage of otoliths with the transparent margin reaches its peak (99–100%) during the winter and early spring. By reading the rings on the otoliths the age and the particular year group to which a fish belongs can be determined without difficulty. Nevertheless, the margins of the rings are very diffuse and the size of the otoliths is subject to great variation. It is very difficult to fix the centre and the line of growth. Although the otolith has been used successfully in reading the age of the fish, it is not practicable to measure it for back-calculation of growth rate. Soon my attention turned towards the other skeletal structures of the fish, since many bones in other fishes have been widely used for age determination (see Menon, 1950).

Age and Rate of Growth from the Second Radial Bone

On the vertebrae, coracoid, hypural and radials of *C. lyra*, there are very obvious alternating transparent and opaque zones, as found also in some bones of other fishes. After careful study, the zones on the second radial were found to be very constant, and the number of zones had a definite relationship with that on the otoliths of the same specimen. This bone was therefore selected for further study.

As a result of the great expansion of the pectoral girdle in *C. lyra*, the second radial bone becomes very flat and greatly enlarged. Its upper and lower surfaces are ossified, and in between the surface layers is the cartilage. During the autumn and winter the margin of the bone becomes almost completely ossified. Thus the narrow, ossified zone (i.e. the slow growth zone or winter ring) is formed.

Method of Preparation

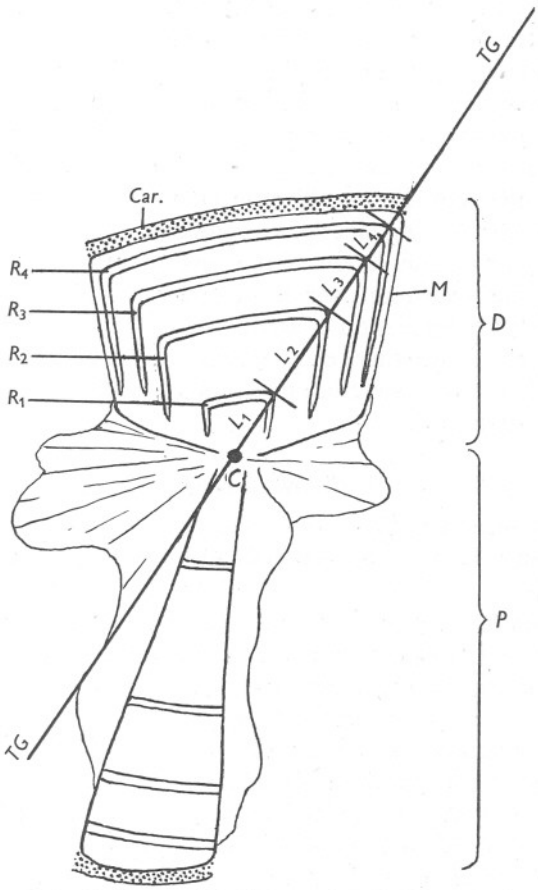
The method of preparing the bone is very easy. After the specimens are measured and numbered, the complete right pectoral girdles are removed one by one with a pair of dissecting scissors, and put into numbered glass dishes. Then they are dipped into a hot bath of water at 70–80° C. for 2-3 min. After this simple treatment, the complete girdle can readily be cleaned with a cloth between the fingers or in a pair of curved forceps. The pectoral girdles of small specimens less than about 80 mm. long are rather delicate, and must be carefully cleaned with forceps under the dissecting microscope, after which they are placed in labelled envelopes and left to dry.

Line of Growth and Method of Measurement

The distal half of the radial bone grows in three directions, as shown in Text-fig. 2. The centre of growth of the bone is very clear. If a straight line is drawn from the centre towards the angle of the bone, it crosses all the angles

of the ossified rings. The measurements taken were along this line, the intervals L_1, L_2, L_3, etc., representing successive annual increments.

Since the radial is a cartilage bone, it shrinks a great deal after drying. The surface is no longer flat and the zones have lost their original shape. Therefore the radial bone of this fish has to be soaked for one to several days before



Text-fig. 2. The second radial bone of *Callionymus lyra* L. Diagram showing the theoretical growth line, and the way of measuring the length of the bone and the annual increments at different ages. *TG*, the hypothetical line of growth; R_1 to R_4, the annual rings; *C*, the centre of growth; L_1, L_2, L_3 and L_4, the first, second, third and fourth years' growth of the second radial bone; *Car.*, cartilage layer; *M*, margin of the bone.

measuring. After this treatment the bone recovers its former size and shape, and the rings become as clear as if the bone were fresh. As the radial bone is not thin enough for projecting, it was measured under a low-power microscope with an eye-piece micrometer.

Validity of the Zones for Age Determination

The main evidence supporting the validity of age determination from the second radial in this fish is that the rings on the bone are formed annually.

Monthly observations on the nature of the margins of the second radial were carried out over one complete year. Altogether 1860 radials were examined specially for this test. The results obtained, together with those of the otoliths, are given in Table I and Text-fig. 1. The data reveal two important points: (i) that there is a nearly complete turning over in a certain period of the year from one kind of margin to the other, and (ii) that there is also a definite agreement between the number of rings on the radial and that on the otoliths. In the second radial bone the narrow ossified zone (the slow-growth zone or winter ring) begins to appear in September. Its percentage occurrence is highest in November and falls rapidly after December. Usually, in young fish, the formation of the first ring takes place comparatively early, whereas in some of the mature fish the completion of the rings is much delayed. Therefore, there is no complete turning over from one kind of margin to the other throughout the entire population at any period.

From the above evidence there can be no doubt that the zones on the second radial are annual, and it is therefore concluded that the second radial is valid for age determination in this fish.

Results of Growth Measurements

Altogether 1059 second radials from male fish and 627 from females were measured. Among the 1059 male radials 876 were from immature and 183 from mature fish.

The mean lengths of both body and radial at each 5 mm. group in the immature males and females are given in Table II. When the mean radial lengths are plotted against the mean body lengths all the points lie practically along a straight line, as shown in Text-fig. 3.

The snout in mature males is greatly prolonged and subjected to great variation. As its length is correlated with the state of sexual maturity and is not in direct proportion to the body length, correction must be made in total length measurements for the excess of snout length (see p. 289). After this correction has been made and the mature males regrouped, the proportion of the mean lengths of the radials and the corrected body length in different length groups came into agreement with those of the immature males and the females (Table VI; Text-fig. 3).

By graphic method the formula for the correlation curve of the radial length and the body length in this fish is as follows

$$R = 0{\cdot}04 + 0{\cdot}0199\,B,$$

where R is the length of the radial and B is the body length; both measured in mm. By using this formula the theoretical lengths of either radial bone or

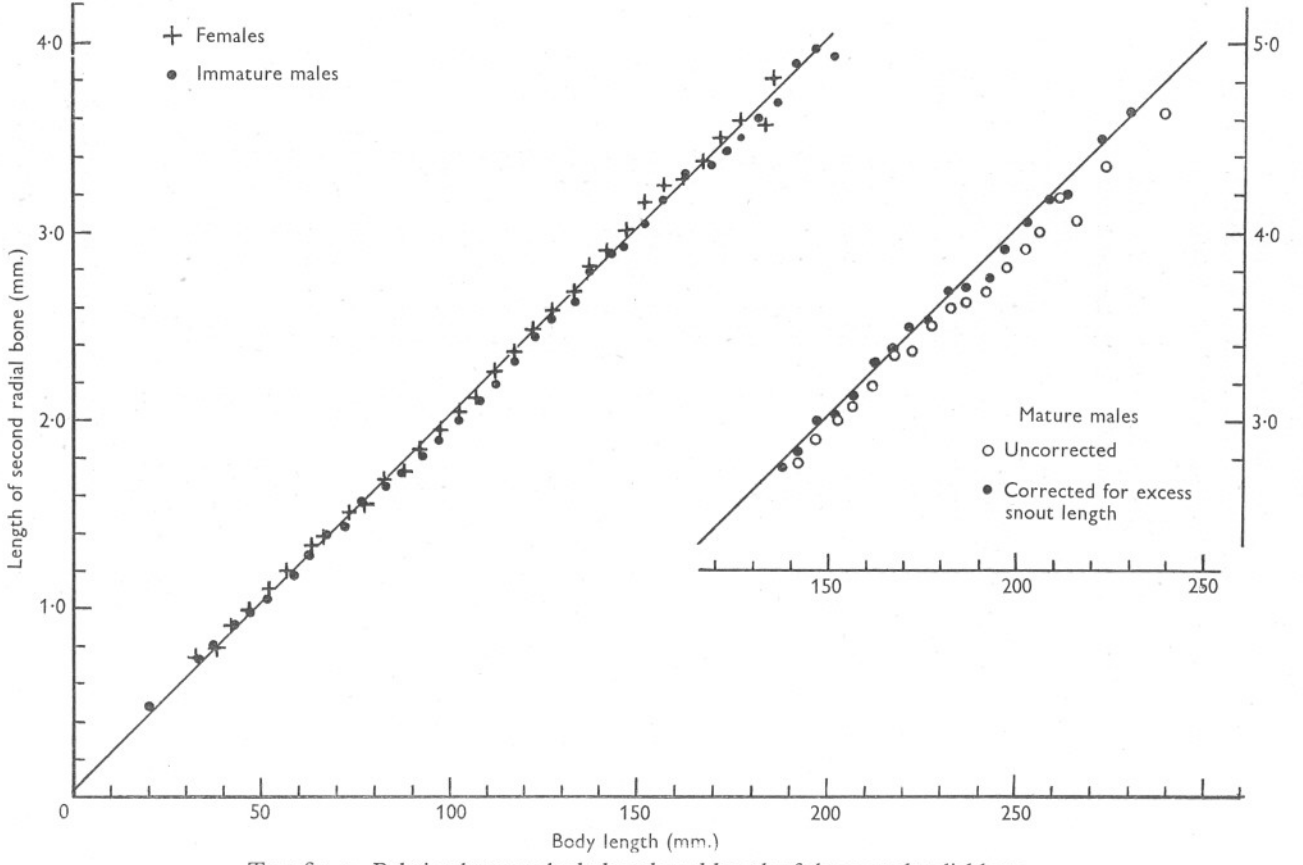
the body length of each 5 mm. group above 35 mm. can be worked out, given one or the other. In Table II the calculated body lengths (given the observed radial bone lengths) are listed for immature males and females, and in Table VI for mature males. The differences between the empirical lengths and

TABLE II. MEAN LENGTHS OF THE SECOND RADIAL IN RELATION TO THE MEAN LENGTH OF FISH AT THE DIFFERENT LENGTH GROUPS

	Immature males					Females				
Length group (mm.)	No. of fish	Mean body length (mm.)	Length of 2nd radial (mm.) mean	σ	Theoretical body length (mm.)	No. of fish	Mean body length (mm.)	Length of 2nd radial (mm.) mean	σ	Theoretical body length (mm.)
20	1	20·0	0·48	—	22·1	0	—	—	—	—
25	0	—	—	—	—	0	—	—	—	—
30	4	33·2	0·73	0·03	34·7	4	32·8	0·74	0·02	35·2
35	5	37·2	0·80	0·06	38·2	12	36·8	0·79	0·07	37·7
40	15	42·8	0·91	0·05	43·7	19	42·1	0·91	0·05	43·7
45	11	47·1	0·98	0·02	47·2	9	46·8	0·99	0·05	47·7
50	7	57·4	1·04	0·05	50·3	6	52·0	1·10	0·05	53·3
55	7	58·6	1·17	0·07	56·8	6	56·7	1·20	0·05	58·3
60	4	62·7	1·28	0·08	62·3	7	63·1	1·33	0·04	64·8
65	6	67·2	1·39	0·06	67·8	10	66·6	1·38	0·06	67·3
70	7	72·0	1·43	0·05	69·8	6	73·0	1·51	0·11	73·9
75	9	76·7	1·56	0·07	76·4	17	76·6	1·56	0·10	76·4
80	9	82·6	1·65	0·07	80·9	14	82·0	1·68	0·08	82·4
85	15	87·0	1·72	0·06	84·4	24	87·2	1·73	0·07	84·9
90	32	92·4	1·81	0·09	88·9	46	91·8	1·84	0·08	90·5
95	43	97·0	1·89	0·07	93·0	46	97·2	1·95	0·09	96·0
100	45	102·0	2·00	0·08	98·5	40	102·0	2·04	0·09	100·5
105	55	107·4	2·10	0·08	103·5	43	106·8	2·12	0·09	104·5
110	50	111·9	2·19	0·10	108·0	32	111·9	2·26	0·10	111·6
115	43	117·0	2·31	0·08	114·1	35	117·0	2·36	0·11	116·6
120	37	122·1	2·44	0·10	120·6	44	121·8	2·48	0·10	122·6
125	25	127·0	2·54	0·10	125·6	38	127·0	2·58	0·11	127·6
130	40	131·6	2·62	0·11	129·6	20	132·3	2·68	0·09	132·7
135	30	137·1	2·79	0·12	138·2	29	136·6	2·81	0·11	139·2
140	23	142·9	2·88	0·09	142·7	25	141·6	2·90	0·14	143·7
145	36	146·8	2·92	0·13	144·7	21	146·8	3·01	0·18	149·2
150	35	151·7	3·04	0·13	150·8	18	151·5	3·16	0·11	156·8
155	30	156·3	3·17	0·10	157·3	13	156·7	3·25	0·13	161·3
160	36	162·0	3·31	0·14	164·3	19	161·5	3·29	0·14	163·3
165	42	166·9	3·36	0·15	166·8	7	167·0	3·38	0·10	167·8
170	49	172·6	3·43	0·14	170·4	10	171·1	3·50	0·18	173·9
175	39	176·4	3·50	0·13	173·9	2	176·5	3·59	0·24	178·4
180	37	181·5	3·60	0·13	178·9	3	183·0	3·57	0·08	177·4
185	25	186·4	3·68	0·15	182·9	2	185·0	3·81	0·04	189·4
190	12	191·6	3·89	0·16	193·5	—	—	—	—	—
195	4	196·5	3·97	0·19	197·5	—	—	—	—	—
200	7	201·2	3·92	0·16	195·0	—	—	—	—	—

the calculated lengths of the body are less than 5 mm. except at a few points.

An analysis of the length frequency in relation to the number of rings on the 2nd radial bone shows that the male may attain an age of 5 years (with four complete ossified winter rings), while the females may live 2 years longer



Text-fig. 3. Relation between body length and length of the second radial bone.

The graph line in the inset is identical with that in the main diagram, showing that the 'corrected' males conform with the females and immatures.

(with six complete ossified winter rings). However, the males grow much more quickly and to a larger size than the females (Table III).

It is also shown that among the 1059 radials from male fish, 1024 have one or more ossified winter rings; 680 radials have two or more; 164 radials have three or more; and only six radials have four complete ossified winter rings. If the mean lengths of the 1st, 2nd, 3rd and 4th rings are worked by back-calculation derived from the radial, the annual increment of the body length can be obtained irrespective of the actual length of the fish. Similarly, the annual increment of the body length in females at different ages can be calculated (Tables IV and V; Text-fig. 4).

The mean lengths of both the body and the radial of the mature males are entered in Table VI. When mean lengths of radials are plotted against the mean fish lengths, nearly all the points are far below the correlation curve for the immature males and the females (Text-fig. 3). If the snout length in the mature males be corrected according to the following averages derived from the immature males, all the points lie very close to the correlation curve of mean radial length and body length of both immature males and females:

$$\text{snout length (mm.)} = -0{\cdot}3625 + 0{\cdot}1275 \times \text{body length.}$$

Natural Death of the Spent Males

During the spring and summer of 1949, some interesting phenomena were observed. First, sexual maturity in the males was not directly related to the length of the body, since the spawning males varied from about 130 mm. to nearly as long as 240 mm., and at the same time many large immature males were found in the catches (Table VII). Secondly, the majority of the spawning males lost weight very quickly after the breeding season started, generally in the latter part of January off Plymouth, and their secondary sexual characters became extremely prominent. Thirdly, the majority of spent males suddenly disappeared after June and July. This sudden disappearance could be explained in three ways: (i) by degeneration or reabsorption of all the secondary sexual characters, (ii) by migration of the spent males to deeper waters after the breeding season, and (iii) by natural death of the spent males after the breeding season. However, there was no evidence to support the first two possibilities.

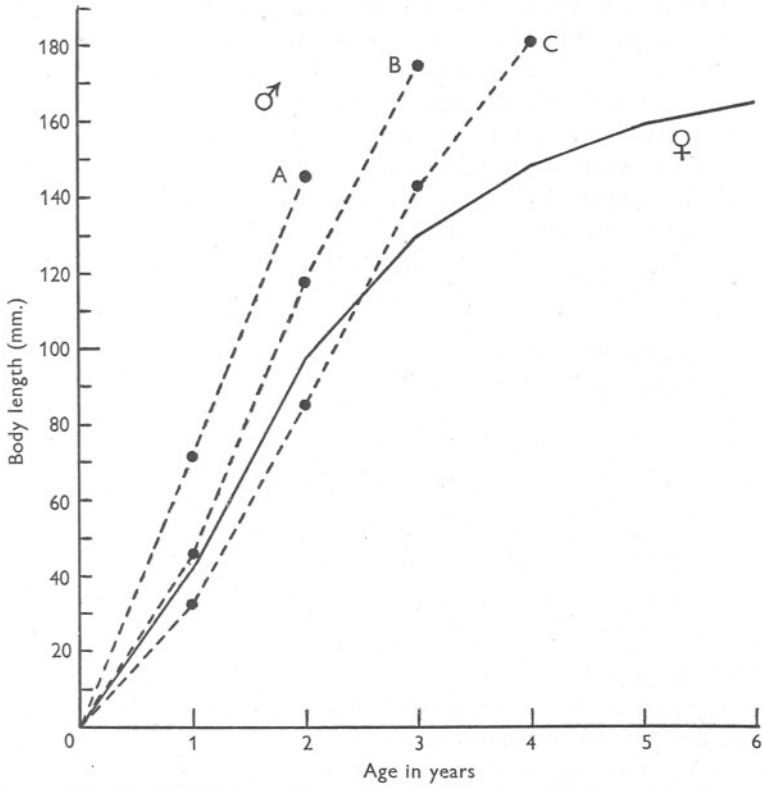
An analysis of the age of all the males collected from April 1949 to March 1950 inclusive, shows that the spawning males clearly fall into three age groups and that all the year round there are mature males; but the percentages of such fish exceeding 120 mm. in length (the size of the smallest mature males) showed a tremendous seasonal change. It was as low as 4·4% in October and as high as 78·1% in March (Table VI). Throughout the 20 months of direct observations on the catches, the gradual changes in the development of the secondary sexual characters in the males could easily be followed, and there was no indication of any recovery of the body condition

TABLE III. LENGTH FREQUENCIES OF FISH AND RELATION TO NUMBER OF OSSIFIED RINGS ON THE SECOND RADIAL BONES

Mature males enumerated separately in heavy type

Length group (mm.)	Males. Ossified rings on second radials						Females. Ossified rings on second radials							
	0	1+	2+	3+	4+	Total	0	1+	2+	3+	4+	5+	6+	Total
20	1	—	—	—	—	1	0	—	—	—	—	—	—	—
25	0	—	—	—	—	0	0	—	—	—	—	—	—	—
30	4	—	—	—	—	4	3	1	—	—	—	—	—	4
35	5	—	—	—	—	5	4	8	—	—	—	—	—	12
40	11	4	—	—	—	15	14	5	—	—	—	—	—	19
45	7	4	—	—	—	11	5	4	—	—	—	—	—	9
50	2	5	—	—	—	7	2	4	—	—	—	—	—	6
55	3	5	—	—	—	8	0	6	—	—	—	—	—	6
60	1	3	—	—	—	4	0	6	1	—	—	—	—	7
65	1	5	—	—	—	6	1	9	0	—	—	—	—	10
70	—	7	—	—	—	7	0	6	0	—	—	—	—	6
75	—	7	2	—	—	9	1	14	2	—	—	—	—	17
80	—	7	2	—	—	9	—	8	6	—	—	—	—	14
85	—	12	3	—	—	15	—	14	10	—	—	—	—	24
90	—	19	13	—	—	32	—	30	16	—	—	—	—	46
95	—	37	6	—	—	43	—	26	20	—	—	—	—	46
100	—	32	13	—	—	45	—	23	17	—	—	—	—	40
105	—	36	19	—	—	55	—	26	17	—	—	—	—	43
110	—	36	14	—	—	50	—	17	15	—	—	—	—	32
115	—	32	11	—	—	43	—	14	15	6	—	—	—	35
120	—	26	11	—	—	37	—	6	30	8	—	—	—	44
125	—	15	10	—	—	25	—	8	24	6	—	—	—	38
130	—	20	20	—	—	40	—	2	14	3	1	—	—	20
135	—	10	17	3	—	30	—	1	18	9	1	—	—	29
140	—	7+1	15+6	1	—	30	—	1	10	14	—	—	—	25
145	—	8+1	26+6	2+1	—	44	—	1	7	12	1	—	—	21
150	—	2+0	32+8	1+3	—	46	—	—	2	15	1	—	—	18
155	—	0+0	30+8	0+5	—	43	—	—	4	6	3	—	—	13
160	—	1+1	30+3	5+6	—	46	—	—	1	11	7	—	—	19
165	—	1	40+3	1+11	—	56	—	—	—	2	5	—	1	7
170	—	—	41+6	8+9	—	64	—	—	—	3	5	1	—	10
175	—	—	35+8	4+17	—	64	—	—	—	—	2	—	—	2
180	—	—	31+0	6+7	1	45	—	—	—	—	1	2	—	3
185	—	—	21+1	4+11	—	37	—	—	—	—	2	—	—	2
190	—	—	10+2	2+10	—	24	—	—	—	—	—	—	—	—
195	—	—	2+3	2+10	—	17	—	—	—	—	—	—	—	—
200	—	—	7+1	9	2	19	—	—	—	—	—	—	—	—
205	—	—	—	7	1	8	—	—	—	—	—	—	—	—
210	—	—	—	3	—	3	—	—	—	—	—	—	—	—
215	—	—	—	5	1	6	—	—	—	—	—	—	—	—
220	—	—	—	4	1	5	—	—	—	—	—	—	—	—
225	—	—	—	—	—	0	—	—	—	—	—	—	—	—
230	—	—	—	—	—	0	—	—	—	—	—	—	—	—
235	—	—	—	1	—	1	—	—	—	—	—	—	—	—
Totals	35	341	461	39	—	1059	—	—	—	—	—	—	—	—
		3	55	119	6	—	30	240	229	95	29	3	1	627

of the spent males or reabsorption of the secondary sexual characters. The most distinct feature of the secondary sexual characters other than coloration is the great prolongation of the snout and of all the median fins, including the



Text-fig. 4. Growth of *Callionymus lyra*. ♂ (broken line), A, third-year breeder; B, fourth-year breeder; C, fifth-year breeder. ♀ (continuous line), mixed.

TABLE IV. MEAN LENGTH OF SUCCESSIVE ANNUAL RINGS

	Male				Female			
	No.	Range	Mean length (mm.)	S.D.	No.	Range	Mean length (mm.)	S.D.
1st ring	1024	0·39–1·90	1·04	0·28	597	0·40–1·90	0·88	0·27
2nd ring	680	0·68–2·08	1·37	0·27	357	0·58–1·98	1·14	0·26
3rd ring	159	0·30–1·88	1·10	0·31	128	0·22–1·40	0·69	0·28
4th ring	6	0·46–1·04	0·81	0·23	33	0·10–0·80	0·41	0·19
5th ring	—	—	—	—	4	0·20–0·36	0·25	0·36
6th ring	—	—	—	—	1	—	0·16	0·00

dorsal, anal and caudal fins. The prolongation of the snout is mainly effected by the elongation of the process of the premaxillary (Günther, 1861, pp. 140–2). For example, the premaxillary of a spent male, 166 mm. long,

collected in May 1949, reached 28 mm., while an immature one 168 mm. long, collected in the same month, was only 22 mm. long. The length of the first dorsal fin-ray of the former was 125 mm. and of the latter 51 mm. In two other specimens, one a mature male, 204 mm. long (May 1949), and the other an immature male, 201 mm. long (November 1949), the lengths of the premaxillary were 40 and 29 mm., and of the first dorsal fin-rays 145 and 95 mm. respectively. Since the fin-ray and the ossified premaxillary are not epidermal structures, they are very different from the nuptial organ (or pearl organs) found in many fresh-water fishes, which can be dropped off after the breeding season (Kimura & Tao, 1937) and the fleshy hump on the forehead of the cichlids (Cichlidae), which is absorbed after the breeding season (Norman, 1947, p. 301). The possibility of sudden absorption of the skeletal structure or of the greatly elongated fin-rays in this fish would seem impossible.

TABLE V. THEORETICAL LENGTHS CALCULATED FROM MEASUREMENTS OF THE SECOND RADIAL BONES (MM.)

	Male		Female	
Towards the end of	Annual increment	Cumulative length	Annual increment	Cumulative length
1st ring	50·3	50·3	42·2	42·2
2nd ring	66·8	117·1	55·3	97·5
3rd ring	53·3	170·4	32·7	130·2
4th ring	38·7	209·1	18·6	148·8
5th ring	—	—	10·6	159·4
6th ring	—	—	6·0	165·4

No marking experiments have been done to elucidate whether or not the spent males migrate into the deeper water after the breeding season. But the age composition of the mature males in the spring of 1949 is similar to that in the spring of 1950. Here we may take the proportion of the spawning males in the month of April 1949 and those of March 1950 for comparison. The numbers of the third-year, fourth-year and fifth-year breeders in April are 10, 21 and 1; and in March 1950 are 24, 52 and 3 respectively. If the spent males did come back to breed again, the age distribution of the spawning males in the spring of 1950 should have included many more fifth-year breeders. Attempts have been made to study the histological structures of the testis at different stages, and to find out how they are correlated with the secondary sexual characters and seasonal changes, but the data so obtained have not yet been analysed.

Turning to the positive evidence that natural death takes place in the males after the breeding season, the age and rate of growth of 153 spent males were studied in detail. These comprised forty third-year breeders (with two complete winter rings), 107 fourth-year breeders (with three complete winter rings), and six fifth-year breeders (with four complete rings). It was found that the rates of growth of these groups were very different (Table VIII, Text-fig. 4). In those males breeding in the third year the rate of growth was

TABLE VI. *CALLIONYMUS LYRA*, MATURE MALES. LENGTHS OF BODY AND SECOND RADIAL BONE AS OBSERVED, AND AS CORRECTED FOR EXCESS SNOUT LENGTH

Length group (mm.)	Total specimens	Mean body length (mm.)	Mean length of 2nd radial (mm.)	Ossified rings on second radial (nos. of fish)			
				1+	2+	3+	4+
			Empirical				
135	—	—	—	—	—	—	—
140	7	141·4	2·78	1	6	—	—
145	8	146·9	2·90	1	6	1	—
150	11	152·5	3·00	—	8	3	—
155	13	156·5	3·07	—	8	5	—
160	10	161·4	3·19	1	3	6	—
165	14	167·1	3·35	—	3	11	—
170	15	172·0	3·37	—	6	9	—
175	25	177·8	3·50	—	8	17	—
180	8	182·4	3·60	—	—	7	1
185	12	186·4	3·63	—	1	11	—
190	12	191·8	3·69	—	2	10	—
195	13	197·2	3·81	—	3	10	—
200	12	202·0	3·91	—	1	9	2
205	8	206·1	4·00	—	—	7	1
210	3	210·7	4·19	—	—	3	—
215	6	215·5	4·06	—	—	5	1
220	5	223·0	4·35	—	—	4	1
225	—	—	—	—	—	—	—
230	—	—	—	—	—	—	—
235	1	239·0	4·64	—	—	1	—
				3	55	119	6
			Corrected				
135	3	138·0	2·75	1	2	—	—
140	9	142·0	2·83	—	9	—	—
145	7	147·4	2·99	1	5	1	—
150	15	151·9	3·03	—	8	7	—
155	11	156·8	3·11	—	5	6	—
160	13	161·9	3·30	1	4	8	—
165	16	167·2	3·37	—	5	11	—
170	9	171·2	3·49	—	3	6	—
175	27	176·9	3·53	—	7	19	1
180	15	181·9	3·69	—	1	14	—
185	7	186·9	3·71	—	1	6	—
190	19	192·4	3·76	—	3	15	1
195	8	196·6	3·91	—	1	6	1
200	15	202·6	4·05	—	1	12	2
205	3	208·2	4·17	—	—	3	—
210	2	213·0	4·20	—	—	1	1
215	—	—	—	—	—	—	—
220	3	220·0	4·49	—	—	3	—
225	—	—	—	—	—	—	—
230	1	230·0	4·64	—	—	1	—
235	—	—	—	—	—	—	—
				3	55	119	6

comparatively very high. Among the forty third-year specimens, the first year's growth of seventeen individuals was higher than the second year's growth, and the mean of the first year's growth is slightly lower than the second year's growth. In those males breeding in the fourth year the rate of twelve specimens was highest in the first year, of eighty highest in the second year, and in only fifteen highest in the third year. The mean of the first year's

TABLE VII. ACTUAL NUMBERS AND PERCENTAGES OF MATURE MALES ABOVE 120 MM., OBTAINED IN DIFFERENT MONTHS THROUGHOUT ONE COMPLETE YEAR

Month	No. of mature males	Percentage of mature males
Apr. 1949	42	62·7
May 1949	90	68·7
June 1949	55	25·3
July 1949	34	15·0
Aug. 1949	10	11·8
Sept. 1949	11	9·8
Oct. 1949	4	4·4
Nov. 1949	16	9·2
Dec. 1949	64	53·3
Jan. 1950	28	52·8
Feb. 1950	39	60·9
Mar. 1950	89	78·1
Total	482	—

TABLE VIII. COMPARISON OF THE RATE OF GROWTH IN THE MALES WHICH BREED AT THE THREE DIFFERENT AGES

Males breeding in	No.	Successive rings	Lengths of successive rings on radial (mm.) mean	σ	Calculated annual increment of body length (mm.)	Calculated mean body length at the end of successive rings (mm.)
3rd year	43	1st ring	1·47	0·32	71·9	71·9
		2nd ring	1·50	0·27	73·4	145·3
4th year	107	1st ring	0·95	0·25	45·7	45·7
		2nd ring	1·48	0·25	72·4	118·1
		3rd ring	1·16	0·28	56·3	174·4
5th year	6	1st ring	0·69	0·17	32·7	32·7
		2nd ring	1·08	0·21	52·3	85·0
		3rd ring	1·19	0·25	57·8	142·8
		4th ring	0·81	0·20	38·7	181·5

growth was much smaller than in the other 2 years. Among the males breeding in the fifth year, two specimens showed their highest growth rate in the first year, three in the second year, and one has its third year's growth almost equal to that of the fourth year. As in their growth rates these three groups of breeding males are so different from each other, they must be very different from each other physiologically.

The presence of large immature males with three complete winter rings on

the radial, and of a considerable number of immature males, further proves that the fifth-year male breeders are neither the same third-year breeders of 2 years earlier nor the fourth-year breeders of 1 year earlier. Similarly, the fourth-year breeders are not the third-year breeders of 1 year before.

The length-weight relation and the secondary sexual characters also show that the males of *C. lyra* breed only once in their life. Besides the well-known example of *Anguilla*, which breeds only once during life, *Callionymus lyra* provides one of the most interesting illustrations of natural death among the large fishes.

Summary

Both the otoliths and the second radials of the pectoral girdle can be used for age determination in *Callionymus lyra* L. Since the latter are far more satisfactory than the former, the second radial was selected for this work and the otolith was used only to check age reading. The radial is here used for the first time for age determination in fish.

The male may live up to 5 years (with four complete ossified winter rings), while the females may live 2 years longer (with six complete ossified winter rings). However, the males grow much quicker and to a larger size (239 mm. long) than the females (185 mm.).

The age of the spawning males falls into three age groups: (A) those that breed in the third year having two complete winter rings; (B) those that breed in the fourth year having three; (C) those that breed in the fifth year having four.

An analysis of the rates of growth of the spent males of three age groups shows that the rate of growth is highest in the third-year breeders, and is lowest in the fifth-year breeders.

Evidence has been given showing that the males of *C. lyra* breed only once and subsequently die. Breeding of the females has not been worked out.

References

Corbin, P. G., 1948. On the seasonal abundance of young fish. IX. The year 1947. *Journ. Mar. Biol. Assoc.*, Vol. 27, pp. 718–22.

Couch, J., 1863. *A History of the Fishes of the British Islands*, Vol. 2. London.

Cunningham, J. T., 1891. The eggs and larva of *Callionymus lyra*. *Journ. Mar. Biol. Assoc.*, Vol. 2, pp. 89–90.

Desbrosses, P., 1949. Dimorphisme sexuel chez quelques Téléostéens de la côte Atlantique française. *Journ. Cons. Int. Explor. Mer*, Vol. 16, pp. 63–73.

Donovan, E., 1808. *The Natural History of British Fishes*, Vol. 1. London. [Plate IX.]

Duncker, G., Ehrenbaum, E., Kyle, H. M., Mohr, E. W. & Schnakenbeck, W., 1929. *Die Fische der Nord- und Ostsee*. Leipzig.

Ehrenbaum, E., 1905–09. Eier und Larven von Fischen. *Nordisches Plankton*, Lief. IV (1905), Teil I; Lief. X (1909), Teil II. 413 pp.

Fage, L., 1918. Shore-fishes. *Rep. Danish Oceanogr. Exped. Medit.*, No. 4, Vol. II (Biology), A. 3, pp. 1–154.

Ford, E., 1937. Vertebral variation in teleostean fishes. *Journ. Mar. Biol. Assoc.*, Vol. 22, pp. 1–60.

FOWLER, H. W., 1936. The marine fishes of West Africa, based on the collection of the American Museum Congo Expedition, 1909–15. *Bull. Amer. Mus. Nat. Hist.*, Vol. 70, pp. 1–1493.

FROST, G. A., 1928. A comparative study of the otoliths of the neopterygian fishes. *Ann. Mag. Nat. Hist.*, Ser. 10, Vol. 1, pp. 451–6.

GALLIEN, L., 1934. Croissance harmonique et dysharmonique des nageoires chez *Callionymus lyra* L. *Bull. Soc. Zool. France*, T. 59, pp. 135–40.

GÜNTHER, A., 1861. *Catalogue of the Acanthopterygian Fishes in the Collection of the British Museum*, Vol. 3. London.

HOLT, E. W. L., 1897. Notes on the reproduction of teleostean fishes in the south-western district. *Journ. Mar. Biol. Assoc.*, Vol. 5, pp. 107–55.

—— 1898. On the breeding of the Dragonet (*Callionymus lyra*) in the Marine Biological Association's Aquarium at Plymouth; with a preliminary account of the elements, and some remarks on the significance of the sexual dimorphism. *Proc. Zool. Soc. London*, 1898, Vol. 1, pp. 281–315.

HOLT, E. W. L. & SCOTT, S. D., 1898. A record of the teleostean eggs and larvae observed at Plymouth in 1897. *Journ. Mar. Biol. Assoc.*, Vol. 5, pp. 156–71.

KIMURA, S. & TAO, Y. S., 1937. Notes on the nuptial coloration and pearl organs in Chinese fresh-water fishes. *Bull. Shanghai Sci. Inst.* Vol. 6, pp. 277–318.

LETACONNOUX, R., 1949. Note sur les caractères sexuels secondaires du *Callionymus lyra*. *Journ. Cons. Int. Explor. Mer*, Vol. 16, pp. 59–62.

MENON, M. D., 1950. Bionomics of the poor-cod (*Gadus minutus* L.) in the Plymouth area. *Journ. Mar. Biol. Assoc.*, Vol. 29, pp. 185–239.

MIELCK, W., 1925. Heringslarven, Eier und Larven anderer Fische und Nahrung der Larven in dem Westlichen Nordsee im Oktober 1922. *Ber. Deutsch. Wiss. Komm. Meeresforsch.*, N.F., Bd. 1, pp. 209–46.

M'INTOSH, W. C., 1885. Notes from the St Andrews Marine Laboratory (under the Fishery Board for Scotland). *Ann. Nat. Hist.*, Ser. 5, Vol. 16, pp. 480–7.

M'INTOSH, W. C. & PRINCE, E. E., 1889. On the development and life histories of the teleostean food- and other fishes. *Trans. Roy. Soc. Edinb.*, Vol. 35, pp. 665–946.

NORMAN, J. R., 1947. *A History of Fishes*, 3rd ed. London.

POLL, M., 1949. Résultats scientifiques des croisières de Navire-École belge 'Mercator'. IV. Poissons. *Mem. Inst. Roy. Sci. Nat. Belg.*, Ser. II, Fasc. 33, pp. 173–269.

RUSSELL, F. S., 1930–47. The seasonal abundance and distribution of the pelagic young of teleostean fishes caught in the ring-trawl in offshore waters in the Plymouth area. I–VIII. *Journ. Mar. Biol. Assoc.*, Vols. 16, pp. 707–22; 20, pp. 147–79 and pp. 595–604; 21, pp. 679–86; 22, pp. 493–500; 23, pp. 381–6; 24, pp. 265–70; 26, pp. 605–8.

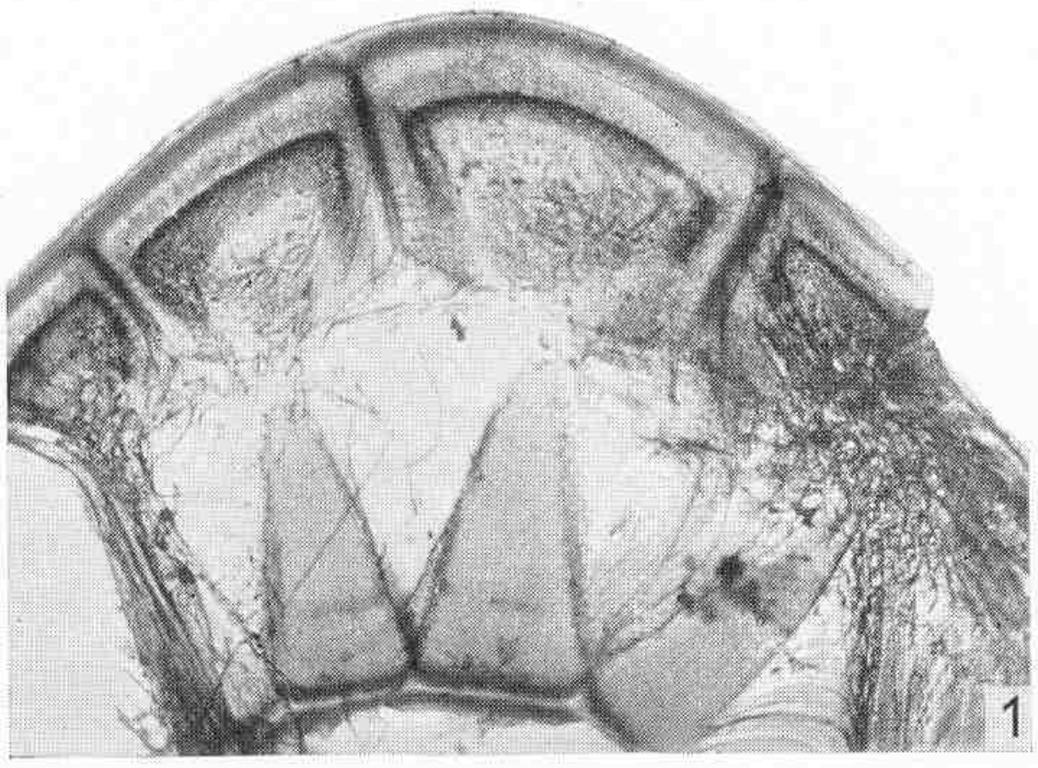
SMITT, F. A., 1892–95. *A History of Scandinavian Fishes*, Parts I and II. 2nd ed. Stockholm.

YARRELL, W., 1859. *A History of British Fishes*, Vol. 2, 3rd ed. London.

EXPLANATION OF PLATE I

PLATE I. Second radials of *Callionymus lyra*

Fig. 1. Female aged 1+, length 69 mm., April 1949.
Fig. 2. Mature male aged 2+, length 168 mm., March 1949.
Fig. 3. Mature male aged 3+, length 198 mm.
Fig. 4. Female aged 4+, length 185 mm., May 1949.
Fig. 5. Male aged 5+, length 172 mm., Oct. 1949.
Fig. 6. Female aged 6+, length 179 mm., Nov. 1948.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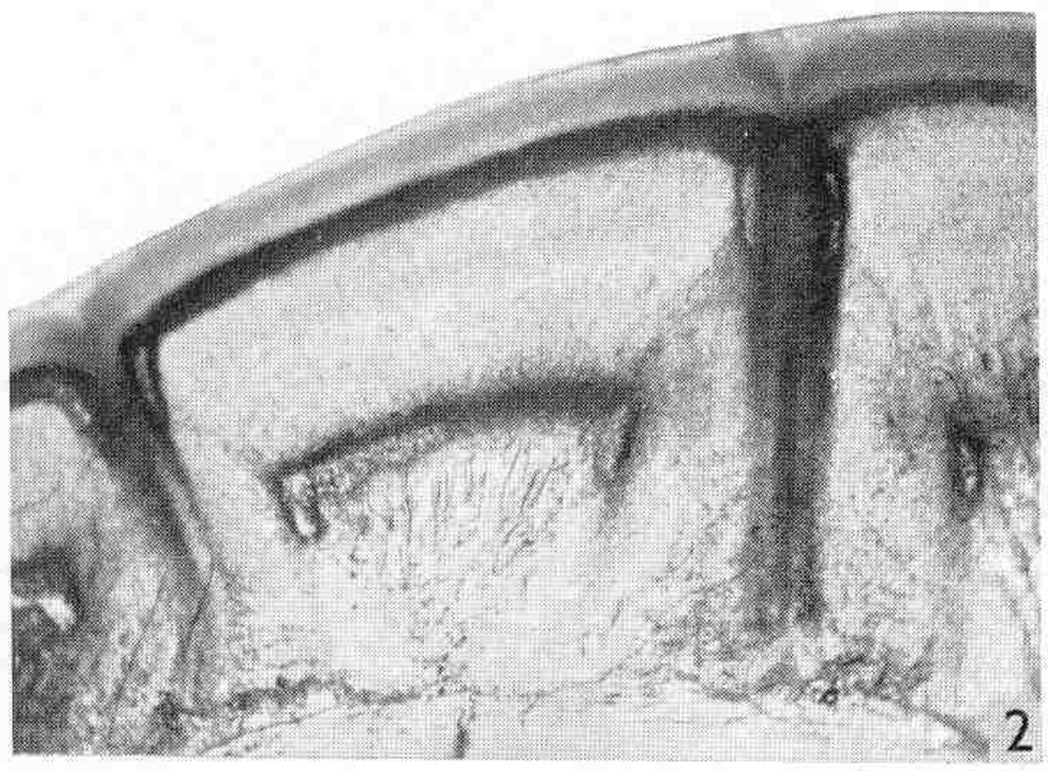
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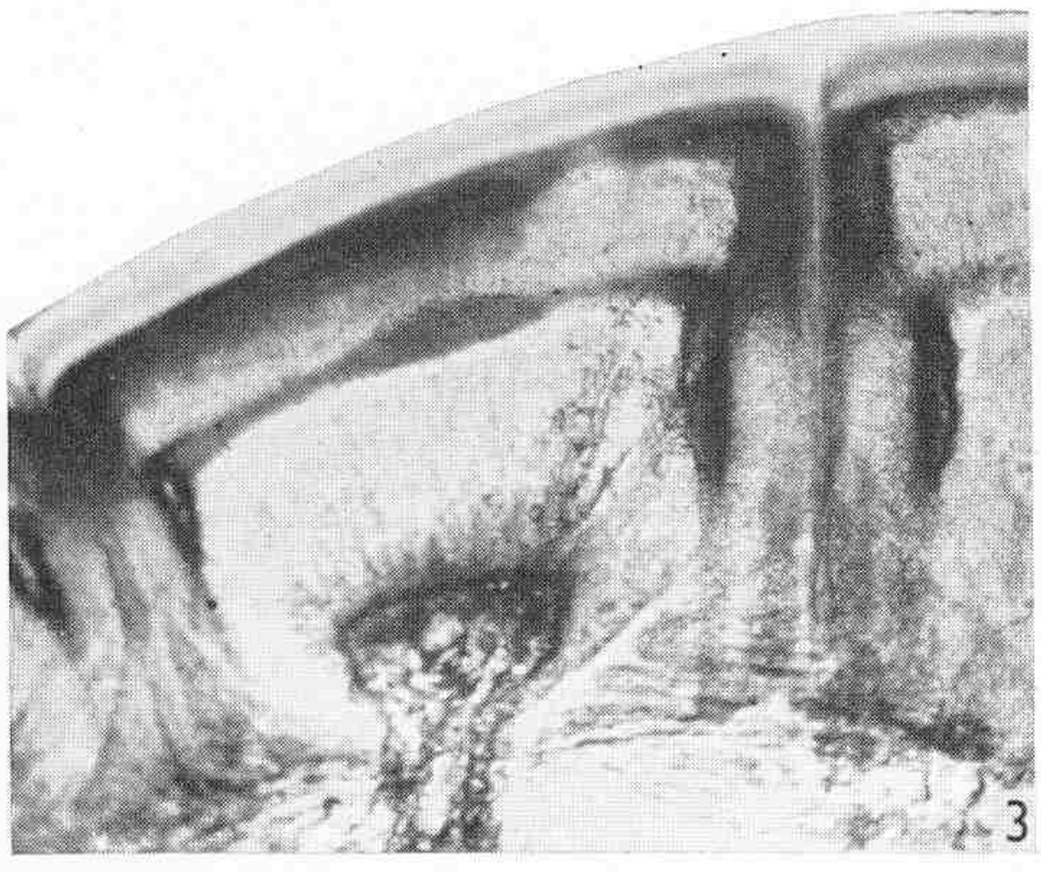
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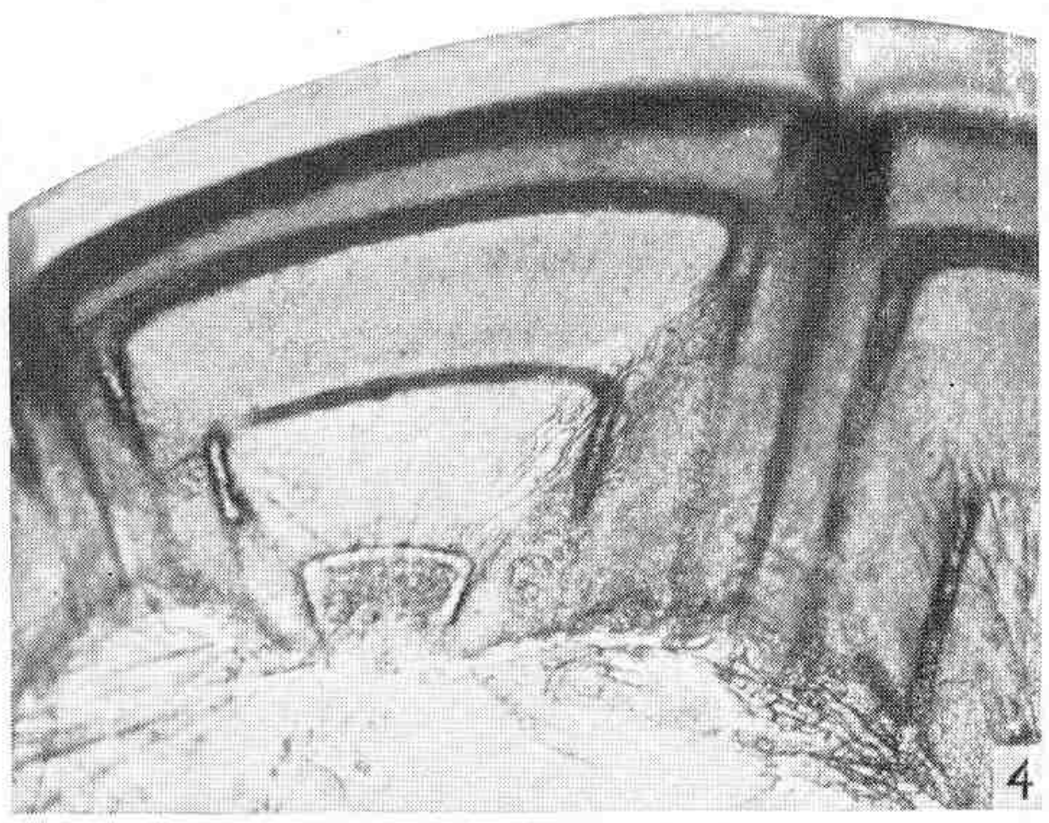
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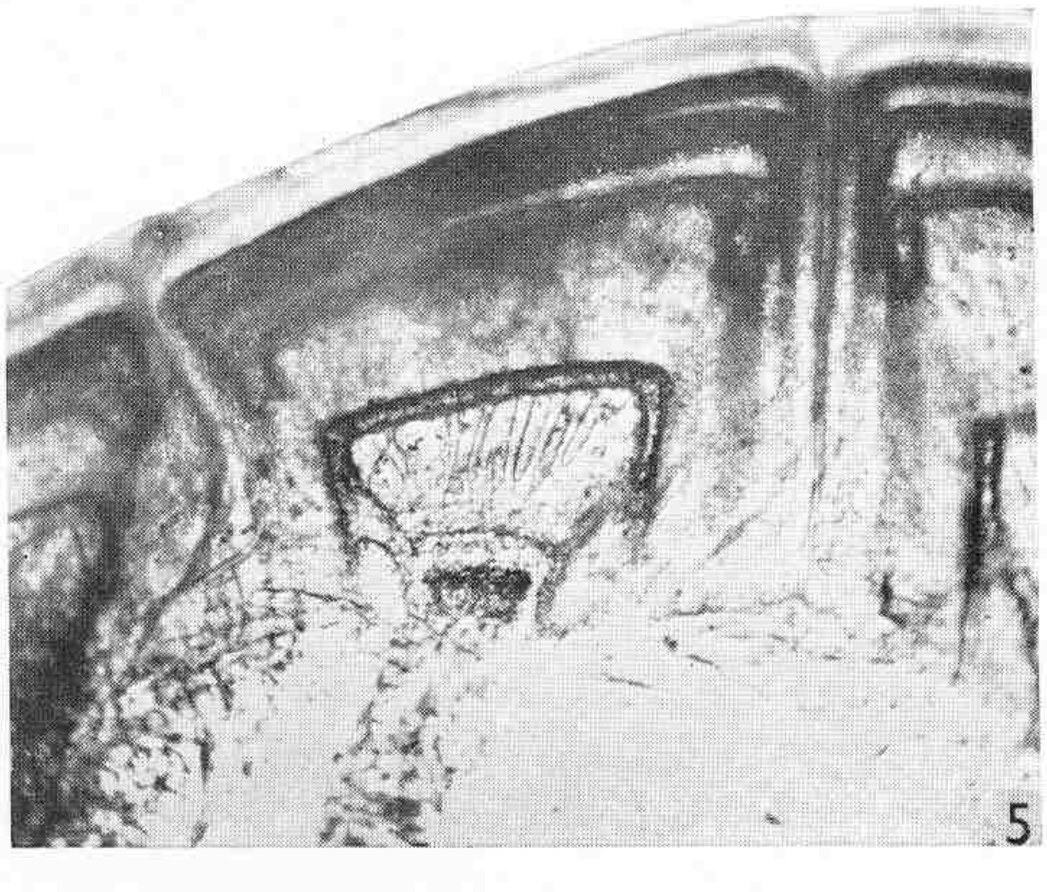
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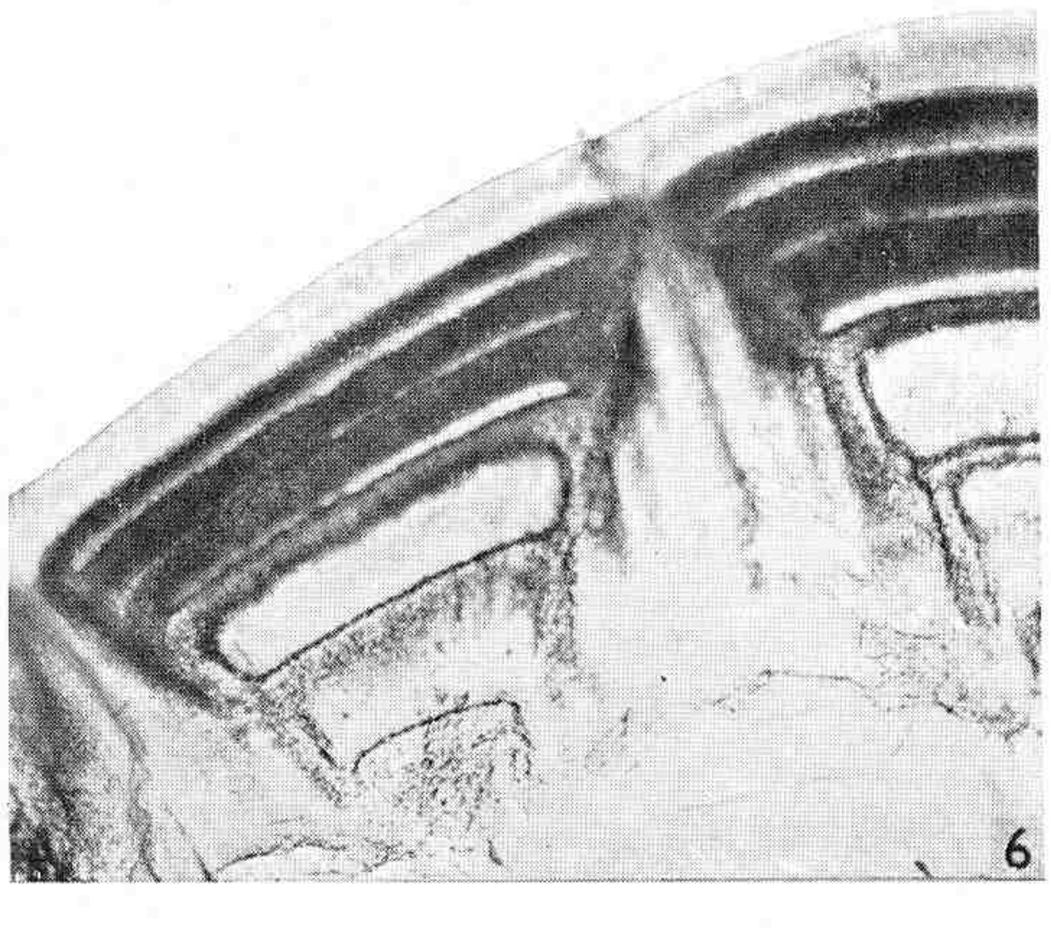
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Figs. 1–6.

ON *CALLIONYMUS RETICULATUS* C. & V. AND ITS DISTRIBUTION IN EUROPEAN SEAS

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(Plates I–III)

CONTENTS

INTRODUCTION

In the course of a study of the age and growth of the Common Dragonet, *Callionymus lyra* L., a number of specimens of another species of *Callionymus* have been collected off Plymouth, differing from both *C. lyra* L. and *C. maculatus* Rafinesque, the only two dragonets previously recorded from the Plymouth area (Mar. Biol. Assoc., 1931), and, indeed, from northern European seas (Andersson, 1942; Day, 1880–84; Duncker, Ehrenbaum, Kyle, Mohr & Schnakenbeck, 1929; Jenkins, 1925; Mohr (in Joubin, 1929–38); Le Danois, 1913; Norman, 1935; Otterstrøm, 1912; Poll, 1947; Redeke, 1941; Sæmundsson, 1949; Smitt, 1892–95).

Through the kindness of Prof. L. Bertin, Muséum National d'Histoire Naturelle, Paris, who gave me full information about the type specimens of both *C. fasciatus* C. & V. and *C. reticulatus* C. & V., this additional species has been identified as *C. reticulatus* Cuvier & Valenciennes (1837).

Specimens in the collections at the Plymouth Laboratory and from other institutions in Great Britain, and other European countries, have yielded some interesting information about this fish.

Historical Review of the European Dragonets

Altogether seventeen 'species' of *Callionymus* have been described from European waters. Their names, together with references to the original descriptions, are listed in chronological order below:

C. lyra Linnaeus	1758, p. 249.
C. dracunculus Linnaeus	1758, pp. 249–50.
C. pusillus Delaroche	1809, pp. 330–31; Pl. 25, fig. 16.
C. maculatus Rafinesque	1810, p. 25; Pl. V, fig. 1.
C. festivus Pallas	1811; pp. 146–47.
C. rissoi Lesueur	1814; pp. 5–6; Pl. I, figs. 16, 16*a*, 16*b*.
C. elegans Lesueur	1814; p. 6; Pl. I, figs. 17 and 17*a*.
C. belenus Risso	1826; pp. 263–64.
C. admirabilis Risso	1826; p. 264; Pl. VI, fig. 11.
C. morrissonii Risso	1826; pp. 265–66; Pl. VI, fig. 12.
C. lacerta Cuvier	1829; pp. 247–48.
C. cithara Cuvier	1829; p. 248.
C. reticulatus Cuvier & Valenciennes	1837; Vol. 12, pp. 284–85.
C. fasciatus Cuvier & Valenciennes	1837; Vol. 12, pp. 285–86.
C. sueurii Cuvier & Valenciennes	1837; Vol. 12, pp. 291–93.
C. phaëton Günther	1861; p. 147.
C. parthenopoeus Giglioli	1883; pp. 397–400.

In addition, Ninni (1934) described two forms, *C. maculatus atlanticus* and *C. festivus giglioli* (=*C. parthenopoeus* Giglioli, 1883); and Gonçalves (1942) recorded two unidentified species of *Callionymus* from Portuguese waters.

Comparatively few of the above have been accepted, however, by such authorities as Cuvier & Valenciennes (1837), who recognized only six of the earlier species (including Cuvier's two), while at the same time they described three new ones. In 1861 Günther, who made an exhaustive study of the European dragonets, established one new species, *C. phaëton*, and recognized four other good ones—*C. lyra*, *C. maculatus*, *C. festivus* and *C. belenus*. *C. fasciatus* and *C. reticulatus* were considered by him as doubtful species and all the others as synonyms.

C. fasciatus was described by Cuvier & Valenciennes (1837) from a single type specimen from Sicily. There was no further record of this species until A. P. Ninni (1877–78) had occasion to examine a number of specimens taken near the Istrian coast. He decided that it was definitely a good species, and worked out a key to the European *Callionymus*, which included the five species recognized by Günther, plus *C. fasciatus*. After A. P. Ninni there were further records of *C. fasciatus*, and its distribution, which is confined to the Mediterranean, was given by Carus (1889–93) and by E. Ninni (1934).

Like Günther, Fage (1918, p. 126) was inclined to think that there are only five good European species. In the same paper he pointed out that *C. parthenopoeus* Giglioli (1883) was a synonym of *C. festivus*, which E. Ninni (1934) redescribed as a form of *C. festivus* Pallas.

The type specimen of *C. reticulatus* Cuvier & Valenciennes (1837) was from Malaga. This species has not been reported since, and has been confused with *C. maculatus* in a number of ways. It was considered as a synonym of *C. maculatus* by Steindachner (1868), Day (1880–84), Carus (1889–93), Smitt (1892–95) and Mohr (in Joubin 1929–38); or identified as *C. maculatus* by Bragança (1903) and Nobre (1935). The descriptions of *C. maculatus* by Moreau (1881) and by Day (1880–84) evidently combined the characteristics of both *C. maculatus* and *C. reticulatus*.

The two unidentified species of *Callionymus* recorded by Gonçalves (1942) are of particular interest (see also p. 302). I was fortunate enough to have the opportunity of examining two specimens, one of each species. One male belonging to the first unidentified species was the identical specimen that had been identified as *C. maculatus* by Bragança (1903) and by Nobre (1935). It proved, however, to be in fact a fully mature male of *C. reticulatus*.

The female specimen belonging to the second unidentified species remains undetermined.

Description

Callionymus reticulatus Cuvier & Valenciennes

C. reticulatus Cuvier & Valenciennes, 1837, Vol. 12, pp. 284–85. *C. maculatus* (part.) Moreau, 1881, Vol. 2, pp. 164, 169–70[1]. *C. maculatus* (part.) Day, 1880–84, Vol. 1, pp. 177–78[1]. *C. maculatus* (nec Raf.) Bragança, 1903, p. 35. *C. maculatus* (nec Raf.) Nobre, 1935, pp. 505–6. *C.* sp. Gonçalves, 1942, p. 80 (fish no. 273, third specimen, male 8·3–11·0 cm.).

Diagnostic Characters

(1) The preopercular spine is tricuspid, but occasionally carries a very rudimentary basal spine. (2) The second dorsal fin always consists of ten rays, of which the last is branched. (3) In the second dorsal fin of the adult male there are eight vertical or oblique rows of dark spots. In between these rows of spots there are dark-margined, bluish, tortuous lines and spots. In the anal fin there exist four or five rows of parallel, but discontinuous, dark-margined bluish lines.

The Plymouth Specimens (Pl. I, figs. 1–4; Pl. II, figs. 1, 4 and 5; Pl. III, figs. 1–4).

The following description is based on the twenty-one males (40–108 mm.)[2] and fifteen females (30–65 mm.) collected in the Plymouth area during a period

[1] The descriptions combine the characteristics of both *C. maculatus* and *C. reticulatus*.

[2] The length of the specimens referred to in this paper is total length; except when specific mention is made of standard length.

of 18 months, from December 1948 to May 1950; and a mature male (the caudal fin had broken, 70 mm. in standard length) collected on 1 August 1900, near Mewstone, Plymouth.

General features. Body of typical dragonet-shape, smooth, devoid of scales and depressed throughout; deepest and broadest in opercular region. Head large, much depressed, and flat on ventral side, contained about 3·2 to 3·6 times (in male) and 3·3 to 3·7 times (in female) in standard length; its width, in opercular region, varying from 1·7 to 2·0 times its depth. Snout spatulate, nearly equal in length to long diameter of eye in females and immature males, but much elongated in mature males. Eyes very prominent, of medium size and closely set on dorsal side; interorbital space very narrow, less than one-half of vertical diameter of eye. Opercular bone very thin; gill opening reduced to foramen; lateral line single, extending to middle of caudal fin, with short branches on trunk and connection with that on opposite side through common dorsal branch near base of caudal fin.

Fin-rays. D1, IV; D2, 10; A, 9–10; V, 1, 5; C, 12. First dorsal fin with four flexible spines; second dorsal fin with ten rays, of which first nine are simple but last one is branched twice. Anal fin with nine to ten rays, last ray also branched. Number of fin-rays in dorsal fin very constant.

Preopercular spines. The preopercular bone ends posteriorly in three small recurved spines. The anterior and the middle ones are rather stout and curved upwards. The posterior one, which is directed backwards, is much smaller than the other two. It is always less than one-half of the middle one, both in length and in thickness. Among the thirty-seven specimens examined, thirty-four have no free basal spine (i.e. there is no fourth preopercular spine projecting forwards). In the other three, however, the basal spine is distinct but very rudimentary; one has a spine on both sides and the other two have one on the left side only.

Coloration. In females and young males the ground colour on the dorsal side varies from orange-brown to orange-red, passing into white or creamy beneath. There are many very light bluish spots scattered over the dorsal side of the body, and six dark orange-red patches along the dorsal median line.

These bluish spots are relatively much larger than those of *C. lyra*, *C. maculatus* and *C. fasciatus*. The six dark, dorsal patches are very similar to those of *C. lyra* and *C. fasciatus;* but in *C. reticulatus* they are very hard in outline, especially the fourth patch which is very characteristic as it has two waves anteriorly and is smooth and rounded posteriorly. On the lateral side of the body there is a series of seven or eight irregular dark spots.

Sexual dimorphism. As in many other dragonets, the male secondary sexual characters develop gradually into a state which makes the fully mature male entirely different from the female and the young male in external features. The largest male examined was 108 mm. long, while no mature female exceeded 65 mm.

In the female the first and second dorsal fins are only as high as, or slightly higher than, the body below them, but in the fully mature male they extend to

more than three times the body depth. The anal fin, which is less than the body depth in the females, may be nearly twice as high as the body in old males. The lower portion of the caudal fin in the mature male is much longer than the upper portion.

On the second dorsal fin of the male there appear gradually three dark spots, of which the uppermost is always very faint, on the membrane between the consecutive rays. As the spots immediately posterior to the odd-numbered rays are arranged somewhat on the same level, and those posterior to the rays of even number on another level, they form eight oblique or vertical rows crossing the rays diagonally, instead of rows running parallel with the dorsal outline as in the male *C. maculatus* (compare Pl. I, figs. 1 and 3 with Smitt, 1892–95, Pl. XV, fig. 1, reproduced in Pl. 36 of Jenkins (1925) and later editions). In between these rows of dark spots, there are eight rows of dark-margined, bluish, tortuous lines and irregular spots. These lines and spots are more or less continuous in young males. On the anal fin of mature males there are four or five parallel rows of dark-margined, discontinuous bluish lines, which turn obliquely downwards on the membrane between the branches of the last ray. The ventral fin of the mature male becomes very dark and the caudal fin bears many dark-margined, bluish spots and lines. In old males, the ground colour between the spots and lines on all median fins is bright yellow.

Other Channel Specimens

Two young specimens, 31 and 34 mm. long (British Museum Fish nos. 1951.2.19.51 and 52), were collected by the *Manihine* in the English Channel at position 50° 35′ 45″ N., 1° 20′ 40″ E., on 28 August 1949. The smaller of these specimens has a rudimentary basal spine on the preoperculum of the right side.

A North Sea Specimen

One adult male (British Museum Fish no. 1950.11.11.1), 74 mm. long, was contained in a bottle labelled 'Mouth of Thames, Mus. Leach'. William Elford Leach, M.D. (1790–1836), was naturalist assistant librarian, and later assistant keeper, at the British Museum, 1813–21. It would thus appear that this specimen was extant before the species was described as new.

A Portuguese Specimen

Dr H. Vilela, Estação de Biologia Marítima, Lisbon, kindly lent me ten valuable specimens[1] of *Callionymus* collected off the Portuguese coast. One of the specimens, a mature male, 110 mm. long, was identified as *C. reticulatus*

[1] A female specimen, 57 mm. long, was collected at the same time and from the same locality as the above male (Gonçalves, 1942, p. 81, Fish no. 274, *Callionymus* sp.). This is a very interesting specimen because the preoperculum has four recurved spines. Like *C. reticulatus*, its second dorsal fin consists of ten rays and there is no basal spine. As none of the European dragonets has four recurved spines, I am uncertain whether this is an abnormality. This specimen remains unidentified. The other eight specimens are all *C. lyra* L.

C. & V., Pl. II, fig. 8. Its colour has been lost; the second dorsal fin consists of ten rays; the preopercular bone has only three recurved spines and no basal spines; the male secondary sexual characters other than coloration are the same as those of the Plymouth specimens. Dr Vilela also informed me that it was collected by Don Carlos de Bragança, the King of Portugal, near Sezimbra (between Cape Espichel and mouth of River Sado), by trawl-net, 110 m. depth, year 1897, and that it is the identical specimen referred to by Bragança (1903, p. 35), Nobre (1935, pp. 505–6) and Gonçalves (1942, p. 80, no. 273, *Callionymus* sp., the third specimen).

The Type Specimen

Prof. L. Bertin has kindly given me the following information about the type specimen of *C. reticulatus* C. & V.:

> Nous avons eu le désagrément de constater qu'il est en très mauvais état. Ses nageoires et sa coloration ne peuvent être connues.
>
> Nous pouvons seulement vous dire que le préopercule comporte seulement 3 épines dirigées vers l'arrière et dont le plus ventrale est presque rudimentaire. Il n'y a pas de 4-ème épine dirigée vers l'avant. C'est à peu près la disposition de votre photographie No. 2 de votre lettre du 9 août.
>
> Dans ces conditions, nous sommes très perplexes au sujet de vos spécimens de Plymouth. Êtes-vous sûr que ce ne sont pas des *C. fasciatus*.—Le type de cette espèce, nous vous le répétons, a 4 épines operculaires.
>
> Fage, dans son travail de 1918, ne parle pas de *C. reticulatus*.

The original description of *C. reticulatus* by Cuvier & Valenciennes (1837) is reproduced here for reference.

> Le Callionyme réticulé.
>
> (*Callionymus reticulatus*, nob.)
>
> Elle nous a été donnée par M. Baillon, qui l'a reçue de Malaga. Son museau est un peu plus long et plus pointu que dans le *Callionymus cithara*: il a aussi trois rayons à la pointe du préopercule. Le premier rayon dorsal s'alonge en fil d'un peu plus du quart de la longueur totale. La seconde dorsale n'a pas trois fois la hauteur du corps, et l'anale est de moitié moindre, en sorte que l'une et l'autre est plus basse que dans le *C. cithara*. La deuxième dorsale a un rayon de plus.
>
> D. 4–10. A. 8, etc.
>
> Il y a sur la seconde dorsale trois rangées de taches brunes semblables à celles du *C. cithara*; mais les intervalles sont remplis de cercles et de rubans ondulés, blancs, avec un liséré mince, brun et blanc; d'où résulte un ensemble très-agréable à l'oeil. La première a deux ou trois de ces rubans ondulés et à double lisérés, et deux ou trois taches noires. Quatre lignes semblables, mais plus étroites, parcourent parallèlement toute la longueur de l'anale, dont le bord est noirâtre. Sur l'anale, entre les rayons supérieurs, sont des traits obliques; entre les autres des longitudinaux, tous blancs, lisérés de brun. On voit aussi quelques taches semblables sur la joue et à la base de la ventrale.
>
> La longueur de notre individu est de trois pouces et demi.

In studying all the material mentioned above, I have found that the specimens from different localities are identical with the original description of

C. reticulatus C. & V. and Prof. Bertin's information about the type, except in one minor point, which is that there are only eight rays in the anal fin in the type. This is probably due to variation. Different from all other European dragonets, *C. reticulatus* is definitely a good species. The details will be discussed under the heading 'Comparison with other European species'.

Distribution

If the above information concerning *C. reticulatus* be accepted it is clear that this species is present in the western portion of the Mediterranean in Spanish waters off Malaga, and in coastal areas off Portugal, and extends into the English Channel and southern North Sea. Recently there have been reports of the spread of southern forms, notably fish and certain algae, into the northern waters of the European seas and into the Northern Atlantic (Cotton, 1935; Fridriksson, 1949; Lund, 1945; Parke, 1948; Tåning, 1949). It appeared at first that the presence of *C. reticulatus* in the Plymouth area might be yet another example of immigration from the south. It now seems more likely that this species has been overlooked and that it may therefore long have been even more widely distributed in European waters than the above records would suggest.

Ecological Notes

The catches of *Callionymus* in the Plymouth area during a period of 18 months, from December 1948 to May 1949 inclusive, indicate the great scarcity of *C. reticulatus* as compared with the other two dragonets. Altogether thirty-six specimens of *C. reticulatus* have been caught (30–108 mm. long); but the total number of *C. lyra* (30–300 mm. long) amounts to over four thousand, and that of *C. maculatus* (30–109 mm.) to 149. The nets used were (*a*) the ordinary otter trawl, (*b*) ordinary otter trawl with small-mesh cod-end, (*c*) fine-meshed Agassiz trawl, and (*d*) small beam trawl (shrimp netting). Owing to their small size[1] *C. reticulatus* and *C. maculatus* would not normally be expected in an ordinary trawl catch, and in fact most of the specimens of these two species were caught in the Agassiz trawl and in the small-mesh cod-end of the otter trawl. The relative abundance of *C. lyra* in the catches is therefore probably due to its presence in greater numbers and to the retention of this large species in the ordinary otter trawl. It is interesting to note, however, that on 18 January 1950, ten specimens of *C. reticulatus* were caught in the Agassiz trawl off Whitsand Bay, and at the entrance of Plymouth Sound, from off Wembury Bay and the Mewstone to Whitsand Bay and Cawsand Bay. This represents a total of about 3 hr. trawling by this net, and indicates considerable numbers of this species on these grounds.

[1] In the Plymouth area *C. reticulatus* reaches at least 108 mm. Although my largest specimen of *C. maculatus* is 109 mm. a preserved specimen of 124 mm. is present in an old collection in the Laboratory of the Marine Biological Association.

Although *C. lyra*, *C. maculatus* and *C. reticulatus* may be trawled from the same grounds in the Plymouth area, it seems that they have their own habitat preferences. *C. lyra*, one of the commonest fishes in this locality, has a very wide distribution. The adults inhabit all the offshore waters off Plymouth. The young fish usually spend the first year very close to the shore and may go as far as 3 or 4 miles up the River Tamar. *C. maculatus*, very scarce inside Plymouth Sound, is sparsely but fairly generally distributed on the trawling grounds off Plymouth. *C. reticulatus*, on the contrary, seems to have a definitely localized distribution in this area. Out of the thirty-six specimens obtained, twenty-seven were caught in or near the entrance to Plymouth Sound, from Wembury Bay to Whitsand Bay, and four from the *Amphioxus* grounds near the Eddystone. Two of the remaining five specimens were obtained from the stomach of a gurnard (*Trigla* sp.). Furthermore, this species does not frequent brackish water as does the young *Callionymus lyra*, and it is very rare in the offshore water on the trawling ground off Plymouth, where *C. maculatus* is common and the adults of *C. lyra* are plentiful.

Unfortunately, no full information about the breeding season has been obtained, but some indications can be derived from the time at which the mature specimens were caught. Among the males there are two specimens with fully developed secondary sexual characters. The first, 72 mm. long, was obtained in May of 1949, and the other, 108 mm. long, collected in 1950, was also obtained in the month of May. Altogether there were four females which could be considered as mature as they contained very large eggs. In the first, 59 mm. long, caught in March 1949, the ova measured up to 0·5 mm. in diameter. The second, 60 mm. long, was obtained in May of 1949, and contained ova measuring up to 0·7 mm., and the third one, 63 mm. long, trawled in May of 1950, contained ova up to 0·5 mm. In another, caught in June of 1949, the ova measured up to 0·4 mm. Several eggs, found lying free in the body cavity of the second mature female, had very large hexagonal reticulations on the vitelline membrane; these reticulations varied from 18 to 26 μ across. It is obvious from the above that the breeding season of *C. reticulatus* in the Plymouth area extends at any rate from March to June.

Comparison with Other European Species

As *C. reticulatus* C. & V. shares many important characteristics common to *C. lyra*, *C. maculatus* and *C. fasciatus*, with any one of which it may easily be confused, I examined the most important characters of a number of specimens of these three species from different localities. The details are described below and shown in Table I.

C. fasciatus C. & V. Prof. L. Bertin has provided the following information about the type specimen:

J'ai examiné le type *Callionymus fasciatus* C. & V. Il possède à chaque préopercule 3 épines dirigées en arrière et une épine dirigée en avant, selon le schema ci-dessous, et

TABLE I. COMPARISON OF THE FREQUENCIES OF THE NUMBERS OF PREOPERCULAR SPINES, AND THE NUMBERS OF RAYS IN THE DORSAL AND ANAL FINS OF *CALLIONYMUS RETICULATUS*, *C. FASCIATUS*, *C. MACULATUS* AND *C. LYRA* EXAMINED FROM DIFFERENT LOCALITIES

		C. reticulatus C. & V.					*C. fasciatus* C. & V.			*C. maculatus* Rafinesque				*C. lyra* L.		
		Malaga (Type)	Plymouth	English Channel	Southern North Sea	Portugal	Sicily (Type)	Genoa	Istria	Port Vendres	Plymouth	Port Erin	Mill-port	Plymouth	Port Erin	Portugal
No. of rays in the dorsal fins	IV+8	..	..	..	..	..	..	..	..	..	..	..	..	4	..	..
	IV+9	..	..	..	..	..	..	..	..	4	107	5	6	277	39	8
	IV+10	1	37	2	1	1	1	2	2	..	2	1	..	1	..	..
No. of rays in the anal fin	8	1	..	..	..	..	..	..	..	..	2	..	..	4	1	..
	9	..	36	2	1	1	1	2	2	4	105	6	6	278	38	8
	10	..	1	..	..	..	..	..	..	..	2	..	..	..	..	..
Preoperculum with three recurved spines and	A basal spine: Well-developed	..	..	..	..	..	1	2	2	4	109	6	6	282	39	8
	A basal spine: Rudimentary	..	3	1	..	..	..	..	..	..	..	..	..	..	..	..
	No basal spine	1	34	1	1	1	..	..	..	..	..	..	..	..	..	..

répondant à peu près à la disposition de votre *C. maculatus* (Photo N. 3). La 3e épine est cependant un peu plus courte et la 4e est moins détachée de l'os préoperculaire.

La coloration de la seconde dorsale—il s'agit d'un mâle—est différente de celle de votre photographie No. I. Il y a en effet 3 ou 4 taches noires dans chaque espace interradiaire, au lieu de 2 ou 3. Le nombre des rangées de ces taches est donc plus élevé que sur votre photo et rappelle la disposition du *C. maculatus* dessiné par Erna Mohr dans la fiche de la Faune ichthyologique de l'Atlantique Nord.

Sur la première dorsale, il n'y a pas de tache prédominante, mais la disposition figurée ci-dessous:

Je ne connais pas le fig. 2. pl. 40 dont parlent Cuvier et Valenciennes.

C. fasciatus me semble tomber en synonymie avec *C. maculatus*. C'est d'ailleurs l'opinion déjà soutenue par Fage, dans un très important travail que vous semblez ignorer: Shore-Fishes, dans Report Danish Oceanogr. Exped. Medit. Adj. Seas- Vol. II- Biology-A. 3. paru en 1918.

Reste à savoir ce que sont vos spécimens ayant seulement 3 épines préoperculaires (et quelquefois 4).

D'après Fage et les auteurs consultés il n'existe dans la Manche et l'Atlantique que *C. lyra* et *C. maculatus* (*fasciatus*) qui ont toutes deux 4 épines.

Les espèces à 3 épines n' existent qu'en Méditerranée.

Il y a donc là un point à éclaircir, mais sur lequel je ne puis vous rendre aucun service à distance.

The four specimens of *C. fasciatus* belonging to the 'Museo Civico de Storia Naturale, Genova', comprise three mature males and one female. The first male was from 'Golfo di Genova' (71[1]) mm. long; the second and third males were from 'Istria', 83 (66[1]) mm. and 90 (71[1]) mm. long respectively (Fish nos. 265A, 468*a*); and the female, from 'Golfo di Genova', was 55 (44) mm. long (Fish nos. 265, 486). D 1, IV; D 2, 10; A, 9; V, 1, 5. The last rays of both the second dorsal and the anal fin are branched. The preopercular bone has always three recurved spines and a very prominent basal spine (Pl. II, figs. 6, 7). There are four dark spots on the membrane between consecutive rays in the second dorsal fin, and the spots on the fin are also arranged in oblique or vertical rows across the fin as in *C. reticulatus*. There are six to eight rows of dark-margined, bluish parallel lines in the anal fin of the male.

C. lyra. Altogether 329 specimens were examined, the length of the specimens varying from 40 mm. to about 300 mm. They all have four very prominent preopercular spines, three recurved spines and a basal spine. The number of the second dorsal fin-rays varies from eight to ten (Pl. I, fig. 5; Pl. II, fig. 3).

C. maculatus. I have examined 125 specimens from different localities. The preopercular bone has constantly four spines. On the second dorsal fin of the males there are four horizontal rows of dark spots; sometimes there are similar numbers of light round spots lying immediately behind the dark spots. There are no parallel lines on the anal fin such as are found in *C. fasciatus* and

[1] The figures in brackets are standard length. The total length of one of the specimens is not known as the tail has been damaged.

C. reticulatus. Dark round spots are present on the sides of the body (Pl. I, fig. 6; Pl. II, fig. 2).

C. reticulatus C. & V. can therefore be distinguished from *C. lyra*, *C. maculatus* and *C. fasciatus* by the following characteristics:

(1) The preopercular spines are the most reliable characteristic. In *C. reticulatus* there are three recurved spines, which project upwards or backwards; but the basal spine, which projects forwards, is normally absent. If present, it is very rudimentary (Pl. II, figs. 4, 5) and is usually on one side only (p. 300). In the other three species, however, there are also three recurved spines, but the basal spine is always present and very prominent (Pl. II, figs. 2, 3, 6 and 7).

(2) The number of the fin-rays in the second dorsal fin is constantly ten in *C. reticulatus* and in *C. fasciatus;* in *C. lyra* and *C. maculatus*, however, there are normally nine rays, but occasionally eight or ten rays. The last rays of the second dorsal fin of all these four species are branched.

(3) The colour patterns on the body of *C. reticulatus* are very similar to those of *C. fasciatus* and of the young *C. lyra*. Nevertheless, in the first species the light bluish spots are much larger than those in the latter two species, in specimens of similar size. As a rule the dorsal patches of *C. maculatus* and *C. lyra* have irregular margins, whereas those of *C. reticulatus* have always very well-defined entire margins, especially the fourth which is very characteristic (Pl. I, figs. 2 and 4–6). As *C. maculatus* has dark spots on the sides of the body, there can be no difficulty in distinguishing it from the other three species, so long as these spots have not been lost in preservation.

(4) The male secondary sexual characters are very different in the mature adults of these four species. There can be no difficulty in distinguishing them when the specimens are fresh, or in preserved material, if the coloration has not been lost completely. The smallest mature male of *C. lyra* is about 180 mm. long. Its greatly elongated first dorsal fin may extend to the base of the caudal fin or beyond it. There are no dark spots on the first and the second dorsal nor on the anal fin (Couch, 1863, pl. CIII; Holt, 1898, pl. XXVI; Jenkins, 1925, pl. 34; Smitt, 1892–95, tab. XIV). The mature males of *C. maculatus*, *C. fasciatus* and *C. reticulatus* are much smaller in size (see pp. 299 and 306). On the second dorsal fin of these three species there are dark spots on the membrane between consecutive rays—four spots in *C. maculatus*, four (the uppermost very faint) in *C. fasciatus*, and three (the uppermost also very faint) in *C. reticulatus*. In *C. maculatus*, these dark spots are arranged in more or less horizontal rows (Smitt, 1892–95, tab. XV, fig. 1; Günther, 1867, pl. V, fig. A; E. Ninni, 1934, tab. II and III, fig. 1). In *C. fasciatus* and *C. reticulatus* these spots are arranged in oblique or vertical rows (Guérin-Meneville, 1829–44, pl. 40, no. 2; E. Ninni, 1934, pl. III, fig. 2; and Pl. I, figs. 1 and 3 in this paper). Furthermore, in both *C. fasciatus* and *C. reticulatus* there are rows of bluish dark-margined, tortuous lines or spots on the second dorsal fin; and

dark-margined bluish parallel lines on the anal fin. In the former there are six to eight lines; while in the latter there are only four or five lines.

As regards the other three European dragonets, *C. phaëton* has two recurved spines, but *C. festivus* and *C. belenus*, like *C. reticulatus*, also have only three recurved spines. Nevertheless, the dorsal fins of *C. belenus* and *C. festivus* have fewer rays than *C. reticulatus*. The fin formula is D 1, IV and D 2, 6–7 in *C. festivus*; and D 1, III, and D 2, 8, in *C. belenus*.

Thus *C. reticulatus* is well distinguished from all other European species, with the possible exception of *C. fasciatus*. These two species present the following incomplete contrasts:

C. reticulatus: preoperculum with three recurved spines; rarely a fourth (basal) spine on one or both sides.
C. fasciatus: preoperculum with three recurved spines and a strong basal spine.
C. reticulatus: mature males with three dark spots on an inter-radial membrane of D2 and four or five horizontal bands on the anal.
C. fasciatus: mature males with four dark spots on an inter-radial membrane of D2 and six to eight horizontal bands on the anal.
C. reticulatus: distributed from Malaga to the North Sea.
C. fasciatus: not known outside the Mediterranean.

The only well-authenticated specimen of *C. reticulatus* from the Mediterranean is the type, but its colour is not known.

The question of geographical variation arises. Fishes with rows of spots on the fins increase the number of rows as the fish grows. The number of mature males of these two species examined by me is too small to admit of generalization, but those named *fasciatus* are not all larger than those assigned to *reticulatus*. In some other species of the northern hemisphere, however, the northern members are known to grow larger and to mature later than the southern, and it is possible that we have here a difference associated with rate of growth. This could hardly account, however, for the presence in one and rarity in the other of a basal spine on the preoperculum; until, therefore, the Mediterranean populations are better known it seems wise to recognize both *C. reticulatus* and *C. fasciatus* as distinct species.

Summary

Three species of *Callionymus* are recognized as occurring in British waters, *C. lyra* L., *C. maculatus* Raf. and *C. reticulatus* C. & V.

The third is here recognized for the first time and is described from an abundant material.

Both *C. reticulatus* and *C. fasciatus* C. & V. in the past have been confused with *C. maculatus*. They are here shown to be quite distinct from that species.

C. reticulatus is closely related to *C. fasciatus*. The value of the characters used to distinguish them is discussed.

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REFERENCES

ALLEN, E. J., 1899. On the fauna and bottom-deposits near the thirty-fathom line from the Eddystone grounds to Start Point. *Journ. Mar. Biol. Assoc.*, Vol. 5, pp. 365–542.

ANDERSSON, K. A., 1942. *Fiskar och Fiske i Norden*. Vol. 1. Stockholm.

BLEGVAD, H. & LØPPENTHIN, B., 1944. Fishes of the Iranian Gulf. *Danish Scientific Investigations in Iran*, Pt. III, pp. 1–247. Copenhagen.

BRAGANÇA, D. CARLOS DE, 1903. *Catalogo das collecções expostas (Palacio de Cristal Portuense)*, p. 15. Lisboa.

BUEN, F. DE, 1935–36. Fauna ictiólogica. *Notas y Resúmenes*, Ser. II, núm. 88 (1935), pp. 1–90; núm. 89 (1935), pp. 91–150; núm. 94 (1936), pp. 151–73. Madrid.

CARUS, J. V., 1889–93. *Prodromus Faunae Mediterraneae*, Vol. 2, pp. 686–8. Stuttgart.

COTTON, A. D., 1935. Changes in the British fauna and flora during the past fifty years. (5) Marine Algae. *Proc. Linn. Soc. London*, 1935, pp. 45–9.

COUCH, J., 1863. *A History of the Fishes of the British Islands*, Vol. 2. London.

CUVIER, G., 1829. *Le Règne Animal*, Tome 2. Paris.

CUVIER, G. & VALENCIENNES, A., 1837. *Histoire Naturelle des Poissons*, Vol. 12. Paris.

DAY, F., 1880–84. *The Fishes of Great Britain and Ireland*, Vol. 1. London.

DELAROCHE, F. E., 1809. Observations sur des poissons recueillis dans un voyage aux îles Baléares et Pythiuses. *Ann. Mus. Hist. Nat.* Paris, Tome 13, pp. 98–122, 313–61.

DUNCKER, G., 1939. *Die Fische der Nordmark*, Lief. 5, pp. 209–12. Kiel.

DUNCKER, G., EHRENBAUM, E., KYLE, H. M., MOHR, E. W. & SCHNAKENBECK, W., 1929. *Die Fische der Nord- und Ostsee*. Leipzig.

FAGE, L., 1918. Shore-fishes. *Rep. Danish Oceanogr. Exped. Medit. 1908–10*. Vol. 2 (Biology), A3, 154 pp.

FOWLER, H. W., 1936. The marine fishes of West Africa, based on the collection of the American Museum Congo Expedition, 1909–15. *Bull. Amer. Mus. Nat. Hist.*, Vol. 70, 2 parts, pp. 1–1493.

—— 1941. New fishes of the family Callionymidae, mostly Philippine, obtained by the United States Bureau of Fisheries steamer *Albatross*. *Proc. U.S. Nat. Mus.*, Vol. 90, No. 3106, pp. 1–31.

FRIDRIKSSON, ÁRNI, 1949. Boreo-tended changes in the marine vertebrate fauna of Iceland during the last 25 years. *Cons. Internat. Explor. Mer, Rapp. et Proc.-Verb.*, Vol. 125, pp. 30–2.

GIGLIOLI, E. H., 1883. Intorno a due nuovi pesci dal golfo di Napoli. *Zool. Anz.*, Bd. 6, pp. 397–400.

GILL, T. N., 1860. On the genus of *Callionymus* of authors. *Proc. Acad. Nat. Sci. Philad.*, 1859 (1860), pp. 128–30.

GONÇALVES, B. C., 1942. Colecção Oceanográfica de D. Carlos I: Catálogo dos peixes. *Trav. Stat. Biol. Mar. Lisbonne*, No. 46, pp. 1–108.

GUÉRIN-MENEVILLE, F. E., 1829–1844. *Iconographie du Règne Animal de G. Cuvier*, Tome 4. Poissons, Pl. 40, no. 2. Paris and London.

GÜNTHER, A., 1861. *Catalogue of the Fishes in the British Museum*, Vol. 3. London.

—— 1867. Additions to the British fauna. *Ann. Mag. Nat. Hist.*, 3rd Series, Vol. 20, pp. 288–91.

HOLT, E. W. L., 1898. On the breeding of the dragonet (*Callionymus lyra*) in the Marine Biological Association's Aquarium at Plymouth. *Proc. Zool. Soc. London*, 1898, Vol. 1, pp. 281–315, pl. XXVI.

HUBBS, C. L. & SCHULTZ, L. P., 1929. The northward occurrence of southern forms of marine life along the Pacific coast in 1926. *Calif. Fish. Game*, Vol. 15, No. 3, pp. 234–41.

JENKINS, J. T., 1925. *The Fishes of the British Isles*. London and New York.

JORDAN, D. S. & FOWLER, H. W., 1903. A review of the dragonets (Callionymidae) and related fishes of the waters of Japan. *Proc. U.S. Nat. Mus.*, Vol. 25, No. 1305, pp. 939–59.

JOUBIN, M., 1929–38. *Faune ichthyologique de l'Atlantique Nord*. 23 pp. Éditée par le Cons. Perm. Intern. pour Explor. de la Mer.

LE DANOIS, ED., 1913. Contribution a l'étude systématique et biologique des poissons de la Manche occidentale. *Ann. Instit. Ocean. Monaco*, Tome 5, Fasc. 5, pp. 1–214.
LESUEUR, C. A., 1814. Note sur deux poissons non encore décrits du genre *Callionymus* et de l'ordre des Jugulaires. *Bull. Sci. Soc. Philom. Paris*, 1814, pp. 5–6, Pl. I, figs. 16, 16*a*, 16*b*, 17 and 17*a*.
LINNAEUS, C., 1758. *Systema Naturae*, Tome I. Regnum Animale. (pp. 249–50.)
LOZANO REY, L., 1919. Los peces de la fauna ibérica en la colección del museo. *Trab. Mus. Ci. Nat.*, Ser. Zoologica, Núm. 39, pp. 1–112. Madrid.
LUND, S., 1945. On *Colpomenia peregrina* Sauv. and its occurrence in Danish waters. *Rep. Danish Biol. Stat.*, 1942, No. XLVII, pp. 3–16.
MARINE BIOLOGICAL ASSOCIATION, 1931. *Plymouth Marine Fauna.* 2nd ed.
MCINTOSH, W. C. & PRINCE, E. E., 1889. On the development and life-histories of the teleostean food and other fishes. *Trans. Royal Soc. Edinb.*, Vol. 35, Pt. 3, No. 19, pp. 665–944.
MOREAU, É., 1881. *Histoire naturelle des poissons de la France*, Tome 2, 572 pp. Paris.
NAVARRO, F. DE P., 1928. Un pez nuevo en la fauna balear *Callionymus phaëton* Günther. *Boletín de Pescas*, Madrid, Núm. 142, pp. 165–8.
NINNI, A. P., 1877–78. Materiali per la fauna veneta. II. Genere *Callionymus*. *Atti. Ist. Veneto. Sci.*, Ser. V, Tome 4, pp. 1043–57.
NINNI, E., 1934. *Callionymus* dei mari d'Europa. *Notas y Resúmenes*, núm. 85, pp. 1–59. Madrid.
NOBRE, A., 1935. *Fauna marinha de Portugal.* 1. *Vertebrados*, pp. 153–6; 505–6. Pôrto.
NORMAN, J. R., 1935. *List of British Vertebrates*. London.
OTTERSTRØM, C. V., 1912. *Danmarks Fauna* VI. *Fisk I. Pigfinnefisk*, pp. 168–72. Kjøbenhavn.
PALLAS, P. S., 1811. *Zoographia Rosso-Asiatica*, Vol. 3, pp. 146–7. Petropoli.
PARKE, M., 1948. *Laminaria ochroleuca* De La Pylaie growing on the coast of Britain. *Nature*, Vol. 162, pp. 295–6.
PERRIER, R., 1924. *La faune de France en tableaux synoptiques illustres.* 10. *Vertebres Poissons*. Paris.
POLL, M., 1947. *Faune de Belgique. Poissons marins.* 452 pp. Bruxelles.
RAFINESQUE SCHMALTZ, C. S., 1810. *Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia, con varie osservazioni soprai medisimi.* 105 pp. Palermo.
REDEKE, H. C., 1941. *De visschen van Nederland.* 331 pp. Leiden.
RISSO, A., 1810. *Ichthyologie de Nice*, pp. 103–6. Paris.
—— 1826. *Histoire naturelle de l'Europe Méridionale*, Tome 3, pp. 262–6. Paris.
SÆMUNDSSON, B., 1949. Marine Pisces. *Zool. Iceland*, Vol. 4, Pt. 72, pp. 1–150. Copenhagen and Reykjavík.
SMITH, J. L. B., 1949. *The Sea Fishes of Southern Africa.* 550 pp. Cape Town.
SMITT, F. A., 1892–95. *A History of Scandinavian Fishes*, by B. Fries, C.U. Ekström, & C. Sundevall, 2nd ed., Pts I and II. Stockholm.
ŠOLJAN, T., 1948. *Fauna i flora Jádrana. Knjiga I. Ribe Jadrana.* (Fishes of the Adriatic). Split.
STEINDACHNER, F., 1868. Ichthyologischer Bericht über eine nach Spanien und Portugal unternommene Reise. V. *Sitzber. Akad. Wiss. Wien*, Vol. 57, 1868, pp. 351–424.
TÅNING, A. V., 1949. On changes in the marine fauna of the north-western Atlantic area, with special reference to Greenland. *Cons. Internat. Explor. Mer, Rapp. et Proc.-Verb.*, Vol. 125, pp. 27–9.
TORTONESE, E. & TROTTI, L., 1949. Catalogo dei pesci del Mare Ligure. *Atti. Accad. Ligure Sci. Lett.*, Vol. 6, pp. 1–118.

VINCIGUERRA, D., 1885. Appunti ittiologici sulle collezioni del Museo Civico di Genova. VII. Spora alcuni pesci nuovi del Golfo di Genova. *Ann. Mus. Civ. Storia Nat.*, Vol. 22, pp. 446–75. Genova.

YARRELL, W., 1859. *A History of British Fishes*, Vol. 2. 3rd ed. London.

EXPLANATION OF PLATES

PLATE I, photographs of *Callionymus*, $\times \frac{2}{3}$

Fig. 1. *C. reticulatus* C. & V., mature male, lateral view of a preserved specimen collected in May 1950, Plymouth.

Fig. 2. Same as fig. 1, dorsal view.

Fig. 3. *C. reticulatus* C. & V., male, lateral view of a living specimen collected in November 1949. Plymouth.

Fig. 4. *C. reticulatus* C. &. V., mature female, dorsal view of a fresh specimen collected in July 1950, Plymouth.

Fig. 5. *C. lyra* L., young female, dorsal view of a fresh specimen collected in September 1950, Plymouth.

Fig. 6. *C. maculatus* (Rafinesque), mature female, dorsal view of a fresh specimen collected in May 1950, Plymouth.

PLATE II, photographs of *Callionymus*, figs. 1–7, $\times 6{\cdot}7$; fig. 7, $\times \frac{2}{3}$.

Fig. 1. The right preopercular bone, lateral view, of a male *C. reticulatus*, 86 mm. long, Plymouth, bearing three recurved spines only.

Fig. 2. The right preopercular bone, lateral view, of a male *C. maculatus*, 86 mm. long, Plymouth, showing three recurved spines and one basal spine.

Fig. 3. The right preopercular bone, lateral view, of a female *C. lyra*, 86 mm. long, Plymouth, showing three recurved spines and one basal spine.

Fig. 4. The right preopercular bone, lateral view, of a male *C. reticulatus*, 60 mm. long, Plymouth, bearing three recurved spines and one rudimentary basal spine.

Fig. 5. Same as the above specimen, dorsal view to show three recurved spines and the rudimentary basal spine.

Fig. 6. A portion of the right preopercular bone, dorsal view, of a male *C. fasciatus*, 71 mm. in standard length, Genoa, showing the well-developed basal spine.

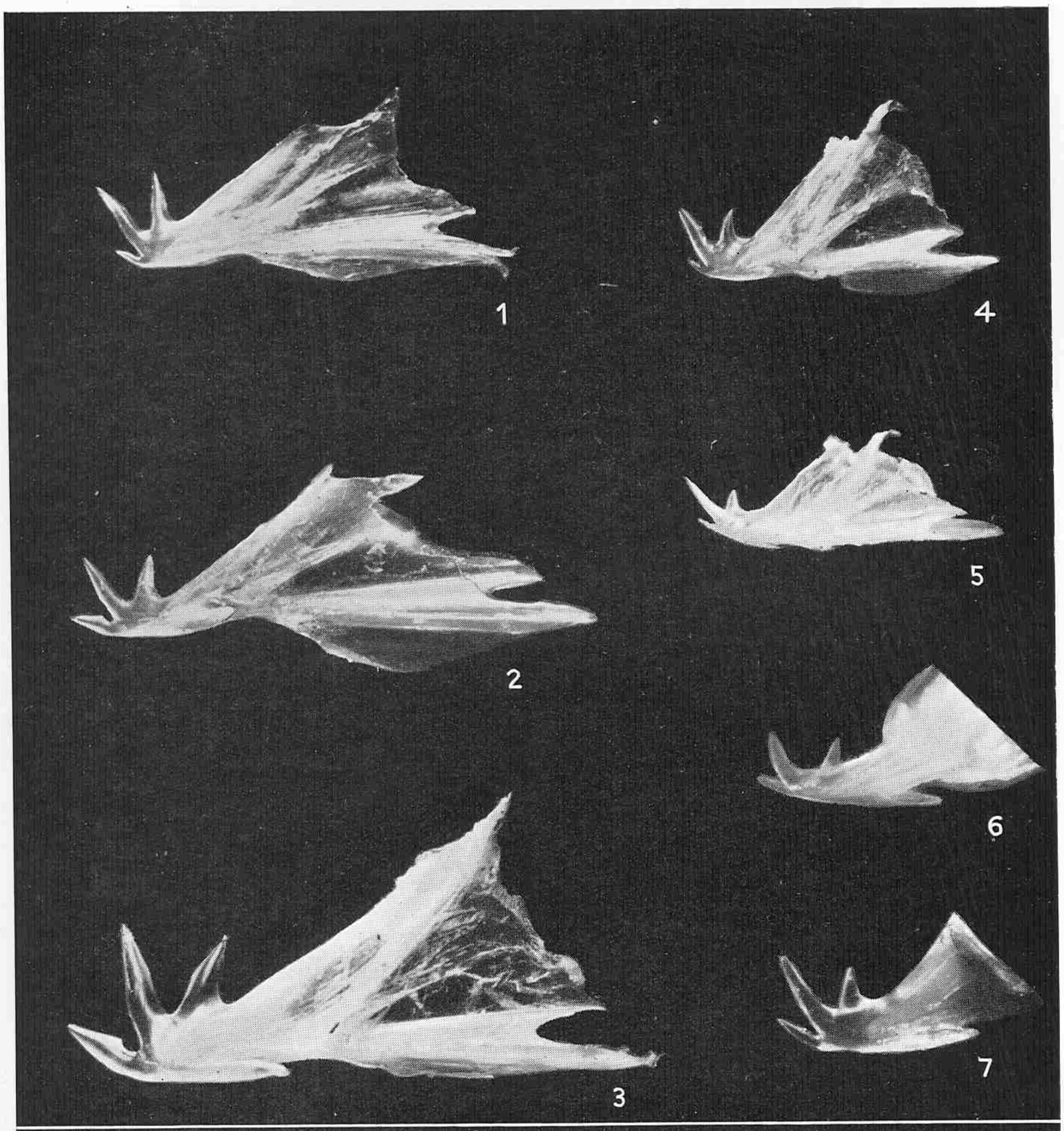
Fig. 7. Same as above, lateral view.

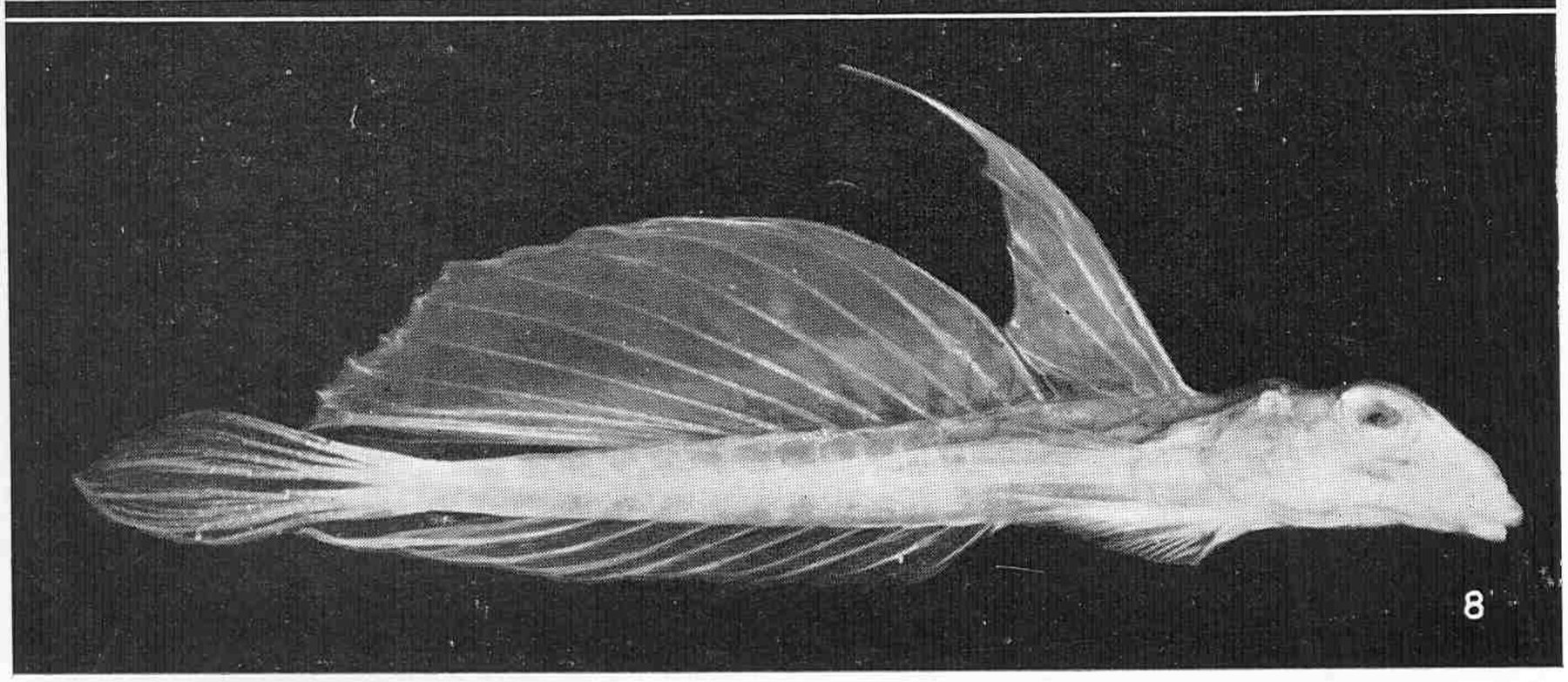
Fig. 8. *C. reticulatus* C. & V., lateral view, mature male, 110 mm. in total length, Portugal.

PLATE III, *Callionymus reticulatus* C. & V.

Figs. 1, 1*a*, male; Figs. 2, 2*a*, female. Fig. 1 is a specimen 108 mm. long caught off Plymouth on 11 May 1950. (*From original coloured drawing by A. Fraser Brunner.*)

Figs. 1–6.





Figs. 1–8.


1
1a
2
2a

A RE-EXAMINATION OF *CALANUS* COLLECTED OFF PLYMOUTH

By F. S. Russell, F.R.S.
Director of the Plymouth Laboratory

Rees (1949) has established significant biological differences between the two forms of the copepod *Calanus finmarchicus*, namely *finmarchicus* and *helgolandicus*. It is therefore necessary that in future research on the behaviour of *Calanus* the two forms should be distinguished.

I have myself published observations on the vertical distribution of *Calanus* and on the seasonal changes in size of individuals of the populations of this copepod off Plymouth. In order to know whether my results have been vitiated by the presence of the two forms in the collections, I have re-examined representative samples of adult females, using the fifth leg as the distinguishing character. The collections in question were those made in 1926 which formed the subject of a paper on seasonal changes in vertical distribution and in the sizes of individuals of different generations (Russell, 1928); and those made in 1931 and recorded in my paper on the vertical distribution of *Calanus* in relation to light intensity (Russell, 1934).

From the collections made between April and September 1926, twelve samples amounting to 240 individuals were examined. Among these, eight specimens were *finmarchicus*, the remainder being *helgolandicus*. The form *finmarchicus* only occurred in the months April, May and June, the maximum number in any one sample being 10 %. Over 300 specimens were examined from the collections made in July and August 1931; all were *helgolandicus*.

I have also examined over 200 specimens scattered through the collections made in 1930 on which the results reported by Bogorov (1934) on seasonal changes in biomass were based. Again *helgolandicus* was found to be similarly predominant. The very large catch recorded on 15 May 1930 (Russell, 1933) contained 4 % *finmarchicus*.

It is thus clear that these published results can be regarded as representative of *helgolandicus*, and the vertical distribution and seasonal changes refer to that form. It is interesting to record that the only specimens of *finmarchicus* occurring in May, in 1926 and 1930, were much smaller than the *helgolandicus* which attained their greatest size in that month. Rees (1949) similarly found *finmarchicus* to be smaller than *helgolandicus* in the North Sea.

I am grateful to Dr C. B. Rees for confirmation of some of my identifications.

Summary

A re-examination of some collections of the copepod *Calanus finmarchicus* made off Plymouth has shown that the conclusions reported in the following publications can be regarded as referring to the form *helgolandicus*.

REFERENCES

Bogorov, B. G., 1934. Seasonal changes in biomass of *Calanus finmarchicus* in the Plymouth area in 1930. *Journ. Mar. Biol. Assoc.*, Vol. 19, pp. 585–612.

Rees, C. B., 1949. Continuous plankton records: the distribution of *Calanus finmarchicus* (Gunn.) and its two forms in the North Sea, 1938–39. *Hull. Bull. Mar. Ecol.*, No. 14, Vol. 2, pp. 215–75.

Russell, F. S., 1928. The vertical distribution of marine macroplankton. VII. Observations on the behaviour of *Calanus finmarchicus*. *Journ. Mar. Biol. Assoc.*, Vol. 15, pp. 429–54.

—— 1933. The seasonal distribution of macroplankton as shown by catches in the 2-metre stramin ring-trawl in off-shore waters off Plymouth. *Journ. Mar. Biol. Assoc.*, Vol. 19, pp. 73–81.

—— 1934. The vertical distribution of marine macroplankton. XII. Some observations on the vertical distribution of *Calanus finmarchicus* in relation to light intensity. *Journ. Mar. Biol. Assoc.*, Vol. 19, pp. 569–84.

LYMPHOCYSTIS TUMOURS IN THE RED MULLET (*MULLUS SURMULETUS* L.)

By J. S. Alexandrowicz

From the Plymouth Laboratory

(Plates I–IV)

A specimen of the red mullet (*Mullus surmuletus* L.), caught in Plymouth waters in October 1949, was found to have tumours projecting externally on the pectoral fins. They were spherical or elliptical in shape, the largest being 20 mm. long. On the suggestion of Mr G. A. Steven some of these outgrowths were cut out and given to the writer for microscopic examination.

When examined fresh they appear to consist of small, spherical, cyst-like bodies of various diameters, but there was no obvious clue as to their nature. After fixing in Bouin and sectioning, they are seen to be composed of cells of various sizes (Pl. I, figs. 1–4). It can be seen that these cells lie close to one another, leaving only a little space for the embedding connective tissue which is characterized by the abundance of its own small cells. The blood vessels met with in this tissue contain fish erythrocytes. The whole is covered by stratified squamous epithelium (Pl. I, fig. 2).

The cells of the tumours have large nuclei very much like those of egg cells, and this resemblance is made still greater by the presence of a distinct cell membrane. On the other hand, they show certain peculiarities which do not occur either in ova or in other cells of normal tissues, viz. their cytoplasm includes bodies in its peripheral layer which stain readily with chromatin stains. Examined under higher power these bodies appear as a network of bars of different shapes, including alveolar spaces, the peculiar arrangement of which can be better seen in tangential sections (Pl. IV, fig. 20).

Although there are certain differences in the appearance of the cells of different sizes, there can be no doubt that they all represent successive stages of development of the same elements growing from the inconspicuous size of a few microns up to giant cells of more than 400 μ in diameter. During this development critical stages must be of common occurrence as many of the cells are obviously in a state of degeneration.

From this description it is clear that these tumours are those characteristic of the lymphocystis disease known to attack various fresh-water and marine fishes, but, apparently, not yet recorded in the red mullet.

The term 'lymphocystis' is a misnomer in both components of the word and may evoke a totally wrong idea as to the nature of the disease; it originates from the time when the cells of these outgrowths were mistaken for parasitic

Sporozoa. The history of the subject is an unusual one, for the peculiar aspect of the cells gave rise to another erroneous assumption that they were eggs laid by some animal under the skin of the fish.

Weissenberg (1914) was the first to recognize that these cells were hypertrophied fibroblasts of the fish itself, and Joseph (1918) came to the same conclusion independently. To Weissenberg goes also the merit of proving experimentally, in his earliest researches on the pike perch (*Acerina cernua*), the infective nature of this disease; he was able to confirm this later on some American fishes (*Stizostedion vitreum*, *Lepomis macrochirus* and *L. gibbosus*).

The bibliography of the subject and a list of species in which the lymphocystis disease has been described are given by Nigrelli & Smith (1939); recent additions have been supplied by Weissenberg (1945). The following species from European waters are so far recorded with the disease: *Pleuronectes flesus*, *P. platessa*, *Acerina cernua* and *Sargus annularis*.

The first data on the characteristic microscopical structure of the lymphocystis tumours have been given by Woodcock (1904). The most detailed histological investigations have been made by Joseph (1918) and Weissenberg (1920). The problem of the nature of the virus responsible for the disease has been touched upon in one of the later papers by Weissenberg (1945).

Among the established characteristics of the disease attention may be drawn to the following. The tumours may grow not only on the skin but also in the internal organs (mesentery, intestine, ovary and spleen). There has been observed a certain periodicity in the development of the disease marked by the eruption of new tumours; it has also been ascertained that the infected fishes can overcome the disease after a longer or shorter period of time and that their skin may resume quite a normal appearance.

It is generally admitted that the disease is due to a virus, although positive proof by virus-test methods has apparently not yet been given. This virus has not proved to be identical in every instance, for in Weissenberg's experiments on *Acerina cernua* and *Stizostedion vitreum* only those fishes belonging to the same species could be infected and he, therefore, assumed that the virus was species-specific. This assumption was, moreover, in agreement with the observations of other investigators that other species of fishes kept for a long time in the same tank with the infected species did not develop similar tumours. However, in 1945, Weissenberg succeeded in transmitting the infection from the bluegill (*Lepomis macrochirus*) to the common sun fish (*L. gibbosus*).

Some Features of the Lymphocystis Tumours in *Mullus surmuletus*

On *macroscopic* examination alone one might hesitate to identify the tumours found in the red mullet with those observed by previous writers. In their descriptions and illustrations the lymphocystis tumours are represented as

numerous small disseminated swellings which, if united into larger bodies, distinctly show their composite structure. Raspberry-, mulberry- and cauliflower-like outgrowths are the terms used to give an idea of their shape. Weissenberg alone mentions that some of them may have a smooth surface.

In *Mullus* no such disseminated nodules were met with, and the few tumours (five in all) had rather smooth surfaces. Only one of them, situated at the corner of the mouth, was comparatively small, being 3 mm. in diameter; the dimensions of the others ranged from 9 to 20 mm.

Microscopically, the lymphocystis cells in *Mullus* show the same characteristic features already recorded in other species, and they closely resemble those of *Sargus annularis*. Many of the details are so much alike that several of Joseph's photomicrographs might have been made from my preparations of *Mullus*. In view of these similarities, it seems superfluous to describe every detail, as they have been discussed at length by Joseph and by Weissenberg. I therefore intend to concentrate only on those points which have given rise to controversy in the past, or which have apparently not been observed in the species previously investigated.

Early Stages of the Lymphocystis Cells and the Development of the Inclusion Bodies

Although in their later stages all the lymphocystis cells look alike, according to Joseph they may have a double origin: some develop from the 'basiepidermoidale Zellen', cells situated in the epidermis, others from the connective tissue elements, especially from the osteoblasts. He thought that the inclusion network originated in the former cells from a dark-staining body noticeable even in very small (*c.* 8 μ) cells; and in the latter he first observed it in the cells which had reached *c.* 30 μ in diameter, in the shape of a small 'calotte' which quickly grows around the nucleus and becomes transformed into a network, moving later into the cortical layer of the cytoplasm.

On the basis of his own observations, as well as on theoretical grounds, Weissenberg rejects the possibility of lymphocystis cells developing from the epidermis elements. He admits their origin from the connective tissue cells and gives a detailed account of the very earliest modifications of the latter as they begin their transformation into the lymphocystis cells.

The origin of the inclusion bodies in the tumour cells of *Acerina cernua* is described by him as follows: 'At first they contained no inclusion body. But about one week later, an inclusion appeared in their cytoplasm as a tiny point which increased in size to a round compact body resembling very much a Guarnieri body of a corneal epithelium cell of a rabbit after inoculation with vaccine virus. The young lymphocystis cells grew larger and larger and corresponding to their growth the inclusion body increased more and more in size

to an oval disc, then to a calotte, which became fenestrated and sprouted into a network of basophilic staining reaction.'

Later investigations by the same writer on various species confirmed his conclusions about the origin of the lymphocystis cells and their inclusion bodies. In every instance the latter were found appearing first as one, or sometimes several, small granules, which in some species could be seen in the youngest stages of the tumour cells. When, however, Nigrelli & Smith (1939) examined a lymphocystis tumour of the orange filefish (*Ceratacanthus schoepfii*) they found features to which the following interpretation was given: 'Certain early stages ($9 \times 24\,\mu$) show cells in what resembles a binuclear condition. In other cells ($20 \times 50\,\mu$) the nuclear material appears in two forms: (*a*) as a deeply pycnotic primary nucleus of the enlarged cell, and (*b*) a secondary nuclear mass, variable in size, which becomes vacuolated and reticulated. The later mass of basophilic material represents probably the forerunner of the system of inclusion bodies which eventually become distributed in the cytoplasm of the enlarged older cell ... .'

The aspect of these cells has therefore much in common with those lymphocystis cells in *Sargus annularis* which Joseph saw developing at the basis of the epidermis, and which contained, apart from the nucleus, another body of such conspicuous dimensions that Joseph said that he hesitated to decide which of these bodies might be the genuine nucleus. But while Joseph lays stress on the point, on which he and Weissenberg are quite unanimous, that the elements of the future inclusion network do not migrate from the nucleus, Nigrelli & Smith suggest that these inclusions may originate from 'one or two nuclear masses formed in certain early stages of the disease'.

My own observations have been made on material less suitable for investigating the origin of the lymphocystis cells than if it had been obtained by experimental transmission of the disease, permitting the evolution of the infected cells to be followed from the very first manifestation of the infection. Nevertheless, the tumours of the red mullet, although they were obviously of long standing, included such a variety of stages that a whole series of them could be established even in the same preparation.

The earliest stages are rather scarce, and were found as single elements, or in small groups in some sections only. The largest of the observed nests is represented in Pl. I, fig. 3.

In this connexion it may be pointed out that the occurrence of all stages of development would favour the assumption that a frequent infection of the host cells has been taking place. On the other hand, since large parts of the tumours are composed of cells of equal size markedly differing from neighbouring areas (Pl. I, fig. 1), it is obvious that they belong to the various eruptive periods of the disease. Hence it seems probable that in the *Mullus* tumours both processes were occurring, viz. a frequent infection of a few cells, and periodical exacerbations of the disease producing simultaneous growth of a greater

number of the hypertrophic elements. The smallest cells which could be distinguished with certainty as early stages of lymphocystis cells measured 9 μ in diameter (Pl. II, fig. 6*a*). They can be recognized by their rounded shape, the deeper staining of their cytoplasm, and, more certainly, by their large spherical nuclei of vesicular appearance, each containing a big nucleolus. There are even smaller cells which, compared with the fibroblasts, show modifications tending in the same direction (Pl. II, fig. 5*a*) and which may be regarded as earlier stages, but the same degree of certainty as to their character cannot be claimed.

In all preparations observed the young stages of the lymphocystis cells appear to originate from among the connective tissue elements. No evidence has been met to support Joseph's contention that the lymphocystis elements evolve from two different kinds of host cells.

The appearance of the next stages of the growing cells may be seen in Pl. II, figs. 5–11. Some of them, if cut at suitable angles, distinctly show a large area of the cytoplasm staining more lightly (called 'sphere' by Joseph).

The inclusion bodies in the young cells have the same appearance as figured by Weissenberg, i.e. of granules surrounded by a halo. The inclusions in this Guarnieri-body stage are found so often to be 3, 4 or even 5 in number that their multiple occurrence seems to be the rule in the red mullet. If one traces the evolution of these bodies retrogressively to the smallest cells, one may see the granules diminishing in size and their halos becoming very faint. In Pl. II, fig. 5*b*, five such granules are present but they are not clearly in focus in the photograph. In the smallest cells the granules are at the limits of visibility. Although there must, of course, be uncertainty as to the nature of elements of so small a size, there are reasons for believing that, even in the smallest lymphocystis cells of the red mullet, the substance of future inclusion networks is already accumulating in particles of microscopical dimensions.

The multiplicity of the inclusions in the early stages of the lymphocystis cells was observed by Weissenberg to be a constant feature in some fishes (Pleuronectidae and *Lachnolaimus*), and in them each of these granular bodies was developing into a fragment of the network; in other species investigated by the same author (*Acerina* and *Stizostedion*) additional granules appeared occasionally but remained rudimentary.

Whether or not some of the initial granules seen in the cells of *Mullus* are disintegrating is difficult to determine, and the diminution in their number may be explained as being, at least partly, brought about by the fusion of two or more into one larger mass. The pictures seen in Pl. II, figs. 8–11, may be best interpreted as the successive stages of this process. In Pl. II, fig. 11, the larger inclusion seems likely to be composed of three granules about to fuse into one. Similar lobed outlines of the inclusions can be observed only in the cells belonging to the same range of size in which the fusion of these bodies is

occurring; they cannot be regarded as the beginning of the later changes of the inclusions, since these do not set in until the cells grow a good deal bigger.

Cells with a 'secondary nuclear mass', as described by Nigrelli & Smith, have not been found. Only in some cases there could be seen a pycnotic conglomeration of the inclusions producing a body of roughly spherical shape, but these cells were unmistakably in a state of degeneration. Binucleated cells are not uncommon, but their nuclei have an identical and typical appearance, and there is not the slightest evidence that one of them may give rise to the inclusion network. I am therefore in agreement with Joseph and Weissenberg in refuting the theory of the migration of the inclusion substance from the nuclei.

The different organization of the younger stages of lymphocystis cells in the orange filefish as described by Nigrelli & Smith would appear anomalous in view of the resemblance of the later stages of the same cells in various species of fish. It seems, however, that this may be explained as follows. From all we know, the infective agent, after penetrating the host cells, will bring about two processes, viz. the hypertrophy of the cells and the development of the inclusion networks; the latter being most probably, as has been postulated by Weissenberg, the aggregation of the virus substance. In the majority of the species investigated the hypertrophy of the cells sets in before the inclusion bodies become visible; if the reverse should occur, as might be the usual course in the species observed by Nigrelli & Smith, the inclusion body would attain a more advanced stage of development in the comparatively small cell. If this suggestion holds good, it is to be expected that in the orange filefish also, if suitable stages of the disease happen to come under investigation, such Guarnieri-like bodies will be found in the small cells.

Centriols. Owing to the multiple occurrence of the inclusion bodies, their variable size and their situation in different regions of the cells, recognition of the centriols described by Joseph in *Sargus annularis* is very uncertain. Some small granules in the lighter part of the cytoplasm, Joseph's sphere, might well be centriols, but in other cases at the same places the granules are of such size that their centriol nature is highly improbable and they are, more likely, to be reckoned among the additional inclusion bodies.

Further Stages of the Development of the Lymphocystis Cells

Inclusion bodies

The development of the inclusion bodies will be considered first, not only because their changes are the most striking feature in the growing lymphocystis cells, but also because they have an obvious bearing on the organization of the cytoplasm.

The changes in the form of the inclusion bodies, which up to now have retained their characteristic appearance of Guarnieri bodies, become noticeable in the cells of *c.* 50 μ; the oval body becomes flattened and assumes the form of a calotte which starts to expand around the nucleus, undergoing at the same time a transformation into a framework made up of irregular bars. The shape of the body during this process is often far from symmetrical: the calotte may be elongated in one direction, some bars of the framework may extend towards the nucleus, others towards the periphery of the cell, so that in the sections they may be seen scattered through the whole cytoplasm. The independence of some fragments makes it probable that some of them may develop from additional inclusion bodies. With the growth of the cells a more orderly arrangement takes place, and eventually the whole inclusion body represents a hollow sphere, the walls of which are irregularly fenestrated. In the sections it appears as a ring made up of several fragments and situated between the nucleus and the periphery of the cell. As the diameter of this spherical basketwork grows faster than that of the cells, the inclusions soon reach their cortical zone, a position maintained during the subsequent life of the cells.

The development of the inclusions and their definite arrangement in *Mullus* may be defined as being of *Sargus* type, as it is much like that described by Joseph for that fish. It differs greatly from what may be called the *Pleuronectes* type which, according to Weissenberg, consists of numerous inclusion networks densely filling the whole cytoplasm; it differs also, but to a lesser degree, from those of other species investigated by the same author.

The extent of the break-up of the framework in any one cell into separate fragments is difficult to ascertain unless all the serial sections of a given cell are examined. In tangential sections one can see how thin may be the connexions between the bars and, moreover, how different the inclusions of the same cell segment may appear if the sections are made in different planes (Pl. IV, fig. 20).

From the stage at which they reach the periphery of the cells up to the time when the latter grow to their maximal dimensions, the inclusion bodies do not show marked differences in their structure. In the large cells the bars are somewhat more widely spaced, but even then their total mass in any one cell must grow as the cell increases in diameter.

In the cells at the peak of their development the inclusions often appear divided into more fragments and show certain changes: the outlines of the bars become more rounded and their structure shows less fine pattern. These changes may be considered as leading to the degeneration of the inclusion bodies which will be discussed later.

It may be mentioned that the staining reactions of the inclusions confirm Weissenberg's opinion that, besides their main basophilic constituent, some other 'ground substance' of acidophilic properties must be present in them.

Cytoplasm.

In the young lymphocystis cells the lighter portion of the cytoplasm has the regular shape of a sphere slightly invaginated by the nucleus (Pl. II, fig. 7). At the time when it is about to start its rapid development, the inclusion body is found situated at the periphery of this sphere, and often on the opposite side to the nucleus. Later on, when it assumes the form of a calotte, it expands in close contact with the 'sphere'. When the inclusion body is seen growing symmetrically around the nucleus the lighter staining cytoplasm may also encircle the nucleus, thus interposing itself between the inclusion body and the nucleus. The frequent deviations from the regular course of development of the inclusion bodies, mentioned above, show their effects on the 'spheres' also, as the latter may be pierced by the growing bars or divided into fragments.

The interpretation of the succeeding sequence of events is uncertain. For when the inclusion body passes through the transitory stage mentioned above, characterized by its irregular growth, the cytoplasm exhibits darker and lighter patches and, moreover, develops a more distinctly reticulated texture. With further growth the reticulum becomes gradually less distinct, while the areas of differently stained cytoplasm merge together into larger patches giving to the cell a chequered appearance (Pl. II, figs. 12, 13). At this time there is also noticeable a certain change in the chemical reaction of the cytoplasm, for it now stains better with the cytoplasmic dyes, whereas in the former stages it showed basophilic tendencies.

The variegated picture of this transitory stage of the lymphocystis cells may be looked upon as reflecting the major disturbances caused by the disproportionately quick growth of the virus substance; the more regular arrangement of various cell constituents in the later stages gives the impression that some equilibrium is being restored among them. Thus, when the inclusion network reaches the stage at which it assumes the form of a more regular basketwork expanding towards the periphery of the cell, the two kinds of cytoplasm begin to accumulate, one in the centre of the cell and the other nearer to the periphery. This process leads to the formation in cells of *c.* 150 μ and upwards, of a cortical ectoplasmic layer, of more homogeneous structure, and more coarsely granulated endoplasm (Pl. II, figs. 12, 13).

The difference in staining properties of the ectoplasm and endoplasm may not be seen equally well in all cells, but, in many of them, the cortical layer appears sharply delimited, exactly as in Joseph's photomicrographs. Thus, in this differentiation of the cytoplasm also, the lymphocystis cells in *Mullus surmuletus* exhibit a great resemblance to those of *Sargus annularis*.

The different appearance of the ectoplasm in which the inclusion networks are situated must obviously reflect its particular properties; the difference in the behaviour of the two cytoplasmic layers is occasionally made evident in some degenerating cells in which the ectoplasm with its inclusion bodies is partly folded and detached from the endoplasm (Pl. III, fig. 16).

The question arises: if in the youngest cells two kinds of distinctly different cytoplasm are present, could the ectoplasm and endoplasm of the older cells be considered as deriving directly one from the lighter and the other from the darker staining portions? The evidence gained from the examination of the preparations stained with different methods is not unequivocal.

In the sections stained with Azan, or haematoxylin and eosin or xylidin red as counterstain, or even with haematoxylin alone, the 'spheres' in the small cells are always lighter in colour. In the transitory stage the direct observation of their fate is uncertain, but it seems more probable that the lighter stained areas of the cytoplasm derive from these spheres. When the differently stained portions become larger and more distinct the lighter ones are moving towards the cell periphery. Hence it may be concluded that the 'sphere' develops into the ectoplasm of the larger cells (Pl. II, fig. 12).

If, however, the preparations are stained with Giemsa the results are contradictory. In the small cells the spheres stain, as with other dyes, in a markedly lighter hue; in the transitory stage the intensively stained reticular structures of the cell dominate the picture, while other cytoplasmic elements are less recognizable; in the later stages the differently stained patches of the cytoplasm become apparent and even show more details of the distribution of the two kinds of the cytoplasm, but in this case the darker staining parts are moving towards the periphery (Pl. II, fig. 13). This phenomenon casts doubt on the former assumption which, moreover, does not agree with Joseph's statement that the endoplasm derives from the sphere. The problem must therefore remain unsettled.

Nucleus

The nucleus, which is very large in the young cells, grows more slowly than the cell itself, so that its volume, in proportion to that of the cell, becomes smaller with the progressing hypertrophy of the latter. It occupies a position at the periphery of the cell, where it is situated immediately under the cell membrane. In the larger cells the original globular form may undergo some modification, becoming ellipsoidal or invaginated, but these alterations do not distort the spherical outlines of the nucleus to such a degree as described by Joseph, and in this respect the lymphocystis cells in *Mullus* appear to differ to a greater degree from those in *Sargus*.

The constitution of the nucleus has a great similarity in both these species. It is characterized by the abundance of the nuclear sap, a very fine and sparse framework, and a large nucleolus. The latter shows great variability in form; it may include vacuolar spaces, become fenestrated, or develop irregular lobes which if detached by deep constrictions are found as separated fragments. Although young cells with two nuclei may sometimes be observed, this fragmentation of the nuclear mass seems to be chiefly responsible for the occurrence of more than one nucleolus in the larger cells.

Abnormal Features in the Lymphocystis Cells

Binucleated Cells

Cells with two nuclei are not infrequently met with in younger stages. In the group of small cells, shown in Pl. II, fig. 5, two such cells are present. Both nuclei have an identical structure, and there is certainly no question of one of them belonging to the category of elements described by Nigrelli & Smith. Larger cells with two nuclei were observed in a few instances only. This rare occurrence may be partly due, as the cells grow larger, to the decreasing chances of meeting both nuclei cut in one section at a suitable angle. It is also possible that the course of development of some of these cells is unusual. An interesting example is seen in Pl. II, fig. 12 (middle right), in which a binucleated cell contains two inclusion bodies in the calotte stage, occupying diametrically opposed positions with the sphere-cytoplasm between them and the nuclei. Further development in this direction may perhaps account for the Siamese-twin monstrosity represented in Pl. III, fig. 14.

Several Cells in One Membrane

Pl. III, fig. 15, shows seven cells, two of them very small, encircled by one collapsed membrane which obviously belonged to a large degenerated cell. In this section only part of the cells included in this way can be seen, and on looking through the following sections it was found that there were fifteen in all. Such cases cannot be very uncommon, as in a second tumour a group of six cells was found similarly enclosed in a membrane. There can hardly be any doubt about the process of formation of such features, in view of the fact that the large cells in their degenerating state (which will be discussed below) are often invaded by the connective tissue elements. If now among the latter there happen to be some carrying the virus infection they must start their development into the lymphocystis cells within the membrane of the old cell, and it may be stated that, in this as in other instances, the membrane can persist a long time after all other cell elements become completely resorbed.

Degeneration of the Lymphocystis Cells

Of all previous writers, Joseph paid most attention to the processes of degeneration of the lymphocystis cells. His findings may be summarized as follows: (i) the degeneration affects exclusively the large cells which have reached the peak of development; (ii) degeneration may proceed in two ways: either the membrane of the cell becomes ruptured at some point and the elements of the surrounding connective tissue penetrate into the lymphocystis cell, or the degeneration proceeds without this perforation of the membrane; (iii) if the cells in the process of degeneration are situated near to the epidermis the latter becomes stimulated and produces buds which extend into the adjacent tissue, filling up the spaces left by the shrinking lymphocystis cells.

The first of these statements certainly does not apply to the lymphocystis cells in the red mullet, for, as has been mentioned, the process of degeneration may be observed in cells in various stages of development. As to the third point, i.e. the part played by the epidermis, nothing of this kind could be seen: the epithelium tissue is everywhere sharply delimited and does not show any tendency to grow towards the connective tissue. However, the small amount of material which I had for investigation was insufficient to enable me to conclude with certainty that no such stimulation of the epidermis would ever occur in *Mullus* tumours.

The remaining statements by Joseph, concerning the two processes of degeneration, could be fully confirmed and, owing to the abundance of the degenerating cells in the tumours of *Mullus*, various stages of their disintegration could be observed even on the same section. Sure symptoms of this process, which are more noticeable than the above-mentioned changes in the appearance of the inclusion bodies, are the irregularities of the outline of the nucleus and of the cell itself. If the membrane is ruptured it soon becomes invaginated in various ways, assuming bizarre outlines in the sections. At the same time, small cells from the surrounding connective tissue invade the opening and contribute to the disintegration of the cell contents. When the membrane remains unperforated it may also exhibit certain deformations while the cell undergoes a gradual resorption, but degenerating cells may often be seen in which the dwindling cytoplasm becomes detached from the membrane and the latter preserves for a long time its spherical outlines. In Pl. III, fig. 17, which shows many cells in different stages of degeneration in which the membranes are selectively stained, there may be seen: (*a*) empty membranes; (*b*) membranes with numerous small infiltrating cells; and (*c*) some in which the shrinking cytoplasm has become retracted from the membrane.

In the larger cells both kinds of degeneration, viz. with and without rupture of the membrane, occur. In those of medium size and in smaller ones the membrane shows more resistance, and the resorption of the cell contents goes on without its perforation.

The course of the degeneration process, the changes in the appearance of the cytoplasm, and the disintegration of the nucleus and of the inclusion bodies, can easily be followed in their various stages, and it can be said that Joseph's description of *Sargus annularis* would apply equally well for *Mullus surmuletus*. It should be added, however, that in the latter not all degenerating cells follow the same course, for there is yet another kind of change which some of the lymphocystis cells may undergo; this is characterized by a peculiar activity of the inclusion bodies, in which there is a series of transformations now to be described.

Transformations of the Inclusion Bodies in the Degenerating Cells

As has been mentioned above, the inclusions of the largest cells may show some modifications in their structure, which have been interpreted as being of a regressive nature, indicating the beginning of the disintegration of these bodies. This assumption seems to be justified, since many intermediate stages have been observed between the typical appearance of the inclusions in medium-sized 'healthy' cells (Pl. IV, fig. 20) and in the degenerating cells (Pl. IV, fig. 21). The process by which these changes are brought about may be compared to a slow melting. At first the outlines of the networks become slightly modified; later on the progressive dissolution of finer linking bars leads to the breaking off of fragments of different sizes, whose finer structure gradually becomes indistinguishable. Finally, the network is reduced to a number of irregularly shaped globular fragments, dwindling in size and number until they disappear altogether.

In some of the degenerating cells of the medium-sized group in which the membrane does not become perforated quite a different process occurs. Not only does the inclusion network not show any signs of this dissolution process, but it starts to grow, developing at the same time a more finely detailed structure. The first recognizable stage may be seen in Pl. IV, fig. 22. In the cell on the right the bars of the inclusions, compared with those of the cell on the left, are beginning to grow a little thicker, and some changes in their fine structure become noticeable. Further transformations in the same direction lead to the appearance shown in Pl. IV, fig. 23, which is markedly different from that in Pl. IV, fig. 20. Even if such 'normal' inclusion networks may sometimes exhibit a more delicate structure than seen in this figure (fig. 20), they always show the same characteristic pattern of alveolar spaces within the bars; whereas in the stage of transformations shown in Pl. IV, fig. 23, the network resembles rather a tridimensional lattice the spaces of which are more or less losing their rounded outlines. Moreover, this lattice does not grow symmetrically; parts of it may become elongated in one direction to form strands of various shapes, sometimes even arranged in lamellae (Pl. IV, fig. 24). If such strands are cut transversely the cross-sections of finer threads might be taken at first sight for separate small bodies (Pl. IV, fig. 25).

Such pictures of networks whose lattice-like structure may be fairly distinguishable on the photomicrographs are comparatively rare; more often the dense and deeply stained networks give a blurred image (Pl. IV, fig. 26).

Whether in the course of this process more inclusion material is produced, or whether that already present at the beginning of the transformations merely becomes rearranged in such a way that its dimensions are increased, is difficult to determine. Anyhow, the inclusions gradually occupy larger areas in the cells, the spreading thread-like bars of the lattice penetrating the cytoplasm

so that in the sections the meshes of the inclusions are seen to enclose the cytoplasmic substance.

At a certain stage the network apparently acquires some new property and begins to exert a lytic action on the cytoplasm. At first small spaces appear around the threads of the network, but soon the cytoplasm enclosed in its meshes breaks up into lumps, which disintegrate in their turn into smaller particles. In consequence of this process larger spaces are formed, filled with the framework of the inclusion bodies and particles of the cytoplasm (Pl. IV, figs. 27–29). As these spaces reproduce the pattern of the growing inclusions they represent a system of irregular canals, some of them ending blindly, others joining to form larger lacunae which assume various shapes in the sections (Pl. IV, figs. 30, 31).

Up to a certain stage of these transformations the inclusion network in the lacunae stains very well. Then, however, a change occurs in its elements, causing them to appear fewer in number, until, finally, they become invisible. Whether the networks are dissolved into submicroscopic particles, or whether their chemical reaction becomes changed, could not be ascertained, since the lacunae are filled with a debris of disintegrated cytoplasm. Without differential staining discrimination of the fine fragments of the inclusions, were they really present, is therefore hardly possible. Whatever may be the true nature of this change it must come about in a comparatively short time, since few cells in transitional stages occur; and there may also be seen, not uncommonly in the same cell, some of the lacunae containing a distinctly stained basophilic network, and others in which it is already disappearing. In Pl. IV, figs. 28–31, are shown cells with inclusions in various stages of this process. The differences in the staining reaction cannot be well rendered in the photographic reproduction, and only parts of the network actually present in the preparations can be focused, but in the original sections stained with contrasting nuclear and plasmatic stains the following contents of the lacunae could be well distinguished. In Pl. IV, fig. 28, there shows a very delicate basophilic network, which later, as seen in Pl. IV, fig. 29, becomes markedly reduced (the large lumps are fragments of the cytoplasm); in Pl. IV, fig. 30, at *a*, are intensely stained basophil elements; at *b* fine basophil threads similar to those in Pl. IV, fig. 29, and at *c*, no basophil elements at all. In Pl. IV, fig. 31, only a very few particles show affinity to chromatin dyes, and practically the whole contents of the lacunae stained the same colour as the surrounding cytoplasm.

As regards the behaviour of other elements of the cells in which all these transformations are taking place it should be noted that often the differences in the appearance of the two protoplasmatic layers, i.e. the ectoplasm and the endoplasm, become more accentuated at the time of formation of the lacunae and, moreover, the territory of the ectoplasm in which the lacunae are situated becomes enlarged at the expense of the receding endoplasm (Pl. IV, fig. 31). It seems, therefore, that the substance which becomes destroyed by the

inclusion bodies must be of ectoplasmic nature, but this cannot be an invariable rule, for at some places the lytic process is seen to encroach on the endoplasm also.

It may be mentioned that the ectoplasm of the cells in which the transformations take place stains more deeply than that of other lymphocystis cells. Only after the stage has been reached at which the basophil substance disappears, and even then presumably not until some further time has elapsed, do the cells lose this property and take on a distinctly lighter tone.

In some of the cells a remarkable increase of the nucleolar substance could be noticed (Pl. IV, figs. 27, 30). As, however, the nucleoli in the lymphocystis cells of *Mullus* may be of different sizes and shapes, it cannot be said for certain whether such increase in volume is a constant feature of the cells having inclusions in process of transformation.

As seen in Pl. IV, fig. 31, very large areas of the cells may be occupied by the widening lacunae, but no cells were found whose whole territory was invaded in this way. It appears, therefore, that the destructive action of the inclusion network comes to an end with the vanishing of the basophilic reaction.

In further stages the number of particles enclosed in the lacunae is diminishing, and finally many of the lacunae appear as empty or nearly empty spaces. At the same time progressive degeneration leads to distortion of the outlines of the cells and the resorption of their contents.

It is tempting to suggest that all these modifications of the inclusions have a particular significance in the life cycle of the virus of the lymphocystis disease, and that they may presumably be regarded as preparatory stages in the transformation of the virus substance into a state in which it is ready to infect new cells of the same host or of other specimens. The rearrangements of the inclusions into a more delicate network may be regarded as transition stages of the dissolution of the compact aggregations of the virus into particles of submicroscopic dimensions. It is interesting to note that transformations of the virus substance are evidently not confined to simple disintegration since, as we have seen, the relations of the virus to the host cells enter a new phase manifested by the lytic action on the cytoplasm of these cells.

Whether the disappearance of the basophilic reaction should be ascribed only to the disintegration of the virus agglomerations into submicroscopic particles, or be due to some chemical changes, is a question to which no definite answer can as yet be given.

The fact that the majority of the lymphocystis cells degenerate without showing any activity of their inclusions leads to the assumption that the transformations may find the right conditions to start in comparatively few of the cells. Indeed, in the tumours investigated only one group of such elements has been found. It appears that even when these transformations are in progress they are liable to be interrupted at any stage. Then the inclusion bodies, instead of increasing still further in size and developing a more delicate

structure, show reverse changes turning into uniformly stained bodies of rounded outlines. Their origin from those inclusions which have started their transformations may be recognized by their dimensions, and the differences in these dimensions indicate the moment at which the process of the transformations was stopped and switched into a degenerative one (Pl. III, figs. 18, 19).

If we take the above interpretation of the behaviour of the inclusion bodies for granted, we have to assume that the completion of the life cycle of the infective agent can only be achieved in a few of the growing lymphocystis cells and apparently only in some of those which have not reached their maximal dimensions; in all others it would meet its doom. The degeneration which overcomes the virus substance during its transformations, as well as all similar processes observed in the lymphocystis tumours, are evidently manifestations of the struggle of the host organism with the infection. A similar struggle must certainly occur in other diseases in which the infective agent is localized in certain cells, and the organism attacked is capable of mobilizing its defensive measures. A remarkable feature of the lymphocystis disease is that, owing to the unusual dimensions of the affected cells and the staining properties of the virus agglomerations, it affords illustrations of the various stages of this struggle.

The importance of certain transformations as essential links in the life cycle of the virus can be related to the periodicity of eruption of new tumours, which could be explained by assuming that the transformations take place at certain times and in relatively few cells only. This would tally with the occurrence of the earliest stages of the infected cells and of the transformations in the same specimen of *Mullus surmuletus*. It would agree also with the view expressed by Weissenberg (1945) that some changes in the lymphocystis virus agglomerations might possibly take place before it is ready to produce new infections.

On the other hand, although the evidence favours the above suggestion, it may be open to dispute on the grounds that similar transformations of the inclusions were not observed by those investigators who made detailed researches with much more abundant material. In the paper of Joseph illustrated by as many as ninety photomicrographs, only one (fig. 43) shows an inclusion body with a swollen appearance somewhat similar to one of the stages I have described. No reference is, however, made to this figure in the text. Further, if such transformations had not been noticed in the lymphocystis cells of *Acerina cernua* or *Stizostedion vitreum* and, nevertheless, Weissenberg's infection experiments with the same material were successful, this would tend to discount their alleged importance. Unless, therefore, one assumes that the transformation of the inclusions did not, owing to their rare occurrence, chance to turn up in the preparations from other fishes, some doubts may legitimately arise as to the interpretation of the phenomena observed in the red mullet. It is desirable, therefore, that particular attention should be given to this point in the future.

I wish to record my gratitude to Mr G. A. Steven for the material which has been worked out in the present paper. Thanks to his observation of the unusual outgrowths, and the suggestion that they would be worth examination, the number of species known to be affected by the lymphocystis disease has been increased by the addition of *Mullus surmuletus*.

I am also greatly indebted to Mr F. S. Russell, F.R.S., for his kind help in preparing the manuscript.

Summary

A case of lymphocystis disease is described in the red mullet (*Mullus surmuletus*) from Plymouth waters. The tumours produced by this disease showed typical lymphocystis cells in all stages of development up to 430 μ in diameter. Apart from minor differences the organization of the cells closely resembled that described by Joseph in *Sargus annularis*; this was especially so as regards the so-called inclusion body, a basophil staining network situated in the cytoplasm and probably representing the agglomeration of the virus agent of the disease.

The development of the lymphocystis cells which originate from the fibroblasts was observed in its earliest stages. The inclusion bodies appear in the young cells as several (up to five) granules which most probably soon merge into larger bodies. They have the characteristic appearance greatly resembling the Guarnieri bodies, as do those discovered by Weissenberg in lymphocystis cells of *Acerina cernua* and other species. Their further development follows the same lines as observed by other writers. There is no evidence in favour of the idea that the inclusion bodies may be derived from the nuclear matter of the cell.

Various forms of the degeneration of the lymphocystis cells have been observed. Special attention has been given to the activity of the inclusion bodies in certain types of degenerating cells. This is characterized by the swelling of these bodies and the rebuilding of their substance into a network exhibiting much more delicate structure and exerting a lytic action on the cytoplasm of the host cell. As these transformations progress the network becomes less distinguishable in the lacunae formed in the cells by the disintegration of the cytoplasm, until finally its elements, previously staining distinctly with chromatin dyes, cannot be discerned any more.

It is suggested that these transformations may represent those stages in the life cycle of the virus agent during which it acquires the ability to transmit the infection to other cells of the same host or other specimens.

REFERENCES

Joseph, H., 1918. Untersuchungen über *Lymphocystis* Woodc. *Arch. Protistenkunde*, Bd. 38, pp. 155–249.

Nigrelli, R. F. & Smith, G. M., 1939. Studies on lymphocystis disease in the orange filefish, *Ceratacanthus schoepfii* (Walbaum), from Sandy Hook Bay, N.J. *Zoologica*, Vol. 24, pp. 255–64.

WEISSENBERG, R., 1914. Über infektiöse Zellhypertrophie bei Fischen [Lymphocystis-erkrankung]. *Sitz.-Ber. Kgl. preuss. Akad. Wiss. Physik. mathem. Cl.*, 1914. [Cited from Weissenberg, 1920].

—— 1920. Lymphocystisstudien. [Infektiöse Hypertrophie von Stützgewebszellen bei Fischen.] I. Die reifen Geschwülste bei Kaulbarsch und Flunder. Lymphocystisgenese beim Kaulbarsch. *Arch. mikr. Anat.*, Bd. 94, pp. 55–134.

—— 1939. Studies on virus diseases of fish. III. Morphological and experimental observations on the lymphocystis disease of the pike perch, *Stizostedion vitreum*. *Zoologica*, Vol. 24, pp. 245–54.

—— 1945. Studies on virus diseases of fish. IV. Lymphocystis disease in Centrarchidae. *Zoologica*, Vol. 30, pp. 169–84.

Woodcock, H. M., 1904. Note on a remarkable parasite of plaice and flounders. *Rep. Lancs. Sea-Fish. Lab.* (for 1903), pp. 63–72.

EXPLANATION OF PLATES

All photomicrographs have been made from preparations of the lymphocystis tumours of *Mullus surmuletus*. The material was fixed in Bouin's fluid and embedded in paraffin. The preparations represented in figs. 1–12 and 17–31 have been stained with Weigert's iron haematoxylin and various counterstains, those in figs. 13–16 with Giemsa's stain.

PLATE I

Fig. 1. Part of a lymphocystis tumour with cells in various stages of development and degeneration. The large upper portion composed of cells of nearly equal size has presumably originated at a later eruption of the disease than the remaining portion of the tumour. The degenerating elements are seen also in the middle of this younger portion.

Fig. 2. Part of the lymphocystis tumour covered by cutaneous epithelium.

Fig. 3. Group of the youngest lymphocystis cells among others advanced in their development. Note in the latter the aspect of the inclusion network cut at various angles.

Fig. 4. Group of lymphocystis cells older than those in the preceding figure with various stages of development of the inclusion bodies. Other cells greatly hypertrophied. Note the shape of the nucleolus in the large cell.

PLATE II

Figs. 5–11. Early stages of the lymphocystis cells. All photographs have the same magnification. Fig. 5: *a*, connective tissue cell showing changes most probably representing the first stages of the evolution of the lymphocystis cells; *b*, cell with five basophil granules (not well distinguishable on the photograph); *c*, cell with a 'sphere'; *d*, binucleated cells; *e*, cells with inclusions in the Guarnieri-body stage. Fig. 6: *a*, small cell with distinct characters of lymphocystis element; *bl*, blood vessel with an erythrocyte. Fig. 7: cells with distinct spheres and inclusion bodies. Fig. 8: cells with inclusion bodies of various sizes; the largest with two bodies which are going to fuse. Fig. 9: further stage of the merging of two inclusion bodies. Fig. 10: cell with three inclusions, one of them as well as the big inclusion in the second cell seemingly representing the final stage of the fusion of two granules. Fig. 11: cell with two inclusions; the larger has a lobed appearance probably caused by the outlines of three granules fusing into one.

Fig. 12. Several cells with lighter and darker portions of the cytoplasm irregularly distributed and becoming arranged, in the larger cells, as ectoplasm and endoplasm. Note on the right side of the figure a small binucleated cell with two 'spheres' and symmetrically developing inclusion bodies. Haematoxylin-eosin staining.

Fig. 13. Cells in the same stages as in the preceding figure but stained with Giemsa's. The darker staining cytoplasm assembles on the periphery of the cells. Note the distinctly delimited ectoplasm in the large cells.

PLATE III

Fig. 14. Two cells united by a cytoplasmic bridge.

Fig. 15. Several cells developing within the membrane of a degenerating cell. Other cells in this and the preceding figure are in various stages of the degeneration.

Fig. 16. Part of the lymphocystis tumour in which nearly all cells have degenerated. *a*, empty membranes; *b*, membrane with invading connective tissue elements; *c*, membrane with shrinking cell contents.

Fig. 17. Degenerating cell in which the ectoplasm containing the inclusion bodies is partly folded and detached from the endoplasm.

Figs. 18, 19. Inclusions of different appearances depending on the stage of their development interrupted by the degeneration. Fig. 18: *a*, degenerating inclusions which have not started their transformations; *b*, those which were in the early and (fig. 19) in the later stage of transformation.

PLATE IV

All photomicrographs have been made with the same magnification (oil-immersion $\frac{1}{7}$ in.).

Fig. 20. Usual 'normal' appearance of the inclusion bodies on a tangential section of a lymphocystis cell of medium size.

Fig. 21. Disintegration of the inclusion bodies in cells degenerating in the manner observed in the majority of the lymphocystic cells.

Fig. 22. First signs of transformations of the inclusion bodies seen in the cell on the right. Note the swelling of the bars and their more delicate structure as compared with the cell on the left.

Fig. 23. Rearrangement of the inclusion networks in a lattice made up of delicate threads.

Fig. 24. Arrangement of the transformed network in strands and lamellae.

Fig. 25. Similar stage as in preceding figure with several strands cut transversely.

Fig. 26. Part of a lymphocystis cell with inclusions in a transformation stage slightly older than in fig. 22, showing increasing area occupied by these bodies. In order to make the inclusions more prominent a red filter was used and therefore in figs. 20–26 the cytoplasm in which the networks are developing is rendered nearly invisible.

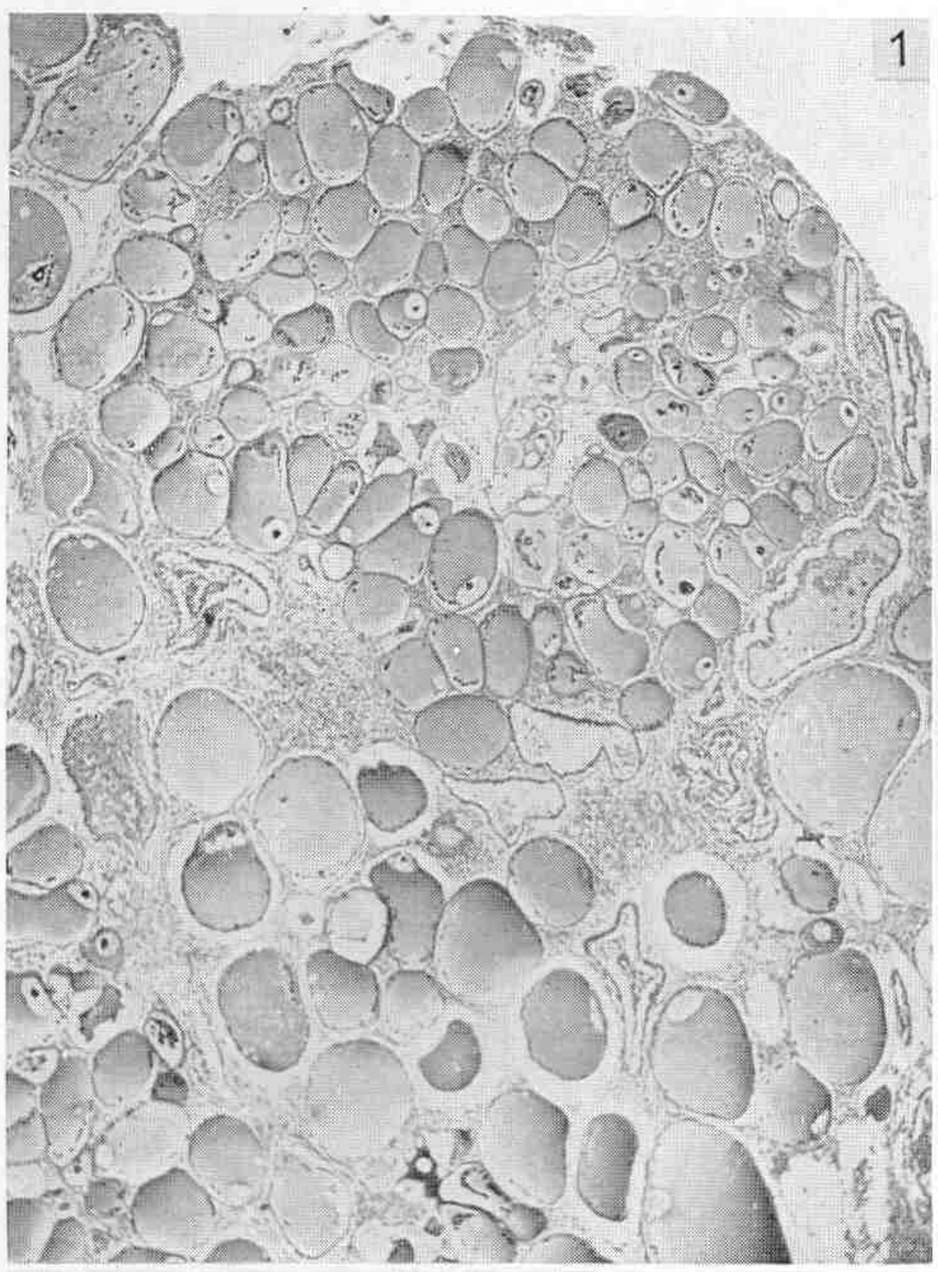
Fig. 27. Part of a lymphocystis cell with the first stages of the dissolution of the cytoplasm. *a*, cross-sections of thicker and very thin threads of the network with narrow spaces around them indicating the beginning of the dissolution seen in more advanced stages round the larger parts of the inclusions; *b*, inclusion network flattened in lamellae which, being forshortened in the picture, do not show their finer structure; *n*, degenerating nucleus with large masses of nucleolar origin.

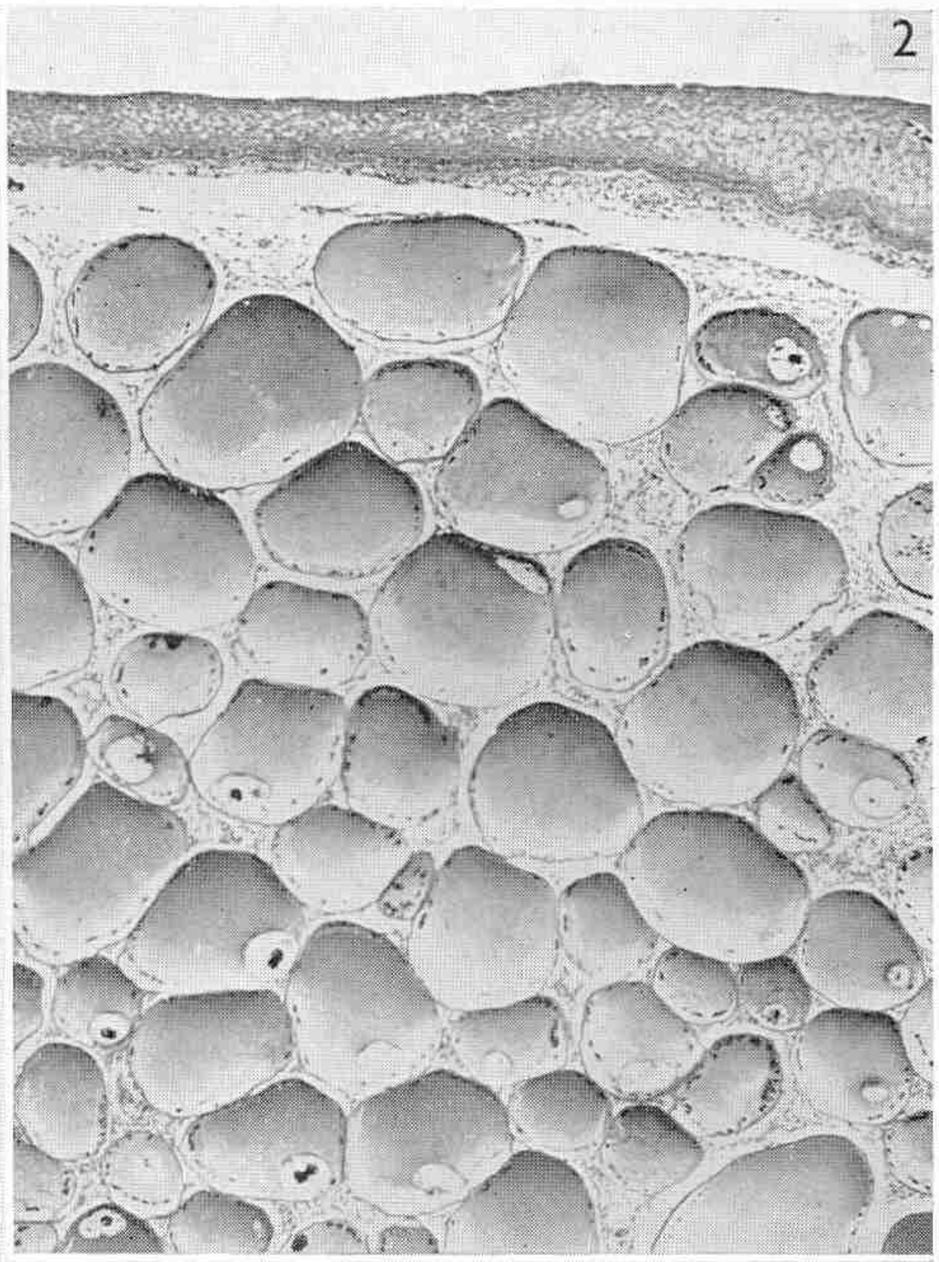
Fig. 28. Lacunae with fine threads of the inclusion network.

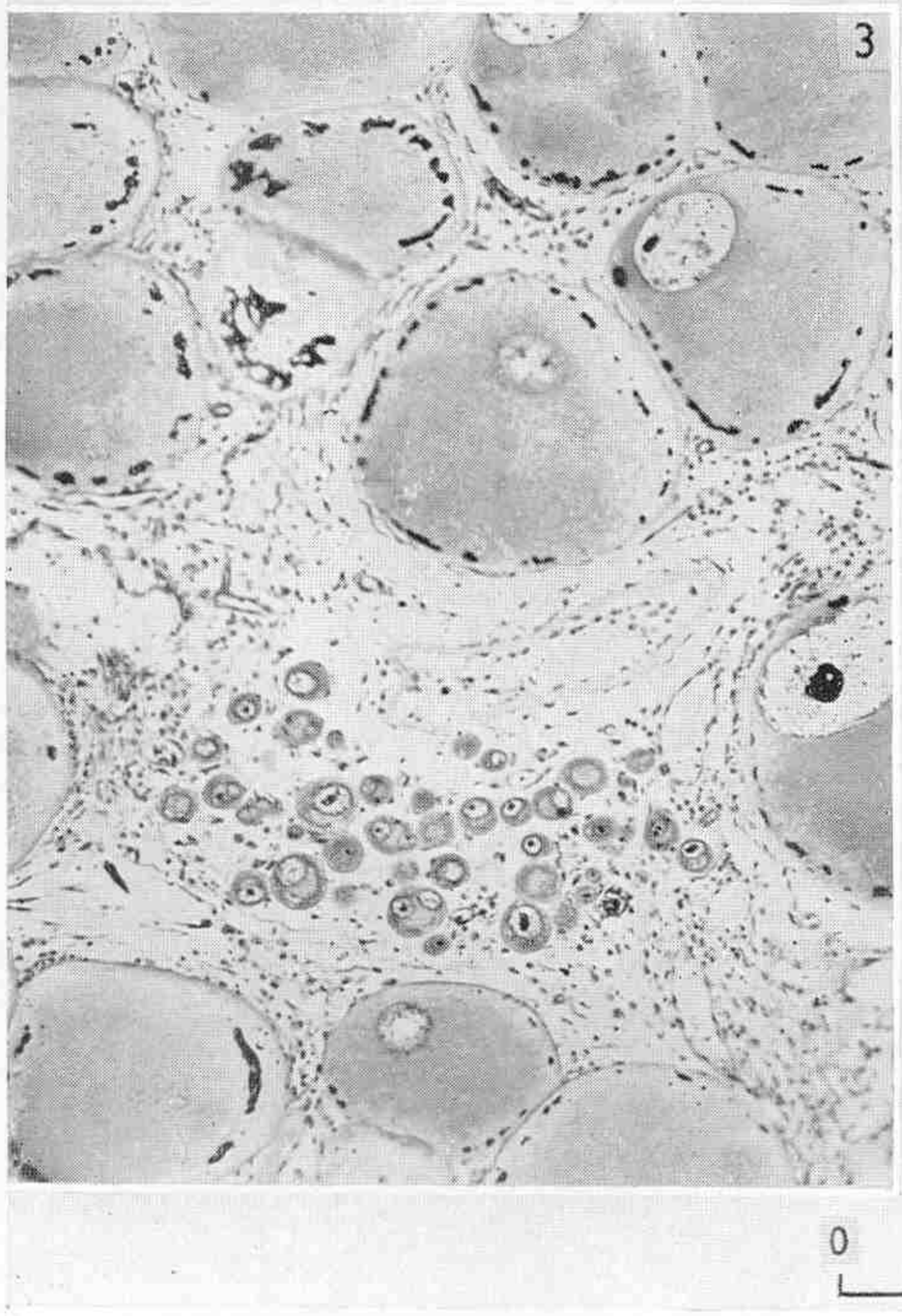
Fig. 29. Lacunae with lumps of disintegrated cytoplasm and threads of inclusion network in the stage when they are becoming less visible.

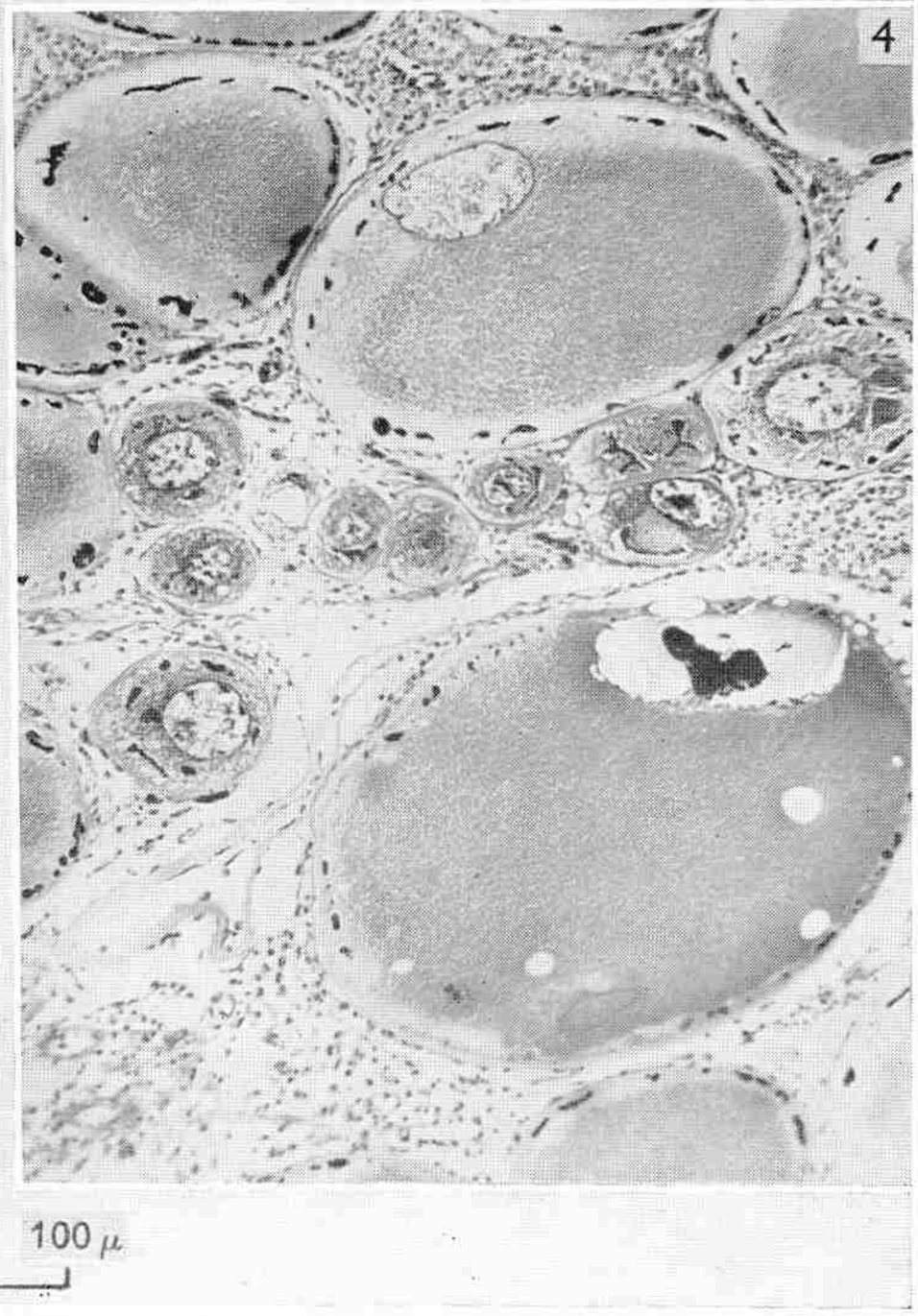
Fig. 30. Parts of three lymphocystis cells with lacunae containing in *a*, deep stained basophil elements; in *b*, lumps of cytoplasm and hardly visible basophil threads; in *c*, particles of various sizes not giving any basophil reaction. Note the shapes of the lacunae cut at various angles and the large nucleolar mass in one of the cells.

Fig. 31. Lymphocystis cell in an advanced stage of the formation of the lacunae filled up with particles not showing basophilic staining reaction. Some of the lacunae are empty. Note the enlarged territory of the ectoplasmic layer.

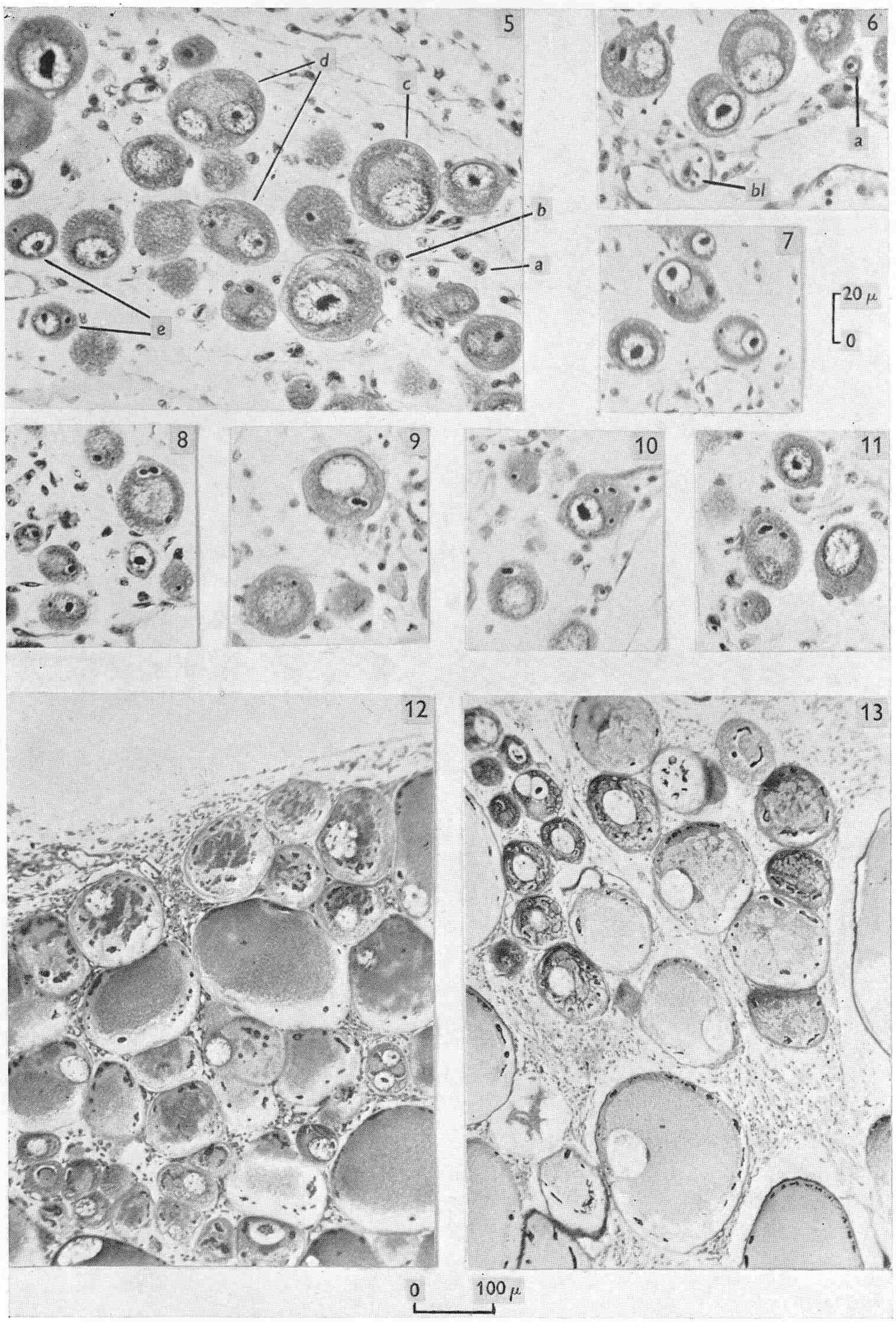




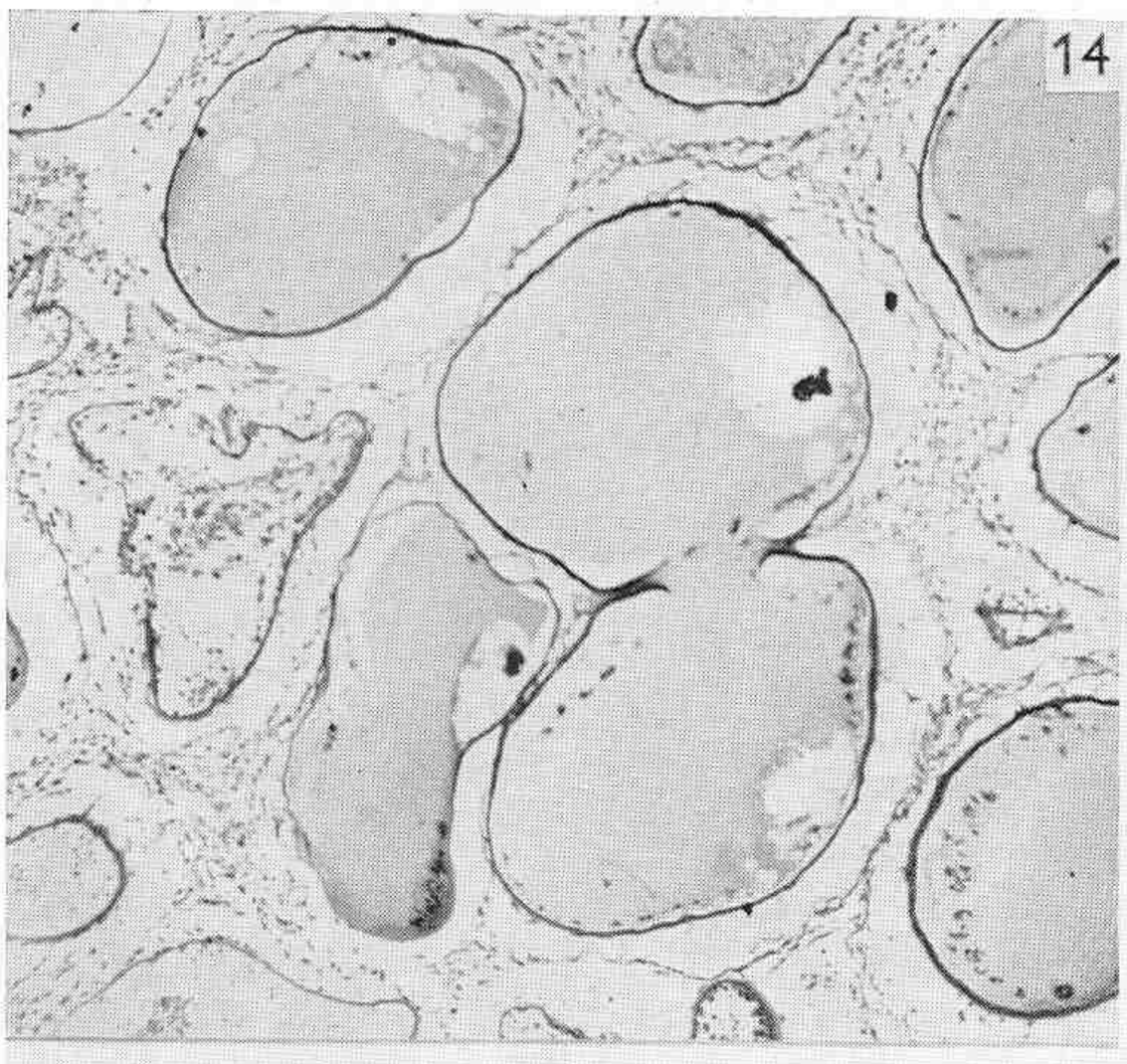


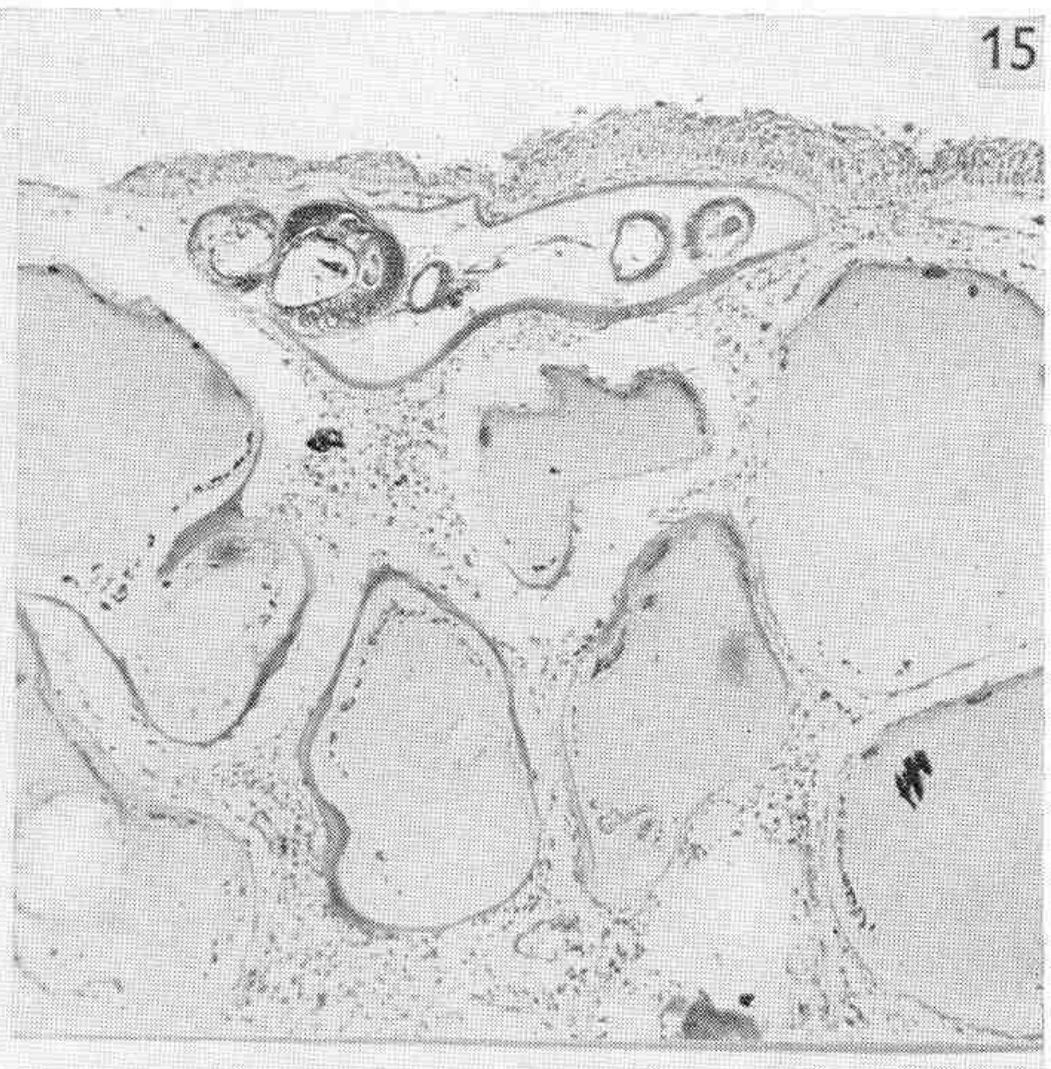


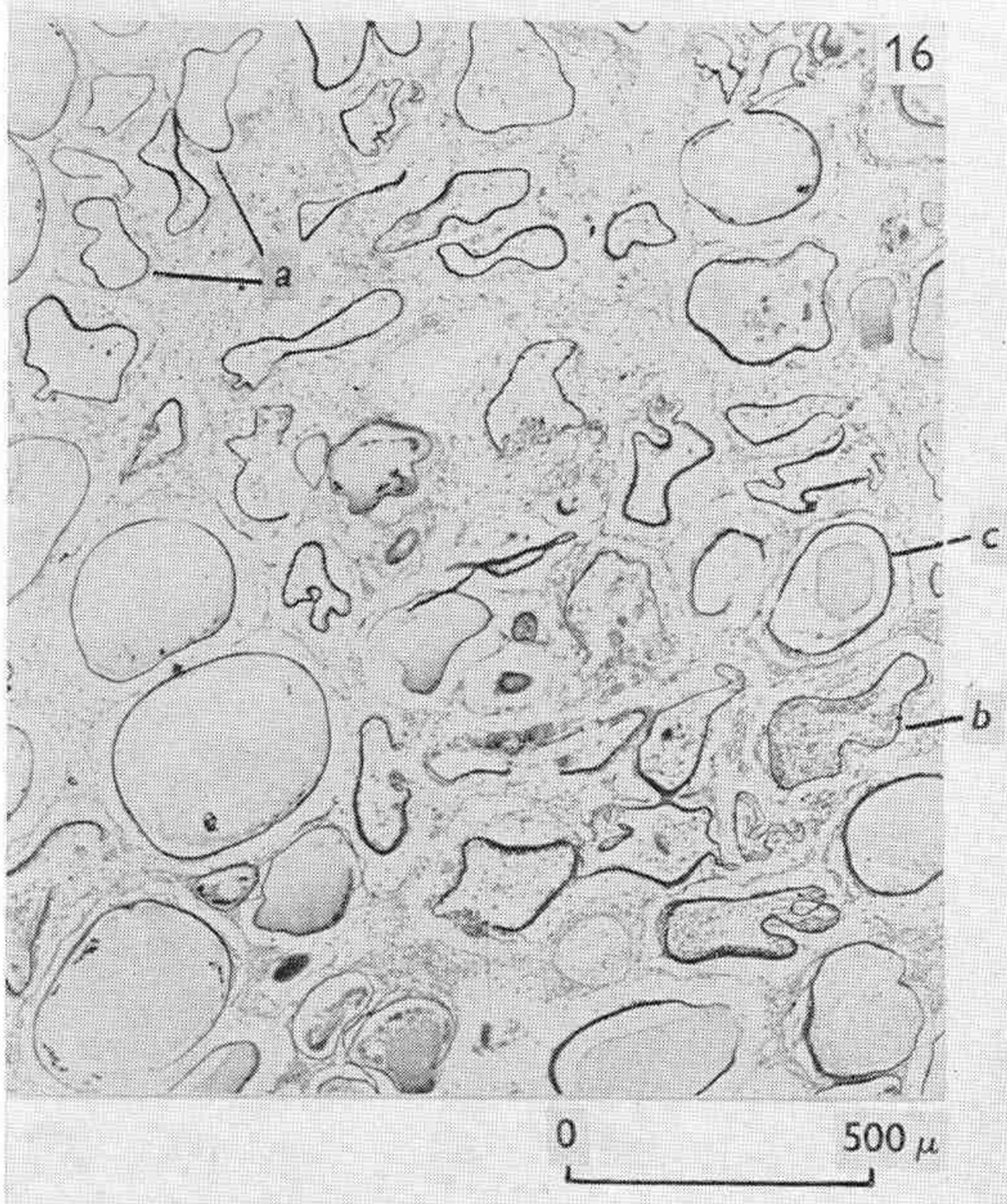
Figs. 1–4.

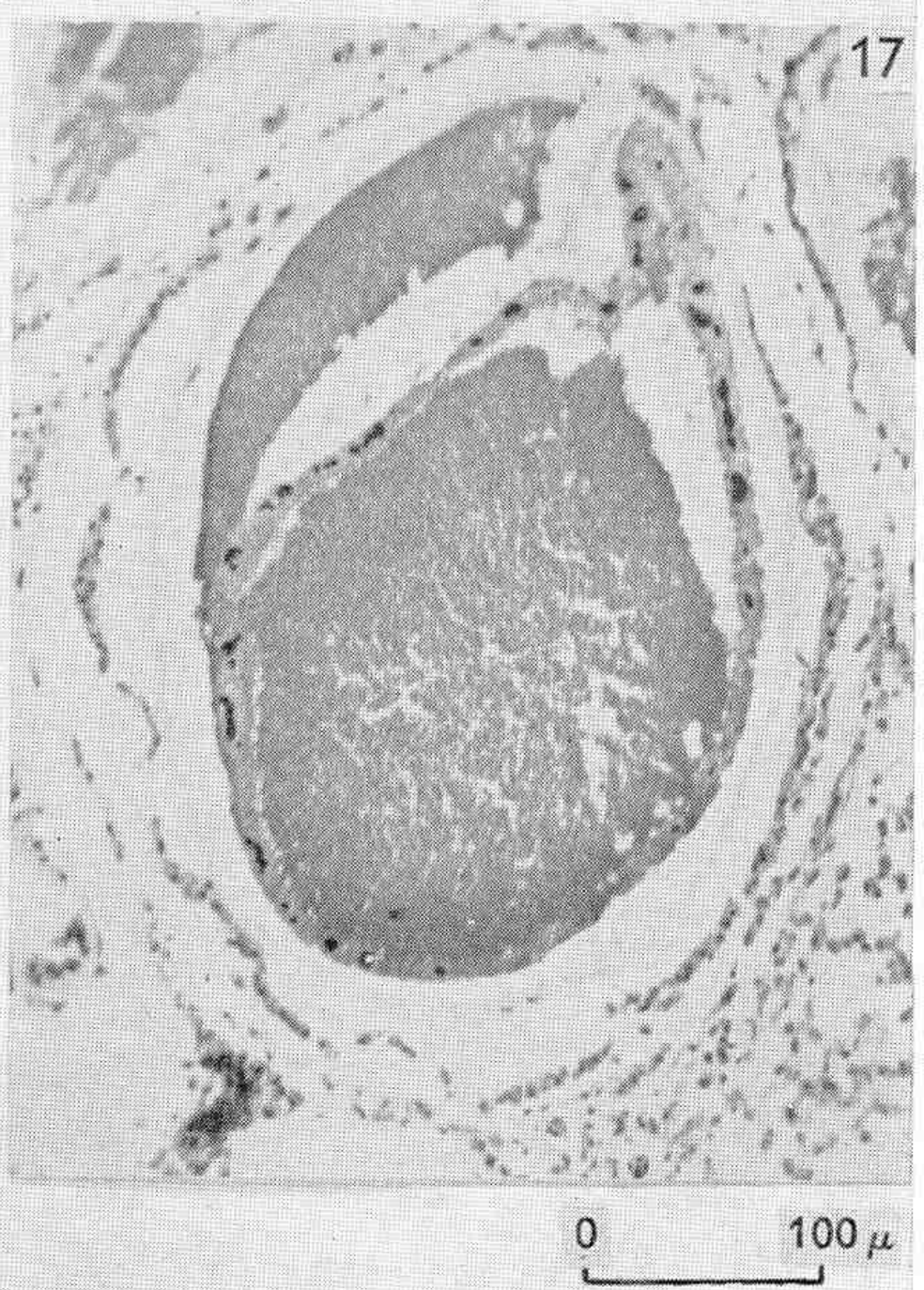


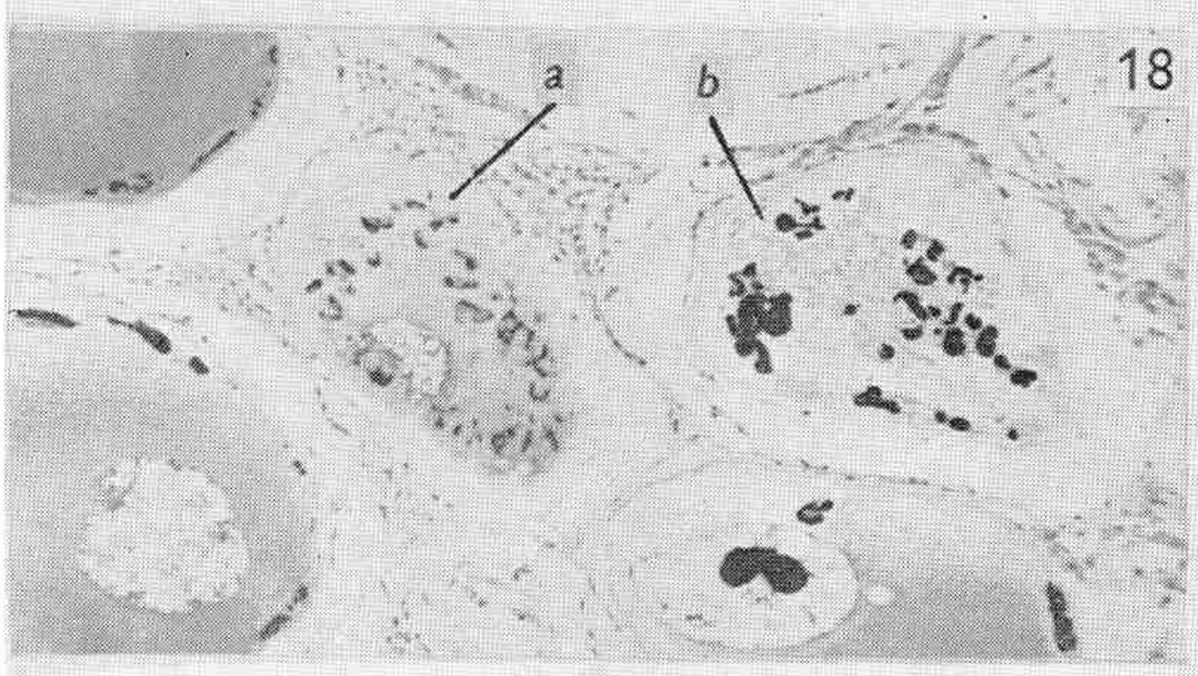
Figs. 5–13.

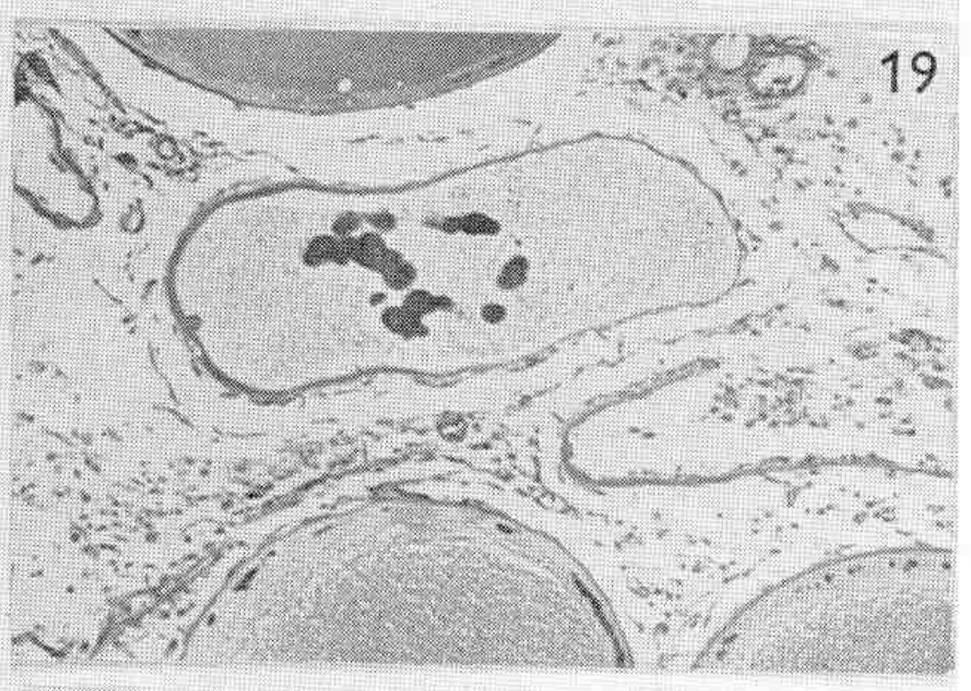






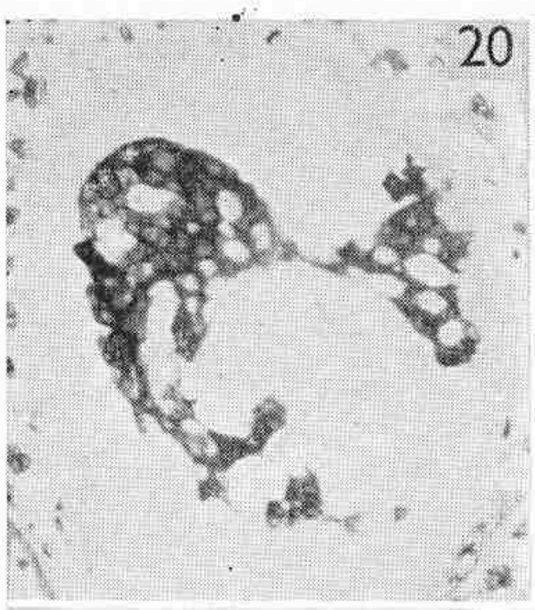


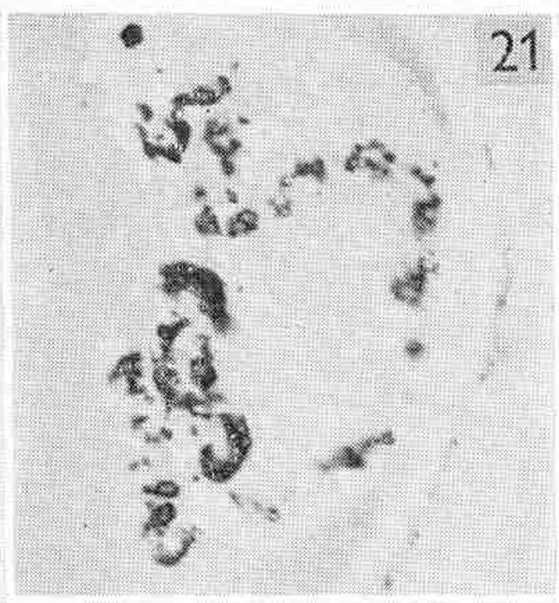


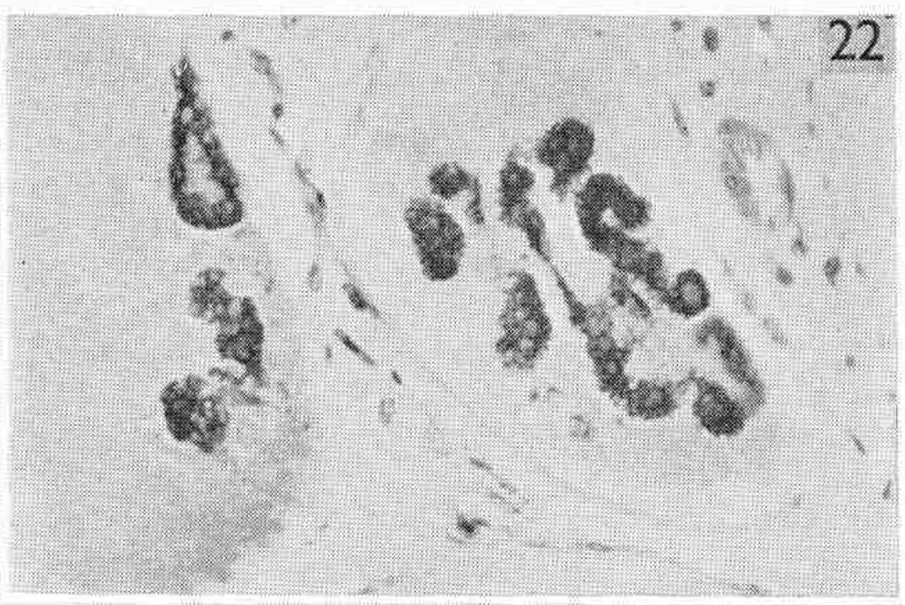


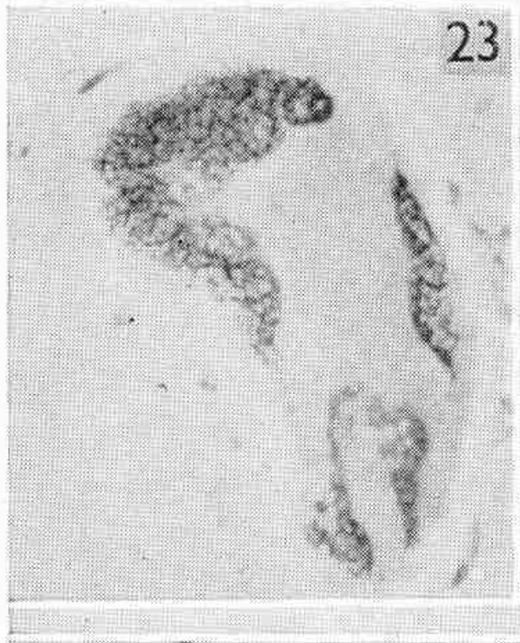
0 100 μ

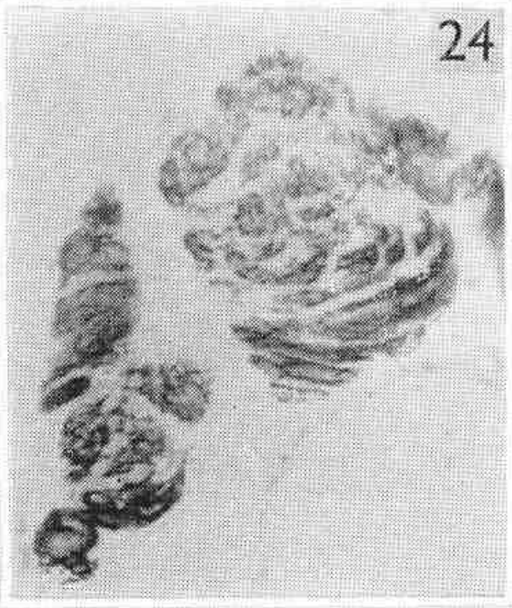
Figs. 14–19.

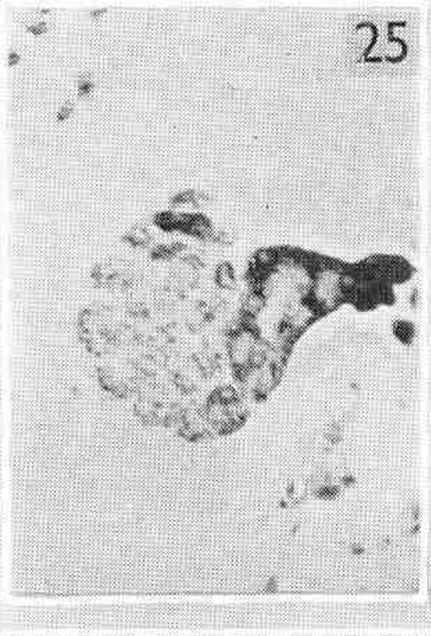


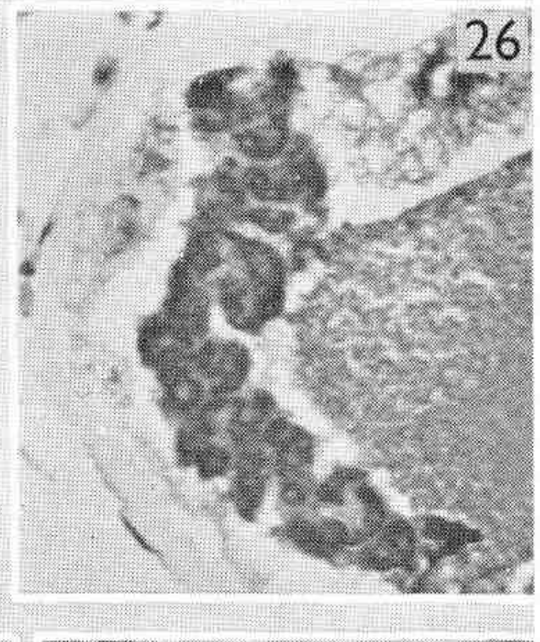


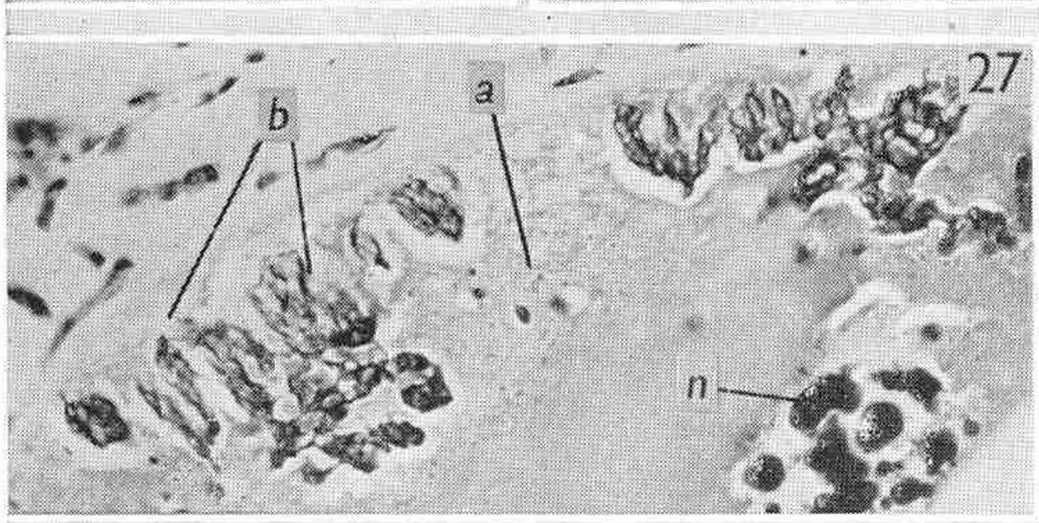


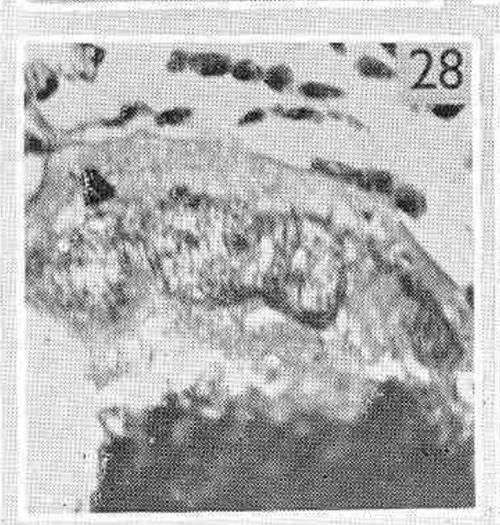


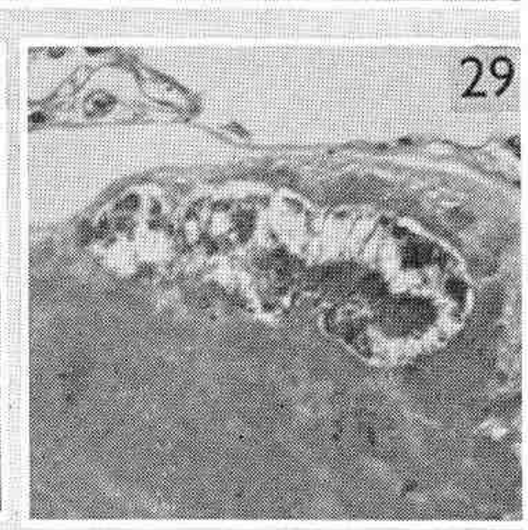


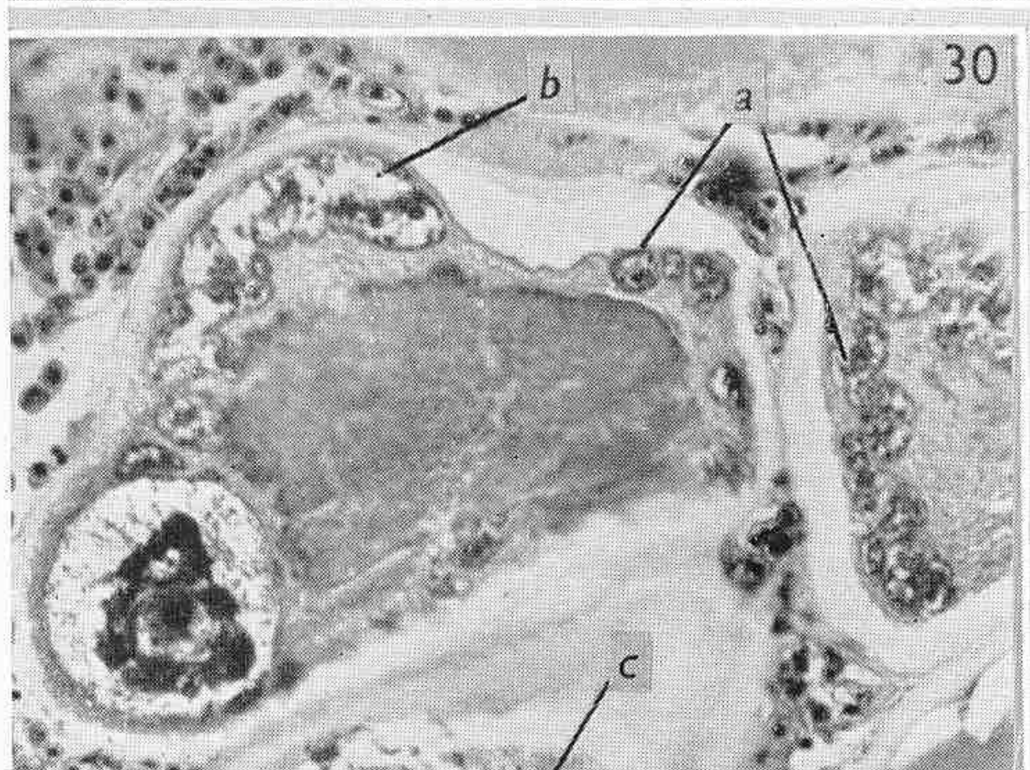


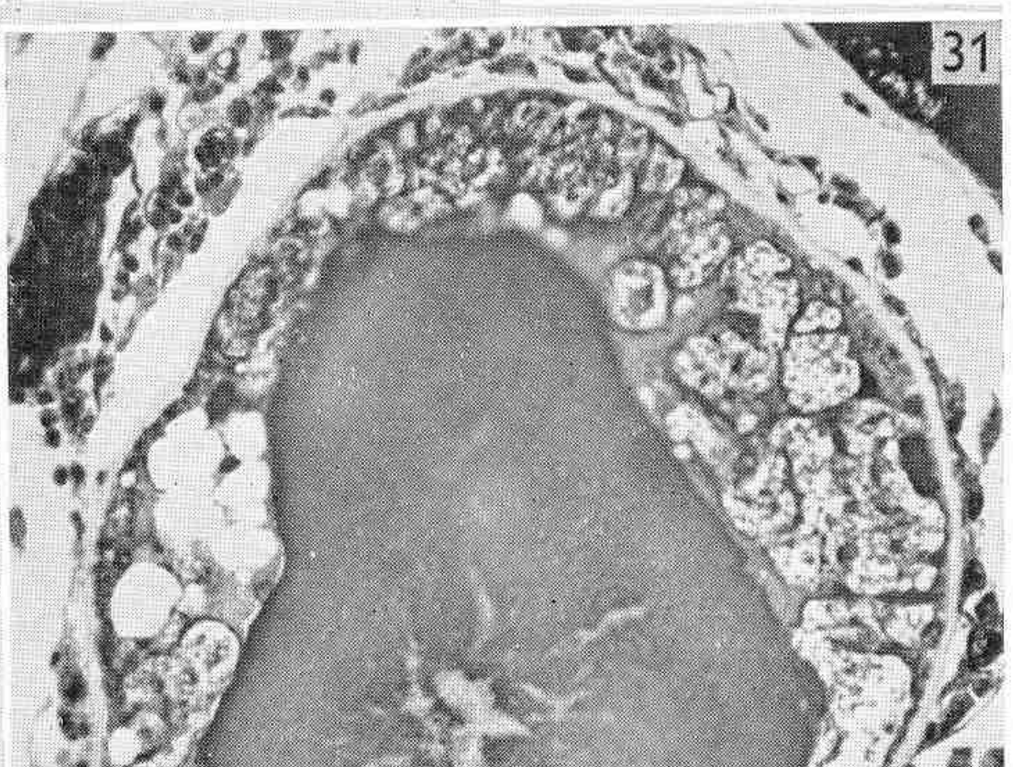












0 50 μ

Figs. 20–31.

THE BIOLOGY OF THE COMMON PRAWN, *LEANDER SERRATUS* PENNANT

By G. R. Forster, B.Sc.

From the Plymouth Laboratory

(Text-figs. 1–14)

CONTENTS

INTRODUCTION

This work was undertaken at the suggestion of Mr F. S. Russell that little was known about the natural history of even so familiar a species as *Leander serratus*, the common prawn of the English Channel, which may at times form a valuable natural resource to the inshore fishermen. Attention was therefore chiefly concentrated on its growth rate and breeding biology. In spite of the long breeding season the statistical method of studying growth was adopted when preliminary results appeared successful. By this method additional data could be obtained on distribution and migration. In the closely related genus *Crangon*, growth has been shown to be retarded under laboratory conditions (Nouvel & van Rysselberge, 1937), so that an experimental study could not be relied on to give a picture of the normal life history. Höglund's recent monograph (1943) has treated *Leander squilla* in great detail. However, this species differs considerably in size and distribution from *L. serratus*,

and it was felt that a similar study in the Plymouth area would not be mere repetition; in fact the biology of *L. squilla* itself differs somewhat in the two districts.

Acknowledgement is due to the Department of Scientific and Industrial Research for financial assistance in the form of a maintenance allowance, and to the crews of the research ships of the Plymouth laboratory for their help in collecting. Mr W. Searle's experience of the Plymouth trawl fishery for prawns also proved a great benefit.

Methods

Prawns were caught by three methods: trawling, hand-netting, and with baited traps or hoop-nets.

The trawl used from the motor boat *Gammarus* was identical with the commercial prawn trawls, consisting of a 6 ft. beam with 1 in. mesh netting on the wings and head and $\frac{3}{4}$ in. mesh in the cod end. Trawling grounds near Plymouth are described in the *Plymouth Marine Fauna* (Marine Biological Association, 1931). The best grounds are located chiefly in muddy areas, sometimes a little rough, or near rocks.

Several patterns of hand-net were used, a pear-shaped frame for rock pools and a rectangular frame for walls or pier piles. Half-inch mesh netting was found suitable for all except the youngest stages, for which stramin was necessary. The most successful places for hand-netting were pier piles and undercut rocky gullies or crevices where the larger brown algae were absent.

Twelve prawn traps were constructed in the form of miniature lobster pots 2 ft. × 1 ft. × 8 in. covered with $\frac{1}{2}$ in. mesh netting. They were laid overnight in fleets of four, baited with fish. Catches varied considerably from trap to trap, usually one out of the four being particularly successful. The largest catch from any one trap was 37 prawns. At Paignton, additional catches were made with hoop-nets kindly loaned by Mr S. Underhay. These nets were set after dark in the *Laminaria* zone and hauled at about quarter-hourly intervals, the technique being to raise the net slowly and gently at first, when the prawns remain clinging to the bait. If the net is given a sharp tug some are disturbed and escape.

Prawns were always measured in fresh condition, owing to the curvature of the abdomen in most preserved specimens. In all cases the total length (tip of the rostrum to base of the spines on the telson) was measured with dividers while the animal rested in its normal upright position on a glass plate. Living prawns could be induced to sit quietly in this position. Although an occasional specimen was taken with a damaged telson or rostrum, with practice it was easy to estimate the extra few millimetres accurately. This method has the advantage of being suitable for all sizes of prawns. Variations in the rostral length, though tending to increase the spread of the results, did not hinder the

separation of different year groups. Measurement of the carapace length alone, though suitable for adults, was virtually impossible for juveniles, and had two further possible disadvantages. An error of $\frac{1}{2}$ mm. is much greater relative to the carapace than to the total length. Secondly, the carapace might undergo heterogonic growth while the ovary is ripening.

Collections were started in October 1949 and the observations continued until October 1950.

Age and Growth

The 1950 O-Group (i.e. Brood of the Year)

Juvenile and post-larval stages belonging to the 1950 O-group of *Leander serratus* were first taken from shore pools at Wembury in the second week of July of that year. They had not been found during the previous spring tides at the end of June, and therefore it is unlikely that many arrived before the first week of July. Very small prawns also appeared in the Sound at about the same time, their size frequencies being shown in Fig. 1. The Wembury catch

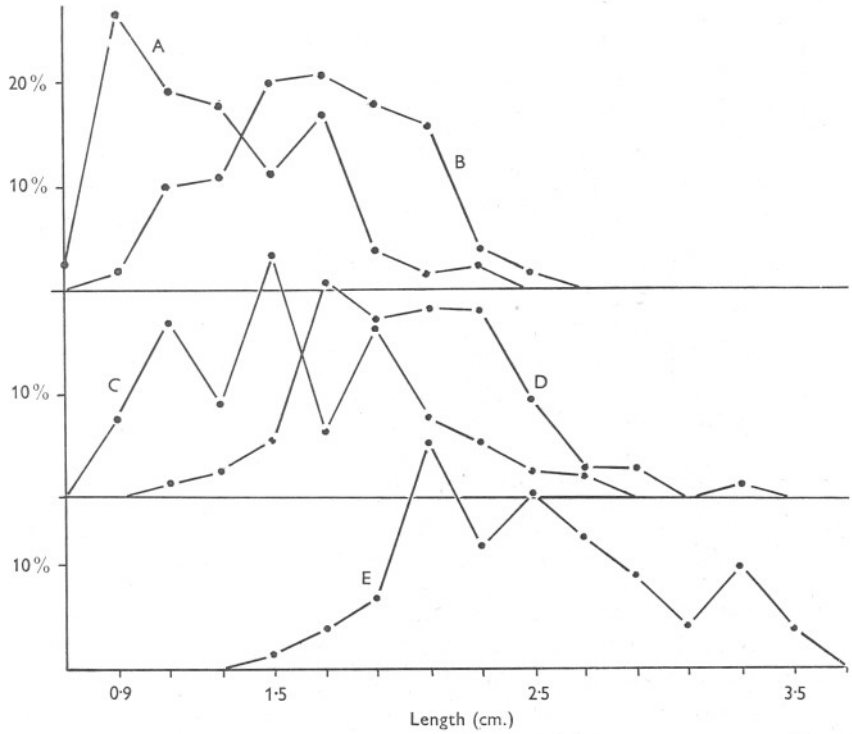


Fig. 1. *Leander serratus*. Percentage size distribution of O-group prawns. A, Rum Bay (Plymouth Sound), 24 July 1950 (136). B, Wembury (outside Sound), 14 July 1950 (127). C, Rum Bay (pool at mid-tide level), 3 Aug. 1950 (77). D, Rum Bay (low-water springs), 3 Aug. 1950 (72). E, Rum Bay (low-water springs), 29 Aug. 1950 (182).

is seen to contain many more prawns in the 1·7, 1·9 and 2·1 cm. groups than those from Rum Bay. At first sight this larger-sized population might be thought to be older or to have grown faster, but catches from Rum Bay on 3 August (Fig. 1) showed that the average size increased towards L.W.S.T. level. Therefore the Wembury catch may have been from a slightly lower level on the shore. The growth of these juvenile prawns is shown in a comparison of the catch of 3 August at L.W.S. with that of 29 August. It is seen that the whole curve has moved roughly 0·5 cm. to the right. There appears to have been only very little further recruitment of small individuals on the left-hand side of the graph.

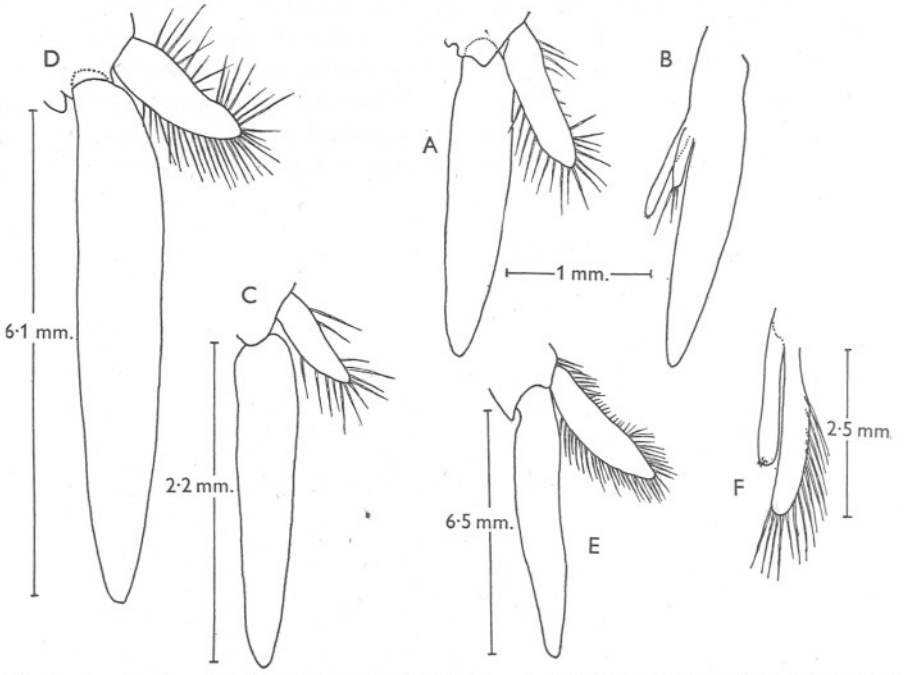


Fig. 2. *Leander serratus*. Secondary sex characters. A, 1st pleopod of immature ♂ (23 mm.). B, setose appendix masculina of the second pleopod of the same. C, 1st pleopod of immature ♀ (24 mm.). D, 1st pleopod of mature ♀ (68 mm.). E, 1st pleopod of mature ♂ (69 mm.). F, appendix masculina of the same. In D and E the inner branch (with setae) has been drawn the same length as in A to show the difference in proportions.

Over a length of 2·5 cm. the males can be distinguished from the females by the shape of the appendix interna of the first pleopod, and by the presence of an appendix masculina on the second pleopod (Fig. 2), these being the normal secondary sex characters. In case some males developed at a slower rate, sexing was not attempted for specimens below 3 cm. in length. This limit had the additional advantages in that it allowed sexing to be done by eye, instead of with a binocular microscope, and also made the stramin net unnecessary

since it was difficult for a 3 cm. prawn to escape through the $\frac{1}{2}$ in. mesh of the hand-net.

Collections in September and October 1950 are illustrated in Fig. 3. Growth is shown by the shift of the peak from approximately 3·75 to 4·75 cm. or over. There appears to be no differential growth rate between the sexes up to this stage, although the catch from Burgh Island shows a tendency in this

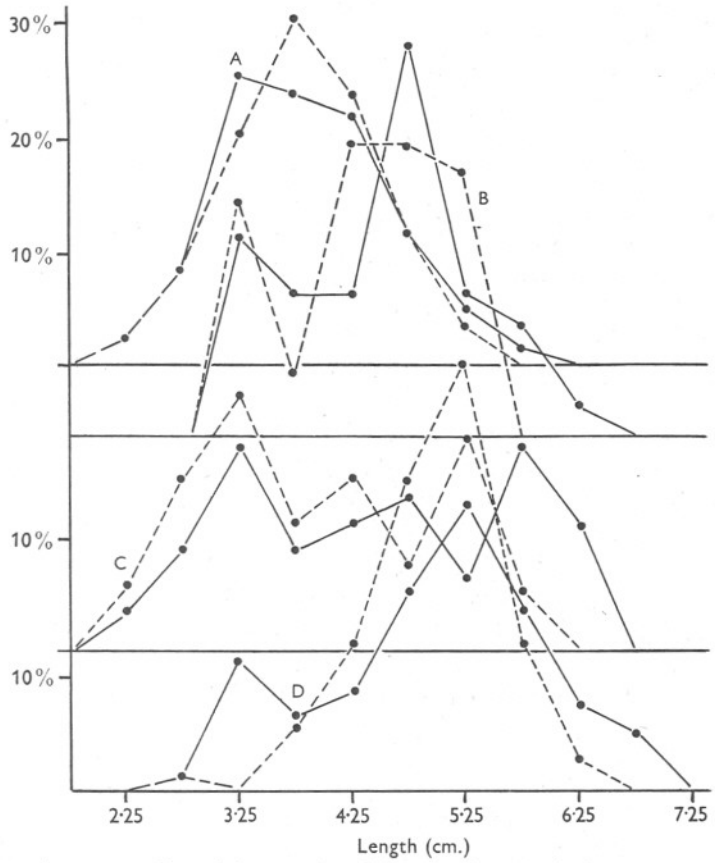


Fig. 3. *Leander serratus*. Length frequencies of the 1950 O-group in the autum. A, Rum Bay, 12 Sept. 1950 (60♀, 60♂). B, Rum Bay, 9 Oct. 1950 (41♀, 39♂). C, Burgh Island (outside Sound), 13 Oct. 1950 (44♀, 27♂). D, Drake's Island, 11 Oct. 1950 (80♀, 70♂). ——, females; – – –, males.

direction. This catch is of interest, since the coast is considerably more exposed to wave action than the Sound. Growth appears to have been just as rapid as in the Sound, but there is a greater proportion of the population in the small-size groups. Possibly in the open sea the chances of the larvae being swept into the littoral zone (where metamorphosis appears to take place) are smaller and therefore the period of settling prolonged.

Comparison of the 1949 *and* 1950 *Year Groups*

The overwintering population of prawns was studied from October 1949 onwards. It was therefore necessary to delimit the 1949 O-group for comparison with that of 1950. This was not difficult for the males (Fig. 5), but as shown in Fig. 4 the females presented a more complex pattern. The first

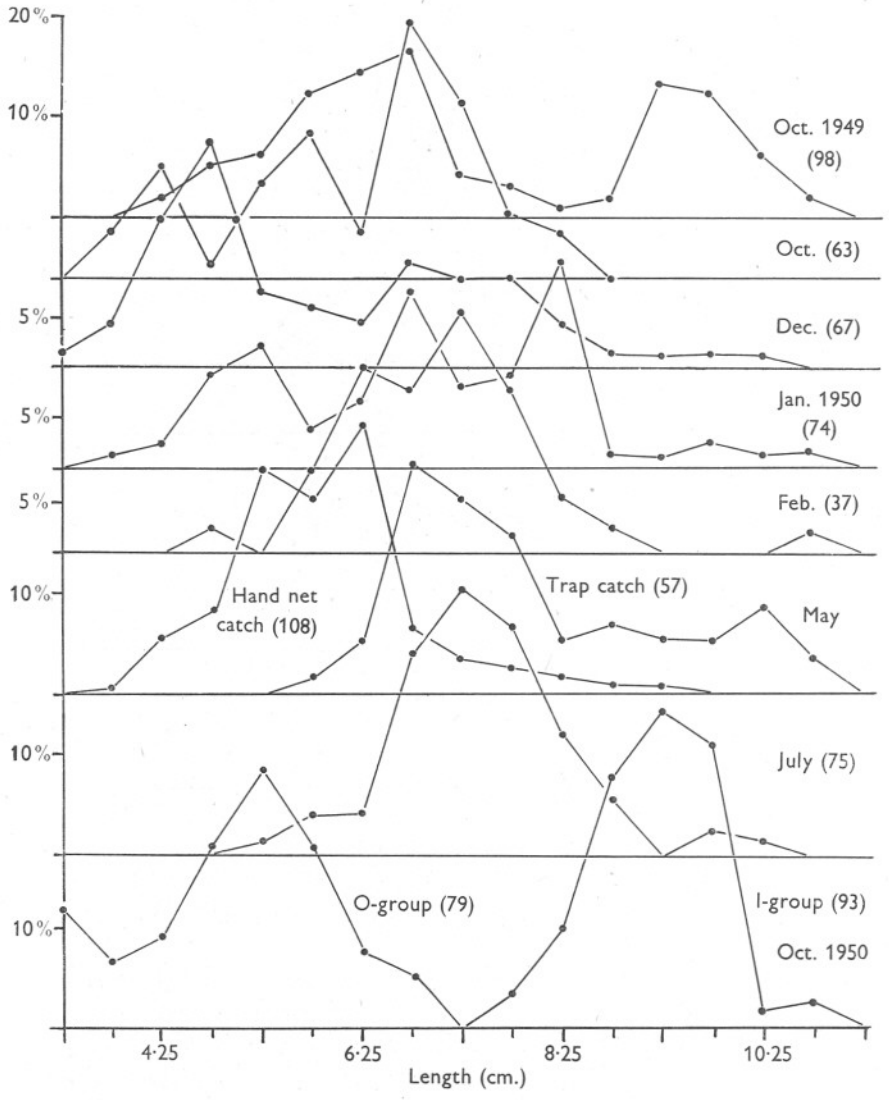


Fig. 4. *Leander serratus*. Length frequencies of selected catches of females, Oct. 1949 to Oct. 1950.

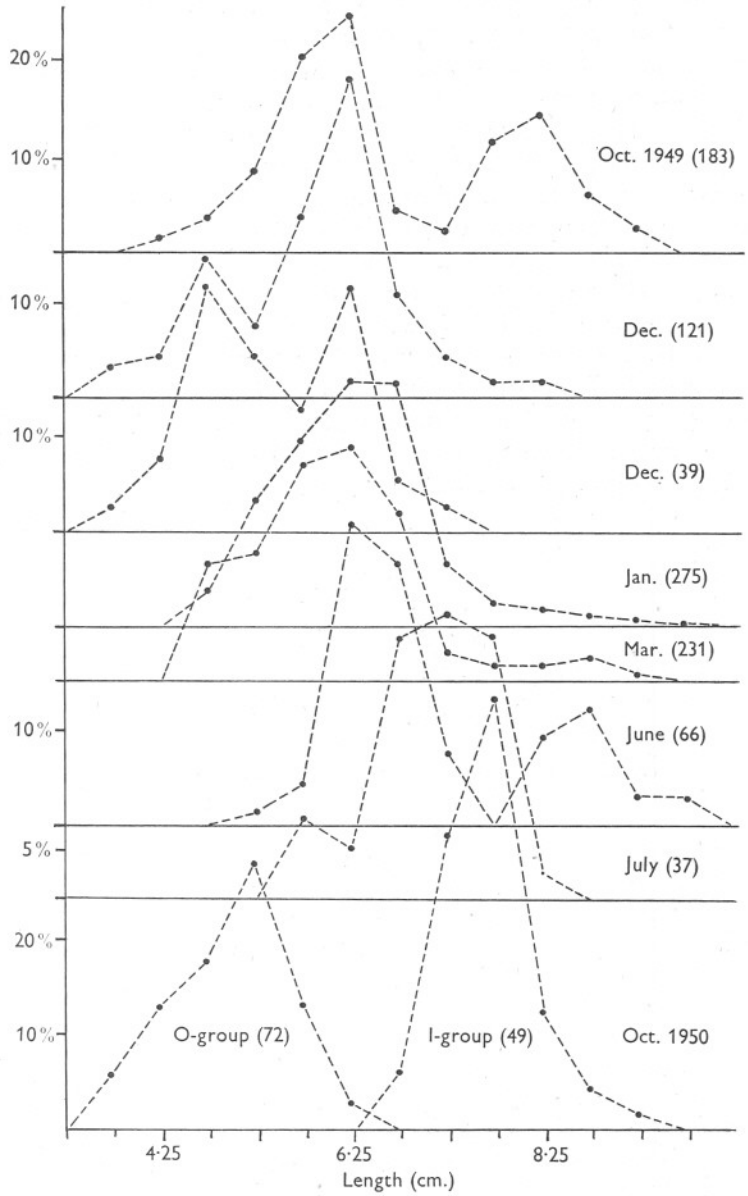


Fig. 5. *Leander serratus*. Length frequencies of selected catches of males, Oct. 1949 to Oct. 1950.

trawl catch (18 October 1949) shows bimodal curves for both sexes, which was a pleasantly simple picture. By December, however, with the males a small secondary peak showed up at 4·75 cm.; while with the females the original peak at 6·25 cm. gave place to a complex pattern with three minor peaks at approximately 4·75, 6·75 and 8·25 cm. This population can only be analysed after a study of catches during the summer of 1950, where it is seen that in 1950 all the prawns from 4·25 to 8·25 cm. groups shift into one peak, which clearly corresponds, although the average length is slightly smaller, to the larger peak at approximately 9·5 cm. in the trawl catch from below the Hoe in October 1949 (Fig. 4). Since the group with the 9·5 cm. peak in October 1950 contains all the smallest prawns before the appearance of the 1950 O-group, it must therefore have been the previous year's brood, i.e. the 1949 year group. By tracing this group back to October 1949, the 1949 O-group can be delimited. It appears to include all males below 7·25 cm. and all females below 7·75 cm. The apparent peak of females at 8·25 cm. in some winter trawl catches is discussed later (p. 342). On this basis, Fig. 6 shows the 1949 and 1950 O-groups superimposed on one another. There is seen to be a

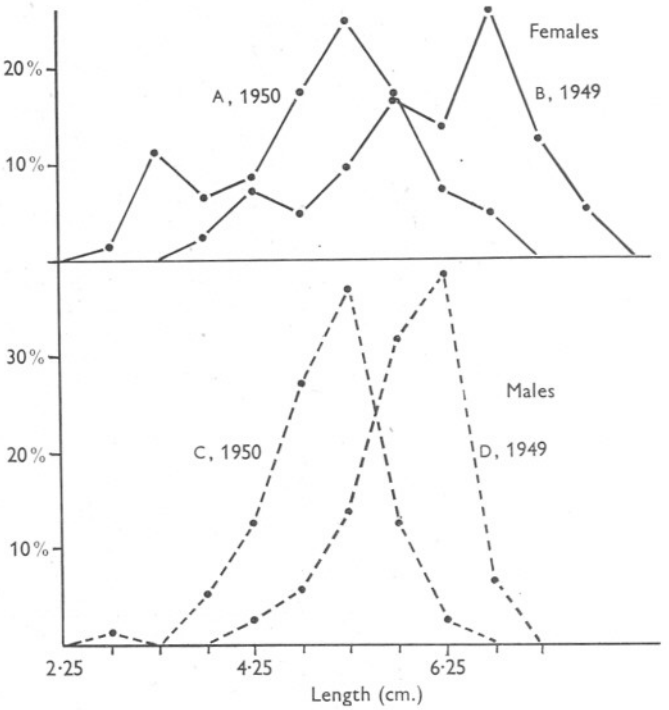


Fig. 6. *Leander serratus*. Comparison of the 1949 and 1950 O-groups in October of the same year. A, Drake's Island, 11 Oct. 1950 (80). B, average of trawls off the Hoe, 21 and 18 Oct. 1949 (60) and Trevol Pier, 20 Oct. 1949 (63). C, Drake's Island, 11 Oct. 1950 (70). D, trawl off the Hoe, 12 Oct. 1949 (116).

difference of about 1 cm. between the peaks for both sexes, the 1949 brood having clearly outgrown the largest October 1950 O-group catch. The year 1949 was exceptional for the warm weather during summer and autumn; whereas 1950 had a much cooler summer. Extraction of the daily sea temperature readings from the bathing station on the Hoe gave the following results. The sea temperature remained above 15° C. for 6 weeks longer in 1949 than in 1950. From 1 July to 12 October inclusive, the average of the daily sea temperature records was 17·27° C. in 1949 and 15·57° C. in 1950. Alternatively, the difference may be expressed as the cumulative (daily) excess temperatures above 15° C., which was 231·8° C. in 1949 and 59·66° C. in 1950.

The problem is therefore to ascertain whether this temperature difference is a reasonable explanation of the difference in mean lengths between the two successive O-groups. Thanks to the assistance of Mr G. M. Spooner, it was possible to calculate the temperature characteristic for growth from the data, and to compare the result with known values.

Assuming that metamorphosis took place at the beginning of July in 1949 and 1950, and that the average length of the post-larva was 9 mm. in both cases, the growth increment (for males only) during the 104 days till 12 October was 49·45 mm. in 1949 and 40·84 mm. in 1950. The average temperatures during this period were 17·27 and 15·57° C. respectively.

The Arrhenius equation for the temperature characteristic μ is taken from Needham (1931, p. 515):

$$\frac{1}{g_1} \div \frac{1}{g_2} = \exp\left[\tfrac{1}{2}\mu\left(\frac{1}{T_1} - \frac{1}{T_1}\right)\right],$$

where g_1 and g_2 are the times to reach a definite state at the high and low temperatures T_1 and T_2 expressed in degrees absolute. In this case the reciprocals of the growth increments during the same period of time are substituted for g_1 and g_2 (assuming uniform growth for the period).

We then have:

$$\frac{1}{40{\cdot}84} \div \frac{1}{49{\cdot}45} = \exp\left[\tfrac{1}{2}\mu\left(\frac{1}{288{\cdot}57} - \frac{1}{290{\cdot}27}\right)\right],$$

therefore
$$\frac{49{\cdot}45}{40{\cdot}84} = \exp\left[\tfrac{1}{2}\mu\,(0{\cdot}000020)\right],$$
$$= \exp\left[\mu \times 10^{-5}\right],$$

therefore
$$\log\left(\frac{49{\cdot}45}{40{\cdot}84}\right) = \mu \times \frac{10^{-5}}{2{\cdot}303},$$

therefore
$$\mu = 2{\cdot}303 \times 10^{5} \times \log\left(\frac{49{\cdot}45}{40{\cdot}84}\right)$$
$$= 19{,}120.$$

Growth increments for the females were 40·74 mm. in 1950 and 52·09 mm. in 1949 from which μ is found to be 24,580. The data for the females is less reliable, however, since it is based on smaller numbers.

From the data shown by Needham (1931, fig. 84), it may be seen that for growth processes a large proportion of the known values of μ lie between 17 and 21,000 with a smaller mode of 27,000, so that the calculated value appears quite reasonable, bearing in mind the scanty nature of the information. Therefore it may be concluded that the difference in mean size between the two year groups was most probably a temperature effect, though it is impossible to tell whether this might be direct or indirect.

Later Growth of the Males

The 1949 *year group.* The form of the 1949 male population remained fairly constant throughout the winter with a clear peak at 6·25 cm. In March and April 1950 there was an increase in the 5·25 cm. group to form a shoulder on the main peak which probably represented the secondary peak at 4·75 cm. shown in the previous December catches (Fig. 5). By June these had grown a little and merged into the main peak. From July to October the graphs show steady growth until the now one-year-old group reached 7·75 cm. This corresponds to the peak at 8·25 cm. found in the trawl catches during October 1949. Again the discrepancy may be explained as a temperature effect, since it would be expected that with larger slower-growing prawns the difference would be less than with the O-group.

The 1948 *year group.* After October 1949 there was no further indication of more than occasional 1-year-olds in the catches, until the hoop-net catch from Paignton in February which shows (Fig. 7) a clear peak with the same mean length of approximately 8·25 cm. Again, in the spring this group was not taken by the trawls but it reappeared in the trap catches during May. In June the mean had risen slightly to about 8·75 cm. Subsequently, during July and August, trap catches were poor and there was no further sign of these prawns continuing their third year of existence.

Later Growth of the Females

The 1949 *year group.* In separating the 1949 O-group from the autumn and winter trawl catches the complexity of the length distributions has already been noted. If we compare the 1949 October catches with those of January 1950 (Fig. 4), we see that a peak at 8·25 cm. has appeared in January, which at first sight might be attributed to growth of the 6·75 and 7·25 cm. groups. Although it showed up in three different catches from the Sound, this peak disappeared from the catches after January, and its origin must therefore remain obscure. By February the trawl catches having lost the 8·25 cm. peak show a pattern which would be expected if there had been little growth since October (Fig. 8). The hoop-net catch from Paignton gave a similar peak though with a slightly higher mean length. This was probably due to the smaller size groups tending to be restricted to the shallowest water, whereas the hoop-nets were shot chiefly around the 5-fathom line. In March and April only the

smaller size groups are well represented in the trawl catches, presumably owing to the larger prawns migrating inshore first. It was not until May, when catches were made with baited traps as well as by hand-net, that a clear picture could be obtained. The population now becomes sharply divided, with the hand-net taking nearly all those below 6·5 cm. and the traps most of those above 6·5 cm., as shown in Fig. 4. Fig. 8B represents the mean of these two

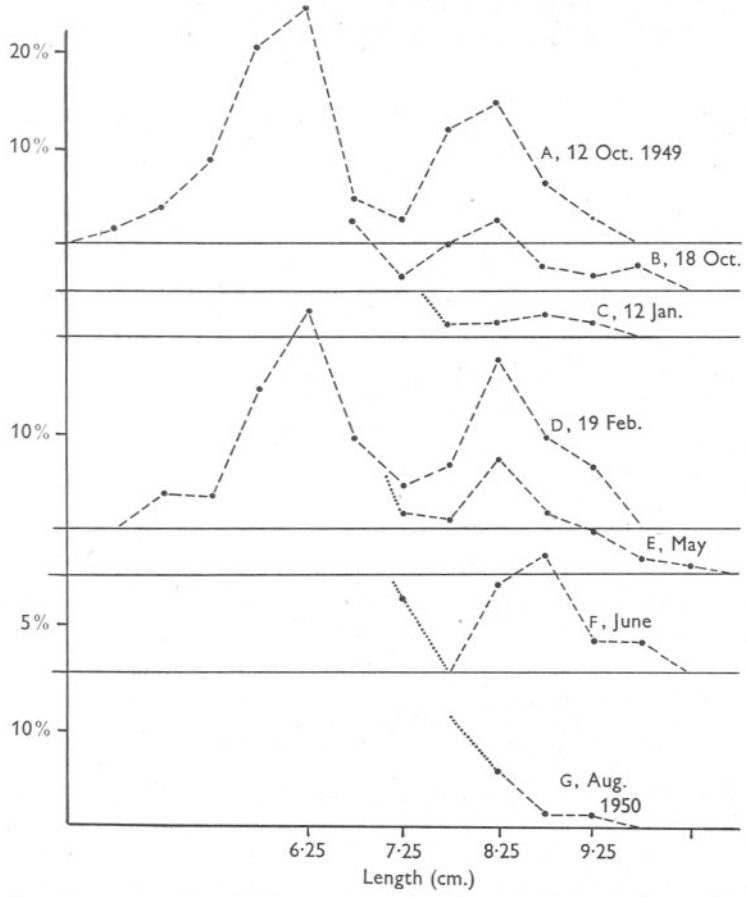


Fig. 7. *Leander serratus*. Length frequencies of 1948-year-group males (except in October and February the 1949 year group has been omitted). Samples taken by: A, trawl (67); B, trawl (16); C, trawl (18); D, hoop-nets (27), Paignton; E, traps (61); F, traps (18); F, hand-net (7).

catches, giving a widely spread peak at 6·75 cm. The length frequencies of the 1949 year group in the trap catches were scaled up to percentages, and these were averaged with percentages in the hand-net catch, so as to eliminate the effect of this second peak in the trap catch. It was not possible to estimate the relative abundance of these two populations, and therefore this curve is by no

means an accurate representation of the whole one-year-old group. It does, however, show the wide spread caused by the long breeding season and serves as a basis for comparison with later catches. A similar combined catch taken 3 weeks later (Fig. 8B) shows a peak at 7·25 cm. implying an average growth of half a centimetre. The difference between the means is actually 0·41 cm. After July the trap catches were poor, possibly due to the population moving closer inshore, so that growth can only be estimated from the hand-net catches (Fig. 10). These may at first be affected by an intermingling of the outer

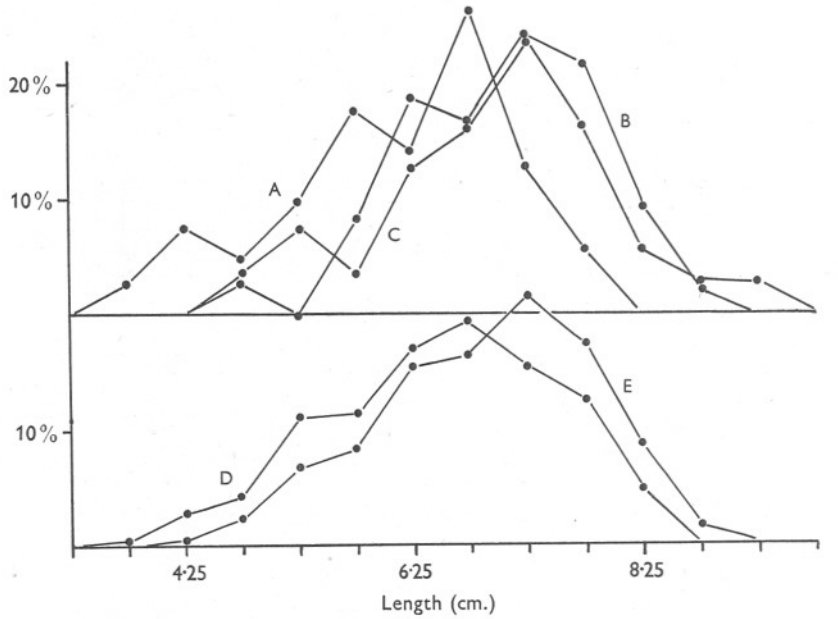


Fig. 8. *Leander serratus*. Above, comparison of catches of 1949 year group in October and February: A, October, trawl (123); B, February, trawl (37); C, February, hoop-nets. Below, two combined trap and hand-net catches. D, May 31; E, 24 and 27 June.

population, but the combined trap and hand-net catch (ratio 43–47) on 28 July (Fig. 9) is almost identical with the previous day's hand-net catch, the means being 7·44 and 7·35 cm. respectively. The hand-net catches may therefore be considered representative of the whole population, for by this time the places where the larger prawns tended to congregate had been discovered. Fig. 10 shows hand-net catches at approximately monthly intervals, chiefly from Rum Bay. By October there appears to have been fairly rapid growth so that the peak has moved up to 9·25 cm., slightly below that of the 1948 year group in October 1949 (Fig. 11), as would be expected.

The 1948 *year group*. After October 1949 the largest size groups (8·75–10·75 cm.) which can now be ascribed to the 1948 year group, disappeared from the trawl catches. During February 1950, however, occasional catches of

prawns were made by the research ships *Sula* and *Sabella*, chiefly after gales. These were composed of very large prawns, whose distribution curves showed peaks in the region of 10·25 cm.

A similar-sized catch was taken with lobster pots from an offshore ground near Dartmouth (see p. 354) and appeared to be representative of a fairly large

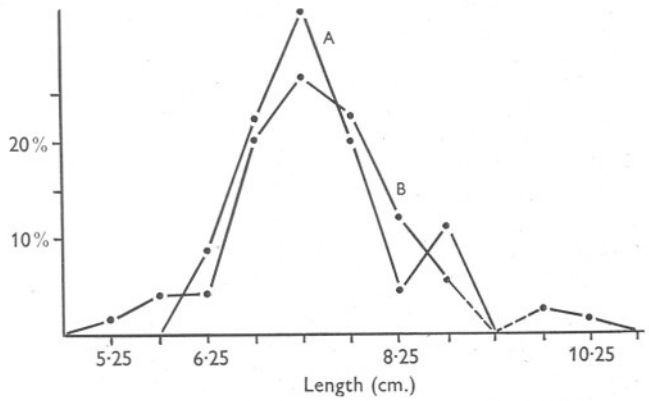


Fig. 9. *Leander serratus*. One-year-old females in July 1950 (see p. 344). A, hand net, 27 July. B, combined trap and hand-net catches, 28 July.

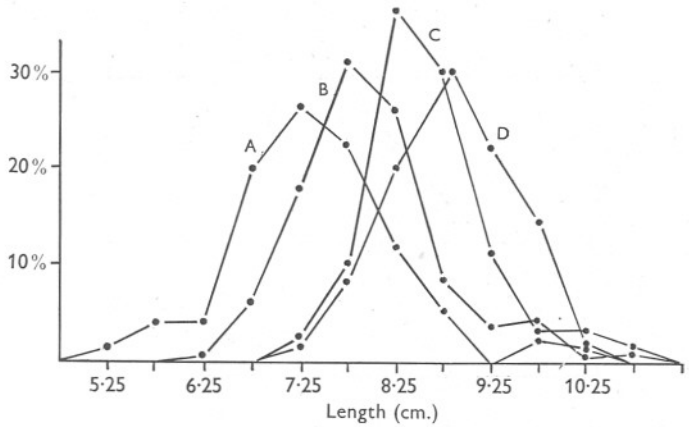


Fig. 10. *Leander serratus*. Length frequencies of the 1949 year group, July to October 1950. A, Rum Bay, July (75); B, Rum Bay, August (161); C, Rum Bay, September (59); D, average of catches from Drake's Island and River Yealm mouth, October (168).

population. The distribution curve presents a clear 'skew' pattern (Fig. 11, February), implying a selection against the smaller size groups. It is therefore likely that there is no significant difference between this group and that caught in October (Fig. 11), so that the bulk of these winter catches were probably composed of the 1948 year group. However, prawns above 11 cm. in length

were almost totally absent from Sound catches and may therefore have been older or have grown more rapidly than the inshore population.

This group also appeared during February in the hoop-net catch from Paignton, but in a rather smaller proportion, not forming a clearly defined peak.

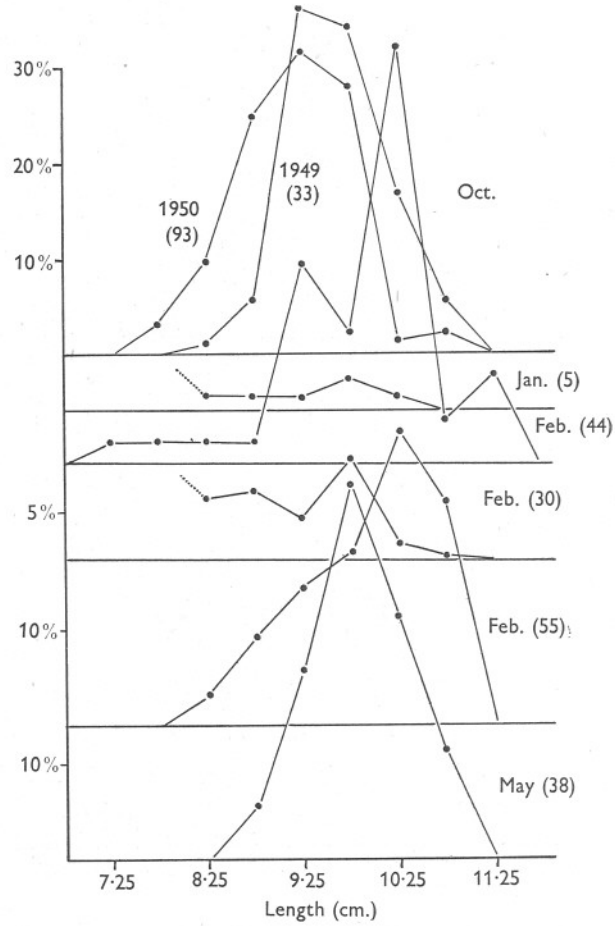


Fig. 11. *Leander serratus*. Length frequencies of 1-year-old females (see p. 345). The October and May figures are from fractions of catches scaled up to 100. October and January catches trawled in the Sound. February catches trawled in 20 fathoms (above), hoop-nets in 4 fathoms (centre) and lobster pots in 11 fathoms (below). May catch from traps inside Sound.

During spring and early summer the group shows up clearly in the trap catches (Fig. 11). There is no sign of any growth during the winter, the peak remaining at 9·75 cm. as in October. After July the trap catches declined and only occasional specimens were taken on the shore, therefore there was probably

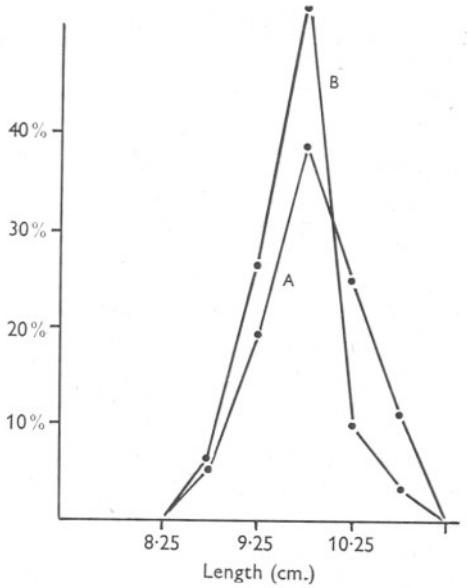


Fig. 12. *Leander serratus*. Comparison of trap catches of 1948 year group on (A) 19 and 31 May 1950 (36) and (B) 23 and 24 June 1950 (60), showing decline from 37% over 10 cm. length in May to only 10% in June.

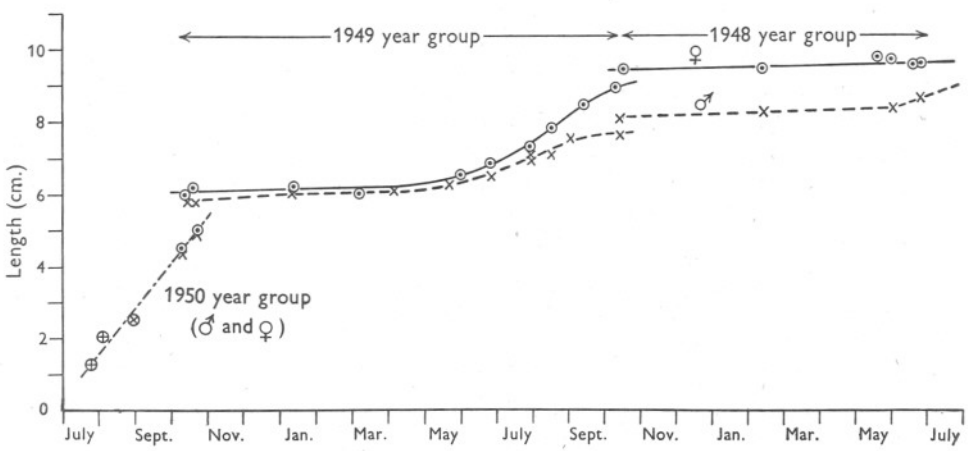


Fig. 13. Mean lengths of the various samples of three year groups, forming an approximate growth curve.

a considerable natural mortality at this time which may be related to the hatching of the last brood. Comparison of trap catches in May with those of June (Fig. 12), reveals a decline in the percentages of the two largest size groups from 37% over 10 cm. in length in May to only 10% a month later. This also appears to show a natural mortality, since there is no reason to presuppose any migration during this period.

The growth data have been summarized in Fig. 13 by plotting the mean lengths of the different year groups from October 1949 to October 1950. These results differ considerably from the conclusions on age and growth of this species reached by Nouvel and Van Rysselberge (1937), based on measurements of growth increments after moulting. At Plymouth there was little growth during the winter, though moulting continued. This may well be the case at Roscoff, since the sea-surface temperatures do not appear to differ by more than 1° C. (Lumby, 1935). Nouvel and van Rysselberge attributed the greater size attained by the females to an extra year's growth, but at least at Plymouth this is incorrect since the males' growth rate is less.

Sollaud (1916) had previously noted both the suppression of growth in the winter and the slower growth rate of the males, so that it is difficult to understand why Nouvel and van Rysselberge apparently took no account of his work. Sollaud, himself, gives a few figures for the growth of female *L. serratus* which he states live for 5 or 6 years. But unfortunately he gives no details of the data on which his conclusions were based.

Owing to variations in the distribution of the size groups no reliable estimates of mortality can be made.

Breeding

Males

Little attention was paid to breeding biology of the male prawns owing to its relative uniformity among the Natantia. Maturity is reached at an early stage, since during December a ripe female prawn in a tank with several small males in the 40–44 mm. group (i.e. 6–7 months old) became berried, showing that one of these had become capable of successful fertilization.

Females

Data on the breeding of female prawns is summarized in Fig. 14; grouping by length is justified since it has been shown that there was little growth during the winter, while towards the end of the breeding season growth shown in the graphs can only be due to those females which have hatched their eggs. In Fig. 14 the 1949 year group is divided into four 1 cm. groups up to 8·4 cm., the 1948 year group being represented by the 8·5–9·4 and 9·5–10·4 cm. groups. Only the two smallest groups show non-breeding or neuter individuals throughout the whole period. In the 4·5–5·4 cm. group only one berried female was taken: therefore those which possessed a maturing ovary probably

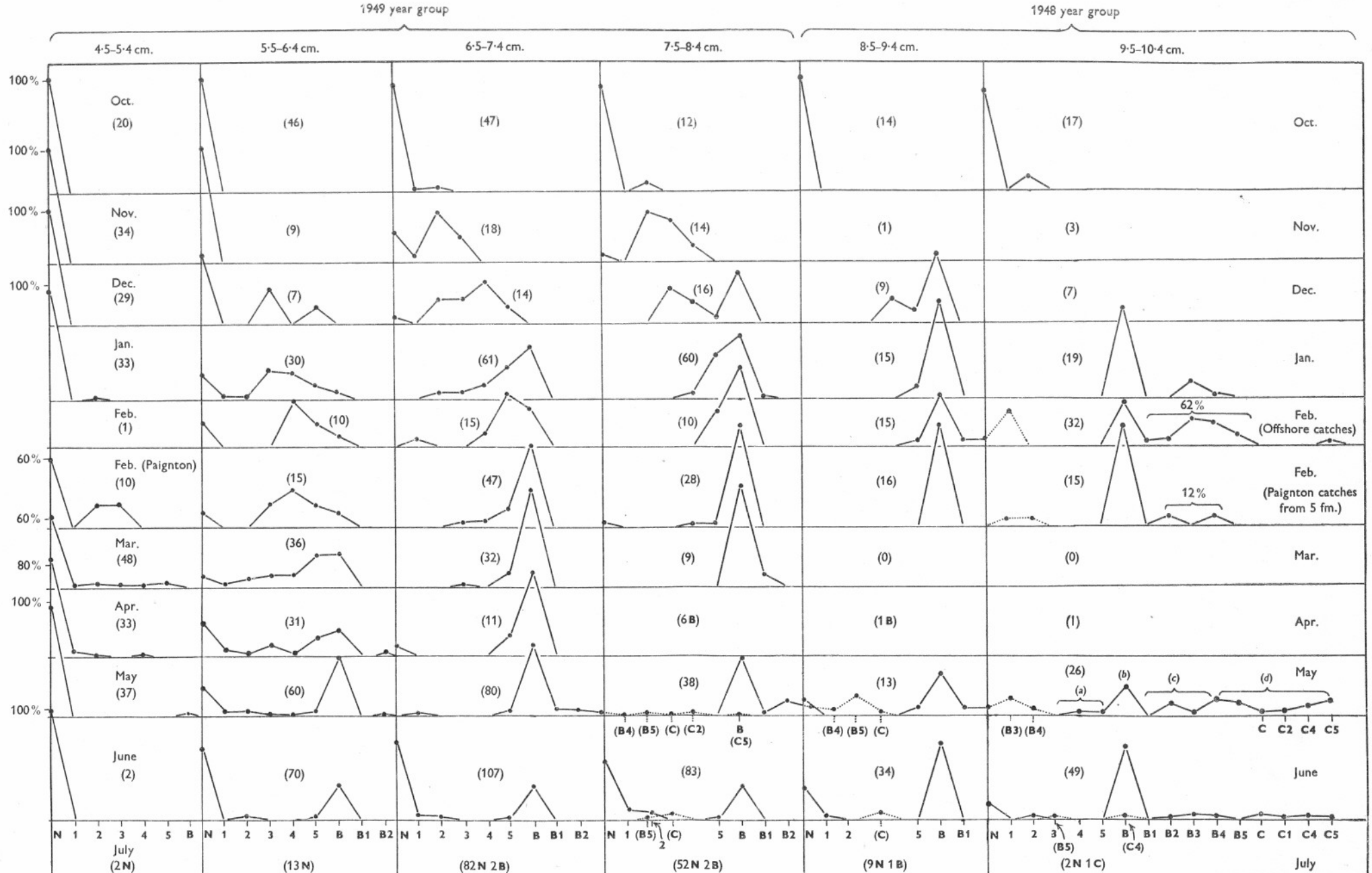


Fig. 14. *Leander serratus*. Seasonal abundance of various breeding stages through the year. **N**, neuter (i.e. ovary not externally visible); **B**, berried (egg-bearing); **C**, cemented (egg membranes attached to pleopods), this stage lasts only about 3 days after the eggs have hatched. **1–5**, arbitrary stages in maturation of the ovary: **1**, ovary small, white and translucent; **2**, ovary green, area about one-eighth of carapace; **3**, ovary green, area about one-third of carapace; **4**, ovary green, area about two-thirds of carapace; **5**, ovary ripe, filling carapace and extending into first abdominal segment. (*a*) neuter intermoult after hatching first brood; (*b*) 2nd brood; (*c*) ovary ripening for third time; (*d*) possibly very late first spawners or advanced second spawners.

grew sufficiently during March and April to be included in the next larger group. The next three sections (5·5–8·4 cm. inclusive) show that with increase in size spawning takes place earlier: February for the 5·5–6·4 cm. group; January for the 6·5–7·4 cm. group; and December for the 7·7–8·4 cm. group. Correlated with this is the increase in the proportion which produce a second brood.

The Period of Egg-Carriage

The approximate time of spawning may be deduced from Fig. 14 as the point where the majority of any one particular group change from showing ripe or ripening ovaries to the 'berried' condition. The end of the period of egg-carriage is more difficult to ascertain since, unless the ovary ripens again for a second brood, the animal may hatch its eggs and moult, remaining 'cemented' for only 2–4 days. Thus only small numbers of cemented females may be expected. Notes were kept of the number of females whose eggs were nearly hatching as shown by the transparency of the eggs, all the yolk having been used up, and by the conspicuous eyes of the embryos. During the first intermoult period after hatching the eggs, the length of the epimera or side plates of the abdomen was considerably greater than that of non-breeding females. This gave a method of separating those of the 5·5–6·4 cm. group which had just finished breeding in June, from non-breeding individuals. The period of egg-carriage for the majority of the 5·5–6·4 cm. group is thus considered to have lasted from the beginning of March till the end of June, or approximately 4 months; bearing in mind that in the spring the smaller prawns were growing and some may have been included in this group. Similarly, for the 6·5–7·4 cm. group about 85% spawned in January and February, whilst the most advanced hatched their eggs in May.

Thus for both these groups the period of egg-carriage appears to have been about 4 months. The 7·5–8·4 cm. group spawned chiefly in December and January, but in April, when some hatching might have been expected, only six specimens were taken. Thus the length of egg-carriage cannot be estimated, for by May some may have spawned a second time.

The two largest size groups comprising prawns of 8·5–9·4 and 9·5–10·4 cm. (including the few specimens which exceeded 10·4 cm.) could not, unfortunately, be caught throughout the whole season, so that results are incomplete. Spawning probably began in November, since the few specimens obtained in December were almost all berried. Information also came from lobster fishermen that they had first taken berried prawns about 7 November. Another lobster pot catch from the same area was examined on 18 February when about 3% had eggs ready to hatch, and altogether 90% showed ovaries ripening for a second brood. From the state of the eggs it seemed unlikely that much hatching would have taken place before the beginning of March. Here again the period of egg-carriage appeared to be about 4 months. Another February sample of these two size groups was obtained with hoop-nets at Paignton in

shallow water (less than 5 fathoms), only 12·1% of which contained ripening ovaries. Development appears therefore to be retarded under inshore conditions, possibly associated with slightly lower temperatures. In March and April no catches were obtained, and it was not until May that the traps supplied reasonable samples. Referring to Fig. 14, the patterns shown in May and June are rather complex, which in part may be due to a mixture of the stock after those from the deeper water had migrated inshore. The non-berried females with a ripening ovary are clearly passing through a neuter intermoult period after hatching their first brood; however, berried females up to stage 3 would be expected to be carrying their second brood of eggs, with the most advanced showing signs of a forthcoming third spawning. Since it has been shown that there was little hatching in February, the berried females in stage 5 in May and the cemented females could not be hatching their second brood and may possibly represent that part of the population which overwintered inshore lagging behind the offshore population by more than a month. It is unlikely that the difference in temperature between 5 and 15 fathoms is more than 1° C. (Dr H. W. Harvey, personal communication) which would account for a lag of only $2\frac{1}{2}$ weeks, taking μ for development as 20,000 (Needham, 1931, p. 521). Therefore other factors are probably involved. One possibility is that the maturation of the ovary is connected with the offshore movement, so that those whose ovaries are slower in development have in some way less tendency to move offshore.

During June the appearance of neuter females marked the end of spawning for 28 and 14% in the 8·5–9·4 and 9·5–10·4 cm. groups respectively. In July the trap catches declined and contained only occasional non-breeding members of these two largest groups. Possibly many died after hatching their eggs, but since Lebour (1947) found larvae in September and October in 1944, a few of these may survive, perhaps hidden away in slightly deeper water.

Sollaud (1916) stated that the females of *L. serratus* did not mature until the end of their second summer, and some not until the spring of their third year. This would imply that on the French coast this species takes very much longer to mature. But, as with his statements on the age and growth, no hint is given of the data on which he based them.

The production of eggs. Only a few egg counts were undertaken to estimate numbers of eggs produced by the largest and smallest females, since Höglund's

TABLE I. NUMBER OF EGGS PRODUCED BY SMALL AND LARGE PRAWNS

Length (cm.)	No. eggs	Length (cm.)	No. eggs
6·2	1523	9·3	3887
6·8	2106	9·6	3150
7·0	1628	10·4	3859
7·1	1989	10·5	4282

extensive egg counts on *L. squilla* have shown a logarithmic relationship between body size and number of eggs, dependent presumably on the increase in volume of the carapace.

Distribution and Migrations

The geographical range of *L. serratus* extends from Britain to the Mediterranean (Kemp, 1910), with the region of the Wash marking its northern limit.

Metamorphosis of the Larvae

No systematic collection of larvae from the plankton was attempted. Instead, watch was kept for the post-larval and young stages. Trawling with a stramin net lashed to the cod-end of a 6 ft. beam trawl worked from the *Gammarus* was carried out in the Sound at the end of May in depths of 4–5 fathoms, fairly close to the rocks. No young prawns were taken. Agassiz trawl catches during June from depths of 10–20 fathoms were also examined without success. Post-larval stages, together with late-stage larvae, were first obtained from pools at or above mid-tide level in July. Sollaud (1916) had previously noted this on the French coast. It was shown while analysing these catches (Fig. 1, C, D) that the smallest stages were virtually absent lower down on the shore at about low-water springs. Since settlement was almost certainly still going on at that time (3 August) it seemed that metamorphosis usually occurred high up in the littoral zone. At first sight it might appear that the larvae, which are fairly widely distributed throughout coastal water, since they have been recorded from near the Eddystone (Russell, 1927) where no prawns are taken by the lobster fishermen, would have to perform a definite migration to reach the shore. But the tidal streams running to and fro along the coast probably set up eddies around headlands and other obstacles sufficient to sweep many larvae inshore at one time or another. It might be possible for the larvae once in the right zone to maintain their position and so avoid being swept away before metamorphosis.

In this respect the work of Sollaud (1923) is of interest. He found, at Roscoff, that laboratory-reared larvae usually had eight stages or moults, but that larvae from townettings almost always metamorphosed at a size similar to his fifth stage. Gurney (1924) drew attention to these facts, but considered the extra stages anomalies associated with the artificial conditions. It seems, however, more reasonable to assume that this is another instance of the capacity of many larvae to postpone metamorphosis until they find suitable conditions.

Distribution of the O-Group

Autumn and winter. From July until the end of October the young O-group prawns remained abundant on the shore. Towards the end of October they

became less numerous and after the beginning of November very few could be taken in either 1949 or 1950. At Trevol pier where conditions, though estuarine, were very sheltered, the larger prawns disappeared in October, but some of those in the smallest size groups remained till the end of November.

Throughout the winter (1949–50) the males of the 1949 year group, 5–7 cm. long, formed the bulk of the trawl catches in the Sound. The best catches were obtained from 'White Patch' area, and from inside the Breakwater, in depths of 4–5 fathoms. The smaller members of this group showed up in the trawls from Mount Batten (shallow water 1–2 fathoms and rocky bottom) as a separate peak, which did not appear with the rest of the trawl catches in slightly deeper water and on softer bottoms.

Perhaps the most notable feature of the winter catches was the relative scarcity of the females. In catches from 'White Patch' and the Breakwater the sex ratio was between three and four males to one female; whereas it will be shown that during the summer when the whole population was close inshore there was a slight majority of females. Therefore it seems probable that during the winter the females, many of which were berried, tended to remain in the greater seclusion of the rocks. This view was supported by the result of the hoop-net catch at Paignton (on rocky bottom) during February when the sex ratio was approximately one male to two females. Poulsen (1946) gave a similar interpretation to the sex ratios obtained with *Nephrops norvegicus*. Conditions at Paignton differed from Plymouth in that the rocks were fringed by fairly clean sand, and sheltered from the south-west. Trawls were made close to the rocks but without taking any prawns. There was thus very little offshore migration at Paignton; the only difference between summer and winter distribution being that part of the population spread into the littoral zone in summer, but not in winter or spring.

On more exposed coasts where the rocks extend into deep water the prawns would doubtless move farther offshore; evidence for this was found only in the older year groups.

The only 1949 autumn catches where the females outnumbered the males were from the Tamar estuary, the results total 36 ♂♂ against 77 ♀♀, which, on the expectation of even numbers, would occur by chance less than one in a hundred times. However, since the males were found to penetrate well into the estuaries during the summer (1950), it appears that they tended to leave them in the autumn before the females. Lloyd & Yonge (1947) found that *Crangon vulgaris* behaved in much the same way, and showed that the males were more susceptible to combined low temperature and salinity (i.e. winter conditions) than the females. Pannikar (1941) found that in *Leander serratus* the osmotic pressure of the blood scarcely differed between males and neuter females, but did not apparently differentiate the sexes in studying the capacity for osmoregulation.

Spring and summer. During March and April the majority of the females from 6·25 to 8·25 cm. gradually disappeared from the trawl catches. Presumably they were the first to migrate inshore. Prawns were first caught with a hand-net from the shore on 3 May, and thereafter in fair quantities. Unfortunately it was not possible to correlate this with a simultaneous disappearance from the trawling grounds, but only occasional prawns were taken during several trawls in June.

In 1949 the inshore migration must have occurred much earlier, since numerous prawns were found in shore pools at Wembury at the end of March. *Aplysia punctata* was also abundant at this time, but only a very few appeared inshore during the spring of 1950. Sea-surface temperatures in the Sound were almost exactly similar for the two years, as were the air temperatures. Temperature therefore cannot have been the decisive factor in the cause of these migrations.

During early summer prawns also moved up the estuaries and by July they were being fished commercially off Saltash. Percival (1929) studied the estuarine distribution of three species of *Leander* including *L. serratus*.

At Rum Bay where most of the trap catches were made, the separation of the females into two apparently distinct populations has been noted (p. 343). For instance, of the females caught on 31 May, the hand-net took specimens almost entirely less than 6·5 cm., while the traps scarcely took any below this length (Fig. 4, May.) This segregation was scarcely noticeable among males, however, except that the hand-net took a much larger proportion of the 5·75 cm. group than the traps.

Distribution of the 1-Year-Old Group

After July the trap catches declined, suggesting that the whole population was close inshore, mostly within the 2-fathom line, and at Paignton within about 4 fathoms. No change in this distribution apparently occurs until October when, according to local tradition, trawling starts (information supplied by Mr W. Searle). However, in the Hamoaze, especially below Saltash, trawling is sometimes successful in late August. Prawn trawling usually started near Saltash and moved downstream to Torpoint, West Muds and Drake's Island, finishing in Jennycliff Bay and inside the Breakwater. Thus there would appear to be, as the fishermen believe, a migration of the prawns away from estuarine conditions; this would be expected from the well-established fact that tolerance of low salinity decreases markedly with temperature in most decapods (Lloyd & Yonge, 1947). Several trawls were made in the Hamoaze during winter with, as might be expected, negative results, but there was always the possibility that the prawns managed to hide away in places inaccessible to the trawl. Preliminary work on tagging was also undertaken to try to verify the migration, but although a method was worked out it proved impracticable to mark sufficient numbers for a reasonable chance of returns.

In the Sound, apart from the first successful trawl in October 1949 (Figs. 4 and 5, October), only very small numbers of the 1-year group (9–11 cm.) were taken throughout the winter. They reappeared, however, in the hoop-net catch from Paignton and the trap catches during the spring, when both sexes seemed to be restricted to a rocky bottom.

During the winter prawns were occasionally caught by *Sula* and *Sabella* on trawling grounds outside the Sound both with the otter trawl and the finer-meshed Agassiz trawl. During February, however, larger catches of up to fifty were made, chiefly after gales. These trawl catches from depths around 20 fathoms, which consisted almost entirely of large female prawns, have been discussed on p. 345. It is unlikely that they represented more than scattered offshoots from the main population. Quite large quantities of prawns were caught by lobster fishermen on various grounds between Berry Head and Start Point from November till the end of March, at a depth of about 10 fathoms. Since these grounds were fished almost all the year round and no prawns were caught during the summer, there must have been an offshore winter migration. The distance between the 5- and 10-fathom lines is about half a mile in this area. The population as sampled by the lobster pots appeared to be wholly female. Out of a catch of some 200 prawns observed during an expedition with the fishermen, all were females. Several pots were partially covered with prawn netting so that if only small males had been present, some at least would have been retained. The disappearance and presumed inshore migration towards the end of March may therefore be related to the hatching of the first brood of eggs, mating, and the second spawning. The basis of the orientation necessary for even a short migration remains inexplicable.

During their third summer this group was well represented in the trap catches in the Sound until July (Fig. 4). Afterwards the catches declined and there was probably a high mortality after the second brood had been hatched.

Food

A note of the stomach contents of *Leander serratus* has been given by Hunt (1925). With further study it was hoped to ascertain how the food varies at different times and in different places. Also it was of interest to see whether the feeding habits differed from those of *L. squilla*, since these two species overlap in their distribution on the shore.

The stomachs were removed within a day or so of collection and preserved in formalin. Most of the contents were lost if the prawns were placed in formalin while still alive. It was usually found possible to distinguish the more freshly filled stomachs from those in which the food had been subjected to a period of grinding or churning, since the latter tended to appear, under a binocular microscope, as a homogeneous mass giving off clouds of minute

particles. Any of this type were discarded. The contents were first examined with a low-power binocular and the larger fragments or animals noted, the remainder being usually subsampled by pipetting a drop of the material on to a slide and examining under higher magnification.

Notes on the Stomach Contents

Debris. This category includes all unidentifiable finely divided material. The name is used in place of the more usual 'detritus', since it was not known whether most of the material was organic or inorganic. It was not always possible to decide whether the debris had been ingested as such or ground up in the stomach. Much churning and grinding does, in fact, take place and may be observed most readily in the smaller and more transparent prawns. Debris was more abundant in the stomach contents of trawl catches from the muddy grounds of the Sound than from elsewhere. Since it was found from aquarium observations that a muddy substratum was not ingested at random, some at least of the debris may well have been eaten accidentally with, say, algal fragments.

Algae. In all inshore catches algae usually formed a considerable part of the stomach contents. During autumn and winter the algal material found in the stomachs of trawl-caught prawns consisted chiefly of unpigmented fragments of thalloid algae which were probably derived from decaying *Laminaria* plants. In hand-net catches, green, thalloid and filamentous fragments were common, also occasionally brown filaments. Hoop-net and trap catches from slightly deeper water showed, as might be expected, more red forms, chiefly filamentous.

Crustaceans. Small crustaceans nearly always formed a significant proportion of the diet. Indeed the stomach contents of one off-shore trawl catch consisted almost wholly of small decapods, while the young O-group prawns very commonly fed on ostracods. Fragments of cuticle were very often found, and when ground up may have formed part of the 'debris'. It was often difficult to distinguish whether cuticle fragments of, say, a small amphipod represented parts of a 'moult' or the remains of a whole animal. When any trace of fleshy material was observed it was assumed that the whole animal had been consumed. Copepods proved particularly difficult in this respect, so that records of them may be unreliable.

Molluscs. Only one species of mollusc appeared in the stomach contents more than occasionally; this was *Odostomia plicata*, which Fretter & Graham (1949) have shown to be probably ectoparasitic on *Pomatoceros*. These tiny gastropods were found chiefly in prawns trawled from near the Hoe. It would seem that they must be fairly common judging from the fact that one large prawn had thirty-one in its stomach. There was a surprising absence of other small gastropods which are usually abundant on algae during the summer.

Other groups. Foraminifera occurred fairly often in the stomach contents

but rarely in significant numbers. Small polychaetes were also found occasionally, rather less frequently than might be expected. Unidentified eggs and algal spores sometimes appeared, often in considerable numbers in just one stomach out of a sample. Doubtless almost any post-larval or young stages under 2 mm. would be taken when available.

TABLE II. THE STOMACH CONTENTS OF *LEANDER SQUILLA* AND O-GROUP *L. SERRATUS* TAKEN FROM THE SAME POOL

(+ + +, 75–100 %; + +, 40–75 %; +, 40 % (all very approximate); tr., very little.)

No.	Debris	Filamentous algae	Thalloid algae	Grit	Cuticle fragments	Foraminifera	Copepods	Ostracods	Mites	Miscellaneous
						L. squilla				
1	+	+ +	..	+ +	..	13	..	..	..	..
2	+	Brown and red	..	..	+	2	..	..	..	..
3	+ + +	+	..	..	..	7		..	..	..
4	+ + +	+	..	+	..	7	..	..	..	..
5	97%	..	..	..	..	1	..	..	..	1 small gastropod
6	85%	+	..	..	+	2	..	..	..	..
7	10%	Few red fils	90% green	..	..	..	..	..	..	..
8	+	+ +	..	tr.	..	..	..	..	..	..
9	80%	+	..	..	+	..	..	..	..	..
10	90%	..	Green	tr.	..	..	..	..	..	..
11	..	..	..	..	..	..	..	..	..	Many? nematode cuticles
12	..	..	..	..	..	..	..	..	..	..
13	tr.	tr.	90% green	..	..	..	..	..	..	..
14	..	+ +	+ +	+	..	..	..	..	..	..
15	..	..	..	..	..	1	Several	..	1	1 chironomid cuticle 1 gastropod
16	+ +	+	..	..	..	..	1 moult	..	..	1 amphipod moult
						L. serratus				
1	tr.	..	..	..	..	..	4 moults	1	1	1 chironomid cuticle, 1 gastropod
2	75%	tr.	..	..	+	..	..	1	1	..
3	80%	..	..	..	+ +	..	..	1	1	1 chironomid cuticle, 1 amphipod head
4	50%	..	..	+	30%	..	2 moults	..	1	..
5	..	..	..	..	+	..	2	1	..	1 amphipod moult
6	tr.	..	..	..	..	..	1 moult	3	..	..
7	+	..	..	..	+	..	12 copepodites	..	..	..
8	tr.	..	..	..	+ +	..	..	4	..	..
9	75%	..	..	tr.	..	..	..	4	..	..
10	85%	..	..	..	..	..	3 moults	1	..	..
11	tr.	..	..	..	+ +	..	..	2	1 2 moults	..
12	+	..	..	..	..	..	..	2	1	1 chironomid cuticle
13	+	..	..	..	..	..	3 moults 1 copepodite	.. 1	..	..
14	..	..	..	..	..	..	..	1	..	..
15	85%	tr.	..	+	..	..	..	4	..	..
16	+	+	..	..	+	..	4 moults	9	..	Pt: of 1 isopod, 1 gastropod, 1 chironomid cuticle
17	70%	..	..	..	+	..	..	2	..	..

The food of the O-group. The food of young prawns less than 30 mm. in length differed rather strikingly from that of larger specimens, notably in the absence of algae and presence of large numbers of ostracods. The exact reverse was found with a sample of *Leander squilla* taken from the same pool (Table II) which fed chiefly on algae, with some foraminifera but no crustaceans. Both species took a fair amount of debris, of which there was ample.

Otherwise there was a clear divergence of feeding habits as would be expected from the theory of closely related species occupying the same habitat.

The stomach contents of about 140 *L. serratus* have been analysed, and show that the food varies from place to place and throughout the year. In general, the species is omnivorous. Algae and small crustaceans appear to form a large proportion of the diet. Much debris was also found in the stomach contents, but there is no reason to consider it of more than slight food value. A great deal more must be known of the digestive powers of prawns and of the composition of the food organisms before definite conclusions can be drawn. It is also impossible to ascertain whether the animals found in the stomach have been captured dead or alive, but since observations have shown that prawns can catch small amphipods and other crustaceans, it may be that their habits are not so much scavenging as is commonly supposed.

Notes on Behaviour

Tidal and diurnal migrations at Rum Bay have been observed. On this shore, which may be taken to represent any fairly sheltered rocky coast, prawns are first seen 2 or 3 hr. before low water walking or swimming over the algae moving out with the tide. Where the shore has been eroded into tiny bays and gulleys they often form a steady procession. The larger prawns usually leave first, keeping in 3 or 4 ft. of water, and creeping under weed or rocky ledges where possible and thus are less easily observed. Last to leave are the baby O-group prawns usually so numerous as to form a steady stream swimming vigorously only a foot or so below the surface. Three-quarters or half an hour before low tide the migration has ceased and most of the prawns are hidden under *Laminaria* fronds or congregated under ledges or in tunnels, anywhere sheltered from light. Very soon after the tide turns the O-group begin to move in with it, but the larger prawns do not usually return until the water is 4 or 6 ft. deep.

During the night there is a migration up the shore, since prawns may be observed with a torch right up in the barnacle zone where they normally never appear by day. The eyes show up very brightly by torchlight, glowing with a fascinating golden iridescence, as was noted almost 100 years ago by Warrington (1855). This is well known to many fishermen since they may be caught readily at neap tides in this way.

Prawns in the deeper part of the *Laminaria* zone can be caught only in hoop-nets or traps after dark, the first being taken usually just after dark. This is a marked difference in behaviour from those on the shore which are definitely attracted to a piece of fish bait in the daytime. It is probable that those in the deeper water remain hidden away during the hours of daylight, which may be related to the presence of predators such as bass, pollack and cuttlefish in this zone.

In spite of their prominent eyes repeated observations show that prawns appear to make little use of them. The antennal signal reaction to a moving object is occasionally shown, but normally there is only a slight twitch or wave of the antennae without relation to the direction of the object. A sudden movement or change in light intensity will often cause the escape reaction, i.e. a series of vigorous backward flaps of the abdomen.

Food seems to be located first by scent and then by the antennae, since a prawn may even walk right over a piece of food if the antennae have failed to touch it.

Mating also appears to be a response to chemical stimuli, associated with the soft cuticle of a recently moulted female with a ripe ovary, since the males are only attracted to females in this state (Höglund, 1943; Burkenroad, 1947). If an 'attractive' female is placed in a tank with males nothing happens until the antenna of a male touches some part of the female. At once the male's behaviour alters, he swims very rapidly in no particular direction, often making small circles, until he again makes contact even though the female had been only a few centimetres away. As this was frequently observed, it seems that the male can have no visual perception of the female.

Summary

An attempt has been made to follow the post-larval life history of *Leander serratus* through 1 year.

Growth has been followed from length measurements of numerous samples of populations obtained by several different methods. Owing to the length of the breeding season and especially to the differing habitat preferences of the various size groups in the littoral and sublittoral zones, estimates of their rate of growth are necessarily approximate.

After metamorphosing, apparently in very shallow water, during July the O-group grew rapidly, their average length by October being in the region of 5 cm. There was a marked difference in length between the October catches of 1949 and 1950, and reasons have been given for correlating this with the differing temperatures of these 2 years. Growth almost ceased during the winter, and not until June was there a marked change in the size frequencies. In their second summer's growth females outstripped the males, the mean lengths in October being approximately 9 and 7·5 cm. respectively. There appeared to be a very high mortality in the third summer, with scarcely any surviving to the autumn. However, occasional extra large prawns were trawled during the winter so that a definite conclusion on the limit of age is impossible.

The breeding season, judged by the presence of berried females, lasted from November until June (1949–50). Maturity in the smallest males of the O-group was reached at least in December and with the smallest females in March.

With increase in size spawning took place earlier and the numbers which spawned twice increased. Some of the largest prawns may have produced a third brood. The period of egg-carriage was about 4 months at roughly 9–11° C.

The distribution in winter varied according to the habitat. There was a tendency to leave the estuaries. In the Sound the male prawns were widely distributed in depths of 4-5 fathoms on a muddy bottom, while the females, judging from their scarcity in the trawl catches, were more restricted to the rocky parts. The spring inshore movement took place during April and May, with some variation between different years, apparently unrelated to temperature. During early summer, trap catches showed a marked discontinuity in the distribution of the size groups, the larger prawns being taken only in the sublittoral zone. On exposed rocky coasts prawns overwintering the second time moved into deeper water, from 10 to 15 fathoms, and have sometimes been of value to lobster fishermen.

Examination of the stomach contents, though often difficult, showed that the species was in general omnivorous. Algae and small Crustacea appeared to be the chief sources of food. Much debris was also taken; this is tentatively considered of lesser importance.

Small-scale tidal and nocturnal migrations have been observed. The results of catches with baited traps show that prawns in the sublittoral zone tend to be markedly nocturnal in their habits, compared to those observed on the shore.

From laboratory and field observations the eyes are thought to be of much less use than their size would suggest. Prawns may perceive sudden movements visually, but they cannot apparently avoid obstacles, locate pieces of food, or in case of the males find a mate, without the use of their antennae.

REFERENCES

BURKENROAD, M. D., 1947. Reproduction activities of decapod Crustacea. *Amer. Nat.*, Vol. 131, pp. 392–8.

FRETTER, V. & GRAHAM, A., 1949. Structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 493–533.

GURNEY, R., 1923. Some notes on *Leander longirostris* M. Edwards, and other British prawns. *Proc. Zool. Soc. Lond.*, Vol. 1, pp. 97–123.

—— 1924. The larval development of some British prawns. II. *Leander longirostris* and *L. squilla*. *Proc. Zool. Soc. Lond.*, Vol. 2, pp. 961–82.

HÖGLUND, H., 1943. On the biology and larval development of *Leander squilla* (L.) forma *typica* de Man. *Svenska Hydrograph. Biol. Komm. Skr.* Ny serie: Biol. Bd. 2, pp. 1–43.

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *Journ. Mar. Biol. Assoc.*, Vol. 13, pp. 560–98.

KEMP, S., 1910. The Decapoda Natantia of the coasts of Ireland. *Dept. of Agriculture and Technical Instruction of Ireland. Fisheries Branch. Sci. Inv.* 1908, pp. 1–190.

LEBOUR, M. V., 1947. Notes on the inshore plankton of Plymouth. *Journ. Mar. Biol. Assoc.*, Vol. 26, pp. 527–47.

LLOYD, A. J. & YONGE, C. M., 1947. The biology of *Crangon vulgaris* in the Bristol Channel and Severn Estuary. *Journ. Mar. Biol. Assoc.*, Vol. 26, pp. 626–61.

LUMBY, J. R., 1935. Salinities and temperatures of the English Channel. *Fish. Invest.*, Series II, Vol. 14, No. 3.

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

NEEDHAM, J., 1931. *Chemical Embryology*, Vol. 1. Cambridge University Press.

NOUVEL, H. & VAN RYSSELBERGE, L., 1937. Contribution à l'étude de la mue, de la croissance, et de la régéneration chez les crustacés natantia. *Rec. Inst. Zool. Torley-Rousseau*, T. 6, pp. 9–161.

PANNIKAR, N. K., 1941. Osmoregulation in some palaemonid prawns. *Journ. Mar. Biol. Assoc.*, Vol. 25, pp. 317–55.

PERCIVAL, E., 1929. A report on the fauna of the River Tamar and the River Lynher estuaries. *Journ. Mar. Biol. Assoc.*, Vol. 16, pp. 81–108.

POULSEN, E. M., 1946. Investigations on the Danish fishery for, and the biology of, the Norway lobster and the deep-sea prawn. *Rep. Danish Biol. Stat.*, Vol. 48, pp. 29–47.

RUSSELL, F. S., 1927. The vertical distribution of marine macroplankton. V. The distribution of animals caught in the ring-trawl in daytime in the Plymouth area. *Journ. Mar. Biol. Assoc.*, Vol. 14, pp. 557–608.

SOLLAUD, E., 1916. Recherches sur la bionomie des 'Palémonides' des côtes de France. *Rec. Fonds. Bonaparte*, T. 1, pp. 69–71.

—— 1923. Le développement larvaire des 'Palaemoninae'. *Bull. Biol. France et Belg.*, T. 57, pp. 510–602.

WARRINGTON, R., 1855. Observations on the natural history and habits of the common prawn. *Ann. Mag. Nat. Hist.* Ser. 2, Vol. 15, pp. 247–52.

NOTES ON *LEANDER SQUILLA* L.

By G. R. Forster, B.Sc.

From the Plymouth Laboratory

(Text-figs. 1–5)

After the monograph on *Leander squilla* by Höglund (1943) in Sweden, any further work might seem repetitive; however, at Plymouth some differences in the biology of this species have been noticed, as might be expected from the different climates. It has been thought worth while to record these additional notes on the age and growth, together with records of the food.

De Man (1915) has separated the Scandinavian population as the forma *typica* from those of Holland, France and Britain, which he grouped into the variety *intermedia*, the only distinctive character being the length of the carpus of the second leg relative (either longer or shorter) to that of the chela. It is not known whether the two forms intergrade.

Habitat

L. squilla is widely distributed in Plymouth Sound and in the river estuaries, being most abundant in the upper shore pools. Around harbour walls and pier piles where it occurs with *L. serratus* there is usually a segregation, *L. squilla* keeping in the shallower water. This was noted many years ago by Sinel (1906) at Jersey. It also appears to be less tolerant of wave action than *L. serratus*, being rare in more exposed localities. During the winter *L. squilla* tends to be scarce in the estuaries but remains in the shore pools and is scarcely ever taken with the prawn trawl. This contrasts with its behaviour in the Clyde (Elmhirst, 1921) and in Sweden (Höglund, 1943) where there is an offshore winter migration; and is presumably related to the warmer temperatures prevailing at Plymouth. (Mean sea surface temperatures in February: Plymouth 7 to 8° C., Millport 6° C., Kristineberg −1 to +1° C.)

Age and Growth

Spawning took place towards the end of April in 1950, and continued well into August, for of the females caught on 16 August 57% were berried. Since the period of egg-carriage would not be more than 6 weeks (data from Höglund, 1943), it is possible that a third brood may be produced. The post-larval stages first appeared on the shore during the second week of August, a month later than those of *L. serratus*. By October the mean lengths for the O-group

were: 1949, males 2·54 cm., females 2·95 cm.; and in 1950, males 1·85 cm., females 2·12 cm. Thus there is a clear divergence between the 1949 and 1950 year groups which, as with *L. serratus* (see Forster, 1951), may be ascribed to the difference in temperature conditions between the two years. As a check, from these figures the temperature characteristic μ can be calculated as shown by Needham (1931). Using the average sea temperatures 17·27° C. in 1949 and 15·57° C. in 1950, values of 34,140 (females) and 31,320 (males) for μ were obtained, instead of 17,000–21,000 as would be expected from the large numbers of already known values of μ for growth processes. However, it must

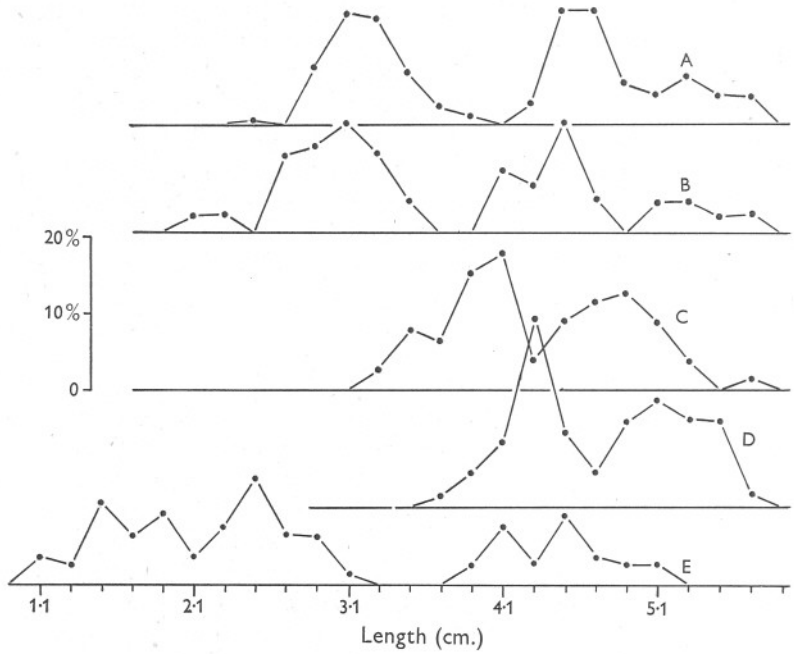


Fig. 1. Length frequencies of female *L. squilla*. A, Trevol, October 1949 (178); B, Rum Bay, October 1949 (50); C, Trevol, April 1950 (79); D, Trevol, August 1950 (73); E, Rum Bay, October 1950 (81).

be remembered that these young *L. squilla* are found in upper shore pools, and at high tide may move into shallow water so that they are affected to a considerable extent by air temperatures. On Plymouth Hoe (height 108 ft.), average air temperature from August to October (inclusive) was found to be 16·50° C. in 1949 and 13·76° C. in 1950. Using these figures μ comes to 20,020 for females and 19,160 for the males. It seems therefore that in the shallow-water conditions under which this species lives the effective temperatures are those of the air rather than of the sea surface.

Figs. 1 and 2 show the percentage size frequencies of various samples of

L. squilla. The samples from Trevol Pier, situated in a creek off the Tamar estuary, differ considerably from those from Rum Bay, in the Sound (Table I).

In 1949 the O-group males, unlike the females, did not penetrate the estuary, being virtually absent from the Trevol catches. This compares with the

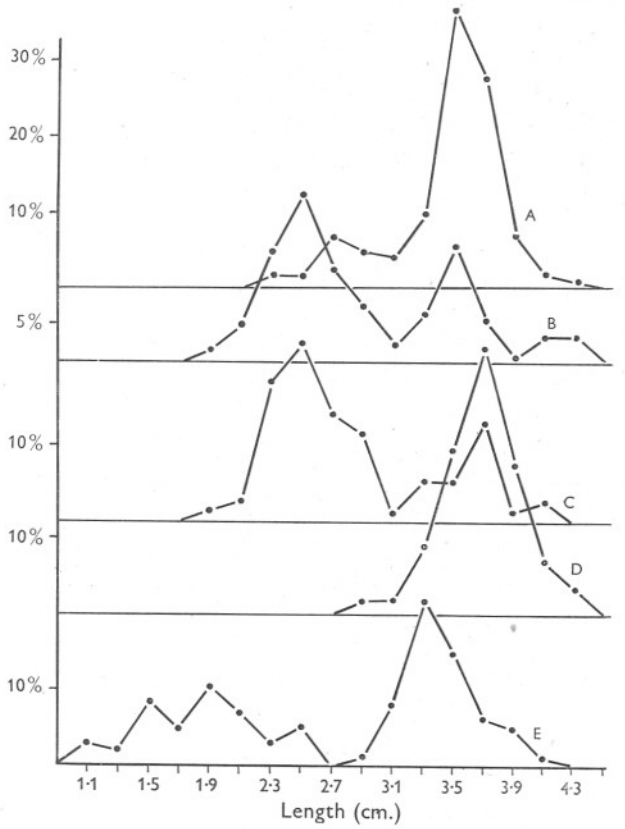


Fig. 2. Length frequencies of male *L. squilla*. A, Trevol, October 1949 (133); B, Rum Bay, October 1949 (123); C, Rum Bay, November 1949 (77); D, Trevol, April 1950 (56); E, Rum Bay, October 1950 (102).

TABLE I. SEX RATIO: PERCENTAGE OF MALES

Trevol Pier	41	13	43	48	—
Rum Bay	63	64	58	73	51

observations of Lloyd & Yonge (1945) on *Crangon vulgaris*, who found that the males tended to inhabit more saline conditions than the females. In an estuary, where the surface water may at times be of low salinity, the males might well keep in slightly deeper water and so affect the sex ratios of samples taken with a hand-net. The sex ratios found in the Gullmar Fjord by Höglund have been

plotted in Fig. 3 as the percentage of males. Although he has attributed the fluctuations solely to chance, it may be seen that this is very unlikely owing to the extent of the fluctuations and the manner in which the points reinforce each other. The two years differ considerably; in 1940 there appears to be a steady decline in the percentage of males, whereas in 1941 there was a more rapid decline partly due to the 1939-year-group males disappearing, followed by further large fluctuations. In both years the females of the O-group appeared at first in much greater numbers than the males. It would have been interesting to compare the rainfall of the two years to see whether any correlation with the sex ratio could be traced, but no data are available.

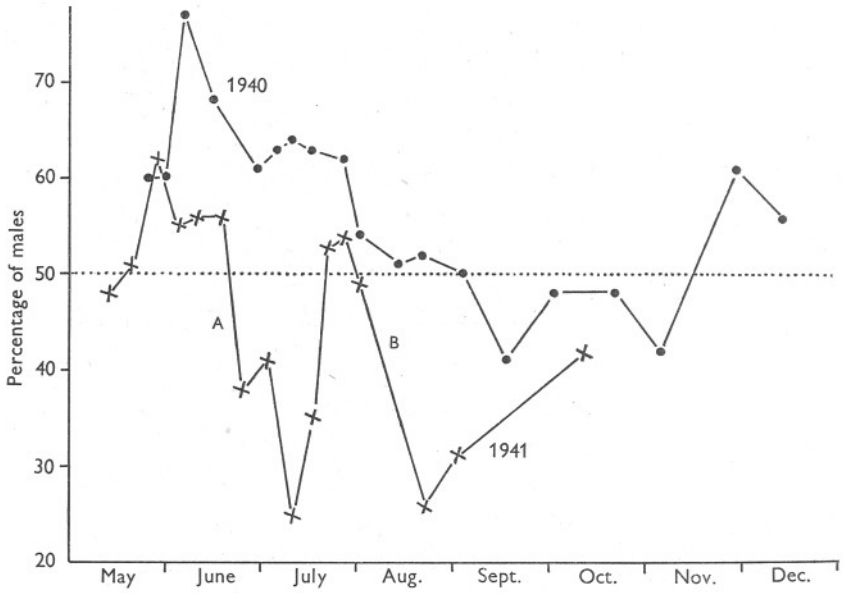


Fig. 3. Percentage of males in catches from Sweden (data from Höglund, 1943). A, 1-year-old males disappearing; B, 'O-group' females arriving.

Variation in the stock from place to place was apparent from a sample from Paignton harbour taken on 21 November which in both males and females showed one very dominant group, presumably the O-group, but with a mean length approximately 4 mm. greater than that of a comparable catch from Trevol Pier (Fig. 5). Whether this represented a difference in growth rate or in the method of sampling is unknown.

The growth of females is shown in Fig. 4, together with Höglund's data on age and growth. Growth is seen to continue throughout the year at Plymouth in contrast with the complete cessation found by Höglund. This appears to be correlated with the temperature of the sea which is much lower in winter and higher in the summer in Sweden. Also the 2-year-olds appear to have grown

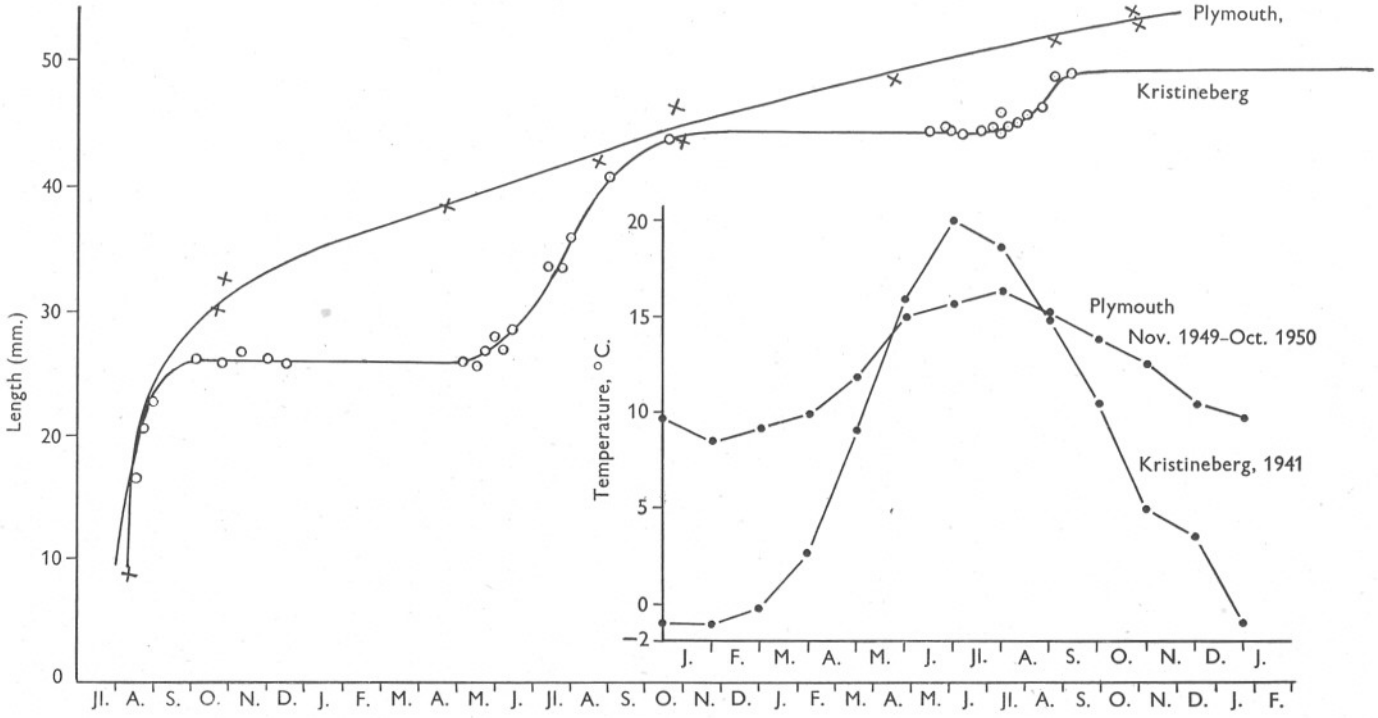


Fig. 4. Age and growth curves of *L. squilla* at Plymouth and in Sweden, together with sea-surface temperatures. Swedish data from Höglund (1943).

somewhat larger at Plymouth. As with *Leander serratus*, both males and females do not appear to survive the third winter, while in 1950, even by the end of October, they had almost disappeared from the catch.

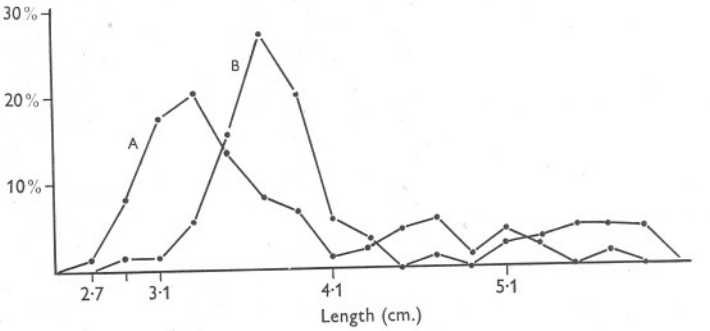


Fig. 5. Comparison of catches from: A, Trevol, 11 November (79); B, Paignton, 21 November (70).

Food

A table of the stomach contents of *L. squilla* appears in the paper on *L. serratus* (Forster, 1951, p. 356). Like *L. serratus* the species is also omnivorous, though in the summer, as the table shows, it seems to select a considerable amount of filamentous algae, especially *Pylaiella littoralis* (kindly identified by Dr M. Parke). In the autumn, however, many crustaceans were also sometimes taken.

Although they did not appear in the stomachs examined, it seems probable that both cypris and nauplius barnacle larvae might commonly be taken in the spring. On one occasion a number of *Leander squilla* in a tank were observed behaving in a peculiar fashion, swimming up to the surface and hovering upside down underneath the surface film. Closer examination showed that they were feeding vigorously on several small swarms of nauplii which had originated from a few large specimens of *Balanus porcatus* attached to a stone on the tank.

Summary

The habitat preferences of *Leander squilla* in the Plymouth area are briefly discussed. Length measurements were taken over a period of 12 months to compare the growth and breeding biology in this area with Sweden.

The 1949 year group grew to a considerably larger average size in October than did the 1950 year group in the corresponding period. The average sea surface temperature was approximately 1·7° C. higher in 1949 and the air temperature about 2·74° C. higher. A more reasonable growth characteristic

was derived by using the mean temperature of the air rather than that of the sea surface, a result consistent with the fact that the species inhabits the upper shore pools.

The O-group males did not move up the estuaries so that the percentage of males was always higher in the Sound. This difference in behaviour, well known in *Crangon vulgaris*, is discussed in relation to the sex ratios found by Höglund (1943).

In contrast with Sweden, growth continued during the winter at Plymouth, and the maximum length attained was slightly higher.

The stomach contents showed a varied diet but with a predominance of filamentous algae.

REFERENCES

ELMHIRST, R., 1921. Faunistic notes and new records. *Ann. Rept. Scottish Mar. Biol. Assoc.*, 1921, pp. 9–13.

FORSTER, G. R., 1951. The biology of the common prawn *Leander serratus* Pennant. *Journ. Mar. Biol. Assoc.*, Vol. 30, pp. 333–60.

HÖGLUND, H., 1943. On the biology and larval development of *Leander squilla* (L.) forma *typica* de Man. *Svenska Hydrograph. Biol. Komm. Skr.*, N.S. (Biol.), Bd. 2, pp. 1–43.

LLOYD, A. J. & YONGE, C. M., 1947. The biology of *Crangon vulgaris* in the Bristol Channel and Severn Estuary. *Journ. Mar. Biol. Assoc.*, Vol. 26, pp. 626–61.

MAN, J. G. DE, 1915. On some European species of the genus *Leander* Desm., also a contribution to the fauna of Dutch waters. *Tijdschr. Ned. Dierkund. Ver.*, Ser. 2, Deel 14, Afl. 2, pp. 115–79.

NEEDHAM, J., 1931. *Chemical Embryology*, Vol. 1. Cambridge University Press.

SINEL, J., 1906. *An Outline of the Natural History of Our Shores*. 344 pp. London.

OBSERVATIONS ON *NEPHROPS NORVEGICUS* (L.) AND ON AN EPIZOIC POPULATION OF *BALANUS CRENATUS* BRUG.

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(Text-figs. 1–4)

The Dublin Prawn or Norway Lobster, *Nephrops norvegicus* (L.), is widely distributed on soft muddy bottoms, usually between 10 and 50 fathoms. It is found as far north as Iceland and the North Cape, is common in the North Sea and off the Atlantic shores of the British Isles, and extends as far south as the coast of Morocco; a variety, v. *meridionalis* (Zariquiey-Cenarro, 1935) is found in the Mediterranean and Adriatic (see Havinga, 1929, and Heldt & Heldt, 1931, for details of its distribution). Some aspects of the general biology of *Nephrops* have been dealt with by Höglund (1942) and Poulsen (1946) for Scandinavian waters, and by McIntosh (1904, 1908) and Storrow (1912) for the waters off north-east England. To a large extent all these workers relied on market catches.

In the Clyde Area, where *Nephrops* is found in very considerable quantities at certain times of the year, a number of collections has been made, and information concerning the size, sex and presence of females in berry has been obtained. In addition, having repeatedly observed the presence of epizoic barnacles (*Balanus crenatus* Brug.) on many of the prawns, it was thought that observations on these might help, not only to interpret the prawn data, particularly with regard to the time and frequency of moulting, but in addition might give some information on the growth of barnacles under these rather unusual conditions.

The Methods and Material

The hauls were taken with a V.D. trawl (see Barnes & Bagenal, 1951, for details) on grounds off the Island of Bute and off Knock Castle (full Kilometre National Grid References 26/1159–1162 and 26/1763–1767). The first collection (June 1950) was taken from the former ground, and the later ones (July, August, October 1950 and January 1951) from the latter ground at a depth of 20 fathoms, where the prawn was found to be more plentiful. Supplementary hauls were taken in these and other places and will be referred to where appropriate.

All the prawns were measured from the point of the rostrum to the telson, to the nearest half centimetre below, and the sex and presence of berried

females noted. At the same time, the small numbers of barnacles on telson, abdomen and cephalothorax were noted. The chelipeds which bore most of the epizoic barnacles were then removed and all the barnacles measured (carino-rostral length, using an eyepiece micrometer) and their distribution on the various parts of the chelipeds, namely meropodite, carpopodite, propodite and dactylopodite together, noted. In all, some 1200 *Nephrops* and 8000 barnacles have been examined and measured.

The Growth Rate of *Balanus crenatus* on *Nephrops norvegicus*

In the Firth of Clyde *Balanus crenatus* liberates nauplii throughout most of the year except for the winter months, but the major liberation and subsequent settlement takes place in the spring followed by a moderate liberation and settlement in August (Pyefinch, 1948).

Table I. The Estimated Mean Sizes (mm.) of *Balanus crenatus* Populations on *Nephrops* Obtained from Size-Frequencies and Analyses by Cumulative Percentages: Each Size-Group of *Nephrops* Analysed Separately and the Range of Means Given.

	Population I		Population II		Population III		Population IV	
Collection	Range of means	Grand mean	Range of means	Grand mean	Range of means	Grand mean	Range of means	Grand mean
June 1950	1·3–1·6	1·5	4·0–4·3	4·1	8·1*	8·1	—	—
July 1950	1·5–2·1	1·8	4·5–4·9	4·7	8·5*	8·5	—	—
August 1950	1·7–3·2	2·5	3·4–5·7	4·9	—	—	—	—
October 1950	4·2–5·1	4·4	—	—	—	—	0·5–1·4	0·7
January 1951	4·4*	4·4	—	—	—	—	1·8*	1·8

* Insufficient numbers to consider size-groups separately.

Size-frequency curves for the barnacles on each centimetre size-group of *Nephrops* have been drawn and analysed, and it has been found that in each collection of *Nephrops* similar barnacle populations were present irrespective of the size-group of the prawn (see later for exceptions and further discussion). In considering the barnacle populations all those collected on a given date are therefore considered together. The range of the estimated mean size from the various *Nephrops* size-groups and the estimated grand mean for these barnacle populations are shown in Table I. They are also plotted in Fig. 1, from which the following construction regarding the settlement and growth of these barnacles is put forward.

During the sampling period, from June to January, four populations (I–IV) of barnacles were found. The large individuals (mean 8·1 mm.) of population III were represented only in the June and July samples and then only as a very small fraction (*c.* 10%) of the whole barnacle population; population IV (small individuals, mean 0·7 mm.) was present only in the October

and January samples and in the latter as a moderate proportion (*c.* 20%) of the whole. It seems clear from Fig. 1 that the growth-curve of population I is that of the spring settlement 1950, since extrapolation indicates its origin during the heavy April settlement. The growth-curve of population IV would seem to correspond to the moderate August settlement noted by Pyefinch (1948). The mean growth-rate in population I was uniform throughout the summer but fell off during the winter (see Pyefinch), whereas in population IV little growth took place before the winter and there was only a small increase in size between October and January. Allowing for this reduced growth rate during the winter and plotting the values as a hypothetical second

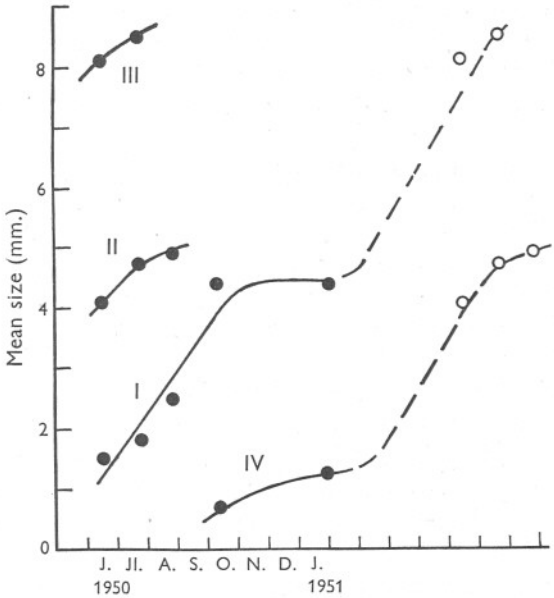

Fig. 1. The mean size of the *Balanus crenatus* populations. I, spat of spring 1950; II, spat of autumn 1949; III, spat of spring 1949; IV, spat of autumn 1950. ——, present year (1950). – – – – –, next year (hypothetical).

year, with 'normal' growth rate established for populations I and IV in the following spring, it seems clear that the spring population of one year gives rise to the third (III) population observed in the spring of the next year; thus the extended curve I is equivalent to two seasons' growth of a spring settlement, so that population III, observed in June and July 1950, is the spring settlement of 1949. The autumn settlement becomes the medium-sized population II (extended curve IV) during the year after settlement; it is smaller since it has had only one period of maximum growth-rate, namely during the spring of its second year. The growth-curve of population IV thus gives rise in the second year to the curve of population II. To these is added the spring settlement in the second year.

On this hypothesis the growth-rates of the spring settlement during the summer months of its first year and also of the settlement of the previous autumn in its second year are very similar, about 2 mm. in 4 months. This is in marked contrast with the growth-rate of *Balanus crenatus* on raft-exposed panels, for which Pyefinch gives up to 0·2 mm. per *day*: it is more comparable with the value given by Topsent (1911) of 5–6 mm. in $3\frac{1}{2}$ months for *B. crenatus* growing on the shore. Other observations have confirmed the high growth-rate on raft-exposed panels, and it would seem that the extremely slow growth-rate of the barnacles settled on *Nephrops* is largely due to the habitat of the prawn since, as Crisp (1951) has pointed out, the growth-rate of barnacles varies enormously in different environments. He finds that growth is greatest in clean water and high currents, while in slow currents, and particularly in the presence of much silt, it is considerably reduced. The latter conditions are, of course, just those which appertain to barnacles epizoic on *Nephrops*.

The above results emphasize the necessity of caution in the use of epizoic fauna as evidence regarding the age of the 'host' (as used, for example, by Foxon, 1940). For *Balanus crenatus* at any rate, and probably other species, only measurements of individuals growing under comparable conditions should be used.

A feature of interest not evident from the discussion may be mentioned. The enormous settlement of *B. crenatus* in 1950 resulted in material such as stones, shells or debris from the sublittoral zone usually being completely covered with living barnacles during the late spring and early summer. By late summer, although large quantities of calcareous remains were left on such material, living barnacles were comparatively few; indeed the whole population had been decimated. In contrast, all the barnacles examined from the *Nephrops* were living. The barnacles on the *Nephrops* may have owed their survival to a 'protective' action of the prawn, or perhaps to the absence of the predators in the muddy bottom habitat of *Nephrops*.

The Moulting of the *Nephrops*

The population of large barnacles (III) was confined to two size-groups of *Nephrops*, 17–18 and 20–21 cm., in the first two collections of June and July (populations I and II being also present), and there were four other examples of a single barnacle population on a given size-group. Apart from these exceptions all the *Nephrops* size-groups at any given date had the same barnacle populations (twenty-five sets in all). It should, of course, be remembered that some *Nephrops* of each size-group were barnacle-free at any time. This suggests that moulting usually takes place irrespective of size-group; at no time did a given *Nephrops* size-group lose all its epizoic barnacles, or carry a distinctive barnacle population. Now it may be seen from Fig. 1 that,

on the hypothesis put forward in the previous section, the spring barnacle settlement of one year is lost by August of the next year (population III, although present in small numbers—2% of the total—in July was absent in August) and further, the summer settlement of one year is lost by the autumn of the next year (population II, although present up to August, was absent in October). The life of a barnacle on a prawn does not exceed one year. This suggests that in any size-group a *Nephrops* moults at least once a year. It cannot be ascertained from these results whether any given prawn moults more frequently, but few individuals appear to moult less than once a year, at any rate not enough to contribute a detectable population of barnacles to that carried by a given size-group. It may be objected that the barnacles

TABLE II. PERCENTAGE OF *NEPHROPS* WITH EPIZOIC *BALANUS CRENATUS*

Size group (cm.)	June	July	August	October	January
7–8	0	0	0	0	—
8–9	0	0	0	0	—
9–10	0	0	0	0	—
10–11	0	0	0	0	0
11–12	0	0	0	0	—
12–13	51	6	13	0	—
13–14	31	10	9	—	—
14–15	50	13	19	57	0
15–16	43	24	41	64	0
16–17	75	37	38	83	(66)
17–18	79	57	46	81	56
18–19	89	45	58	83	50
19–20	100	51	76	88	92
20–21	100	35	(100)*	79	86
21–22	100	43	—	100	(100)

* A bracketed value signifies only very small number (< 10) of individuals.

themselves do not live after the first year; this, however, would seem unlikely, since *Balanus crenatus* is known to live for much longer periods in its normal habitat, and, further, had this been so, their calcareous bases would have been left and observed.

The above results indicate that some moulting takes place in the late spring and also in the early autumn. Table II gives the percentages, at each collection, of the *Nephrops* in the various size-groups with barnacles (for a consideration of the barnacle-free size-groups see p. 377), and the drop in these percentages between the June and July collections indicates that some moulting also took place at this time. There is little change in these percentages in August, and the increase in October is presumably due to the moderate barnacle settlement in August. The numbers of *Nephrops* in the January collection were small, and the values are therefore less reliable, but the decrease in the 17–19 cm. size-groups may be due to moulting or perhaps mortality of the smaller barnacles during the winter months. Storrow (1912) found that soft or 'recovering'

males were found mainly in the spring catches (April), but some were present throughout the year except in the late autumn and early winter (September–November).

Maturity and Breeding Season of *Nephrops*

In all these collections only seventy-eight female *Nephrops* have been taken, compared with 1200 males, and on no occasion were the females ever more than 9% of the catch (Table III): Poulsen gives the proportion of females in his material as about 30%. In the Scandinavian material berried females were found in all the size-groups greater than 12 cm., but Havinga (1929) gives the size at sexual maturity, presumably for material in more southerly regions, as 10 cm., with rare individuals at 8 and 9 cm. Storrow (1912, 1913) records berried females at 9–11 cm. but the majority were in the size-groups greater than 11 cm. Most of the berried females collected in the supplementary hauls in this region were in the size-groups of 10 cm. and above, but a small number have been found berried at 7·5 cm.

Table III. Size-Frequency (nos.) Distribution of Female *Nephrops*

Size group (cm.)	June	July	August	October	January
8–9	0	1	2	0	
9–10	1	2	5	0	
10–11	0	10	10	1	Nil, but very small catches
11–12	5	11	6	2	
12–13	1	8	3	1	
13–14	3	0	1	1	
14–15	1	1	0	2	
Total	11	33	27	7	—
Berried	0	0	2	0	—
Percentage of total catch	8·0	5·6	8·8	3·4	—

Berried females have been taken from August to January, and in all these eggs were in the unripe green state. This is in agreement with Storrow's observation that spawning takes place from July to September and, according to this worker, the female carries the eggs for a long period, and then hatching takes place in the following spring and summer. It would seem that the spawning and hatching take place somewhat later than in Scandinavian waters (see Poulsen, 1946).

Poulsen has suggested that the fact that the percentage of berried females sometimes approaches 50% of the adult female population indicates a biennial spawning, and that any reduction below this value is due to the berried females burrowing more deeply. There seems little evidence to support the view that the females in general burrow more deeply (see p. 377), and the very small proportion of berried females recorded here and also by Storrow would indicate a very infrequent spawning. An alternative possibility is that the females, after fertilization, tend to migrate away from the grounds usually

fished, for Meek (1903, 1913, 1914) has suggested that the well-known migration of crabs is related to the ripening of ova: it seems clear from his work that female crabs migrate far more readily than males. In Meek's work the percentage of female *Nephrops* dropped from June-August and this would agree with fertilization in late spring and subsequent migration.

The Size and Sex Ratio of *Nephrops*

The frequency of the various size-groups is given in Tables III and IV, and for the males the appropriate histograms have been drawn in Fig. 2. The range in size both for males and females, 7–24 and 7–15 cm., respectively, is similar to that given by both Poulsen and Storrow, although the former records occasional males as large as 25 cm., while the latter's records do not show any larger than 22 cm. (The lower size limit is, of course, determined in part by the trawl-mesh size used).

Table IV. Percentage-Size-Frequency of all the Male *Nephrops* Collected

Size group (cm.)	June	July	August	October	January
6–7	0·0	0·0	0·4	0·0	0·0
7–8	0·7	0·2	0·0	0·0	0·0
8–9	0·7	0·6	0·4	0·5	0·0
9–10	0·7	1·3	2·8	0·5	0·0
10–11	2·9	3·1	6·0	2·0	2·6
11–12	5·8	3·2	5·2	1·5	7·7
12–13	9·5	3·1	6·4	1·5	0·0
13–14	11·7	7·8	9·2	0·0	0·0
14–15	13·1	6·7	12·4	7·0	2·6
15–16	5·1	14·5	10·8	5·5	2·6
16–17	8·8	17·0	13·6	17·6	7·7
17–18	17·5	15·4	9·6	16·1	0·0
18–19	13·1	11·4	9·6	17·6	20·5
19–20	5·8	7·8	12·0	17·6	20·5
20–21	2·2	4·4	1·2	9·6	25·6
21–22	1·5	1·3	0·0	2·0	10·3
22–23	0·0	0·2	0·0	1·0	0·0
23–24	0·7	0·2	0·4	0·0	0·0

There is no separation into distinct year-classes, but unlike Poulsen's data there is an indication of an increase in the proportion of the larger size-groups throughout the season. The reason for this difference is not entirely clear. Both Poulsen's and Storrow's data were obtained from material obtained over a wide area, or from market catches, while the present catches were always taken in the same place. The simplest explanation would be to assume that differential changes took place (as a result of migrations) with respect to size-groups in the present collections which were taken in one locality, and that this resulted in an accumulation of the larger size-groups in the area sampled.

In these and all other records the great proportion of males in all the catches and at all times of the year has been stressed. Poulsen has suggested that this

is due to the females burrowing more deeply, and therefore being less likely to be taken in a trawl. The explanation seems hardly adequate; although the smaller animals may, as is suggested later (p. 377) lead a more secluded life, and may therefore be inadequately represented in the catches; an examination

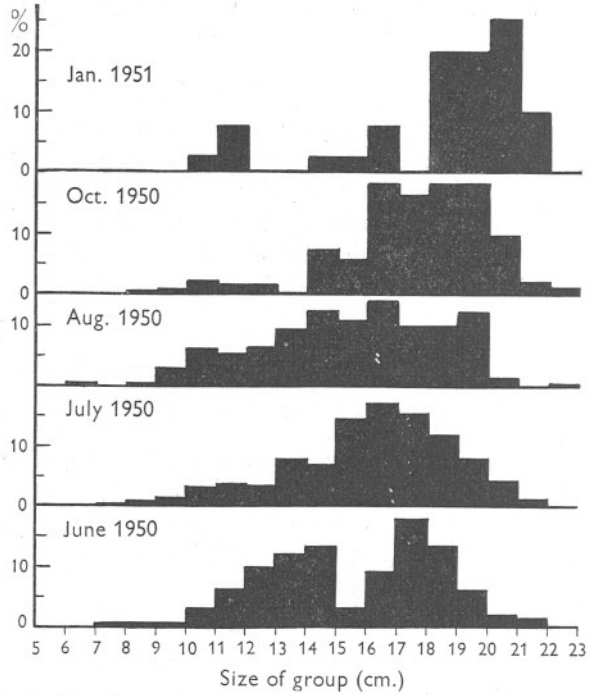

Fig. 2. Size-frequency histograms of *Nephrops* (collections I–V).

TABLE V. SIZE-FREQUENCY (NOS.) OF MALE AND FEMALE *NEPHROPS* FOR 7–15 CM. GROUPS

Size group (cm.)	June		July		August		October	
	♂	♀	♂	♀	♂	♀	♂	♀
7–8	0	0	0	1	1	1	0	0
8–9	1	0	1	1	0	4	0	0
9–10	1	1	5	6	5	5	0	0
10–11	3	0	11	11	9	9	1	1
11–12	4	5	18	18	12	8	1	2
12–13	14	1	15	11	12	3	0	1
13–14	14	3	25	2	7	1	3	1
14–15	14	1	40	0	9	1	0	0

of the numbers for the smaller size-groups shows that the males and females are caught in roughly equal numbers (Table V): this is also seen in the figures given by Poulsen (1946), Storrow (1912, 1913) and McIntosh (1904). The inequality in the sex ratio only becomes apparent when the large size-groups are considered.

The above facts, together with the smaller size-range of the females, suggests that the sexes are present in the same number up to 12 cm., but beyond this size there is differential mortality—the females living little longer than the time required to reach sexual maturity, while the males continue to grow for some years (contrast Punnett, 1904).

The Habitat of *Nephrops* and the Barnacle Settlement

Poulsen remarks that it is well known that adult *Nephrops* hide in the bottom mud and that this has not been demonstrated for the younger individuals. It has now been shown that these young individuals do indeed burrow in the mud. Fig. 3 is a photograph of a burrow of a 5 cm. *Nephrops* kept on mud in a tank; the burrow was 10–12 cm. in length, shallow and open at both ends, and

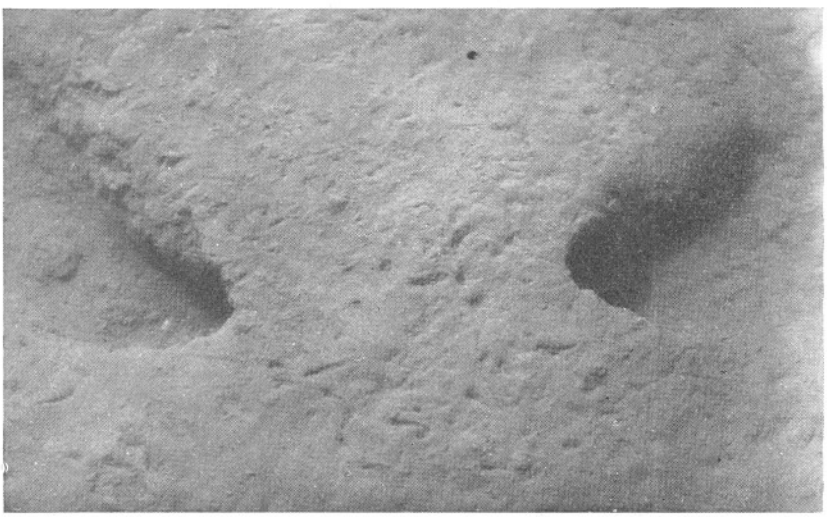

Fig. 3. Burrow made by a 5 cm. *Nephrops* kept on mud in an aquarium tank. Length of burrow 10–12 cm., with entrance on the left and the *Nephrops* completely hidden. ×0·7.

sufficiently long to hide the entire animal (including chelipeds) which indeed had retracted into the burrow before the photograph was taken. Although larger *Nephrops* have not been seen to make burrows in laboratory experiments it seems unlikely that a burrow proportional in length could readily be maintained in soft mud: this would require a burrow 60 cm. long for a 24 cm. *Nephrops*.

The above observations suggest that the smaller animals, males and females, may lead a more secluded life than the larger ones and this is borne out by the fact that all the *Nephrops* up to 11 cm. were completely free from barnacles, although it must be remembered that the larger individuals present a larger surface area for settlement.

The distribution of barnacles on these larger *Nephrops* is illustrated in Table VI and Fig. 4, and these results suggest quite clearly that the animals spend much of their time in their relatively smaller burrows, with the thorax just protruding and the heavy chelipeds resting with their lower surfaces on the mud.

TABLE VI. DISTRIBUTION OF BARNACLES (ALL SIZES), JULY COLLECTION

	Nephrops with barnacles (%)	Total barnacles present (%)
Cephalothorax	12	3·5
Meropodite	5	1·2
Carpopodite	41	18·4
Pro- + dactylopodite	74	76·6
Telson	1	0·1
Abdomen	—	0·3

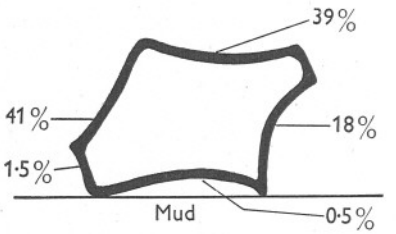

Fig. 4. Diagrammatic cross-section of the right propodite of *Nephrops*, showing the percentage distribution of barnacles on the various regions for the July collection.

SUMMARY

Data have been given concerning the size-frequencies, sex and presence of females in berry of *Nephrops norvegicus* in the Clyde Sea Area, and also the numbers, size and distribution of *Balanus crenatus* epizoic on the prawns. This entailed the examination of *c.* 1200 *Nephrops* and *c.* 8000 barnacles, taken in June, July, August and October 1950 and January 1951.

Four barnacle populations were found during the sampling period, and these have been shown to originate from two periods of settlement (April and August).

The growth-rates of the spring barnacle spat-fall during its first summer, and the settlement of the previous autumn in its second year, are both shown to be about 2 mm. in 4 months, and reasons for, and consequences of, this slow growth-rate are put forward.

It is suggested from the evidence provided by the examination of epizoic barnacles on the prawns that the moulting of the latter takes place irrespective of the size-group, at least annually and largely between late spring and early autumn.

Females may attain sexual maturity (as evidenced by the carrying of eggs) at 7·5 cm., but the 10 cm. size-group is more commonly mature. Berried females are observed between August and January.

Size-frequency histograms are given for the males, and the shift in the population towards the higher size-groups is tentatively explained on the basis of differential migration.

It is pointed out that the differences in proportion of males and females is only evident when the higher size-groups are included; below 12 cm. the numbers for both sexes are similar for these and other observations. The suggestion that the females burrow more deeply is therefore open to question.

It is shown by laboratory experiments that young *Nephrops* are able to construct burrows $2\frac{1}{2}$ times their length; the entire absence of barnacles on prawns of less than 11 cm. is correlated with the ability of these smaller animals to become completely hidden. The distribution of *Balanus crenatus* on the larger *Nephrops* suggests that these also live in the mud but that the burrows are not proportionately as large; they spend much of their time at the entrance of their relatively smaller burrows with the heavy chelipeds resting on the mud surface.

REFERENCES

BARNES, H. & BAGENAL, T. B., 1951. A statistical study of the variability in catch obtained by short repeated trawls taken over an inshore ground. *Journ. Mar. Biol. Assoc.*, Vol. 29, pp. 649–60.

CRISP, D. C., 1951. The habitat of *Balanus amphitrite* var. *denticulata* in Britain. *Nature*. (In the press.)

FOXON, G. E. H., 1940. Notes on the life history of *Sacculina carcini* Thompson. *Journ. Mar. Biol. Assoc.*, Vol. 24, pp. 253–64.

HAVINGA, B., 1929. Krebse und Weichtiere. *Handb. Seefisch. Nordeuropas*. Bd. 3, pp. 1–147.

HELDT, H. & HELDT, H., 1931. Des langoustines (*Nephrops norvegicus* Leach) dans les mers Tunisiennes. *Stat. Ocean. de Salammbô*, Vol. 16, pp. 1–16.

HÖGLUND, H., 1942. De matnyttige kräftdjuren. *Fisker och Fiske i Norden*. Stockholm, 1942.

MCINTOSH, D. C., 1904. On variation in number and arrangement of the male genital aperture and on the proportion of the sexes in the Norway lobster (*Nephrops norvegicus*). *Proc. Camb. Phil. Soc.*, Vol. 12, pp. 441–4.

—— 1908. Variation in the Norway Lobster. (*Nephrops norvegicus*). *Proc. Roy. Phys. Soc.*, Vol. 17, pp. 129–42.

MEEK, A., 1903. The migration of crabs. *Northumberland Sea Fish. Comm. Rept. Sci. Investig.*, 1903, pp. 33–5.

—— 1913. Migration of crabs. *Dove Mar. Lab. Rept.*, 1912–13, pp. 13–20.

—— 1914. Migration of the crab. *Dove Mar. Lab. Rept.*, 1913–14, pp. 73–6.

POULSEN, E. M., 1946. Investigations on the Danish fishery for and the biology of the Norway lobster and deep sea prawn. *Rept. Danish Biol. Stat.*, Vol. 48, pp. 27–49.

PUNNETT, R. C., 1904. Note on the proportion of sexes in *Carcinus maenas*. *Proc. Camb. Phil. Soc.*, Vol. 12, pp. 293–6.

PYEFINCH, K. A., 1948. Notes on the biology of Cirripedes. *Journ. Mar. Biol. Assoc.*, Vol. 27, pp. 464–503.

STORROW, B., 1912. The prawn (Norway lobster, *Nephrops norvegicus*) and the prawn fishery of North Shields. *Dove Mar. Lab. Rept.*, 1911–12, pp. 10–31.

—— 1913. The prawn (Norway lobster, *Nephrops norvegicus*) and the prawn fishery of North Shields. *Dove. Mar. Lab. Rept.* 1912–13, pp. 9–12.

TOPSENT, E., 1911. Croissance et mort des balanes à Luc-sur-Mer. *Ann. Inst. Océanogr.*, T. 2, Fasc. 6, 3 pp.

ZARIQUIEY-CENARRO, R., 1935. Crustaceás del Mediterranéo (Decapodos Macruros) *Nephrops norvegicus* L. var. *meridionalis*. *Bull. Inst. Catalana Hist. Nat.*, Vol. 35, pp. 26–32.

THE GROWTH-RATE OF JUVENILE *ASTERIAS RUBENS* L.

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(Text-fig. 1)

The general biology of the common starfish, *Asterias rubens* L., has recently been studied by Vevers (1949), whose investigations included observations on the growth-rate of this species. His material was collected in the Plymouth area largely by means of an otter trawl, supplemented by a few Agassiz and dredge hauls, and there are therefore few records in his data of the growth-rate of juvenile *A. rubens* under natural conditions. Vevers also made observations on the growth-rate of individuals kept in laboratory tanks, but again the results apply mainly to fairly large animals. The only other direct observations on growth-rate appear to be those of Bull (1934), who dealt with three small individuals kept in aquarium tanks, while Orton & Fraser (1930) give measurements made on a natural population taken, after one year's growth, from a buoy.

While examining barnacle-covered panels, suspended 2 ft. below the surface from a raft moored at Millport, large numbers of very small (about 4·5 mm. radius) *A. rubens* were noted during early August 1950 and, in view of the lack of information on the growth-rate of such small starfish, samples of this population were collected and measured at intervals over a period of 6 months; the data so obtained supplement those given in the investigations referred to above.

The panels (each 15 × 10 in.) had only been exposed since March 1950 and by the beginning of August, when the collections of starfish were first made, were covered with close-packed elongated barnacles—mixed *Balanus crenatus* Brug. and *B. balanoides* (L.), similar to the growth described and figured by Barnes & Powell (1950, Pl. I)—together with numerous young *Mytilus edulis* L. Although many of the barnacles had already been killed by the predatory activities of the nudibranch *Onchidoris fusca* (O. F. Müller), large numbers of which were present on the panels at this time, the panels would appear from the point of view of food supply to have been an ideal site for the growth of young starfish.

There were a number of such panels all carrying this young *Asterias* population, and each subsequent sample of the latter consisted of all the starfish that could be found on one or more of the densely colonized panels. No panel

was sampled more than once, errors due to selection being thereby minimized. The animals were brought into the laboratory and killed in weak alcohol with the arms fully extended, and measured immediately. In view of the difficulty of determining the exact position of the anus in the smallest specimens, the distance from the centre of the disk to the tip of the longest ray was measured and this measurement was adhered to in all subsequent samples; a vernier measuring microscope was used for the smaller, and ordinary dividers for the larger animals. Table I gives the dates of collection, the numbers of starfish measured, the mean length (radius), the standard error of each mean, together with the increase in the mean length per 30 days. The mean lengths and the monthly mean surface sea-water temperatures near the site are plotted in Fig. 1.

In the subsequent discussion of growth-rate our results can be considered comparable with those of Bull (1934), who gives the mean radius of all the arms of normal length of each individual measured from the centre of the disk, and with those of Vevers, although the latter author in accordance with general convention for adult asteroids, measured the distance from the anus to the tip of the longest arm. In considering the results of Orton & Fraser (1930), the conversions made by Vevers are used.

It is well known that large starfish wander over considerable distances, and they may climb on to rafts and moored buoys via the mooring chains. It is considered that the population under consideration, which was under observation at frequent intervals (in addition to the occasions when the starfish samples were being taken) received no influx in this way. The possibility exists that some of the larger starfish may have dropped off the panels but this is considered unlikely, although late in the season there was some evidence that the larger starfish did move from panel to panel. It is almost certain that the young starfish had developed from larvae which settled directly on the panels since, according to Gemmill (1914), spawning of *A. rubens* takes place at Millport from April to June, varying somewhat from year to year, and development of the larva to the youngest adult form takes from 9 to 10 weeks.

Irregularities in the growth-rate curves of *A. rubens* seem to be common, as is emphasized by Vevers in discussing his results; a similar irregularity is seen in our growth-curve which is that of mean sizes. However, there were two periods of uniform growth: in the first period (7 August 1950 to 5 October 1950) the average of the separate estimates of the rate of increase of the mean size is 4·4 mm. per 30 days, while in the second period (2 November 1950 to 22 January 1951) the value is somewhat lower: 3·2 mm. per 30 days (see Table I and Fig. 1).

Vevers (1949, p. 172) points out that starfish up to at least 6 cm. radius can grow at a monthly rate of rather more than 10 mm. in the summer and rather less than 5 mm. in winter. However, his two smallest animals, B8 and B9,

show the variation in growth which can take place between individuals kept under apparently identical environmental conditions. Thus B8 grew from 13 to 39 mm. from 6 July to 21 September 1947, that is an overall rate of 10 mm.

TABLE I. ANALYSIS OF GROWTH-RATE OF SAMPLES OF YOUNG *ASTERIAS RUBENS*

Date of collection	No. in sample	Mean length (r) of sample (mm.)	Standard error	Increase in mean length (r), mm. per 30 days
7 Aug. 1950	33	4·6	0·39	
19 Aug. 1950	31	5·9	0·33	3·3
30 Aug. 1950	48	7·5	0·29	4·4
16 Sept. 1950	38	10·6	0·47	5·5
5 Oct. 1950	50	13·4	0·66	4·4
18 Oct. 1950	71	17·9	0·58	10·4
2 Nov. 1950	75	18·9	0·56	2·0
5 Dec. 1950	73	23·1	0·64	3·8
28 Dec. 1950	68	25·3	0·71	2·9
22 Jan. 1951	78	27·8	0·97	3·0

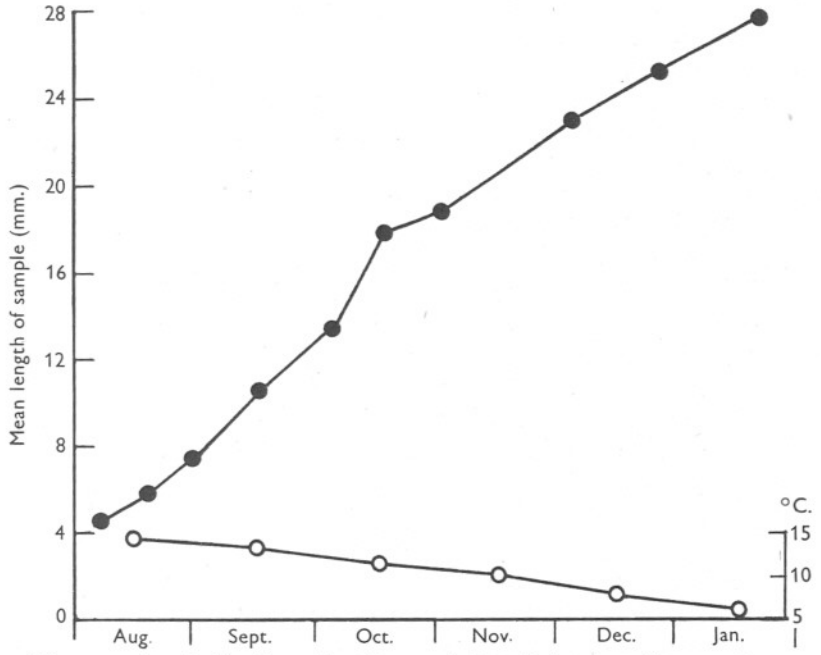

Fig. 1. The mean length (r) of samples of a population of *Asterias rubens* growing on raft-exposed panels (●), together with the monthly mean surface sea-water temperatures near the site (○).

per 30 days, and B9 increased from 10 to 27 mm. during roughly the same period (20 July to 21 September 1947), an overall rate of 8·1 mm. per 30 days. The latter animal (B9) increased its overall rate to 11 mm. per 30 days during the late autumn and winter (26 October to 14 December 1947).

The present results are for the means of a series of samples, and therefore supply no information regarding variation in growth-rate of individuals of the same age or size, but in view of the variations discussed above there would not seem to be any great discrepancy between our results and those of Vevers, although in general the latter are somewhat greater; indeed he estimated that the animals used in his experiments had grown at an average rate of 5 mm. per month over the 12 months before capture.

The results are also in agreement with those of Bull (1934) for the growth of two of his animals, 2(*b*) and 3, reared in the laboratory; these showed an overall growth rate of 3·3 and 6·0 mm. per 30 days respectively during comparable periods of the summer and early autumn months (May to September 1929).

Orton & Fraser (1930) measured 1805 small *A. rubens* taken from a buoy in Liverpool Bay, and estimated that the modal size attained by this population after 1 year's growth was 22 mm., with a range from 6 to 52 mm.; this corresponds to a monthly increment of approximately 2·5 mm. around the mode (which was little different from the mean). The growth of this population, therefore, was much less than that observed either in the experimental animals of Bull and Vevers or in those of the present investigation.

Vevers's results have clearly shown the great effect of reduced food supply in lowering the growth-rate of animals of such voracious habits as *A. rubens*. Both his and Bull's animals were reared in laboratory tanks with a plentiful supply of the appropriate kind and size of food, and the present results show that a similar growth-rate can be attained under natural conditions. The discrepancy between these results and those of Orton & Fraser would hardly seem to be due to a temperature effect, since the surface seawater temperatures at Millport (Fig. 1) are very similar to those of Liverpool Bay (Proudman, Lewis & Dennis, 1937). It is considered that a shortage of food of the appropriate kind at some stage in their growth, rather than possible loss of some of the larger animals due to wind and wave action, is more likely to account for this discrepancy.

Summary

A population of young *Asterias rubens* settled on raft-exposed panels has been sampled at frequent intervals over a period of 6 months.

The conditions under which this population developed are described, and from the measurements obtained estimates are given of the growth-rate during the period of the observations.

These values are compared with those obtained by other workers.

REFERENCES

BARNES, H. & POWELL, H. T., 1950. The development, general morphology and subsequent elimination of barnacle populations, *Balanus crenatus* and *B. balanoides*, after a heavy initial settlement. *Journ. Anim. Ecol.*, Vol. 19, pp. 175–9.

BULL, H. O., 1934. Aquarium observations on the rate of growth and enemies of the common star-fish, *Asterias rubens* L. *Report of the Dove Marine Laboratory*, Third Series, No. 2, pp. 60–5.

GEMMILL, J. F., 1914. The development and certain points in the adult structure of the starfish *Asterias rubens* L. *Phil. Trans. Roy. Soc.*, B, Vol. 205, pp. 213–94.

ORTON, J. H. & FRASER, J. H., 1930. Rate of growth of the common starfish, *Asterias rubens*. *Nature*, Vol. 126, p. 567.

PROUDMAN, J., LEWIS, H. M. & DENNIS, A. L., 1937. On the temperature of the surface waters of the Irish Sea. *Phil. Trans. Roy. Soc.*, A, Vol. 236, pp. 261–302.

VEVERS, H. G., 1949. The biology of *Asterias rubens* L.: growth and reproduction. *Journ. Mar. Biol. Assoc.*, Vol. XXVIII, pp. 165–87.

OBSERVATIONS ON *SPHENIA BINGHAMI* TURTON

By C. M. Yonge, F.R.S.

From the Department of Zoology, University of Glasgow

(Text-figs. 1, 2)

The family Myidae is represented in British seas by the two large and well-known species, *Mya arenaria* L. and *M. truncata* L., which burrow deeply into muddy sand and into stiff mud or clay respectively, and by the much smaller *Sphenia binghami* Turton. Interest in members of this family of Eulamellibranchia was aroused during 1949 when studying two species which are common along the coast of California, namely *Platyodon cancellatus* (Conrad) and *Cryptomya californica* (Conrad). These differ most interestingly in habitat. The former bores into soft rocks between tide marks, and the latter, although practically devoid of siphons, burrows deep in the mud of tidal estuaries, always in association with burrows made by various large invertebrates. The species most usually involved is *Callianassa californiensis*, a large anomuran prawn (McGinitie, 1935). The almost sessile siphons open into the sides of the burrows. Accounts of both species have been prepared for publication in California.

The desire to survey more widely the range of form and habit in this family led to this inquiry into *Sphenia binghami*. This has been described and also figured, although indifferently, by Forbes & Hanley (1853) and Jeffreys (1865). They record a wide distribution in depths of between 5 and 25 fathoms in the Mediterranean, along the Atlantic coasts of Spain and France, and around the British Isles as far north as Scarborough on the east and Skye on the west. But there is a surprising paucity of recent records, and certainly during the present century this species has been almost completely overlooked. By the kindness of Dr W. J. Rees, shells collected off Weymouth by Canon A. M. Norman and now in the possession of the British Museum (Natural History) were examined. But no complete specimens were available. *S. binghami* is not listed in the Plymouth Marine Fauna (Marine Biological Association, 1931), although it very probably occurs in that area. There are a number of early records from the Clyde sea area but no specimens have as yet been found in the course of the faunal survey at present being conducted from Millport. Dr H. O. Bull states that it is not recorded from the Cullercoats area. It is, however, listed in the Marine Fauna of the Isle of Man as occurring in various localities around the island at depths of between 12 and 26 fathoms.

Specimens were finally obtained from the marine station at Port Erin through the kindness of Dr N. Sumner Jones who identified them during the course of his work on the bottom fauna and sent them living, by air, to Glasgow. This work would have been impossible without his help and care.

Habitat

Some ten specimens were eventually received, the majority alive, the largest being only about 1 cm. long. Dr N. S. Jones writes saying that 'all the specimens I have found came from an area about one to two miles south of the Calf of Man, in about 20 fathoms. The bottom consists of gravel and fairly large stones, many of which are limestone. I picked out all the stones brought up in the dredge that showed holes bored by *Hiatella*. The *Sphenia* inhabit these holes, presumably after the death of the original occupant. I obtained them by breaking up the stones with a hammer and picking them out of the cavities. For each *Sphenia* there must have been about 50 living *Hiatella*.' The accounts given by Forbes & Hanley and by Jeffreys are very similar, the latter writing of the occurrence of *Sphenia* within cavities of limestone rocks and oyster shells perforated by *Saxicava rugosa* (*Hiatella rugosa*) and *Cliona celata* and also within the holdfasts of *Laminaria*.

There is no doubt that *Sphenia* has been overlooked for so long owing to confusion with the much commoner *Hiatella* with which it normally lives and which it superficially resembles. When the siphons are extended distinction between the species is easy, those of *Hiatella* being red and also relatively longer with the two openings the more widely separated. But when contracted only careful examination of the ligament, external in *Hiatella* and internal in *Sphenia*, provides certain indication. Both *Sphenia* and the two species of *Hiatella*, no matter whether the latter are boring or byssal-attached individuals (Hunter, 1949) are frequently very iregular in shape. Jeffreys refers to the frequent distortion of the shell in *Sphenia* and this was also noted in all the specimens personally examined, three of which are shown in Fig. 1. This irregular shape is certainly associated with the mode of life, namely attachment by byssus (see Fig. 1 B) within crevices, often those initially formed or else increased in size by *Hiatella*, in stones or shells. The lack of mobility and the nature of the habitat was further indicated by the presence of encrusting growths of hydroids, polyzoa, etc., both on the shell and on the periostracum at the base of the siphons.

Description

Shell. This is white in colour and typically almost twice as long as broad, seldom much exceeding 1 cm. in length. It has been well described by Forbes & Hanley and by Jeffreys, and only points of special interest demand mention. The hinge is situated about one-third of the distance from the anterior end

and possesses the chondrophore characteristic of the Myidae with the associated condensed internal ligament (Fig. 2, *l*). The asymmetry of the hinge mechanism is responsible, as in all Myidae, for the shell being inequivalve, the right valve being the larger as shown in Fig. 1, A–C. This asymmetry is still more marked in *Aloidis* (*Corbula*), a genus included with the Myidae in the Myacea (see Thiele, 1935). There is a slight posterior gape but the shell valves in this region as indicated in Fig. 1 A–C, are very weakly calcified, another point of resemblance with *Aloidis*, although calcified plates occur in this region in *A. gibba* (Yonge, 1946).

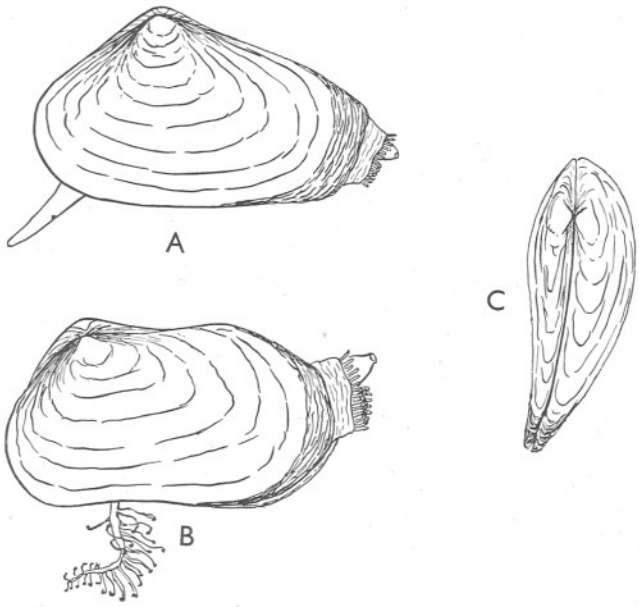

Fig. 1. *Sphenia binghami*, sketches of living specimens showing variation in form. A, specimen viewed from left side with foot and siphons projecting, × 10; B, specimen showing byssus, also siphons projecting, × 8; C, specimen viewed from dorsal aspect showing asymmetry of valves, right being the larger, and also twisting of the shell valves posteriorly due to environmental influences, × 4. In all the posterior area of uncalcified shell is indicated.

Intact animal. The only previous description of the living animal appears to be that furnished by Forbes & Hanley, and based on an account by a Mr Clark who observed the animal at Exmouth. It is substantially correct. As shown in Fig. 1 A, the small foot is thin and wedge-shaped, its prime function being to plant the stout byssus thread shown in Fig. 1 B. The short siphons are united and, as in Myidae generally, surrounded with periostracum. The siphons are thus composed of the inner and middle lobes of the mantle edge, together with the periostracal groove (Yonge, 1948). There is a common outer row of simple tentacles, numbering about 36 in a specimen of shell length 8·0 mm. The inhalant siphon is fringed with an inner row of tentacles, about 12 in number

in the specimen mentioned above. These arch inwards, acting as strainers. The exhalant siphon has a tubular membrane which is very mobile. Although slightly longer, the siphons of *Sphenia* closely resemble those of *Aloidis gibba* (Yonge, 1946). But they are less sensitive, probably because there is much less danger of any sudden intake of sediment than in the mud-living *A. gibba*.

Organs in the mantle cavity. The appearance after removal of the left shell valve and mantle lobe is shown in Fig. 2. Owing to the greater portion of the shell being posterior to the hinge and umbo—associated presumably with byssal attachment—the anterior adductor (*ad*) is much smaller than the posterior adductor (*pd*) and is also displaced ventrally. The same is true of the

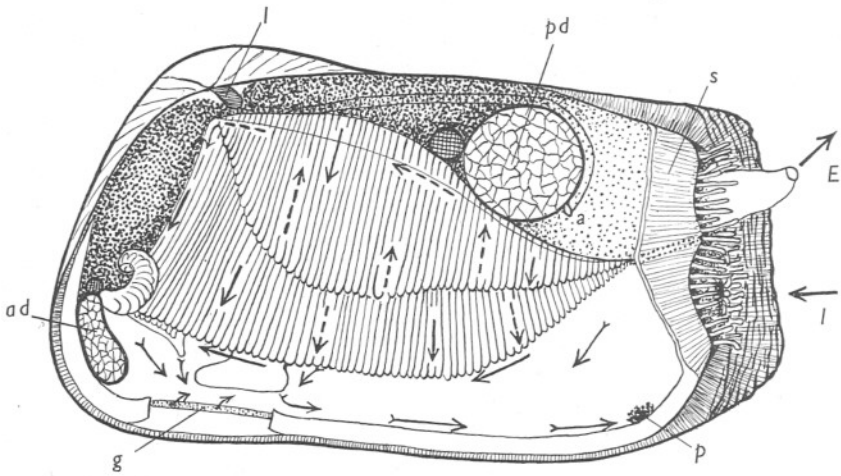

Fig. 2. *S. binghami*, animal viewed from left side after removal of left shell valve and mantle lobe, × 10. *a*, anus; *ad*, anterior adductor; *E*, *I*, exhalant and inhalant currents; *g*, glandular area; *l*, internal ligament; *p*, pseudofaeces; *pd* posterior adductor; *s*, base of siphons covered with periostracum. Complete arrows show currents on exposed surfaces on gills, broken arrows currents on under surfaces, feathered arrowas rejection currents.

anterior pedal retractor. The pedal gape is small, in correspondence with the small size of the foot, and the inner opening is bounded laterally by glandular areas (*g*), just as described in *Mya* (Vlès, 1909) and *Aloidis* (Yonge, 1946), and also present in *Cryptomya* and *Platyodon*. This appears to be a characteristic feature of the Myacea. Apart from the pedal gape the mantle edges are united ventrally, the inner and middle lobes being fused and also the periostracal grooves so that, as in the siphons, the mantle tissues exposed ventrally when the valves separate are everywhere covered with periostracum. But the mantle edges are not thickened as they are in the deep-burrowing *Mya arenaria* or *M. truncata* where, as previously suggested (Yonge, 1949), they probably assist in separating the shell valves. The gills, which are not plicated, are large, the inner demibranch being the larger but the outer one having a short supra-axial

extension. There is a food groove along the edge of the inner demibranch only. The palps are small but typical in form; they have few but relatively large ridges.

The only point of interest in the internal anatomy is the style which lies in a sac separated from the mid-gut as in all other Myidae (though not in *Aloidis*). It is stout and straight and can be seen through the translucent walls of the visceral mass in the intact animal after removal of the shell. It was observed to rotate at a speed of about 60 revolutions a minute.

Ciliary currents. The nature of these is indicated by the arrows on Fig. 2. Frontal cilia carry particles ventralward on the outer surface of the outer demibranch but dorsalward, to the axis, on the inner surface. Along the axis there is a weak forward current. Although there is no food groove along the ventral margin of the outer demibranch, and most particles are carried around it to the inner surface, there is some slight forward movement due probably to large cilia such as those described in *Aloidis* where the course of the ciliary currents is identical in other respects (Yonge, 1946). On the inner demibranch frontal cilia beat ventralward on both faces and particles are carried oralward in a marginal food groove. Particles on the mantle surface are conveyed ventrally and eventually accumulate at the posterior end as masses of pseudo-faeces (*p*). The cilia on the glandular patches lateral to the pedal gape are especially dense and long, and beat away from the pedal gape; there are small vortices on either side of the posterior end of the pedal gape, although not as well developed as those in *Mya* (Kellogg, 1915; Yonge, 1923). This again indicates that the problem of removing excess material from the mantle cavity is much less in byssally attached bivalves living in clean water than in those that burrow in soft substrata.

Discussion

Sphenia binghami is revealed as a member of the sublittoral fauna specialized for life at moderate depths under rather restricted conditions, but clearly less successfully than the two species of *Hiatella*, individuals of which are so very much the more common. The shell in *Sphenia* can certainly be modified to suit the constraint of the habitat while the foot, though doubtless capable of enabling the animal to move, is primarily concerned with planting the byssus. This mode of attachment is probably responsible, as in *Mytilus*, for the reduction of the anterior in relation to the posterior portion of the body. Unlike the other members of the Myidae, *Sphenia* is heteromyarian. The short siphons are adequate for the collection of suspended matter on which the animal feeds.

After publication of the accounts of *Cryptomya* and *Platyodon*, it is hoped to prepare a comparative account of the Myacea (i.e. the Myidae and also

Aloidis) showing how the basic form possessed by this group of Eulamellibranchia has been variously modified in connexion with specialization for life under a surprising variety of conditions.

Summary

Sphenia binghami Turton is a small member of the Myidae with the characteristic features of this family but adapted for life attached by a byssus within crevices in stones or shells at moderate depths. It is usually found with species of the much more numerous *Hiatella*. It has been confused with these, all having characteristically irregular shells. But examination of the ligament (internal in *Sphenia*, external in *Hiatella*) is enough to distinguish between them. Byssal attachment is probably responsible for the reduction of the anterior in relation to the posterior portion of the body and so for the heteromyarian condition not found in other genera of the Myidae.

References

Forbes, E. & Hanley, S., 1853. *A History of British Mollusca, and their Shells*, Vol. I. London.

Hunter, W. R., 1949. The structure and behaviour of *Hiatella gallicana* (Lamarck) and *H. arctica* (L.), with special reference to the boring habit. *Proc. Roy. Soc. Edinb.*, B, Vol. 63, pp. 271–89.

Jeffreys, J. G., 1865. *British Conchology*, Vol. III. London.

Kellogg, J. L., 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. *Journ. Morph.*, Vol. 26, pp. 625–701.

McGinitie, G. E., 1935. Ecological aspects of a California marine estuary. *Amer. Midl. Nat.*, Vol. 16, pp. 629–765.

Marine Biological Association, 1931. *Plymouth Marine Fauna*. 2nd ed.

Thiele, J., 1935. *Handbuch der systematischen Weichtierkunde*. Dritter Teil. Classis Bivalvia. Jena.

Vlès, F., 1909. Monographie sommaire de la Mye (*Mya arenaria* Linné 1767). *Mém. Soc. Zool. France*, T. 22, pp. 90–141.

Yonge, C. M., 1923. The mechanism of feeding, digestion, and assimilation in the lamellibranch *Mya*. *Brit. J. Exp. Biol.*, Vol. I, pp. 15–63.

—— 1946. On the habits and adaptations of *Aloidis* (*Corbula*) *gibba*. *Journ. Mar. Biol. Assoc.*, Vol. 26, pp. 258–76.

—— 1948. Formation of siphons in Lamellibranchia. *Nature*, Vol. 161, p. 198.

—— 1949. On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Phil. Trans. Roy. Soc. Lond.*, B, Vol. 234, pp. 29–76.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Nitrogenous Excretion of Amphipods and Isopods

By E. I. B. Dresel and V. Moyle

Journ. Exp. Biol., Vol. 27, 1950, pp. 210–25

The nitrogen excretion of eleven species of amphipods and isopods, including marine, fresh-water and terrestrial forms, has been studied. All species are essentially ammonotelic, since more than 50% of the total soluble non-protein nitrogen of the excreta was present in the form of ammonia throughout. The level of nitrogen excretion is appreciably lower in the terrestrial species than in any of the others, indicating that, in this group, adaptation to terrestrial conditions has been attended by a general suppression of nitrogen metabolism rather than by a transformation to other, less toxic products.

Some 5–10% of the total soluble non-protein nitrogen was present as urea in the case of the fresh-water amphipod, *Gammarus pulex*, and as uric acid in the terrestrial isopods as well as the fresh-water isopod, *Asellus aquaticus*. It is suggested that these minor excretory components might originate from purines as a result of the loss of one or more uricolytic enzymes. In association with the excretion of uric acid some retention of this compound usually occurs, and it was found that the amount so stored in the terrestrial species parallels the degree of morphological and physiological adaptation to terrestrial conditions. The greatest accumulation of uric acid was, however, observed in the fresh-water species, *A. aquaticus*, and although such a storage cannot necessarily be taken as evidence for a partially uricogenic metabolism, this possibility must be borne in mind. E.I.B.D. & V.M.

The Effect of Stimulation on the Opacity of a Crustacean Nerve Trunk and its Relation to Fibre Diameter

By D. K. Hill

Journ. Physiol., Vol. 111, 1950, pp. 283–303

It was known previously that the opacity of a crustacean nerve trunk undergoes a change when the nerve is stimulated. The present paper describes experiments for a further examination of the phenomenon. The effect is measured by making photoelectric recordings of the intensity of light scattered by the nerve. It was found that the opacity of the nerve is very sensitive to changes

in fibre diameter, brought about by altering the osmotic pressure of the solution. When the fibres swell the opacity decreases, when they shrink it increases: a quantitative relation can be obtained.

Under some conditions there may be an initial, transitory, increase in opacity following stimulation; but the main effect is a decrease, and recovery takes 10–15 min. On the basis of known figures for ionic movements across a nerve membrane it is argued that this change in opacity is attributable to an increase in fibre diameter.

The dependence of opacity upon fibre size has been made use of in studying the permeability of the fibre membrane to certain solutes. D.K.H.

The Volume Change Resulting from Stimulation of a Giant Nerve Fibre

By D. K. Hill

Journ. Physiol., Vol. 111, 1950, pp. 304–27

There are reasons for supposing that stimulation of a nerve fibre causes an increase of the osmotic pressure in the interior. The fibre should therefore swell. The present paper describes a method for measuring the swelling of a cuttlefish nerve fibre following repetitive stimulation.

The relation between length and volume of a giant nerve fibre is also investigated; the volume is varied by altering the osmotic pressure of the external medium.

A study is made of the kinetics of water exchange across the fibre membrane resulting from a sudden change in the external osmotic pressure. D.K.H.

The Males of *Canthocamptus bidens* Schmeil

By Ashley G. Lowndes

Proc. Zool. Soc. London, Vol. 120, 1950, pp. 395–403

There has been a considerable difference of opinion over the taxonomy of this species. In 1929 Chappuis created a new genus *Elaphoidella* and placed *Canthocamptus bidens* (Schmeil) in that genus. All taxonomists, so far as I am aware, followed Chappuis, with the single exception of Gurney, who not only disagreed with the creation of the new genus but also pointed out that there was no justification whatsoever for placing *C. bidens* in that genus, especially since the genus was founded almost entirely on the characteristics of the males. The recent discovery of the males and the structure of the fifth foot, among other things, have shown beyond question that Gurney was right so far as the taxonomy of *C. bidens* is concerned. A.G.L.

The Autonomic Nervous System of the Chimaeroid Fish *Hydrolagus colliei*

By J. A. Colin Nicol

Quart. Journ. Micr. Sci., Vol. 91, 1950, pp. 379–99.

The autonomic nervous system of the chimaeroid fish *Hydrolagus colliei* has been investigated by dissections and histological methods. It consists of a cranial parasympathetic portion and a sympathetic portion confined to the trunk. The latter extends from the level of the heart to the anus, and consists of segmentally arranged ganglia on each side of the dorsal aorta. These ganglia are closely associated with small accumulations of suprarenal tissue. Two axillary bodies are the largest of the sympathetic and suprarenal structures. They lie about the subclavian arteries and are made up of a gastric ganglion and a relatively large mass of chromaffin tissue. The sympathetic ganglia lie in an irregular plexus of longitudinal and crossing sympathetic strands but there is no regular sympathetic chain or commissure between ganglia. There are white rami communicantes which connect the sympathetic ganglia with spinal nerves. A small pregastric ganglion lies on the rami communicantes to the gastric ganglion. The visceral nerves arising from the sympathetic ganglia proceed to blood vessels, genital ducts, chromaffin tissue, and gut. The latter is supplied by large splanchnic nerves which originate in the gastric ganglia and proceed along the coeliac axis to the intestine, pancreas, and liver. Prevertebral ganglia are absent. A mucosal and a submucosal plexus are present in the intestine. The cranial component of the autonomic system comprises a midbrain and a hindbrain outflow. In the former there is a ciliary ganglion on the inferior oblique branch of the oculomotor nerve. Short ciliary nerves proceed from this branch to the eyeball. A radix longa is absent. Sensory fibres go directly to the eyeball from the profundus nerve as anterior and posterior long ciliary nerves. The hindbrain outflow comprises scattered nerve cells and ganglia on post-trematic branches of the glossopharyngeal and vagus nerves. These autonomic fibres in the branchial nerves innervate smooth muscle in the pharyngeal region. A visceral branch of the vagus innervates the heart, oesophagus, and intestine; it also establishes a connexion with the pregastric ganglion. In general, the autonomic nervous system of *Hydrolagus* is very similar to that of selachians. It appears that the autonomic systems of these two groups have undergone little alteration since their origin in the Palaeozoic from some common form. Their autonomic systems reflect a simple and primitive level of organization from which more complex systems of the bony fishes and amphibians have evolved. J.A.C.N.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1950–51

The Council has to record with deep regret the deaths of the following former members of Council: Mr H. G. Maurice, C.B., who had represented the Ministry of Agriculture and Fisheries and the Zoological Society, and was a Vice-President; Prof. H. Gordon Jackson who had represented the British Association for the Advancement of Science; Dr R. S. Clark, a former member of the Scientific Staff of the Plymouth laboratory; and Sir Sidney F. Harmer, F.R.S., a member of Council since 1895, who had represented the Royal Society, and was a Vice-President; also Mr R. Hansford Worth and Mr Arthur W. W. Brown, Founders of the Association, the latter having been the last survivor of the original members.

The Council and Officers

Four ordinary meetings of Council were held during the year, three in the rooms of the Royal Society, and one at the Plymouth laboratory. At these the average attendance was sixteen.

During the year the following new Governors have been appointed: Mr H. J. Johns, C.B., M.B.E., Fisheries Secretary, representing the Ministry of Agriculture and Fisheries in place of Mr P. D. H. Dunn, C.M.G., O.B.E.; Dr H. W. Parker, representing the Zoological Society; and Dr Edward Hindle, F.R.S., representing the British Association.

The Plymouth Laboratory

The laboratory buildings have been kept in a good state of repair, and during the year all exterior wood and metal work has been painted. The dog-fish store house has been renovated; new fencing has been erected round the laboratory grounds. New wooden gates have been built at the entrance to the laboratory yard; these were made in the workshop from wood salved from the partitions between the cubicles in the original main laboratory.

During the year permission has been given by the Admiralty for the use of the facilities at Pier Cellars by certain members of the staff of the Plymouth laboratory; this small harbour in Cawsand Bay, with its storage accommodation, used to be rented by the Association before the war from the War Department.

The Aquarium

The aquarium has continued to attract large numbers of adults and school children. All the tanks have been fully stocked and more pictorial labels, drawn by past and present students of the Plymouth City School of Art, have been added. There are now few exhibits in the large tanks without such labels.

Unusual animals to be seen in the tanks this year were Trigger-fish (*Balistes capriscus*), Boar-fish (*Capros aper*), Smelt (*Atherina presbyter*), and Sting-rays (*Trygon pastinaca*); a small Loggerhead Turtle (*Caretta caretta*) proved an attraction for several months.

Research Ships

The research vessels *Sabella* and *Sula* and the motor-boat *Gammarus* have worked regularly throughout the year, except for brief periods necessary for general maintenance of hulls and machinery.

The Association is indebted for the loan from the Admiralty of an improved type of transportable outboard echo-sounder to replace that at present fitted in the *Sabella*.

The Staff

Mr G. A. Steven has been promoted to the grade of Senior Principal Scientific Officer as from 1 April 1950. Mr J. Lowy was appointed to the staff of the Plymouth laboratory in October 1950, in the grade of Scientific Officer.

Dr D. P. Wilson attended a meeting of the Centre National de la Recherche Scientifique in Paris in February 1950, where he was invited to read a paper on 'Larval Metamorphosis and the Substratum'.

Dr W. R. G. Atkins, F.R.S., and Dr Mary Parke attended the meeting of the Seventh International Congress of Botany at Stockholm in July 1950, at which Dr Atkins was invited to read a paper.

Mr G. A. Steven attended the thirty-eighth meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1950.

Mr H. W. Chang, of the Academia Sinica, Shanghai, was appointed Ray Lankester Investigator to work at the Plymouth laboratory during 1950.

Occupation of Tables

The following one hundred and five workers have occupied tables at the Plymouth laboratory during the year:

E. ADAMS, Plymouth (Library).
Miss E. B. ALBRECHT, London (Behaviour of *Arenicola*).
Mrs F. R. ALLISON, Oxford (Cocoon formation in Platyhelminths).
Miss G. AUDY, London (Islets of Langerhans in *Lophius*).
Rev. B. AUSTIN, Southsea (Marine bacteriology).
R. BAINBRIDGE, Oxford (Plankton interrelationships).
Miss D. BALLANTINE, Development Commission (Taxonomy and culture of marine Flagellates).
Dr D. BARKER, Oxford (General).
Prof. E. J. W. BARRINGTON, Nottingham (Cytology of Elasmobranch pancreas).
Dr ELIZABETH J. BATHAM, Cambridge (Nerve net of sea anemones).
L. G. E. BELL, London (Library).

A. C. G. BEST, London (Histology of marine animals).
Prof. M. L. BHATIA, Delhi (Morphology of *Pontobdella muricata*).
Dr ANNA M. BIDDER, Cambridge (Library; digestive mechanism in Cephalopods).
M. BLACKBURN, Australia (General).
Dr H. BLASCHKO, Oxford (Amine oxidase in *Octopus* liver).
Dr B. P. BODEN, Cape Town (Phytoplankton and Euphausians).
B. B. BOYCOTT, London (Brain of *Octopus* and *Sepia*).
Prof. T. BRAARUD, Oslo (Phytoplankton).
L. R. BRIGHTWELL, East Horsley (General).
Miss E. M. BROWN, London (Parasitic Dinoflagellates).
Dr P. BUFFA, Oxford (Effects of sodium fluoracetate on marine animals).
Dr M. BULJAN, Split, Yugoslavia (Chemical oceanography).
Dr T. H. BULLOCK, Los Angeles (Comparative neurophysiology).
A. BURSA, Kenton (Dinoflagellates; *Prorocentrum*).
G. W. CAMBRIDGE, London (Comparative physiology).
H. W. CHANG, Academia Sinica, Shanghai (British Council Scholar and Ray Lankester Investigator) (Biology of *Callionymus*).
Dr P. N. J. CHIPPERFIELD, Brixham (Library).
Miss E. CLAY, Brixham (Phytoplankton culture methods).
Dr K. W. CLELAND, London (*Echinus* eggs).
Miss M. COLLIS, London (*Sabellaria*).
J. S. COLMAN, Port Erin (General).
R. H. COOK, Cambridge (General).
F. R. COOMBE, Plymouth (Physical properties of sand grains).
B. G. CRAGG, Cardiff (Neurophysiology of *Sepia* and *Loligo*).
Miss G. CREEK, London (Lamellibranchs).
Dr D. J. CRISP, Brixham (Library).
Dr DORIS R. CROFTS, London (Muscle morphogenesis of Archaeogastropods).
R. I. CURRIE, National Institute of Oceanography (Physical Oceanography).
Dr R. PHILLIPS DALES, London (Ecology of *Nereis diversicolor*).
J. H. ELGOOD, Nigeria (Library).
D. ETHERINGTON, London (Coccidian parasites).
Dr P. FATT, London (Nerve muscle system in Crustaceans).
Dr MARIA FELINSKA, Cheltenham (Ciliates).
G. R. FORSTER, D.S.I.R. (Biology of prawns).
H. C. FOUNTAIN, Torpoint (Marine Acarines).
Dr VERA FRETTER, London (*Turbonilla elegantissima*).
Dr HELEN GOODRICH, Oxford (Parasites in *Leander* and Polychaetes).
Dr ISABELLA GORDON, British Museum (Nat. Hist.) (Decapod Crustaceans).
C. O. GRANGER, Ministry of Supply, Windscale (Chemical Oceanography).
Dr J. J. GROEN, Utrecht (Physiology of Elasmobranch labyrinth).
Dr J. P. HARDING, British Museum (Nat. Hist.) (Chromosomes in *Calanus*).
Dr T. J. HART, National Institute of Oceanography (Plankton).
Miss J. HAWKINS, Oxford (Amine oxidase in *Octopus* liver).
M. N. HILL, Cambridge (Geology of the English Channel).
G. A. HORRIDGE, Cambridge (Nervous system of Polychaetes).
G. HOYLE, London (*Phallusia*).
O. D. HUNT, Newton Ferrers (Fouling organisms).
F. R. JOHNSON, Accra, Gold Coast (General).
Dr F. R. HARDEN JONES, Cambridge (Swim bladder of fishes).
W. C. JONES, Cambridge (Spicule formation in *Leucosolenia*).

K. A. JOYSEY, London (Geographical distribution of *Echinoderms*).
Dr ELIZABETH M. KAMPA, Cape Town (Retinal pigment in fish).
Dr B. KATZ, London (Nerve muscle system in Crustaceans).
R. S. KEIR, Development Commission (Development of Lamellibranchs).
Dr G. Y. KENNEDY, Sheffield (Porphyrins in marine animals).
Prof. W. B. R. KING, F.R.S., Cambridge (Geology of the English Channel).
Dr F. G. W. KNOWLES, Marlborough (Library).
Miss P. KOTT, Australia (Biology of Tunicates).
Dr MARIE V. LEBOUR, Cawsand (Decapod Crustaceans).
Miss H. M. LLOYD, London (Reproductive systems of Tectibranchs and Nudibranchs).
Dr O. LOWENSTEIN, Glasgow (Physiology of Elasmobranch labyrinth).
Dr A. G. LOWNDES, Wells (Entomostraca).
J. LOWY, Cambridge (Muscular contraction in Lamellibranchs).
M. F. MAHMOUD, Manchester (*Scalpellum* and *Balanus*).
A. W. MANSFIELD, Cambridge (General).
Dr SIDNIE M. MANTON, F.R.S., London (Locomotion of Arthropods).
N. A. MITCHISON, Oxford (Regeneration in *Sycon* and *Grantia*).
Miss M. MORRIS, Oxford (Luminescent Copepods).
J. E. MORTON, London (Marine Pulmonates).
Inst.-Lieut. H. C. PARMENTER, R.N., Admiralty, London, (Physical Oceanography).
Miss G. D. PARRY, Glasgow (Sea anemones).
Prof. R. A. PETERS, F.R.S., Oxford (Effect of sodium fluoracetate on marine animals).
Dr L. E. R. PICKEN, Cambridge (Comparative study of chitins).
Dr W. J. REES, British Museum (Nat. Hist.) (Larvae of Cephalopods).
G. A. ROBINSON, Development Commission (Phytoplankton).
D. J. ROCHFORD, Australia (General).
Miss H. G. Q. ROWETT, Plymouth (Library).
P. SEVENSTER, Oxford (Breeding and behaviour of *Spinachia*).
Prof. J. E. SMITH, London (Nervous system of Polychaetes).
S. E. SMITH, Oxford (Regeneration in *Sycon* and *Grantia*).
A. J. SOUTHWARD, Port Erin (General rock fauna).
C. P. SPENCER, Bangor (Physiology of Diatoms).
Miss F. A. STANBURY, Plymouth (Library).
Miss M. F. SUTTON, London (Regeneration in *Ciona*).
Dr N. TINBERGEN, Oxford (Breeding and behaviour of *Spinachia*).
E. R. TRUEMAN, Hull (Elasticity of bivalve ligaments).
D. VAUX, Lowestoft (Physical oceanography).
Dr A. J. H. VENDRIK, Utrecht (Physiology of Elasmobranch labyrinth).
Dr P. VONWILLER, Zurich (Nervous system of prawns).
P. R. WALNE, Conway (Chemistry of sea water).
G. P. WELLS, London (*Arenicola*, *Chaetopterus* and *Sabella*).
Dr Kr. Fr. WIBORG, Bergen (Fisheries and plankton).
W. WIESER, Vienna (Ecology of free-living Nematodes).
Dr J. D. H. WISEMAN, British Museum, (Nat. Hist.) (Geology of the English Channel).

As in recent years, since the war, there has been a regular flow of scientists making visits of a day or so to see the work of the laboratory, or discuss problems with the staff. The following visitors have come from overseas: Dr C. O'D. Iselin, Woods Hole; Dr P. Ullyott, Istanbul; Prof. Bernhard Peyer, Zurich; A. E. Greenberg, U.S.A.; Dr V. Tonolli, Pallanza; Dr W. F.

Clapp, Duxbury, Mass.; Dr Herbert Graham, Miami; Mr and Mrs A. R. A. Taylor, Prince Edward Island; Dr H. A. L. Trampusch, Amsterdam; Prof. H. Grundfest, Columbia University; Prof. Gilbert M. Smith, Stanford University; B. Komarofsky, Haifa; B. S. Kisch, St Jean de Luz; Dr C. G. Schmitterlöw, Stockholm; Dr L. A. Walford, Washington, D.C.; Dr V. D. Vladykov, Quebec; Dr E. Wubben, Leiden; Lt.Cdr. W. J. Perry, U.S.N.; and Capt. S. Mahagita, Thailand.

A number of research ships have visited Plymouth during the year and afforded opportunities for members of staffs to meet and take part in useful discussions. These have been R.R.S. *Discovery II*, F.R.V. *Sir Lancelot*, H.M.S. *Challenger*, R.R.S. *William Scoresby*, and H.M. Danish Ship *Galathea*, which left in October for a two-year research voyage under the scientific leadership of Dr Anton Fr. Bruun.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-four students from the following Universities and University Colleges: Oxford, Cambridge, Glasgow, Edinburgh, London, Liverpool, Leeds, Sheffield, Reading, Southampton, Cardiff, Bangor, Newcastle, Hull and Leicester, and from Medway Technical College.

Also during the Easter Vacation, Mr R. J. Jones brought a party of seven boys from Whitgift School and Mrs F. B. J. Sewell a party of two teachers and nine pupils from Southampton Girls' School.

An Advanced Course in Experimental Zoology, conducted by Dr J. A. C. Nicol, was held during the Summer Vacation and was attended by fourteen students.

A meeting of the Society for Experimental Biology was held at the Plymouth laboratory from 27 to 29 September 1950. Some ninety members attended the meeting, at which there were a number of contributions from members of the laboratory staff.

Scientific Work of the Plymouth Laboratory Staff

Physics and Chemistry of Sea Water

Measurements of daylight have been continued by Dr W. R. G. Atkins, assisted by Miss P. G. Jenkins, and the results for 1947, 1948 and 1949 are nearly ready for publication. These can now be compared with similar measurements made during 1947 and 1948 by Dr J. M. Stagg at Kew, using a selenium instead of a sodium cell.

Work on the scattering of light in water has been continued by Dr H. H. Poole, Dr Atkins and Mr F. J. Warren, and the seasonal changes in sea water have been studied. Scattering is greatest at the surface and shows no marked change at a thermocline. Forward and back scatter are closely the same in distilled water, but in tap water and in sea water forward scatter may be from three to ten times the greater, according to the angle and quantity of suspended

matter. For distilled water the scattering in the minimum position, a little less than 90° with respect to the beam, is about one in two or three million of the total flux, so every possible precaution has to be taken to avoid the smallest trace of light from unwanted sources.

During trials following recommissioning the two vessels of the National Institute of Oceanography collected water and plankton samples from over the continental slope of the Celtic Sea for Dr L. H. N. Cooper. The first determinations of total phosphorus in the deep water of the Atlantic slope collected by R.R.S. *Discovery II* gave around 1·3 mg.-atom/m.^{3}P between 800 and 2000 m. depth rising to 1·6 mg.-atom/m.^{3} at 3800 m. Between 2000 m. and the bottom at 4000 m., in water that otherwise has markedly uniform properties, the silicate content was found to be doubled—from 20 to 41 mg.-atom/m.^{3} Si.

In the upper waters south-west of Ireland examined by R.R.S. *William Scoresby* in January the content of total and inorganic phosphorus was lower than had been expected. Partly to follow this information up and partly to supplement the investigations of the Lowestoft research vessel *Sir Lancelot* in the English Channel, cruises were made in the Celtic Sea by R.V. *Sabella* in April and June, and by R.R.S. *Discovery II* in May. Much of the Celtic Sea proved to be relatively poor in total phosphorus ($<0{\cdot}6$ mg.-atom/m.^{3}). In the neighbourhood of the Labadie Bank regeneration of inorganic phosphate seems to be occurring from the muddy bottom deposits which occur there.

Water relatively rich in nutrients was found off the Pembrokeshire shore of the Bristol Channel. This enrichment is probably due to the sewage of urban South Wales and to the run off from the fertile watershed of the Severn, Usk and Wye. The Smalls fishing ground comes within the area of enrichment.

Information on the nature of the currents which run round Ushant, Land's End and St David's Head was obtained. Observations crucial for the completion of the papers on themes put forward in previous Reports have been made.

Close and fruitful co-operation has been maintained with Dr J. N. Carruthers and his colleagues at the Hydrographic Department of the Admiralty, and with the Ministry of Agriculture and Fisheries Laboratory at Lowestoft who have this year carried out extensive hydrographical and biological investigations in the English Channel.

In all this work the method for the analysis of total phosphorus in sea water developed by Dr H. W. Harvey has proved of much value. With it one may not only assess potential productivity but also the movements of water masses.

Mr F. A. J. Armstrong has continued the monthly cruises to Station E 1 and has made the routine determinations of inorganic phosphate and total phosphorus. In addition, since May 1950, silicate has been determined by a sensitive molybdenum-blue method, of which an account is being prepared for publication. Mr Armstrong has also undertaken all the analyses of inorganic phosphate, total phosphorus and silicate on the samples collected from the

Celtic Sea by R.V. *Sabella*, R.R.S. *William Scoresby* and R.R.S. *Discovery II*, and a number for total phosphorus collected in the English Channel by F.R.V. *Sir Lancelot*.

He has confirmed that long storage of sea water in glass bottles causes leaching of iron from the glass. Some bottles used for the total phosphorus estimation have been found to release phosphate into the water kept in them. These effects have been overcome by the use of small polyethylene bottles, of which the laboratory has acquired some 300.

Dr H. W. Harvey has collaborated in several investigations bearing upon the production of plant life in the sea. With Dr R. A. Cox, of the National Institute of Oceanography, a photoelectric instrument was built which allows accurate colorimetric estimations to be made on board ship. This was required, and has been in use in R.R.S. *Discovery II*, for estimating phosphate, nitrate, nitrite, silicate and iron in the sea. With Dr M. Buljan of the Institute for Oceanography and Fisheries, Split, Yugoslavia, the several methods of estimating ammonia in sea water have been examined; a development of the principle used by Teorell promises to allow reasonably rapid and precise estimations of ammonia in sea water without distillation.

Many experimental observations have been made concerning the effect of added substances other than available nitrogen, phosphorus, iron and manganese on the growth rate of diatoms, and concerning their different growth rates when sown in waters collected from different positions in the sea. For an interpretation of these observations it has become necessary to evolve techniques to control conditions, such as the presence of associated bacteria and the intensity and periodicity of illumination. Control of these conditions is also necessary to confirm observations of direct utilization of organic phosphorus compounds by these plants; to ascertain the range of organic nitrogen compounds which can be utilized directly without prior breakdown by bacteria; and to investigate the adaptation which takes place when a diatom is transferred from a nitrate to an ammonium source of nitrogen supply and vice versa. In connexion with this, Dr C. P. Spencer, of the Bangor Marine Laboratory, has made observations on the growth of diatoms and, by growing the diatoms for several seven-day periods in culture media containing both streptomycin and penicillin, has obtained cultures from which no bacterial growth can be induced in a variety of media. He has also started observations on the kinetics of diatom growth of a similar type to those which have already thrown light upon various bacterial metabolic processes.

Estimations of the chlorophyll content of surface water at E 1 have been made by Dr Atkins during 1948 and 1949. The maximum weight of chlorophyll found was 4·3 mg./m.^{3}, comparable with Krey's figure of 5 mg. for Kiel, and Riley's 3·6 mg. for the North Atlantic. In collaboration with Dr Parke he used pure algal cultures to determine the number of cells required to give 1 mg. of chlorophyll. Trouble due to yellowish tints was eliminated by using

red colour filters. Of nine cultures six were between 50 and 750 million cells per milligram. These results, which were published in Vol. 29, No. 3, of the *Journal*, show that only the very smallest and therefore most numerous species would provide one cell in each haemocytometer field (0·1 mm.^{3}) of natural sea water, if all the chlorophyll found at the maximum, 4·3 mg./m.^{3}, were contributed by that one species.

Plankton

Dr Mary Parke has continued the study of the small flagellate and non-motile forms in the sea water off Plymouth. About seventy different organisms, not including diatoms, have now been isolated and are being maintained in species-pure culture for further study. The contamination of some of the cultures by fungal growth brought in by tiny flies during the summer has caused considerable trouble, and a great deal of time has been and is being spent in clearing the cultures of the fungus.

This year organisms present in samples of sea water which were taken at least 70 miles offshore have been cultured and examined. These samples were kindly collected by Dr L. H. N. Cooper, and by Dr D. H. Cushing of Lowestoft. The few samples that have been examined show that the same flagellate forms occur frequently in both inshore and offshore waters. A number of these organisms have been obtained from the Tamar Estuary, Knap Buoy, Stations L4 and E1, and the offshore stations worked by Dr Cooper and Dr Cushing. The forms recognized so far are *Pyramimonas grossii*, *Stichococcus* n.sp., *Prorocentrum micans*, *Massartia rotundata*, *Hemiselmis rufescens*, a 1–2 μ yellow green flagellate containing chlorophyll, *Dicrateria gilva*, *Pseudopedinella* n.sp., and three other undescribed chrysomonads, two of which possess three flagella.

At least six different species of flagellates possessing three flagella have been isolated from sea water off Plymouth. These flagellates belong to the Chrysophyceae but cannot be placed in the only described genus with three flagella, *Prymnesium*. Experiments, set up by Mr S. M. Nunn, are now in progress to see if these flagellates, like *Prymnesium parvum* Carter, produce a specific poison which is fatal to fish.

Mr G. A. Robinson, on a Development Commission Fisheries Research Training Grant, has been studying the production and vertical distribution of phytoplankton in the Plymouth area throughout the year 1950. The spring outbreak was late and prolonged, and lasted until the end of April when the numbers fell very low. There was an outburst of *Skeletonema costatum* in June and of *Rhizosolenia alata* Brightwell var. *genuina* Gran. in July. The autumnal outburst occurred at the end of August, carrying on until the beginning of October.

Results of samples taken at various depths showed that in a depth of approximately 50 m. the diatoms were evenly distributed throughout the water

column when vertical mixing occurred in winter and spring. In the summer, when the thermocline was established, there was some evidence to show that there may have been two communities of diatoms, above and below the thermocline.

Mr Robinson has assisted Dr Parke in the maintenance of the stock cultures of marine flagellates and diatoms, paying special attention to the latter. Subcultures have been sent to a large number of institutions in this country and abroad for research purposes.

The weekly half-hour oblique hauls with the 2 m. stramin ring-trawl have been continued during the year. Preliminary inspection of the catches by Mr P. G. Corbin shows a possible slight improvement in macroplankton production over the low level of the last few years; there has not, however, been a pronounced increase. A note by Mr Corbin on pilchard spawning in the central English Channel in 1947 and 1948 was published in Vol. 29, No. 1, of the *Journal*. The material was taken in plankton collections made by members of the scientific staff of the British Museum (Natural History) during cruises of Major H. W. Hall's M.Y. *Manihine*, and was very kindly placed at the disposal of the Marine Biological Association by the Director and Trustees of the Museum.

During the last two years Dr D. P. Wilson has examined the possibility of the existence of a difference in biological properties between waters from the English Channel off Plymouth and from the Celtic Sea west of the Isles of Scilly. He has succeeded in showing that such a difference exists. Experiments made mainly with eggs and larvae of the sea urchin, *Echinus esculentus*, but also with those of the worms, *Ophelia bicornis* and *Sabellaria alveolata*, have shown that they often develop abnormally, or are in poor condition, in filtered sea water from the region of the Eddystone, but that they are usually normal and healthy in filtered water from the Celtic Sea. They also develop normally and healthily in a mixture of equal proportions of both kinds of water. The indications are that the Eddystone water lacks some constituent essential for healthy development, which is present in Celtic Sea water. The result accords with the hydrographical and biological changes which have taken place in the area within recent years. A paper on this work has been prepared for the *Journal*. In this research Dr Cooper has been of great assistance in collecting and interpreting the hydrographic data.

Fauna and Flora of the Sea Floor

Dr D. P. Wilson has spent some time, partly with the assistance of Mr F. R. Coombe of Devonport High School for Boys, investigating the physical properties of sands from the Exmouth district, with the aim of finding some clue to the means whereby the larvae of the polychaete worm, *Ophelia bicornis*, distinguish the sand of their adult habitat from similar sands in which the species does not live. The data obtained are fairly extensive but it is as yet

uncertain how far they will prove applicable to the problem. During the breeding season further experiments on the settlement reactions of the living larvae gave substantial support to the idea that certain sands repel the larvae by virtue of some substance strongly adhering to the grains and that this substance can be removed only by drastic treatments. There is some evidence, not yet conclusive, that this substance can be transferred to 'clean' grains by physical contact. There is, at present, little indication of its nature. The ability of the larvae to distinguish between sands inhabited and uninhabited by the adults was strikingly demonstrated in a number of experiments in which they were given the opportunity to choose between them. The results of many experiments are only now being critically examined.

Mr N. A. Holme has been making a survey of the bottom fauna in the immediate neighbourhood of Plymouth, using the new 'scoop' sampler. Samples are being taken at twenty stations both inside and outside the Eddystone, an area of $\frac{1}{2}$ m.^{2} being sampled at each station. The survey will provide data on the biomass of the macrofauna to provide a basis for following long-term changes in the productivity of the area. At the same time, information is accumulating on the distribution of certain lamellibranch molluscs in relation to the grade of soil. This is being supplemented where possible by observations on the same species living on the shore.

Further attempts are being made by Mr Holme to devise more efficient and more easily handled bottom-sampling gear.

He has now completed a paper on the systematics of the genus *Ensis*. Shell characters alone have been found insufficient for certain identification, and a reliable character has been found in the form and arrangement of the papillae surrounding the fourth pallial aperture. The results were published in Vol. XXIX, No. 3, of the *Journal*.

Over the past two years the small-scale distribution of *Tellina tenuis* on a sandbank in the Exe estuary has been studied. The distribution of individuals was found to be non-random, tending towards an even distribution. It is possible that each shell delineates a 'territory' on the soil surface as a result of the activities of its long inhalent siphon, and this may be the cause of the observed distribution. The results of this work have been published in Vol. 29, No. 2, of the *Journal*. It is hoped to continue this line of study through observations in tanks in the laboratory, but attempts to keep *Tellina* in a healthy condition under artificial conditions have not yet been successful.

Dr H. G. Vevers has continued his work on underwater photography as a means of estimating the density of epifaunal invertebrates. The main technical problem has been the provision of an adequate light source. This has now been solved by fitting to the apparatus an increased number of large photoflood lamps. With this array many photographs have been taken on the trawling grounds off Plymouth and Looe. An interesting series of pictures taken in an area near Looe Island shows a very rich population of *Ophiothrix*

fragilis; these are not distributed evenly on the sea-bed, but are massed in groups, with the individual brittle-stars often lying one above the other. A paper describing the apparatus and some preliminary results is in preparation. In addition, a paper has been published in Vol. 29, No. 3, of the *Journal*, giving observations on the parasitization of *Asterias rubens* gonads by the ciliate *Orchitophrya stellarum*, and also a note confirming the presence in the Plymouth area of the rare ophiuroid *Ophiopsila annulosa* (*Journal*, Vol. 29, No. 1).

In an attempt to analyse the very varied coloration found in natural populations of *Asterias rubens*, Dr Vevers has done some preliminary work on carotenoid pigments of starfish, and in collaboration with Dr G. Y. Kennedy, of the Department of Cancer Research, University of Sheffield, on the porphyrin pigments in the starfish integument.

Mr G. M. Spooner has continued to study the amphipod crustaceans with a view to acquiring more complete knowledge of the fauna of south-west Britain, and to deal with outstanding taxonomic problems. A paper has been prepared for publication giving further information on *Gammarus zaddachi oceanicus*. From an examination of fresh British material its status in Britain could be described for the first time. It occurs in the north—on the west coast of Scotland southward to the Clyde area, and on the east coast to north-east Yorkshire. This matches its occurrence on the Continent (southward to Heligoland, east Denmark and Baltic Sea). In sheltered west Scottish habitats it may be plentiful amongst brown algae in the lower half of the tidal zone. Additional information on its world range is also presented, partly from re-identifications of records in the literature where biometric data or illustrations render this possible. The form has a wide range in subarctic regions, occurring from the Siberian coasts, at least from 140° E., westward to Greenland and north-eastern America. Until recently it had passed under the all-embracing name of *G. locusta*, which strictly should refer to a species which is abundant on the shore of temperate and southern Europe, but does not spread into the arctic-boreal regions. Nor does *G. locusta* seem to occur in America, and it is emphasized that the common species which normally goes under that name on the Atlantic seaboard of Canada and the U.S.A. is really *G. zaddachi oceanicus*.

An investigation by Mr Spooner of the composition of populations of *Jassa falcata* is throwing new light on the old problem of its so-called polymorphism.

Mr G. R. Forster, on a D.S.I.R. maintenance grant, has studied the biology of *Leander serratus*, the common prawn. Samples have been taken throughout one year to provide data on the rate of growth and the breeding season. The youngest prawns appeared inshore during July in pools at or above mid-tide level. By October their mean length was approximately 5·25 cm. in 1950 and 6·25 cm. in 1949. This difference may possibly be related to the fact that in 1949 the sea temperature remained above 15° C. six weeks later than in 1950. Subsequently, the females showed a slightly higher growth rate than

the males. It is unlikely that more than occasional prawns survive their third summer. A paper is now being prepared on the age and growth, breeding, and food of this species.

Collections of ascidians, especially the compound forms, have been made by Miss P. Kott of C.S.I.R.O. Fisheries Division, Australia, who has been working at the Plymouth laboratory during the past year. Species not already represented have been added to the laboratory museum, and a new species, *Corella halli*, has been described showing an interesting intermediate condition of the branchial sac. This specimen was obtained by Major H. W. Hall's yacht *Manihine* when collecting for the British Museum (Natural History) in the English Channel.

Dr Parke has devoted much time to the revising of the late Mr G. F. Tregelles's manuscript on the marine algae of Devon for part II of the 'Flora of Devon', published by the Devonshire Association. This manuscript was prepared in 1939, and a great deal of work is needed to bring the nomenclature up to date and to add the recent records.

Physiology of Marine Organisms

Dr J. S. Alexandrowicz has continued his study of the special muscle units in crustaceans which, together with their associated nerve cells, may be regarded as muscle receptor organs. By means of methylene-blue staining it has been found that these organs are represented on each side of the abdominal segments by two units, and from the different structure of the muscle elements of each unit of a pair it may be concluded that they are not physiologically identical. In the nerves supplying the muscle receptor organs three kinds of fibres could be distinguished, most probably having different functions.

In the embryonic lobster stained with methylene blue it has been possible to identify the nerve cells of these muscle receptors and to establish that the axons of these cells, after reaching the ganglionic cord, form a tract running through all the abdominal and thoracic segments. It was found that the nerve cells of the muscle receptor organs are identical with elements described as sensory cells by E. J. Allen in his work on the nervous system of the lobster embryo. A paper on the results of this research has been accepted for publication in the *Quarterly Journal of Microscopical Science*.

In October and November 1950, Dr Alexandrowicz spent his leave working at the Stazione Zoologica, Naples, where he continued researches made before the war on the nervous system of stomatopods.

Further work on the nervous system of the sabellid, *Branchiomma vesiculosum*, by Dr J. A. C. Nicol, has revealed that this worm has a giant axon arrangement very similar to that of *Myxicola*, which has already been described. Electrical stimulation of the axons gives rise to single muscle twitches, one per stimulus, with no evidence for facilitation. These results are being published in the *Journal of Experimental Biology*. The action of drugs on the neuro-muscular

junction has also been investigated. This study has revealed an essentially cholinergic system, with acetylcholine and nicotine giving the greatest effects.

Additional observations on photosensitivity in *Branchiomma*, continued by Dr Nicol from last year, have shown that these animals can give all their usual light responses without their eyes. Some preliminary observations to the contrary, reported earlier, are ascribed to sensory fatigue.

Mr J. Lowy, who joined the staff in October, has continued work started at Cambridge concerning the mechanism of contraction in the muscles of *Mytilus edulis*. In an attempt to discover whether both tonic and phasic contractions in these muscles are accompanied by expenditure of energy, experiments are now in progress in which simultaneous records are made of the electrical and mechanical activity in the adductor muscles of intact animals.

Miss P. Kott has been attempting to measure the rates of oxygen consumption of the ascidian, *Ascidiella aspersa*; difficulties have been encountered in removing the plant material from the test and results are as yet inconclusive. Results obtained from Winkler titrations using the whole animal are being supplemented by results obtained from tissue slices in Warburg manometers. There are indications that the strength of illumination may inversely affect the amount of oxygen used by the animal. Later the effects of various respiratory inhibitors on different species are to be tested.

Kymograph drum recordings of movements in the species *Ciona intestinalis*, *Ascidia aspersa* and *Molgula manhattensis* have been made; there are rhythms in contraction of both the atrial and branchial siphons, but these are extremely variable and as yet no specific distinctions have been made, either in type of movement, magnitude or frequency; it is hoped to continue this work.

Mr Spooner has maintained contact during the year with the Radiobiological Research Unit and the Chemistry Department of A.E.R.E. Harwell, whom he has supplied with annotated abstracts of the literature dealing with the concentration of certain minor constituents of sea water.

Fish and Fisheries

Mr G. A. Steven has continued his work on the biology of the mackerel. Owing to the very long spawning period of these fish (March–September inclusive) and their extremely rapid growth during early life it has proved difficult to extract precise results from the mass of data collected. Most difficulties have now been largely overcome and more definite assessments of age and growth have been made. These agree very closely with the provisional results given in last year's report. It now also appears that about half a full year's growth takes place during the four months May to August inclusive.

The gonads of nearly ten thousand fish have been examined, the sex determined and maturity stages recorded. From these and other data it appears that many fish reach maturity during their second year of life at lengths of about 28 cm. and upwards. There is, however, great variability, spent fish of smaller

size being not uncommon while others are still in virgin condition at lengths of 32 cm. or more. There is also some evidence to suggest that in relatively small fish of not more than 28 cm. the gonads often progress a considerable way towards ripeness and then assume a spent appearance without any actual spawning having taken place. A paper embodying the results of this work is nearing completion.

Further study by Mr P. G. Corbin of the sand-eels of the Plymouth area has resulted in the identification of a new species, *Ammodytes immaculatus* (*Nature*, Vol. 166, p. 525). This is evidently the adult of a post-larva which when first described in Vol. 28, No. 1, of the *Journal* could not be attributed to a known adult. An adult specimen of *A. marinus* Raitt was taken in Start Bay in May 1950. The post-larva of this species occurs in the Plymouth area but not in such large numbers as in Scottish waters, the southern North Sea and the eastern Channel. Several years' collections of *Ammodytes* post-larvae from those areas have now been examined by Mr Corbin through the courtesy of the Directors of the Fisheries Laboratories of Aberdeen and Lowestoft. The occurrence of only one adult *A. marinus* in the Plymouth area during twelve months' employment of a trawl with a fine-mesh cover on the cod-end, together with the comparative scarcity of the post-larvae, tend to confirm the suggestion that the range of the species does not extend southwards of a line joining south-west Ireland, Land's End and the Channel Islands.

Mr H. W. Chang, British Council Scholar and Ray Lankester Investigator, has continued to work on the biology of *Callionymus* in the Plymouth area. The rate of growth of the male *C. lyra* falls into three main categories. In those males which breed in the third year the growth rate in the first year is normally higher than that of the second year; but in those which breed in the fourth year it is usually highest in the second year. The growth rate of those males breeding in the fifth year is highest either in the second or in the third year. These differences are probably related to sexual maturity rather than racial characters. Similar differences in growth rate also occur in the female, but it is difficult to find the proper standard by which to treat them separately.

After obtaining detailed information on the type specimens from the Museum National d'Histoire Naturelle, Paris, and examining Mediterranean specimens, it is evident that the third species of *Callionymus* found off Plymouth is not *C. fasciatus* as was originally thought. It is *C. reticulatus* C. & V., which hitherto has not been recorded from British waters and has now been shown to occur also in the southern North Sea as well as off the coast of Portugal.

Mr P. G. Corbin, in co-operation with Mr Steven, has developed a bridle suspension for a conical tow-net which eliminates the necessity for a metal ring at its mouth. When fishing, the net maintains a rigidly circular opening, and it is towed from a single warp. The principle is applicable to tow-nets of the usual sizes, and successful trials have been made with a net of 9 ft.

(*c.* $2\frac{3}{4}$ m.) diameter at the mouth, towed at speeds from 1 to $4\frac{1}{2}$ knots: it seems probable that this net could be fished at greater speeds. It is hoped at the next stage to work a net of this design having a diameter of some 30 ft. Experiments on the development of a depressor for use with this net are also being made.

Now that access to the Pier Cellars basin is again available some further experiments on rope and net preservation have been started. Contrary to expectation, it has been found that the supposed waterproofing of ropes, claimed for a commercial treatment, leads to little or no diminution in the uptake of water when equilibrium has been attained. Rather more water was in some cases taken up, especially with waterproofed sisal. All ropes sank immediately except untreated coir. The sum of the residual preservative, after evaporation of solvent, and of water uptake was found to be approximately constant.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated. We are grateful to Mrs E. W. Sexton for the gift of thirty volumes of the *Transactions of the Devonshire Association*; to Dr A. G. Lowndes for the gift of Rideal's *Introduction to Surface Chemistry*, Alexander and Johnson's *Colloid Science* (2 volumes) and Kanthack and Goldsmith's *Table of Refractive Indices* (2 volumes), as well as for many reprints; and to Messrs Macmillan and Co. for six books, all of which are valuable additions to the Library.

The Library has lately been much used by visiting members of the Association.

Published Memoirs

Volume 29, No. 1, of the *Journal* was published in April 1950, No. 2 in September 1950, and No. 3 in February 1951.

The following papers, the outcome of work done at the laboratory, have been published elsewhere than in the *Journal* of the Association:

ALEEM, A. A., 1950. The occurrence of *Eurychasma Dicksonii* (Wright) Magnus in England and Sweden. *Medd. fr. Göteborgs Bot. Trädgärd*, XVIII, pp. 239–45. (*Contr. Mar. Bot. Inst. Göteborg*, No. 2.)

BATHAM, E. J. & PANTIN, C. F. A., 1950. Muscular and hydrostatic action in the sea-anemone *Metridium senile* (L.). *Journ. Exp. Biol.*, Vol. 27, pp. 264–89.

BATHAM, E. J. & PANTIN, C. F. A., 1950. Inherent activity in the sea-anemone, *Metridium senile* (L.). *Journ. Exp. Biol.*, Vol. 27, pp. 290–301.

BATHAM, E. J. & PANTIN, C. F. A., 1950. Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *Journ. Exp. Biol.*, Vol. 27, pp. 377–99.

BIDDER, ANNA M., 1950. The digestive mechanism of the European squids, *Loligo vulgaris*, *Loligo forbesii*, *Alloteuthis media* and *Alloteuthis subulata*. *Quart. Journ. Micr. Sci.*, Vol. 91, pp. 1–43.

COOPER, L. H. N., 1950. The nitrogen cycle. *Chambers's Encyclopædia:* 1950.

CORBIN, P. G., 1950. *Ammodytes immaculatus*, a new species of sand-eel found in European seas. *Nature*, Vol. 166, pp. 525–6.

DRESEL, ELISABETH I. B. & MOYLE, VIVIEN, 1950. Nitrogenous excretion of Amphipods and Isopods. *Journ. Exp. Biol.*, Vol. 27, pp. 210–24.

HANSON, JEAN., 1950. The blood-system in the Serpulimorpha (*Annelida, Polychaeta*). II. The anatomy of the blood-system in the Sabellidae, and comparison of Sabellidae and Serpulidae. *Quart. Journ. Micr. Sci.*, Vol. 91, pp. 369–78.

HAYWOOD, C. A. & MOON, H. P., 1950. The mechanics of the blood vascular system of *Ascidiella aspersa*. *Journ. Exp. Biol.*, Vol. 27, pp. 14–28.

HILL, D. K., 1950. The effect of stimulation on the opacity of a crustacean nerve trunk and its relation to fibre diameter. *Journ. Physiol.*, Vol. 111, pp. 283–303.

HILL, D. K., 1950. The volume change resulting from stimulation of a giant nerve fibre. *Journ. Physiol.*, Vol. 111, pp. 304–27.

HOLME, N. A., 1951. Sampling the sea-bed. *Discovery*, February 1951, pp. 59–63.

HÖRSTADIUS, SVEN, LORCH, I. J. & DANIELLI, J. F., 1950. Differentiation of the sea urchin egg following reduction of the interior cytoplasm in relation to the cortex. *Exper. Cell Res.*, Vol. 1, pp. 188–93.

KEYNES, R. D. & LEWIS, P. R., 1950. Determination of the ionic exchange during nervous activity by activation analysis. *Nature*, Vol. 165, pp. 809–10.

KING, W. B. R., 1950. Floor of the English Channel. *Geolog. Mag.*, Vol. 87, pp. 383–4.

LEBOUR, M. V., 1950. Some Euphausids from Bermuda. *Proc. Zool. Soc. Lond.*, Vol. 119, pp. 823–37.

LEBOUR, M. V., 1950. Notes on some larval Decapods (Crustacea) from Bermuda. *Proc. Zool. Soc. Lond.*, Vol. 120, pp. 369–79.

LEBOUR, M. V., 1951. Notes on some larval decapods (Crustacea) from Bermuda. II. *Proc. Zool. Soc. Lond.*, Vol. 120, pp. 743–7.

LOWNDES, A. G., 1950. The males of *Canthocamptus bidens* Schmeil. *Proc. Zool. Soc. Lond.*, Vol. 120, pp. 395–403.

MENON, M. D. (with an appendix by T. J. HART), 1950. The use of bones, other than otoliths, in determining the age and growth-rate of fishes. *Journ. du Conseil*, Vol. 16, pp. 311–35, appendix pp. 335–40.

NICOL, J. A. C., 1950. Autonomic nervous system of the ratfish. *Nature*, Vol. 165, p. 854.

NICOL, J. A. C., 1950. The autonomic nervous system of the Chimaeroid fish *Hydrolagus colliei*. *Quart. Journ. Micr. Sci.*, Vol. 91, pp. 379–99.

RUSSELL, F. S., 1950. Hydromedusae—Family: Tubulariidae (contd.). Family: Margelopsidae. *Cons. Internat. Explor. Mer, Zooplancton.* Sheet 28.

RUSSELL, F. S., 1950. Hydromedusae—Family: Corynidae. *Cons. Internat. Explor. Mer, Zooplancton.* Sheet 29.

STEVEN, G. A., 1950. Swimming of dolphins. *Sci. Progress*, Vol. 151, pp. 524–5.

STEVEN, G. A., 1950. *Nets. How to Make, Mend and Preserve them.* 128 pp. London: Routledge and Kegan Paul.

Membership of the Association

The total number of members on 31 March 1951 was 612, being 69 more than on 31 March 1950; of these the number of life members was 80 and of annual members 532. The number of Associate members is now six, Mr A. T. A. Dobson, C.B., C.V.O., C.B.E., having been elected during the year.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledge the following generous grants for the year:

From the Fishmongers' Company (£500), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s.*), Leeds (£20), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Nottingham (£10. 10*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), Hull (£10. 10*s.*), Southampton (£10. 10*s.*), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1951–52:

President

Prof. JAMES GRAY, C.B.E., M.C., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of IVEAGH, C.B., C.M.G.
Viscount ASTOR
Sir NICHOLAS E. WATERHOUSE, K.B.E.
Col. Sir EDWARD T. PEEL, K.B.E., D.S.O., M.C.
The Rt. Hon. TOM WILLIAMS, M.P.
G. P. BIDDER, Sc.D.
W. T. CALMAN, C.B., D.Sc., F.R.S.
Vice-Admiral Sir JOHN A. EDGELL, K.B.E., C.B., F.R.S.
Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S.
E. S. RUSSELL, O.B.E., D.Sc.
Sir EDWARD J. SALISBURY, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir AUBREY C. H. SMITH, K.B.E., C.B., M.V.O.
A. T. A. DOBSON, C.B., C.V.O., C.B.E.
Major E. G. CHRISTIE-MILLER
MORLEY H. NEALE

COUNCIL

To retire in 1952

MICHAEL GRAHAM, O.B.E.
C. E. LUCAS, D.Sc.
L. HARRISON MATTHEWS, Sc.D.
J. D. H. WISEMAN, Ph.D.
Prof. V. C. WYNNE-EDWARDS

To retire in 1953

G. E. R. DEACON, D.Sc., F.R.S.
E. FORD
F. C. FRASER, D.Sc.
Prof. J. E. HARRIS, Ph.D.
Prof. C. M. YONGE, D.Sc., F.R.S.

To retire in 1954

J. S. COLMAN
H. CARY GILSON
Prof. ALASTAIR GRAHAM, D.Sc.
N. A. MACKINTOSH, C.B.E., D.Sc.
Prof. J. Z. YOUNG, F.R.S.

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2

Secretary

F. S. RUSSELL, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

G. P. BIDDER, Sc.D.
H. J. JOHNS, C.B., M.B.E. (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
Prof. J. E. SMITH, Ph.D. (Cambridge University)
E. HINDLE, Sc.D., F.R.S. (British Association)
H. W. PARKER, D.Sc. (Zoological Society)
Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S. (Royal Society)

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31ST MARCH 1951

31st March 1950		£	£
	CAPITAL RESERVE ACCOUNT:		
32,688	As at 31st March 1950		32,688
	SURPLUS ACCOUNT:		
	As at 31st March 1950	4,680	
4,680	*Add:* Excess of Income over Expenditure for the year	2,223	
			6,903
37,368			39,591
	AQUARIUM SINKING FUND:		
	As at 31st March 1950	196	
	Add: Donations for rebuilding Aquarium Tanks ...	28	
196			224
		£	
	E. T. BROWNE BEQUEST FUNDS:		
	Building Fund, as at 31st March 1950 ...	25	
	Less: Expenditure on Extension of Workshops	25	
	Library Fund, as at 31st March 1950... ...	1,154	
	Add: Interest on Investment	35	
		1,189	
	Special Apparatus Fund, as at 31st March 1950	2,510	
	Add: Interest on Investment	77	
		2,587	
	Scientific Publications Fund, as at 31st March 1950	2,105	
	Add: Interest on Investment	64	
		2,169	
5,794			5,945
	COMPOSITION FEES FUND:		
	As at 31st March 1950	762	
	Add: Fees received during year	95	
762			857
	BUILDINGS RECONSTRUCTION FUND:		
	As at 31st March 1950	426	
	Add: War Damage Compensation received during year	540	
	Estimated War Damage Compensation receivable (per contra)...	1,000	
		1,540	
		1,966	
	Less: Expenditure during year		
	Buildings reconstruction	1,181	
	Rebuilding East Wing...	515	
	Equipment for East Wing	94	
		1,790	
426			176
	SUNDRY CREDITORS:		
1,051	Accrued Expenses	890	
183	Subscriptions and Grant received in advance ...	180	
			1,070
	BANK OVERDRAFT:		
	Coutts & Co., London		776
£45,780			£48,639

31st March 1950		£	£
	FIXED ASSETS, at valuations as estimated by the Director at 31st March 1951:		
	Boats and Equipment:		
11,000	F.V. 'Sula'	11,000	
200	Motor Boat 'Gammarus'	200	
100	Nets, Gear and General Equipment	100	
11,300		11,300	
7,342	Laboratory Apparatus, Equipment and Machinery ...	8,851	
	Library at valuation of Mr Ridgill Trout in January 1941, plus additions at cost	17,887	
35,829			38,038
	STOCKS ON HAND as valued by the Director:		
	Specimens	600	
	Chemicals	250	
	Journals	400	
1,250			1,250
232	**GENERAL FUND INVESTMENT** at Book Value £352. 2*s*. 3*d*. 2½ % Treasury Stock ... ... (Market Value £237. 13*s*. 6*d*. Last year £239. 8*s*. 9*d*.)		232
5,795	**E. T. BROWNE BEQUEST FUND INVESTMENTS**, at cost £5901. 8*s*. 7*d*. British Transport 3 % Guaranteed Stock 1978/88 ... ... (Market Value £5,222. 15*s*. 4*d*. Last year £5,252. 5*s*. 3*d*.)		5,795
	COMPOSITION FEES FUND INVESTMENTS, at cost:		
	£18. 8*s*. 6*d*. 2½ % Treasury Stock	16	
	£872. 4*s*. 9*d*. British Transport 3 % Guaranteed Stock 1978/88	841	
731	(Market Value £784. 7*s*. 4*d*. Last year £662. 15*s*. 2*d*.)		857
	SUNDRY DEBTORS:		
739	Sale of Specimens, etc.	785	
—	War Damage Commission—Estimated compensation receivable (per contra)	1,000	
			1,785
104	**PREPAYMENTS**		89
	BALANCE AT BANK AND CASH IN HAND:		
	Lloyds Bank Ltd., Plymouth	519	
	Cash in Hand	74	
1,100			593
	Note: The Association had a capital commitment at 31st March 1951, in respect of a new building amounting to £2,200 which will be recoverable under a Treasury Grant.		
	JOHN E. HARRIS O. D. HUNT } *Members of the Council*		
£45,780			£48,639

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1951, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

57 York Street, Plymouth.
16 May 1951.

PRICE, WATERHOUSE & Co.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1951

31st March 1950		£	£
	SALARIES, including Association's contributions to Superannuation Scheme and National Insurance		
15,558			17,275
	LABORATORY AND BOATS' CREWS' WAGES, including National Insurance, contributions to Superannuation Scheme, War Bonus and Employer's Liability Insurance		
12,786			12,828
584	UPKEEP OF LIBRARY		765
	SCIENTIFIC PUBLICATIONS, *less* sales	1,743	
	Less: Grant from Dr M. Knight 30		
	Grant from Birkbeck College 7		
		37	
1,202			1,706
	UPKEEP OF LABORATORIES AND AQUARIUM:		
785	Buildings and Machinery	479	
556	Electricity, Oil, Gas, Coal and Water	651	
918	Chemicals and Apparatus	1,159	
135	Insurances, Tithe, Ground Rent and Rent of Store ...	136	
589	Travelling Expenses	587	
10	Audit Fees (2 years)	74	
577	Stationery, Postages, Telephone, Carriage and Sundries	764	
180	Specimens	207	
			4,057
	MAINTENANCE AND HIRE OF BOATS:		
605	Petrol, Oil, Paraffin, etc.	633	
1,884	Maintenance and Repairs to Nets, Gear and Apparatus	1,486	
143	Boat Hire, Collecting Expenses and Upkeep of Truck	224	
668	Insurances	690	
1,380	Hire of R.V. 'Sabella'	1,380	
			4,413
33	ENTERTAINMENT EXPENSES		40
27	BANK CHARGES		35
2,218	BALANCE, being Excess of Income over Expenditure for the year		2,223
	Note: No provision is made for replacement of Fixed Assets.		
£40,838			£43,342

31st March 1950		£	£
	GRANTS AND TABLE RENTS:		
	Ministry of Agriculture and Fisheries grant from		
34,000	Development Fund	36,350	
500	Fishmongers' Company	500	
	Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s.* 0*d.*, Leeds £20, Durham £10. 10*s.* 0*d.*, Exeter £10. 10*s.* 0*d.*, Leicester £10. 10*s.* 0*d.*, Manchester £10. 10*s.* 0*d.*, Nottingham £10. 10*s.* 0*d.*, Southampton £10. 10*s.* 0*d.*, Hull £10. 10*s.* 0*d.*, Sheffield £10. 10*s.* 0*d.*, Imperial		
1,276	College £10, and Ministry of Works £104.) ...	1,273	
			38,123
	SUBSCRIPTIONS (excluding subscriptions received in		
473	advance)		514
12	FEES FOR TESTS OF MATERIALS		65
	SALES:		
2,521	Specimens	2,350	
361	Fish	410	
	Nets, Gear and Hydrographical Apparatus ... 1,922		
	Less: Cost of Materials 1,494		
225		428	
			3,188
29	INTEREST ON INVESTMENTS		31
8	SALES OF DR M. V. LEBOUR'S BOOK		6
9	SALES OF 'PLYMOUTH MARINE FAUNA'		7
	AQUARIUM:		
	Admission Fees	1,601	
	Sales of Guides and Postcards	108	
		1,709	
	Less: Maintenance of Building 52		
	Printing Tickets and Guides 47		
	Food 105		
	Wages 97		
		301	
1,424			1,408
£40,838			£43,342

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £23,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the Laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. An account of the Laboratory and the scope of the work undertaken there will be found in Vol. XV (p. 735) and Vol. XXVII (p. 761) of this *Journal*.

The Laboratory is open throughout the year and its work is carried out under the supervision of a Director and with a fully qualified research staff. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology and physiology. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat and these also collect the specimens required in the Laboratory.

TERMS OF MEMBERSHIP

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

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

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

The Council of the Marine Biological Association wish it to be understood that they do not accept responsibility for statements published in this *Journal* excepting when those statements are contained in an official report of the Council.

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