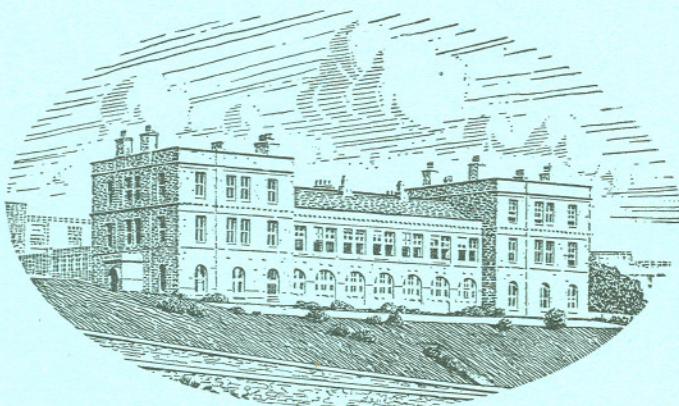


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## STRONTIUM AND BARIUM IN SEA WATER AND MARINE ORGANISMS

By H. J. M. Bowen

Radiobiological Research Unit, A.E.R.E.,  
Harwell, Berks

(Text-fig. 1)

Since the advent of nuclear fission, considerable interest has been aroused in the biological fate of strontium, as two isotopes of this element are among the longer-lived fission products of uranium. Strontium 90, in particular, constitutes the major hazard in old fission products because it is readily taken up by all kinds of plants and may then be concentrated in the bones of animals feeding on the plants. Strontium 90 contamination may be derived from fall-out from atomic explosions or from uncontrolled disposal of fission products. The oceans should receive more than twice as much Strontium 90 from fall-out as the continents because 70% of the surface area of the globe is covered by sea. The oceans are also used, though to a very limited extent at present, for the disposal of fission products from reactor wastes. Hence it is obviously important to study the behaviour of strontium in marine organisms in order to evaluate the health hazard caused by controlled or uncontrolled radioactive contamination of sea water. Barium has only relatively short-lived fission-product isotopes, but can readily be studied at the same time as strontium because of its similar chemistry.

Strontium and barium are elements which have long been known to occur in marine organisms and the sea. In the past, however, there have been considerable difficulties in the analytical determination of these two elements. Strontium, especially in trace amounts, is very difficult to separate from calcium, and while barium is more easily separated it is present in amounts so small as to tax the ingenuity of the conventional analyst. Spectrometric analysis has been used by several workers to determine these elements, but is not always sufficiently sensitive nor very precise. Recently, Harrison & Raymond (1955) and Bowen & Dymond (1955) have described the method of activation-analysis for these elements in animal and vegetable tissues respectively. In this work the same technique has been applied to determine strontium and barium in sea water and in various marine organisms.



## METHODS

*Collection and preparation of specimens*

*Sea water* was collected and stored in polythene aspirators and all chemical manipulations were carried out using polythene apparatus. The chlorinity was determined by the Mohr titration method. Strontium was determined by activating 2 ml. samples of sea water sealed in polythene tubes with neutrons in the Harwell pile, BEPO. The associated activity, largely  $^{24}\text{Na}$  and  $^{38}\text{Cl}$ , after activation was considerable, and repeated precipitation with sodium carbonate was used to ensure complete decontamination from active sodium and chlorine.  $^{139}\text{Ba}$  was separated from the  $^{87}\text{Sr}$ , but could not be counted from such a small volume of sea water. Hence barium was concentrated from 1.5 l. samples of sea water using purified Zeo-Karb 225, sulphonated polystyrene resin. Columns of this resin, 15 cm long and 1 cm diameter, were set up in polythene tubes and were washed successively with 500 ml. 2N- $\text{NH}_4\text{Cl}$  at pH 3.0, 500 ml. 6N redistilled HCl and finally 200 ml. redistilled  $\text{H}_2\text{O}$ . The  $\text{NH}_4\text{Cl}$  was made by passing cylinder ammonia into redistilled HCl in a polythene bottle. After this treatment samples of the resin left no residue after ashing at 500° C. Sea water 'spiked' with  $^{140}\text{Ba}$  was then used to determine the break-through-volume of barium for the columns used. It was established that 88% of the barium in 1.5 l. of sea water was retained by the resin-column using a flow-rate of 2 ml./min. The losses were made up as follows:

Passed through resin	Percentage Ba lost
500 ml. 'spiked' sea water	0.4
1000 ml. 'spiked' sea water	1.9
1500 ml. 'spiked' sea water	8.3
500 ml. N/5 $\text{HNO}_3$ wash	3.8

After passing 1.5 l. of sea water through the column the resin was washed with 500 ml. of 0.2N redistilled  $\text{HNO}_3$  and allowed to drain. Practically all the sodium, potassium and chlorine were removed in the eluent. The resin was then transferred to a silica basin and ashed at 500° C for 24 hr. The barium in the ash was determined by neutron-activation, taking care to remove sulphate ions from the activated ash by repeatedly boiling with saturated sodium carbonate solution and extracting the residue with hydrochloric acid. A 'blank' analysis of the clean resin showed no detectable barium.

*Marine algae* were collected in polythene bags and analysed as soon after collection as possible. Samples of tissue free from encrustations and parasitic algae were selected and washed with dilute choline chloride solution. They were then blotted dry and ashed in silica basins at 500° C: samples of the ash were used for activation-analysis.

*Corals and mollusc shells* were washed with distilled water, dried and ground in an agate mortar. They were not ashed before analysis.



*Analytical methods*

The method of activation-analysis has already been described (Harrison & Raymond, 1955; Bowen & Dymond, 1955). In this work  $^{87}\text{Sr}$  was counted in liquid form in a scintillation counter as previously described, but  $^{139}\text{Ba}$  was generally slurried on to a counting tray as the solid chromate, dried, and counted with an end-window EHM2 Geiger-Muller counter. Chemical yields were better than 95% for strontium and 90% for barium. Determinations of calcium were made by flame-photometry as described by Bowen & Dymond (1955).

## RESULTS

Table I gives the strontium and barium contents of sea water from the English Channel corrected to 19.0‰ chlorinity.

TABLE I. STRONTIUM AND BARIUM CONTENT OF SEA WATER

Vol. sea water taken	mg Sr/l.	Vol. sea water taken	$\mu\text{g Ba/l.}$
400 ml. abs. on resin	8.66	900 ml. abs. on resin	5.8
2 ml. abs. on resin	8.69	1500 ml. abs. on resin	5.9
2 ml. abs. on resin	8.30	1500 ml. abs. on resin	6.8
2 ml. abs. on resin	8.36		
Mean	8.50 <sub>2</sub>	Mean	6.2

These figures should require a small correction because of the presence of uranium in sea water:  $^{235}\text{U}$  undergoes nuclear fission on neutron activation and  $^{92}\text{Sr}$  and  $^{139}\text{Ba}$  are produced. The concentration of uranium in sea water is

TABLE II. ALKALINE EARTH CONTENT OF MARINE ALGAE

Species	Where collected	p.p.m. dry wt.			wt./wt.	
		Ca	Sr	Ba	Ca/Sr	Ca/Ba
Brown						
<i>Fucus serratus</i> L.	Plymouth	8,430	804	14	10.5	800
<i>F. serratus</i> L.	Weymouth	8,270	833	< 23	9.9	> 360
<i>F. vesiculosus</i> L.	Weymouth	8,180	702	9.5	11.6	860
<i>Laminaria digitata</i> Lam.	Plymouth	9,520	1,045	6.5	9.1	1,500
<i>L. digitata</i> Lam.	Weymouth	7,080	783	< 23	9.0	> 310
<i>L. saccharina</i> Lam.	Weymouth	7,720	698	11.3	11.1	680
<i>Ascophyllum nodosum</i> Le Jol.	Weymouth	8,320	428	5.6	19.5	1,500
<i>Chorda filum</i> Lam.	Weymouth	16,300	1240	18.6	13.2	880
Mean		9,228	817	11	12	1,040
Red						
<i>Gigartina stellata</i> Batt.	Plymouth	2,810	133	2.1	21	1,300
<i>Chondrus crispus</i> Lyngb.	Plymouth	6,420	131	5.6	49	1,100
<i>Rhodomenia palmata</i> Grev.	Weymouth	1,050	18.8	0.6	56	1,700
Mean		3,427	94	2.8	42	1,370
Green						
<i>Enteromorpha compressa</i> Grev.	Plymouth	10,300	87	—	120	—
<i>E. intestinalis</i> Link.	Weymouth	4,590	54.8	0.4	84	11,000
<i>Ulva lactuca</i> L.	Weymouth	3,840	38.2	2.5	100	1,500
<i>U. lactuca</i> L.	Plymouth	4,560	67.7	—	68	—
Mean		5,822	62	1.45	93	6,250



about  $1.5 \mu\text{g/l.}$  (Föyn, Karlik, Pettersson & Rona, 1939), and this would give rise to an apparent  $0.0008 \text{ mg/l.}$  of strontium and  $0.2 \mu\text{g/l.}$  of barium.

Hence the correction is negligible for strontium and within the accuracy of the analysis for barium.

Tables II–IV give calcium, strontium and barium analyses for sea weeds, molluscan shells, and corals respectively.

TABLE III. ALKALINE EARTH CONTENT OF MOLLUSC SHELLS

Species	Where collected	p.p.m. dry wt.			wt./wt.	
		Ca	Sr	Ba	Ca/Sr	Ca/Ba
Amphineura						
<i>Acanthochitona crinitus</i> Penn.	Weymouth	239,000	3,950	< 10	60	> 24,000
<i>Lepidochitona cinereus</i> L.	Weymouth	317,000	5,600	20	57	16,000
Mean		278,000	4,775	—	58.5	—
Gastropoda						
<i>Patella vulgata</i> L.	Weymouth	402,000	2,320	< 4	173	> 100,000
<i>Littorina littoralis</i> L.	Weymouth	388,000	1,720	< 5	225	> 80,000
<i>Haliotis tuberculata</i> L.	Sark	412,000	2,340	< 7	176	> 60,000
<i>Rissoa parva</i> Da Costa	Lands End	313,000	1,520	< 6	206	> 50,000
<i>Bittium reticulatum</i> Da Costa	Kimmeridge	361,000	1,420	< 4	248	> 90,000
Scaphopoda						
<i>Dentalium vulgare</i> Da Costa	Herm	406,000	3,550	7.2	114	60,000
Lamellibranchiata						
<i>Arca tetragona</i> L.	Lands End	353,000	3,260	< 4	108	> 90,000
<i>Cardium echinatum</i> L.	Weymouth	386,000	2,260	< 1	170	> 400,000
<i>Pecten maximus</i> L.	Weymouth	404,000	1,630	< 3	247	> 130,000
<i>Lasaea rubra</i> Mont.	Kimmeridge	375,000	1,820	< 25	206	> 15,000
<i>Teredo megotara</i> F. & H.	Weymouth	355,000	1,810	48	196	7,000
Mean		377,700	2,150	—	188	—
Cephalopoda						
<i>Sepia elegans</i> D'Orbigny	Lands End	243,000	2,910	20	84	12,000

TABLE IV. ALKALINE EARTH CONTENT OF RECENT CORALS

Species	Where collected	p.p.m. dry weight			wt./wt.	
		Ca	Sr	Ba	Ca/Sr	Ca/Ba
<i>Millepora</i> sp.	Indian Ocean, 8 fm.	323,000	7,360	8	44	40,000
<i>Allopora norvegica</i>	N. Atlantic, 87 fm.	303,000	7,760	18	39	17,000
<i>Heliopora coerulea</i>	China Sea, 13 fm.	325,000	6,520	85	50	4,000
<i>Caulastrea furcata</i>	China Sea, 32 fm.	324,000	13,400	11	24	30,000
<i>Hydnophora polygonata</i>	China Sea, 6 fm.	310,000	10,700	24	29	13,000
<i>Pocillophora verrucosa</i>	China Sea, 13 fm.	327,000	12,900	11	25	30,000
<i>Psammocora planipora</i>	Indian Ocean, 60 fm.	295,000	5,590	23	53	13,000
<i>Seriatopora elegans</i>	China Sea, $\frac{1}{2}$ fm.	332,000	12,500	11	27	30,000
<i>Leptoseria solida</i>	Indian Ocean, 60 fm.	326,000	8,680	12	38	27,000
<i>Acropora</i> sp.	Persian Gulf, 63 fm.	306,000	8,970	56	34	5,500
<i>Alveopora</i> sp.	Indian Ocean, 39 fm.	296,000	7,840	210*	38	1,400
<i>Merulina</i> sp.	China Sea, 30 fm.	337,000	8,360	450*	41	750
<i>Stylopora</i> sp.	Muscat, 1 fm.	323,000	8,490	43	37	7,500
<i>Montipora</i> sp.		345,000	9,290	—	37	—
<i>Amphihelia</i> sp.		345,000	10,650	—	32	—
Mean		321,100	9,267	29.5	36	16,860

N.B. The two starred barium figures were omitted in taking the mean.

Fossil corals were also investigated and the results are collected in Table V.



TABLE V. ALKALINE EARTH CONTENT OF FOSSIL CORALS

Species	Where collected	Geological age	p.p.m. dry wt.			wt./wt.	
			Ca	Sr	Ba	Ca/Sr	Ca/Ba
<i>Dendrophyllia elegans</i>		Oligocene	361,000	8300	66	44	5,500
<i>Balanophyllia calyculata</i>		Upper chalk	326,000	6850	251	47	1,300
<i>Caryophyllia cylindracea</i>	Wilts	Upper chalk	335,000	640	<5	523	>67,000
<i>Caryophyllia</i> sp.		Upper chalk	345,000	526	35	658	10,000
<i>Coelosmilia regularis</i>	Wilts	Upper chalk	310,000	524	<9	591	>34,000
<i>Holocystis elegans</i>	Isle of Wight	Lower greensand	83,900	278	<21	301	>4,000
<i>Micrabacia coronula</i>	Yorks	Upper chalk	270,000	273	8	990	34,000
<i>M. fittoni</i>	S. England	Gault	265,000	4230	160	63	1,700
<i>Parastraea stricta</i>		Cretaceous	309,000	7640	18	41	17,000
<i>Placosmilia rudis</i>	Lérída, Spain	Cretaceous	312,000	1760	<15	177	>21,000
<i>Trichosmilia granulata</i>		Cretaceous	369,000	530	15	695	25,000
<i>Chromoseris porpites</i>	Boulogne, France	Jurassic	309,000	209	<19	1040	>16,000
<i>Dimorphoseris terquemi</i>	Glos	Jurassic	384,000	295	<6	1300	>64,000
<i>Goniocera socialis</i>	Wilts	Jurassic	335,000	1860	<220	180	>1,500
<i>Isastraea explanata</i>	Berks	Jurassic	391,000	1500	<19	260	>21,000
<i>I. explanata</i>	Berks	Jurassic	365,000	1130	—	322	—
<i>I. limitata</i>	Dorset	Jurassic	337,000	730	<10	461	>34,000
<i>Montlivaltia delabecki</i>	Glos	Jurassic	313,000	538	<28	583	>11,000
<i>M. dispar</i>	Berks	Jurassic	259,000	1356	—	184	—
<i>Oppelismilia mucronata</i>	Leics	Lower Lias	269,000	972	20	276	13,000
<i>O. victoriae</i>	Oxon	Middle Lias	254,000	548	<66	465	>4,000
<i>Rhabdophyllia phillipsii</i>	Berks	Jurassic	386,000	219	<4	1760	>100,000
<i>Stylina tubulifera</i>	Wilts	Jurassic	375,000	1540	<210	243	>1,800
<i>Thamasteria arachnoides</i>	Wilts	Jurassic	351,000	437	—	802	—
<i>T. concinna</i>	Berks	Jurassic	398,000	1180	<2	338	>200,000
<i>T. defranciana</i>	Glos	Jurassic	330,000	575	<3	574	>110,000
<i>Thamasteria</i> sp.	Berks	Jurassic	353,000	810	—	435	—
<i>Thecosmilia annularis</i>	Wilts	Jurassic	371,000	678	<11	549	>34,000
<i>Dibunophyllum bipartitum</i>	Glos	Carboniferous	277,000	418	8	661	35,000
<i>Lithostrotion decipiens</i>	Glos	Carboniferous	196,000	155	<31	1270	>6,000
<i>L. martini</i>	Pembs	Carboniferous	367,000	154	11	2380	33,000
<i>L. pauciradiale</i>	Somerset	Carboniferous	362,000	383	<22	944	>16,000
<i>L. portlocki</i>	Westmorland	Carboniferous	365,000	398	4	917	91,000
<i>Lithostrotion</i> sp.		Carboniferous	353,000	304	—	1160	—
<i>Lonsdaleia floriformis</i>	Glos	Carboniferous	358,000	537	<27	658	>13,000
<i>Michelinia favosa</i>	Pembs	Carboniferous	241,000	<850	17	233	14,000
<i>M. megastoma</i>	Westmorland	Carboniferous	305,000	902	5	338	61,000
<i>Palaeosmilia murchisoni</i>	Glos	Carboniferous	375,000	308	<6	1250	>62,000
<i>P. regia</i>	Glos	Carboniferous	356,000	733	<44	486	>8,000
<i>Alveolites billingsi</i>	W. Ontario	Devonian	—	364	6	—	—
<i>A. cervicornis</i>	Devon	Devonian	389,000	157	7	2480	56,000
<i>Cystiphyllum vesiculosum</i>	Devon	Devonian	113,000	489	35	230	3,200
<i>Favosites goldfussi</i>	Devon	Devonian	400,000	675	3	592	130,000
<i>F. stricklandi</i>	Salop	Silurian	354,000	842	—	418	—
<i>Halysites catenularius</i>	Worcs	Silurian	305,000	1780	<26	172	>12,000
<i>H. catenularius</i>	Salop	Silurian	327,000	973	10	336	33,000
<i>Kodonophyllum truncatum</i>	Salop	Silurian	340,000	828	7	411	50,000

## DISCUSSION

Our value for the amount of strontium in sea water is 5% higher than recent flame spectrometric determinations by Odum (1951*a*) and Chow & Thompson (1955), and an activation determination by Hummel & Smales (1956). The discrepancy may arise since our sample was collected near the shoreline whereas the other workers used oceanic water, but it is within the limits of accuracy of both methods. Our value for barium is considerably lower than has been previously reported. Thus Thompson & Robinson (1932) report 200  $\mu\text{g/l.}$ , and von Engelhardt (1936) found 54  $\mu\text{g/l.}$  by a spectrometric method. The discrepancy is not surprising in view of



Goldschmidt's remarks (1937); 'In several cases we have found that in traditional methods of analytical determination of minor constituents a higher percentage of, for instance, barium, has been contributed from the reagents than from the specimen'. The mean of the published results for calcium in sea water of 19‰ chlorinity is 403 mg/l. (Harvey, 1955), so we find the Ca/Sr and Ca/Ba ratios in sea water to be 47.4 and 65,000 (wt/wt) respectively.

Relatively little work has been reported on the alkaline earth contents of marine organisms. Forchhammer was the first worker to detect strontium (1859) and barium (1884) in the ash of *Fucus vesiculosus*, a species which later work has shown to be an accumulator and a discriminator for both elements. All the later analytical work has been spectrometric in nature. Fox & Ramage (1931) found strontium in nearly all the soft tissues of marine invertebrates they examined, but could not detect barium. Webb (1937) showed that two brown algae, *Fucus serratus* and *Saccorhiza bulbosa*, both discriminate for strontium and barium, while most of the seven molluscs studied discriminated against strontium. The mantle of *Archidoris britannica* is remarkably rich in strontium, which is interesting in view of the recent observations of Fretter (1953) and Rao & Goldberg (1954) that alkaline earths are absorbed largely through the mucus of marine invertebrates. McCance & Masters (1937) also record concentration of strontium by *Archidoris*. Analyses of algae by Borovik-Romanova (1939), Wilson & Fieldes (1941), and Black & Mitchell (1952) are of the same order as those found here. The last authors are responsible for the interesting observation that the strontium-content (p.p.m. dry matter) of all the algae studied diminished by a factor of 3-6 between January and June.

Shells of marine molluscs have been analysed for strontium by Vinogradov (1937) and for barium by Borovik-Romanova (1939) (see also Vinogradov, 1953). Earlier, Thomas, quoted in Thompson & Robinson (1932), stated that the Ca/Sr ratio in shells is roughly equal to the ratio in sea water. Our results do not support this contention, nor do those of Odum (1951*b*) who found a mean Ca/Sr ratio of 174 in a number of recent mollusc shells, in good agreement with our figures. Odum also showed that the shells of brachiopods discriminate against strontium about as much as molluscs: recent corals were found to have a mean Ca/Sr of 43, again in agreement with the present work.

The terms 'accumulation' and 'discrimination' as applied to the uptake of nutrient elements need clear definition, since different authors use these words in different senses. Black & Mitchell (1952) define an 'accumulation factor' which we will call  $A$  here, as

$$A = \frac{\text{p.p.m. element in dry organism}}{\text{p.p.m. element in sea water}},$$

while Spooner (1949) uses a more complex definition based on the effective dilution of sea water in the element per g of organism. In this work we shall

define a 'discrimination factor  $D$ ' for the uptake of a pair of related elements  $X$  and  $Y$  as:

$$D = \frac{X/Y \text{ in dry organism}}{X/Y \text{ in sea water}},$$

so that clearly

$$D(X, Y) = A(X)/A(Y).$$

The rather unwieldy data in Tables II–IV can be summarized in terms of mean accumulation and discrimination factors for the different groups (Table VI). Hence we see that all the organisms studied concentrate calcium, strontium and barium. Marked discrimination for strontium and barium occurs in the brown algae; the other algae discriminate for barium but not strontium. Molluscan shells, unlike corals, discriminate against both strontium and barium.

TABLE VI. MEAN ACCUMULATION AND DISCRIMINATION FACTORS FOR MARINE ORGANISMS

Group	No. studied	A (Ca)	A (Sr)	A (Ba)	D (Sr, Ca)	D (Ba, Ca)
Brown algae	8	23	96	1800	4.2	78
Red algae	3	8.5	11	450	1.3	53
Green algae	4	14.5	7.3	—	0.50	16
Chiton shells	2	690	560	2400	0.81	3.5
Cephalopod bone	1	600	340	3200	0.57	5.3
Other mollusc shells	11	940	250	650	0.27	0.7
Corals	15	800	1090	4400	1.4	5.5

The amount of  $^{90}\text{Sr}$  which will ultimately reach the ocean from all atomic explosions to date is estimated to be about 0.8 megaCurie. If this were uniformly mixed into the ocean, which it certainly is not, this would amount to  $5 \times 10^{-12} \mu\text{C/ml}$ . Since  $10^{-1} \mu\text{C}$  is about the smallest amount of  $\beta$ -activity that can be detected experimentally, 1000 kg of sea water would have to be concentrated down to determine this isotope. However, it should be possible to measure  $^{90}\text{Sr}$  in kilogram samples of coral, since corals accumulate the element by a factor of 1000. This would constitute a very delicate test for the isotope in any given area of ocean. It might be possible to determine the rate of mixing of the oceans by measuring the  $^{90}\text{Sr}$  content of corals from different depths. The hazard from contamination of the ocean by radioactive strontium is negligible at present, in view of the enormous quantities of naturally radioactive substances already existing there, e.g. nearly 500,000 megaCuries of  $^{40}\text{K}$  alone.

The mechanism of accumulation of ions is fairly obvious. Large volumes of sea water are strained of their ions by natural ion-exchange systems in animals and sea weeds, and these ions may then be precipitated as insoluble inorganic structures (as is calcium in molluscs and corals) or retained in the ion-exchange systems (as in sea weeds). In this connexion it is interesting to notice that in

order to increase in weight by 1 g., a coral needs to filter all the calcium ions from 800 ml. of sea water. The barium content of the coral shows that it must have taken up all the barium from 4400 ml. of water, so that the efficiency of calcium uptake must be less than or equal to  $800/4400$  or 18 %. The accumulation factors of calcium and barium for molluscan shells are nearly equal, so that for these organisms the efficiency of calcium uptake may be as high as 100 %. It is much more difficult to account for the discrimination between similar ions which we have found here. It is known, however, that ion-exchange resins always discriminate for the rarer of a pair of similar ions, and it is noteworthy that the very scarce ion, barium, is retained more strongly than the more common ion strontium in almost every case.

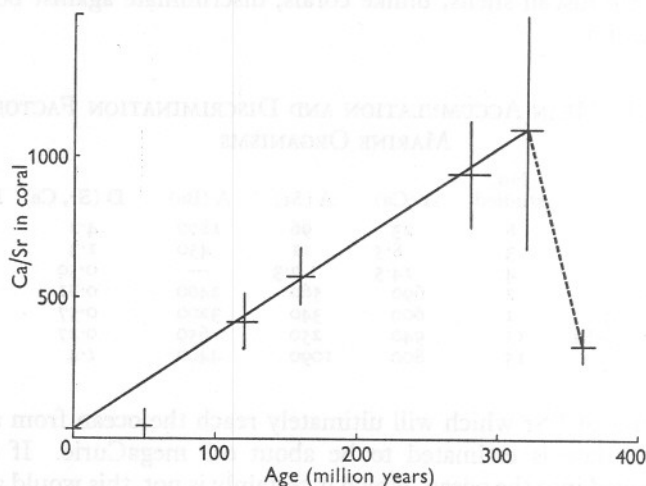


Fig. 1. Apparent Ca/Sr in corals plotted against geological age.

During inorganic precipitations traces of foreign ions are generally left in the mother liquor so that the precipitate is purer than the original solution. Barium and strontium are likewise excluded in the biochemical precipitation of calcium carbonate by molluscs, but not by corals. Some of the molluscs studied were constructed of calcite and others of aragonite, but we could find no consistent difference in strontium and barium content between the two forms. Theoretically one would expect strontium to prefer the aragonite structure to calcite.

The fossil corals were studied in order to check the results of Odum (1951*b*) and Kulp, Turekian & Boyd (1952) regarding the constant level of strontium in the ocean during geological time. These authors analysed a number of recent and fossil molluscs and brachiopods by a spectrometric method and reported that the Ca/Sr ratio remained constant at roughly 175/1 in all the specimens. We have two comments to make on this work.



In the first place molluscs and brachiopods are perhaps not the most suitable organisms for this kind of work since they discriminate against strontium by a factor of four. We have chosen corals since they appear scarcely to distinguish strontium from calcium. Secondly, the present day Ca/Sr ratio in sea water (50/1) is much smaller than in any common igneous or sedimentary rock, where it ranges from 120/1 to 1000/1. This suggests that strontium, like sodium and magnesium, is an element which has accumulated in the sea during the earth's history.

Our results are summarized in Fig. 1. They indicate a progressive increase in the Ca/Sr ratio with geological age, passing backwards in time as far as the Devonian period. The Devonian and Silurian results are of limited value because few samples of this age could be obtained. In addition, it is obvious that in the older samples the Ca/Sr ratios may have been drastically altered from their original values by recrystallization. This may well apply to the four Silurian corals analysed, as there is no reason to suppose a catastrophic change in the Ca/Sr ratio in the ocean between Devonian and Silurian times. Otherwise it appears that strontium has been steadily accumulating in the ocean during geological time.

#### SUMMARY

Strontium and barium have been determined in sea water. The strontium content of sea water was found to be 8.5 mg/l., in agreement with recent work, but the barium content (6.2  $\mu$ g/l.) is lower than previously reported values.

Strontium and barium have also been determined in a number of marine algae, mollusc shells and corals. All the organisms studied concentrate these elements from sea water, but whereas brown algae discriminate markedly for them in preference to calcium, mollusc shells discriminate against them.

The amounts of strontium found in fossil corals are very much smaller than they are in recent corals, and there is an approximately linear relation between the Ca/Sr ratio and geological age as far back as the Devonian period: the Silurian results are anomalous. It is suggested that strontium is an element which accumulates in sea water, though further work is needed to establish the point.

I should like to thank Dr E. J. Bowen, Mr J. M. Edmonds, Dr H. D. Thomas and Mr E. White for supplying most of the specimens for analysis, and Miss J. A. Dymond for much technical assistance.

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## PLANKTONIC EVIDENCE FOR IRREGULAR FLOW THROUGH THE IRISH SEA AND NORTH CHANNEL IN THE AUTUMN OF 1954

By D. I. Williamson

Marine Biological Station, Port Erin

(Text-fig. 1)

During the autumn of 1954 plankton samples were taken in the northern Irish Sea and the North Channel from R.V. *Clupea* (Scottish Home Department) as part of a larger survey of the distribution of herring larvae. At the same period similar samples were taken in the central Irish Sea from R.V. *William Herdman* (Marine Biological Station, Port Erin). The present paper concerns the distribution, as shown by both sets of samples, of a few selected species.

I am indebted to all those on both vessels who assisted in taking the samples and to the Marine Laboratory, Aberdeen, for lending me for analysis the samples taken from R.V. *Clupea*.

### METHODS

In each vessel oblique hauls were taken with a 1 m diameter silk net of 60 meshes to the inch (24 meshes per cm), weighted with a 56 lb (25 kg) iron weight. The relationship between towing depth and length of warp was determined by previous experiment with 'Sealax' sounding tubes. Each haul was taken with the vessel travelling at about 2 knots (3.5 km/h); the net was lowered rapidly to within a few fathoms of the bottom, then hauled steadily at about 9 fathoms (16 m) of warp per minute.

The dates of sampling are shown in Fig. 1 (top left). Within each sub-area shown no pair of neighbouring samples was separated by more than 24 h. Excluding the area to the south of the Isle of Man, all samples were taken within 9 days, and no pair of neighbouring samples was separated by more than 5 days. The stations to the south of the Isle of Man were added after a considerable time lag, so that the samples taken on 14 October were separated by an interval of 17 days from those immediately to the north of them, and the most southerly samples were added another 8 days later. Intervals of more than 7 days between neighbouring samples are signified by broken lines in the three distribution charts (Fig. 1).



The distributions of *Biddulphia sinensis* (Greville), *Sagitta setosa* J. Müller and *Isias clavipes* Boeck are indicated by contouring (Fig. 1). In each chart each contour level is set at 10 times that below it. With the contour levels set at such extremely wide intervals (cf. Cushing, 1953) sampling errors are likely to have little effect on the apparent distributions.

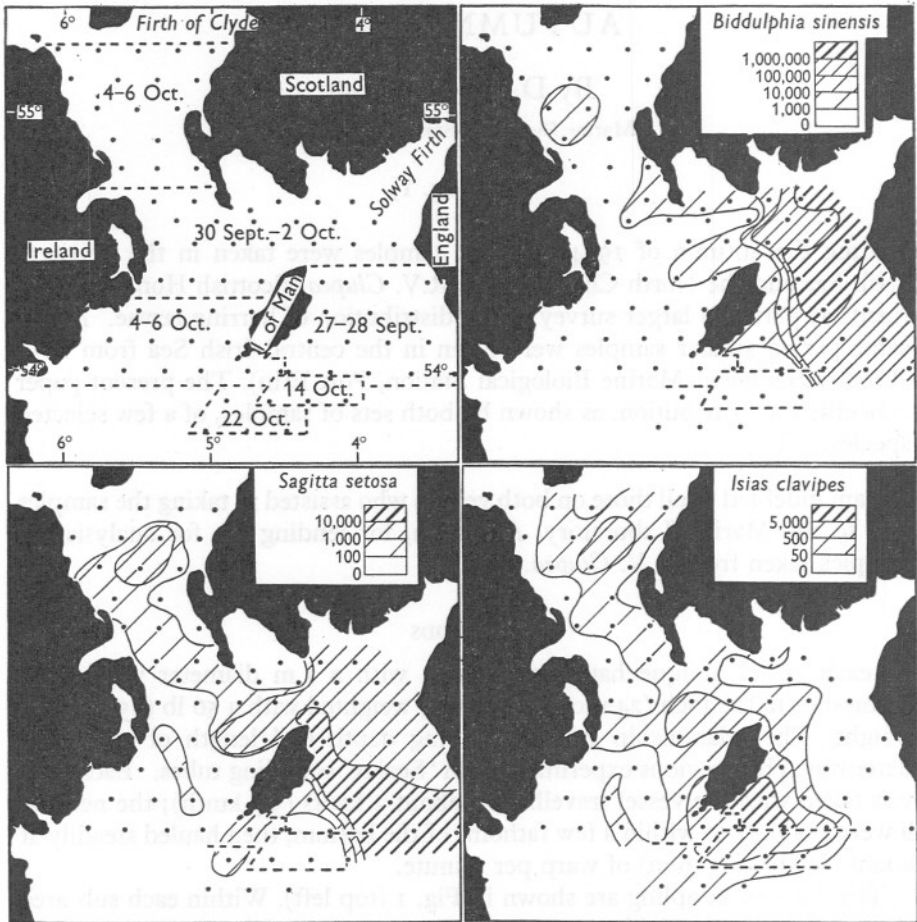


Fig. 1. Top left: North Channel and part of Irish Sea, showing positions and dates of samples. Top right: distribution of *Biddulphia sinensis*. Bottom left: distribution of *Sagitta setosa*. Bottom right: distribution of *Isias clavipes*. Keys relate to numbers of specimens per haul.

## RESULTS AND DISCUSSION

Results of the present and earlier plankton surveys (Williamson, 1952, 1956) are all consistent with the following generalized picture of conditions in the Irish Sea in the autumn. Water enters the southern Irish Sea from St George's

Channel and carries with it *Sagitta elegans*, large numbers of *Isias clavipes* and smaller numbers of other species including *Centropages typicus* and the late larvae of *Nyctiphanes couchii*. The main flow passes to the east of the Isle of Man, where it comes into contact with water very rich in *Sagitta setosa* and *Biddulphia sinensis*. There is considerable mixing in this region, and the north-flowing water itself becomes rich in *Sagitta setosa* and *Biddulphia sinensis*. Beyond the north of the Isle of Man the flow continues westward and northward into the North Channel. Previous surveys have not extended into the North Channel.

The present results (Fig. 1) show that at the time of sampling in the autumn of 1954 there was an area of water containing relatively high concentrations of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* near the north end of the North Channel. The regions of maximum local concentration of *Sagitta setosa* and *Isias clavipes* coincided almost exactly; that of *Biddulphia sinensis* was about 10 miles to the south-west, but the patch of this species overlapped with those of the other two. Altogether the distributions of fifteen species were analysed, but only the three mentioned showed local concentrations in the northern North Channel; as other species were not affected, it is unlikely that the concentrations were formed in the region where they were recorded. These same three species also showed considerable similarity of distribution in a lobe extending westward from the north of the Isle of Man, and their concentrations in this area were similar to those in the patch in the North Channel. From the distributions shown in Fig. 1 it appears that the lobe off the north of the Isle of Man consisted of mixed water from the east of the Island, and it seems probable that the patch in the North Channel was of the same origin. The distributions of *Sagitta setosa* and *Isias clavipes* appear to indicate the path that this body of water had followed from the north of the Isle of Man to the northern North Channel.

The fact that the patch of *Biddulphia sinensis* did not quite coincide with the patches of *Sagitta setosa* and *Isias clavipes* may have been the result of uneven horizontal distribution of the three species in the detached body of water, or it may be related to differences in vertical distribution and indicate some shearing of the body of water.

The results also show a distinct patch of *Isias clavipes* off the south-east of the Isle of Man, but the time intervals in the samples make it impossible to estimate accurately its size and shape. Small numbers of *Centropages typicus* and of cyrtopia larvae of *Nyctiphanes couchii* were also present in this patch but otherwise absent from the Isle of Man area. From previous results (Williamson, 1956) the main source of these three species appearing in this region at this season is likely to be in St George's Channel or farther south.

Seasonal and other long-term variations in the rate of the north-going current through the Irish Sea have been previously suggested, but apart from these the flow has been assumed to be fairly regular (see Bowden, 1955;

Williamson, 1956). The distributions recorded in the present paper would, however, be almost impossible to explain in terms of a steady flow. Reasons have already been given for believing that the body of water distinguished in the North Channel was produced by mixing of waters to the east of the Isle of Man and transport of the mixed water so produced. A steady rate of mixing and a steady flow would have produced a tongue of water whose planktonic character changed gradually through further mixing with increasing distance from the north of the Isle of Man. A temporary increase in the rate of mixing would have produced a corresponding increase in the concentrations of *Biddulphia sinensis* and *Sagitta setosa* and a decrease in *Isias clavipes* in the north-going water, because the former two species are introduced into the north-going water by mixing, while the concentration of *Isias clavipes* is reduced by the same process. Similarly, a temporary decrease in the rate of mixing would have produced a corresponding decrease in the numbers of *Biddulphia sinensis* and *Sagitta setosa* and an increase in the numbers of *Isias clavipes* in the north-going water. Only a sudden and temporary increase in the rate of flow from the Isle of Man area could have produced a marked and simultaneous increase in the numbers of all three species in the north-going water by producing a faster moving body of water which would mix less with surrounding water in a given distance and so maintain its planktonic character for a greater distance. It is suggested that such a 'puff' of water was responsible for the local concentrations of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* recorded in the North Channel.

The patch off the south-east of the Isle of Man containing *I. clavipes* and smaller numbers of *Centropages typicus* and the larvae of *Nyctiphanes couchii* suggests that the flow into the Isle of Man area from the south may also have been irregular.

Salinity measurements are available for all stations except those sampled on 14 and 22 October (Smed, 1955). These show high salinity water off the south-east of the Isle of Man corresponding with the area of greatest abundance of *Isias clavipes*, but they show no patches of high salinity water in the North Channel. As the north-flowing water undergoes sufficient mixing off the Isle of Man to alter drastically its planktonic character, it is not surprising that it should also lose its characteristically high salinity in this region.

On existing information it is not possible to say whether the flow through either the Irish Sea or the North Channel is commonly intermittent. Previous plankton surveys in the Irish Sea (Williamson, 1952, 1956) have given no indication of intermittent flow, but the coverage of the area has never been sufficiently complete to rule out the possibility. Also the sampling area has not previously included the North Channel, and it seems possible that the flow through the North Channel may be intermittent at times even when the flow into the Irish Sea from the south is regular. This could result from a period of south-westerly winds causing an accumulation of water in the Solway Firth



region, followed by the release of the water on a reduction in strength or a change in direction of the wind. (Winds were generally south-westerly throughout September 1954; Smed, 1955.) It may also be relevant that the region off the north-east of the Isle of Man consists of shallow water (much of it less than 10 fathoms, 18 m) and is the meeting place of flood tides from both north and south. These two factors, however, are probably more important in promoting mixing than as temporary barriers to the non-tidal flow.

It is not known how common the occurrence may be, but it is improbable that conditions at the time of the 1954 autumn survey were unique. It seems probable, therefore, that the northward flow through the North Channel may at times consist of comparatively fast-moving pulses of water separated by periods of reduced flow. Under these conditions, each pulse of Irish Sea water seems likely to penetrate much farther north before losing its identity through mixing than the slow average rate of flow would suggest. Bowden (1955) has estimated the mean flow across a section at the southern end of the North Channel to be about  $\frac{1}{4}$  mile (400 m) per day, although this figure is likely to be exceeded in parts of the section.

The Irish Sea has been suggested as the source of populations of *Sagitta setosa* recorded in upper Loch Fyne in the Clyde Sea area (Barnes, 1950) and off the Scottish west coast as far north as the Minches (Fraser, 1952). The first of these examples implies the transport of Irish Sea water through about 120 miles (145 km), of which the last 30 miles (48 km) are a narrow cul-de-sac; the second implies the transport of the water through more than 200 miles (320 km). In both cases the Irish Sea origin of the water seems highly improbable if the rate of transport were only of the order of Bowden's figure (see above), but much more probable if the water were transported in pulses travelling at many times this rate.

#### SUMMARY

The distributions of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* in late September and early October 1954 suggest that the flow through the Irish Sea and North Channel was intermittent at that period.

Some effects of intermittent flow on the transport of Irish Sea water are discussed.

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## TWO NEW MARINE SPECIES OF *GYMNODINIUM* ISOLATED FROM THE PLYMOUTH AREA

By Dorothy Ballantine

The Plymouth Laboratory

(Text-figs. 1-17)

The two new species, *Gymnodinium vitiligo* and *G. veneficum*, which are described below, are both small and highly motile, and in other respects very similar. The greatest difference between them is physiological, as *G. vitiligo* is harmless, whereas *G. veneficum* produces a very powerful toxin which is lethal to fish. There are also small morphological differences which are visible when the organisms are in culture, confirming the existence of two separate species.

I wish to thank Dr Mary Parke of this Laboratory for giving me these two organisms which she isolated, and for her unfailing help and advice, and Dr J. E. Morton, of Queen Mary College, for translating the English diagnoses into Latin.

### SPECIFIC DESCRIPTIONS

#### *Gymnodinium vitiligo* n.sp. (Figs. 1-5)

##### Diagnosis

Cells broadly oval, small, not flattened. Epicone slightly smaller than the hypocone, cell rounded at the apex and antapex. Girdle deeply impressed, narrower on the ventral side, displaced about two girdle widths. Anterior pore at the junction of the left side of the girdle and sulcus, posterior pore at the junction of the right side of the girdle and sulcus. Sulcus extends on to epicone and hypocone, deeply impressed at the junction with the girdle and gradually decreasing in depth towards apex and antapex. Transverse flagellum extending all around the body, longitudinal flagellum very fine,  $1-1\frac{1}{4}$  times the cell length. The right ventral side of the epicone is produced into a hook-like flap which protrudes and overlaps the girdle and sulcus to some extent. Nucleus median, indistinct except just prior to cell division. Cytoplasm hyaline, containing refractile oily bodies. Periplast rigid, but without apparent structure, cell slightly metabolic. Chromatophores golden brown, irregular in shape, usually four (varying from 2 to 8 and occasionally more). Cell size  $7-18\mu \times 7-14\mu$ , most common size  $11.5 \times 9.5\mu$ . Not toxic to fish or other animals.



Isolated by Dr Mary Parke from a sample of sea water from 4 fathoms from the Knap Buoy region off Plymouth Sound (lat. N.  $50^{\circ} 19' 30''$ , long. W.  $04^{\circ} 10'$ ) on 8 June 1949. Type culture (Plymouth collection no. 102) at the Marine Biological Association, Plymouth.

Cellula late ovata, parva, non planata, rotundata ad extremitates apicalem et antapicalem; parte epiconica parum minore quam hypoconica. Cingulo profunde impresso, angustiore ad aspectum ventralem, summoto fere bis suum latitudinem. Foraminibus ad conjunctionem utriusque partis cinguli cum sulco, anteriore sinistrae, posteriore dextrae. Sulco extendente in partem epiconicam necnon hypoconicam, profunde impresso ad conjunctionem cingulo, paulatim altitudine decrescente ut accedit ad extremitates apicalem et antapicalem. Altero flagello transverso totam cellulam circumstante, altero longitudinali tenuissimo, longiore  $1-1.25$  quam cellula. Dextro latere partis epiconicae producto ex aspectu ventrali in processum fere hamuliformem sed et rotundatum qui super cingulum sulcumque nonnullomodo extendit. Nucleo medio, inconspicuo nisi ante fissionem cellulae. Cytoplasmate hyalino, abundante corporibus oleacibus refractilibus; periplasto rigido, clara structura defecta; cellulae forma paulum mutante; chromatophoris aureo-brunneis, forma irregularibus, ex norma 4 (numero variantibus 2-8, nonnunquam pluribus). Cellulae magnitudine  $7-18 \mu \times 7-14 \mu$ , plerumque  $11.5 \times 9.5 \mu$ . Non toxica piscibus et ceteris animalibus.

Isolata a Maria Parke exemplo aquae marinae ad regionem Knap Buoy prope Plymouth Sound, profund. c. 8 metr., 8 jun. 1949. Cultura typica conservata (Collectio Plymouth num. 102) ad laboratorium Marine Biological Association, Plymouth.

### **Gymnodinium veneficum** n.sp. (Figs. 6-17)

#### *Diagnosis*

Cell ovoid, small, not flattened. Epicone and hypocone equal, but epicone more pointed. Girdle deeply impressed, displaced about one girdle width. Anterior pore at the junction of the left side of the girdle and sulcus, posterior pore at the junction of the right side of the girdle and sulcus. Sulcus extends on to the epicone and to the antapex, not deeply impressed, gradually decreasing in depth towards the apex and antapex. Transverse flagellum extending all round body, longitudinal flagellum fine,  $1\frac{1}{2}$  times the cell length. Nucleus median, indistinct except just prior to cell division. Cytoplasm hyaline, containing numerous refractile oily bodies. Periplast rigid, but without apparent structure, cell slightly metabolic. Chromatophores golden brown, irregular in shape, varying in number from 2 to 8, usually 4. Cell size  $9-18 \times 7-14 \mu$ , most common size  $12 \times 9.5 \mu$ . This species produces an exotoxin which is lethal to fish and other animals.

Isolated by Dr Mary Parke from a sea-water sample from the Hamoaze, over Rubble Bank, off King William Point, South Yard, Devonport (lat. N.  $50^{\circ} 21' 50''$ , long. W.  $04^{\circ} 10' 55''$ ) on 23 June 1950. Type culture (Plymouth collection no. 103) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association, Plymouth.

Cellula ovoidali, parva, non planata, partibus epiconica et hypoconica aequalibus, epiconica tamen acutior; cingulo profunde impresso, summoto fere aequae latitudini

suo. Foraminibus ad conjunctionem utriusque partis cinguli cum sulco, anteriore sinistrae, posteriore dextrae. Sulco extendente in partem epiconicam et ad extremitatem antapicalem, non profunde impresso, paulatim altitudine decrescente ut accedit ad extremitates apicalem et antapicalem. Altero flagello transverso totam cellulam circumstante, altero longitudinali et tenui, longiore 1.5 quam cellula. Nucleo medio, inconspicuo nisi ante fissionem cellulae; cytoplasmate hyalino, abundante numerosis corporibus refractilibus oleacibus; periplasto rigido, clara structura defecta, cellulae forma paulum mutante. Chromatophoris aureo-brunneis, forma irregularibus, numerosis 2-8, ex norma 4. Cellulae magnitudine  $9-18 \times 7-14 \mu$ , plerumque  $12 \times 9.5 \mu$ . Cellula substantiam exotoxicam producente quae letalis sic piscibus et ceteris animalibus.

Isolata a Maria Parke exemplum aquae marinae ex Hamoaze, supra Rubble Bank, prope King William Point, South Yard, Devonport 23 jun. 1950. Cultura typica deposita in collectionem culturarum typicarum Cantabrigiensem, sed et ad laboratorium Marine Biological Association, Plymouth (Collectio Plymouth num. 103).

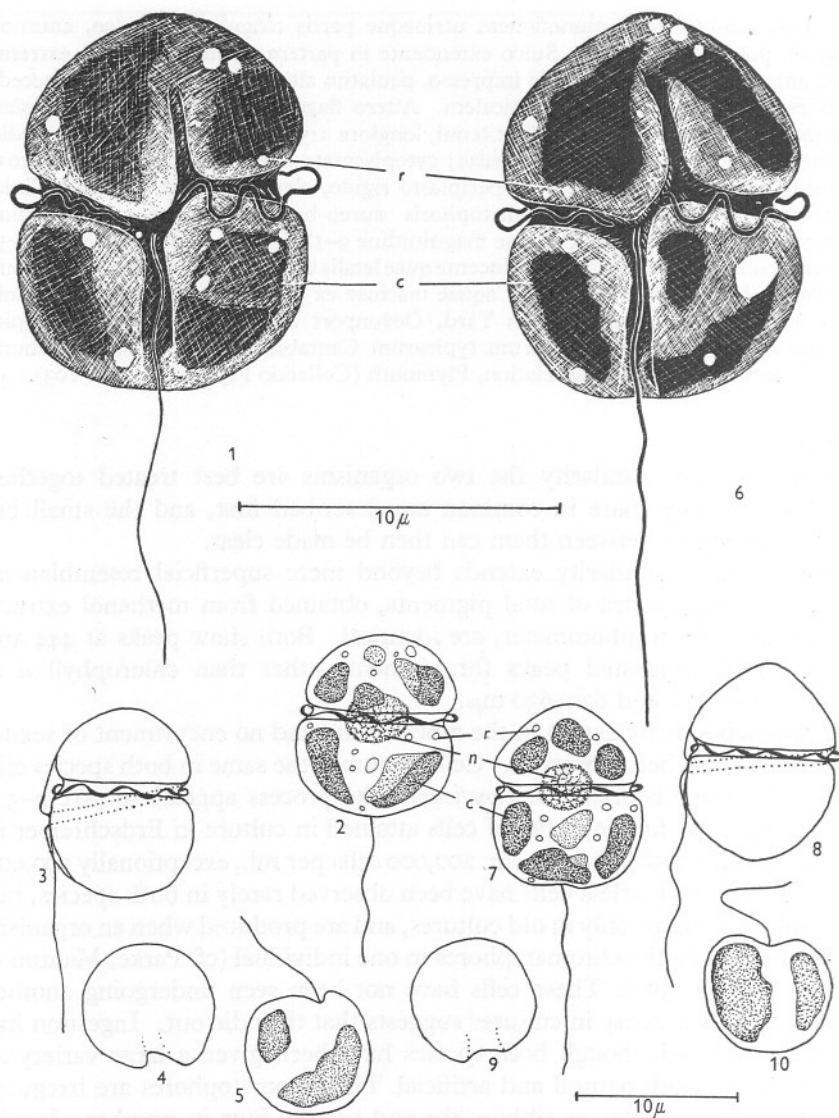
### Description

Owing to their similarity the two organisms are best treated together. The features they share in common are described first, and the small but critical differences between them can then be made clear.

Their obvious similarity extends beyond mere superficial resemblances. The absorption spectra of total pigments, obtained from methanol extracts in a Unicam spectrophotometer, are identical. Both show peaks at 444 and 673  $m\mu$ , with suggested peaks for pigments other than chlorophyll *a* at 425-430, 460-470 and 620-630  $m\mu$ .

Reproduction is by fission in the motile state, and no encystment or sexual reproduction has been observed. Cell division is the same in both species and will be described later for *G. veneficum*. The process appears to take 2-3 h to complete. The final number of cells attained in culture in Erdschreiber in both species is about the same (ca. 200,000 cells per ml., exceptionally 500,000 cells per ml.). Colourless cells have been observed rarely in both species, but these appear to occur only in old cultures, and are produced when an organism divides leaving all the chromatophores in one individual (cf. Parke, Manton & Clarke, 1955, p. 592). These cells have not been seen undergoing another division and their rarity in cultures suggests that they die out. Ingestion has not been observed, though both species have been given a large variety of possible foods, both natural and artificial. The chromatophores are irregular in shape, usually plate- or ribbon-like and usually four in number. In old cultures, however, the chromatophores may split up giving an almost reticulate appearance.

These organisms move in a characteristic fashion. They swim forward in a jerky irregular spiral, usually with the ventral side downwards. Movement is quite rapid and the cell rotates from time to time. The transverse flagellum undulates rapidly in the girdle during movement and the longitudinal flagellum either vibrates rapidly (during active swimming) or remains almost still, trailing behind (sluggish movement).



Figs. 1-5. *Gymnodinium vtiligo*.

Fig. 1. Ventral view. Fig. 2. Dorsal view. Fig. 3. View from the left side, showing the displacement of the girdle and projection of the right side of the epicone. Fig. 4. Apical view, showing sulcus. Fig. 5. Antapical view, showing sulcus and longitudinal flagellum.

Figs. 6-10. *Gymnodinium veneficum*.

Fig. 6. Ventral view. Fig. 7. Dorsal view. Fig. 8. View from the left side, showing displacement of the girdle. Fig. 9. Apical view, showing sulcus. Fig. 10. Antapical view, showing sulcus and longitudinal flagellum. c, chromatophore; n, nucleus; r, refractile bodies.

Satisfactory fixation of these naked dinoflagellates has been found to be impossible. They lose shape or burst in many fixatives, and become absolutely unrecognizable in formalin. The greatest success has been obtained with the vapour from 4% osmic acid, but only small quantities of material can be fixed at one time, and even then many cells round off and throw off their flagella. This means that almost all observations have to be made on living material. Both vital staining and staining after osmic acid fixation have been used to try to determine the position of the nucleus and the composition of the refractile bodies present in both species. These bodies appear to be composed of a variety of substances. Starch appears to be formed in very small quantities by some, but not all, individuals in culture, as occasionally small scattered bodies staining blue-black with dilute iodine are observed. After prolonged contact with iodine the whole cell becomes brown. The remaining, usually very numerous, refractile bodies, which are scattered throughout the cytoplasm show up as orthochromatic granules, or vacuoles, absorbing cresyl blue and neutral red without altering the colour of the stain. They also stain with methylene blue and Nile blue. It is felt that these bodies have a lipid base, as osmic acid and Sudan black B both give a black stain. The periplast shows up with trypan blue or iodine staining, but no structure can be seen in either species. *Gymnodinium vitiligo* appears to have a firmer periplast than *G. veneficum*, as it stays a better shape on fixation and survives longer when examined living under the microscope.

In neither species has a pusule been observed, this being either lacking, or, possibly, obscured by the many other cell contents.

The characters which distinguish these two species are few, and undoubtedly the most important is the toxicity of *G. veneficum*. Whereas all marine animals so far tested have lived for days in *G. vitiligo* culture, fish are killed within a short time by *G. veneficum* (10 min for *Gobius ruthensparri*). This toxicity also affects molluscs, which die in a few hours, and to a lesser extent arthropods and echinoderms. This work will be discussed separately and more fully, in collaboration with Dr B. C. Abbott. The slow toxicity to animals other than fish has already been reported by Bainbridge (1953, p. 393—*Hemimysis lamornae*), Marshall & Orr (1955, p. 510—*Calanus*) and Ballantine & Morton (1956, p. 246—*Lasaea rubra*).

The other distinguishing characters between the two species are morphological and can be seen by comparing the figures (1-10). In general outline *G. vitiligo* is more rounded at the apex (Figs. 1 and 6), has a more deeply impressed sulcus (Figs. 1, 4, 5 and 6, 9, 10) and more displaced girdle (Figs. 3, 8) than *G. veneficum*. The greatest distinction between them under the microscope is the prominence of the hook-like right side of the epicone in *G. vitiligo*. This structure is at times very pronounced, and even if not very well developed shows up the girdle and sulcus very clearly when the cell is living. By contrast there is no protrusion in *G. veneficum*, and the sulcus and girdle



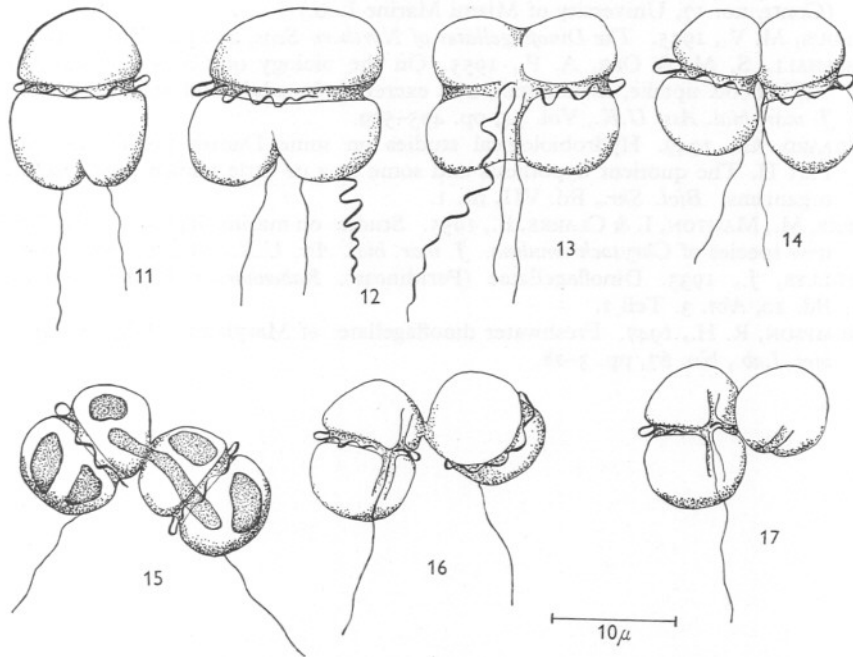
are not so clearly defined, nor so deep. The shape of the sulcus also differs when seen in section, as in Figs. 4 and 5, 9 and 10. These views from the apex and antapex show the influence on the sulcus shape of the hook on the right side of the epicone and the less prominent outward development of the left side of the hypocone of *G. vitiligo* as compared with the almost equal development at each side of the sulcus in *G. veneficum*. In addition to these features the longitudinal flagellum of *G. veneficum* is longer and coarser.

With regard to the relationships of these two species within the genus, little can be said. The nearest species appear to be *G. simplex* (Lohmann) Kofoid & Swezy, *G. pygmaeum* Lebour, *G. marylandicum* Thompson and *G. mirum* Ütermohl. *G. brevis* Davis (1948), which is also toxic, differs greatly morphologically, being lobed and flattened, and the remaining species listed all have quite marked distinguishing characters. *G. simplex* has a straight girdle, a sulcus that does not extend to the epicone (Lebour, 1925) and much smaller cells ( $1.7-7\mu$ ). The sulcus in *G. pygmaeum* Lebour (1925) extends over both apex and antapex and is overlapped on the left side by a tongue-like process, absent in the new species. The remaining two species are freshwater. In *G. mirum* Ütermohl (= *G. rotundatum* Schiller non Klebs and *G. obesum* Schiller, 1933, see Nygaard, 1949) the general shape is similar, but the girdle is straight or only very slightly displaced, the sulcus is very oblique on the epicone and the chromatophores are numerous and small. *G. marylandicum* Thompson (1947) somewhat resembles *G. veneficum* and *G. vitiligo* from the ventral aspect, but differs in being dorso-ventrally flattened. Thus apparently there are no very closely related species in the genus, which though large is very diverse.

#### NOTES ON CELL DIVISION

Cell division was observed closely in both species, is the same in each, and has been illustrated for *Gymnodinium veneficum* (Figs. 11-17). The first stage of division is observed as a doubling of the longitudinal flagellum and indentation of the antapex (Fig. 11). Shortly after this stage the organism becomes somewhat quiescent. Indentation of the hypocone continues and the new transverse flagellum appears, one remaining in the girdle and the new flagellum free and undulating, usually directed towards the hypocone (Figs. 12, 13). When division of the cell has reached a point above the girdle the new transverse flagellum encircles the body and goes into position in the girdle (Fig. 14). Division of the epicone now proceeds from all directions, until only a thin connexion of cytoplasm is left (Fig. 15). Division of the nucleus is complete at this stage, but nuclear division has not been observed at all stages as the nucleus, though large and median in position, does not show clearly except just prior to division. It was found impossible after fixation to

ascertain the stage to which division had proceeded, and thus alcoholic stains could not be used. Normally there are four chromatophores, but an early stage after division may contain only two, which later divide. The chromatophores may also divide prior to, or during, cell division, and organisms giving the appearance of Fig. 15 are not uncommon. The joined individuals are by now again rotating rapidly and irregularly, and during this gyration they pivot



Figs. 11-17. Cell division in *Gymnodinium veneficum*.

Fig. 11. Early stage, two longitudinal flagella and divided antapex. Fig. 12. Dorsal view, both flagella duplicated. Fig. 13. Slightly later stage than 12, ventral view. Fig. 14. Hypocones completely separate. New transverse flagellum is now in the girdle. Figs. 15-17. Two new individuals still joined, but twisting round prior to separating. Fig. 15 shows chromatophores in a pair at this stage.

about the connecting strand until they are lying in planes at right angles (Figs. 16, 17). They then part, probably as a result of this jerky movement breaking the connecting strand.

#### SUMMARY

Two new species of dinoflagellate, *Gymnodinium vitiligo* and *G. veneficum*, have been described and the toxicity of *G. veneficum* is discussed briefly. Notes and figures on cell division in these organisms are also included.

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## ON A PELAGIC PENAEID PRAWN, *FUNCHALIA* *WOODWARDI* JOHNSON, NEW TO THE BRITISH FAUNA

By Isabella Gordon, D.Sc. and R. W. Ingle

British Museum (Natural History), London

(Text-fig. 1)

On 30 July 1955 Dr J. H. Fraser, of the Scottish Home Department, Marine Laboratory, Aberdeen, sent to the British Museum for determination two adult females of a penaeid prawn collected at 01.56-02.0 h B.S.T. near the surface (15 fm.) at Rosemary Knoll, to the north-west of the Outer Hebrides, on 22 July 1955. The actual depth at this locality, 59° 12' N., 10° 09' W., is 229 fm (419 m). These specimens were identified by one of us (R. W. I.) as *Funchalia woodwardi* Johnson. Later the senior author confirmed this determination and wrote to Dr Fraser as follows:

This is the first British record of the genus *Funchalia*. . . For some reason not understood adult specimens of *Funchalia* are rarely captured, unless by fish. . . In 1936 Burkenroad showed that two species had been confused under *Funchalia* (*Funchalia*) *woodwardi* Johnson and in 1940 he added two new species in the *Dana* collection, one from the Indian Ocean, the other from the Canaries. The older references do not, therefore, necessarily refer to *woodwardi*. Authentic adult specimens of *F. woodwardi* are known from Madeira (holotype, ♀ in B.M.); off South Africa (Calman, 1925, 3 ♀♀, 2 in B.M.; Barnard, 1950, 16 ♂♂, 6 ♀♀, all from stomachs of stock-fish, *Merluccius*); and off Nice in the S. of France (2 ♀♀ in Villefranche-sur-mer Colln. Roger, 1938).

She also asked to see the third specimen, which proved to be an adult male. Dr Fraser's reply was interesting:

I had no idea, when we caught them [*Funchalia*], that they would turn out to be anything so unusual. We only retained three, out of a total of 84 in a half-hour haul, thinking a quick glance through the literature at Aberdeen would tell us that they were something common enough. (I must confess the remainder of the catch went to prove their comestible value!) Indeed it seems as though they are common enough, given the right type of gear to catch them and used in the right place. Our programme in July was specifically arranged to look into the identification of traces made on the echo sounder and these prawns were the cause of one of them. . . The gear was an Icelandic Pelagic Trawl specially fitted with a small mesh cod-end (the net designed for catching herring), and I expect we are the first to use such a net near the surface over these oceanic banks.

I do not know yet for certain if they are subject to diurnal migration, but I strongly suspect they are distributed in the deeper layers during the day, where they are caught by the deeper living fish, such as *Merluccius*, and swarm at the surface at night as we found them. [Letter to I. G. dated 17 November 1955.]



Later in September Dr Fraser sent a photo copy of the appropriate part of the echo trace.

It starts at 00.40 h on 21/vii/55 with a distinct double trace, at 15–20 fm. and at 35–40 fm. approximately, and we chose to sample the lower one first. This gave a haul of small fish only—*Gadus poutassou* and scopolids, mostly *Myctophum glaciale*. By the time we had completed the first haul the two traces had merged—at about midnight (01.40 h B.S.T. at that longitude)—and our second haul thus sampled the mixture. This caught similar numbers of *G. poutassou* and scopolids, and in addition 84 *Funchalia*, so that it seems justifiable to infer that the upper trace prior to midnight was *Funchalia*. This was first seen to appear as a scattered trace at about 23.00 h the previous evening, i.e. about 2½ h before midnight, either because we steamed into the shoal at that time, or because the prawns had started shoaling in the upper layers.

The colour, in life, of the specimens was a pleasant yellowish-orange with a very bright scarlet area (still to be seen in the preserved specimen, though not so brilliantly) on the back of the cephalothorax. [Letter to I. G. dated 26 November 1955.]

### *Funchalia* Johnson

For references and synonymy see Burkenroad, 1936, p. 126; Barnard, 1950, p. 608.

At present the genus *Funchalia* comprises five species, namely *F. woodwardi* Johnson, 1867, *F. villosa* (Bouvier, 1905), *F. danae* Burkenroad, 1940, *F. taaningi* Burkenroad, 1940, and *F. balboae* (Faxon, 1893). Burkenroad (1934, pp. 76–7, and 1936, p. 136) thought it necessary to establish a new subgenus *Pelagopenaeus* for the last-named species, which differs in several minor details from the first two species. But this difference breaks down, at least in part, since the thelycum of his species *Funchalia danae* resembles that of *Pelagopenaeus*, and as moreover there are so few species in the genus, it seems to us that subgenera are unnecessary.

For many years all specimens of *Funchalia*, whether adult or immature, were referred to *F. woodwardi*, but, as stated in the letter to Dr Fraser, Burkenroad (1936, pp. 128–35) found that two species had been confused under that name. Roger (1938, p. 25) arrived independently at the same conclusion, for he writes: 'Il semble bien que le genre *Funchalia* d'après les auteurs présente au moins deux espèces différentes actuellement connues. *Funchalia vanhoeffeni*, que Lenz et Strunck ont décrit, différerait assez notablement de *Funchalia woodwardi* Johnson'. Roger was apparently unaware of Burkenroad's paper when he described the two females of *F. woodwardi* in the Villefranche-sur-mer collection. Burkenroad used Bouvier's name *Hemipenaeopsis villosus*, later retracted in favour of *Funchalia woodwardi*, for this second species. Roger's record of *F. woodwardi* from the Mediterranean would seem to confirm Burkenroad's suggestion that larvae referred to *Aristeus antennatus* by Monticelli and Lo Bianco (1902) and to *Aristeomorpha foliacea* by Stephensen (1923) are probably referable to *Funchalia woodwardi*. Where no information has been given as to the number of rostral spines and other characters, or where the material is immature, it is probable that either

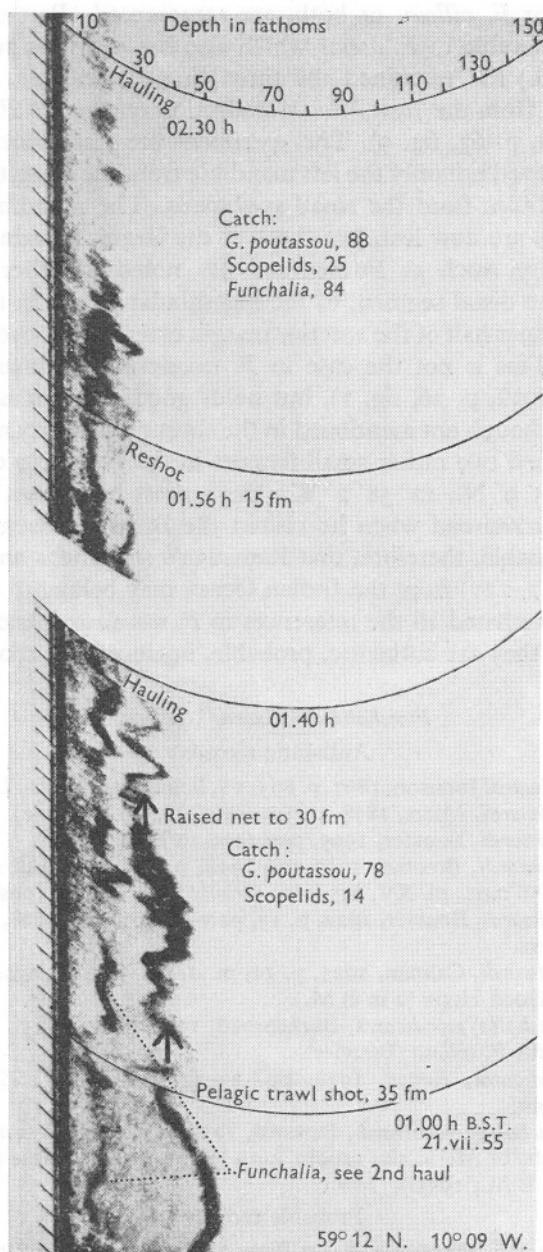


Fig. 1. Echo trace prepared by Dr Fraser. The actual depth is 229 fm and the bottom does not therefore appear on this trace, phased at 0-160 fm. The time scale on left-hand edge is in 2 min ticks. The time at the start and finish of each of the two hauls is given in B.S.T. and the natural midnight at 10° W. is at 01.40 hr. The first catch comprised 78 *Gadus poutassou* and 14 scopelids; the second 88 *G. poutassou*, 25 scopelids and 84 *Funchalia woodwardi* Johnson.

*F. woodwardi*, or *F. villosa*, or both, are represented. Part of the material may even be referable to *F. danae* which also has a similar rostral formula. One of us (I.G.) has examined the three small specimens, the largest an immature male, from the *John Murray* collection referred to *F. woodwardi* by Ramadan (1938, p. 63, fig. 9). The specimens are imperfect and Ramadan apparently removed not only the left mandible from the largest specimen, but also both mandibles from the small specimens. The mandibles of the two small specimens are now lost, but those of the largest remain. They show a slight asymmetry, such as Burkenroad has noted in other specimens of *Funchalia*, in the distal segment of the mandibular palp. In the left mandibular palp the inner half of the anterior margin extends far beyond the shallow emargination. This is not the case in *F. woodwardi* (Calman, 1925, pl. 3, fig. 7; Roger, 1938, p. 26, fig. 1), but holds good for *F. danae* Burkenroad (1940, p. 36), although not mentioned in the short preliminary note. However, we have examined two rather small females in the *Discovery* collection from St. 1594, 04° 15' 9" N., 12° 58' 2" W.; these must have been determined as *F. danae* by Burkenroad when he visited the British Museum in 1938-39. It is highly probable, therefore, that Ramadan's specimens and perhaps that of Balss (1925, p. 227) from the Indian Ocean may belong to *F. danae*.

Below we have listed all the references to *F. woodwardi* known to us indicating whether they are authentic, probable, or uncertain, records.

*Funchalia woodwardi* Johnson

Authentic records

- Funchalia woodwardi* Johnson, 1867, p. 895. 1 ♀, holotype in B.M. Locality Madeira.  
*Funchalia woodwardi*, Miers, 1878, p. 309 (reference to holotype).  
*Funchalia woodwardi*, Bouvier, 1905, part (acc. to Burkenroad).  
*Funchalia woodwardi*, Bouvier, 1907 and 1908, p. 93, part. For example ♂ from St. 1856,  $l = 56$  mm., pl. XV, figs. 1-2; *Grimaldiella richardi* (post-larval) in part.  
*Funchalia woodwardi*, Bouvier, 1922, p. 13, part—♂ from St. 3028,  $l = 100$  mm. and perhaps others.  
*Funchalia woodwardi*, Calman, 1925, p. 10, pl. 2, fig. 5, pl. 3, figs. 6-8. 3 ♀♀ from off Cape of Good Hope (2 in B.M.).  
*Funchalia* (*Funchalia*) *woodwardi*, Burkenroad, 1936, pp. 129-35. No new records; compared with *F. villosa* (Bouvier).  
*Funchalia woodwardi*, Roger, 1938, pp. 23-32, figs. 1-4. 2 ♀♀ from Western Mediterranean.  
*Funchalia* (*Funchalia*) *woodwardi*, Barnard, 1950, p. 609, fig. 112 a-h. 16 ♂♂, 6 ♀♀, from stomachs of *Merluccius* caught Jan.-Feb. 1943, off Table Bay. (3 ♀♀, 6 ♂♂ presented to B.M., 1955.)

Probable records

- Aristeus antennatus*, Monticelli & Lo Bianco, 1902, p. 30. Probable larvae of *F. woodwardi* acc. Burkenroad, 1936, p. 135.  
*Aristeomorpha foliacea*, Stephensen, 1923, p. 15, fig. 6. Probable larvae of *F. woodwardi* acc. Burkenroad, 1936, p. 135.  
 ? Larva of *Funchalia woodwardi*, Stephensen, 1923, p. 26, fig. 10.

## Uncertain records

## (a) Atlantic Ocean

*Funchalia woodwardi*, Murray & Hjort, 1912, p. 668 in Table.

*Funchalia woodwardi*, Sund, 1920, p. 32, 67 immature specimens—the majority from night hauls and 45 from between 50 and 150 m depth.

*Funchalia woodwardi*, Stephensen, 1923, p. 17. 17 specimens—small ♂♂ and ♀♀ from 45 to 70 mm in length, but no details given; the rest young. (8 in 8 hauls at 7 stations in Mediterranean and 9 in 4 hauls at 3 stations in Atlantic.)

*Funchalia* sp., Lenz & Strunck, 1914, p. 307, fig. 3a and b. One immature specimen, off St Helena. The number of rostral teeth suggests either *F. woodwardi* or *F. danae*.

## (b) Indian Ocean

*Funchalia woodwardi*, Balss, 1914, p. 593; 1925, p. 227. 1 ♀ about 50 mm long at 16° 8' S., 97° 14' E.

*Funchalia* (*Funchalia*) *woodwardi*, Ramadan, 1938, p. 63, fig. 9. Three young, the largest an immature ♂. Perhaps referable to *F. danae* Burkenroad (I.G.).

## REMARKS

Between 1867 and 1938 adult specimens of *Funchalia woodwardi* were captured sporadically in very small numbers (1 to 3) at various localities in the eastern Atlantic, from the Cape region to approximately 40° N., and in the western Mediterranean. Good descriptions of the female have been given by Calman (1925) and by Roger (1938). For the first time a considerable number of adults were obtained in January–February 1943 from the stomachs of *Merluccius* by Mr Rattray of the Low Temperature Laboratories, Cape Town, while he was studying the food and diseases of the stock-fish. Although many of the specimens were mutilated, Barnard was able to describe and figure for the first time the petasma and appendix masculina of the adult male (Barnard, 1950, p. 609, fig. 112e and f). The petasma is asymmetrical, either the left or the right half being the longer.

The haul mentioned by Dr Fraser in the letters quoted above (see p. 475) is of special interest because for the first time free-swimming adults were obtained in quantity—84 in a half-hour haul. The three specimens selected at random as a sample for determination are a male and two females measuring from 120 to 130 mm in length, neither female being impregnated. The catch, therefore, apparently consisted of adults of both sexes. Moreover, this is the first British record and by far the most northerly locality for the species. The depth at which the adults were swarming, as indicated by the upper trace on the echo sounder, was approximately 15–20 fm or less than 40 m.

Little is known of the bathymetric range, habits or development of *Funchalia*. Bouvier regarded the genus as bathypelagic, though capable of ascending to the upper layers; 'from the catches of the *Michael Sars* it would appear that the young at least are denizens of the upper water layers though not of the



very surface' (Sund, 1920, p. 32). Most of the *Michael Sars* specimens were collected in night hauls at 50–150 m, but of the seven taken in day hauls four were also from 50 and 100 m (Murray & Hjort, 1912, table on p. 668)\*. Burkenroad says that *Funchalia* is a pelagic genus with autogenous statolith in the statocyst chamber which, however, retains an opening to the exterior (1936, p. 128). Sund regarded all his material as immature, although the largest specimen measured 75 mm in length; Bouvier and Stephensen sexed their specimens of about 45–70 mm but the majority of these, if not all, were probably not fully adult. The maximum recorded length is approximately 157 mm (holotype, rostrum damaged). It is quite likely that *F. woodwardi* may exhibit vertical diurnal migrations of considerable magnitude such as have been described for *Gennadas* and other deep-water Crustacea Malacostraca (Waterman *et al.*, 1939).

The British Museum authorities are indebted to the Director of the South African Museum for nine specimens obtained from stomachs of *Merluccius* and to the Director of the Scottish Home Department, Marine Laboratory, Aberdeen, for permission to retain the male and one female from Rosemary Knoll.

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\* This work lists sixty-two specimens, but the total given in Sund, 1920, p. 32, is sixty-seven.

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## THE NANSEN CLOSING METHOD WITH VERTICAL PLANKTON NETS

By R. I. Currie and P. Foxton

National Institute of Oceanography, Wormley, Surrey

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

Several methods have been devised for sampling plankton in the different water layers in the ocean, but for many years vertically hauled nets closed by a method devised by Nansen (1915) have fulfilled this purpose. Recently, however, an investigation by Barnes (1949) cast doubt on the validity of this method for quantitative work, and as in his paper he specifically referred to nets of the type used on the 'Discovery' Expeditions, the present observations were conducted to determine whether Barnes's findings were of general application to the method.

The Nansen closing principle, which will be described in more detail later, employs a throttling rope which encircles the net some distance behind the mouth, and which becomes the towing line when the front towing bridles are released, and closes the net. Although the method is adaptable to almost any medium-sized net, with vertical hauls it is most frequently used with the Nansen net (Künne, 1929), and in this manner it has been employed extensively. Hjort in the Norwegian plankton investigations (Hjort & Ruud, 1927, footnote, p. 32; Murray & Hjort, 1912, p. 46), the 'Discovery' Expeditions in the Antarctic, the British, Australian and New Zealand Antarctic Research Expedition, the Australian, South African and many other fishery research organizations have all used the method.

More recently, in view of the claims made for the superiority of obliquely hauled nets (mostly on the grounds that they are less liable to be affected by patchiness of the plankton distribution), many workers have tended to replace the vertical net with such devices as the Clarke-Bumpus sampler and other obliquely towed apparatus (Wiborg, 1948). In the collection of zooplankton in deep oceanic waters, however, practical problems arise. Oblique hauls in several thousand metres of water become a tedious and time-consuming operation, whereas vertical hauls can conveniently be carried out at the same time as water-sampling and other such operations. Furthermore, one can be much more certain of fishing a specific depth horizon with a vertical haul.

It is also desirable in plankton collection to use standardized methods as far as is possible. This simplifies greatly the comparison between observations made by different workers, at different times and in different places. It was,

therefore, not only with regard to the vast amount of earlier work which has been done with the method, but also in view of future work which is anticipated, that it was felt a matter of some importance to undertake the present observations.

It was originally intended to study the closing of the net in an experimental tank, watching, by means of coloured dyes or streamers, the movement of water in the net during the closing process. It was at once apparent, however, that it would be impossible to simulate in the laboratory the actual working conditions which prevail at sea. The effects of the rolling of the ship and the action of the special davit from which the net is used would be extremely difficult to reproduce in the laboratory. In view of this, it was decided that direct observation of the normal performance of the net at sea would be the only satisfactory way of studying the closing process and then a record could be made of it by means of underwater cine-photographs. Suitable opportunity for doing this occurred in September and October–November of 1955, when the R.R.S. *Discovery II* was working in the region of the Canary Islands. Preliminary observations were first made by swimming with face masks while the net was being operated from the ship, and later, for the underwater cine-photography, the ship went to the Salvage Islands. Here, the water was warm and exceptionally clear, presenting ideal conditions for photography, and it was possible to anchor the ship in the calm water under the lee of the islands.

As far as we are aware these photographs are the first attempt to record the performance of a plankton net under actual working conditions at sea.

#### BARNES'S OBSERVATIONS

The data which were analysed by Barnes (1949) were obtained by Marshall, Nicholls & Orr (Nicholls, 1933) in their study of the Clyde Sea Area in 1932. The net hauls were taken by means of a modified International net fitted with a Nansen (1915) closing mechanism. The net, according to Nicholls (1933), differed from the standard pattern of Ostenfeld & Jespersen (1924) in that 'the cylindrical part of the net was made of canvas instead of netting and the conical part of fine-meshed bolting-silk (77 strands in 1 cm). An ordinary townet bucket was used. A hand winch was used for hauling the net, the rate at first being kept as nearly as possible to half a metre per second.' Dr Barnes informs us that no accumulator springs were used on the davits.

From a statistical analysis of the composition of the catches of undivided and divided hauls (divided meaning that the net was hauled through a specific depth horizon and then closed), Barnes reached the conclusion that a significant loss of catch took place from the divided haul or, in other words, when the Nansen closing method was brought into use. He illustrated this in particular from the catches of *Microcalanus pygmaeus*, a copepod which was present only *below* the level of closure of the divided haul. Of this organism



the catches in the divided hauls were significantly less than those in the undivided hauls.

To determine the cause of this loss from the divided haul, he carried out some experiments with a model net in the mouth of which was hung a bag of powdered potassium permanganate. An electric motor was used to tow the model horizontally in a tank, and closing was simulated by stopping the motor and reducing the forward speed of the model by a line attached to the tail of the net. A colour film taken of those experiments showed that when the net was stopped the water in the net tended to flow out of the mouth of the net. Recently Motoda & Anraku (1955) have observed this to occur with a full-scale net. Thus it seemed that in the full-scale net it must be the deceleration or falling back of the net while it closed that accounted for the loss of catch.

The net which Barnes considered, and a model of which he used in his experiments, he refers to as the 'Discovery' net. However, the measurements show that it is actually a model of the 'Discovery' phytoplankton net, the N 50 V, a net which is practically never used with the Nansen closing mechanism. Only on a few occasions has the N 50 V ever been closed, and this only to prevent ripping of the net as it comes out of the water in very bad weather.

The net which is normally used with the Nansen closing mechanism on board the R.R.S. *Discovery II* is the so-called N 70 V. This net is larger and of much coarser mesh than the N 50 V, and this fact combined with the method of operation led us to expect that its behaviour might be quite different from the International net, and models of the N 50 V which Barnes used.

#### THE 'DISCOVERY' NET (N 70 V)

This net was based on that used by Hjort (Hjort & Ruud, 1927, p. 32, footnote), and is described by Kemp, Hardy & Mackintosh (1929). It is similar to the Nansen net of Künne (1929). It is 70 cm. in diameter at the mouth and is constructed of four sections, the first three of which are almost cylindrical while the final section tapers conically to the net bucket. The first section is of  $\frac{1}{4}$  in. mesh netting, and this is followed by a canvas band bearing six brass rings through which the throttling rope is led. The third section, which is almost cylindrical, is a length of bolting-silk having 40 meshes to the linear inch, and the fourth section which tapers conically to the net bucket is a length of bolting-silk having 74 meshes to the linear inch. The net bucket has on its side a gauze window of 140 meshes to the linear inch. The throttling rope is  $16\frac{1}{2}$  ft. long, which allows 4 in. of slack when the net is properly rigged. It is threaded from the release gear down and around the canvas band and back up to the release gear, so that the net when it closes is caught up in a bight of this rope. The net is towed by three bridles,  $\frac{1}{4}$  in. brass rods 32 in. long joining into a single ring which fits into the closing mechanism. Below the net is a weight of about 40 lb. supported on three phosphor-bronze stays, which

run the full length of the net and are attached both to the net bucket and the net ring.

With the ship hove to, the net is lowered to the required depth on a wire running over a metre counter on a specially designed davit. The latter incorporates a long accumulator spring on a movable sheave (Text-fig. 1). With the rolling of the ship this spring helps to keep an even strain on the wire, preventing any undue slackening as the net is paid out. On reaching the necessary depth, the steam winch is put in gear, and a messenger on a long line is clipped on the wire. Hauling is commenced, and a steady rate of 1 m/sec (compared with  $\frac{1}{2}$  m/sec of the International net) can easily be maintained by regulating the steam valve, while a stopwatch is used to check the readings of the metre counter. As the net is hauled upwards, the winch and accumulator spring maintain a tension on the wire to the net which never relaxes, even in the very worst weather conditions. One can be fairly certain, therefore, that the net travels upward at quite a uniform speed. At a predetermined instant the messenger is released from the line—e.g. if it is desired to close the net at 500 m the messenger would be released at a metre counter reading of 660 m, since it travels at 3.12 m/sec and would take 160 sec to reach 500 m—the hauling time required for 160 m. The closing of the net can easily be seen by a sudden drop of the accumulator sheave, and only very rarely is the depth of closure more than 1–2% different from the calculated depth even on a 500 m haul.

As the messenger hits the release mechanism, the brass towing bridles are released, and the towing point transfers to the throttling rope which closes the net by tightening the bight behind the mouth of the net. When this takes place the accumulator spring suddenly extends, taking in the slack on the wire, while at the same instant the steam winch accelerates momentarily until the wire to the net is once more taut.

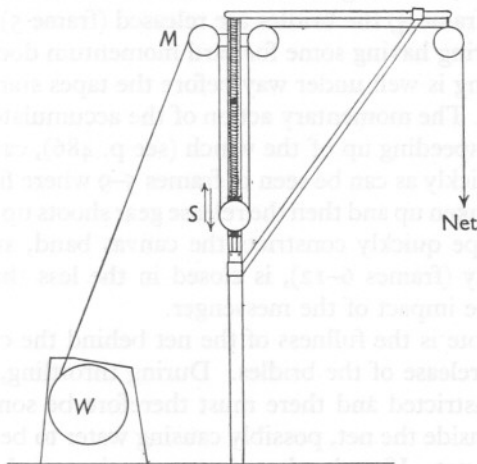
#### THE UNDERWATER OBSERVATIONS AND PHOTOGRAPHS

For the purpose of observing the net closing underwater the ship was anchored in about 15 fathoms of water to the leeward of Selvagem Grande (in September) and Selvagem Pequena (in November). The net was fished from the port forward well-deck winch to facilitate the photography, which was controlled from a rubber dinghy, the swimmers being equipped with face masks and frogmen's feet. In a calm sea and with the ship at anchor, the net-wire led vertically into the water. It was normally paid out to 25 m, at which depth, in the clear waters of the Salvage Islands, it was perfectly visible, and it was thus possible to observe the net throughout the whole of its operation.

To ensure that the net would close just below the surface, two large stream-lined messengers were clipped on the wire and suspended about 1 m below the surface. The bore and weight of the messengers were such that the

wire could be hauled through them, so to speak, without any tendency for the messengers to ride up the wire out of the water. The net, on the impact of the release mechanism and messenger, closed in the normal manner. In the hope that they would indicate direction of flow, tapes were attached on cross-wires inside the mouth and at several points further down the net. The movements of the tapes in the mouth were clearly seen (see Pls. I and II).

The attachment of tapes, the suspension of the messengers and the anchoring of the ship were the only deviations from the normal routine method of fishing the N70V, none of which could in any way alter the performance of the net. In the course of these investigations some thirty vertical hauls were made in this manner.



Text-fig. 1. Diagrammatic arrangement of sheaves and accumulator spring on davit. *M*, measuring sheave; *S*, sliding sheave; *W*, winch.

Observations of the net as it is lowered show that both the silk section and the canvas throttling band are collapsed by the pressure of water forcing them up towards the ring, where the tapes can be seen leading upwards. The filtering part of the net is thus constricted, making it virtually impossible for the net to catch plankton on the way down. The shape of the net is, in fact, identical to that figured in Murray & Hjort (1912, fig. 29*a*, p. 46). On hauling, the pressure of water fills out the net. The net rises steadily in the water until the release mechanism strikes the messengers and closing takes place. The bridles are released and the net ring starts to fall in the water; at the same time the net appears to decelerate slightly and the throttling rope tightens, closing the net, which then continues on its upward path. The closing takes but an instant and seeing it for the first time one is immediately struck by the speed and smoothness of the whole operation.

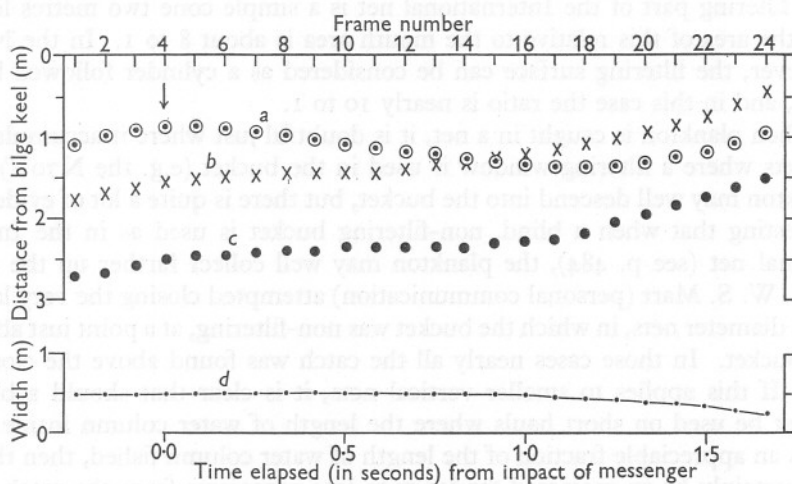
The quickness of the throttling makes it impossible to see anything more than has just been described. Of the hauls that were made, however, some twenty were successfully photographed, including two in which the messenger was released in the normal manner (see p. 486) and allowed to run down the wire to close the net at a predetermined depth—in this case 5 m. The resulting cine-film makes it possible to analyse and measure the sequence of events in greater detail, and Pls. I and II show a series of stills made from a typical section of the film. They are of alternate frames, the time interval between each shot being  $\frac{1}{12}$  sec, so that the whole series represents 2.0 sec of the haul.

The first three frames in Pl. I show the net being hauled vertically through the water at a speed of 1 m/sec, with the silk filtering section filled out, and the tapes in the mouth leading down into the net. On the impact of messenger and release gear (frame 4) the bridles are released (frame 5) and the net starts to close. The net ring having some forward momentum does not fall immediately, and throttling is well under way before the tapes start to fall out of the net ring (frame 7). The momentary action of the accumulator spring (p. 486), together with the speeding up of the winch (see p. 486), causes the throttling rope to tighten quickly as can be seen in frames 5–9 where first the slack in the throttling rope is taken up and then the release gear shoots up out of the picture. The throttling rope quickly constricts the canvas band, and the net, which decelerates slightly (frames 6–12), is closed in the less than 1.0 sec (Pl. II, frame 16) after the impact of the messenger.

Of particular note is the fullness of the net behind the canvas band which persists after the release of the bridles. During throttling, the mouth of the silk section is constricted and there must therefore be some decrease in the volume of water inside the net, possibly causing water to be expelled from the upper part of the net. If such a loss does occur it must be very small since any appreciable loss of water would cause the back section of the net to collapse very rapidly. The reverse in fact appears to occur and the action of the throttling rope seems to be to maintain or even increase the internal pressure of water in the net. Even after complete throttling there is still a considerable bulge in the silk which disappears only when the net has once more resumed its upward path through the water (frames 16–24).

The bilge keel of the ship, which is clearly shown in each frame of the sequence, provides a convenient point of reference from which it is possible to measure, and, thereby follow closely, the successive positions of the various parts of the net. Such measurements, taken from Pls. I and II, can be converted into metres using the known dimensions of the net—in this case the diameter of the ring. Text-fig. 2 shows the distances in successive frames from the bilge keel to (a) the net ring, (b) the throttling point, and (c) the end of the first silk section (the white horizontal band in the photographs), together with (d) the width of the end of the first silk section. This diagram confirms the previous description of the process of throttling. It will be seen that the

filtering part of the net, represented by curves (b) and (c), does not drop in the water, although it does decelerate and is practically stationary for about a  $\frac{1}{4}$  sec (frames 8–11). It then accelerates and by the 15th frame the point of throttling has passed the falling net ring (curve a) and the net is then completely throttled. It is only then, as it is once more hauled rapidly through the water, that the bulge in the silk, represented by the width of the white band (curve d), starts to decrease. The time scale shows that complete throttling occurs about 0.75–1.0 sec after the impact of the messenger, and similar measurements taken from two other vertical closing hauls selected at random from the film confirm this time.



Text-fig. 2. Measurements taken from Plates I and II, showing the distance from the bilge keel of the ship to various parts of the net, plotted against time. The width of the end of the first silk filtering section is also shown. The arrow indicates the point of impact of the messenger on the release gear. *a*, net ring; *b*, throttling band; *c*, end of first silk section; *d*, width of end of first silk section.

### DISCUSSION

To understand the difference in behaviour between the 'Discovery' net (the N70V) and the modified international net used by Barnes, we must take into consideration the different construction and method of operation of the nets.

If a net has a low filtration coefficient, then only a small quantity of water actually passes through the meshes, and the water which does not escape in this way must be carried along by the net, creating a back pressure against the inflow of water at the mouth of the net. As the filtration coefficient increases, so more water escapes through the meshes and the back pressure must reduce until it reaches zero at 100% filtration coefficient.

Suppose now that the net is stopped instantly, there will be a tendency for any back pressure to manifest itself in an outflow of water from the mouth



of the net, and this tendency will become greater the lower the filtration coefficient is. It is, therefore, a point of great importance that the N70V with its relatively wide mesh (40–74 meshes/in. compared with 200 meshes/in. of the International net) has a very much higher filtration coefficient than the fine-meshed International net. We have made experiments in which vertical hauls were made with a flow-meter mounted in a net of the N70V type and compared with hauls in which the meter alone was used, and these show that the filtration coefficient of the 'Discovery' net is of the order of 80–90%. Not only does the filtration coefficient depend on the mesh of the net, but also on the ratio of the area of the filtering surface to that of the mouth opening. The filtering part of the International net is a simple cone two metres long, and the area of this relative to the mouth area is about 8 to 1. In the N70, however, the filtering surface can be considered as a cylinder followed by a cone, and in this case the ratio is nearly 10 to 1.

When plankton is caught in a net, it is doubtful just where it accumulates. In nets where a filtering window is used in the bucket (e.g. the N70V) the plankton may well descend into the bucket, but there is quite a lot of evidence suggesting that when a blind, non-filtering bucket is used as in the International net (see p. 484), the plankton may well collect farther up the net. Mr J. W. S. Marr (personal communication) attempted closing the very large  $4\frac{1}{2}$  m diameter nets, in which the bucket was non-filtering, at a point just above the bucket. In those cases nearly all the catch was found above the closing line. If this applies to smaller vertical nets, it is clear that should a blind bucket be used on short hauls where the length of water column inside the net is an appreciable fraction of the length of water column fished, then there will certainly be an increased tendency to lose organisms from the catch.

We have emphasized the importance of the accumulator spring and steam winch ensuring the rapid closing of the N70V. Although it is realized that the human arm in the operation of a hand winch such as used by Nicholls (1933) is a fairly effective accumulator, the reaction time of a strong spring must be considerably less than that of a man's arm released from a great strain, and it is no doubt on account of this, and the great flexibility of the steam winch, that the N70 throttles so rapidly compared with the International net (Barnes, 1949, fig. 6). (It should of course be remembered that the hauling speed of the 'Discovery' net is twice that of the International net.)

One final point that might be mentioned is the form of the closing rope. In the N70, this is a *bight* of rope extending down from the release gear and encircling the net. Some authors (e.g. Ostenfeld & Jespersen, 1924), recommend the use of a single line descending from the release gear and forming a *running noose*, around the net. With this, obviously, there is much more slack to be taken in before the net is throttled, and this method should certainly be avoided.

## CONCLUSIONS

The observations which are presented here cannot be regarded as conclusive proof of the infallibility of the Nansen closing method. They do show, however, that it is highly improbable that any significant loss of catch can occur if the method is applied in the way it has been to the 'Discovery' net. The latter differed from the International net in respect of its high filtration coefficient. The other mechanisms which help to avoid loss of catch are the filtering window in the bucket and the rapidity of closure which is effected by the efficient accumulator and the steam engine's capacity to adjust the speed of the winch automatically to the tension of the wire. We would, however, stress that we are in entire agreement with Barnes's findings as applied to the International net as Nicholls used it, and this must be looked upon as a warning against the use of catches, made with the Nansen method, for quantitative work, before ascertaining in what manner the hauls have been made.

It will be obvious that this work would have been impossible without the assistance of all our colleagues on board the R.R.S. *Discovery II* in the September and October–November cruises in 1955. To them we should like to express our gratitude, and in particular to Mr Edgar French who made the majority of the net hauls while the photographs were taken. We are also indebted to the Director and Lieut.-Commander Terrell of H.M. Underwater Countermeasures and Weapons Establishment for the loan and guidance in the use of the underwater camera.

## SUMMARY

The object of the work described in this paper was to find out if the Nansen closing method used with vertical net hauls can be reliable in quantitative plankton investigations.

Barnes has shown that the International net, which is a fine-meshed net, can lose (through the mouth) a significant part of the catch at the moment of closure by the Nansen method.

The net used by Barnes, however, is not comparable to the 'Discovery' net which has a much higher filtration coefficient and is used with gear which ensures that there is no relaxation of tension on the towing wire when the net is closed.

Underwater observations and films have demonstrated that closure is very rapid and that there is little doubt that the Nansen closing method can be quite reliable provided certain precautions are observed.

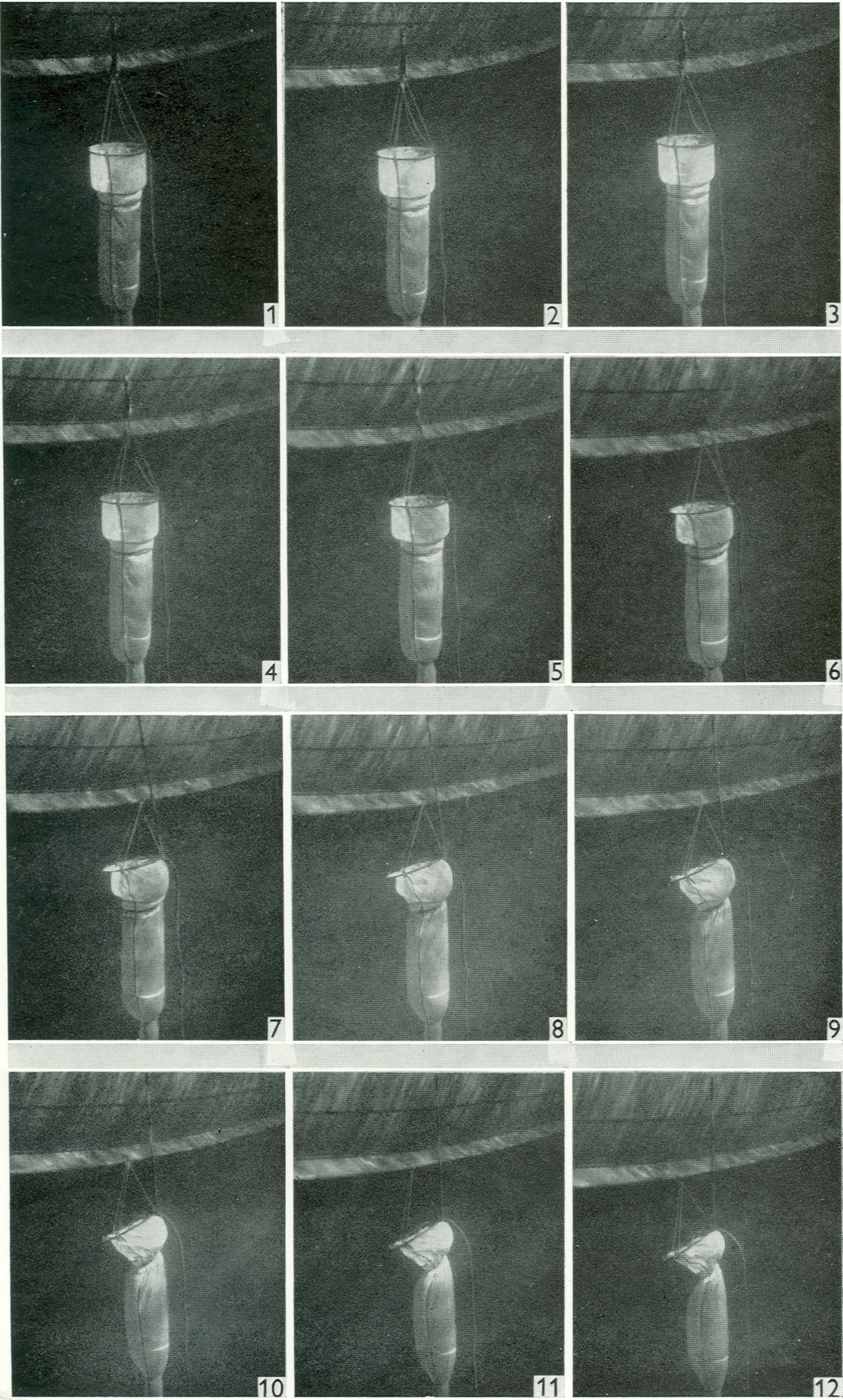
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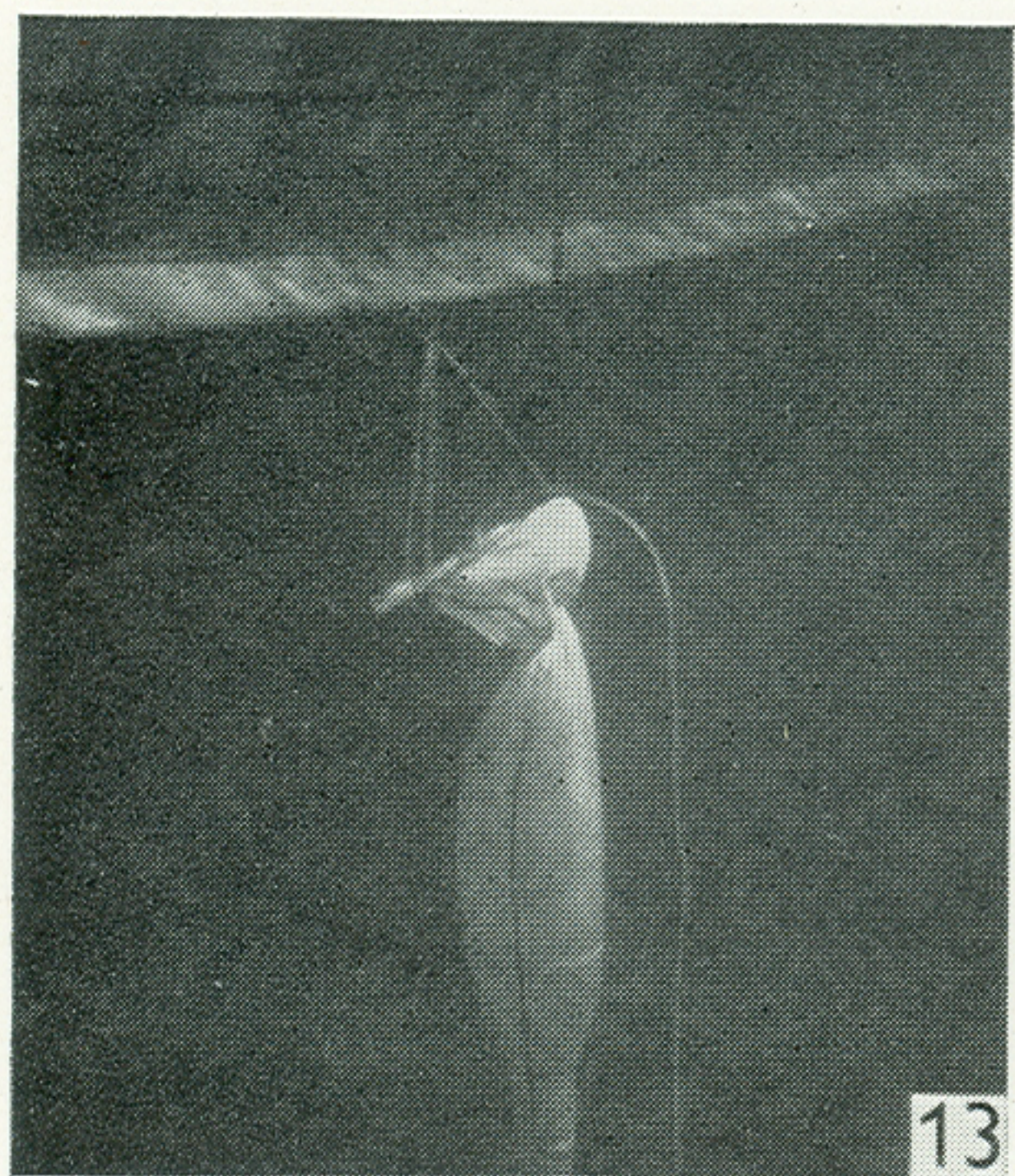
## EXPLANATION OF PLATES I AND II

These photographs are alternate frames from a 35 mm cine-film, taken at a speed of 24 frames/sec. The time interval between each photograph is therefore  $\frac{1}{24}$  sec. The point of impact of the messenger is on the 4th frame.

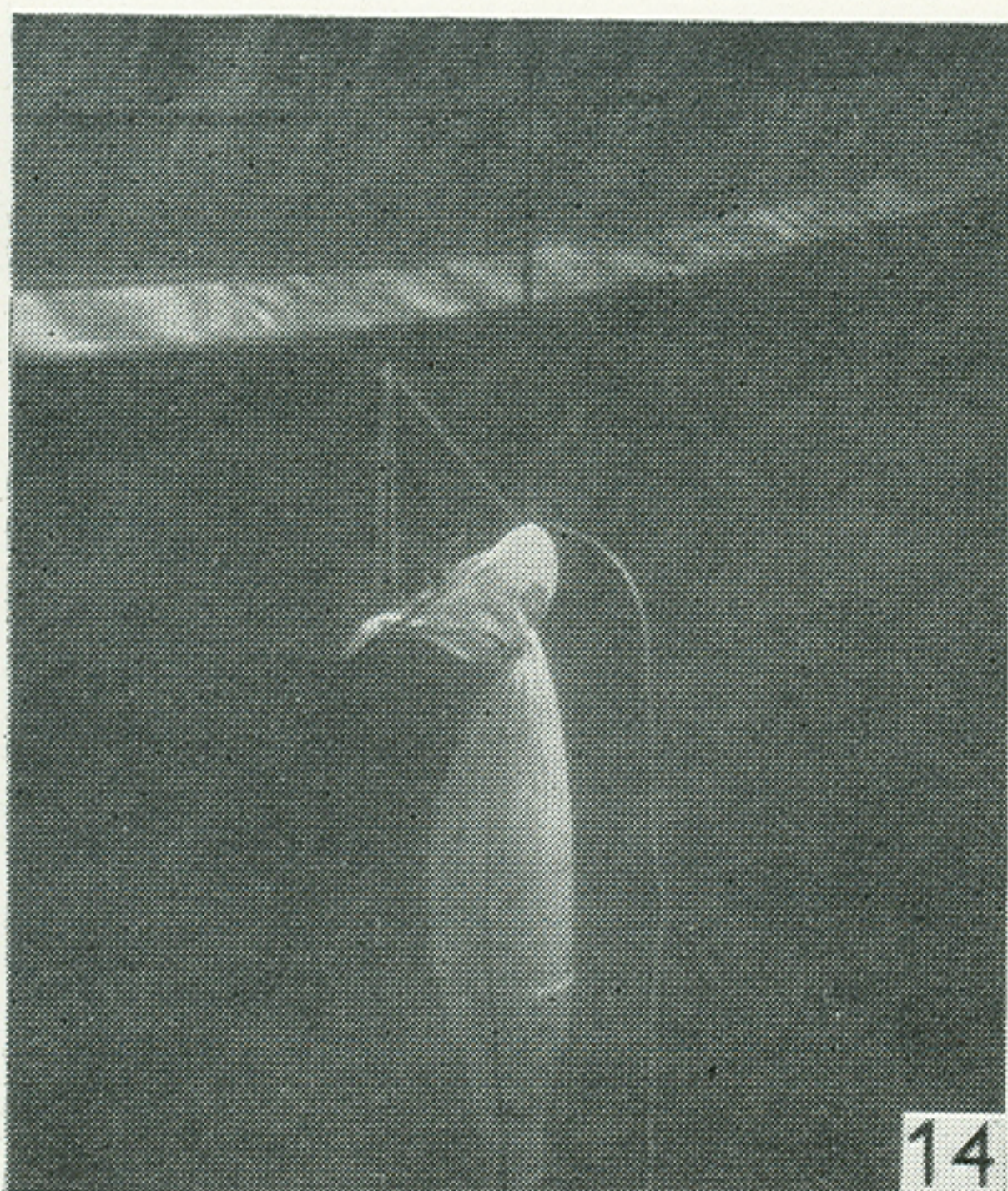




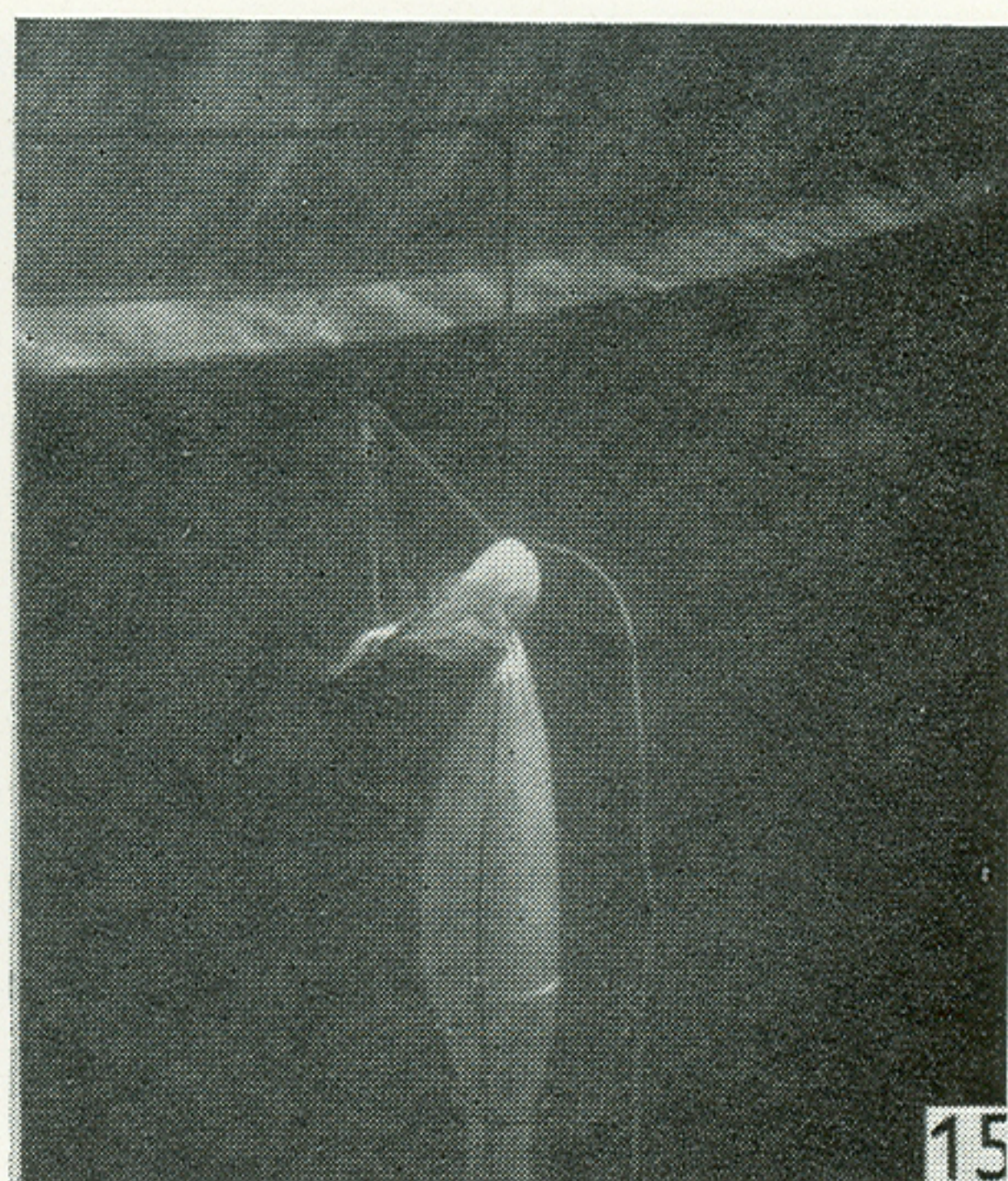




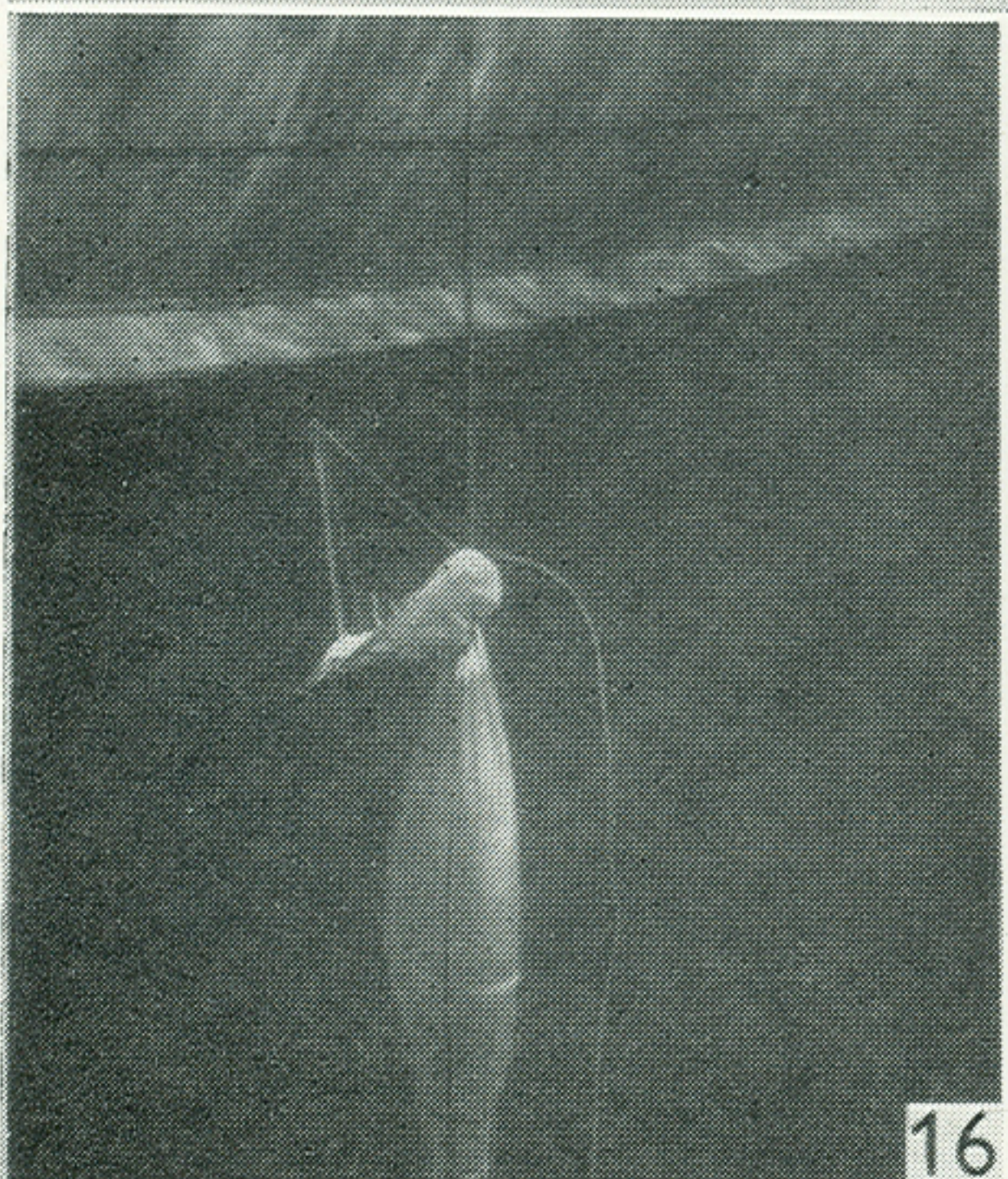
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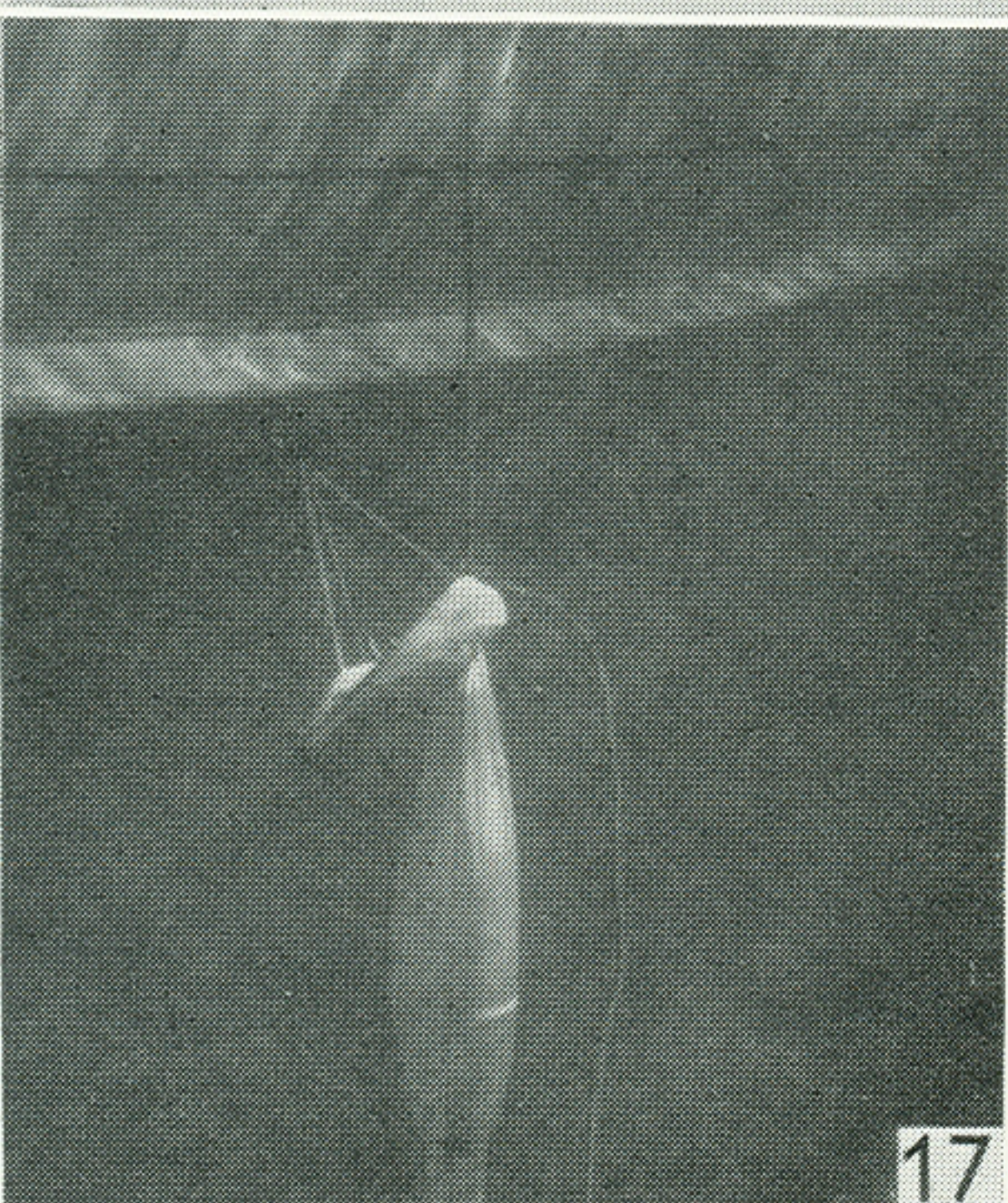
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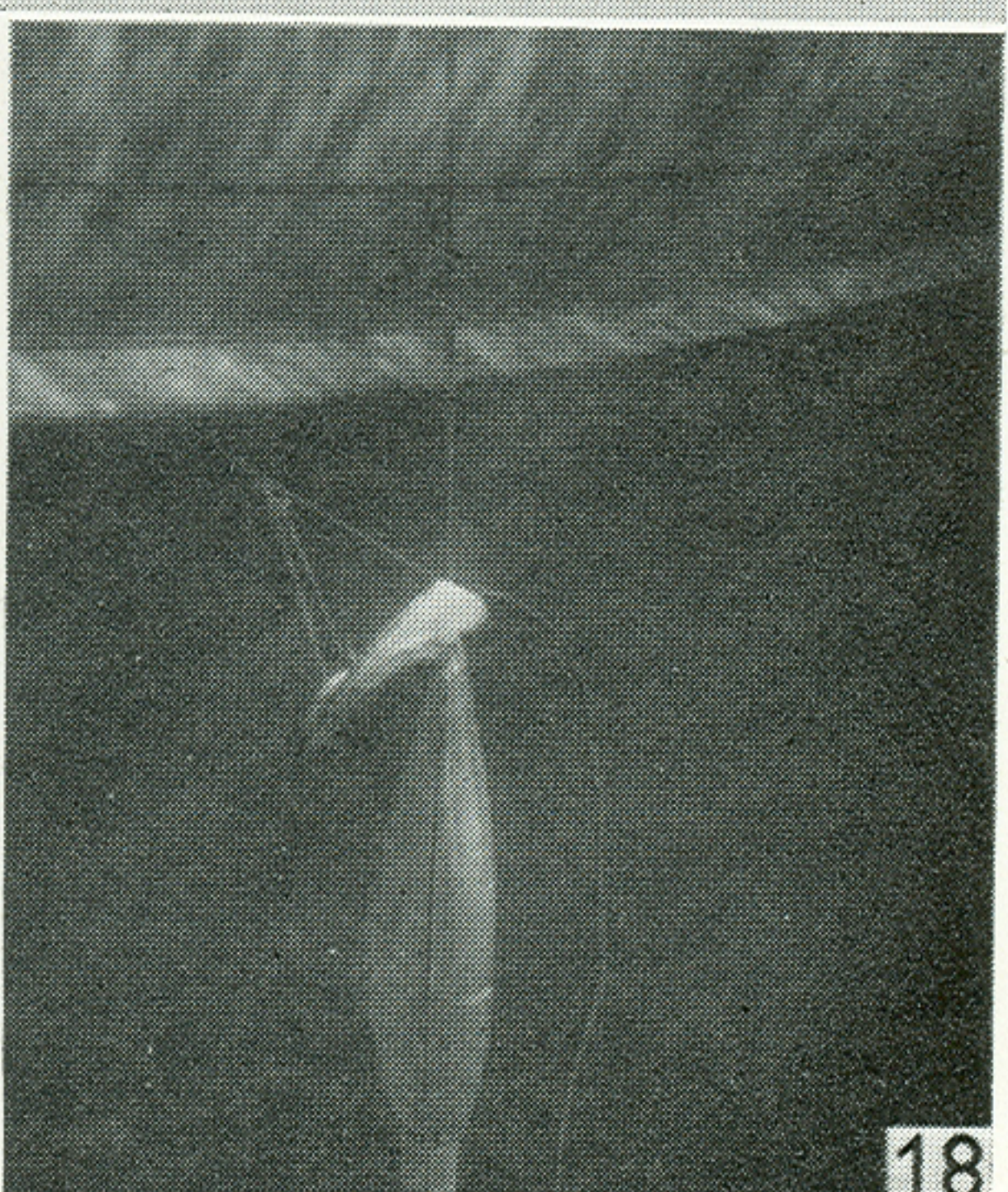
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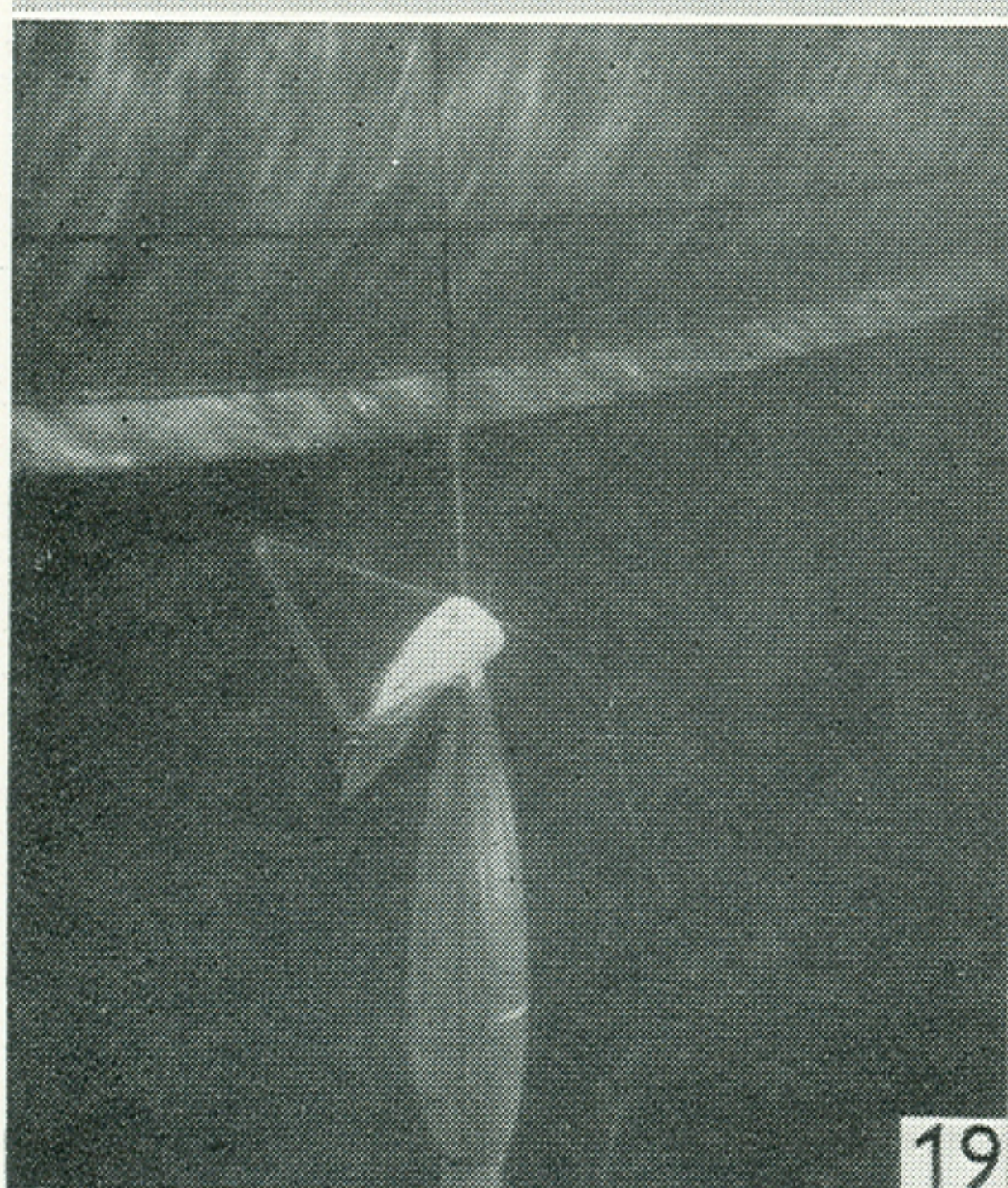
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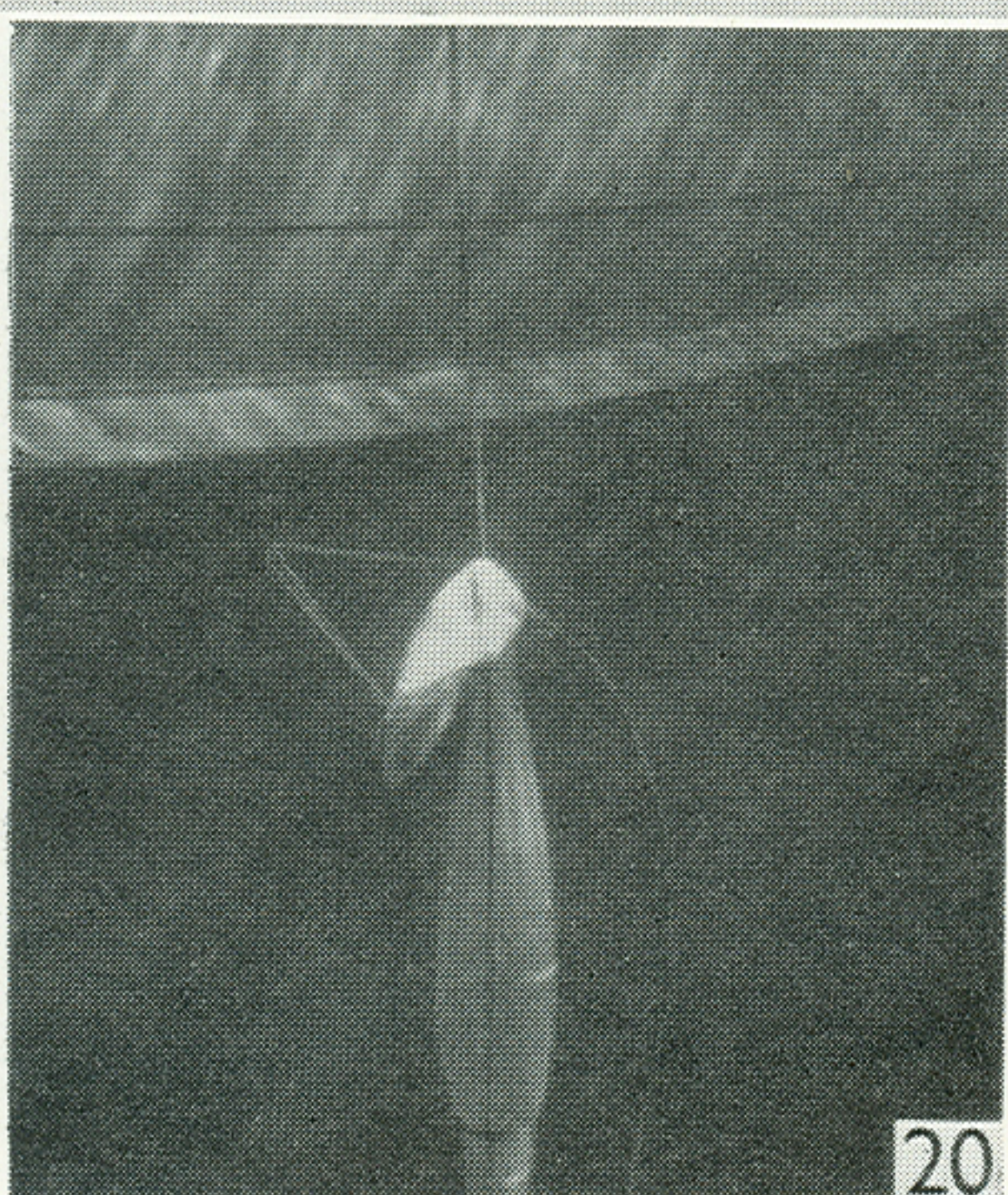
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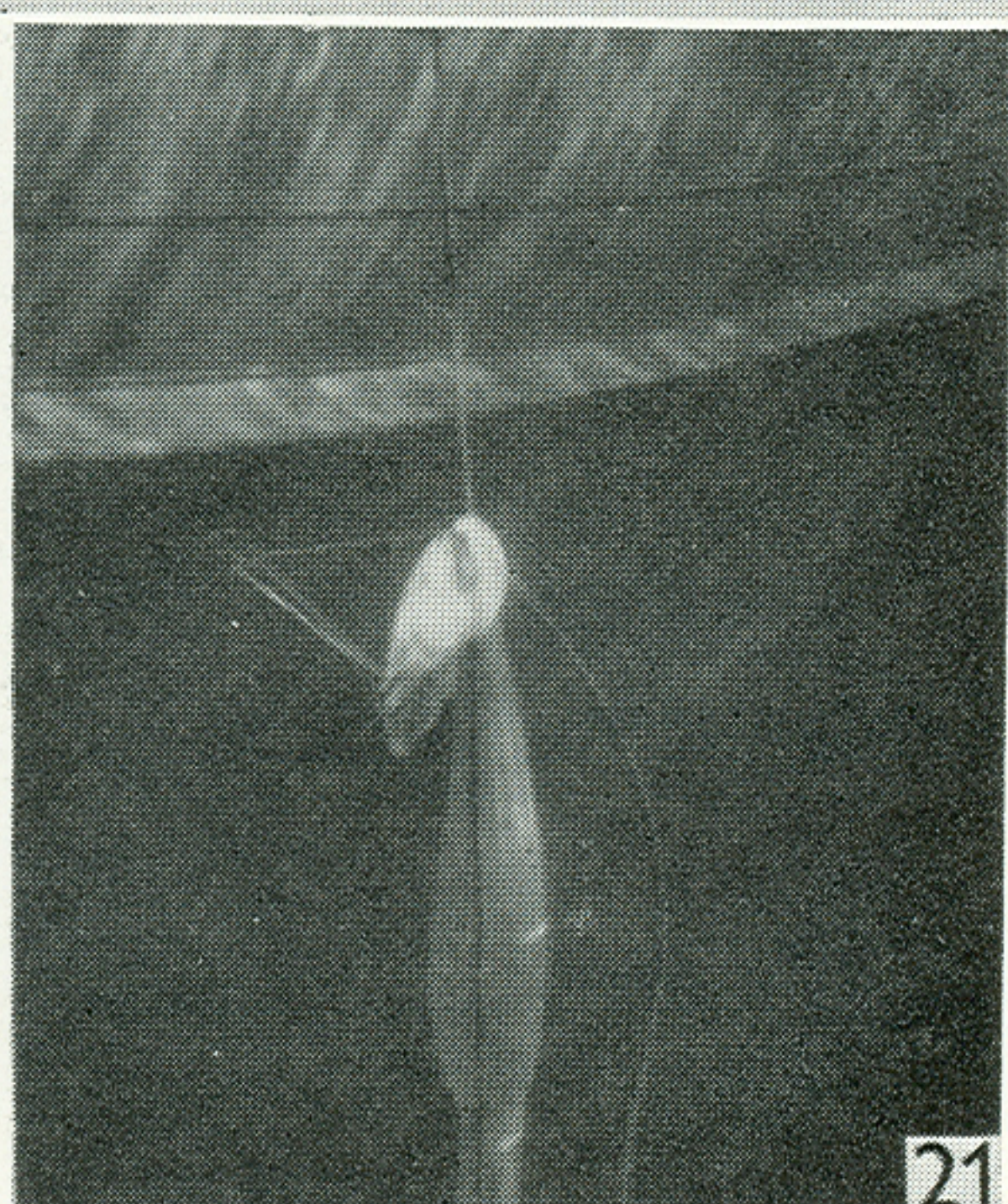
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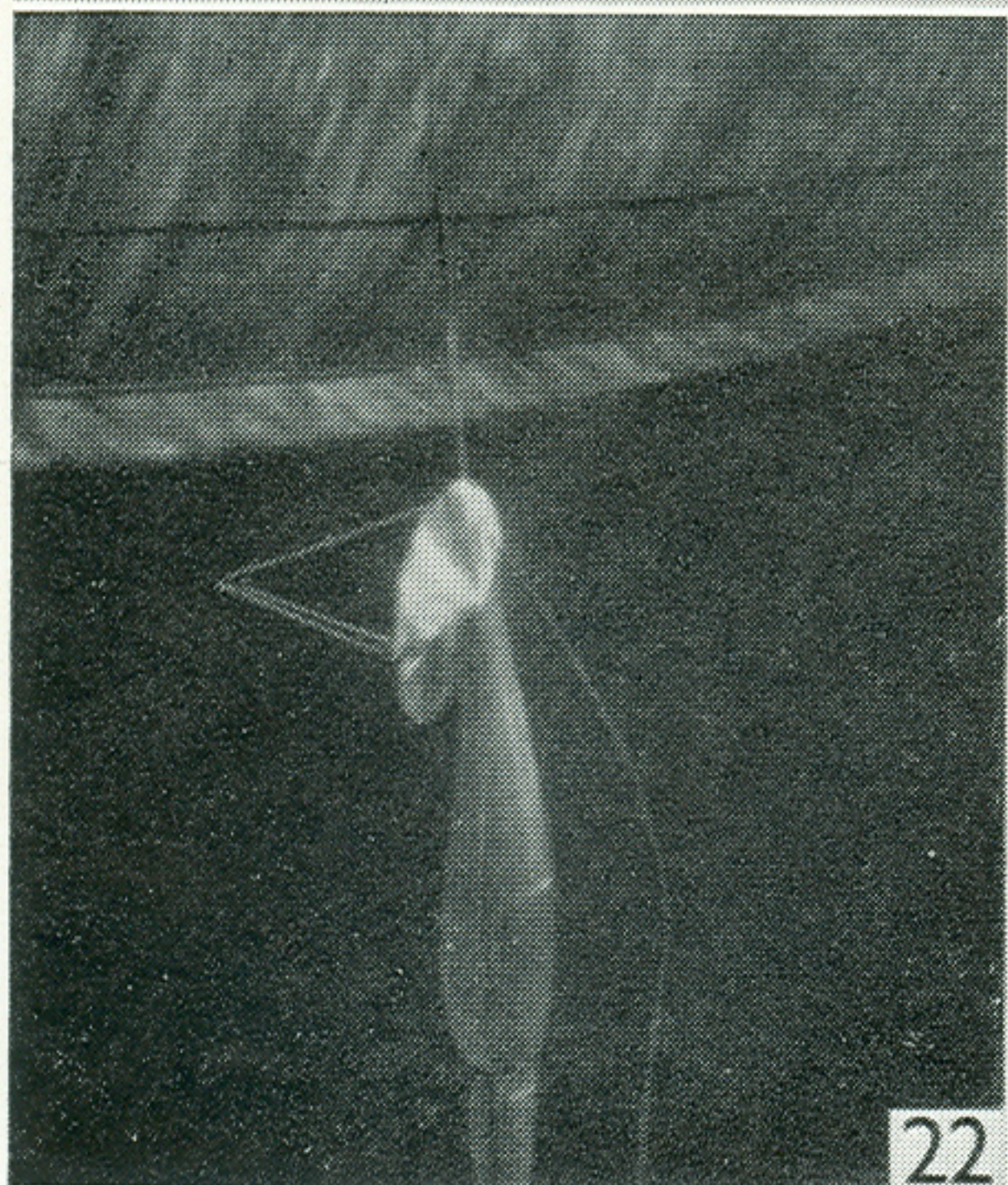
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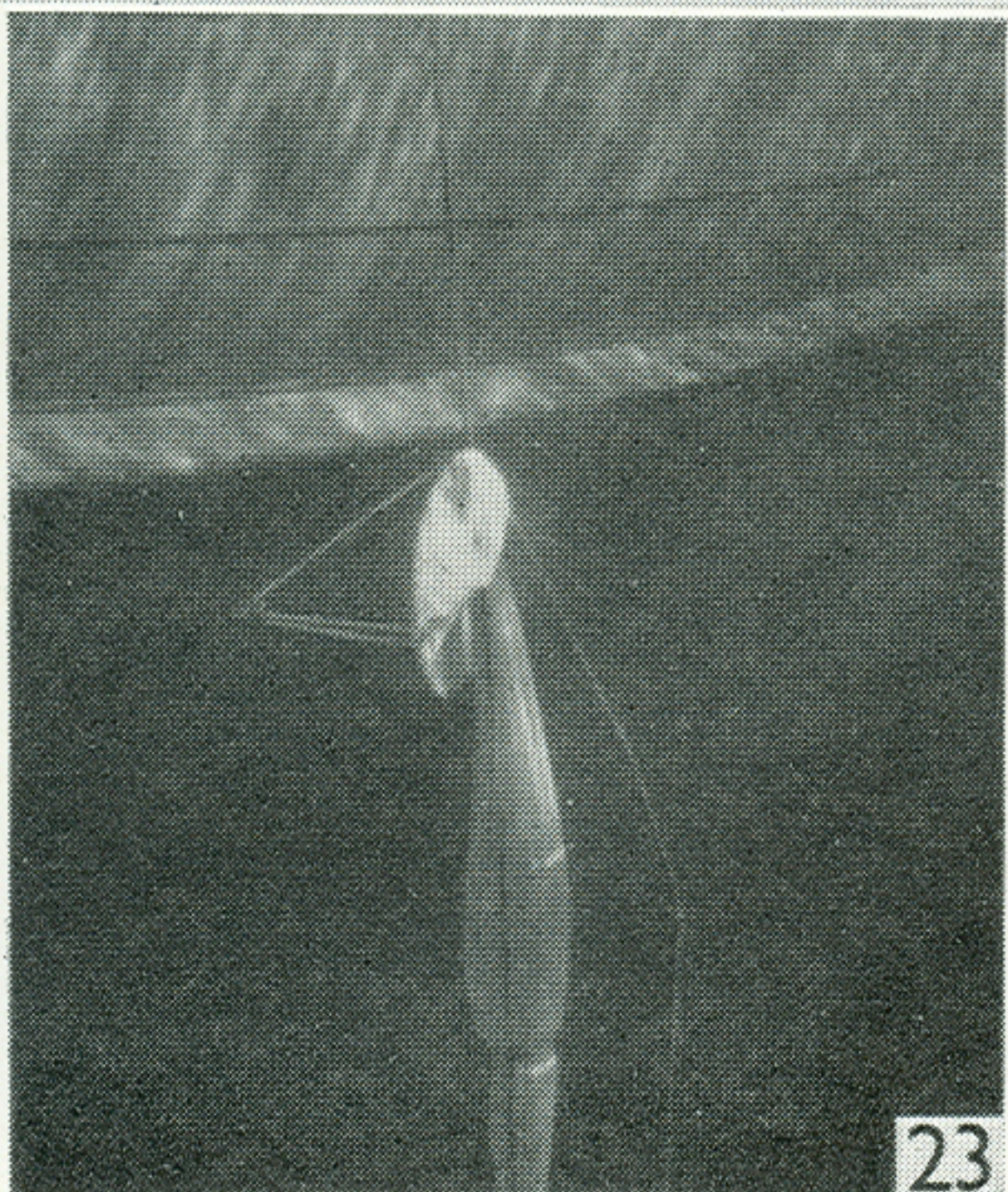
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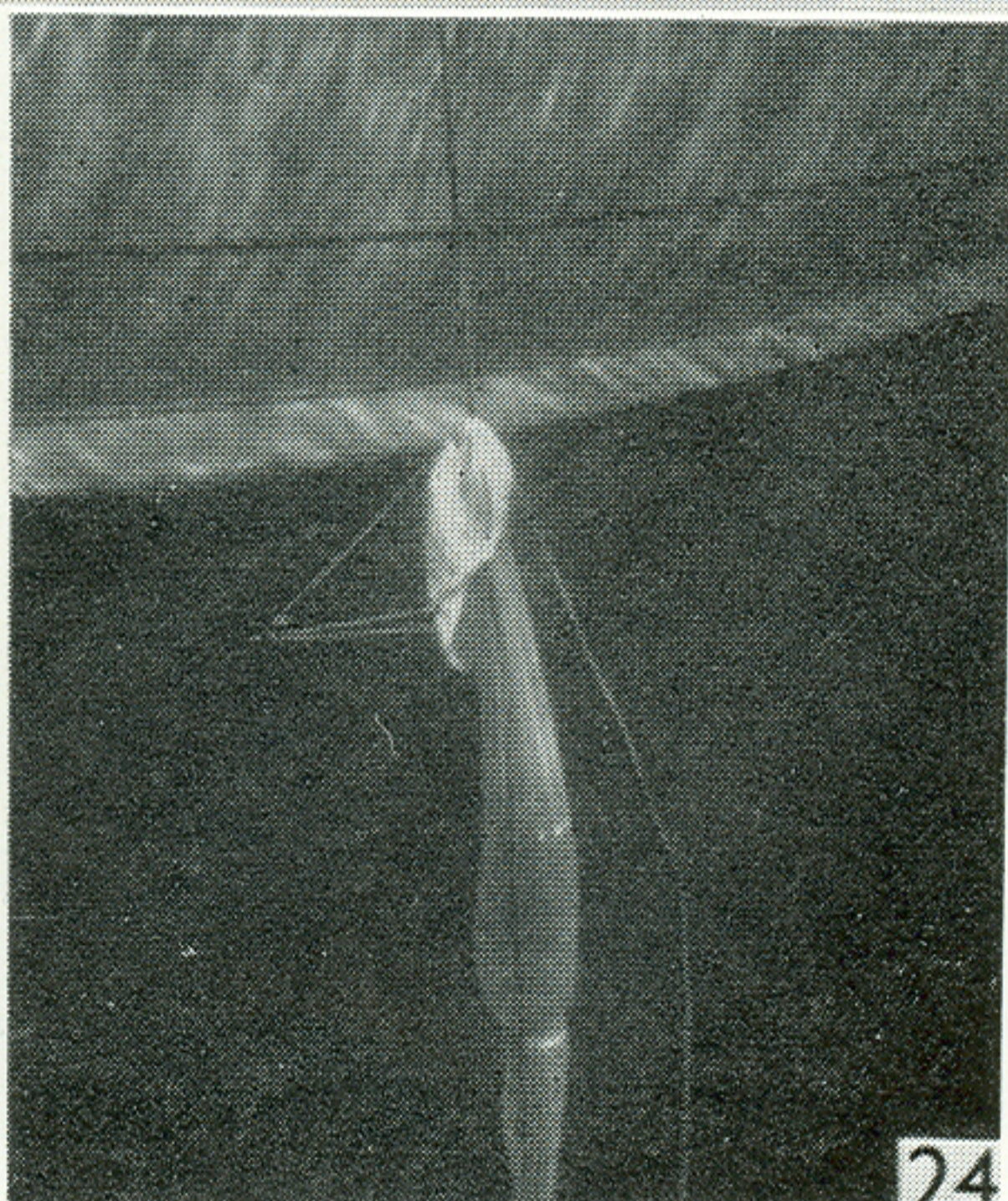
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# ON TWO NEW MEDUSAE, *MERGA REESI* N.SP. AND *TIAROPSIDIUM* *ATLANTICUM* N.SP.

By F. S. Russell, F.R.S.

The Plymouth Laboratory

(Text-figs. 1-3)

In a collection made with a 2 m stramin ring trawl at 47° 12' N., 7° 40' W. on 21 July 1955, with 450 fathoms of wire out, I have found two new species of medusae. These were each represented by a single specimen, one of which was an anthomedusa and the other a leptomedusa.

The anthomedusa was much crushed and contracted, but it was nevertheless intact and it has been possible to make a fairly complete description of it. It is apparently a pandeid, but I have had difficulty in placing it in a known genus. It is evident that the classification of some pandeids needs revision, but until more is known it seems premature to attempt this. In the meanwhile I am provisionally placing this new species in the genus *Merga*, as Kramp (1956) has recently done for another new pandeid. I propose to call it *M. reesi* n.sp. after my colleague Dr W. J. Rees, whose researches on hydroids and their medusae are well known.

The medusa, of which a reconstruction is given in Fig. 1, can be described as follows.

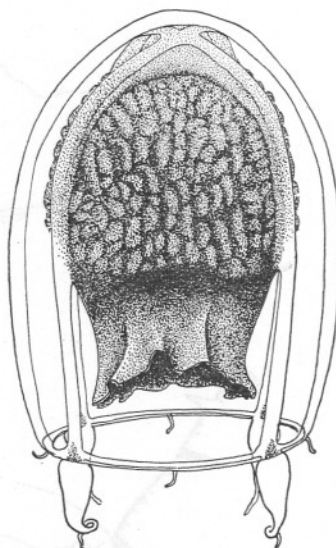


Fig. 1. *Merga reesi* n.sp. Reconstruction from damaged specimen.

## *Merga reesi* n.sp.

Umbrella bell-shaped, higher than wide, no apical process. Stomach flask-shaped, not extending beyond umbrella margin, with broad base. Mouth with four slightly crenulated lips. Four radial canals and ring canal broad, with smooth outlines. Radial canals attached to stomach over more than half its length to form 'mesenteries'. Gonads interradial, irregularly folded to form numerous small raised corrugations. Four perradial marginal tentacles with

swollen elongated basal bulbs, without exumbrellar spurs. Four small tentaculæ, one in each interradius. No ocelli. Height 10 mm. Colour of stomach and gonads dark chocolate red; basal bulbs of marginal tentacles colourless, except for slight internal pigmentation at junction of radial canal and ring canal.

The specimen has been deposited in the British Museum (Natural History) and has been given the registered number B.M. 1956.4.14.1.

The other specimen was a large leptomedusa. I showed it to Dr P. L. Kramp, when he was in Plymouth, and he immediately identified it as a species of the mitrocomid genus *Tiaropsidium*. The medusa has the distinctive characters typical of that genus.

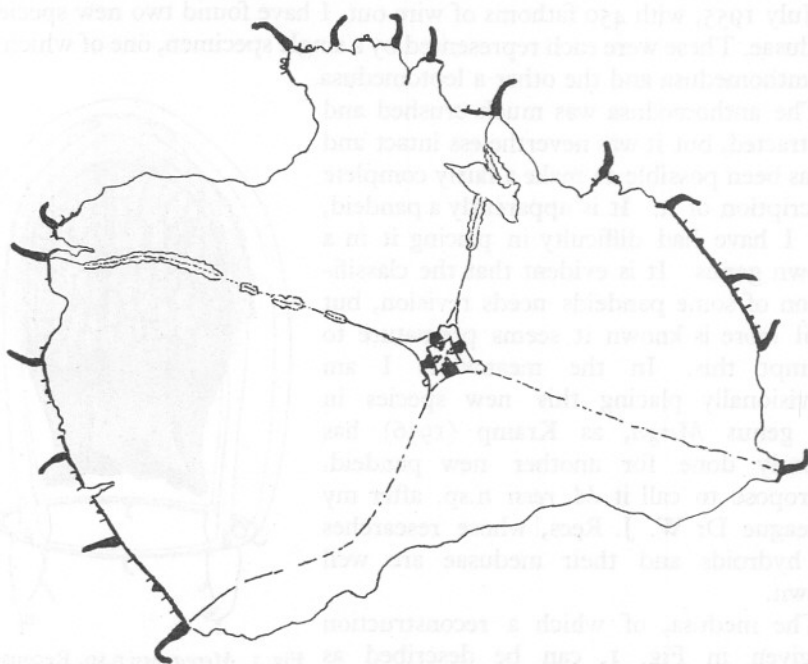


Fig. 2. *Tiaropsidium atlanticum* n.sp. Drawing of specimen showing actual state of preservation.

The specimen is somewhat damaged; the margin of the umbrella is not entire and some of the gonads are missing. Nevertheless, the portions that remain are in a good state of preservation. The umbrella is about 60 mm in diameter and flatter than a hemisphere. The most striking feature is the black pigment on the stomach and on the large marginal tentacles.

The medusa most closely resembles *T. japonicum* Kramp. It differs from

that species in its greater size, its higher number of marginal tentacles and vesicles, and the presence of the black pigment.

In outline the stomach resembles exactly the drawing given by Kramp (1932, text-fig. 1) for that of *T. japonicum*, but on each of its interradiial walls it has black areas forming four triangles on the base of the stomach each of which is continued as a black streak down each mouth lip (Fig. 3a, b). The gonads are linear and extend along the middle three-quarters of the radial canals; they have median longitudinal divisions. The specimen is a mature male and the gonads are somewhat follicular in appearance (Fig. 3d).

The large marginal tentacles appear to be similar in form to those of *T. japonicum*. Each tentacle has a narrow abaxial and a broader adaxial streak clearly demarcated from the black pigmented lateral areas; these may be indications of a similar arrangement of muscles to that described by Kramp (1932, p. 328) for *T. japonicum*. There is a triangular prolongation of the exumbrella extending a short way down the abaxial side of the base of each tentacle (Fig. 3c, d).

The medusa has the small tentacles on the umbrella margin characteristic of the genus, and the open marginal vesicles are typical, each having a large black ocellus and containing 12 to 20, or possibly more, concretions.

A drawing of the whole medusa, showing the missing portions of the umbrella margin, is given in Fig. 2. I have counted the marginal organs in those parts of the margin which are complete and the sequence in each quadrant is as follows:

Quadrant

- 1 *T t t v t (T) t v t T t v t v t T t -- t v t T t --- t*
- 2 *T t ---- T t v t v v T t v ---- T - v ---- T ---- T ---- t t*
- 3 *T ---- t T - v ---- T t t (T) t t T t v t v t T ----*
- 4 *T ----*

*T*=large marginal tentacle; (*T*)=bulb of developing large tentacle; *t*=small marginal tentacle; *v*=marginal vesicle. The dashes indicate missing portions. The first *T* in each quadrant is opposite a radial canal.

It is to be seen that the greatest number of large tentacles in any one quadrant is six, the number in the remaining two complete quadrants being 4 plus 1 developing and 5 plus 1 developing, respectively. A drawing of the first two sections of the first quadrant is given in Fig. 3c. In the centre of the first section between two large tentacles (on the left) there is a bulb of a developing large tentacle; this is flanked on either side by two small tentacles with a marginal vesicle between them, and on one side a third small tentacle developing. In the second section (on the right) there are two marginal vesicles each flanked on the outer side by a small tentacle and having a third small tentacle between them. There are evident abnormalities such as two marginal vesicles adjoining one another in the second section of the second quadrant, and no marginal vesicles in the third section of the third quadrant.

It seems reasonable to suppose that the sequences in the second sector of the first quadrant and the fourth sector of the third quadrant represent the final

development, that is two marginal vesicles and three small tentacles between two large tentacles. On this basis a fully developed specimen could have

$$\begin{aligned}4 \times 6 &= 24 \text{ large tentacles} \\24 \times 3 &= 72 \text{ small tentacles} \\24 \times 2 &= 48 \text{ marginal vesicles}\end{aligned}$$

In my damaged specimen there are three quadrants complete as regards the large marginal tentacles. The number of large tentacles in these three quadrants was 15 plus 2 developing. If the specimen had been complete it is therefore likely that it would have had at least twenty fully developed large tentacles.

This medusa therefore differs from all known species of the genus *Tiaropsidium* in having a considerably higher number of large marginal tentacles and marginal vesicles, the greatest number so far being 8 tentacles and 16 marginal vesicles, in *T. japonicum*. The combination of higher numerical characters, striking black pigment, and locality of capture supplies sufficient grounds for regarding this specimen as belonging to a new species to which I give the name *T. atlanticum*.

Since this new species may perhaps have as many as 48 marginal vesicles it will be necessary to amend the generic diagnosis given by Kramp (1932, p. 366) accordingly.

The new species may be described as follows:

***Tiaropsidium atlanticum* n.sp.**

Umbrella flatter than a hemisphere with fairly thick jelly. Velum narrow. Stomach small, about one-twelfth the diameter of the umbrella, attached to subumbrella along arms of perradial cross, leaving small flat triangular pouches between dorsal wall of stomach and subumbrella. Mouth with four short broad lips with slightly folded margins. Four straight radial canals and ring canal narrow. Gonads along four radial canals, linear, with median division; along middle three-quarters of radial canal, not reaching to umbrella margin. Large marginal tentacles hollow, probably up to 24 in number, with elongated swollen bases. Small marginal tentacles solid, probably up to 72 in number, three in each space between two large marginal tentacles. Probably up to 48 open marginal vesicles, two in each space between two large marginal tentacles, each with *c.* 12 to 20, or more, concretions and one black ocellus at base. Diameter *c.* 60 mm. Colour on interradial walls of stomach and large marginal tentacles black.

The specimen has been deposited in the British Museum (Natural History) and has been given the registered number B.M. 1956.4.14.2.

My thanks are due to Captain C. A. Hoodless and the crew of R.V. *Sarsia* who collected these specimens; and to Dr P. L. Kramp for reading through the manuscript.

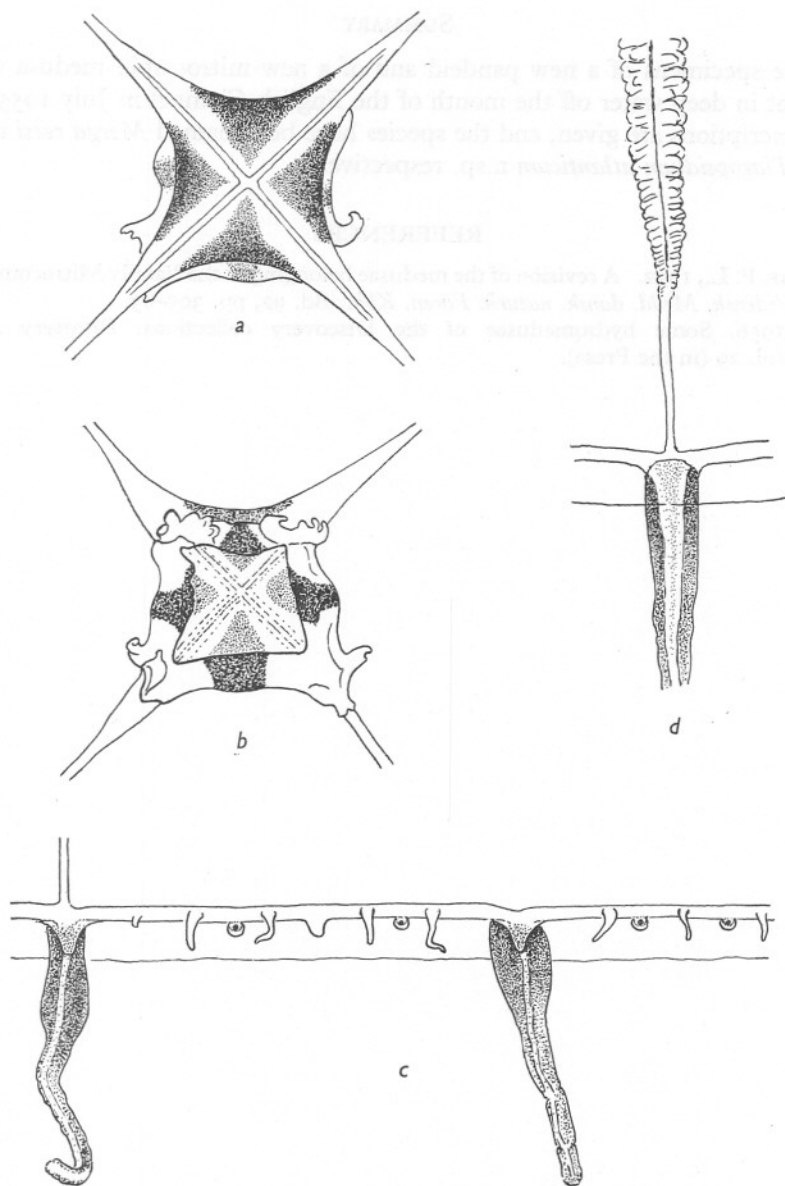


Fig. 3. *Tiaropsidium atlanticum* n.sp. Outline drawings in which dotted areas indicate black pigmentation: *a*, Dorsal view of stomach; *b*, ventral view of stomach; *c*, portion of umbrella margin seen from abaxial side showing two large tentacles, one bulb of large tentacle developing, small tentacles and marginal vesicles; *d*, adaxial view of large tentacle and portion of gonad.



## SUMMARY

Single specimens of a new pandeid and of a new mitrocomid medusa were caught in deep water off the mouth of the English Channel in July 1955.

Descriptions are given, and the species have been named *Merga reesi* n.sp. and *Tiaropsidium atlanticum* n.sp. respectively.

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## ON THE HYDROID *MERONA CORNUCOPIAE* (NORMAN)

By W. J. Rees

British Museum (Natural History), London

(Text-figs. 1-3)

### INTRODUCTION

During a short visit of seven days to the Station Biologique, Roscoff, in June 1955, I was able to make some observations on living colonies of *Merona cornucopiae* (Norman) and on other hydroids. The object of my visit was to see living polyps of *Tricyclusa singularis* which were also found, but unexpectedly *Merona* was found during dredging and provided the opportunity for a much needed re-description from living material.

I am grateful to Professor Georges Teissier for kindly placing a table at my disposal at Roscoff, and also to Dr Claude Levi and Dr Bertil Swedmark for many kindnesses during my stay.

### A RE-DESCRIPTION OF *MERONA CORNUCOPIAE* (NORMAN)

There has been no adequate description of *Merona cornucopiae* since Norman (1864) described the species from preserved material from the Shetlands, although Garstang (1892) described some features of a large colony from Plymouth.

#### Material

Colonies found on *Glycimeris glycimeris* (L.) (Fig. 1) and *Turritella communis* Lamarck, dredged from 15 to 25 m off Callot in the Baie de Morlaix on 23 June 1955, were examined alive, and the following description is based mainly on this material, supplemented by a re-examination of preserved material in the British Museum (Natural History).

The Museum material examined is noted below.

#### Shetland

On *Dentalium entale* inhabited by *Phascolion*, fertile. B.M. No. 1912.12.21.255. Coll. A. M. Norman (Syntype). This colony is in alcohol; the hydranths have partially disappeared but the perisarcal tubes and the reproductive polyps with their mulberry-like bunches of gonophores remain.

On two shells of *Dentalium entale* with *Phascolion*, sterile, 1867. B.M. No. 1912.12.21.254. Coll. A. M. Norman. There are no polyps and the perisarcal tubes are well covered with adhering sand grains. This colony was probably dead when collected.

*Plymouth*

On *Aporrhais pes-pelecani* inhabited by *Phascolion strombi*, sterile. B.M. No. 1910.10.1.1. Coll. A. M. Norman. The hydranths are well retracted into the perisarcal tubes which have a height of 3.0–4.0 mm. The tubes have a diameter of about 0.14 mm at the base and expand gradually distally and have a diameter of about 0.35 mm at the rim.

On two living *Dentalium entale*, sterile, Eddystone Grounds, 26 May 1898, B.M. No. 1948.9.8.53. Coll. E. T. Browne. No hydranths or gonophores are present. The taller perisarcal tubes have lengths of 1.98–3.34 mm. The stolons have diameters of 0.06–0.08 mm.

On one living *D. entale*, fertile, Eddystone Grounds, 4 June 1898, B.M. No. 1941.3.20.516. Coll. E. T. Browne. This colony occurs right at the tip of the *Dentalium* shell. The polyps have been killed in an expanded condition and are very life-like. The reproductive polyps have long stalks like the nutritive ones and are further discussed on p. 502. Measurements of this colony are given in Table I.

On a dead *Aporrhais pes-pelecani*, sterile; off Stoke Point, 4 November 1913, B.M. No. 1948.9.8.18. Coll. E. T. Browne. Sterile colony with short stems and polyps moderately extended.

Fragments from the shell of *Glycimeris glycimeris* (L.), sterile, 18 March 1914. B.M. No. 1948.10.1.26. Coll. E. T. Browne. Fragments with few polyps; stems 1.0–3.5 mm high.

Three colonies each on *Dentalium entale*, fertile. B.M. No. 1921.8.31.5 (purchased). Colonies on the apices of the shells; each colony with tall reproductive polyps of the same length as the nutritive polyps.

*Eastern Channel, position, 50° 28' 45" N., 00° 05' W.*

On *Glycimeris glycimeris* (L.), sterile, 49 m., 24 July 1947. Manihine Collections. A small sterile colony with retracted polyps.

*Description*

All the colonies seen by me have consisted of 10–40 nutritive polyps, with or without a small number of reproductive polyps, but Garstang (1892) noted a very large colony of 90–100 polyps covering almost the whole upper surface of a shell of *Aporrhais pes-pelecani* inhabited by the sipunculid *Phascolion strombi*. In this colony the hydrorhiza formed a continuous crust, whereas in Norman's colonies and others in the Museum, the stolons sometimes form a crust and sometimes a reticulate network. When the stolons can easily be seen (as on *Dentalium*), they are seen to be covered with smooth, non-annulated, horn-coloured perisarc; they have diameters of 0.06–0.08 mm.

The unbranched polyp stems have a height (to hypostome) of 2.0–5.0 mm when the polyp is expanded. The stems are not usually vertical but slope in the direction of the ciliary currents of the host mollusc; they arise directly from the hydrorhiza with a diameter at base of about 0.14 mm. They expand gradually distally to a diameter of 0.4–0.56 mm. The distal margin is often flared into a trumpet-shape but this feature is more noticeable in young polyps than in old ones which become coated with sand grains or fine mud. When the

polyps die down and regenerate, new tubes arise within the mouths of the old ones as noted by Norman, but this feature is not always apparent and caused Garstang to remark: 'The lines of growth encircling the hydrothecae nowhere project so as to assume a ridge-like form, as shown in Norman's figure.'

The large polyps have a length of up to 5 mm when expanded, and have about twenty long filiform tentacles scattered over the anterior half (Figs. 1, 2). Tentacles proximal to the mouth seem to be arranged in an oral whorl of four to seven in number. The proboscis is bluntly rounded and of an intense white

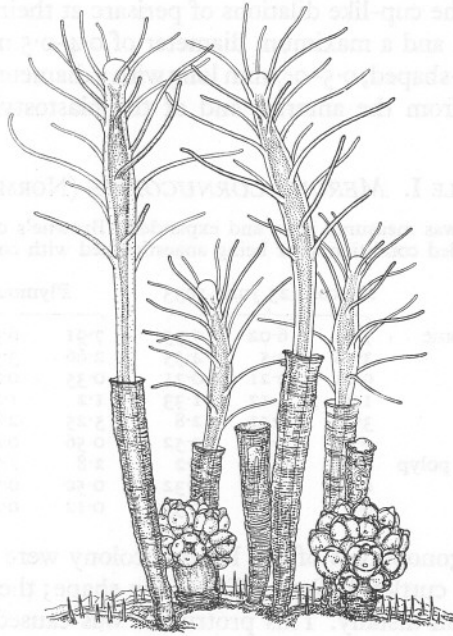


Fig. 1. *Merona cornucopiae* (Norman): a portion of a fertile colony dredged off Callot, Baie de Morlaix, 23 June 1955; drawn from life.

colour but posterior to it the digestive endoderm is orange brown to scarlet in colour, and this fades distally to an uniformly pale tint in the non-tentacular portion of the polyp. When brought in from the dredgings the polyps were contracted into their perisarcial tubes with only the tip of the hypostome and the tips of the oral tentacles visible. They did not expand into full length until nearly 12 hours had passed. In the Roscoff colonies the perisarc is of a pale horn colour and rather opaque; it was usually covered with a fine layer of mud particles. The dimensions of the Roscoff and Plymouth colonies are given in Table I.

The gonophores in one of the Roscoff colonies were, as Norman stated, borne in the form of mulberry-like masses on very short stalks arising from

openings in the stolons. These reproductive polyps have short collars of perisarc at their point of origin on the stolons. It was noticed by Garstang (1892) that 'the blastostyles invariably possess a slender terminal portion, with a slightly dilated apex, which is prolonged beyond the mulberry-like mass of gonophores except when contracted. It is an interesting vestigial structure being homologous with the tentacular portion of a digestive polyp....'

In one of E. T. Browne's well-preserved colonies (B.M. No. 1941.3.20.516) these reproductive polyps had long stalks so that they had a total height of 1.75–3.5 mm, but the cup-like dilations of perisarc at their base had a height of only 0.4–0.6 mm and a maximum diameter of 0.4–0.5 mm (Fig. 2). Each gonophore was pod-shaped, 0.5–0.7 mm long with a diameter of 0.12–0.22 mm. They arise single from the anterior end of the blastostyle and not in any particular order.

TABLE I. *MERONA CORNUCOPIAE* (NORMAN)

The Roscoff colony was measured alive and expanded. Browne's colony from Plymouth was killed in an expanded condition after being anaesthetized with cocaine. Measurements in mm.

	Roscoff, 23 June 1955			Plymouth, 4 June 1898			
Total height to hypostome	7.0	6.02	7.35	7.91	6.3	9.59	7.28
Length of polyp	3.78	3.5	4.55	2.66	3.5	5.04	3.08
Diameter of polyp	0.21	0.21	0.21	0.35	0.28	0.28	0.28
Length of tentacle	1.36	1.12	1.33	1.2	1.25	1.3	1.1
Height of stem	3.22	2.52	2.8	5.25	2.8	4.55	4.2
Max. diameter of stem	0.56	0.5	0.52	0.56	0.49	0.56	0.42
Height of reproductive polyp	0.84	0.84	1.2	2.8	1.75	3.5	—
Diameter of gonophore	0.24	0.28	0.32	0.50	0.7	0.6	0.6
Length of gonophore	0.4	0.32	0.35	0.12	0.22	0.22	0.2

By contrast the gonophores of the Roscoff colony were styloid and rather like the eggs of the cuttlefish *Sepia officinalis* in shape; they were round with a teat-like protrusion distally. This protrusion was caused by the tip of the scarlet-coloured spadix. The eggs were pinkish in colour and few in number (two to six). Male gonophores were not seen by me.

#### Distribution

Although *Merona cornucopiae* is probably not a rare species in our waters, records of its occurrence are few, but these indicate that it may be found all round our coasts except perhaps in the southern North Sea. The species is known from the following localities: Shetland (Norman, 1864), Northumberland (Alder, 1867), 2 miles north-east of Bamborough Castle (Schulze, 1875), the Plymouth area (Garstang, 1892, 1894; Allen, 1899; Crawshay, 1912), the Isle of Man (Moore, 1933, 1937), Blacksod Bay, Ireland (Duerden, 1895) and the Eastern Channel (this paper, p. 500).

Outside British waters the hydroid has been taken at Roscoff (Teissier, 1950), the Faroes (Broch, 1916), the Eastern Kattegat (Kramp, 1935) and in the



Mediterranean (Graeffe, 1884; Motz-Kossowska, 1905). On the North American coast it has been recorded from New England by Verrill (1879) and by Fraser (1936) from Queen Charlotte Islands, British Columbia.

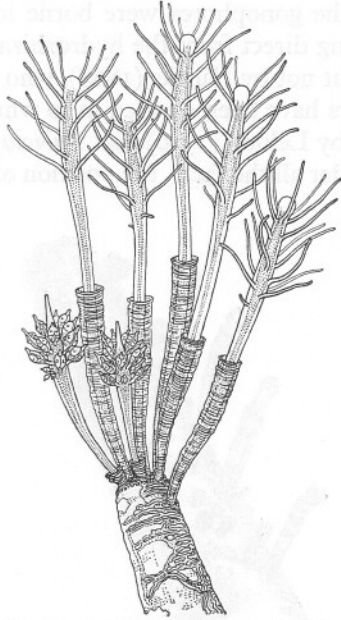


Fig. 2. *Merona cornucopiae* (Norman): fertile colony at the tip of a shell of *Dentalium entale*, Eddystone Grounds, Plymouth, 4 June 1898; E. T. Browne Collection (Brit. Mus. No. 1941.3.20.516). This colony was anaesthetized with cocaine.

### Biology

*Merona cornucopiae* exhibits a preference for the shells of molluscs as a substratum. Where the mollusc is a bivalve like *Glycimeris glycimeris*, the colonies are found at the posterior or postero-ventral margin of the valves, and the stems always lean towards this edge, so that the polyps when expanded project into the ciliary feeding currents of the host. Similarly, the colonies on *Dentalium* are found strategically placed close to the tip of the shell.

In British waters *Merona* has been found on *D. entale* L., *Astarte sulcata* (da Costa), *Glycimeris glycimeris* (L.) and *Venus fasciata* (da Costa), but it has also been found on dead gastropod shells of *Aporrhais*, *Turritella* and *Natica* inhabited by the sipunculid *Phascolion strombi*. Farther afield it has been reported on *Cardium* from the Faroes by Broch (1916) and on an old skeleton of a gorgonian at Banyuls by Motz-Kossowska (1905).

Fertile colonies of *Merona* have been taken at Plymouth in May and June. The species has a bathymetric range of 10–182 m in British waters, but it has been recorded from a depth of 274 m in the Faroes (Broch, 1916).

## DISCUSSION

When Norman first described his species *cornucopiae* he placed it in *Tubiclava* Allman 1863, but later decided (1865) that a new genus, *Merona*, must be created for it, because the gonophores were borne in clusters at the tips of short gonoblastidia arising direct from the hydrorhiza. *Merona* was accepted by Allman (1871-72), but not by Hincks (1868), who preferred to retain it in *Tubiclava*. Later authors have been divided as to what genus to use, and the conclusion was reached by Leloup (1930) that *Tubiclava* should be employed. As Leloup did not consider all the facts, the position of the species *cornucopiae* is discussed below.

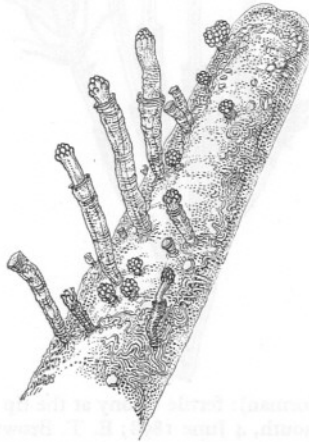


Fig. 3. *Merona cornucopiae* (Norman): a sketch of one of Canon Norman's type colonies on *Dentalium entale* from the Shetlands (Brit. Mus. No. 1912.12.21.255).

The re-description of *M. cornucopiae* shows that the reproductive polyps are sometimes tall and may resemble nutritive polyps in which the tentacles had been resorbed through reproductive exhaustion (comparable to what takes place in species of *Hydractinia*, *Coryne* and *Zanclaea*). If this were so, it would narrow the distinction between *Merona* and the more typical *Clava*-like hydroids, but there is no evidence that the reproductive polyp ever possesses tentacles, and it is very significant that the *Merona* reproductive polyp never possesses more than a short collar of perisarc at the base. This last feature indicates that *Merona* possesses two distinct types of polyp and that the reproductive ones are not derived by reduction from the long-stemmed nutritive ones.

Leloup pointed out that *Merona* was distinguished from *Tubiclava* only by '(a) les gonophores prennant naissance sur des polypes reduits au blastostyle; (b) le perisarque lisse des ses hydranthes et assez large à sa partie pour recevoir l'hydranthe retractoré'. Evidence has already been presented which

indicates that the reproductive polyps of *Merona* are special ones, not derived from the ordinary nutritive ones. Leloup based his case on the fact that the hydranths of a Mediterranean species, *Tubiclava pusilla* Motz-Kossowska, became reduced to blastostyles, so narrowing (in his opinion) the gap between the two genera.

Leloup, however, did not consider the status of *T. lucerna* Allman 1863; this, the type species, is a problematic one whose identity is uncertain, for Allman himself later doubted whether he had actually observed fixed gonophores in this species (Allman, 1872, p. 256). *T. lucerna* has a closely adherent perisarc below the hydranth, so that the latter is not retractile as in *Merona*, and more properly belongs to the group of hydroids which have at various times been referred to *Corydendrium*, *Dendroclava* and *Turritopsis*. Allman's second species *Tubiclava fruticosa*, described without knowledge of its gonophores, also belongs to this group (of which only *Turritopsis nutricula* McCrady is known from British waters from its medusa only). I propose to regard *Tubiclava* as a possible synonym of *Turritopsis*, but it should be noted that *Tubiclava pusilla*, on which Leloup based his arguments, does not appear to belong to the *Turritopsis* group, and its status will have to be clarified when it is found again.

*Merona* Norman may therefore be confidently accepted as a well-constituted genus for *Tubiclava cornucopiae*.

#### SUMMARY

*Merona cornucopiae* (Norman) is re-described from living material from the Baie de Morlaix, supplemented by a re-examination of material in the British Museum (Nat. Hist.). *Merona* Norman is demonstrated to be a valid genus while *Tubiclava* Allman (in which *M. cornucopiae* is placed by some authors) is shown to have affinities with the *Turritopsis*-*Corydendrium* group of clavid hydroids.

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## THE ADHESIVE MECHANISMS OF MONO-GENETIC TREMATODES: THE ATTACHMENT OF *PLECTANOCOTYLE GURNARDI* (V. BEN. & HESSE) TO THE GILLS OF *TRIGLA*

By J. Llewellyn

Department of Zoology and Comparative Physiology, University of Birmingham

(Plate I and Text-figs. 1 and 2)

Little is known of the mechanisms whereby trematode parasites of fish gills attach themselves to their hosts. The adhesive apparatus consists of a posterior set of suckers or clamps supported by skeletal bars (sclerites) whose arrangement varies considerably in the different species. Yet though the pattern of these sclerites forms the main basis for the classification of the 200 or so species of the Diclidophoroidea into its six families, the only attempts to describe the mechanism of attachment of these parasites to their hosts appear to be those of Cerfontaine (1896), who described the attachment of *Diclidophora denticulata* to the gills of *Gadus virens*, and of Sproston (1945*a*) and Llewellyn (1956*a*), both of whom described the attachment of *Kuhnia scombri* to the gills of *Scomber scombrus*. In the present paper the mechanism by which *Plectanocotyle gurnardi* (v. Ben. & Hesse) adheres to its hosts *Trigla cuculus* L., and *T. lineata* Gmelin will be described.

Material was collected at Plymouth during the summers of 1953-55, and investigated by the techniques previously described (Llewellyn, 1956*a*) for *Kuhnia scombri*. In addition, the tendon connecting each extrinsic muscle to its respective clamp was further characterized in histological sections by its positive response to the periodic acid-leucofuchsin routine for the demonstration of polysaccharides (Pearse, 1953), after treatment with ptyalin to remove glycogen from the muscles, and followed by treatment with picric acid to counterstain the muscles and sclerites.

### THE ADHESIVE MECHANISM

The adhesive apparatus of *Plectanocotyle* consists of three pairs of clamps and a variable number of hooks, all borne ventrally in the posterior region of the body (Pl. I, fig. 1). A pair of anteriorly placed suckers is also present, but these suckers are essentially a component of the feeding mechanism, and will be described elsewhere. The clamps and hooks are all applied to the secondary gill lamellae of one surface of a primary lamella in a manner I have described



previously in a comparative account of the adhesive attitudes of gill trematodes (Llewellyn, 1956b).

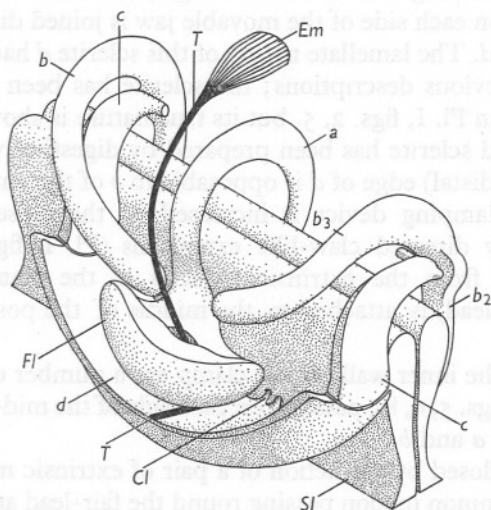
The hooks are borne on a posterior appendage (Pl. I, fig. 1) and function as anchors; such is their efficiency that after separating the clamps from the host tissue during the collection of specimens, it is often impossible to remove the parasite without rupturing the posterior appendage and leaving the hooks still engaged in the secondary gill lamellae. There are invariably two pairs of anchor hooks of which the hooks of the outer pair are about twice as long as those of the inner pair, and sometimes a third much smaller pair of hooks is also present. It is possible that this third pair of hooks may be surviving larval hooks, but an examination of fifteen specimens (histologically fixed and flattened) of *Plectanocotyle gurnardi* revealed no correlation between the size of the specimen and the presence of the third pair of hooks.

Each clamp consists of a postero-ventrally directed pair of hinged opposable jaws with the hinge axis lying approximately transversely across the body, so that one of the jaws lies anteriorly, and the other posteriorly. When the clamp grasps the secondary lamellae of the host, it is the distal end of the posterior jaw that is swung anteriorly about the proximal hinge axis, so that the posterior jaw may be conveniently referred to as the 'movable jaw' and the anterior one as the 'fixed jaw'.

The walls of the clamp consist mainly of fibrous tissue (*Ft* in Pl. I, fig. 4) supported by a framework of skeletal bars or sclerites (*a*, *b*, *c*, *d* and *e*, in Pl. I, figs. 1-8, and Text-fig. 1). Of these sclerites, a single J-shaped one (*a*) lies in the sagittal plane of the clamp. The curved region of the J lies proximally in the clamp, with the short arm extending about half-way into the anterior jaw, and the longer arm extending about three-quarters of the way into the posterior jaw. Sclerite *a* is V- or Y-shaped in transverse section, with the groove facing away from the cavity of the clamp. It is the presence of this groove which gives sclerite *a* the appearance of being hollow when seen in optical section as in Pl. I, fig. 6.

The terminal region of sclerite *a* lying in the posterior jaw is elaborately fashioned to form an important part of the clamping mechanism: the lateral portions of the sclerite, i.e. the arms of the Y, are continued distally and expand to form a lamellate structure that in surface view is approximately semicircular in outline, with the diameter lying proximally and transversely in the posterior jaw of the clamp (Pl. I, figs. 6, 7). The basal region of the sclerite, i.e. the stem of the Y, continues its distal course for a short distance anteriorly to the lamellate expansion, i.e. it forms a short peg running beneath (= nearer to the cavity of the clamp) this lamellate expansion. The lamellate end of *a* does not lie in one plane in the posterior jaw, but curves down over the short peg-like extension of the base of the grooved sclerite, i.e. it curves anteriorly in towards the cavity of the clamp, and then its distal margin is folded posteriorly through 180° so that a transverse groove is formed. In its

median region the posterior wall of this groove is greatly thickened, and opposite the centre of the thickened region the groove itself is perforated to permit the passage of a slender tendon (Pl. I, fig. 4) which connects the extrinsic muscles of the clamp with the hinged jaw. From the antero-distal surface of the thickened margin arises a pair of ridges running parallel to the median longitudinal axis of the clamp and which are continuations of the side walls of the perforation (Pl. I, fig. 2). They project towards the cavity of the clamp, and between them enclose a groove which serves as a guide for the tendon. The posterior distal end of sclerite *a* thus constitutes a 'fair-lead' such as I have described (Llewellyn, 1956*a*) in *Kuhnia scombri*.



Text-fig. 1. Stereogram of clamp of *Plectanocotyle gurnardi* grasping secondary gill lamellae of host. *a*, *b*, *b*<sub>2</sub>, *b*<sub>3</sub>, *c*, *d*, sclerites (see text, pp. 508–10); *Cl*, claw; *Em*, extrinsic muscle; *Fl*, fair-lead; *Sl*, secondary gill lamella; *T*, tendon.

Two systems of sclerites support the peripheral regions of the jaws of the clamp; one of these systems (*c*, *d*) lies wholly within the posterior, movable jaw, while the other (*b*) lies mainly in the anterior, fixed jaw, but extends also into the proximal region of the posterior jaw.

Sclerite *b* passes, on each side of the clamp, along the margin of the anterior jaw (*b*<sub>1</sub>), then curves through the proximal, hinge region of the clamp (*b*<sub>2</sub>), and finally curves again, obliquely medianly into the proximal region of the posterior jaw (*b*<sub>3</sub>) towards its fellow from the opposite side. The distal ends of the two *b*<sub>1</sub> regions meet each other in the anterior jaw apparently without any actual fusion of sclerite material, while the distal ends of the *b*<sub>3</sub> regions in the posterior jaw are separated from each other by the median sclerite *a*. Sclerite *b* consists of a flattened bar that in its anterior distal region *b*<sub>1</sub> presents

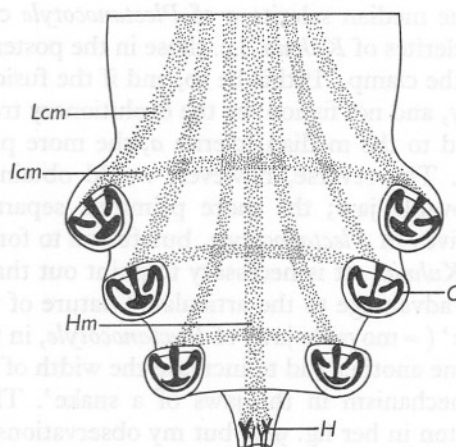
its narrow edge towards the opposable margin of the posterior jaw; in its proximal region  $b_2$  it bears an outwardly directed shelf-like projection which is grooved on its posterior surface to receive the articulating region of the movable jaw; and in its posterior distal region  $b_3$ , sclerite  $b$  presents a narrow edge distally and a wider surface towards the cavity of the clamp.

The margins of the posterior jaw are supported by three sclerites. In the proximal region of each side is a sclerite  $c$  consisting of a flattened tapering bar with its wider end lying distally, and with its narrower edges facing anteriorly and posteriorly. The proximal end of  $c$  bears a peg-like extension directed laterally inwards towards the sagittal plane of the clamp, and which serves as an articulating surface in the hinge joint it makes with the groove of  $b_2$ . Sclerite  $c$  on each side of the movable jaw is joined distally to a median lamellate sclerite  $d$ . The lamellate nature of this sclerite  $d$  has usually not been recognized in previous descriptions; the sclerite has been represented by a bar as it appears in Pl. I, figs. 2, 5, but its true nature is shown in Pl. I, fig. 3, where the isolated sclerite has been prepared by digestion with trypsin. The narrow anterior (distal) edge of  $d$  is opposable to  $b$  of the anterior jaw, and its efficiency as a clamping device is increased by the presence of a pair of minute anteriorly directed claw-like extensions (Pl. I, figs. 3, 7, 8). The common tendon from the extrinsic muscles of the clamp, after passing through the fair-lead, is attached to the middle of the posterior (proximal) border of  $d$ .

Lastly, lining the inner walls of the clamp are a number of slender rib-like sclerites  $e$  (Pl. I, figs. 5, 6, 8), passing, on each side of the mid-line, transversely between sclerites  $a$  and  $b$ .

The clamp is closed by the action of a pair of extrinsic muscles, operating by means of a common tendon passing round the fair-lead and attached to the posterior margin of sclerite  $d$ . On contraction of the muscles, the movable jaw swings anteriorly about its hinge joint with  $b_2$ , and secondary gill lamellae are grasped between the opposable edges of  $b$  and  $d$ . The course of the extrinsic muscles is illustrated in Text-fig. 2: each clamp is served by two muscles, one of which, the inter-clamp muscle, runs directly across the body to the fellow clamp on the other side, and the other, the longitudinal muscle, turns anteriorly in the body to join with the corresponding muscles from all the other clamps to form a broad longitudinal band of muscle running to the anterior end of the parasite. This pattern of musculature whereby paired clamps are connected to each other by a common inter-clamp muscle obviously increases the efficiency of both the clamps, since each clamp acts as a firm origin for the muscle inserted on the other clamp. It is questionable whether the primary function of the longitudinal muscles is to operate the clamps; they could do so efficiently only when they have a relatively firm origin, otherwise the effects of their contraction would be dissipated into shortening the body rather than bringing about the closing of the clamp. On the other

hand, when the anterior end of the parasite is attached to the host during feeding, the efficiency of both the posterior clamping mechanism and the anterior feeding mechanism would be increased. Another possible function of the longitudinal clamp muscle could be the manoeuvring of the anterior end of the body.



Text-fig. 2. The musculature of the adhesive organs of *Plectanocotyle gurnardi*. C, clamp; H, hook; Hm, hook muscle; Icm, inter-clamp muscle; Lcm, longitudinal clamp muscle.

#### DISCUSSION

*Plectanocotyle gurnardi* has been shown to have an adhesive mechanism similar to that of *Kuhnia scombri*. The main differences lie in the number of clamps, there being three pairs in *Plectanocotyle* and four pairs in *Kuhnia*, and in the relative sizes of the hooks in the two trematodes. The large hooks of *Kuhnia* are about twice as long as the proximal-distal axis of one of its clamps, but the large hooks of *Plectanocotyle* are only about half as long as the corresponding length of one of its clamps. Again, while the hooks of *Kuhnia* are borne on the ventral surface of the body proper, those of *Plectanocotyle* are borne on what appears to be a slender posterior appendage, but an assessment of the significance of this is probably best left until the larval development of these parasites has been studied.

Though the clamping mechanisms in *Plectanocotyle* and *Kuhnia* are so similar to each other, there are differences in the detailed arrangement of the supporting sclerites that are of considerable phylogenetic interest. The support given to the median region of the clamp of *Plectanocotyle* by the single sclerite *a* is provided in *Kuhnia* by three quite distinct lamellate sclerites, the two in the anterior and posterior jaws lining the fibrous walls of the clamp, and the one in the proximal region of the clamp lying outside the fibrous wall, i.e. lying between the clamp wall and the remainder of the body. It is probable

that a detailed investigation of the development of sclerites and their relationship with other tissues would contribute much to the establishment of the precise homologies of diclidophoroidean sclerites, but here it is suggested tentatively that the rib-like sclerites *e* lining the wall of the clamp of *Plectanocotyle* are represented by the single lamellate sclerite lining the anterior jaw of *Kuhnia*, and that the median sclerite *a* of *Plectanocotyle* corresponds to the other two median sclerites of *Kuhnia*, i.e. those in the posterior jaw and in the proximal region of the clamp. If this be so, and if the fusion of parts to promote greater rigidity, and not fission, be the evolutionary trend, then it would follow that in regard to the median sclerite *a*, the more primitive condition survives in *Kuhnia*. The reverse, however, would obtain in regard to the sclerites of the movable jaw; the more primitive separate nature of the sclerites *c*, *d*, *c* survives in *Plectanocotyle*, but fusion to form a single sclerite has taken place in *Kuhnia*. It is necessary to point out that Sproston (1946, p. 414) ascribed an advantage to the articulated nature of the lateral regions of the 'middle piece' (= movable jaw) of *Plectanocotyle*, in that it enabled the parts to slide over one another and to increase the width of the grasp, 'faintly analogous to the mechanism in the jaws of a snake'. This condition was illustrated by Sproston in her fig. 95*f*, but my observations on *Plectanocotyle* have convinced me that what Sproston has drawn is a clamp skeleton in which the jaws have been forced open and the sclerites disarticulated, possibly by pressure of the cover glass during microscopy.

It is unnecessary to repeat here the criticisms I have made previously (Llewellyn, 1956*a*) of Sproston's (1945*b*) general theory of the evolution of clamping mechanisms in the Diclidophoroidea; it is sufficient to state that in *Plectanocotyle* I have been quite unable to reconcile for example Sproston's description of the 'middle piece' (= movable jaw sclerites) having its 'anterior' (= proximal) border cuticularized and joined with the 'median spring' (= sclerite *a*) by a median cuticularized band, with anything that I have observed in *Plectanocotyle*. As for Sproston's observation that the clamp structure of the Plectanocotylinae is discocotyloid in general pattern, it appears to me to be much more like *Kuhnia scombri* than *Discocotyle sagittata*. Furthermore, a preliminary examination of the adhesive mechanisms of the thirteen species of Plymouth diclidophoroideans that I have studied for other purposes (Llewellyn, 1956*b*), indicates that *Plectanocotyle gurnardi*, *Kuhnia scombri* and *Mazocraes alosae* all have very similar mechanisms involving a fair-lead, and all differ substantially from the remainder of these trematodes. If, then, clamp structure is of major phylogenetic significance, *Kuhnia*, *Mazocraes* and *Plectanocotyle* are closely related to each other. Acceptance of this idea would, however, present a new problem: in general, among the Polyopisthocotylea, groups of nearly related parasites are restricted in their distribution to groups of nearly related hosts, e.g. the Diclidophorinae on the Gadidae, and the Cyclocotylinae (= 'Choricotylinae') on the Sparidae; but



the three closely related (?) genera *Mazocraes*, *Kuhnia*, and *Plectanocotyle* would then form a group parasitizing such widely divergent host families (Berg, 1947) as the Clupeidae (Clupeiformes), Scombridae (Perciformes, Scombroidei), and Triglidae (Perciformes, Cottoidei).

It is a pleasure to record my thanks to the Director and Staff of the Plymouth Laboratory for collecting facilities, and to Miss Barbara Williams for technical assistance.

#### SUMMARY

The mechanism by which the monogenetic trematode *Plectanocotyle gurnardi* attaches itself to the gills of various species of *Trigla* has been shown to consist of hooks borne on a posterior appendage, together with three pairs of clamps which, although differing somewhat in structure, act by an extrinsic muscle-tendon-fair-lead-hinged jaw mechanism in exactly the same manner as *Kuhnia scombri* on *Scomber scombrus*. On the basis of clamp structure, *Plectanocotyle* appears to be more nearly related to the Mazocraeidae than to the Discocotylidae in which it is included at present.

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## EXPLANATION OF PLATE I

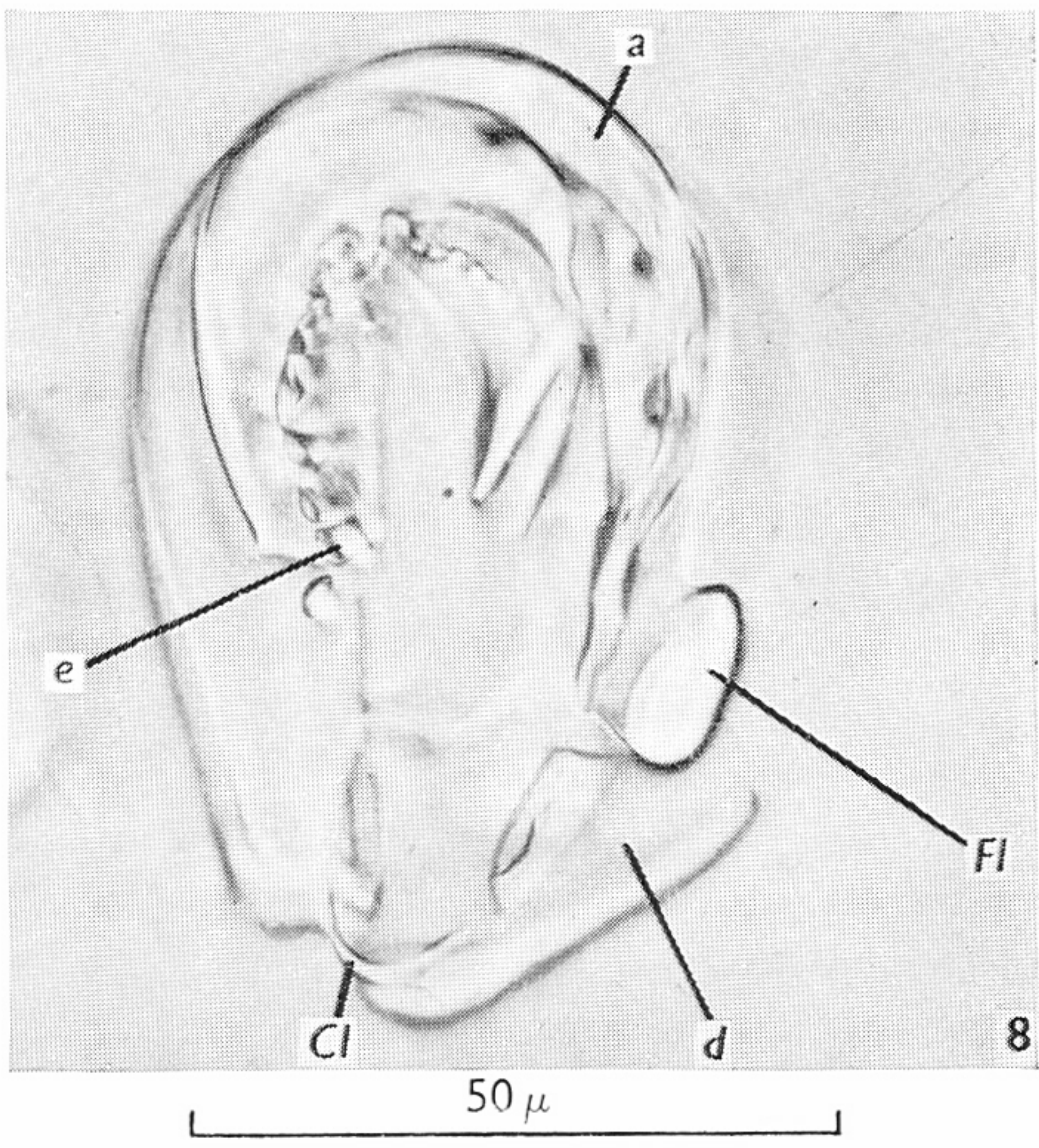
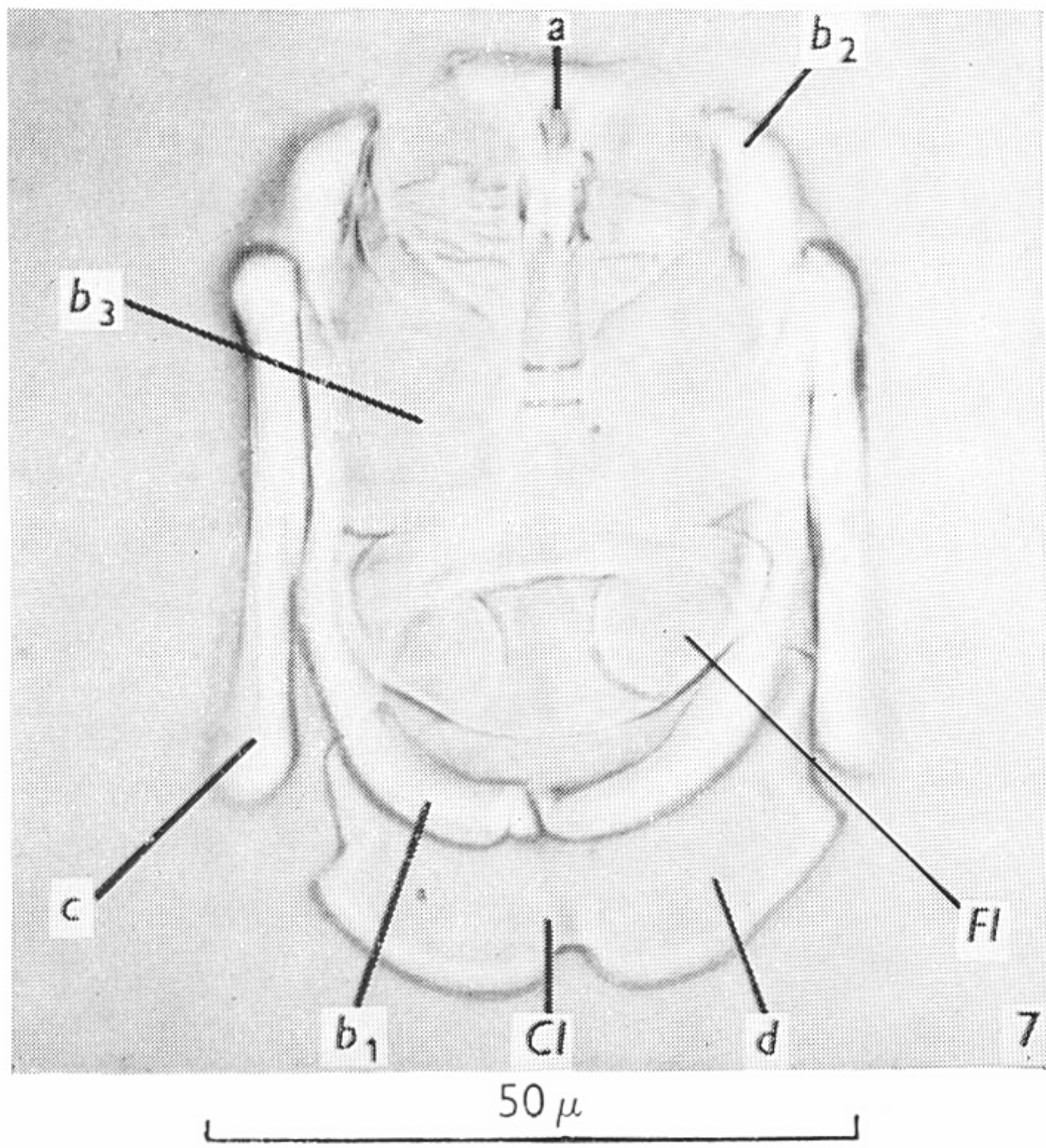
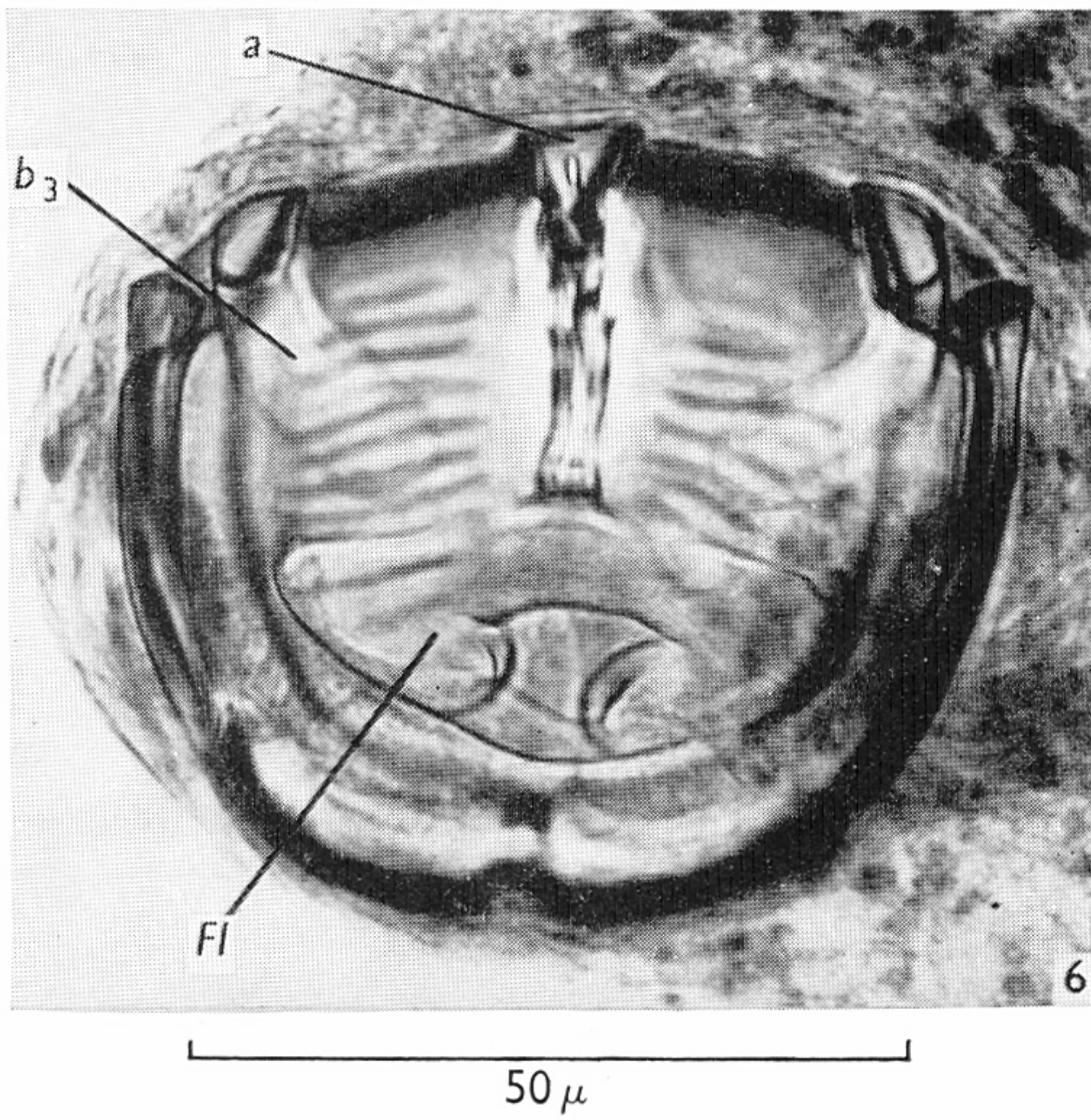
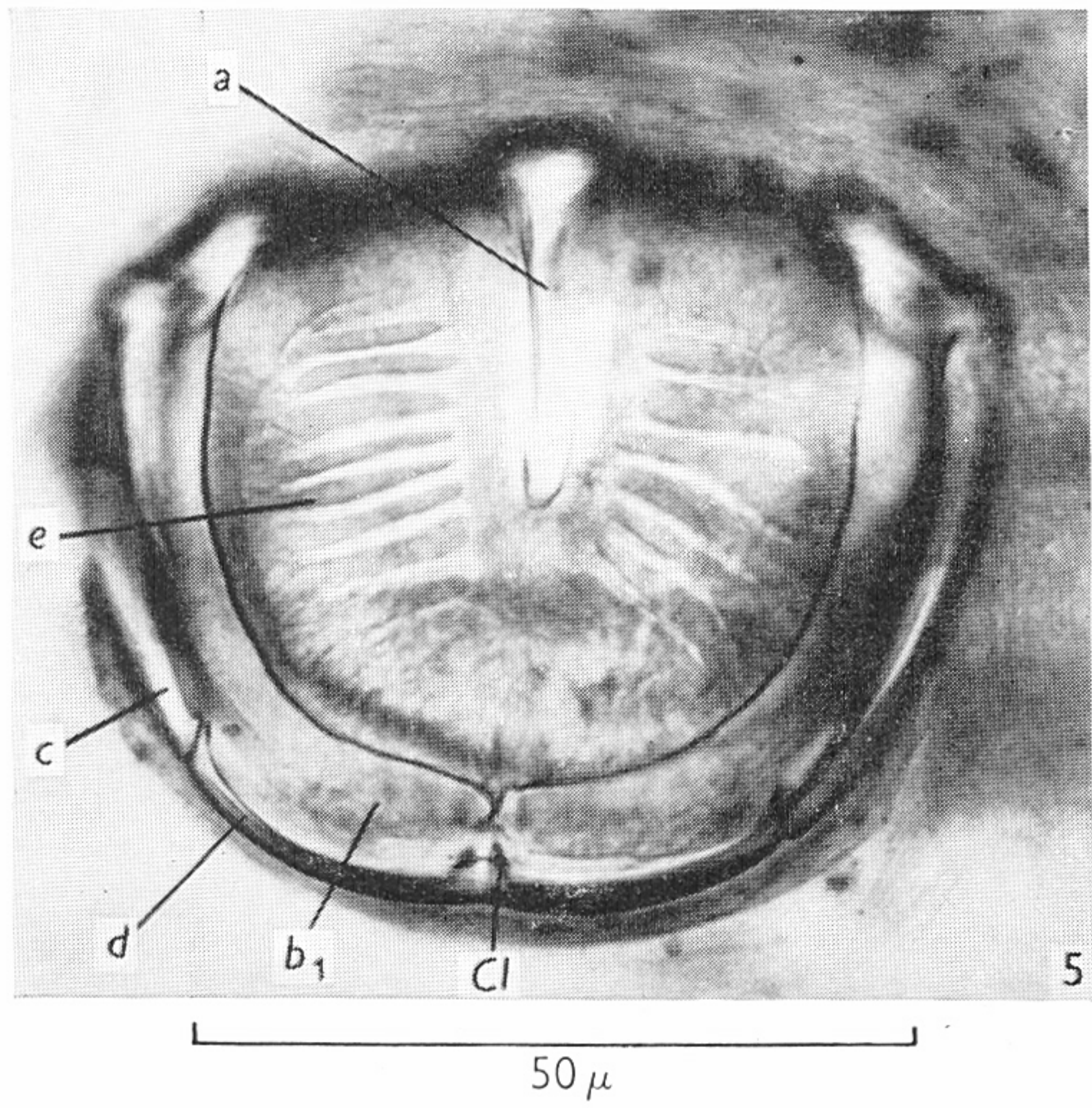
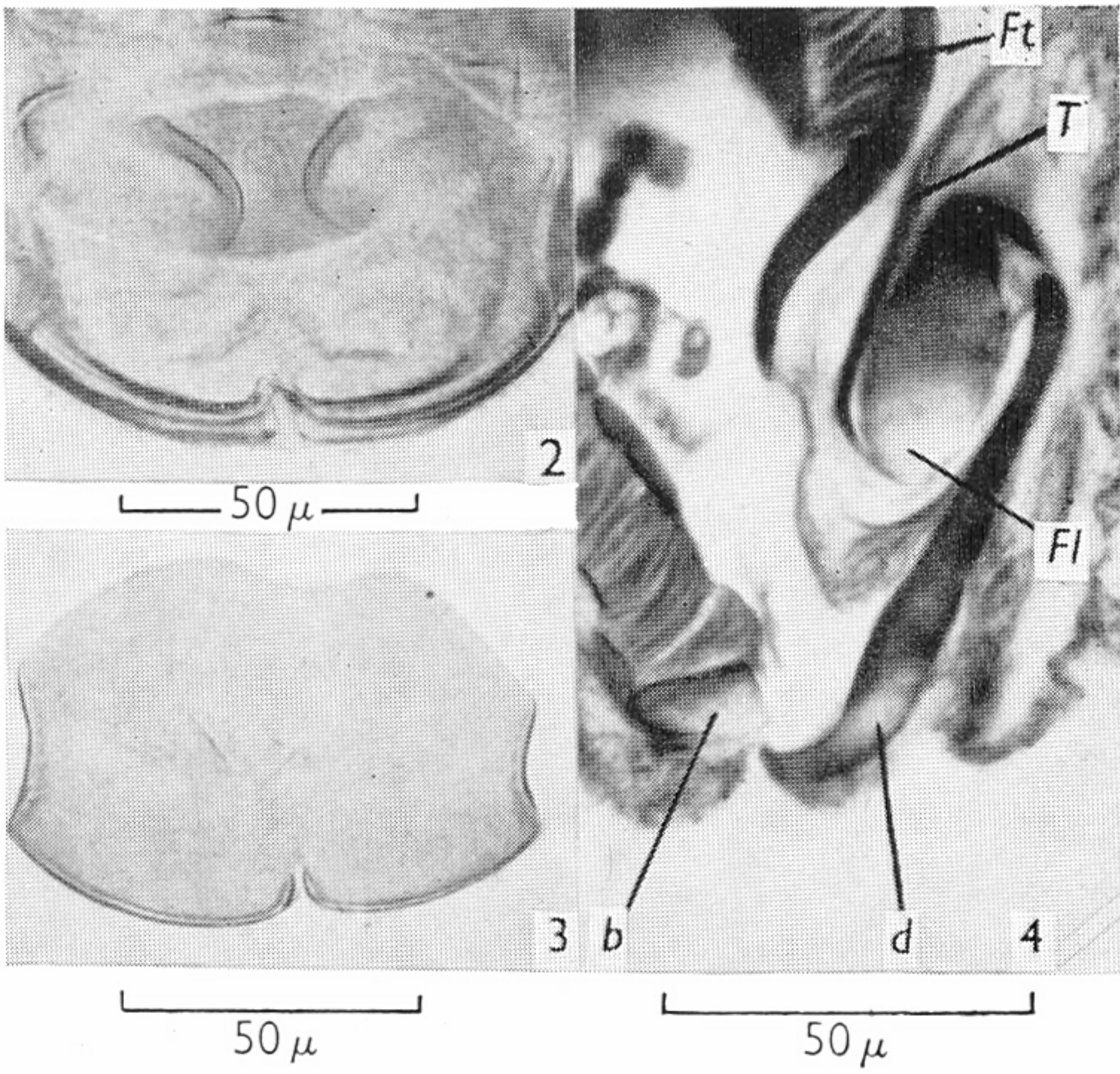
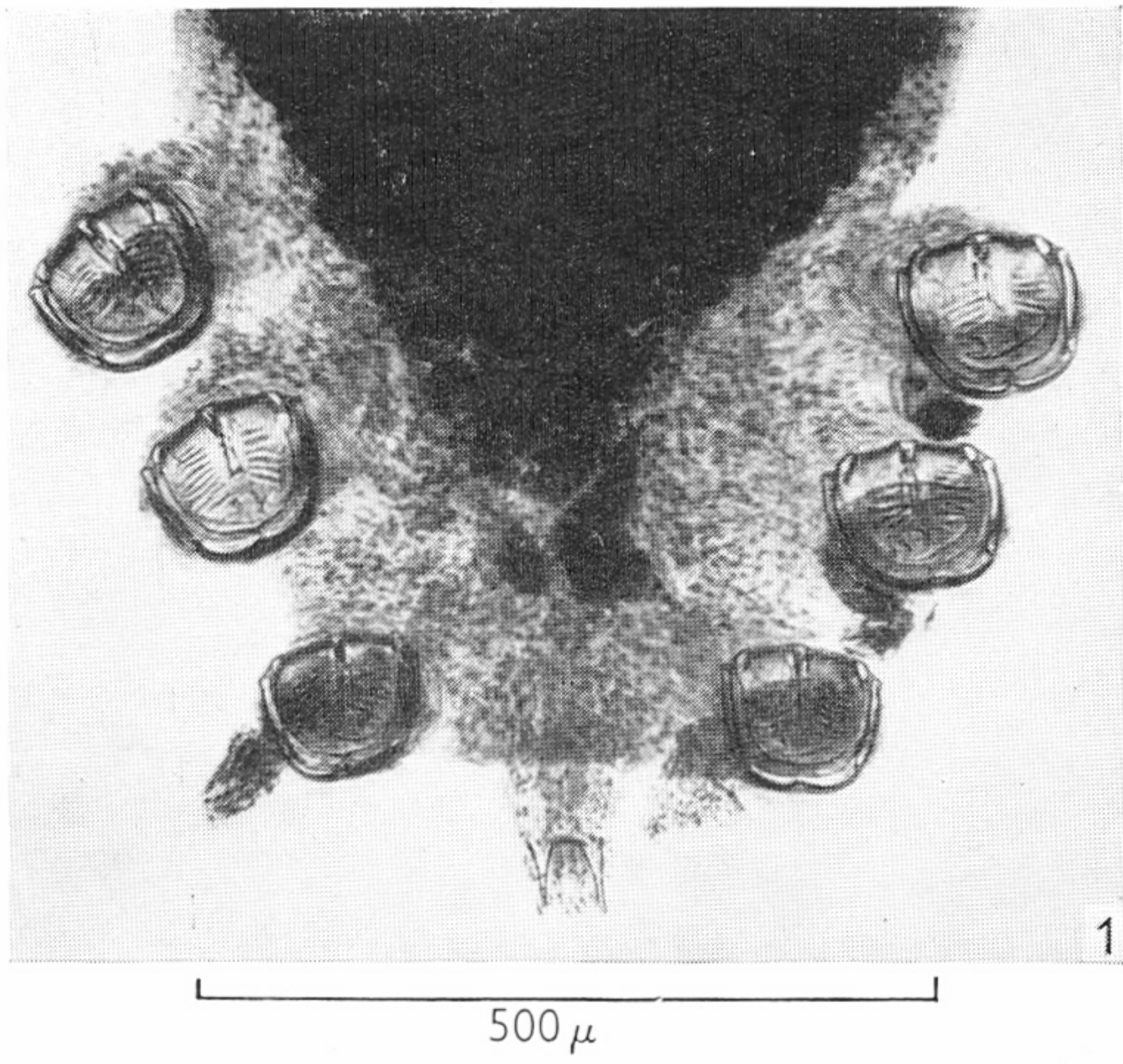
The adhesive mechanism of *Plectanocotyle gurnardi*

- Fig. 1. Posterior end of the parasite in ventral view, showing the three pairs of clamps and two pairs of hooks. Due to the flattening of the specimen beneath a cover-glass, the clamps are seen in anterior view.
- Fig. 2. The pair of ridges on the anterior surface of the fair-lead which act as a guide for the tendon. ( $\times 40$  phase contrast objective used with  $\times 95$  substage annulus.)
- Fig. 3. The distal median sclerite ('d') of the posterior movable jaw, isolated by digestion of the other tissues with trypsin.
- Fig. 4. Sagittal section of distal region of clamp, showing the tendon passing around the fair-lead to the movable jaw.
- Fig. 5. Clamp in anterior view. (Stained preparation in Canada Balsam.)
- Fig. 6. Clamp in posterior view. (Same clamp as in Fig. 5.)
- Fig. 7. Sclerites of a whole clamp remaining after other tissues have been digested with trypsin. The sclerites of the posterior jaw have become disarticulated. Posterior view.
- Fig. 8. Another clamp from the same specimen as in Fig. 7, similarly treated. Side view.

## Abbreviations

*a, b, b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, c, d, e*, sclerites (see text, pp. 508-10); *Cl*, claw; *Ft*, fibrous tissue; *Fl*, fair-lead; *T*, tendon.





(Facing p. 514)



## STUDIES ON THE ENDOCRINOLOGY OF ISOPOD CRUSTACEANS. MOULTING IN *LIGIA OCEANICA* (L.)

By D. B. Carlisle

The Plymouth Laboratory

(Text-fig. 1)

Experiments performed upon decapod crustaceans have produced much information on the hormonal control of moulting in this group. So far as I am aware, however, no one has tried to discover how moulting in other groups of Crustacea is controlled. This note represents the beginning of an attempt to investigate this aspect of moulting in the Isopoda.

### OBSERVATIONS ON THE MOULTING PROCESS IN *LIGIA OCEANICA*

It is well known that isopods, unlike most Malacostraca, moult in two portions, shedding the old cuticle of the posterior part of the body sometime before that of the front end. This seems to have been noticed first in *Porcellio* by Schöbl (1879). A detailed account of moulting in some Oniscoidea is given by Herold (1913); Numanoi (1934) describes the process in *Ligia exotica*; Tait (1917) describes it in *L. oceanica*. Briefly, in this species, after a premoult period of 6 or 7 days, a complete separation takes place between the integument of the fourth and fifth thoracic segments; the tergites of the fifth, sixth and seventh segments split longitudinally at the sides (dorso-laterally), and the animal extracts itself from the hinder part of the cuticle by a series of writhing movements. This process takes 10-12 min. The animal walks away using only the anterior walking legs since the posterior ones are not stiff enough to use for at least 12 h. In my experience moulting usually takes place during the hours of daylight. After a variable period (Tait states 4 days at a temperature of 10.5-14.5° C) the anterior thoracic segments split dorso-laterally. Once more the animal extracts itself from the old integument by writhing movements, taking about 30-40 min. I have found that the anterior integument is usually cast in one piece except in underfed animals which have been for sometime in the laboratory. Tait, however, found that this part of the integument was most usually torn into fragments, while the dorsal portions of segments 2, 3 and 4 often remained attached to the animals for some days. This condition I have only seen in animals which moulted in difficult conditions after a period of shortage of food in the laboratory. Indeed, it seems likely that most of Tait's animals were underfed and observed after some

lengthy period of captivity, for he states: 'It is a curious fact that in *Ligia*, subsequent to posterior and prior to anterior moult, no increase in the girth of the posterior part of the body can be detected with the eye. . . . Even when moult is complete *Ligia* is not obviously larger than before'. I find this is only true of starved animals.

The interval between posterior and anterior moult is not nearly so constant as Tait implies. He states that at a temperature of  $10.5-14.5^{\circ}\text{C}$  the interval is 4 days; he makes no mention of any variability. I have found that at a temperature of  $15.7-17.4^{\circ}\text{C}$  the interval is usually 2 or 3 days, but on occasion it may be only 1 day, in either large or small individuals, while periods of 4, 5 or 6 days between the two halves of the moult are not unusual. In five of the animals which I have observed anterior moult has been delayed to the day immediately before the succeeding posterior moult, while in two it has been delayed even beyond this, so that a posterior moult has been followed within 3 days by two anterior moults, one corresponding to the posterior moult of some weeks previously, and one corresponding to the more recent posterior moult.

The average intermoult period at a temperature of  $15.7-17.4^{\circ}\text{C}$  was 47.44 days, corresponding closely with the periods recorded by Nicholls (1931).

#### THE EFFECT OF EXTRACTS OF *LEANDER* EYESTALKS ON MOULTING RATE IN *LIGIA OCEANICA*

In these experiments about 340 specimens of *Ligia*, freshly collected from Drake's Island, Plymouth Sound, in December 1953, were placed singly in numbered 450 ml. containers in the laboratory each with a piece of *Fucus vesiculosus* for food. Each day the containers were rinsed out with sea water and the weed damped. All moults, deaths and escapes were recorded daily. After a preliminary observation period of 1 week one-third of the animals were injected with an extract of whole eyestalks of female *Leander serratus*. These eyestalks had been excised in August, at a period when they contain a high titre of moult-accelerating hormone, and stored in the refrigerator in absolute acetone. The extract was prepared by draining off the acetone, evaporating the remainder at room temperature, until there was no longer any perceptible aroma of acetone, and grinding the eyestalks with distilled water, acidified to pH 3.8 with hydrochloric acid, in a mortar with washed sand as an abrasive. The extract was filtered and the residue washed with a small measured quantity of acidified water. The total filtrate represented forty eyestalks per millilitre. Each *Ligia* that was injected received 0.05 ml., i.e. two eyestalk equivalents. The injection was made with a tuberculin syringe fitted with a no. 28 needle, inserted in a forward direction between the tergites of the sixth and seventh free thoracic segments into the lateral musculature. No animals died immediately after the injection, but some were found dead 24-48 h later;



these are recorded in Table I. One-third of the animals received no injection; one-third received an injection of acidified distilled water. All animals which had moulted either half of the body during the preliminary observation period were rejected from the experiment.

TABLE I. THE NUMBERS OF DEAD AND OF MOULTS IN EACH GROUP

Days after injection 0	Group 1. Injected eyestalk extract 96 alive		Group 2. Not injected 92 alive		Group 3. Injected distilled water 94 alive	
	Dead	Moulted	Dead	Moulted	Dead	Moulted
1	5	3	1	3	6	2
2	11	13	0	2	8	2
3	0	4	0	2	1	2
4	0	5	0	2	0	3
5	0	2	0	2	0	2
	16	27	1	12	15	11

The dead recorded are those which died without initiating posterior moulting; the moults are the posterior moults which were at least begun, even if the animals died before completion of the moult. The numbers are given only for the 5 days after injection, when there was a significant difference in moult rates; Fig. 1 illustrates the moult rate over a longer period of time.

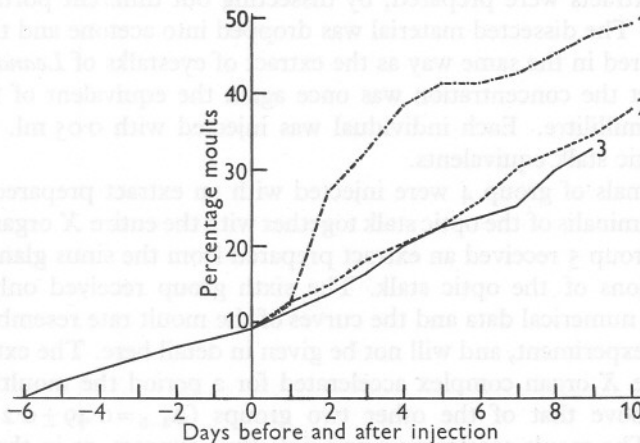


Fig. 1. The percentage moult-rates in the three groups of animals of the first experiment. For explanation see text.

The numbers which died without first moulting and the numbers which initiated the moult of the posterior half of the body are recorded in Table I; some of these latter died without completing the moult; the criterion adopted was simply the initiation of the moult process and the partial withdrawal from the exoskeleton of the posterior half of the body. Parallel probit lines were computed for the moulting rate in the three groups of animals by the method described by Carlisle & Dohrn (1953). The value of  $\Delta_{1-2}$  was  $0.57 \pm 0.23$ ,

which, with 180 degrees of freedom, gave  $P < 0.01$ . The rate of moulting in the group injected with eye-stalk extract was thus significantly greater at the 1% level of probability from that in the uninjected control group and also from that in the control group injected with distilled water. The percentage moulting rate is illustrated graphically in Fig. 1, where it will be seen that after an initial period of accelerated moulting the moult rate in the injected group drops back to normal again, giving a line parallel to that of the controls.

#### THE EFFECT OF EXTRACTS OF THE OPTIC STALKS OF *LIGIA* UPON MOULTING RATE IN *LIGIA OCEANICA*

I have previously shown (Carlisle, 1953) that the moult-accelerating hormone of *Natantia* is produced in the eyestalks, within the *X* organ complex. Isopoda, however, are sessile-eyed Crustacea and lack eyestalks. The elements of the *X* organ complex are located instead in the optic stalks of the brain within the head, where also is found the sinus gland. Experiments using extracts of the optic stalks of *Ligia oceanica* were therefore made. They followed the same plan as those of the previous section. Three groups of animals were used, each group consisting of about ninety individuals. Two different extracts were prepared, by dissecting out different portions of the optic stalk. The dissected material was dropped into acetone and the extracts were prepared in the same way as the extract of eyestalks of *Leander*. In the final extract the concentration was once again the equivalent of forty optic stalks per millilitre. Each individual was injected with 0.05 ml. of extract, i.e. two optic stalk equivalents.

The animals of group 4 were injected with an extract prepared from the medulla terminalis of the optic stalk together with the entire *X* organ complex. Those of group 5 received an extract prepared from the sinus glands and the distal portions of the optic stalk. The sixth group received only acidified water. The numerical data and the curves of the moult rate resemble those of the earlier experiment, and will not be given in detail here. The extract of the parts of the *X* organ complex accelerated for a period the moulting rate of group 4 above that of the other two groups ( $\Delta_{4-5} = 0.49 \pm 0.21$ , whence  $P < 0.01$ ). The moult accelerating principle thus appears, as in the *Natantia*, to reside in the *X* organ complex and to be absent from the sinus gland.

#### DISCUSSION

The hormonal control of colour change in Isopoda is similar in many respects to that of *Natantia* (Kleinholz, 1937; Ståhl, 1938*a, b*; Okay, 1945; Suneson, 1947; see also Knowles & Carlisle, 1956). It appears from the experiments reported here that this resemblance extends also to some aspects at least of the hormonal control of moulting. Thus the moult-accelerating principle of the

eyestalk of *Leander*, which is known to be active also on the natantian *Lysmata* (Carlisle & Dohrn, 1953) has a similar effect in accelerating the processes of the premoult in *Ligia*. Moreover, extracts of the *X* organ complex of *Ligia* have had the same effect, whereas the sinus-gland extract has had no effect on the moult rate. This agrees well with the conclusion that the moult-accelerating principle in *Lysmata* is present in both the ganglionic and the sensory papilla *X* organs and absent from the sinus gland (Carlisle, 1953). It seems probable then that essentially the same factor is present in the *X* organ complex both of Isopoda and Natantia and is responsible for the control of the course of proecdysis in both.

I have found no evidence that any of my experimental procedures have interfered at all with the interval between the two halves of the moult. It is reasonable to suppose that this somewhat variable interval is under some kind of hormonal control, but if so this is entirely separate from the control exerted by the moult-accelerating principle of the *X* organ complex.

#### SUMMARY

The moulting process of *Ligia oceanica* is briefly described, and mention is made of the great variability of the period between the two halves of the moult.

The moulting rate of *Ligia* is increased temporarily by a single injection of eyestalk extract of female *Leander*.

The moulting rate is similarly increased by a single injection of extract of the *X* organ complex of *Ligia*, but is unaffected by an extract of the sinus gland and the distal positions of the optic stalk, when this is injected at equivalent concentrations.

The moult accelerating principle of the *X* organ complex of the optic stalk is probably responsible for the control of the course of proecdysis in *Ligia*.

No evidence has been found that eyestalk or optic stalk extracts of the kind used interfere in any way with the length of the interval between the two halves of the moult.

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## MECHANICAL PROPERTIES OF *PINNA* ADDUCTOR MUSCLE

By B. C. Abbott and J. Lowy

From the Plymouth Laboratory and Department of Zoology, University of Belfast

(Text-figs. 1-6)

### INTRODUCTION

It is well known that the smooth adductors of lamellibranch molluscs can hold the shells closed against the tension exerted by the elastic hinge ligament for prolonged periods without visible signs of fatigue. Two opposing hypotheses have been put forward to explain this phenomenon. One postulates that tonic contraction is a tetanic phenomenon and that the economy of lamellibranch smooth muscles is due to their slow speed of relaxation (Ritchie, 1928); whilst the other proposes the existence of a 'catch mechanism' which enables tension to be maintained without expenditure of energy, so that no excitation is needed during tonic contraction (Jordan, 1938).

The mechanical properties of the smooth adductor of *Pinna* have recently been studied in detail by Reichel (1952, 1955) and Bandmann & Reichel (1954), who take the view that though there exists a 'plastic catch-tonus' (based on a mechanism different from that for phasic contraction) continuous excitation is needed to maintain that tonus.

In the experiments described by Bandmann & Reichel, treatment of the smooth posterior muscle of *Pinna* with acetylcholine (ACh) leads to the development of tonus, but it takes about 1 h for the tension to reach maximum. In similar experiments with *Mytilus* muscle we found that maximum tension was reached within 15 sec. This discrepancy, together with Bandmann & Reichel's observation that the *Pinna* muscle was always strongly contracted after isolation—whereas this is not true of *Mytilus* preparations—led us to investigate the *Pinna* adductor.

### METHODS

The posterior adductor muscle of *P. fragilis* (Pennant, 1777, Edition 4, Vol. 4, p. 97 and pl. 69) was used. It consists of a small white portion facing the hinge and of a larger portion, darker in appearance and containing the longest fibres. Bandmann & Reichel (1954), who worked with *P. nobilis*, do not mention the presence of two macroscopically distinct portions in the posterior adductor. Strips of the muscle containing the longest fibres (dark portion) have been used in the present experiments and these correspond to the preparation described by Bandmann & Reichel.



Histologically, both portions are composed of unstriated fibres and the only distinction between them under the light microscope is that the fibres of the dark portion have a slightly smaller diameter than those which compose the white portion.

Specimens of *P. fragilis* were collected from the region of the Grande Vasière near Concarneau (Brittany). They were kept in running sea water in the Plymouth aquarium and survived satisfactorily for up to 6 months. The posterior adductor muscle was exposed by removing parts of the shells with bone forceps. The visceral ganglia were quickly extirpated. After soaking in aerated sea water for about an hour the muscle relaxed completely under a small load. The length of the muscle with closed shells was from 25 to 30 mm; and relaxed with shells gaping, from 35 to 45 mm. Strips about 4 mm broad and 2 mm thick were prepared from the dark part, tied at both ends and cut. Sea water was used as a bathing medium.

These muscle strips relaxed under a light load to the longest length reached by them in the animal with shells gaping (*reference length*). If the muscle strips were pre-shortened by stimulation below the reference length, allowed to relax, and then stretched, a tension developed which decayed completely within about 1 min. This phenomenon was observed in all smooth and striated muscles which we have investigated (Abbott & Lowy, unpublished) and shows that no resting tension is developed below reference length.

Muscle strips were mounted on a multielectrode stimulating assembly (Hill, 1949) and connected by a light silver chain to an isometric or isotonic lever. Stimulation was by square wave pulses from an electronic stimulator (Attree, 1950). The strength-duration curve for single twitches (threshold responses) was obtained in order to find a suitable pulse width for stimulation. The width generally used was 3 msec, with a voltage of 10 V for maximal stimulation. Contraction was recorded photoelectrically and displayed on a cathode-ray tube.

Records at room temperature were taken of isometric twitches, isometric tetani and of after-loaded isotonic contractions. The stimulus frequency for tetanus was determined from fusion frequency experiments.

The mechanical properties of the resting muscle were investigated by stretches which were applied by lowering a Palmer stand. The resulting tension changes with time were recorded.

Responses to acetylcholine were studied. A final concentration in the bath of  $10^{-5}$  or  $10^{-6}$  g/ml. was adequate to produce maintained tonic contractions.

Some preliminary experiments have been done on the mechanical properties of the small white portion of *Pinna's* posterior adductor: it is much slower than the darker part of the muscle. The latter will from now on be referred to as the fast part of the adductor.

## RESULTS

*Excitability*

Isolated strips of the fast part of *Pinna*'s posterior adductor muscle respond to a single stimulus with a twitch. The strength-duration curve for the system is shown in Fig. 1. On the evidence available it is not possible to decide whether this represents excitation of nerve or muscle. Using either multiple-point or one-end stimulation, a maximal isometric twitch can be obtained (Fig. 2). At the recording sensitivity used, the first sign of tension appears at 40 msec (latent period) and the peak twitch tension of 250 g/cm<sup>2</sup> is reached in 200 msec. Relaxation is very slow compared with the rising phase, tension falling to half-value in 2 sec.

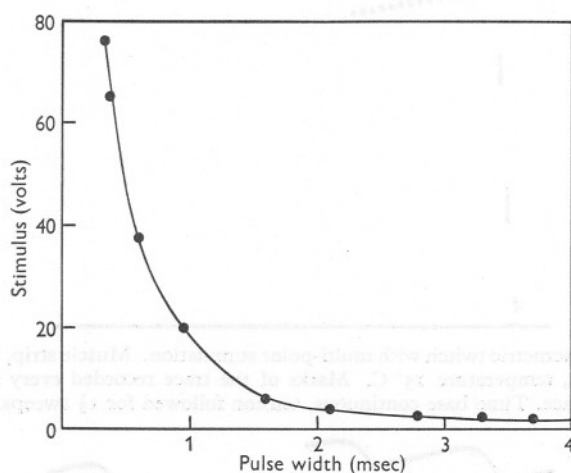


Fig. 1. Strength-duration curve: threshold responses to single stimuli.

Repeated stimuli give responses which summate. There are no signs of facilitation (Fig. 3). The fusion frequency is 7/sec at room temperature and the tetanus tension is about 6 times that developed during a twitch. An isometric tetanus at 7/sec shows a smooth rise of tension which reaches maximum within 4 sec. When stimulation ends the muscle relaxes completely.

*Isotonic Shortening*

The speed of shortening under afterload conditions varies with load as shown in Fig. 4, the load for zero speed being represented by the isometric tension. The curve is a hyperbola and can be described by Hill's (1938) characteristic relation between force and velocity:

$$(P + a)(v + b) = b(P_0 + a),$$

where  $P$  is load on the muscle,  $P_0$  is isometric tension,  $v$  is speed of shortening, and  $a$  and  $b$  are constants.

In the muscle illustrated maximum speed of shortening is 8.5 mm/sec or 0.3 length/sec; it is faster than the *Mytilus* adductor (0.1 length/sec). The

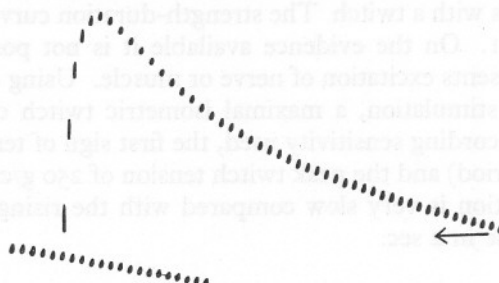


Fig. 2. Record of isometric twitch with multi-point stimulation. Muscle strip, weight 240 mg, length 37 mm, temperature 15° C. Marks of the trace recorded every 20 msec, shock at origin of trace. Time base continuous, tension followed for  $1\frac{1}{2}$  sweeps.

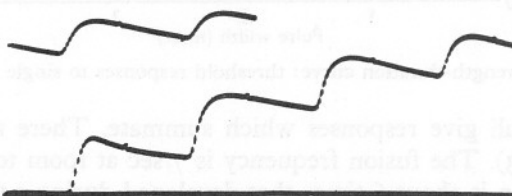


Fig. 3. Repeated twitches, 2/sec. Muscle strip as in Fig. 2.

maximum tension developed by the *Pinna* muscle is only 1.5 kg/cm<sup>2</sup> compared with about 2 kg/cm<sup>2</sup> for many other muscles (Wilkie, 1954), but this is probably due to damage during preparation. The value for  $a/P_0$  is 0.35—well within the range found for a variety of muscle types (Wilkie, 1954).

### Length-Tension Relations

The relaxed muscle at reference length shows no resting tension. Tetanic stimulation produces active tension and the value of peak isometric tension decreases on either side of an optimum, at about reference length (Fig. 5).

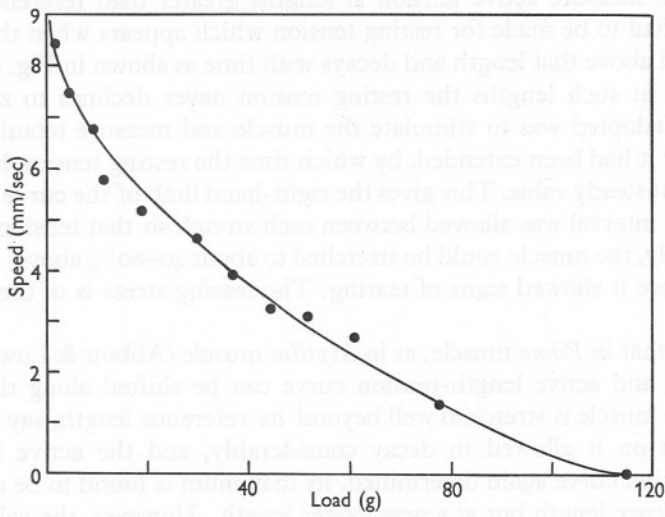


Fig. 4. Force-velocity curve for muscle strip. Weight 250 mg, length 30 mm, temperature 15° C.

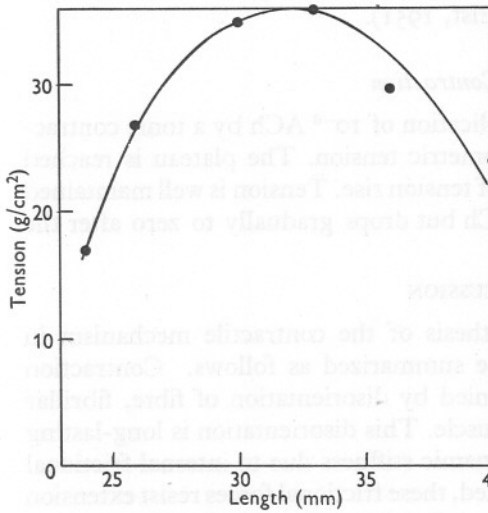


Fig. 5

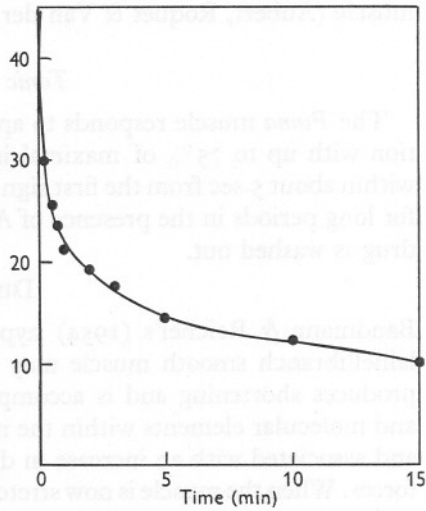


Fig. 6

Fig. 5. Isometric tension-length curve for muscle strip. Temperature 15° C.

Fig. 6. Decay of tension following stretch of resting muscle strip above reference length. Reference length 35 mm, muscle stretched from 35 to 40 mm.

In order to measure active tension at lengths greater than reference length allowance had to be made for resting tension which appears when the muscle is stretched above that length and decays with time as shown in Fig. 6. It was found that at such lengths the resting tension never declines to zero. The procedure adopted was to stimulate the muscle and measure tetanic tension 5 min after it had been extended, by which time the resting tension had fallen to an almost steady value. This gives the right-hand limb of the curve in Fig. 5.

If a long interval was allowed between each stretch so that tension decayed considerably, the muscle could be stretched to about 40–60% above reference length before it showed signs of tearing. The tearing stress is of the order of 6 kg/cm<sup>2</sup>.

We find that in *Pinna* muscle, as in *Mytilus* muscle (Abbott & Lowy, 1953), the resting and active length-tension curve can be shifted along the length axis. If the muscle is stretched well beyond its reference length, say by 25%, the tension on it allowed to decay considerably, and the active isometric length-tension curve again determined, its maximum is found to be no longer at the reference length but at a new longer length. However, the value of the peak tension is still the same, i.e. the curve has evidently moved along the length axis. This shift is irreversible and probably due to a 'slip' within the muscle. A similar phenomenon has been observed in vertebrate skeletal muscle (Aubert, Roquet & Van der Elst, 1951).

#### *Tonic Contraction*

The *Pinna* muscle responds to application of  $10^{-6}$  ACh by a tonic contraction with up to 75% of maximal isometric tension. The plateau is reached within about 5 sec from the first sign of tension rise. Tension is well maintained for long periods in the presence of ACh but drops gradually to zero after the drug is washed out.

#### DISCUSSION

Bandmann & Reichel's (1954) hypothesis of the contractile mechanism in lamellibranch smooth muscle may be summarized as follows. Contraction produces shortening and is accompanied by disorientation of fibre, fibrillar and molecular elements within the muscle. This disorientation is long-lasting and associated with an increase in dynamic stiffness due to internal frictional forces. When the muscle is now stretched, these frictional forces resist extension and a state is set up which Bandmann & Reichel define as 'plastic tonus'.

In the animal, excitation causes the adductor to contract against the elastic tension exerted by the hinge ligament. It is assumed that when excitation stops, the frictional forces due to disorientation produced by contraction can 'take over' and resist the pull of the ligament by tension due to 'plastic tonus' (Spertronus or catch mechanism). This tension is believed to be the same as that which results from stretch of a resting, shortened, disorientated muscle;



it decays extremely slowly and therefore in the animal very little re-excitation is needed to maintain it.

Evidence for the above hypothesis comes from Bandmann & Reichel's experiments on the mechanical properties of *Pinna* muscle. Length-tension relations of the isolated resting muscle were studied. Stretch produced a tension which decays very slowly. The object of this experiment was to demonstrate the existence of 'plastic tonus'. Next, the decrease in tension as the muscle was allowed to shorten from the stretched condition was investigated. A well-defined length-tension curve could be obtained on which zero tension was reached at the original starting length. But the experiment also showed that 'plastic tonus' can give only a limited amount of tension: if too great a stretch is applied, the muscle 'slips', i.e. if the length-tension curve is again determined, zero tension is reached at a new, longer length. This process, called 'plastic lengthening', is believed to be associated with re-orientation of muscle structures. It is of critical importance to Reichel's hypothesis, for 'plastic tonus' can only disappear by such re-orientation. Thus, in the animal, when the shells gape the muscle is supposed to be plastically lengthened; contraction to result in closure; and 'plastic tonus' to keep the shells closed. That this sequence of events is possible was demonstrated by Bandmann & Reichel in experiments where a plastically lengthened muscle was treated with acetylcholine. Tension was developed and when such an acetylcholine-activated muscle was allowed to shorten in steps and the recovery of tension observed, the length at which no tension was re-developed proved to be the original starting length, i.e. the length before 'slip' occurred.

Taking Bandmann & Reichel's experimental findings as they stand, their hypothesis is both attractive and consistent. But from our experience with other molluscan muscles we were concerned about three observations reported by Bandmann & Reichel. First, that after isolation the *Pinna* muscle was stated to be in a very contracted state, and, even after stretching with a heavy load for a matter of hours, could never be brought back to its body length. Secondly, that at all lengths stretch of the resting muscle resulted in a very long-lasting tension. Thirdly, that the time taken for development of maximum isometric tension following treatment with acetylcholine was about 1 h.

Our experimental results described above indicate that the *Pinna* muscle does in fact behave very much like other molluscan muscles. After suitable dissection it relaxes to maximum body length (reference length); any tension produced by stretch of the resting muscle at lengths below reference length decays completely within a minute; lastly, the time taken to reach maximum isometric tension, whether induced tetanically or by acetylcholine, is only of the order of seconds.

It would thus seem that the muscles used by Bandmann & Reichel were not in good condition. This is substantiated by our observation that if the ganglia are not extirpated rapidly, molluscan smooth muscles often go into

a state of 'contracture' and then give much the same responses as those described by Bandmann & Reichel. We have also found that when muscle strips are prepared complete with shell attachments, similar 'contractures' may develop—presumably due to violent excitation whilst cutting the shell close to the muscle strip.

Similar considerations possibly apply to the experiments of Brecht, Utz & Lutz (1955) with *Anodonta* adductors in which the muscles were slow to contract and failed to relax completely after tetanic stimulation. These workers investigated oxygen consumption polarographically and found that the increased rate of metabolism associated with contraction returns to its resting value whilst tension still persists. Brecht and his collaborators conclude from this that a 'freezing' of the contractile elements (catch mechanism) could be responsible for maintenance of tension without increased oxygen consumption. This is contrary to our own findings with *Mytilus* muscle where increased energy expenditure (heat production) accompanies tonic contraction (Abbott & Lowy, 1955).

In our view, the economy of lamellibranch smooth muscle is due to its slow rate of relaxation. The present work on *Pinna* serves to support this hypothesis which has been mainly derived from experiments on *Mytilus* muscle (Lowy, 1953; Abbott & Lowy, 1953, 1955; Hoyle & Lowy, 1956). The fast *Pinna* adductor proved to be much quicker than *Mytilus* muscle; its intrinsic speed is actually comparable to that of some vertebrate striated muscles. On the other hand, as is the case in most other molluscan smooth muscles, the *Pinna* adductor relaxes relatively slowly. Peak twitch tension is reached by 200 msec, but relaxation is complete only after 12 sec. These findings can be considered in terms of the concept of active state (Hill, 1949). The level of active state in a muscle is equated to the tension which the contractile component can exert if it is neither shortening or lengthening. Full activity is developed very soon after stimulation (as shown by quick-stretch experiments). This active state decays with time and at the peak of an isometric twitch, when the contractile and series elastic elements are in equilibrium, the tension exerted represents the level of active state remaining. Since in *Pinna* muscle the tetanus-twitch ratio is about 6, only 1/6 of the full active state remains 200 msec. after the stimulus. Tension, however, decays to zero only after 12 sec. Thus the contractile component returns to a state of complete rest a very long time after the active state has disappeared.

From the above considerations we conclude that tension in the intact *Pinna* adductor cannot be identified with tension due to stretch of isolated resting muscle, i.e. 'plastic tonus' is not a physiological phenomenon. Bandmann & Reichel (1954) and Reichel (1955) agree with the view (Lowy, 1953) that re-excitation is needed to maintain tonic contraction but their concept of the mechanism involved differs basically from our own. Whereas their 'plastic

tonus' reflects an internal state of disorientation in resting muscle, we explain the economy of tonic contraction in lamellibranch smooth muscle by the slowness with which the contractile component returns from the active to the resting state.

We wish to thank the officers and crew of *R.V. Sarsia* for their enthusiastic help in locating and collecting *Pinna*. We also wish to acknowledge our gratitude to M. Cailleau of Lesconil, Finisterre who, with his colleagues, guided us to the *Pinna* grounds.

#### SUMMARY

The mechanical properties of the fast portion of *Pinna*'s posterior adductor muscle at rest and during activity have been studied.

The muscle responds to a single shock with a twitch and to repeated shocks with a tetanus. Maximum speed of shortening is about 0.3 length/sec. and peak isometric twitch tension is reached at 200 msec. Relaxation is slow, tension dropping to half in 2 sec and to zero in 12 sec.

Acetylcholine induces a tonic contraction, peak tension being reached in 7 sec. Tonus is only maintained as long as the drug is present.

Contraction in lamellibranch smooth muscle is discussed in the light of Reichel & Bandmann's (1954) hypothesis. On this hypothesis, tonus in the intact muscle is supposed to be maintained by the same mechanism which produces long-lasting tension when resting isolated muscle is stretched ('plastic tonus'). The present experiments show that tension produced by stretch of isolated resting muscle within the limits of body lengths is long-lasting only if the muscle is in poor condition. From this evidence on time relations of muscles in good and poor condition, it is concluded that 'plastic tonus' is not a physiological phenomenon.

The tetanus hypothesis of tonic contraction in lamellibranch smooth muscles is supported, and the economy of these muscles is explained in terms of their slow relaxation rate.

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## THE MODES OF ACTION OF TOXIC AGENTS

### I. OBSERVATIONS ON THE POISONING OF CERTAIN CRUSTACEANS BY COPPER AND MERCURY

By E. D. S. Corner and B. W. Sparrow

From the Plymouth Laboratory and the International Paints Research Laboratory,  
Newton Ferrers, Devon

(Text-figs. 1-5)

Reports of recent attempts to discover how copper and mercury act as poisons to crustaceans are to be found in papers by Clarke (1947), Pyefinch & Mott (1948), Barnes & Stanbury (1948), Hoffmann (1950) and Russell Hunter (1950). Most of the evidence obtained has been interpreted in the light of two general theories (cf. Pyefinch & Mott, 1948). One is that these poisons exert their toxic effects by inactivating vital processes which occur at the animal's surface: the other is that they are absorbed by the animal and act internally by inhibiting metabolic changes. However, conclusive evidence supporting or excluding either possibility has yet to be obtained.

The present account describes some preliminary experiments which have formed part of a series on the effects of toxic agents on crustaceans, and is concerned with testing the importance of penetration in the toxic action of a heavy metal. In these experiments the relative toxicities of copper (as copper sodium citrate) and mercury (as mercuric chloride, ethylmercuric chloride and mercuric iodide) have been tested using (1) larvae of the brine shrimp *Artemia salina* (L.), a species which has been found to be very resistant to heavy metal poisons, and (2) adults of the marine copepod *Acartia clausi* (Giesbrecht) and larvae of the barnacle *Elminius modestus* (Darwin), two crustacean species which, compared with *Artemia*, show little resistance to these poisons. Copper sodium citrate ( $\text{NaCuC}_6\text{H}_5\text{O}_7 \cdot n\text{H}_2\text{O}$ ;  $\text{Cu} = 17.5\%$ ) was used in this investigation because, of the various copper salts tested in experiments with *Artemia*, this was the only one which gave lethal concentrations of copper which were soluble in sea water at pH 8.1. Mercuric chloride was tested because of its widespread use by other workers in this field of research. Ethylmercuric chloride was used because studies of its action as a bactericide and fungicide have led to the view that it possesses a rapid rate of penetration (cf. Sexton, 1953). Finally, mercuric iodide was tested because of the results of further studies carried out in this laboratory (to be published later) concerning the influence of iodine compounds (e.g. sodium iodide and sodium iodoacetate) on the susceptibilities of certain crustaceans to mercury poisoning.



Experiments have also been carried out in which the toxic effects of bipartite mixtures of copper and mercury have been tested, and the results of these studies have provided further information on the modes of action of these poisons which, in some respects, appear to be different.

## METHODS

### *Animals*

*Artemia salina* was reared in sea water. Two days after the larvae had hatched they were removed by filtration on a fine gauze, transferred to a small volume (5–10 ml.) of filtered sea water, and samples (0.01–0.02 ml.) of the thick suspension of animals so formed were then added to the various solutions under test.

*Acartia clausi* was collected on the day of each experiment with a medium tow-net in Wembury Bay. Separation of these animals from unwanted material was facilitated by their swarming in an area of high light intensity at the surface of the sample, from which they were pipetted first into filtered sea water and then into the toxic solutions.

*Elminius modestus* larvae were obtained from adults collected from rocks at Tinside in front of the Plymouth Laboratory. The animals were placed in fresh sea water and the larvae which they liberated were collected after 1 h and added to the toxic solutions.

### *Toxic agents*

Solutions of the poisons in sea water were prepared immediately before each experiment and adjusted to pH 8.1. The amounts of mercuric iodide and ethylmercuric chloride which dissolved in sea water were small and limited the range of toxic concentrations which could be studied. However, by shaking suspensions of these compounds in sea water overnight, it was possible to dissolve a concentration of each equivalent to 25 mg  $\text{Hg}^{++}/\text{l}$ .

The concentrations of the mercury and copper compounds used in the present work are expressed throughout in terms of mg  $\text{Hg}^{++}$  and  $\text{Cu}^{++}/\text{l}$ ., a terminology used purely for the sake of convenience in comparing toxicities and not intended to imply that these are the ions to which the compounds give rise in sea water.

### *Toxicity measurement*

Glass tubes (10 by 2 cm), each containing the solution to be tested (5 ml.), and 50–100 animals, were placed on a glass plate marked in squares ( $0.25 \text{ cm}^2$ ) and examined under a binocular microscope. Sufficient replicates were used to give a total of 200–300 animals in each of several concentrations of the same poison and in the control solutions. With the three species used it was found that when the animals lost their activity they settled on the bottom of the tube so that the number which lay above each square section of the glass

plate could readily be counted. In this way the total number of animals which had lost all signs of movement, including that of their appendages, was estimated at suitable time intervals. These experiments were usually conducted until at least 90% of the test animals had become sufficiently quiescent to be presumed dead. The remaining 10% were then killed (by adding one or two drops of a solution of sodium azide in dil. HCl to the sea-water medium), the total number of animals was estimated, and the percentage of the total which were dead at each time interval was calculated. These percentage values plotted against time gave sigmoid curves, from which the time required for 50% of the test animals to die was calculated. Such '50% death' values were found for each of several concentrations of a given poison. Usually duplicate determinations of the time of 50% death in a particular concentration of any of the poisons used gave values not differing by more than 5% of the mean.

No experiment on *Acartia* and *Elminius* lasted for more than 24 h. Some on *Artemia*, however, were continued for 4 days, during which time animals immersed in toxic solutions and in sea water moulted from the first to the second instar (Heath, 1924). As yet, however, no quantitative examination has been made of the effects of the poisons used on the mechanism of moulting or on the toxicities of these poisons to animals at different stages of development. No food was given to the animals during the toxicity experiments, but both *Acartia* and *Elminius* remained normally active for one day and *Artemia* for at least 5 days in filtered sea water.

## EXPERIMENTS AND RESULTS

### *Comparison of Toxicities*

When the time of 50% death was plotted against the concentration the toxicity curves shown in Fig. 1 (*Artemia*), Fig. 2 (*Elminius*) and Fig. 3 (*Acartia*) were obtained. It seemed that the most satisfactory way of estimating the relative toxicities of the poisons to each species was to determine the ratios of the concentrations which caused 50% death in the same time. Because, however, of the marked differences between the toxicities of the poisons used, it was not possible to find a time of 50% death which was common to all four toxicity curves. Accordingly, times of 50% death which were common to the toxicity curves of mercuric chloride, ethylmercuric chloride and mercuric iodide were used to estimate the equitoxic concentrations and hence the relative toxicities of these three mercury compounds; and times of 50% death which were common to the toxicity curves of copper sodium citrate and mercuric chloride were used in the same way in order to calculate the relative toxicities of these two poisons. The toxicities of the four poisons tested with each species, expressed in terms of that of mercuric chloride as a standard, are shown in Table I.

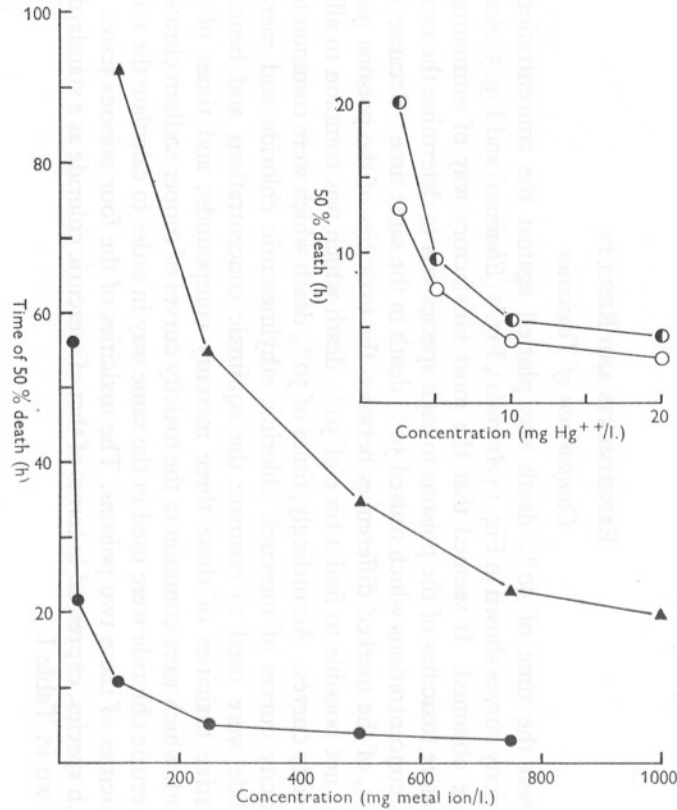


Fig. 1. Survival of larvae of *Artemia salina* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐) and mercuric iodide (○—○).

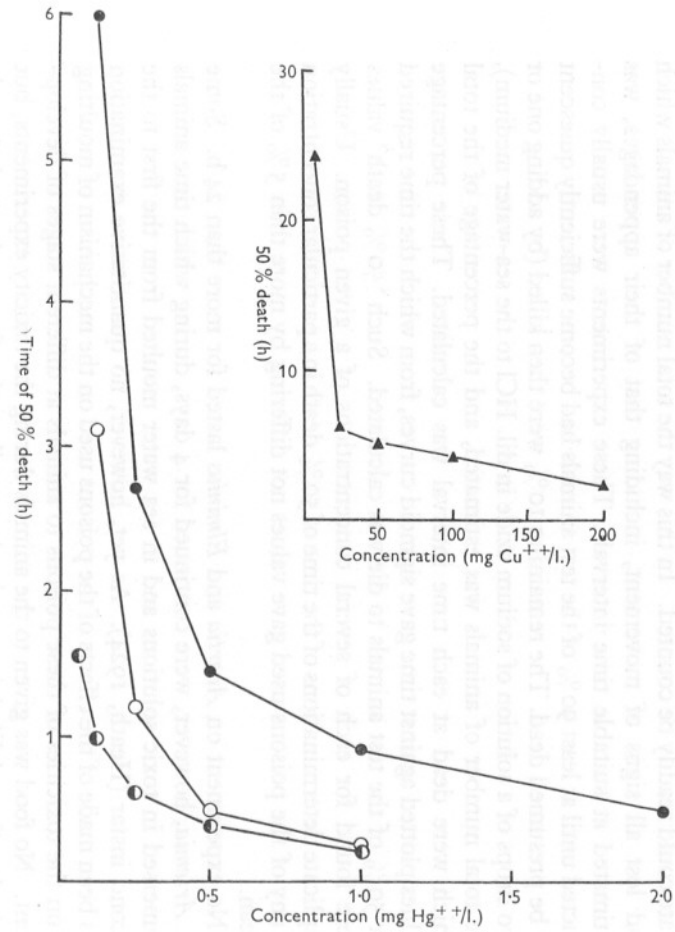


Fig. 2. Survival of larvae of *Elminius modestus* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐), and mercuric iodide (○—○).

The results showed that differences between the toxicities of mercuric iodide and ethylmercuric chloride on the one hand and mercuric chloride on the other varied with the resistance to mercury poisoning of the species used as test animal, being minimal with the least resistant *Acartia* (50% death in 2.5 h in 0.05 mg  $\text{Hg}^{++}/\text{l.}$ ), slightly greater with *Elminius* (50% death in 2.5 h in 0.30 mg  $\text{Hg}^{++}/\text{l.}$ ) and maximal with the very resistant *Artemia* (50% death in 2.5 h in 800 mg  $\text{Hg}^{++}/\text{l.}$ ). No such trend was observed, however, in the differences between the toxicities of copper sodium citrate and mercuric chloride: the results simply indicated that copper sodium citrate was much less toxic than mercuric chloride to the three species examined.

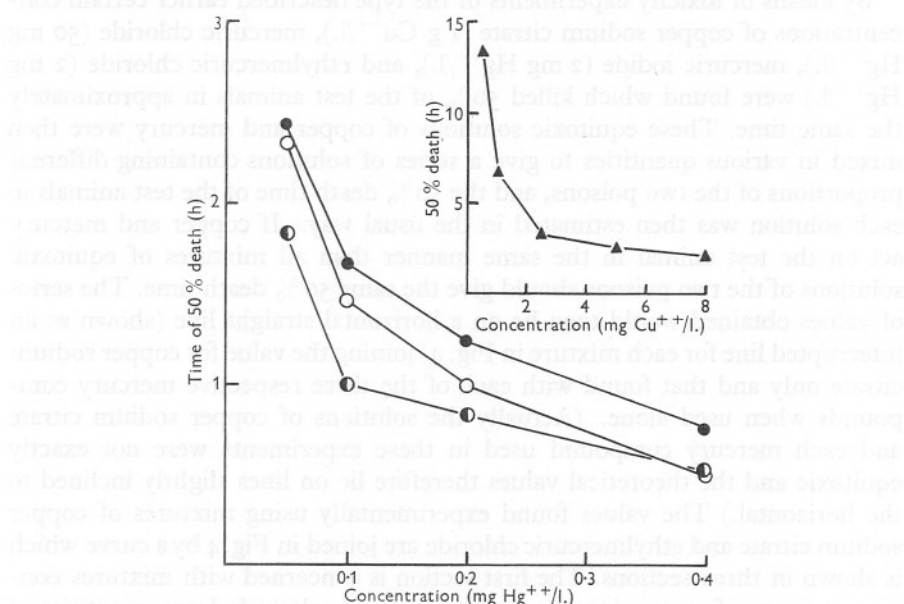


Fig. 3. Survival of *Acartia clausi* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐) and mercuric iodide (○—○).

TABLE I. RELATIVE TOXICITIES OF COPPER AND MERCURY POISONS

Compounds in sea water, pH 8.1. Experiments conducted at room temperatures between 21 and 25°C. Toxicity of each poison relative to that of mercury as mercuric chloride, expressed as unity.

Poison	Relative toxicity		
	<i>Artemia</i>	<i>Elminius</i>	<i>Acartia</i>
Mercuric chloride	1.0	1.0	1.0
Ethylmercuric chloride	24	4.4	2.0
Mercuric iodide	31	2.6	1.7
Copper sodium citrate	0.005	0.002	0.01

*Studies with Bipartite Mixtures of Poisons*

Previous workers have reported that toxic mixtures of copper and mercury show marked supplemental synergisms (more-than-additive toxic effects) when used against crustaceans (Barnes & Stanbury, 1948; Pyefinch & Mott, 1948; Hoffmann, 1950; Russell Hunter, 1950). These observations have led to the view that copper and mercury act on the animals in different ways and that the changes induced by one poison reinforce those caused by the other. To examine this possibility with *Artemia* as the test animal the following procedure was used.

By means of toxicity experiments of the type described earlier certain concentrations of copper sodium citrate (1 g  $\text{Cu}^{++}/\text{l.}$ ), mercuric chloride (50 mg  $\text{Hg}^{++}/\text{l.}$ ), mercuric iodide (2 mg  $\text{Hg}^{++}/\text{l.}$ ), and ethylmercuric chloride (2 mg  $\text{Hg}^{++}/\text{l.}$ ) were found which killed 50% of the test animals in approximately the same time. These equitoxic solutions of copper and mercury were then mixed in various quantities to give a series of solutions containing different proportions of the two poisons, and the 50% death time of the test animals in each solution was then estimated in the usual way. If copper and mercury act on the test animal in the same manner then all mixtures of equitoxic solutions of the two poisons should give the same 50% death time. The series of values obtained would then lie on a horizontal straight line (shown as an interrupted line for each mixture in Fig. 4) joining the value for copper sodium citrate only and that found with each of the three respective mercury compounds when used alone. (Actually the solutions of copper sodium citrate and each mercury compound used in these experiments were not exactly equitoxic and the theoretical values therefore lie on lines slightly inclined to the horizontal.) The values found experimentally using mixtures of copper sodium citrate and ethylmercuric chloride are joined in Fig. 4 by a curve which is shown in three sections. The first section is concerned with mixtures containing traces of copper (0-0.05 mg  $\text{Cu}^{++}/\text{l.}$ ) and relatively large quantities of mercury (*ca.* 2 mg  $\text{Hg}^{++}/\text{l.}$ ) and in this region of the curve it will be seen that the differences between the theoretical and experimental values are insignificant. The second section of the curve includes a value obtained using a mixture of approximately equal quantities of the two poisons (2.0 mg  $\text{Cu}^{++}/\text{l.}$ ; 1.9960 mg  $\text{Hg}^{++}/\text{l.}$ ), and this again is not significantly different from the theoretical value. The third and largest section of the curve deals with mixtures containing small quantities of mercury and large amounts of copper, and in this region of the curve values found experimentally are consistently lower than the theoretical values. Similar results were obtained when mixtures containing mercuric iodide and copper sodium citrate were studied (central curve of Fig. 4).

The lowest curve in Fig. 4 describes the results of experiments using toxic mixtures of copper sodium citrate and mercuric chloride. The first section of



this curve is concerned with mixtures which contained traces of copper ( $1.0$ – $2.5$  mg  $\text{Cu}^{++}/\text{l.}$ ) and a large excess of mercury ( $49.75$ – $49.95$  mg  $\text{Hg}^{++}/\text{l.}$ ), and it will be seen that, unlike the corresponding sections of the curves described earlier, more-than-additive effects are already manifest. Results depicted in the other section of the curve show that as the quantity of copper in the mixture exceeded that of mercury the differences between theoretical and experimental values became greater until a ratio of  $20:1$  in favour of copper was reached; thereafter the differences became less marked except for the mixture which contained  $975$  mg  $\text{Cu}^{++}/\text{l.}$  and  $1.25$  mg  $\text{Hg}^{++}/\text{l.}$  which again showed a large more-than-additive effect. This latter mixture has been used many times in the course of these studies and has always been found to show a marked supplemental synergism.

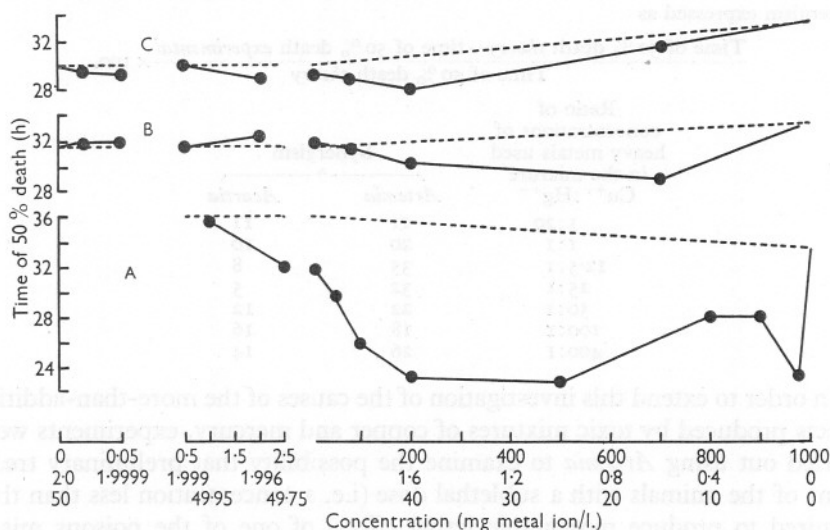


Fig. 4. Effects of bipartite mixtures of equitoxic concentrations of copper sodium citrate and mercuric chloride (A), mercuric iodide (B), and ethylmercuric chloride (C) on the survival of *Artemia* larvae in sea water (●—●, curves obtained experimentally; ---, theoretical curves expected if toxic effects of the mixed poisons were exactly additive). Concentrations of the metal ion measured as follows:  $\text{Cu}^{++}$  as citrate (upper row, 0...1000),  $\text{Hg}^{++}$  as  $\text{C}_2\text{H}_5\text{HgCl}$  or as  $\text{HgI}_2$  (middle row, 20...0), and  $\text{Hg}^{++}$  as  $\text{HgCl}_2$  (lower row, 50...0).

Further experiments of a similar nature were carried out with *Artemia* as the test animal in order to examine the toxic effects of bipartite mixtures of mercuric chloride, mercuric iodide and ethylmercuric chloride. In these experiments, however, theoretical and experimentally determined values were always found to coincide and more-than-additive effects were not observed.

For the purposes of comparison, attempts were made to carry out studies similar to those just described for *Artemia* with the much less resistant species *Acartia*. Because of a shortage of test material, however, experiments with

this species were restricted to an examination of the toxic effects shown by mixtures of equitoxic concentrations of copper sodium citrate and mercuric chloride. The results showed that although the mixtures studied gave more-than-additive effects, in nearly every instance the sizes of the effects produced were considerably smaller than those observed when mixtures containing the same proportions of copper and mercury were examined using *Artemia* (see Table II).

TABLE II. COMPARISON OF THE SIZES OF THE SUPPLEMENTAL SYNERGISMS SHOWN BY TOXIC MIXTURES OF COPPER SODIUM CITRATE AND MERCURIC CHLORIDE WHEN TESTED WITH *ARTEMIA* AND *ACARTIA* IN SEA WATER

Synergism expressed as

$$\frac{\text{Time of 50 \% death theory} - \text{time of 50 \% death experimental}}{\text{Time of 50 \% death theory}} \times 100.$$

Ratio of concentrations of heavy metals used in the mixture $\text{Cu}^{++}:\text{Hg}^{++}$	Synergism	
	<i>Artemia</i>	<i>Acartia</i>
1:20	11	11
1:1	20	10
12.5:1	35	8
25:1	32	5
50:1	22	12
100:1	18	16
400:1	26	14

In order to extend this investigation of the causes of the more-than-additive effects produced by toxic mixtures of copper and mercury, experiments were carried out using *Artemia* to examine the possibility that preliminary treatment of the animals with a sublethal dose (i.e. a concentration less than that required to produce measurable lethal effect) of one of the poisons might influence the rate of death of the animals in toxic solutions of the other.

*The effect of mercury on Artemia previously treated with copper.* A large number of animals were immersed in sea water containing 1 g  $\text{Cu}^{++}/\text{l.}$  as copper sodium citrate. After 1 h in this solution the animals were removed by filtration on a fine gauze, washed several times with plain sea water and then transferred to a series of solutions containing different concentrations of mercuric chloride (250, 125 and 62.5 mg  $\text{Hg}^{++}/\text{l.}$ ) mercuric iodide (10, 7.5 and 2.5 mg  $\text{Hg}^{++}/\text{l.}$ ) and ethylmercuric chloride (10, 7.5 and 2.5 mg  $\text{Hg}^{++}/\text{l.}$ ). For comparison with this copper-treated series, animals were immersed for 1 h in sea water to which no copper had been added and were then transferred to each of a second and similar series of the toxic solutions just described, while samples of these untreated as well as of the copper-treated animals were also placed in plain sea water. The 50% death time of animals present in each solution under test was then estimated in the usual way.

It was found in this experiment that animals which had been immersed in sea water containing 1 g  $\text{Cu}^{++}$ /l. as copper sodium citrate did not die at a rate significantly faster than that of the untreated animals in either sea water itself or in sea water to which various concentrations of copper had been added. They did, however, die at a much faster rate in each of the sea-water solutions which contained mercury as mercuric chloride and at a slightly faster rate in sea-water solutions of mercuric as mercuric iodide and ethylmercuric chloride (see Table III). Consequently, it seemed that although the amount of poison

TABLE III. THE EFFECT OF A PRELIMINARY TREATMENT OF *ARTEMIA* WITH COPPER ON THE RATES OF DEATH OF ANIMALS SUBSEQUENTLY IMMERSSED IN TOXIC SOLUTIONS OF VARIOUS MERCURY COMPOUNDS AND OF COPPER IN SEA WATER

Time of preliminary treatment 1 h. Effect expressed as:

$\frac{\text{Time of 50 \% death untreated} - \text{time of 50 \% death treated}}{\text{Time of 50 \% death untreated}} \times 100.$		
Poison used to test copper-treated animals	Concn. (mg/l.)	Effect
$\text{Hg}^{++}$ as chloride	250	33
	125	48
	62.5	47
$\text{Hg}^{++}$ as ethylmercuric chloride	10	21
	7.5	15
	2.5	18
$\text{Hg}^{++}$ as iodide	10	18
	7.5	24
	2.5	23
$\text{Cu}^{++}$ as citrate	1000	4
	500	3
	250	11

which was taken up by the animals which were given a preliminary treatment with copper was insufficient to render them less viable in plain sea water or more susceptible to subsequent copper poisoning, it did markedly lower their resistance to the toxic effects of mercury.

A period of 1 h was used for the preliminary treatment with copper, but

TABLE IV. THE INFLUENCE OF TIME OF PRELIMINARY TREATMENT OF *ARTEMIA* WITH COPPER ON THE RATES OF DEATH OF ANIMALS SUBSEQUENTLY IMMERSSED IN TOXIC SOLUTIONS OF MERCURY IN SEA WATER

Concentration of copper = 1 g  $\text{Cu}^{++}$ /l. as citrate: concentration of mercury = 125 mg  $\text{Hg}^{++}$ /l. as chloride. Effect expressed as in Table III.

Time of preliminary treatment in copper solution (min)	Effect
5	12
20	34
60	41

later experiments showed that it was possible to induce a considerable influence on the susceptibility of the animals to mercury poisoning by initially exposing them to a copper treatment lasting only 5 min. (see Table IV). On the other hand, prolonging the copper treatment to more than 1 h produced only a slight further increase in the death rate of the animals in the mercury solutions, and in some experiments it was found that after this prolonged treatment the animals died at a rate faster than that of untreated animals in plain sea water.

*Effect of copper on Artemia previously treated with mercury.* These experiments followed a pattern similar to that described previously. The test solutions contained copper sodium citrate (250, 500, 750 and 1000 mg  $\text{Cu}^{++}/\text{l.}$ ) and before they were immersed in these solutions, the animals were given a preliminary treatment with either mercuric chloride (250 mg  $\text{Hg}^{++}/\text{l.}$ ), ethylmercuric chloride (10 mg  $\text{Hg}^{++}/\text{l.}$ ) or mercuric iodide (10 mg  $\text{Hg}^{++}/\text{l.}$ ) for 1 h. It was found that animals treated with each mercury compound died at the same rate as that of untreated *Artemia* in sea water and in a series of solutions containing different concentrations of the mercury compound. They died, however, at a rate significantly faster than that of the untreated animals in sea

TABLE V. THE EFFECTS OF PRELIMINARY TREATMENTS OF *ARTEMIA* WITH DIFFERENT MERCURY COMPOUNDS ON THE RATES OF DEATH OF THE ANIMALS IN TOXIC SOLUTIONS OF COPPER IN SEA WATER

Time of preliminary treatment 1 h. Effects expressed as in Table III.

Concn. of $\text{Cu}^{++}$ as citrate in solution used to test mercury-treated animals (mg/l.)	Mercury compound used		
	$\text{HgCl}_2$ (250 mg $\text{Hg}^{++}/\text{l.}$ )	$\text{HgI}_2$ (10 mg $\text{Hg}^{++}/\text{l.}$ )	$\text{C}_2\text{H}_5\text{HgCl}$ (10 mg $\text{Hg}^{++}/\text{l.}$ )
1000	51	23	26
500	30	25	23
250	42	33	28

water to which various amounts of copper sodium citrate had been added (see Table V). Apparently, therefore, the quantity of mercury taken up by the animals during their preliminary treatment with either mercuric iodide, mercuric chloride or ethylmercuric chloride was insufficient to render them less viable in sea water or to increase their susceptibility to subsequent mercury poisoning. It did, however, make them far less resistant to copper poisoning.

In the experiments just described the concentrations of the mercury compounds used to 'sensitize' *Artemia* to copper poisoning had approximately 5 times the potential toxicity of the concentration of copper used in earlier experiments to 'sensitize' the animals to mercury poisoning. Preliminary experiments, however, showed that although concentrations

of the mercury compounds of the same potential toxicity as 1 g  $\text{Cu}^{++}/\text{l}$ . 'sensitized' the animals to copper poisoning, the extent to which they so acted varied greatly from experiment to experiment and in some instances the effect was not detected.

*Experiments with cysteine and reduced glutathione.* It seemed possible that the effect of non-toxic amounts of copper on the susceptibility of *Artemia* to mercury poisoning was sufficiently large to be used as a means of examining the way in which copper acted on this animal. Thus, the possibility was borne in mind that if copper exerted this influence when adsorbed on the surface of the animal, the effect might be abolished if the animal were treated with cysteine, or reduced glutathione, before it was placed in solutions containing mercury. Accordingly, the following experiments were carried out.

TABLE VI. THE INFLUENCE OF CYSTEINE AND REDUCED GLUTATHIONE ON *ARTEMIA* 'SENSITIZED' BY COPPER TO MERCURY POISONING

Concn. of mercury as mercuric chloride in test solution (mg/l.)	Effect expressed as in Table III.		
	Effect after copper-treated animals washed with		
	Sea water	Glutathione	Cysteine
250	40	27	20
125	49	45	27
62.5	33	25	20

*Artemia* were immersed for 1 h in sea water containing copper sodium citrate (1 g  $\text{Cu}^{++}/\text{l}$ ). After the animals had been removed from the solution by filtration, approximately equal numbers were added to sea-water solutions of 0.01 M cysteine, and 0.01 M glutathione. Others were added to plain sea water. The animals were suspended in these solutions for 5 min, filtered off, washed several times with sea water and then transferred to solutions of mercuric chloride in sea water equivalent to 250, 125 and 62.5 mg  $\text{Hg}^{++}/\text{l}$ . The rates at which the four sets of animals died in the mercury solutions were compared with those shown by animals which had not been immersed in the copper solution, but simply treated with either cysteine, glutathione or sea water before they were added to sea water containing mercuric chloride. The results of this experiment (see Table VI) showed that animals treated with copper and then washed with sea water died at the same enhanced rate in the mercury solutions. However, copper-treated animals which had subsequently been washed with cysteine or glutathione, died at a rate which, though still enhanced, was usually considerably closer to that shown by animals which had not been given an initial immersion in the copper solution. These findings, therefore, were consistent with the view that some at least of the copper which was instrumental in lowering the resistance of *Artemia* to mercury poisoning might be localized on the surfaces of these animals.



Further, this attached copper was removable with cysteine and glutathione but not with sea water.

Similar experiments were carried out in which animals were immersed for 1 h in sea water containing mercuric chloride (250 mg  $\text{Hg}^{++}/\text{l.}$ ), and then washed with either sea water, cysteine or glutathione and transferred to solutions of copper sodium citrate (1000, 750 and 500 mg  $\text{Cu}^{++}/\text{l.}$ ) in sea water. The results of this experiment (see Table VII) showed that the mercury-treated animals which had been subsequently washed with cysteine or glutathione died at a rate considerably slower than that of the animals which had been washed in sea water. This finding, therefore, indicated that some, at least, of the mercury responsible for raising the sensitivity of *Artemia* to copper poisoning was attached to the surfaces of these animals, and could be removed with cysteine or glutathione.

TABLE VII. THE INFLUENCE OF CYSTEINE AND REDUCED GLUTATHIONE ON *ARTEMIA* SENSITIZED BY MERCURY TO COPPER POISONING

Concn. of copper as citrate in test solution (mg/l.)	Effect expressed as in Table III.		
	Effect after mercury-treated animals washed with		
	Sea water	Glutathione	Cysteine
1000	64	42	27
750	61	24	26
500	50	15	25

#### RESPIRATION EXPERIMENTS

The experiments carried out to investigate the toxic effects of bipartite mixtures of copper sodium citrate and different mercury compounds provided evidence in support of the view that the toxic action of copper reinforces that of mercury, and that when mixtures of copper sodium citrate and mercuric chloride are tested, using *Artemia*, large supplemental synergisms are produced. In order to extend these studies it was considered worth while to compare the effects, if any, of copper (as copper sodium citrate) and mercury (as mercuric chloride) on a fundamental biological activity of the animals, namely their respiration.

#### *Manometric Procedure for Measuring the Respiration Rate of Artemia Treated with Copper and Mercury*

Oxygen consumption was measured in the conventional Warburg apparatus at a temperature of 25° C and the gas phase used was air. Flasks of approximately 15 ml. capacity equipped with single sidearms were used. Filtered sea water (2 ml.) containing 100–150 mg wet weight of larvae was placed in the main compartment of each flask, samples of filtered sea water (1 ml.) to which no additions had been made or to which an appropriate quantity of copper

sodium citrate, sodium citrate, or mercuric chloride had been added were pipetted into the sidearm, and 0.2 ml. 20% KOH together with 3 cm<sup>2</sup> of starch-free filter-paper were placed in the centre well. Equilibration was for 30 min., after which time the rate of oxygen consumption was measured at 30 min. intervals for 2 h. The contents of the sidearms were then tipped into the main compartments of the flasks and measurements of respiration rate were continued for a further 5 h. It was found that the rate of oxygen consumption was independent of the shaking rate over the range 70–100 oscillations/min. (5 cm traverse) and determinations of  $qO_2$  ( $\mu\text{l. O}_2/\text{mg wet wt./h}$ ) were unaffected by the quantities of animals used between 100 and 300 mg wet wt. The pH of the contents of the flasks was measured at the beginning and the end of each experiment and was usually found to have changed from 8.1 to 7.5 during the 7 h experimental period.

### *The Effects of Copper and Mercury on Respiration and Motility*

During the time of these manometric experiments (7 h) the respiration rate of the animals in ordinary sea water, or sea water to which an appropriate quantity of sodium citrate had been added (as a control in the experiments in which copper sodium citrate was used), remained linear, and when the animals were inspected at the end of the experiment they appeared to have suffered no loss of motility and showed no signs of damage. In the course of these studies some twenty determinations of the  $qO_2$  of *Artemia* larvae were made and the values obtained were between 1.01 and 1.15  $\mu\text{l. O}_2/\text{mg wet wt./h}$ . The respiration rates of the animals which had been subjected to poisoning by copper and mercury, however, decreased considerably during the experiment and the percentage reduction in oxygen consumption was estimated over each hour subsequent to tipping. To compare these changes in respiration rate with changes in motility the following method was used. Exactly 1 h after tipping took place one of the flasks to which a solution of the poison had been added was detached from its manometer, representative samples (0.2 ml.) of its contents were withdrawn and the numbers of animals which had lost all signs of motility in these samples were determined as percentages of the total numbers present. Other flasks were removed exactly 2, 3, 4 and 5 h after tipping took place and their contents were examined in the same way for percentage loss of motility. By this means percentage reductions in respiration and motility were compared during the same time intervals.

Results of typical experiments are shown in Fig. 5. In these experiments the quantity of copper used was 1 g  $\text{Cu}^{++}/\text{l.}$  and the quantity of mercury was 250 mg  $\text{Hg}^{++}/\text{l.}$ , concentrations identical with those employed in the 'sensitizing' studies described earlier. The results showed that whereas copper caused a marked and immediate decrease in respiration, this poison had only a small effect on motility. By contrast, mercury reduced motility faster than it inhibited respiration. A reduction in motility might be expected to precede

a fall in respiration on the grounds that animals in a completely motionless state might still possess a small but measurable oxygen consumption. Consequently the action of mercury was considered to be typical of many poisons. The effect of copper on the animals, however, appeared to be one of specific inhibition of respiratory mechanisms, and this finding indicated a difference between the modes of toxic action of the two heavy metals. Similar conclusions have been reached by Russell Hunter (1950) from studies using *Marinogammarus marinus*.

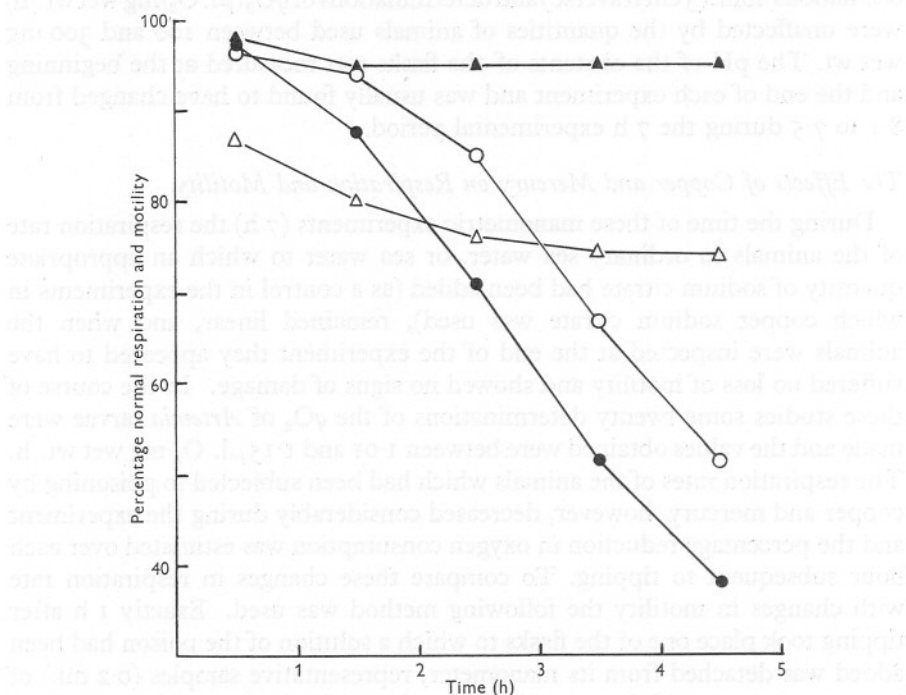


Fig. 5. Effects of copper sodium citrate and mercuric chloride on the motility and respiration rate of *Artemia* larvae in sea water (▲—▲, % decrease of motility in copper sodium citrate; △—△, % decrease of respiration in copper sodium citrate; ●—●, % decrease of motility in mercuric chloride; ○—○, % decrease of respiration in mercuric chloride).

### DISCUSSION

The experiments described above were mainly of an exploratory nature and therefore only tentative conclusions may be drawn from their results. Even so the findings reported are worth examining in the light of current theories concerning the mechanisms of action of heavy metal poisons.

At the outset of the present work it was found that *Artemia* is far more resistant than either *Elminius* or *Acartia* to poisoning by mercuric chloride,

mercuric iodide, ethylmercuric chloride, and copper sodium citrate. In addition it was found that, whereas mercuric iodide and ethylmercuric chloride are far more toxic than mercuric chloride to *Artemia*, all three mercury compounds are of the same order of toxicity to *Elminius* and to *Acartia*. It appears, therefore, that when the test animal used is one which possesses a considerable resistance to mercury poisoning the differences between the toxicities of the mercury compounds are far greater than those observed when these compounds are tested on an animal which is much more readily poisoned by mercury. These results would be expected if differences between toxicities were closely allied with differences between rates of penetration, for the latter probably play an important part in determining relative toxicities to a highly impermeable animal, but exert a much smaller influence when the test animal used is one which is rapidly penetrated. The results of these toxicity studies therefore are consistent with the view that rates of penetration are, in fact, important factors influencing the toxicities of mercury compounds, and a corollary of this is that these poisons act inside the test animal.

This view assists interpretation of the results of studies made in the present work concerning the toxic effects shown by bipartite mixtures of mercury compounds and copper. Thus it has been found, using *Artemia* as the test animal, that whereas large more-than-additive toxic effects are shown by mixtures of copper sodium citrate and mercuric chloride these effects are much less marked when the mercuric chloride used in the mixtures is replaced by the more toxic iodide or ethylmercuric chloride. A possible explanation of these findings is that the copper used in the mixtures enables the mercury compounds to penetrate the test animal more readily, for it might be expected that this effect of the copper would enhance considerably the toxicity of mercuric chloride but influence to a much smaller extent that of the faster penetrating mercuric iodide or ethylmercuric chloride.

Furthermore, if copper increases the permeability of the test animal to mercury poisons it might be expected that this would have more pronounced effects when mixtures of copper and mercury are tested on a species highly impermeable to mercury than when the test animal used is one which this poison penetrates rapidly. Concerning this aspect of copper poisoning, therefore, interest attaches to further studies made in the present work which have demonstrated that the more-than-additive toxic effects shown by mixtures of copper (as citrate) and mercury (as chloride) when tested on *Artemia* are usually far greater than those observed when similarly proportioned mixtures of the two poisons have been applied to *Acartia*.

Although the results so far discussed support the view that mercury compounds are poisons which act internally, other findings during the present work have suggested that additional toxic effects of mercury as mercuric chloride may be induced at the surfaces of the test animals. Thus it has been found that *Artemia* which have been treated with a sublethal dose of mercuric

chloride become more sensitive to poisoning by copper, and that this 'sensitizing' effect can be appreciably reduced if the mercury-treated animals are washed with cysteine or with reduced glutathione. These experiments were similar in principle to those carried out by various workers in the course of studies of enzyme inhibition in which it was observed that the inactivation of enzymes such as urease (Hellerman, Chinard & Deitz, 1943), papain (Hellerman & Perkins, 1934) and yeast carboxylase (Stoppani, Actis, Deferrari & Gonzalez, 1953) by small amounts of copper, mercury and various 'mercaptide-forming' substances derived from the latter (e.g. phenylmercuric chloride) could be partially reversed by thiol compounds such as cysteine. These findings have led to the view that heavy metal poisons inhibit these enzymes by attaching to the surfaces at sulphhydryl groups responsible for catalytic activity. The results of analogous experiments carried out with *Artemia* in the present investigation, therefore, lend support to the view that mercury (as chloride) becomes attached to the surfaces of these test animals, possibly by interaction with sulphhydryl groups, and that when it is adsorbed at these surface sites it effectively lowers the resistance of *Artemia* to copper poisoning. Evidence consistent with the view that at least part of the toxic action of copper may be explained in a similar way has been obtained from complementary studies in which this poison has been found to 'sensitize' *Artemia* to the toxic effects of mercuric chloride: and in which this 'sensitizing' effect has been observed to diminish after the copper-treated animals have been washed with cysteine and reduced glutathione.

Two further significant observations have been made in the course of these 'sensitizing' experiments using *Artemia*. One is that sublethal doses of mercuric chloride, mercuric iodide, and ethylmercury chloride which have been found to render *Artemia* more susceptible to copper poisoning have been observed to show no marked influence on the susceptibility of the animals to poisoning by the respective mercury compound: and the other is that sublethal doses of copper which have been found to lower markedly the resistance of *Artemia* to mercuric chloride and, to a lesser extent, mercuric iodide and ethylmercuric chloride have no significant effect on the susceptibility of these animals to copper poisoning.

These findings, therefore, together with the results of other experiments discussed earlier in which bipartite mixtures of copper and mercury have been used, provide considerable evidence in support of the view that these two poisons act on *Artemia* in different ways, and evidence of one such difference has been obtained from an investigation of the effects of copper (as citrate) and mercury (as chloride) on the respiration rate of *Artemia* larvae. In these studies it has been found that copper depresses the respiration of the animals by approximately 25% without significantly affecting their motility, whereas mercury reduces their motility at a rate much faster than that at which it inhibits their respiration. As the concentrations of copper and of



mercury used in these respiration experiments were equivalent to those used in the 'sensitizing' experiments, there can be little doubt that the animals which were treated with copper in these studies had a reduced respiration when they were later placed in the mercury solutions. It seems possible, therefore, that one of the toxic effects ascribed to copper earlier, namely an ability to increase the permeability of *Artemia* to mercury compounds, may be correlated with its effect as a respiratory depressant.

It is clear that further experimental work is needed before a proper conspectus of heavy-metal poisoning in crustaceans can be formed. In experiments already begun attempts are being made to apply histochemical methods to the problem of determining the sites, either on or in these animals, at which heavy metal poisons exert their toxic effects. Other studies are contemplated in which it is hoped that further information concerning the causes of more-than-additive toxic effects will be gained by testing mixtures of poisons as inhibitors of isolated enzymes and multi-enzyme systems.

The authors are indebted to Dr H. W. Harvey, F.R.S., for his continuous interest in the work. They also wish to record their thanks to Prof. J. E. Harris, F.R.S., Mr O. D. Hunt and Mr R. Robinson for valuable advice and criticism, and to Dr P. C. Croghan for allowing them to study an account of his work on osmotic and ionic regulation in *Artemia* prior to its publication. Part of the expenses of the work reported in this paper were defrayed by apparatus grants from International Paints Ltd., to whom one of us (E. D. S. C.) is indebted for a Research Fellowship, and these, together with the many facilities provided by the Plymouth Laboratory of the Marine Biological Association, are gratefully acknowledged.

#### SUMMARY

Studies have been made of the toxicities of copper (as copper sodium citrate) and mercury (as mercuric chloride, mercuric iodide and ethylmercuric chloride) to the three crustaceans *Artemia salina*, *Elminius modestus* and *Acartia clausi*.

Compared with *Artemia* the other two species examined have been found to be much more easily poisoned by each of the four toxic agents tested. Moreover, the differences between the toxicities of mercuric iodide and ethylmercuric chloride on the one hand and mercuric chloride on the other have been found to be far greater with *Artemia* than when *Elminius* and *Acartia* were used as the test animals.

Bipartite mixtures of copper (as citrate) and mercury (as chloride, iodide and ethylmercuric chloride) have been found to show more-than-additive toxic effects (supplemental synergisms) when tested with *Artemia*. Whereas the effects shown by mixtures of copper and mercuric chloride were found to be considerable, those displayed by mixtures of copper and either mercuric iodide or ethylmercuric chloride were, by comparison, very small. In

addition, it has been observed that the more-than-additive effects shown by mixtures of copper and mercury (as chloride) to *Acartia* were significantly smaller than those observed when corresponding mixtures were tested using the more resistant species *Artemia*.

*Artemia* have been found to be markedly 'sensitized' by pretreatment with a sublethal dose of copper to poisoning by mercuric chloride and, to a lesser extent, to poisoning by mercuric iodide and ethylmercuric chloride. Animals so treated, however, were not rendered more susceptible to copper poisoning. Similarly, it has been found that *Artemia* can be 'sensitized' to copper poisoning by pretreatment with mercuric chloride, mercuric iodide or ethylmercuric chloride in sublethal doses which do not render the animals less resistant to the respective mercury compound. These 'sensitizing' effects induced by copper and mercury (as mercuric chloride) have been found to be partially eliminated by washing the treated animals with solutions of either cysteine or reduced glutathione.

Further experiments with *Artemia* larvae have shown that copper depresses their respiration without significantly affecting their motility. Mercury, however, does not appear to have this effect.

The findings made in this investigation have been discussed in relation to current theories concerning the mechanisms of action of heavy metal poisons, and future studies have been indicated.

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## THE DENSITY OF THE EGGS OF *CALANUS FINMARCHICUS*

By E. A. Salzen

Department of Zoology, University of Edinburgh, and Marine Station, Millport

(Text-fig. 1)

The vertical distribution of plankton organisms and their eggs depends largely on their surface area and density (Eyden, 1923). Gross & Raymont (1942) observed that the eggs of *Calanus finmarchicus* had a density (1.045-1.049 g/cc) greater than that of sea water (1.0235-1.025 g/cc), and that they sank at the rate of approximately 2.5 cm/min at 13° C. These workers pointed out that since the embryo takes 24 h to complete development, the eggs will sink in still water to a depth of 36 m before hatching and yet the data available, although not extensive, suggests that both eggs and nauplii are found most abundantly nearer the surface than this (Nicholls, 1933; Kraefft, 1910). If these observations are correct, then in addition to density and viscosity, there must be a third factor affecting the distribution of these eggs. Unfortunately, Gross & Raymont did not consider the possible effect of viscosity in their method for determining density, and they themselves commented on the disturbing degree of variation in some of their results. Their measurements of the rate of sinking of the eggs in sea water also showed considerable variation. It seemed worth while, therefore, to measure the density of these eggs using the density gradient technique of Linderstrøm-Lang (1937), a method which enables the individual and precise measurement of the density of small living objects, independent of their shape, i.e. surface area. This method was being used in work on *Psammarchinus* eggs at the Marine Station, Millport, and, at the suggestion of Dr A. P. Orr, the following measurements were made on the eggs of *Calanus finmarchicus*.

### METHODS

The principles of the gradient-tube method have been fully described elsewhere (Linderstrøm-Lang & Lanz, 1938; Løvtrup, 1950; Linderstrøm-Lang, Jacobson & Johansen, 1938). The medium used was a thorotrast-distilled water mixture made isotonic with sea water by the addition of 0.092 g KCl + 3.46 g NaCl per 100 cc of mixture. (Thorotrast, a stabilized colloidal solution of thorium oxide, has a high density, low viscosity, a negligible osmotic pressure, and is relatively transparent. It has been used for gradients in the Carlsberg Laboratory.) An artificial sea-water medium was used because natural sea water was found to cause precipitation of the thorotrast.

The gradient was about 10 cm in height and covered linearly a density range of 1.048–1.098 g/cc. Thus a difference in height of 1 mm in the gradient corresponded with a density difference of 0.0005 g/cc. The density standards were small hollow glass beads (of 1–2 mm diameter) previously made and standardized against droplets of standard potassium chloride solutions in bromobenzene-kerosene gradients. These standards were accurate to  $\pm 0.0005$  g/cc and the positions of the eggs in the gradient could easily be measured to 0.1 mm with a cathetometer.

Measurements were made at  $22 \pm 0.01^\circ$  C on mixed batches of eggs which had been laid during the night and were approximately 12 h old at the time of the experiments. Two determinations on separate days were made, 20–30 eggs being used on each occasion. The eggs fell as a group in the gradient, and their rate of fall was measured with reference to one egg chosen at random. The small amount of water introduced with the eggs from a braking pipette remained at the top of the gradient tube. The gradient itself was not affected because of the large amount of medium contained in the upper reservoir of the gradient tube. The positions of all the eggs and the standards were measured at suitable intervals for up to  $2\frac{1}{2}$  h after entry into the gradient. The diameters of several mixed batches of eggs were measured, with an ocular micrometer, with an accuracy of  $\pm 3\mu$ .

## RESULTS

The results of the two experiments are shown in Table 1. Fig. 1 shows a typical set of measurements of the position of an egg throughout the hour following its entry into the gradient. This suggests that the eggs should have reached their definitive positions (i.e. the position at which egg and medium have the same density) within 1 h of entry. This is a slow fall and is due to the small size of the eggs, i.e. high surface-area/volume ratio, giving a high viscosity effect. (In the gradient method viscosity may affect the rate of fall of the material but it does not affect its final position in the gradient.) It is clear from Fig. 1 that the  $\frac{1}{2}$  h readings are likely to give density values a little too low. The values for 2 h in Expt. 1 and  $1\frac{1}{2}$  h in Expt. 2 may be of doubtful value because the eggs had become opaque, i.e. cytolysed, and may have changed in density. However, this seems unlikely, because if the egg shell is intact internal changes will not affect the egg density; and indeed the opaque eggs were quite steady in the gradient. The progressive decrease in sample size in the second experiment was due to eggs which burst and sank rapidly to the bottom of the gradient. Control eggs remained unchanged for 2 h in the thorotrast-saline mixture at room temperature, while others in the artificial sea water showed signs of deterioration.

Thus from Expt. 1 the density of *Calanus* eggs at  $22^\circ$  C appears to be 1.0735 g/cc with a standard deviation for the population of 0.0005 g/cc. The final value in Expt. 2 supports this, although the standard deviation is much

larger. If all the measurements be included, except those of the first half-hour, then the results for the two experiments are:

Expt. 1. Mean density = 1.074 g/cc. Standard deviation = 0.00075.

Expt. 2. Mean density = 1.074 g/cc. Standard deviation = 0.0018.

Since the standards were accurate to  $\pm 0.0005$ , then the density of *Calanus* eggs may be taken as 1.074 g/cc with a population standard deviation of 0.002 g/cc.

TABLE I. DENSITY OF EGGS OF *CALANUS FINMARCHICUS* AS DETERMINED IN THE DENSITY GRADIENT

Expt.	Time since eggs entered gradient (h)	No. of eggs measured	Mean value of density (g/cc)	Standard deviation	Condition of eggs
1	$\frac{1}{2}$	22	1.0725	0.00075	Normal
	1	22	1.0735	0.0005	Normal
	2	22	1.0745	0.0006	Opaque
2	$\frac{1}{2}$	28	1.0730	0.00025	Normal
	1	13	1.0750	0.0005	Opaque
	1 $\frac{1}{2}$	11	1.0750	0.002	Opaque
	2 $\frac{1}{2}$	8	1.0735	0.00125	Opaque

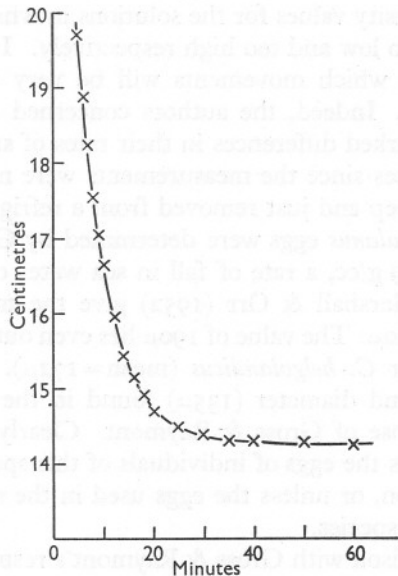


Fig. 1. The fall of an egg of *Calanus finmarchicus* in a thorotrast gradient. The height of the egg above an arbitrary fixed point has been plotted against the corresponding time from the moment of entry of the egg into the gradient.



The results of the diameter measurements are given in Table II. The eggs were, within the accuracy of the measurements, quite spherical. If all the measurements be taken together, the mean diameter for the population becomes  $135\mu$  with a standard deviation of  $4\mu$ .

TABLE II. DIAMETER OF EGGS OF *CALANUS FINMARCHICUS*

Expt.	No. of eggs measured	Mean value of diameter ( $\mu$ )	Standard deviation ( $\mu$ )
1	26	140	3.0
	20	129	3.5
2	25	134	4.0

#### DISCUSSION

Gross & Raymont (1942) determined the density of *Calanus* eggs by measuring their rates of movement in gum solutions of increasing density, and found that the rate of fall decreased linearly, the egg and the medium having the same density at zero velocity. This, however, only applies if the effect of viscosity is constant or can be neglected, for the rate of movement will be reduced by the increasing viscosity as well as the increasing density of the gum solutions. If the retardation of movement due to viscosity is considerable, as may well be with such small bodies as *Calanus* eggs, then the method becomes very insensitive, giving density values for the solutions in which the eggs just sink or just rise that are too low and too high respectively. In fact there will be a range of solutions in which movements will be very slow and subject to extraneous influences. Indeed, the authors concerned report that different specimens showed marked differences in their rates of sinking—perhaps due to extraneous influences since the measurements were made in glass cells or watch-glasses 2 cm deep and just removed from a refrigerator.

Three values for *Calanus* eggs were determined by Gross & Raymont—a density of 1.045–1.049 g/cc, a rate of fall in sea water of 2.5 cm/min, and a diameter of  $190\mu$ . Marshall & Orr (1952) give the mean diameter of *C. finmarchicus* eggs as  $140\mu$ . The value of  $190\mu$  lies even outside the range which these authors give for *C. helgolandicus* (mean =  $172\mu$ ). The values for egg density (1.074 g/cc) and diameter ( $135\mu$ ) found in the present work differ considerably from those of Gross & Raymont. Clearly both sets of values cannot be right, unless the eggs of individuals of this species differ markedly in size and constitution, or unless the eggs used in the separate experiments were from a different species.

For further comparison with Gross & Raymont's results it is of interest to calculate, using Stokes's equation, the rate of fall in sea water of an egg with diameter  $135\mu$  and density 1.074 g/cc, i.e. with the values determined in the present work. Values for the temperature and salinity of the sea water must

be assumed in order to obtain values for density and viscosity. If the sea water has a temperature of 15° C and a salinity of 35‰, then the viscosity will be  $12.2 \times 10^{-3}$  c.g.s. units (Sverdrup *et al.*, 1942, p. 69) and the density 1.026 g/cc (Knudsen, 1901; Matthews, 1932). Inserting these values in the equation

$$v = \frac{2}{9} \frac{a^2 g (d_1 - d_2)}{\eta},$$

where  $v$  = rate of fall (cm/sec),

$a$  = radius of egg (cm) = 0.00675 cm,

$g$  = acceleration due to gravity (dynes/cm<sup>2</sup>) = 981 dynes/cm<sup>2</sup>,

$d_1$  = density of egg (g/cc) = 1.074 g/cc,

$d_2$  = density of sea water = 1.026 g/cc,

$\eta$  = viscosity of sea water (c.g.s. units) =  $12.2 \times 10^{-3}$  c.g.s. unit,

we obtain

$$v = 0.0393 \text{ cm/sec} = 2.36 \text{ cm/min.}$$

Thus the theoretical rate of fall in sea water is in fair agreement with the experimentally determined value of Gross & Raymont, and their comments on the vertical distribution of *Calanus* eggs are still valid. If the previously mentioned reports concerning the abundance of eggs near the surface are accurate, then there must be some factor which materially slows down the rate of sinking of these eggs in the sea. A detailed analysis of their distribution might help in determining the part played by turbulence in this respect.

The results also show that although measurements of the rate of fall of spherical bodies, such as eggs, in sea water can give values for their density, the method is much less sensitive than direct measurements as in the Linderstrøm-Lang gradient method. Furthermore, the possible effect of viscosity must be considered when measuring rates of movement in solutions of different density, and for this reason Gross & Raymont's work on the nauplii and adults of *Calanus* awaits confirmation by methods independent of viscosity.

#### SUMMARY

The Linderstrøm-Lang density gradient technique has been used to determine the density of the eggs of *Calanus finmarchicus*. The mean density for the population was 1.074 g/cc with a standard deviation of 0.002 g/cc. Measurements of egg diameter gave a mean of 135  $\mu$  with a standard deviation of 4  $\mu$ . This would give a rate of fall in sea water (salinity 35‰ and temperature 15° C) of the order of 2.4 cm/min.

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## UNDERWATER OBSERVATIONS ON ESCALLOP (*PECTEN MAXIMUS* L.) BEDS

By R. H. Baird

Fisheries Experiment Station, Castle Bank, Conway, North Wales

and F. A. Gibson

Department of Agriculture, Fisheries Branch, Dublin

(Text-figs. 1-5)

### INTRODUCTION

In an age-frequency distribution plot of a biological population, it is axiomatic that there should be, in general, a preponderance of young. This expected distribution is not found when sampling some marine animals, notably lobsters and scallops. Many workers, including Priol (1930), Tang (1941), Elmhirst (1945), Baird (1952), Fairbridge (1953), and Mason<sup>1</sup> have noted the absence of young scallops (*Pecten* sp.) in the expected numbers in dredge hauls.

A dredge without teeth, lined with sprat netting, was used at Brixham in an attempt to catch the young scallops, but this dredge filled so quickly with sand, gravel and shell that very few scallops were caught, although those taken did have a generally lower size-range than the scallops caught with a standard toothed dredge. At Castletownbere (Co. Cork) a naturalist's dredge similarly failed to catch many scallops.

The relative scarcity of the young scallops may reasonably be explained in one of two ways:

(1) Settlement may occur in feeder beds, probably inshore, from which, upon reaching a certain size or age, the young scallops migrate back to the parent beds to effect the necessary recruitment.

(2) The young may be present on the beds, but for some reason are not caught.

The first explanation has been fairly widely held, but in some cases, notably in the English Channel beds off Newhaven (Baird, 1952) very long migrations from inshore waters would be necessary. Moreover, recaptures of tagged scallops have never shown any appreciable evidence of migration (Gibson, 1953; Mason<sup>1</sup>). To investigate the second possibility it was decided that direct observations of the scallops on the beds by the use of diving apparatus would be necessary.

The commercial scallop beds in Berehaven Sound, between Bere Island and the mainland, County Cork, were chosen for the initial investigation

<sup>1</sup> J. Mason, Ph.D. Thesis, Liverpool University, 1953, 42 pp.



because scallops were found there in depths ranging from 3 to 14 m (10–46 ft.). The Sound is nearly 7 miles long and from one-half to three-quarters of a mile wide. The beds in the Sound had formed the basis for population studies during the preceding 4 years, and a series of tagging experiments had been made (Gibson, 1953).

An exceptionally high proportion of o-group scallops had been taken during 2 days commercial fishing in December, 1949, at Castletownbere. Of 400 scallops dredged on this occasion 51 were o-group. This high proportion has not been noted since, and the percentage of o-group in commercial catches seldom exceeds 2%, but the suggestion remained that the young were present.

It was later found desirable to continue the work at Port Erin, where scallops are found in depths ranging from 17 to 60 m (56–198 ft.), although observations were limited to a maximum of 37 m (120 ft.), and collection and dredging to a maximum of 26 m (85 ft.). Scallops on these beds had been studied by Tang (1941) and Mason.

#### EQUIPMENT AND METHODS

A Siebe Gorman Aqualung compressed air, self-contained, diving apparatus and a Dunlop underwater swimsuit and swim fins were used.

The standard Irish scallop dredge was used at Castletownbere. This dredge was 1.68 m (5 ft. 6 in.) wide, with a toothed blade. The teeth had an average gap of 8.0 cm, were about 7.0 cm long, and entered the sand to a depth of 1.0–2.0 cm. The dredge bag and belly were made of sisal netting, 6.5 cm between knots, hung square on the dredge frame. The dredge was towed from an 18 ft. boat, without motor, winched by hand up to an anchor.

At Port Erin a standard 1.17 m (4 ft.) dredge completely lined with shrimp netting was used. This dredge had a toothed blade, with a gap of 7.5 cm (3 in.) between the teeth, which projected 5 cm (2 in.) from the bar. The dredge was towed from a 25 ft. motor boat.

The sampling at Castletownbere was done in September 1953. All the sampling was done within a radius of 1 mile (1600 m) at depths ranging from 4 to 14 m (13–46 ft.). Scallops of all sizes were fairly easily seen at all depths in spite of being recessed in the sand and covered with a layer of silt. The overlap of the bottom valve was visible when the valves were closed, and the extruded tentacles were seen when the valves were open. All scallops seen while diving were collected. Consumption of air by a diver is much increased at greater depths, and owing to a shortage of compressed air approximately 70% of the collection by diving was done at the lesser depths; most of the dredging was done at the greater depths because of fouling by annual sea weeds in the shallower water. Thus a possible source of bias was introduced in the sampling; the results of this sampling appear in Fig. 1.

To determine whether the results obtained at Castletownbere resulted from the bias introduced by the distribution of sampling, a further investigation was undertaken at Port Erin, Isle of Man, in July 1955. The diving and dredging were done at random on a bed of scallops off Fleshwick Bay. This bed was approximately half a mile (800 m) square, in depths ranging from 17 to 26 m (56–85 ft.). Although underwater visibility was exceptionally good the scallops were not so easily seen as at Castletownbere. This was probably due to the general reduction of light at the greater depths and to the nature of the bottom, which was of a much rougher and coarser texture than that at Castletownbere. Again all scallops seen were collected and measured. Diving was alternated with dredging, both, as far as possible, being done in the same area. The results of this sampling appear in Figs. 2 and 3.

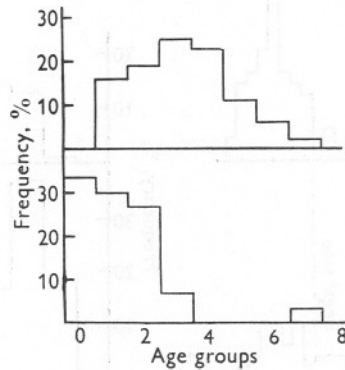


Fig. 1. Age-frequency distribution of collected and dredged samples at Castletownbere. Above: dredged sample, 100 scallops; below: collected sample, 30 scallops.

#### MOVEMENT AND DISTRIBUTION

The scallops normally lie recessed in the bottom in a self-formed depression, the flat valve roughly in the plane of the bottom. Recent swimmers lie unrecessed. The time that elapses after swimming before the recessing is done is not known and is probably very variable. The recessing, which has been observed in action only once, is performed by ejecting water downwards; this is accompanied by a rocking movement. A great deal of the disturbed silt resettles on the upper valve.

The amount of swimming by scallops was very variable but in all cases limited, and occurred most frequently among the smaller ones. In general, not more than 5% of scallops collected by diving swam on the approach of the diver, although on one occasion 10 out of 12 scallops collected swam, but the swimming did not appear to be directional. An scallop could easily be caught whilst swimming. Having swum, and come to rest, no further swimming could be induced. Most frequently a closure of the valves occurred



on the initial approach of the diver. Buddenbrock & Möller-Racke (1953) have shown that the reaction of *Pecten* and *Chlamys* to moving objects depends upon the velocity of the object—a slow movement results in a stretching of the tentacles towards the object—while a more rapid movement results in a retraction of the tentacles and closure of the valves. In neither case does the capacity to swim appear to result in an escape reaction and this was confirmed during these observations. Smaller scallops tended to swim more frequently than the larger ones, but even so the very low proportion that were found un-recessed indicated that very little recent swimming had occurred on the grounds examined.

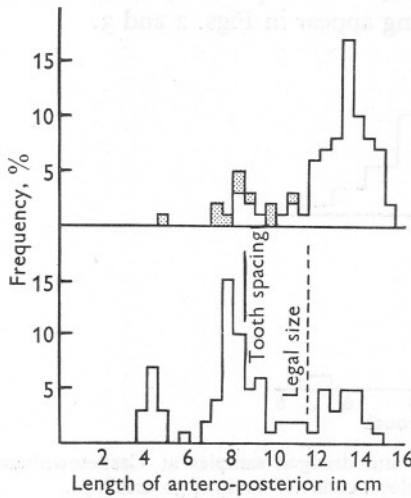


Fig. 2

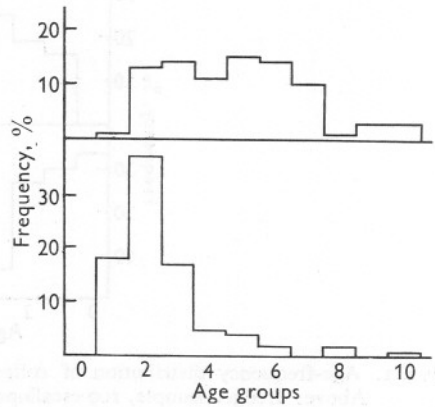


Fig. 3

Fig. 2. Size-frequency distribution of collected and dredged samples at Port Erin. Above: dredged sample, 85 scallops; below: collected sample, 86 scallops.

Fig. 3. Age-frequency distribution of collected and dredged samples at Port Erin. Above: dredged sample, 85 scallops; below: collected sample, 86 scallops.

In an attempt to determine whether any escape reactions occurred among the scallops the dredge was observed in action on the bottom at Castletownbere at a depth of 14 m. In order to see what was happening at the teeth of the dredge it was necessary to look at the bottom immediately in front of the dredge from a height of only half a metre, and this resulted in a high apparent speed over the bottom when the dredge was being towed. Under these conditions it was very difficult to see scallops in the path of the dredge. In the course of the dredge observations only two scallops were positively seen in the path of the dredge, which was moving along in a series of long shallow leaps. Neither of these scallops was taken. On other occasions scallops

were taken that were not seen. One 1-group scallop was seen to escape outside the edge of the dredge and was captured by hand. The leaping movement of the dredge must result in a very low dredge efficiency.

The distribution of scallops on the beds was very patchy, estimated densities ranging from one per 2 sq. yd. ( $1.65 \text{ m}^2$ ) to one per 100 sq. yd. ( $84 \text{ m}^2$ ). There was evidence of localized age grouping which would be heavily masked in dredge sampling. This patchiness appeared to be particularly evident among the lower age groups, though this may have been due to greater ease of identification of the age group of the younger scallops underwater. At Port Erin the 2-group, resulting from a good settlement year, was found almost everywhere but at varying densities.

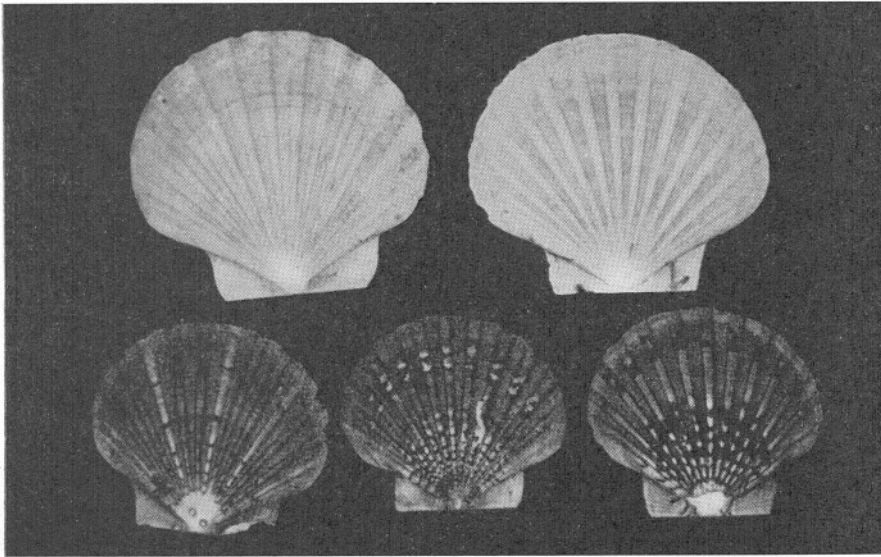


Fig. 4. Contrast in pigmentation of scallops from adjacent areas. The larger scallops are from deeper water and muddy bottom; the smaller from shallow water and gravel bottom.

At Castletownbere a wide range of sizes was found in shallow water on a gravel bottom, but in an area within 200 m (220 yd.) in deeper water on a muddy bottom, the few scallops found were all large. The pigmentation of the shallow water scallops was characteristic and different from that of the adjacent deeper water scallops (Fig. 4). There had obviously been no interchange of populations. A difference in pigmentation was also observed on scallops collected from gravel and muddy bottoms within a mile (1600 m) of each other at Cornwall. The pigmentation in all cases was uniform from the earliest stages of shell growth, indicating that the scallops had been in the same local environment since settlement.



At various intervals from April 1952 until September 1953, 719 scallops have been tagged at Castletownbere, of which 200 (27·8%) were recaptured by 15 April 1955. Only two have been recaptured in places other than where they were released, and then only within 1 mile (1600 m). Neither can be regarded, therefore, as having made a significant migration. The periods at liberty, ranging from 2 days to 34 months, were adequate for migrations to have taken place, if such migrations are characteristic of scallops. Tagging experiments at Port Erin by Mason have shown a complete absence of migratory movement.

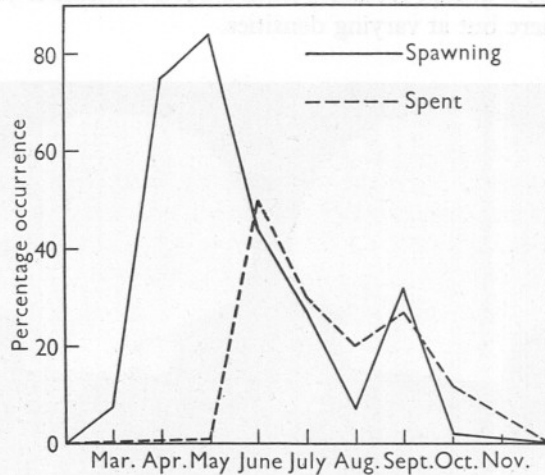


Fig. 5. Spawning and spent scallops in monthly samples.

At Castletownbere o-group scallops were collected in September. An analysis of the 1953 material has shown that spawning commenced in March, and reached a peak in May, fell away in July and August, and resurged during September (Fig. 5); the early spawned o-group had attained a size range of 2·9–5·8 cm by September. None were embryoned. At Port Erin, Mason (1953) has shown that the major spawning occurs in the autumn—between 90 and 95% of scallops on these beds are autumn-spawned—and this will account for the absence of o-group in the collected sample at Port Erin in July (Fig. 3).

#### COMPARISON OF CATCHES BY DIVING AND DREDGING

It can be seen (Figs. 1–3) that collection by diving yielded much smaller sizes and lower age-groups than collection by dredging. That this was not due to mesh selection in the bag of the dredge is manifest at Port Erin, where the bag was completely lined with shrimp netting that would retain all scallops. The teeth, which are the only other selective feature of the dredge, must be the effective selection agent.

The shaded squares on the histogram of the dredged sample at Port Erin (Fig. 2) represent scallops caught when the dredge filled with gravel and stopped the boat, i.e. they were caught when the dredge was acting as a complete bottom sampler and the teeth were ineffective as a selecting agent. If the legal size limit (11.5 cm or  $4\frac{1}{2}$  in.) is taken as an arbitrary line of size division, the actual distribution as indicated by diving shows 74.4% of the scallops on this bed to be below the legal size, while the dredge retained only 23.5% below this size. The teeth on the dredges, the length and spacing of which are not regulated, are therefore seen to constitute a highly effective saving gear.

Truly representative sampling of scallop beds is difficult. The low densities of scallops makes grab sampling impracticable. Dredges that effectively sample the whole size-range of the population have to have a considerable 'bite' because the scallops are recessed. They thus retain much of the bottom material and are effective for only a short distance. In consequence of the low densities at which scallops occur, such small catches are obtained under these conditions that sampling becomes extremely laborious.

Underwater photography and underwater television have been developed in recent years as methods of observing bottom animals. There are limitations to their use in assessing scallop populations. The area of the bottom observed is small and the scallops would not always be easily seen. The equipment required is relatively expensive. The advantages of the methods lie in their capacity to operate at greater depths and lower temperatures than is possible for a diver using self-contained breathing apparatus.

We would like to acknowledge, with thanks, the co-operation of the Director and staff of the Marine Biological Station at Port Erin in making boats and facilities at the laboratory available.

#### SUMMARY

Present methods of dredge sampling of scallop beds are unsatisfactory where all age-groups require to be studied. Selectivity by the dredge is continued above the point that might reasonably be expected from mesh size and tooth spacing, the latter being the primary selecting agent. A dredge without teeth and with a fine mesh bag fills with sand and bottom material within a short distance. As the mean density of scallops is low, even on good commercial beds, this results in very small catches.

Scallops of all age-groups are present together on the beds with a tendency towards very localized age grouping, which would not be apparent in dredge sampling. There is no evidence that migration from feeder beds occurs.

There is little apparent escape reaction; the limited reactions seen occurred most often among 0- and 1-group scallops.



Dredge efficiency is low, the Irish dredge used being on the bottom for only a part of the time that it was moving, progression occurring in a series of long shallow leaps.

Conservation of stocks of scallops would be most effective if based on dredge-tooth spacing and size rather than on the size of mesh or rings forming the belly of the dredge.

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## THE TIDAL RHYTHM AND ACTION OF THE DIGESTIVE SYSTEM OF THE LAMELLIBRANCH *LASAEA RUBRA*

By J. E. Morton

Department of Zoology, Queen Mary College, University of London

(Text-figs. 1-8)

It has been well established that some of the features of the feeding and digestive process in molluscs are rhythmic in character. Notably Hirsch (1915, 1917, 1931) has demonstrated a rhythmic periodicity of secretion in the salivary glands and digestive glands of some carnivorous Gastropoda, and Krijgsman (1925, 1928) has done similar work on the land pulmonate *Helix*. In all of these animals—and in cephalopods, too, where there is a more elaborate nervous control of secretion—discontinuous feeding is the rule. In the other and perhaps larger category of molluscs—those continuously feeding on fine particles—the central mechanism of the gut is the crystalline style, or its forerunner the protostyle. Here the need is, in Yonge's words (1937), 'to the extent to which they depend on extracellular enzymes for digestion, continuous secretion'. In Yonge's view, now classical, the style was regarded as 'an ideal mechanism for the continuous liberation of small quantities of an amylolytic enzyme'. Graham (1939), in his work on style-bearing gastropods, showed that the style is in general confined to animals with a continuous feeding habit, whether by ciliary means or by using the radula to graze on and rasp off fine particles. It is known that style secretion stops and the style is frequently dissolved when animals are removed from the water and cease feeding (see Yonge, 1925).

It has also been suspected in recent years that the digestive gland in continuous feeders displays a well-marked periodicity, with phases of absorption and of ejection of cell contents into the stomach. To some extent the action of the digestive gland in molluscs is still a subject of controversy. Yonge (1926*a, b*) made the first thorough study of this gland in a lamellibranch, and held it to be an absorbing organ, ingesting fine solid particles for intracellular digestion. In the opisthobranch gastropods, Fretter (1939) and Graham (1938) elucidated the nature of the digestive gland in detail, and found it to be an organ which, in addition to its ingesting role, had an important accessory role in excretion. Numerous authors, too, have found it to secrete into the stomach. This has always been assumed in carnivores, for example from the work of Hirsch (1915); and Millott's observations (1937) on the nudibranch *Forumna* showed



the formation of what the present writer (1955*a*) later called 'fragmentation phagocytes'. It was further established that the digestive gland in the primitive pulmonates had a secretory role, which, it was suggested, might be general in microphagous as well as macrophagous gastropods.

In the lamellibranchs, Mansour (1946) made claims for the secretory action of the digestive gland which seemed to go to the length of denying its ingesting role. In this class the problem is simpler, for we are concerned with a gland whose single function appears to be digestive. Several writers on gastropods have suggested an alternation of absorption and secretion in the digestive gland; but the present view, most generally accepted by workers on lamellibranchs, is that such extracellular digestion of proteins and fats as may occur in the stomach is performed by the enzymes from burst or cytolysed phagocytes, and that the 'secretory' role of the digestive gland is confined to the elimination of effete and rejected particles. In a recent investigation of *Lasaea rubra* (Ballantine & Morton, 1956) the authors noted the extreme suitability of that species for a contribution to this problem. *Lasaea* was found to carry out preliminary extracellular digestion of naked or thin-walled flagellates, *Phaeodactylum* ('*Nitzschia*') and diatoms. It furthermore has no gut phagocytes. *Lasaea* lives at a high tidal level, and the periodicity that must thus be imposed on its feeding was thought to offer good scope for an investigation of possible periodicity, both in crystalline style secretion and in the action of the digestive gland. Oldfield (1955) has recently completed a detailed account of the structure of *L. rubra*, including a full description of the gut; and Owen (1955) has published a review of certain aspects of the lamellibranch digestive gland, which is principally concerned with other problems than are discussed here. The writer has benefited greatly by discussion with Mr Owen and from being allowed to read his paper in manuscript.

This work was done while the author was the holder of the University of London table at the Plymouth Laboratory of the Marine Biological Association. His thanks are due to Dr Mary Parke for the generous provision of some of the organisms used in experiments, to Dr G. Y. Kennedy, for much assistance in the investigation of pigments, to Mrs E. Oldfield for a helpful discussion and to Miss D. Ballantine for useful criticisms and suggestions. Finally, the writer has had the great assistance of many discussions with Prof. C. M. Yonge, to whose knowledge of the lamellibranchs and kindly encouragement and criticism of this manuscript he is especially indebted.

#### THE INGESTION OF FOOD AND THE SECRETION OF THE CRYSTALLINE STYLE

The specimens of *Lasaea rubra* used in this work were gathered from crevices and between barnacle shells near the Hoe (Tinside) bathing pool, at a point that is covered by normal tides for approximately 3 h out of 12. During this

time they were able to filter and feed. The relation of the condition of the gut to the tidal cycle was investigated by the daily collection of animals over fortnightly periods and by making routine observations during these periods at the same time each day. An alternative method was to collect animals at hourly intervals during a single day's tides, and also to examine animals experimentally fed after known periods of filtering.

The presence and position of food in the gut was first accurately determined by the fixing, clearing and examining by transparency of sufficiently large samples of animals. This feature was found to show a very constant relation to the position of the tide, so that the immediate past history of a given animal could be fairly reliably ascertained by such an examination of its gut. The nature of the contents of the gut have already been discussed by Ballantine & Morton (1956). Table I sets out the relation between the tidal level and the gut contents of some of the animals considered.

TABLE I. POSITION OF FOOD IN THE DIGESTIVE TRACT OF  
*LASAEA RUBRA* AT VARIOUS TIMES OF THE TIDAL CYCLE

Sample no.	Hours submerged	Stomach only	Stomach and anterior intestine	Loop of intestine only	Rectum only (faeces in pallial cavity)	Gut empty	No. of specimens examined
1	1	.	16	2	1	5	24
2	2	.	8	4	3	5	20
3	3	3	27	.	.	.	30
4	3½	.	24	1	1	1	27
	Hours exposed						
5	0.3	.	.	11	4	3	18
6	1	.	.	6	7	6	19
7	2	.	.	8	5	.	13
8	8½	.	.	1	1	14	16
9	9	.	.	3	3	16	22

Equally predictable, in relation to the tide, was found to be the size of the crystalline style, and these two features, gut contents and style size, are treated in the next section as the foundation for a third rhythmic feature of the digestive process, the condition of the digestive gland.

From Fig. 1 it becomes clear that during the normal digestive cycle the crystalline style becomes partly dissolved, after the tide has withdrawn, to be rapidly secreted again on the return of the tide. When the stomach is filled with food the style is large and robust, filling the whole length and breadth of the style sac, in which it rotates, with a coiled food string attached to its free end. The style performs perfectly in *Lasaea* the role of a capstan first suggested by Orton (1923, p. 54) and later observed by Yonge (1949) in the Tellinacea, and by Morton (1952) in a number of gastropods. An observation sometimes possible in *Lasaea* strikingly illustrates this role: the style with the coiled string attached could by dissection be slipped from the grip of the



style sac, whereupon it was rapidly rotated in the reverse direction to its previous movement by the uncoiling of the tight twist it had imparted to the food string.

The style is reduced to a very small size in the last hours before the return of the tide, and sometimes—though not often—it seems to disappear completely. This was so with the animals in column 12 of Fig. 1. These were at the top of the tidal range during a neap tide, and the visit of the tide must have been too short to re-establish the style. This cannot happen often, and as a rule a style of full dimensions reappears in less than 1 h.

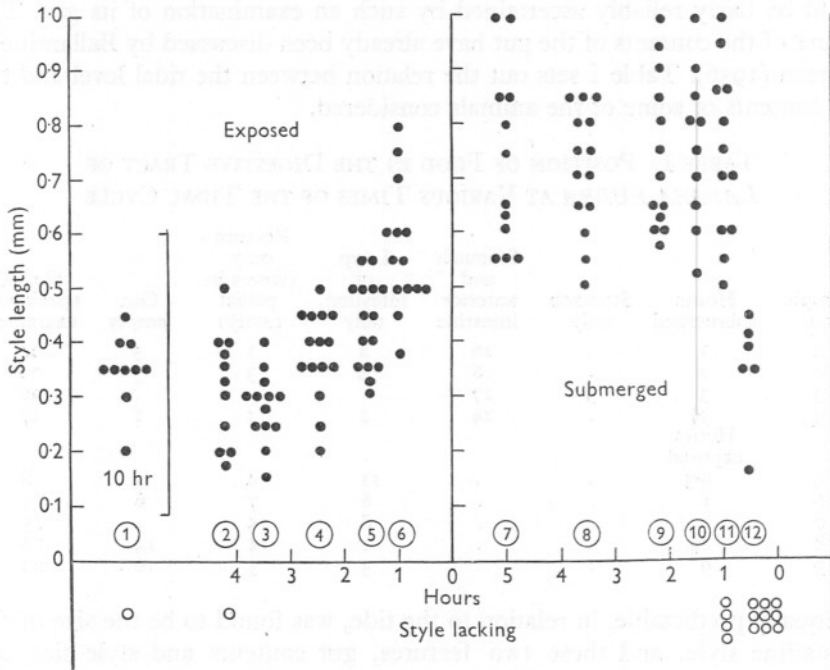


Fig. 1. Diagram showing the relation of the length of the crystalline style to the duration of exposure or submersion of the animal. Clear circles below the zero line represent animals in which the style was found to be absent.

From such observations as were made, the stimulus to the resecretion of the style appears to be the mere resubmersion of the animals. The presence of food in the stomach seems unnecessary, for the style was found to be restored to full size after  $1\frac{1}{2}$  h in filtered sea water (Fig. 1, column 10). Here, there may have been some small entry of particles such as debris dislodged from the animal's shell; but even after submergence in cultures of the toxic flagellate *Gymnodinium veneficum*, in which (see Ballantine & Morton, 1956) filtration is prevented, the resecretion of the style was not impeded (Fig. 1, column 11).

## SOME EXPERIMENTS ON FEEDING AND DIGESTION

*Lasaea rubra* is without gut phagocytes at any stage. During this work serial sections have been carefully examined from more than 200 animals, and these amoebocytic cells, which in other lamellibranchs emerge into the lumen to take part in digestion, could never be found. This is possibly a consequence of the small cell size of a minute animal, which may render the phagocytic mode of ingesting particles unsuitable. In this feature *Lasaea* is almost certainly exceptional among the eulamellibranchs. There is as yet no reason to suppose that other lamellibranchs of the Erycinacea may share in this peculiarity; as well as being of small size, *Lasaea* has further specializations peculiar to itself in this group, in particular its extremely high upper tidal limit and the periodicity thus imposed upon its feeding and digestion.

Extracellular digestion of carbohydrates has long been known to take place by the action of the enzymes of the crystalline style. In *Lasaea rubra* there is evidence, from two sources, of extracellular digestion of other substances as well. First, Ballantine & Morton (1956) have already reported in detail the preliminary digestion of *Phaeodactylum* ('*Nitzschia*'), the flagellate *Isochrysis*, and—more slowly—the diatom *Thalassiosira*. All these organisms were digested extracellularly within the lumen of the stomach. With *Phaeodactylum*, digestion could be quantitatively estimated at intervals over a 2 h period by direct counting of organisms in the stomach. The thin cell wall appears to require no cellulase to break it down, and the cell contents, consisting in large part of protein and lipid reserves, were completely digested out. The cell wall was left as an empty but recognizable 'ghost', sometimes containing traces of the complex carbohydrate leucosin, which appeared to resist digestion longer. The cellulose-armoured dinoflagellate *Peridinium trochoideum* was also fed to *Lasaea*, but was not digested at all.

Evidence of extracellular digestion was also gained from feeding experiments with dogfish erythrocytes. This food, like flagellates, could easily be fed in filterable suspension and had the advantage that it could be readily recognized in the gut after sectioning, and stages in the digestive breakdown of the corpuscles could be followed over the succeeding 8 h, until absorption was complete. Digestion could be carefully localized, and was found to take place in the lumina both of the stomach and of the digestive diverticula. Table II sets out the histologically visible signs of erythrocyte digestion over a period of 10 h after feeding.

For the first 30 min after feeding, the cytoplasm of the erythrocytes stained blue with azan. With the onset of digestion, the cytoplasm was attacked first, and during the first 2 h the outlines of the cells became more irregular until, at length, the nuclei were alone recognizable. Azan staining had the great advantage that its reaction with the erythrocyte changed from light blue to yellowish brown or orange as digestion proceeded. Eroded corpuscles were



TABLE II. PROGRESS OF DIGESTION OF DOGFISH ERYTHROCYTES BY *LASAEA RUBRA* DURING 11 H, AFTER FEEDING WITH A PALE SUSPENSION OF BLOOD IN SEAWATER

(Animals were removed after feeding for various lengths of time, and in some experiments were kept in filtered sea water for varying times before fixing. Fixed Bouin's, stained azan.)

Specimen no.	Time in blood suspension	Time in filtered sea water after feeding	Condition of erythrocytes after azan staining of sections	Condition of digestive gland (see pp. 571 <i>et seq.</i> )			
				I	II	III	IV
1	30 min	—	Many in the oesophagus and stomach, all intact, with cytoplasm blue. Majority in diverticula intact and blue, though a few eroded, and cytoplasm yellowish	×	×	—	—
2	30 min	—	Only a few in diverticula, none digested. Many in stomach not digested.	×	×	×	—
3	1 hr	—	Majority in stomach and diverticula undergoing digestion, cytoplasm orange yellow, margins usually much eroded. Nuclei persist longest. Final stage is of dispersion of yellowish granular cytoplasm released from the cells. Free nuclei visible (See Fig. 2)	×	×	—	—
4	1 h 50 min	—	All in stomach staining yellow to orange; much erosion in diverticula, and dispersion of amorphous yellow material. Yellowish droplets appearing in distal cytoplasm of digestive cells	×	×	×	—
5	1 h 50 min	—	Very eroded in diverticula, intensely orange. Much diffused yellowish material in lumen, some being absorbed by digestive cells. No free nuclei detected	×	×	—	—
6	2 h	1 h	All now in the diverticula, much more digested than previously; very few intact, but released yellowish material freely taken up along upper third of digestive cells	×	×	×	—
7	2 h	1 h	Much diffuse yellow from digestion. No whole corpuscles. Digestive cells absorbing	×	×	×	—
8	2 h	1 h	A few erythrocytes, almost wholly eroded in diverticula, and a great deal of diffuse yellow	×	×	×	—
9	2 h	1 h	Identical with (8)	×	×	×	—
10	2 h	2 h	No corpuscles and little diffuse yellow material in lumina of diverticula, but the upper half of the digestive cells loaded with orange-red droplets. Digestion and absorption appear complete	—	×	×	×
11	2 h	3½ h	A few areas of yellowish material remain in the lumen, but digestion is mostly completed. Orange-red granules now deeper in the digestive cells, with a superficial layer of blue-staining vacuoles (? material absorbed after blood)	—	×	×	×
12	2 h	3½ h	Similar to (11)	—	×	×	×
13	2 h	8½ h	No trace of yellowish material in lumina of diverticula. Digestive cells and cut off spherules filled with reddish brown vacuoles	—	×	×	×
14	2 h	8½ h	Identical with (13)	—	×	×	×

always yellowish brown, and after the complete breakdown of the cytoplasm, large amounts of yellowish granules appeared in the lumen. Absorption by the digestive gland could also be traced by staining with azan. At 3 h from first feeding, droplets of yellowish material were being taken up, after 4 h the vacuoles of the upper layer of the epithelium were strongly reddish brown after azan staining. After 5½ h these vacuoles lay more deeply and there was a superficial layer of light-blue staining vacuoles, which had evidently arisen from material later absorbed (see Fig. 2).

Azan staining may not be ideal for the tracing of erythrocyte digestion, since its coloration is apt to be unpredictable and uncertain in interpretation. In *Lasaea*, however, orange or reddish stained digestive vacuoles were never obtained save after feeding with blood, and the method by good fortune allowed digestion and absorption to be reliably traced. Another staining technique that might be attempted after digestion of erythrocytes is the Prussian blue detection of iron after its possible release as 'haemosiderin'. While haemoglobin specific stains are sometimes lacking in definiteness, the writer has found Van Gieson's to be a general stain that picks out haemoglobin rather distinctively (see also Dunn & Thompson, 1945).

Although occurring *in vivo*, these experiments were subjected to as careful as possible control. It was suspected that the cells might have been disintegrated by the mechanical action of the surrounding mucus or ruptured by the effect of the lower pH of the stomach contents, rather than by enzyme action. Cells were therefore examined that had passed through the mucus of pseudofaeces, and were found to show no erosion. Comparison was made with blood cells in other locations in the gut of ciliary feeders where enzymes were not active. It was impossible to measure the stomach pH of *Lasaea*, but this was assumed (see Yonge, 1925), to be somewhat greater than 5. Suspensions of dogfish erythrocytes and of *Phaeodactylum* were therefore immersed in salt solutions isotonic with sea water and buffered at pH 5.3, 5.5 and 5.9. Inspection at hourly intervals over the period of the experiments never showed any evidence of rupture or disintegration. Smears of dogfish blood were also subjected to digestion by mammalian trypsin at pH 7, after which the erythrocytes showed erosion in a similar way to those fed to *Lasaea*.

References to 'extracellular digestion' in this paper must clearly be taken to imply digestion in its widest sense. Preliminary digestion of relatively large cellular particles in the stomach proceeds at least as far as the breakdown of the cell wall; the cell contents are thus liberated in finely divided form, able—with or without further digestion—to be ingested by the free surface of the digestive cells. No one to-day would doubt that the digestive gland has an important function of intracellular digestion.



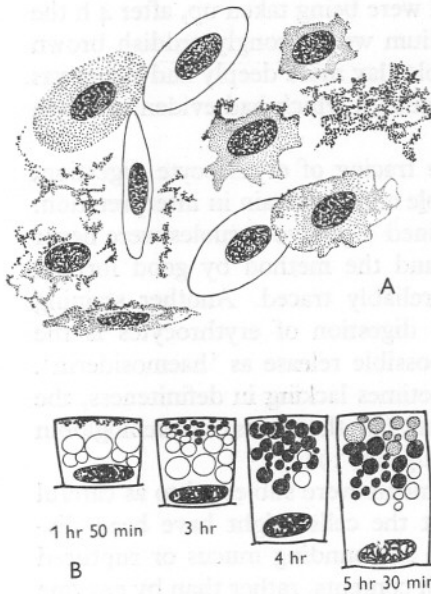


Fig. 2

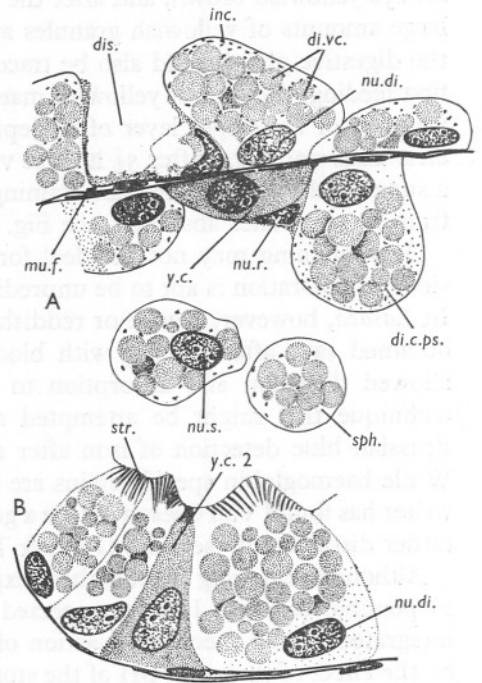


Fig. 3

Fig. 2. A group of dogfish erythrocytes in the lumen of the digestive diverticula of an animal that had been fed for 1 h with a suspension of blood corpuscles, and fixed in Bouin's fluid immediately. Erythrocytes with clear cytoplasm stained blue in azan, the nuclei red. After digestion an orange colour was shown by the cytoplasm, stippled in the figure, of both the intact and the eroded erythrocytes. Digestive cells, represented diagrammatically at 1 h 50 min, 3 h, 4 hr, and 5 h 30 min, respectively, after feeding with a suspension of blood. Absorbed material staining orange or brown with azan is represented black.

Fig. 3. (A) Portion of the wall of two adjacent tubules of the digestive gland. The digestive cells are at the beginning of the fragmentation stage, following absorption, and two spheres released into the lumen are shown in the lower part of the figure. Fixed Flemming's without acetic. (B) A group of cells from a tubule of the digestive gland during or after absorption, but before fragmentation. The striated layer at the free border is well shown. Fixed Bouin's fluid. Both drawings are from sections stained with azan. *di.c.ps.*, digestive cell which has become pseudopodial prior to the formation of a sphere; *di.vc.*, vacuole of absorbed material within a digestive cell, blue-staining with azan; *dis.*, space left in the epithelium after the discharge of a sphere; *inc.*, small inclusions, red-staining in azan, in the cytoplasm of a digestive cell; *mu.f.*, muscle fibre; *nu.di.*, nucleus of a digestive cell; *nu.r.*, nucleus, (?) remaining after discharge of a sphere, displaced to the surface of the epithelium; *nu.s.*, nucleus of a free sphere, lying in the lumen; *sph.*, sphere within the lumen, with no nucleus apparent; *str.*, layer of striations at the free surface of the digestive cell; *y.c.*, young cell; *y.c. 2*, young cell, compressed to a narrow shape between adjacent digestive cells.

## THE CYCLE OF THE DIGESTIVE GLAND

The structure of the digestive gland was studied in two ways: by the examination of stained serial sections, of which many were cut; and by the viewing of the whole gland in living condition immediately after removal from the animal. For this purpose digestive glands were isolated from other tissues, quickly mounted in sea water, lightly covered and immediately sketched under  $\frac{1}{12}$  in. oil-immersion objective. For quickness and accuracy this method was by far the more useful: the whole gland could be carefully scrutinized, while avoiding all fixation artifacts and allowing reliable interpretation of the living cell. This is another of the advantages of working with a small animal. Furthermore, large numbers of preparations could be made with no laborious procedure.

On the whole, the best stained preparations were obtained after fixing with Flemming's without acetic. Though penetration of Flemming's was usually poor and microanatomy distorted, yet in cell detail the best preparations with Flemming's were the finest of any obtained. Both aqueous Bouin's and Susa were found suitable for microanatomy, and both penetrate well. In spite of what is sometimes alleged against it as a cytoplasmic fixative, I have not found Bouin's, in this or in other molluscs where I have used it, much inferior to Flemming's. It has the great incidental advantage that it perfectly removes the shell. Ten per cent. formalin always gave poor results with *Lasaea*, penetrating badly and yielding poor staining. Heidenhain's azan was used for staining throughout.

The tubules or follicles of the digestive gland are lined wholly with glandular cells, which, as usual in bivalves, are essentially of one kind. No cilia were found in the tubules anywhere beyond the exit of the diverticula from the stomach, and in this feature *Lasaea* differs, probably again on account of its small size and functional simplification, from the numerous lamellibranchs recently studied by Owen (1955).

The digestive cell is a versatile structure and passes through several forms during its history. We should first refer to the nests of small cells, which correspond to the 'crypts of young cells' described by Yonge (1926*a, b*) and by Owen (1955). These cells in *Lasaea* occupy the tips of the older tubules of the digestive gland, and have a very different appearance from the absorbing cells. They are coloured pale yellow in life and appear to lack all forms of visible inclusions. They generally form groups of half a dozen or more cells at the tubule tip (see Figs. 5 and 6); and small spherical clusters of young cells are usually also to be found, forming smaller club-shaped branches that evidently represent the rudiments of new tubules. Owen describes the 'young cells' in eulamellibranchs in general as extending from the tubule tip in two or more tracts or 'crypts' along the whole length of the lumen of the tubule. Isolated young cells could often be seen in *Lasaea*, mingled with the more mature



absorbing cells, along the length of the tubule, but their most usual position is always in clusters at the tip. In correlation with the small size of the animal, and the relatively small number of cells constituting the whole tubule, replacing cells would seem as a rule to be concentrated at the tip of the tubule rather than diffusely scattered. A well-known parallel to this distribution of young cells is found in the nests of cells at the tips of the digestive tubules in the crayfish, *Astacus*, described and figured by Jacobs (1928).

Difficulty was encountered in *Lasaea* with the fixing and staining of the contents of the terminal cells. Oldfield (1955) states that, on fixing with Susa, the cell contents contract from the periphery and form a sphere of heterogeneous consistency. With Bouin's and Flemming fixation, the writer's results were generally unsatisfactory, the cells appearing as almost empty vesicles with their cytoplasmic contents barely recognizable or wholly shed. The isolated young cells dispersed irregularly among the digestive cells (see Fig. 3, *y.c.*) were, however, usually well fixed, and their cytoplasm stained pinkish brown with azan.

Oldfield (1955) remarks upon the great difference in appearance of the terminal cells from the absorbing cells, and gives evidence for the presence of two distinct types of cell in the digestive tubules of *Lasaea*. A short statement is thus necessary of the present writer's reasons for adopting a different interpretation on this point, and for taking a unitarian view of the terminal cells, as the youngest stages of a single versatile cell type, the absorptive and digestive cell. In specimens examined during this work, it seemed that the cell contents, 'of semifluid yellow material', rather than forming a vacuole, constituted in fact the whole of the cytoplasm of the cell, uniformly light yellow coloured and devoid of any stainable inclusions. As Oldfield points out, there is no response to tests for calcium, glycogen, fats or lipides. In the examination of digestive glands dissected living, these terminal cells can be shown to be wholly similar in form and appearance to the light-coloured 'young cells' interspersed with mature cells along the length of the tubule. Furthermore, in digestive glands examined alive immediately after feeding, pale-coloured cells in the terminal groups were seen to be absorbing droplets of pigmented food material along their free surface, while otherwise identical in appearance with non-absorbing cells alongside them. At times the whole cell group at the tip of a tubule was found to be carrying out absorption. In other specimens, soon after feeding, a single non-absorbing 'young cell' alone remained at the tip of the tubule; all its neighbours had been pressed into service as absorbing cells. This was the picture most generally seen within an hour or two of feeding; by contrast, in animals that had remained dry for some hours, the most conspicuous feature of the almost colourless digestive gland was the appearance of small rudimentary tubules, with no contents in their

lumina, and composed wholly of 'young cells' that had not absorbed (see sketches from life in Fig. 6).

The position of young cells at the tip of the tubule is in agreement with Owen's results on a wide range of eulamellibranchs, and also, as was pointed out above, with the condition in the digestive gland of *Astacus*. It would be desirable, but was not attempted during the present work, to investigate the incidence of mitotic divisions in these cells in *Lasaea*. Yonge (1926a) found numerous mitotic figures during histological studies of similar cells in other lamellibranchs. It is remarkable that the terminal cell groups should fix and stain so poorly as compared with the similar cells irregularly scattered among mature digestive cells. One must suppose that the uniform, semi-fluid cytoplasm of the groups of young cells is peculiarly susceptible to disruption by the tensions set up by diffusion currents in fixing and dehydrating. The isolated cells, surrounded by mature absorbing cells packed with contents, would thus be better protected against distortion arising during fixation.

The young cells are at first typically dome-shaped or pyramidal (see Figs. 5 and 6) and bulge from a flat base towards the lumen. Although they were never seen to be ciliated in *Lasaea*, either by the present writer or by Oldfield (1955), they seem with little doubt to be cells of the same type as were found in other lamellibranchs (see Owen, 1955; Potts, 1923) to bear long lash-like cilia at this stage of their history.

The digestive cells do not long remain dome-shaped or colourless, and the first transformation seems to be brought about by the flow of food material into the tubules. The lumina now become widely distended, and—though certainty is difficult on this point—the impression is gained that the young cells constituting some of the tubules are actually stretched by the mechanical pressure of contents, and that as the tubule is enlarged, so each cell becomes wider based and flattened. The surface appears at this stage to absorb material freely from the lumen, and there is often a continuity of staining between the cytoplasm of the cell and the contents of the lumen. At the same time there is a gathering of yellow vacuoles in the cytoplasm.

Absorption was studied by several experiments with natural and artificial foods. A suspension of colloidal graphite ('Aquadag') was fed to the animal, by mixing it with *Phaeodactylum*. After 2 h, absorption was freely in progress, and the surface of the digestive epithelium was dark grey. Closer examination revealed that graphite particles lay freely in the superficial cytoplasm, and were not at this stage contained in vacuoles. After feeding with *Phaeodactylum* alone for 1 h, immense numbers of tiny colloidal droplets could be detected both in the lumen and in the cytoplasm surrounding the vacuoles. They were being rapidly absorbed by the cells. Pigment introduced with food organisms, as well as probably indigestible waste materials, seems to aggregate in the vacuoles. After feeding for 2 h on *Phaeodactylum* made pink with neutral red, a pink coloration was found in the vacuoles in the upper half of the cell,



those at the base remaining yellow. In addition, minute red-stained granules lay freely in the cytoplasm between the pink vacuoles. These probably represent particles derived from the preliminary extracellular digestion of *Phaeodactylum* and now being taken up by the cells. A similar absorption of pigment by the vacuoles could be detected after feeding with a culture of *Dunaliella*, a thin-walled chlorophycean flagellate (6–12  $\mu$ ) (Plymouth no. 83). After 1½ h feeding a long rope of flagellates was found attached to the head of the style and each digestive cell showed a conspicuous absorption of chlorophyll in all the vacuoles of its superficial half. Preliminary digestion of *Dunaliella* took place extracellularly. The cells—as with *Phaeodactylum*—are much too large for direct ingestion; in one case where the lumen of a tubule was clogged with undigested flagellates no ingestion took place.

In addition to the absorption of graphite and plant pigments, the ingestion of soluble and insoluble iron saccharate by the digestive cells of *Lasaea* has been well demonstrated by Oldfield (1955).

Absorbing cells increase rapidly in height to columnar or bulging form (Fig. 3). During absorption the cytoplasm becomes loaded with conspicuous orange-yellow vacuoles which impart their colour to the living digestive gland. Such vacuoles seem to occur very widely in the molluscan digestive gland. They are essentially similar in *Lasaea rubra* to those recently described in the ellobiid pulmonates by Morton (1955*a*) in an account of the cyclic changes of the digestive gland. Their staining reactions in various Mollusca seem very uniform. They are coloured blue after azan (except in some rather exceptional cases, as after feeding with blood, p. 569); they become green with Masson's trichrome and do not stain at all with haematoxylin, or with the mucus stains alcian blue or mucicarmine. They appear to be loaded with lipid material which they probably store and which is responsible for their strong staining reactions with Sudan scarlet and with osmic acid. The vacuoles are not, however, simply droplets of lipoids: they have a permanence in the cell quite distinct from their occupation by fatty inclusions, and after treatment with acetone or ether they can be seen to persist as colourless spheres. Staining reactions with azan and with Masson's stain are well elicited in sections from which stored fat has been removed by routine treatment.

In the pulmonate *Otina* (Morton, 1955*b*) these vacuoles were studied in their 'empty' colourless condition towards the base of the cell, and were shown to be covered by a surface layer or membrane, stainable with thionin, but with no other stains tried. Attempts to detect it in *Lasaea* failed. This layer has many of the properties of that described by Barrington (1951) surrounding the 'chromophobe bodies' in the islet cells of the frog's pancreas. Colourless vacuoles are present in the digestive cells before the ingestion of food, and the writer earlier suggested (1955*a*) that the cell enzymes might be contained in or around them, or absorbed on the 'surface layer' when it occurs. There is as yet little positive evidence of this, and it would be of interest to examine

further the enzyme localization of the digestive cell. The small size of *Lasaea* does not, however, make it ideal for such tests.

The vacuoles of the digestive cell owe their coloration to mixtures of pigments taken into the cell from the materials fed upon. These pigments, originating in phytoplankton and fragments of plant detritus, included large amounts of chlorophyll. For extraction of the total pigments from *Lasaea*, pale or colourless low-tidal individuals were selected, with a minimum of algal

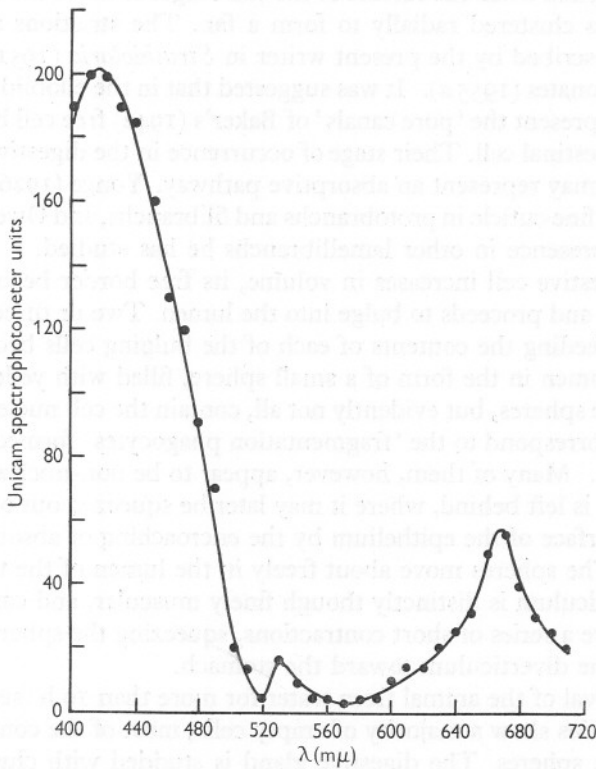


Fig. 4. Light-absorption curve obtained from the pigments extracted from the digestive gland, measured by the Unicam absorptiometer.

contamination of the shell. The spectral curve obtained after extraction with methanol of a greenish yellow solution from the soft parts is shown in Fig. 4. With peaks at 670, 535 and 420, it corresponds fairly closely with that of chlorophyll. Examination of macerated digestive glands with the spectroscopic microscope was difficult because of the animal's minute size; the 670 band, however, seemed fairly clearly identifiable. Pigments of the fucoxanthin type might also be expected to appear in the digestive gland, deriving with chlorophyll from the phytoplanktonic food (see Heilbron, 1942), and these are



probably chiefly responsible for the yellow coloration. No attempt has yet been made to extract separately the pigments present in *Lasaea*, and in the absorption curve shown in Fig. 4, fucoxanthin bands at *c.* 450 and 480 would—if present—be concealed (Strain, Manning & Hardin, 1944).

During the absorption of food the free border of the digestive cell may often be obscured by the passage of particles from the lumen. At other times, following soon after absorption, its free surface possesses a narrow border of fine striations, and when the surface of the cell bulges towards the lumen, these are sometimes clustered radially to form a fan. The striations are identical with those described by the present writer in *Struthiolaria* (1951) and in the ellobiid pulmonates (1955*a*). It was suggested that in the ellobiid *Leucophytia* they might represent the 'pore canals' of Baker's (1942) free cell border in the vertebrate intestinal cell. Their stage of occurrence in the digestive cell makes it likely they may represent an absorptive pathway. Yonge (1926*a*) speaks of this layer as a fine cuticle in protobranchs and filibranchs, and Owen (*in litteris*) confirms its presence in other lamellibranchs he has studied.

As the digestive cell increases in volume, its free border becomes bluntly pseudopodial and proceeds to bulge into the lumen. Two or three hours after cessation of feeding the contents of each of the bulging cells become nipped off into the lumen in the form of a small sphere, filled with yellow vacuoles. Some of these spheres, but evidently not all, contain the cell nucleus, and they would then correspond to the 'fragmentation phagocytes' formed in this way in gastropods. Many of them, however, appear to be non-nucleated, and the basal nucleus is left behind, where it may later be squeezed out or pressed up to the free surface of the epithelium by the encroaching of absorbing cells at either side. The spheres move about freely in the lumen of the tubules. The wall of diverticulum is distinctly though finely muscular, and can sometimes be seen to give a series of short contractions, squeezing the spheres and other contents of the diverticulum toward the stomach.

After removal of the animal from water for more than 10 h (see Fig. 5) the digestive tubules show a majority of empty cells, most of the contents having passed out as spheres. The digestive gland is studded with clusters of new tubules, consisting of young cells that have not yet absorbed.

At most times, spheres can be identified in the lumen (see Fig. 3*a*). They are generally present in some numbers during the absorbing phase of the digestive gland (Fig. 5*a*), though their time of formation appears always to be at the final stage of the cell's history, after absorption has been completed. They mark the end of the life of the cell, and their formation is a type of holocrine 'secretion'. The production of free spheres from the digestive cells has generally been regarded in lamellibranchs as a form of excretion. By this means, much pigmented material and indigestible remains are returned to the stomach for disposal as faeces. Owen (1955) has shown in detail how the arrangement of the typhlosole of the stomach and the excurrent tracts of

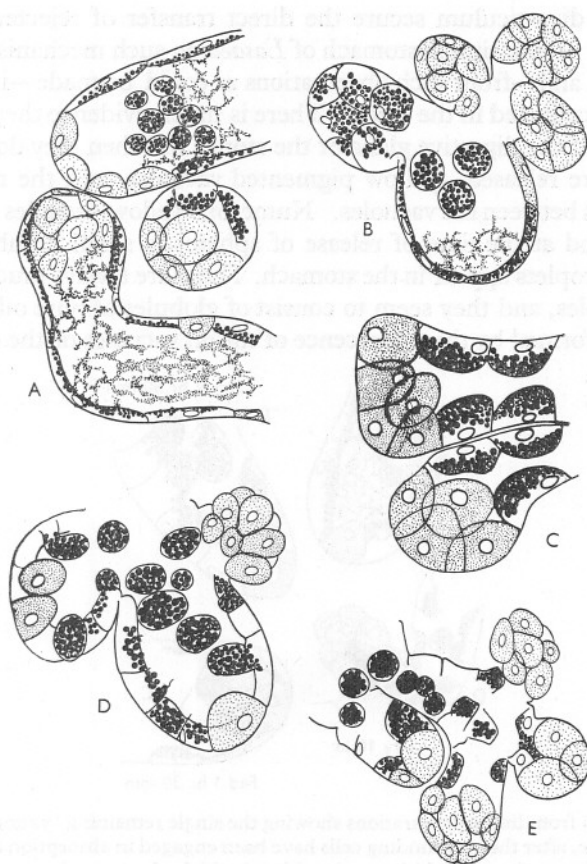


Fig. 5. Sketches made from living digestive glands, showing the features typical of various stages in the cycle. The portions of tubules illustrated are drawn from preparations examined as transparent objects under  $1/12$  objectives, the cover-slip resting lightly to prevent distortion of the cells. Young cells are present singly, in groups, or as young tubules and are lightly stippled in the figures; the coloured vacuoles in the mature cells are represented black. (A) Stage of absorption, with wide stretched tubules and flattened cells. Some free spheres are still present intact within the lumen of one of the tubules. Fed with *Phaeodactylum* for 1 h and examined immediately. (B) A later stage, from an animal exposed for 3 h after tidal withdrawal. The sketch shows three immature tubules composed of young cells, and, rather untypically for 3 h exposure, a tubule containing one young, non-absorbing cell, and the rest of its cells stretched flat and taking up contents from the lumen. (C) Stage of intact cells filled with absorbed contents, from a specimen exposed for  $3\frac{1}{2}$  h after feeding with *Phaeodactylum*. The two tubules show very typically the appearance of groups of young cells at the rounded tips. (D) Stage of fragmentation of digestive cells, to produce free spheres. The majority of the digestive cells have discharged their contents, and are represented by colourless spaces. One cell is shown as a pseudopodial projection about to be cut off into the lumen. (E) Stage of fully discharged epithelium, from a specimen exposed for more than 12 h after feeding on *Phaeodactylum*. The majority of the cells are represented by empty spaces, and there are numerous spherules lying freely in the lumen. Three immature tubules are shown, composed of colourless young cells.



the digestive diverticulum secure the direct transfer of rejected material to the intestine. In the simple stomach of *Lasaea* no such mechanism appears to be developed, and—from such observations as could be made—intact spheres were never recognized in the faeces. There is much evidence they disintegrate in the lumen of the digestive gland or the stomach. When they do so, two sorts of contents are released—yellow pigmented vacuoles, and the residual cytoplasm that lies between the vacuoles. Numerous yellow vacuoles are found in the lumen, and at the time of release of spheres, a small number of golden brown, oily droplets appear in the stomach. These are always much larger than the cell vacuoles, and they seem to consist of globules of pure oil or fat. They are probably formed by the coalescence of the oil vacuoles of the disintegrated spheres.

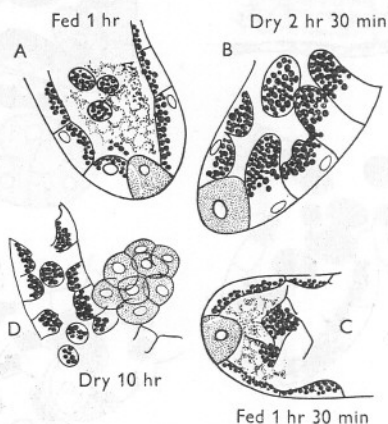


Fig. 6. Sketches from living preparations showing the single remaining 'young cell' at the tip of the tubule, after the surrounding cells have been engaged in absorption for 1 h or longer (A, B, C). Comparison should be made with (D) which shows a cluster of 'young cells' forming the rudiment of a tubule, in a specimen that has been several hours exposed; and with the group of young cells forming the whole tip of a tubule in Fig. 5 (c).

These large droplets were occasionally found in living preparations in such proximity to a recently discharged digestive cell as to suggest that they had been formed *in situ* by the coalescence of vacuoles before discharge. Such large vacuoles would then resemble those of the 'excretory cells' found in the digestive gland of gastropods. They were never found in sections of *Lasaea*.

Disintegration of the spheres may also release traces of free enzymes for extracellular digestion. It is suggested that these enzymes originate in the residual cytoplasm of the cells after the vacuoles are shed, and are responsible for the preliminary digestion of flagellates, *Phaeodactylum* cells and dogfish blood corpuscles shown to occur before absorption by the digestive gland. In other lamellibranchs, the free enzyme action detected in the stomach has been suspected to be due to the action of amoebocytes. In *Lasaea*, there would appear to be a different mechanism whereby food is able to be broken

down by preliminary digestion to particles minute enough to be absorbed by the digestive cells. The digestive cycle is primarily one of absorption and excretion; but from the contents of previously fragmented cells there appear to remain sufficient free enzymes to initiate a first phase of extracellular digestion.

### TIDAL RHYTHM

The sequence of activity of the digestive cells was investigated by the examination of 225 animals, at known times after feeding or exposure by the tide. Some of these were fed experimentally, with *Phaeodactylum*, *Dunaliella*, colloidal graphite ('Aquadag') or dogfish erythrocytes. Others were collected at regular intervals from the field, and examined after natural feeding. Examination was by dissection of the living digestive gland, as described above (p. 573) or from stained serial sections. Table III presents in condensed form the methods used in this survey, and its results; while Fig. 7 shows graphically the condition of the digestive gland from hour to hour during feeding and subsequent exposure. Three stages of activity of the digestive gland have been recognized, namely, (I) *absorption*, with flattened epithelial cells of low height and widely distended tubules; (II) *digestion*, with the majority of cells fully loaded and columnar; (III) *excretion*, with the production of free spheres by rounding off and liberation of the epithelial cells. 'Secretion' may be regarded as a subordinate activity taking place together with excretion. A further condition (IV) may be recognized where the epithelium is almost completely discharged of coloured cells, after several hours' exposure, and the whole digestive gland is pale or colourless.

Tables III and IV and Fig. 7 disclose a periodicity of the digestive gland, in relation to feeding and exposure. Like the presence of contents in the stomach and the size of the crystalline style, this is under ultimate tidal control. It is thus possible to speak of a tidal rhythm in the digestive system of *Lasaea*. Processes that may take place more or less continuously in bivalves mostly covered by the tide are, in high-tidal *Lasaea*, compressed into the short period of 3 or 4 h out of the 12, the period to which filtering is restricted.

There is no period when it is possible to say that all the cells of the digestive gland are at a particular stage, and it is perhaps unlikely that all the cells or tubules of a digestive gland ever take part together in one cycle of activity. Nevertheless, it is possible to recognize a well-marked *predominance* of activity, during various times of feeding and exposure. Thus, immediately after feeding the cells are with few exceptions at the flat, actively absorbing stage. And later in the tidal cycle, subsequent stages can be very satisfactorily followed, each having a maximum period, varying in length with the duration and amount of feeding. Successive phases overlap, and in all cases young cells are present in clusters at the tips of the tubules.



TABLE III. CONDITION OF DIGESTIVE GLAND AFTER FEEDING

(The condition of the digestive gland is given in relation to time of feeding or exposure in 232 specimens of *Lasaea rubra*. The stages recognized are (I) absorption, (II) intracellular digestion, (III) formation of spheres, (IV) post-spherulation (see pp. 579 *et seq.*). A bold numeral indicates a predominance of a given stage. A bracketed numeral indicates a stage that was also frequent.)

Series 1 (4/54). Fed *Phaeodactylum* 1½ h, transferred to filtered sea water. Fixed at intervals shown. Examined in stained sections. (20 specimens.)

Hours after feeding	I	II (numbers of specimens)	III	IV
1	8	3 (3)	I	.
2	(2)	5	I (2)	.
3	I (1)	3 (2)	I (2)	.

Series 2 (4/54). Fed erythrocytes up to 2 h, transferred to filtered sea water, and fixed at intervals shown. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
.	12 (1)	4 (10)	(3)	.
1	I (2)	5	(1)	.
2	.	2 (4)	I (4)	.
3½	(2)	5 (1)	(3)	.
6½	I	2 (2)	2 (2)	.
8	(1)	4 (2)	3 (3)	(1)

Series 3 (4/54). Fed *Peridinium trochoideum*, 1½ h. No digestion. Fixed immediately. (5 specimens.)

I	II	III	IV
I (2)	5	I	.

Series 4 (4/54). Fed *Isochrysis galbana*, removed into filtered sea water. Fixed after intervals shown. (5 specimens.)

Hours after feeding	I	II	III	IV
7	I	I (1)	I (1)	.
8½	I	I	(1)	.

Series 5 (4/55). Fed 'Aquadag',\* *Phaeodactylum* with neutral red,† and *Dunaliella*,‡ for times shown, and examined alive immediately after feeding. (7 specimens.)

Hours fed	I	II	III	IV
2*	3	.	.	.
2†	2	(1)	.	.
2‡	I (1)	(1)	.	.
3‡	.	.	I	.

Series 6 (7/54). Fixed in the field daily over 8 days. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
½	5	(4)	.	.
¾	I (2)	5 (1)	3	.
1	2 (2)	3 (2)	I	.
3	(1)	2	I (2)	.
3½	I (1)	2 (1)	I (1)	.
6	(2)	6	(1)	.
7	I (1)	4 (2)	I (3)	.
7½	(1)	4 (2)	3 (1)	.

Series 7 (8/54). Fed *Phaeodactylum* for times shown and fixed while still feeding. Examined in stained sections. (38 specimens.)

Hours fed	I	II	III	IV
2 $\frac{1}{2}$	6 (4)	8 (3)	1 (5)	.
2 $\frac{3}{4}$	(1)	(3)	4 (1)	.
3 $\frac{1}{2}$	2 (4)	3 (2)	1 (2)	.
4	.	.	1 (2)	.
5	1 (1)	4 (1)	2 (4)	.

Series 8 (8/54). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours after feeding	I	II	III	IV
1 $\frac{3}{4}$	(1)	1 (2)	2 (6)	1 (3)
2 $\frac{1}{2}$	.	.	1 (5)	(4)
3 $\frac{1}{2}$	(1)	1	2 (2)	1 (1)
4 $\frac{1}{2}$	.	(1)	4 (2)	2 (2)
5 $\frac{3}{8}$	.	.	2 (3)	1 (4)

Series 9 (4/55). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours fed	I	II	III	IV
1	2	.	(1)	.
1-2	1	.	.	.
2-3	(1)	1 (1)	.	.
3-4	2	(2)	(2)	.
4-5	.	2 (1)	1 (2)	1
Hours since feeding	I	II	III	IV
2-3	.	(5)	5	1 (1)
3-4	1 (1)	2 (4)	3 (4)	4 (3)
4-5	.	1 (2)	5	2
12-13	.	.	2 (6)	9 (1)

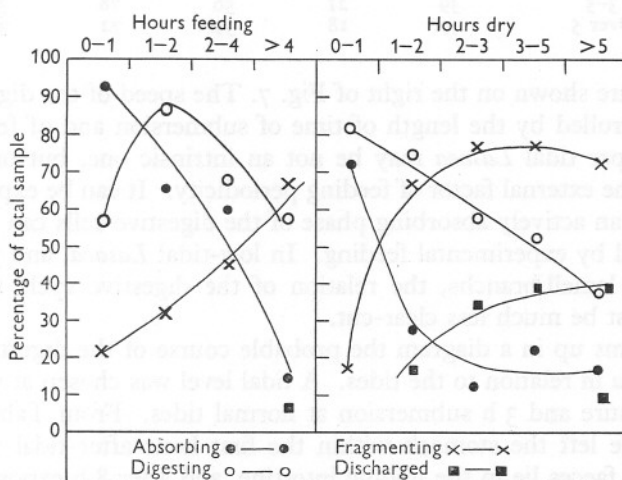


Fig. 7. Graph of the data set out in Tables III and IV showing the relation between the condition of the digestive gland, and the hours for which the animal had fed (left) or the hours for which it had remained exposed, following upon feeding (right).



Fig. 7 makes a comparison between two series of experiments in which animals were rather differently treated.

In the first series (results shown on the left) animals had been experimentally fed with *Phaeodactylum*, or with dogfish erythrocytes, in much greater abundance than that of natural food. After 4 h, the stages of 'absorption' and 'digestion' have generally been passed through, and 'fragmentation' predominates. Although the animals were still immersed in a suspension of food particles, the digestive cycle had—after overfeeding—advanced to a further stage than is normally found when animals in the field are left dry by the retreat of the tide. Animals which had spent 2 h feeding in natural conditions in the field were examined in a second series of experiments, and

TABLE IV. CONDITION OF DIGESTIVE GLAND

Percentage of occurrence of stages I to IV of the digestive gland, in relation to time after feeding or exposure, from results obtained with 225 animals. (In many cases a single animal is represented under more than one stage, and the calculations have been made by adding together the bold and bracketed entries in Table III.)

Hours	Total no. of animals	Percentages			
		I	II	III	IV
Fed					
0-1	14	93	57	21	.
1-2	9	66	89	33	.
2-4	33	60	69	48	.
Over 4	13	15	61	69	8
Exposed					
0-1	26	73	81	19	.
1-2	25	28	76	68	16
2-3	17	12	59	77	35
3-5	39	21	56	78	38
Over 5	49	18	39	72	39

the results are shown on the right of Fig. 7. The speed of the digestive cycle is thus controlled by the length of time of submersion and of feeding. The cycle in upper tidal *Lasaea* may be not an intrinsic one, but one imposed merely by the external factor of feeding periodicity. It can be experimentally varied, and an actively absorbing phase of the digestive cells can at any time be produced by experimental feeding. In low-tidal *Lasaea*, and probably in most other lamellibranchs, the relation of the digestive cycle to the tidal rhythm must be much less clear-cut.

Fig. 8 sums up in a diagram the probable course of the digestive cycle of *Lasaea rubra* in relation to the tides. A tidal level was chosen at which there is 9 h exposure and 3 h submersion at normal tides. From Table I food is seen to have left the stomach within the first hour after tidal withdrawal. After 1-2 h faeces lie in the middle intestine, and after 8 h exposure the last remains of faeces are in the rectum or discharged into the pallial cavity. The style is largest while food is in the stomach, drawing in the food string by its

rotation. During exposure it becomes too small to be rotated in its sac, but is probably not often lost altogether. It is rapidly rebuilt during the first hour after the tide returns. The sequence of the four phases of the digestive gland extends throughout the 12 h of the tidal period, and is evidently controlled primarily by the presence of food in the stomach.

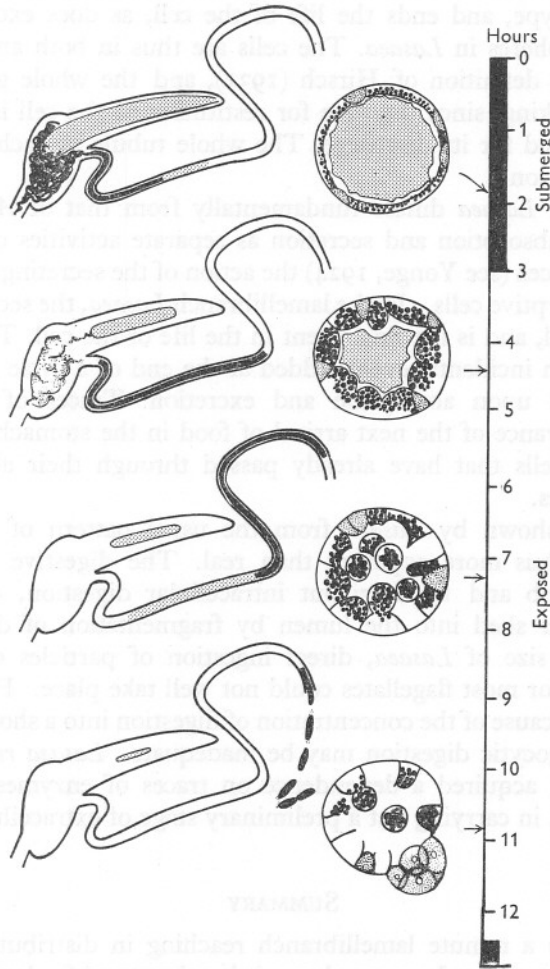


Fig. 8. Diagram showing the relationship between the periodicity of the digestive system, and the submergence or exposure of the animal by the tides. The outline drawings of the stomach, style sac and intestine indicate the size of the crystalline style, and the position of food in the various parts of the gut. The diagrams of digestive tubules, in transverse section, show the following stages: (I) absorption; (II) fully loaded cells, presumably with intracellular digestion in progress; (III) fragmentation, and formation of spherules; (IV) discharged epithelium, with spherules in the lumen, and a developing tubule composed of young cells.



Comparison may be drawn between the cycle of the digestive gland in *Lasaea rubra* and that of the digestive diverticula of the decapod crustacean *Astacus*, which was carefully investigated by Hirsch & Jacobs (1928, 1930). In decapods, the young cells forming the tip of the tubule give rise to two separate types of cell, secretory and absorptive. The secretory cells are under rhythmic control, which is speeded up greatly after feeding. Secretion is of the holocrine type, and ends the life of the cell, as does excretion by the formation of spheres in *Lasaea*. The cells are thus in both animals 'monophasic', by the definition of Hirsch (1931), and the whole gland shows a rhythmical working, since the time for restitution of the cell is greater than the time required for its discharge. The whole tubule in each constitutes a 'field of restitution'.

The cycle in *Lasaea* differs fundamentally from that of *Astacus*, in the occurrence of absorption and secretion as separate activities of one type of cell. In Crustacea (see Yonge, 1924) the action of the secreting cells precedes that of the absorptive cells. In the lamellibranch *Lasaea*, the secretory activity comes last of all, and is the final event in the life of the cell. This is because 'secretion' is an incidental event, added at the end of a cycle that is fundamentally based upon absorption and excretion. Traces of enzymes are provided in advance of the next arrival of food in the stomach, by the fragmentation of cells that have already passed through their absorptive and excretory phases.

Differences shown by *Lasaea* from the usual pattern of lamellibranch digestion are thus more apparent than real. The digestive cell has been shown to absorb and to carry out intracellular digestion, and excretory material is later shed into the lumen by fragmentation of digestive cells. From the cell size of *Lasaea*, direct ingestion of particles of the size of *Phaeodactylum* or most flagellates could not well take place. For this reason too, and also because of the concentration of digestion into a short time during each tide, phagocytic digestion may be inadequate. *Lasaea rubra* has thus, it is suggested, acquired a dependence on traces of enzymes shed by the digestive gland, in carrying out a preliminary stage of extracellular digestion.

#### SUMMARY

*Lasaea rubra* is a minute lamellibranch reaching in distribution almost to high-water spring tides. In some places, it is submerged for less than an hour at each tide and a tidal periodicity is thus imposed upon its feeding. The effect of this periodicity is reflected in the mode of action of the gut. The amount and position of food varies regularly with the state of the tide. The crystalline style undergoes a regular cycle, being partly dissolved and becoming vestigial at low tide, and being resecreted at the high tide. A sequence of four phases was established in the digestive gland, by the study of large numbers

of animals, living and after sectioning, and after both natural and artificial feeding. While the animal is feeding, and shortly afterwards, finely divided material is absorbed into the cells of the digestive gland. Intracellular digestion then takes place, with the loaded digestive cell attaining its maximum size. Fragmentation of the digestive cells next occurs, with the discharge of rounded spheres, filled with vacuoles of rejected pigmented material. Finally, just before the tide returns, the digestive gland shows a discharged epithelium, with the digestive cells being replaced by young cells. Evidence is given of the close relation of this cycle to the tides. It is also shown that extracellular digestion of dogfish erythrocytes and phytoplanktonic organisms takes place within the stomach before absorption, and it is suggested that this is brought about by traces of enzymes in the fragmented spheres issuing from the digestive gland.

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## ON THE BIOLOGY OF *CALANUS* *FINMARCHICUS*

### IX. FEEDING AND DIGESTION IN THE YOUNG STAGES

By S. M. Marshall, D.Sc. and A. P. Orr, D.Sc.

The Marine Station, Millport

(Text-figs. 1-3)

Little is known about the food of the nauplii and early copepodite stages of copepods, although it has usually been assumed that the food particles must be smaller and perhaps more concentrated than for adults. A few observations have been made on the feeding of the young stages of *Calanus* in the laboratory, but none are available on feeding under natural conditions. It has been suggested (Marshall, Nicholls & Orr, 1934) that the success or failure of a brood of *Calanus* in the sea might depend on the presence of a rich food supply during its development. Experiments were therefore undertaken to find what organisms could be ingested by the nauplii and early copepodite stages, how much of the different foods could be digested, and how much water they could filter in a day.

#### METHODS

The use of food cultures labelled with radioactive phosphorus ( $^{32}\text{P}$ ) enables an accurate measure to be made of the amount taken in, and the methods used in earlier experiments on feeding in adult and Stage V *Calanus* (Marshall & Orr, 1955*b*) were found to be useful for the younger stages also. When photosynthetic organisms are grown in a medium containing inorganic  $^{32}\text{P}$  it is rapidly taken up and the culture can then be used in different concentrations to measure the amount filtered and the amount assimilated in a given time. A variety of food organisms of different sizes and belonging to different systematic groups was used. In each experiment the copepods were put in bottles filled with a culture of known concentration of the food to be tested and the bottles were then tied in dark cloth bags and attached to a wheel revolving slowly so that the culture was kept in suspension. All experiments were carried out in a cool aquarium. At the end of the experiment, which lasted from 16 to 24 h, the bodies, and sometimes the faecal pellets also, were removed and washed. They were then dried and exposed on a disc or planchette under the end window of a Geiger counter to measure the radioactivity. The results are shown as counts per minute. Specimens were also kept in filtrate from the radioactive culture to measure the uptake, if any, of  $^{32}\text{P}$  in solution.



If faecal pellets were to be collected, the early stages of *Calanus* were kept in bottles which might be as small as 2 ml. capacity. For experiments in which faecal pellets were not collected, as many as 20 Nauplius III were put in a bottle of about 30 ml. capacity and the bodies were usually read in batches of about 10 at a time. The figures given for volume filtered by these are therefore averages. Occasionally, however, a Nauplius III was read singly and it was clear that this stage showed as much variation as any other. The later stages were usually read singly, although several might be kept in one bottle.

It is not so easy to judge the health of the early stages as of the adults. The amount of food taken up varied much in different experiments with the same stage, and it seems likely that those with the higher feeding rate represent the normal individuals. Experiments were therefore usually made with two species of food organism, one of which was known to be a satisfactory food. If this was eaten freely it was assumed that the copepods were in good condition.

Nauplius Stages I, II, III and sometimes IV were usually obtained from eggs hatched in the laboratory. The later stages were usually picked out from tow-nettings taken on the day of the experiment. They were commonest during the spring and summer in fine and medium tow-nettings, but a small number could be obtained throughout the autumn and even part of the winter. Nauplius I usually moults to Nauplius II in less than 24 h and Nauplius II to Nauplius III about 24 h later. Nauplius III remains as such for several days (Lebour, 1916) and mortality at this stage is usually high. The interval between moults is short in the early stages and at the end of an experiment some had remained in the same stage, some had moulted to the next and some were in process of moulting. Only the results from the first group are included in Table IV.

The food organisms were most often used in concentrations such as are found in the sea. Counts were made either with a haemocytometer or, more usually, in 0.2 ml. samples on a ruled slide. The number of cells present does not affect the calculation for the number of ml. filtered, but it can give an idea of the number of cells which the copepod ingests in a given time (Table VIII).

The following organisms were used:

*Nannochloris oculata* Droop (M. R. Droop's strain 66)

*Monochrysis lutheri* Droop (M. R. Droop's strain 60)

*Dicrateria inornata* Parke (Plymouth strain B)

*Syracosphaera elongata* Droop (M. R. Droop's strain 62)

*Skeletonema costatum* (Grev.) Cleve (Plymouth strain 106)

*Chaetoceros decipiens* Cleve (Plymouth strain 107)

*Ditylum brightwellii* (West) Grun. ex Van Heurck (Plymouth strain 110)

*Prorocentrum micans* Ehrenberg (Plymouth strain 97)

*P. triestinum* Schiller (Plymouth strain 98)

*Peridinium trochoideum* (Stein) Lemm. (Plymouth strain 104)

The dilution of the cultures was always made with water which had been filtered through a 'membrane' filter. This removed particles down to about  $1\mu$  and so ensured that a negligible quantity of non-radioactive food was added. Filtering through filter-paper makes the water harmful to at least the early nauplius stages of *Calanus*. It was noticed that nauplii in Stage I mostly died or at least failed to moult when kept in paper-filtered water. The fine threads which come from the filter-paper probably get entangled in the nauplius limbs and prevent free movement. Rejecting the first portion of the filtrate and so reducing the number of fine threads led to an improvement in survival and moulting and a complete removal of these threads by filtering again through a 'membrane' filter resulted in normal survival and moult.

#### THE FILTERING APPENDAGES

##### *Nauplii*

Some observations on the feeding mechanisms of copepod nauplii have been made by Storch (1928). He states that the nauplius feeds like the adult, i.e. if the adult copepod is filter-feeding so is the nauplius, and, if the adult is predatory, the nauplius is predatory too. The limbs used in feeding by *Calanus* nauplii must be different from those used by the adult, for the maxilla, which is the main filtering appendage, does not appear until Nauplius VI and

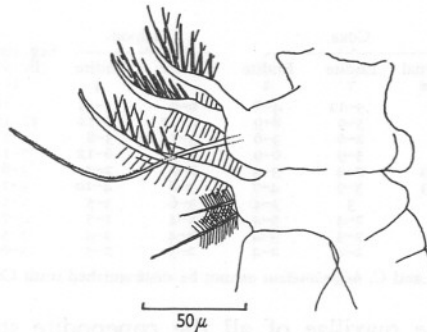


Fig. 1. Coxa and basipod of the antenna of Nauplius V.

is then small and weak. Storch says that in *Diaptomus gracilis* nauplii the filtering is done by the long masticatory processes of the coxa and basipod of the antenna and mandible. In Nauplius I and II of *Calanus* these processes are short and weak and have no setules. As will be shown later, it seems certain that neither of these stages feeds. In Nauplius Stages III to VI the masticatory spines on the coxa and basipod of the antenna have developed further, are longer and stronger and lie close together. Each of the three (Fig. 1) has a double row of strong setules on the distal half about  $10\text{--}30\mu$  long and  $1.5\text{--}4\mu$  apart. These are the only setules present on the proximal



coxal seta, but on the other two the lower halves have single rows of fine setules on each side. There is a long thin seta on the coxa and two shorter on the basipod, but these may play no part in filtration. It seems possible that the 'masticatory processes' are wrongly named and that their function is filtration rather than mastication. The function of the separate setae is not obvious.

The maxilla, which appears in Nauplius V as a knob, has in Nauplius VI almost the complete number of setae, but these are relatively very short and have no setules.

### *Copepodites*

It may be assumed that in the copepodite filtration is done mainly by the maxilla (Cannon, 1928; Lowndes, 1935). This is a uniramous appendage bearing a series of setose endites. Following Gurney (1931), it may be taken as formed by a coxa bearing two endites and an external seta, a basipod bearing two endites, and a five-segmented endopod, the first two segments of which are produced as endites. The maxilla of *Calanus gracilis* (which differs only slightly from that of *C. finmarchicus*) has been beautifully figured by Giesbrecht (1892, Plate 7, fig. 17).

TABLE I. DISTANCE APART OF SETULES IN COPEPODITE STAGES (IN  $\mu$ )

	Stage	Coxa			Basipod		Endopod			
		External seta	Endite 1	Endite 2	Endite 3	Endite 4	Segment 1 (Endite 5)	Segments 2-4	Segment 5 'Feather'	Others
<i>C. finmarchicus</i>	♂	—	5-12	4-8	6-9	5-11	4-16	5-21	4-5	6-17
<i>C. helgolandicus</i>	♂	—	5-9	8-9	8-10	8-11	12-17	11-19	7-9	13-22
<i>C. finmarchicus</i>	♀	2	2-6	3-6	3-7	3-8	4-7	5-14	3-5	5-14
<i>C. helgolandicus</i>	♀	2	5-9	6-9	4-9	6-12	7-13	11-19	3-9	12-20
<i>C. finmarchicus</i>	V	1-3	2-4	3-4	2-4	2-4	3-6	4-8	2-5	4-10
<i>C. helgolandicus</i>	V	1-3	3-9	4-7	3-8	4-10	3-15	5-17	3	7-20
	IV*	2	3	3-4	3-6	3-5	3-5	4-7	3-4	5-9
	III*	2	2-4	3-4	3-4	2-5	4-7	4-8	3-4	4-11
	II*	—	2-3	3-4	2-4	3-4	3-5	3-7	3-4	3-7
	I*	—	2-4	2-4	2-5	2-5	3-6	4-7	2-4	4-8

\* *C. finmarchicus* and *C. helgolandicus* cannot be distinguished until Copepodite V.

The setules on the maxillae of all the copepodite stages and of both *C. finmarchicus* and *C. helgolandicus* were examined and measured to give some idea of the size of organism which could be retained (Fig. 2 and Table I).

There are altogether twenty-nine setae, of which twenty form a sheet approximately in the same plane, the gaps between them being partly filled by closely set setules. Usually each seta bears two rows of setules set at an angle of about 90° to one another, sloping forward and making an angle of about 60° with the seta. All but one (the external) of the remaining setae are on the inner side of the limb, one to each endite except the fifth and one to each of the three terminal segments; they usually lie at an angle to the main setae. Those on endites 1-4 are shorter and have irregularly spaced setules projecting in all directions. That on the first endite is like the others on the

upper half only; on its lower half there are two regular rows of setules at  $180^\circ$  in the plane of the setae. That on the terminal segment of the limb has two opposite rows of closely set setules (like a feather) but it is naked in the upper half. Finally, there is a short external seta on the coxa with opposite rows of very fine and closely set setules.

Apart from the setules most of the main setae are serrated towards the tip, the serrations sometimes enlarging to become a series of short spines between the setules.

It is not easy to understand the purpose of all this elaboration. Obviously the setules forming a close network between the setae must be the main instruments of filtration, and the fact that the setules and serrations are both directed forward will make it easy for a particle to pass towards the mouth. The function of the special internal setae and of the external seta is unknown. The whole limb is slightly concave viewed from the outer side.

With the exception of the first and sixth endites on which one or two setae are added during development, the main setae are present in all the copepodite stages and indeed almost all are present in Nauplius VI.

The maxillae were dissected off from several specimens of each stage and the distance apart of the setules measured on one or more setae of each endite of the coxa and basipod as well as on most of the segments of the endopod. The smallest divisions on the micrometers used measured  $1.86$  and  $1.67 \mu$ . It was not always possible to make measurements on the same part of the setae or even on the same setae on different individuals, so that the results which are shown in Table I indicate the variations only approximately.

It can be seen that the smallest distance between the setules lies between  $2$  and  $3 \mu$ . In general the setules are closest towards the lower half of the setae and widest towards the tip; one distance may be double the other. There is a gradual but less regular widening of the distance between setules from endite 1 of the coxa to segment 5 of the endopod. On the two terminal setae the spacing is much wider and may reach  $20 \mu$ . Although the minimum distance of  $2-3 \mu$  is found also in the adult (chiefly at the base of the coxal setae) there is a tendency for the setules to be closer in the younger stages.

There seems also to be a real difference between the adults of the *finmarchicus* and the *helgolandicus* forms; the setules are between  $50$  and  $100\%$  wider apart in the latter. This difference is less marked in Stage V. The distances apart are considerably smaller than Ussing's (1938) estimate. According to him the smallest distance apart of the setules in the adult female is  $5.7 \mu$ , in Stage V,  $3.8 \mu$ , and in Stage IV,  $3.2 \mu$ . His specimens were from the Arctic where the *Calanus* are much larger than ours and the size difference may account for the greater minimum distance apart.

It is not possible to say how efficient the maxilla of *Calanus* is as a filter but we should expect considerably smaller particles to be retained than have been shown experimentally to be important as food. The size of some of the cells



used in the feeding experiments is shown in Fig. 2. It is obvious that the small flagellate *Nannochloris* can easily pass the filter over most of its area.

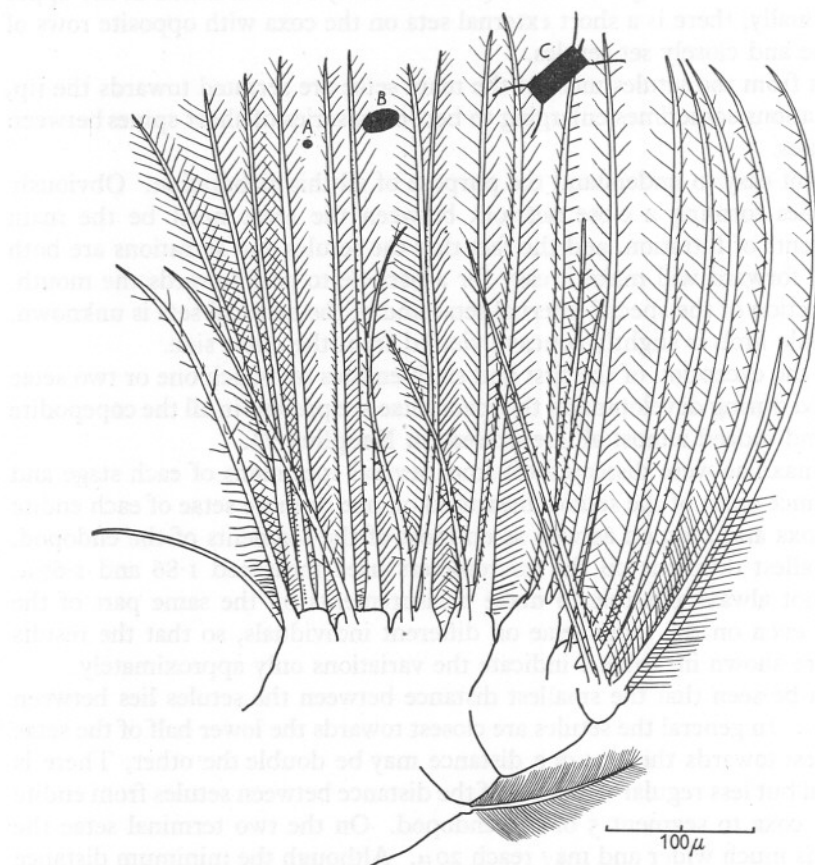


Fig. 2. Left maxilla of *Calanus helgolandicus* ♀ from the right. A, B and C represent the sizes of food organisms used. A, *Nannochloris oculata*; B, *Syracosphaera elongata*; C, *Chaetoceros decipiens* (from culture).

#### FEEDING EXPERIMENTS

One certain test of whether a nauplius can eat any particular food is to find the skeleton in the faecal pellets. This is sometimes very difficult if the skeleton has no obvious sculpturing. It is probable, for instance, that Nauplius III can eat *Chaetoceros decipiens* but the frustule of this diatom is thin-walled and has no striking features and it has not been identified in faecal pellets. *Skeletonema* frustules are found complete in the faecal pellets of Nauplius III showing that this common spring diatom is a food for even the earliest feeding stages of *Calanus*. Coccoliths are shed in large numbers when a culture of

*Syracosphaera* is getting old, and it is possible that some may be taken loose. However, the faecal pellets examined from nauplii which had been feeding on a culture of this species were packed full of coccoliths, which had probably come from ingested cells.

These and other observations for the later nauplius stages are shown in Table II and the size of some of the faecal pellets given. It shows a gradual increase with the size of the nauplius, as might be expected. The pellets were usually ovoidal but were very pale compared with those from adults on the same food. The rate of production with a suitable food is somewhat similar to that of the adult *Calanus*.

TABLE II. SIZE OF FAECAL PELLETS IN NAUPLIUS STAGES

(In those marked with an asterisk, the food organism was identified in the faecal pellet.)

Food organism	Size of food organism ( $\mu$ )	Size of faecal pellets ( $\mu$ )			
		N III	N IV	N V	N VI
<i>Skeletonema costatum</i>	4-5	48 × 24*	46 × 25*	83 × 33*	87 × 30*
<i>Chaetoceros decipiens</i>	14-21 × 20-78	33 × 20	—	—	—
<i>Syracosphaera elongata</i>	18-30 × 12	31 × 17*	39 × 21*	48 × 28*	*
<i>Prorocentrum micans</i>	43 × 27	—	—	60 × 29*	87 × 32*
<i>P. triestinum</i>	10 × 14	*	—	—	*
<i>Peridinium trochoideum</i>	25 × 19	—	—	—	*
<i>Ditylum brightwellii</i>	20-60 diam.	—	—	—	*

The earlier the stage of the *Calanus* the more difficult are the faecal pellets to recognize. When Janus green is added to the water, they take up the stain and are slightly easier to see, but the risk of losing some is much greater with nauplius stages than with copepodites. In the quantitative experiments in which the faecal pellets were picked out, the percentage of the phosphorus-containing portion digested was always high and it was felt that little was gained by a time-consuming and perhaps unsuccessful attempt to find all the faecal pellets produced. In many of the experiments therefore faecal pellets were ignored and a larger number of individuals could thus be tested. The total variation was so great that in comparing volume filtered with faecal pellets included and excluded, the first does not show a consistently higher figure; the reading of the body alone is therefore justifiable.

Grobben (1881) has stated that the first nauplius stage has no anal opening and this has been confirmed by Miss Judith C. Perryman of King's College, London (personal communication). She finds that the mouth does not open until Nauplius II and the anus not until Nauplius III. From our experiments also it seems certain that neither Nauplius I nor Nauplius II feeds. When Nauplius II were used some of them always moulted during the course of the experiment, and from the results shown in Table III it is clear that only those nauplii which reached Stage III had taken up an appreciable amount of food. The slight radioactivity shown by the Nauplius II may be due to  $^{32}\text{P}$  absorbed from solution.



The results of twenty-two out of the thirty-nine experiments done on the late nauplius and early copepodite stages are shown in Table IV. The rest are omitted for one reason or another. In nine the copepods had eaten badly; it was considered that if Nauplius III had filtered less than an average of 0.05 ml. in 24 h they must have been unhealthy. If, however, two food species were tested in the same experiment and the copepods did well in one and badly in the other, both results were accepted. Four experiments on *Nannochloris* and *Monochrysis* are excluded; these are very small flagellates, not likely to be taken freely, but no check against a good food was made. Two experiments on Nauplius II (which does not feed) and two on *Bacillus globigii*, which gave negative results, are also omitted.

TABLE III. UPTAKE OF  $^{32}\text{P}$  IN LATE N II AND EARLY N III

(Duration of experiments between 18 and 24 h)

Culture	Age of culture (days)	Concentration cells/ml.	Nauplii put in	Nauplii as read	Counts/min	Counts/nauplius/min
<i>Skeletonema</i> (contaminated) 26. viii. 54	7	275	20N II	2N II	112	56
				17N III	7096	417
			20N II	5N II	90	18
				14N III	4405	315
<i>Chaetoceros</i> <i>decipiens</i> 21. iv. 55	6	1670	20N II	2N II	122	61
				9N III	10146	1127
				8N III	8134	1017
			23N II	5N II	78	16
				12N III	11133	928
			20N II	4N II	19	5
				8N III	9139	1142
				9N III	5649	628
<i>Ditylum</i> <i>brightwellii</i> 6. vi. 55	18	57	20N II	5N II	241	48
				12N III	1176	98
			20N II	7N II	177	25
				10N III	5567	557
<i>Dicrateria</i> <i>inornata</i> 21. iv. 55 26. viii. 54	6	71000	20N II	1N II	5	5
				13N III	5218	401
	7	43000	20N II	5N II	1	0
				13N III	4180	322
			20N II	9N II	27	3
				9N III	2910	323

In any one experiment also the result was rejected for any individual which had done very much worse than its fellows. Sometimes these were noted as being inactive or unhealthy, but sometimes they were apparently in as good condition as the rest. They may, however, have been near moult (see Tables V and VII). The results given in the table are therefore selected, but as explained above it is thought that they probably show the normal behaviour.

On one or two occasions Nauplii III and IV reared in the laboratory were separated into *Calanus finmarchicus* and *C. helgolandicus* and read separately. The former is always smaller and gave, as might be expected, lower results.

TABLE IV. FEEDING EXPERIMENTS ON NAUPLII AND COPEPODITES

Number of copepods used in each experiment and volume filtered in 24 h

Food species and size	Date	Age of culture (days)	Culture cells/ml.	NIII		NIV		NV		NVI		CI		CII		CIII	
				No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h
<i>Skeletonema costatum</i> , 4-5 $\mu$	9. vi. 54	7	10,200	—	—	—	—	—	—	—	—	5	0.53	—	—	—	—
	29. iii. 55	6	9,440	29	0.08	—	—	3	0.26	14	0.30	2	0.40*	4	2.04	2	3.49
	4. iv. 55	12	1,290	61	0.07	—	—	—	—	—	—	—	—	—	—	—	—
	26. viii. 54	7	275†	8	0.24	—	—	2	0.72	3	0.72	5	0.92	—	—	—	—
<i>Chaetoceros decipiens</i> , 14-21 $\mu$ diam., 20-78 $\mu$ long	17. viii. 54	19	4,090	—	—	—	—	1	0.12*	—	—	—	—	—	—	—	—
	4. x. 54	8	68	49	0.28	15	0.21	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	2,260	—	—	—	—	—	—	1	0.54*	—	—	—	—	—	—
	19. iv. 55	4	2,260	—	—	—	—	—	—	—	—	2	0.51*	7	1.87	7	1.94
	21. iv. 55	6	1,670	33	0.05	6	0.22	5	0.32	1	0.48	1	0.77	—	—	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0.31*	1	0.47*	—	—	2	0.51*	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0.23	1	0.23	—	—	—	—	—	—
	27. vi. 55	4	315	17	0.09	3	0.66	2	0.78	5	1.04	2	1.44	9	3.19	6	5.67
<i>Ditylum brightwellii</i> , 20-60 $\mu$ diam.	6. vi. 55	18	57	38	0.05	7	0.14	7	0.15	3	0.35*	9	0.61	3	0.83*	—	—
	6. vi. 55	18	57	—	—	—	—	—	—	9	0.37	—	—	5	1.07	—	—
<i>Nannochloris oculata</i> , 2-4 $\mu$	26. viii. 54	17	32,000	15	0.03	5	0.07	5	0.04	6	0.05	3	0.07	7	0.27	—	—
	4. iv. 55	12	19,600	59	0.01	1	0.01	4	0.00	4	0.00	—	—	3	0.11	2	0.07
<i>Dicrateria inornata</i> , 3-5.5 $\mu$	23. viii. 54	4	130,000	51 <sup>f</sup>	0.04	7	0.05	—	—	—	—	18	0.14	—	—	—	—
	23. viii. 54	4	130,000	35 <sup>h</sup>	0.05	—	—	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	2	0.56*	3	1.83	4	1.28
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	3	0.44	—	—	—	—
	21. iv. 55	6	71,000	38	0.07	1	0.12	4	0.15	1	0.07*	—	—	—	—	—	—
	21. iv. 55	6	71,000	—	—	—	—	4	0.15	4	0.04	—	—	—	—	—	—
<i>Syracosphaera elongata</i> , 18-30 $\times$ 12 $\mu$	9. viii. 54	11	1,250	1	0.04*	1	0.04*	1	0.44*	1	0.67*	—	—	1	0.80*	—	—
	9. viii. 54	11	1,250	9 <sup>f</sup>	0.11	3 <sup>f</sup>	0.17	—	—	—	—	—	—	—	—	—	—
	9. viii. 54	11	1,250	9 <sup>h</sup>	0.25	2 <sup>h</sup>	0.25	—	—	—	—	—	—	—	—	—	—
	11. viii. 54	13	570	6	0.08	1	0.41*	3	0.59*	1	0.22*	2	1.08	—	—	—	—
	28. iv. 55	13	64	24	0.08	—	—	—	—	—	—	—	—	—	—	—	—
	9. v. 55	24	370	20	0.05	3	0.06	4	0.10	10	0.12	—	—	—	—	—	—
	27. vi. 55	4	320	26	0.17	3	0.75	11	1.01	11	1.36	7	2.12	13	2.95	14	4.45
<i>Prorocentrum triestinum</i> 10-14 $\mu$	8. vi. 55	22	1,515	21	0.01	5	0.05	10	0.07	6	0.10	9	0.18	8	0.30	—	—

\* Faecal pellets included. *h* = *helgolandicus*, *f* = *finmarchicus*. † contaminated with flagellates.



TABLE V. UTILIZATION OF *SYRACOSPHAERA ELONGATA*

(Culture 4 days old. 14.5° C. 27-28 June 1955)

Concentrations	Vol. of bottle ml.	Time (h)	<i>Calanus</i>			Total	ml. filtered in 24 h	
			Put in	As read	Counts/ min		Total	<i>Calanus</i>
5120 c/ml./min 320 cells/ml.	34.86	24½	21N III	8N III	6,613	14,288	2.89	0.17
				8N III	7,384			0.19
				IN IV	291			0.02
	35.28	24	20N III	10N III	7,330	10,854	2.20	0.15
				9N III*	3,524			0.08
	37.50	16	4N IV*	IN IV	3,953	6,233	1.84	1.17
				IN IV-V	195			0.06
				IN IV-V	447			0.13
				IN V	1,638			0.48
	37.50	24	4N IV	IN IV	2,283	5,501	1.09	0.45
				IN IV	3,147			0.62
				IN IV-V	71			0.01
	69.54	22½	10N V	IN V	4,068	27,524	5.96	0.88
				IN V	4,746			1.03
				IN V	4,142			0.90
				IN V	4,706			1.02
				IN V-VI	273			0.06
				IN V-VI	1,704			0.37
				IN V-VI	1,202			0.26
				IN V-VI	404			0.09
				IN VI	2,025			0.44
				IN VI	4,254			0.92
	70.68	19½	10N V	IN V	6,294	43,835	11.39	1.64
				IN V	3,044			0.79
				IN V	1,607			0.42
				IN V	5,286			1.37
				IN V	4,867			1.27
				IN V	6,587			1.71
				IN V	3,346			0.87
				IN V-VI	3,623			0.94
				IN V-VI	5,929			1.54
				IN V-VI	3,252			0.85
	70.26	21½	10N VI	IN VI	6,803	65,128	15.68	1.64
				IN VI	1,708			0.41
				IN VI	5,063			1.22
				IN VI	4,647			1.12
				IN VI	8,254			1.99
				IN VI-C I	7,558			1.82
				IN VI-C I	6,213			1.50
				IN VI-C I	10,570			2.55
	70.93	22½	10N VI	IC I	6,798	53,658	12.23	1.64
				IC I	7,514			1.81
				IN VI	6,894			1.57
				IN VI	6,207			1.41
				IN VI	6,938			1.58
				IN VI	4,703			1.07
				IN VI	6,263			1.43
				IN VI*	6,690			1.52
				IN VI-C I	5,529			1.26
				IN VI-C I	4,155			0.95
				IN VI-C I	4,020			0.92
				IN VI-C I	2,259			0.52

\* Sluggish.

TABLE V (continued)

Concentrations	Vol. of bottle ml.	Time (h)	<i>Calanus</i>			ml. filtered in 24 h	
			Put in	As read	Counts/min	Total	Total / <i>Calanus</i>
5120 c/ml./min 320 cells/ml.	72.79	21 $\frac{3}{4}$	4C I	1C I	8,107	39,577	9.02
				1C I	10,064		
				1C I	12,307		
				1C I	9,105		
	74.70	21 $\frac{3}{4}$	5C I	1C I	8,225	35,922	8.14
				1C I	10,079		
				1C I	7,300		
				1C I*	2,522		
				1C I-C II	7,796	93,579	28.12
	66.20	18 $\frac{1}{2}$	10C II	1C II	7,818		
				1C II	9,662		
				1C II	14,305		
				1C II	2,476		
				1C II	13,967		
				1C II	21,461		
				1C II	12,210		
				1C II-C III	11,680	74,031	17.30
	68.72	22 $\frac{1}{2}$	10C II	1C II	15,751		
				1C II	8,570		
				1C II	671		
				1C II	9,292		
				1C II	10,004		
				1C II	14,403		
				1C II-III	282		
				1C II-III	3,940	127,284	45.91
	75.73	15 $\frac{3}{4}$	12C III	1C III	11,118		
				1C III	4,304		
				1C III	13,802		
				1C III	11,032		
				1C III	21,817		
				1C III	11,322		
				1C III	17,568		
				1C III	15,765	92,284	22.68
				1C III	25,540		
				1C III*	3,866		
				1C III-IV	2,268		
	76.76	21 $\frac{3}{4}$	12C III	1C III	6,990		
				1C III	5,744		
				1C III	16,078		
				1C III	5,837		
				1C III	7,912	33,109	8.14
				1C III	5		
				1C III	33,109		
				1C III*	4,490		
				1C III*	5,549		
				1C III-IV	6,570		
Filtrate	32.38	23	5N III	4N III	0	—	—
35 counts/ml./min			1N V	1N V	0	—	—
			2N VI	2N VI	0	—	—
			2C II	1C II	2	—	—
			2C III	1C II	0	—	—
				1C III	3	—	—
				1C III	2	—	—

\* Sluggish.



As Table IV shows, the volumes filtered vary much even with the same species of food organisms. On the whole, as is natural, the volume filtered rises from one stage to the next. The very small flagellate *Nannochloris* is clearly used no better by the nauplii and early copepodite stages than it is by the adult. Thus in two experiments the utilization of *Nannochloris* was compared with that of *Skeletonema*. In the first a fairly long range of stages was compared in *Nannochloris* and in a contaminated *Skeletonema* culture. In every one the volume filtered in *Nannochloris* culture was much smaller. In the second a long range of stages was tested in *Nannochloris* and this was checked only by Nauplius III in *Skeletonema*. Both experiments showed that the low results in *Nannochloris* were not caused by unhealthy copepods.

To test whether really small particles could be filtered off, an experiment was made with radioactive bacterial spores (*Bacillus globigii*,  $0.7\mu$  in volume). But, as with adults, none was retained.

It is surprising to find that a large diatom such as *Ditylum* is apparently taken up by even the small nauplii, but possibly debris or broken cells, some of which are always found in old cultures, are being ingested.

The maximum filtration by Nauplius III found in any experiment was 1.0 ml. in 24 h; by Nauplius IV, 1.2 ml.; by Nauplius V, 1.7 ml.; by Nauplius VI, 2.0 ml.; by Copepodite I, 2.8 ml.; by Copepodite II, 6.4 ml.; and by Copepodite III, 9.2 ml. Most of these maxima were reached in one experiment comparing the utilization of *Chaetoceros decipiens* and *Syracosphaera elongata*. Details of the second may be seen in Table V. This experiment, done at the end of June, gave quite exceptionally high figures for all the stages tested. The results also show the great variation which may occur even among copepods of the same stage.

In this experiment, as in others, there were many moults. As a rule animals found in the process of moulting gave a low reading, whereas those which had successfully moulted might be as high as the rest, but this was not invariable. Although several individuals were used in each bottle, the total volume filtered was not a high proportion of its contents except with Copepodite III. In this case the animals may have been too crowded and might have filtered more had they been put singly in similar bottles.

Not all the *Calanus* put in were recovered. Some nauplii may die and disintegrate during an experiment, and occasionally one or two individuals are lost when the stopper of the bottle is put in.

Fig. 3 shows the average results obtained in all the experiments of Table IV for a number of different food species. There appears to be no abrupt change in the volume filtered with the change from nauplius to copepodite, indeed the change from the first to the second copepodite is usually more marked.

The results of three experiments in which faecal pellets were collected are shown in Table VI. That on *Skeletonema costatum* indicates that the percentage of  $^{32}\text{P}$  retained is considerably lower than with the other two species.

This comparatively low assimilation of *Skeletonema* was found also with adults. In the experiments on *Ditylum brightwellii* the Nauplius VI have retained a larger proportion than the Copepodite II. This may be partly because not all the faecal pellets were found; even in the unlikely event of a 50% loss, however, the digestion would still be higher than in Copepodite II.

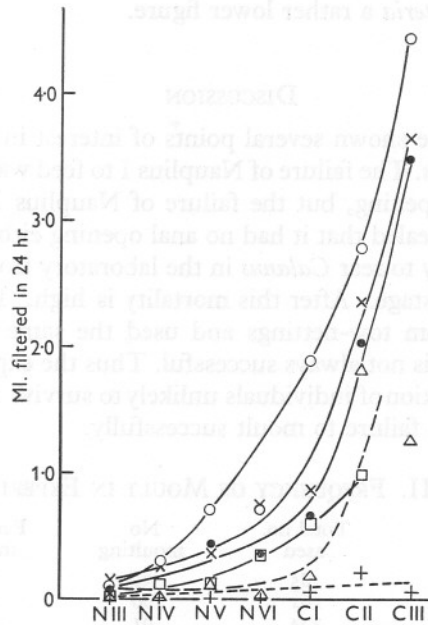


Fig. 3. Average volumes filtered in 24 h by stages Nauplius III to Copepodite III in various food species. ○, *Syracosphaera*; ×, *Chaetoceros*; ●, *Skeletonema*; □, *Ditylum*; △, *Dicrateria*; +, *Nannochloris*.

TABLE VI. UTILIZATION, INCLUDING FAECAL PELLETS,  
OF THREE FOOD ORGANISMS

Concentration	Age of culture (days)	Date	Time in h	Stage of <i>Calanus</i>	Faecal pellets		Body less filtrate	Total removed, c/min	Percentage used	ml. filtered in 24 h
					No.	Counts/min				
<i>Skeletonema</i> 91,125 c/ml./min 266,000 cells/ml.	7	9. vi. 54	18	N III	11	72	772	844	91.5	0.39
				C I	40	355	569	924	61.6	0.43
				C I	30	293	895	1,188	75.3	0.56
				C I	35	562	538	1,100	48.9	0.51
				C I	57	531	1,092	1,623	67.3	0.78
				C II	16	558	10,536	11,094	95.1	0.38
<i>Ditylum</i> 36,610 c/ml./min, 57 cells/ml.	18	6. vi. 55	20.5	N VI	18	612	8,529	9,141	93.3	0.31
				N VI	13	425	9,886	10,311	95.9	0.35
				C II	48	5339	24,270	29,609	82.0	1.10
				C II	43	4210	14,733	18,943	77.8	0.68
				C II	33	3396	16,095	19,491	82.6	0.72
				C II	1	3	228	231	98.7	0.04
<i>Syracosphaera</i> 9725 c/ml./min 1250 cells/ml.	11	9. viii. 54	16	N IV	7	8	2,351	2,359	99.7	0.38
				N V	9	9	2,644	2,653	99.7	0.44
				N VI	26	76	3,873	3,949	98.1	0.67
				C II	15	86	4,442	4,528	98.1	0.80
				C II						

In the experiment with *Syracosphaera elongata* the faecal pellets of the smaller nauplii were probably not all found. The Nauplius III filtered only a small volume and was probably not in good condition.

In general the proportion assimilated of the phosphorus-containing part of the different food species used varies as it does in adults. *Syracosphaera* and *Chaetoceros* show a high figure (over 90%), *Ditylum*, *Skeletonema* and the small flagellate *Dicrateria* a rather lower figure.

#### DISCUSSION

The experiments have shown several points of interest in the feeding of the early stages of *Calanus*. The failure of Nauplius I to feed was expected because its gut has no anal opening, but the failure of Nauplius II was unexpected until examination revealed that it had no anal opening either. This, however, explains why it is easy to rear *Calanus* in the laboratory from egg to Nauplius III, the first feeding stage. After this mortality is high. In the experimental *Calanus* taken from tow-nettings and used the same day, moulting, as is shown in Table V, is not always successful. Thus the experimental *Calanus* contain a high proportion of individuals unlikely to survive and this shows in a failure to feed or in a failure to moult successfully.

TABLE VII. FREQUENCY OF MOULT IN EXPERIMENTS

Stage	Total no. used	No. moulting	Percentage moulting
NIV	85	30	35.3
NV	161	66	41.0
NVI	234	91	38.9
CI	155	28	18.1
CII	92	21	22.8
CIII	84	12	14.3

Table VII shows the numbers of *Calanus* used in all the experiments made between April and August and the percentage which reached moult. Since the experiments lasted less than 24 h it may be deduced that *Calanus* remains as Nauplii IV, V and VI only 2-3 days each, as Copepodites I and II about 5 days each and as Copepodite III about 7 days. Since Nauplius III was used as a rule immediately after its moult from Nauplius II, nothing can be deduced about the duration of this stage. The times agree fairly well with those given by Nicholls (Marshall & Orr, 1955a, p. 78) but are much less than the times suggested for the North Sea by Rees (1949) and Cushing (1955).

One result of these experiments on the young stages of *Calanus* is to throw doubt on the assumption that the earlier the stage the smaller will be the food organism which it requires. Although from a study of the setulation it seems that even an adult *Calanus* should be able to retain particles of 2-3  $\mu$  by at least some part of its filtering apparatus, in practice it hardly does so at all. In the



nauplii the distance between setules is not much smaller than in the adult, the filtering screen looks less efficient, and in fact the nauplius seems to feed on much the same as the adult except for really large cells. The filtering apparatus has not a rigid mesh and it would be easy for particles which could be retained by the closest setules to slip through where they are wider, or to escape between the setae.

Cushing (1955) has seen a *Pseudocalanus* take a large *Biddulphia* cell, break it and filter off some of the contents. If *Calanus* nauplii can also break diatoms and remove the contents without ingesting the cell, they may be able to feed on even the largest species.

TABLE VIII. MAXIMUM UPTAKE OF PLANT CELLS BY ONE *CALANUS* IN 24 h IN SEA-WATER CONCENTRATIONS\*

Species	No. cells/ml.	Counts/cell	NIII		NIV		NV		NVI	
			Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
<i>Skeletonema costatum</i>	10,200	1.5	—	—	—	—	—	—	—	—
	9,440	0.31	2845	0.32	—	—	2539	0.28	4515	0.51
	1,290	5.7	77	0.09 <sub>18</sub>	—	—	—	—	—	—
<i>Chaetoceros decipiens</i>	68	87.0	{ 64	0.98	—	—	—	—	—	—
	1	—	{ 2	0.34 <sub>11</sub>	—	—	—	—	—	—
	2,260	22.4	—	—	—	—	—	—	—	—
	1,670	27.8	98	0.06 <sub>7</sub>	479	0.29	847	0.52	733	0.48
	1,480	29.0	—	—	—	—	448	0.31	652	0.47
	315	125.0	30	0.10 <sub>10</sub>	211	0.69	251	0.82	385	1.29
<i>Ditylum brightwellii</i>	57	642.0	3	0.05 <sub>10</sub>	12	0.20 <sub>2</sub>	15	0.27	31	0.56
<i>Syracosphaera elongata</i>	64	38.0	71	0.11 <sub>10</sub>	—	—	—	—	—	—
	320	16.0	57	0.19 <sub>8</sub>	371	1.17	513	1.71	576	1.99

Species	No. cells/ml.	Counts/cell	CI		CII		CIII	
			Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
<i>Skeletonema costatum</i>	10,200	1.5	6979	0.78	—	—	—	—
	9,440	0.31	5454	0.65	23,800	2.72	35,140	3.90
	1,290	5.7	—	—	—	—	—	—
<i>Chaetoceros decipiens</i>	68	87.0	{ —	—	—	—	—	—
	1	—	{ —	—	—	—	—	—
	2,260	22.4	1602	0.78	5,339	2.77	5,632	3.06
	1,670	27.8	991	0.60	—	—	—	—
	1,480	29.0	—	—	—	—	—	—
	315	125.0	481	1.56	1,272	4.39	2,353	8.24
<i>Ditylum brightwellii</i>	57	642.0	53	0.96	74	1.35	131	2.44
<i>Syracosphaera elongata</i>	320	16.0	849	2.81	1,764	6.45	2,283	8.14

\*When more than one copepod was used at one time (NIII and NIV) the number is shown as a suffix.

The data in Fig. 3 and Table IV (p. 595) show that the average volume filtered in 24 h by healthy individuals varies from about 0.1 ml. for Nauplius III up to between 2 and 4 ml. for Copepodite III. The rate increases rapidly with development but the variation is great. Compared with adults and Stage V copepodites the volumes filtered are relatively high.

The results can also be expressed as cell equivalents. Table VIII shows the maximum intake of cells in experiments where the culture concentration was such as might be found in the sea, although the numbers in the first two *Skeletonema* cultures would be found only during a rich diatom increase. A single Nauplius III took in *Skeletonema* cells at the rate of about two a

minute and in the same experiment Copepodite III took them up at the rate of twenty-five a minute. Remains of *Ditylum* cells were not recognized in any stage below Nauplius VI so the figures for the earlier nauplii probably mean little.

No data on respiration are available so that the volumes filtered cannot be related to food requirements nor these to the food available in the sea.

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#### SUMMARY

Nauplii and early copepodites of *Calanus* were fed on radioactive cultures of diatoms and phytoflagellates and the food uptake and volumes filtered were measured.

Nauplius Stages I and II do not feed; Nauplius III can filter up to a maximum of 1 ml. in 24 h. There is an increase of volume filtered with development up to a maximum of about 9 ml. in 24 h for Copepodite III.

Organisms of up to  $20\mu$  (*Skeletonema costatum*, *Prorocentrum triestinum*, *Syracosphaera elongata*) can be eaten by Nauplius III but, as with adults, the very small flagellate *Nannochloris oculata* ( $2-4\mu$ ) is not taken in to any extent by the young stages.

The filtering appendages are described. The minimum distance between the setules is about  $2\mu$  but for the main filtering surface it varies from 2 to  $11\mu$ , and shows little variation with stage.

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## *AMALOSOMA EDDYSTONENSE* SP.N., A NEW SPECIES OF BONELLIIDAE

By A. C. Stephen, D.Sc.

Royal Scottish Museum, Edinburgh

In October 1921, a specimen of an echiuroid was taken in the otter trawl of the S.S. *Salpa*, in the neighbourhood of the Eddystone lighthouse. The specimen, a female, was provisionally identified by Mr E. Ford as *Hamingia arctica* Kör & Dan. The pharynx and uteri were opened in an unsuccessful search for males, after which the specimen was preserved in alcohol and put in the museum at the Plymouth Laboratory. In the following month, two more specimens were taken in the dredge by Dr R. S. Clark, a few miles from the same locality, and placed in the museum without further examination. No more specimens were obtained until April 1925, when one was taken in a trawl off Rame Head, near the entrance to Plymouth Harbour. This specimen was examined by O. D. Hunt, and was found to be a female with uteri containing ripe eggs. Twenty-eight males of minute size were discovered attached externally to the female in a groove in the skin which extended for a short distance in the median line just posterior to what appeared to be a single genital orifice.

The males described by Lanchester from the pharynx of *Hamingia arctica*, and those from the uterus of *Acanthohamingia* described by Ikeda, closely resemble those of *Bonellia* in possessing a ciliated epidermis and in their nematoform shape. The males found by Hunt, however, were neither ciliated nor of such an elongated form. This difference showed at once that the species was not *Hamingia arctica* as supposed. Hunt therefore re-examined all the specimens in the Museum, and in each case males of the new type were found attached externally in a similar fashion and in a similar position.

Partially embedded in the thick epidermis of the female, these males are easily overlooked, as they had been on the specimen examined by Ford, on which Hunt subsequently found a single male. The two other specimens provided thirty-eight and thirty males respectively.

Four more specimens have been obtained since then from the same locality, two with attached males and two with none.

Since it was decided to include the records of this capture in the new edition of the *Plymouth Marine Fauna*, it finally became necessary to name it. Hunt, in manuscript 1925, recognized that the animals belonged to a new genus and species, but owing to pressure of work was unable to complete his observations. To him I am indebted for his generous gesture in placing his notes,

drawings and specimens at my disposal, and to Dr D. P. Wilson for the suggestion that I should have the privilege of examining and reporting on the material.

#### CLASSIFICATION OF THE BONELLIIDAE

The classification of the Bonelliidae has always presented difficulties, and several attempts have been made to clarify it. A revision undertaken by Fisher summarized the state of the accepted classification (1946, pp. 249-62) and he described three new genera. New material, and the fact that he had overlooked an important paper by Monro (1927), caused him to study the family again, and in the following year (Fisher, 1947), he published a fresh revision, adding three more new genera, including the genus *Amalosoma*, which interests us here. Most of his new genera contain but a single species, and it may be felt that more genera have been created than necessary, but at least he has put the classification on a reasonable basis. I have not had the opportunity of examining many animals belonging to this family and so have no personal comments to make on his classification, which I have adopted.

#### GENUS *AMALOSOMA* FISHER, 1947

Fisher (1946, pp. 260-2, pls. 31, 32) described a new species of *Acanthohamingia* Ikeda, from Japan, which he named *A. paradola*, and which differed in several important particulars from the two previously described species of this genus, *A. shiplei* and *A. ijimai*. In his second revision, he recognized that these differences were sufficient to warrant the creation of a new genus which he called *Amalosoma* (Fisher, 1947, p. 856), the generic criteria being the absence of minute setae in the genital groove of the female; the anal trees being numerous, slender, sparsely branched, arising for the most part independently from the very thin wall of the cloaca; there being two nephridia; and the male possessing a pair of curved setae.

A second species, so far known only as occurring in the Plymouth area, is now added to the genus.

#### *Amalosoma eddystonense* n.sp.

##### *Holotype—female*

The body is 144 mm in length, and no proboscis is preserved. Judging, however, by the appearance of the anterior portion there has been one, but probably broken off and lost during dredging. The body wall on the anterior and posterior thirds is solid, cylindrical and heavily papillated, and the diameters of these two portions are some 13 and 15 mm respectively. The middle third is swollen to the appearance of a thin-walled balloon, some 25 mm in diameter, through which the viscera can be seen.

The skin is covered, except in the thin portion, with numerous irregularly shaped flattened papillae, which are most numerous and largest anteriorly and posteriorly, less numerous in the middle portions of the body, and smallest ventrally.

The internal anatomy, so far as can be made out in its slightly macerated state, closely resembles that of *A. paradola*. The pharynx is similarly enlarged, and the arrangement of the neuro-intestinal blood vessels, so far as can be seen, is much the same. The gut is also long, as in the previous species. In *A. paradola*, Fisher described the nerve cord as being extremely thin, only 0.135 mm in diameter, but in this species it seems normal and the average diameter is 0.5 mm.

The two nephridiophores are packed with large ova, which have an average diameter of 0.45–0.5 mm. The ova are not enclosed in a follicle.

The anal trees, in all specimens, are much macerated, but arise as single stems from the cloaca, as described for *A. paradola*. They are, however, fewer, larger and more branched than in that species, to judge by Fisher's figure (1946, pl. 31, fig. 6). In the specimen dissected and drawn by Hunt, but not included in the material I have seen, they seem to have filled the posterior third of the body with a solid mass of tubules. Each branch ends in a conical-shaped wide-mouthed cup.

#### Male

The male is small, usually just under 2 mm in length, planiform or oblong. It is attached externally to the female in the nuptial groove by two hooked genital setae, which have comparatively large and well-developed muscular attachments. The body wall has two muscle layers, circular and longitudinal. The cuticle is not ciliated.

It agrees in most respects with Fisher's description of *A. paradola*.

The other females are very much macerated internally. Most are cylindrical in shape, but one resembles the type in having a thin expanded middle portion. In these the papillae are largest and most numerous anteriorly and posteriorly, less numerous in the middle region and least numerous ventrally. The colour of the spirit specimens is a uniform greyish yellow; in life these animals are a dull mid-green on the papillae with a similar but paler colour between them.

The species is referable to the genus *Amalosoma*, and there is not a great deal of difference between *A. paradola* and *A. eddystonense* but I hesitate to consider them as belonging to a single species. The chief differences are shown in Table 1.

*Distribution.* Eddystone grounds Oct. and Nov. 1921, Sept. 1926, May 1927. (Marine Biological Association, 1931, p. 148). Eddystone grounds and Rame Head, April 1925, July 1929 (specimen submitted by O. D. Hunt).



*Breeding.* Of the specimens examined only the type carried ova. This specimen, taken in April 1925, has the nephridia occupying about one-eighth of the body cavity; full of ova.

TABLE I

	<i>A. paradola</i>	<i>A. eddystonense</i>
Body	Pear-shaped	Sausage-shaped
Body wall	Very thin, viscera visible	Body-wall very thick: in only two specimens is there a thin-walled median enlargement
Nerve cord	Very thin, 0.135 mm	Thick, 0.5 mm diameter
Anal trees	Arising, one presumes, as numerous individual stems from the cloaca; branches bearing funnels few, short, not branching. Funnels vase-like, widest at the base	Arising from a few individual stems from the cloaca; branches bearing funnels numerous, long and sometimes themselves branching. Funnels ciliated, conical, widest at the mouth

## SUMMARY

The animals recorded as *Hamingia arctica* Kören & Danielssen in the *Plymouth Marine Fauna* (Marine Biological Association, 1931) were recognized some years ago by O. D. Hunt as belonging to a, then, new genus and species. His description was, unfortunately, never published, and his genus must be suppressed in favour of *Amalosoma* created by Fisher in 1947. His species remains valid but the name he originally suggested, *eddystonia*, from the locality in which it was first taken, has been changed to *eddystonense* in accordance with accepted practice.

*A. eddystonense* differs from the other described member of the genus, namely *A. paradola*, in having a cylindrical instead of a pear-shaped body; generally a thick cuticle; a thick instead of a thin nerve cord, and strong anal trees ending in conical cups.

The males, minute in size and deeply embedded in the thick epidermis of the genital groove of the female, are easily overlooked.

The species has been taken on several occasions near the Eddystone Light-house and off Rame Head, near Plymouth.

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## A PRELIMINARY INVESTIGATION OF THE VARIATION OF VITAMIN B<sub>12</sub> IN OCEANIC AND COASTAL WATERS

By C. B. Cowey

National Institute for Research in Dairying, University of Reading

(Text-figs. 1-4)

The presence in the sea of vitamin B<sub>12</sub>, or of its analogues, has been found necessary for the growth of several species of unicellular plants (e.g. Provasoli & Pintner, 1953; Droop, 1954, 1955).

Bioassays of the quantities in inshore waters during winter have been made by Lewin (1954) and by Droop (1954). The present investigation, carried out in 1955, was designed to extend this study and compare different seasons of the year.

### EXPERIMENTAL

In the work reported here the vitamin B<sub>12</sub> content of oceanic and coastal waters in areas investigated regularly by vessels of the Scottish Home Department laboratory at Aberdeen was examined over a period of several months. Surface sea water and plankton samples were collected from the Butt of Lewis (58° 40' N., 6° 10' W.), the Norwegian Deep (60-61° N., 3-4° E.), the Northern North Sea (58-60° N., 0-2° W.), the Faroe Channel (60° N., 7° W.) and the North Atlantic (58° N., 17° W.) during 1955. These areas contrast markedly in their content of planktonic indicator organisms (see for example, Fraser, 1952), and it seemed feasible that their biological differences might be causally related to differences in their content of micro-nutrients, including vitamin B<sub>12</sub>. Thus it was hoped that if spatial and temporal differences in the vitamin B<sub>12</sub> content of sea water occur, both would become evident during the course of the work.

In general three 4 l. samples were collected at each station and treated as follows: (a) filtered and preserved, (b) unfiltered and unpreserved (stored in darkness at 15° C), and (c) unfiltered and preserved. In addition, when circumstances permitted, samples were Seitz-filtered, thus removing any bacteria present, and preserved. The preservative used was that recommended by Hutner & Bjercknes (1948), consisting of a mixture by volume of 1 part *o*-fluorotoluene, 2 parts *n*-butyl chloride, and 1 part 1:2-dichloroethane. Plankton hauls were made at each station with vertically towed Hensen and Standard non-closing nets.

The Hensen hauls were made with a 70 cm diameter net of 60 meshes to the inch, from 100 m (or near the bottom in shallower water) to the surface. The Standard hauls were made with a 50 cm diameter net of 180 meshes per inch, towed from 50 m to the surface.

Preliminary experiments indicated that none of the organisms that can conveniently be used for assaying vitamin B<sub>12</sub> grew in normal sea water, probably because of the salinity and the low concentration of the vitamin. Hence it was necessary to reduce the concentration of salt in the sea water and to concentrate the vitamin.

After experimenting with several approaches the following standardized procedure was adopted:

The pH of a 4 l. sample of sea water was adjusted to 5 by the addition of 8 g of potassium dihydrogen phosphate, 0.2 ml. of a 1% solution of sodium cyanide were added and the mixture was maintained at 80–90° C for 20 min. After cooling the solution was saturated with liquid phenol (about 230 ml.) and the whole extracted with 10 successive 30 ml. portions of liquid phenol.

The phenolic extracts were combined and 150 ml. portions were shaken with 300 ml. diethyl ether. The aqueous layer that separated at this stage was collected and the phenolic phase was further extracted with ten 10 ml. portions of distilled water. The combined aqueous extracts so obtained were buffered with sodium- $\beta$ -glycerophosphate at the rate of 0.5 g/100 ml., and the pH was brought to 7.4 by the addition of a drop or two of concentrated hydrochloric acid. The extract was then concentrated under reduced pressure (at 45° C) to about 70 ml. Traces of phenol were removed from the solution by shaking it with three 20 ml. portions of diethyl ether. Ether was then removed from the aqueous solution by distillation under reduced pressure and the solution was made to the desired volume (40–60 ml.). This extract was then suitably diluted for assay.

Plankton, which was preserved in 60% acetone, was extracted by steaming off the acetone, adding a few drops of a 1% solution of sodium cyanide, reducing the pH to 5.0 with 0.1N-HCl and maintaining at 80° C for 20 min. The extracts were made to a desired volume, filtered through a Whatman no. 42 filter-paper and then diluted appropriately for assay.

Extracts were assayed with *Ochromonas malhamensis* (Ford, 1953) and with *Lactobacillus leichmannii*, ATCC 4797, by the method of Skeggs, Nepple, Valentik, Huff & Wright (1950) modified as described by Ford (1953).

It has been known for some time that, besides vitamin B<sub>12</sub> (cyanocobalamin) itself, there exist in natural materials several analogues of vitamin B<sub>12</sub> (see, for example, Kon, 1955). These are active to different extents for various organisms (Coates, Ford, Harrison, Kon & Porter, 1953; Droop, 1955) and organisms requiring 'vitamin B<sub>12</sub>' differ in the extent to which they can utilize different analogues. Thus higher animals are generally narrowly specific in their



utilization of vitamin B<sub>12</sub> analogues but certain micro-organisms (e.g. *Escherichia coli*) are non-specific. Table I (compiled by Dr J. E. Ford) indicates the relative potencies of vitamin B<sub>12</sub> analogues of natural occurrence for *Lactobacillus leichmannii* and *Ochromonas malhamensis*. Clearly, if the vitamin B<sub>12</sub> activity of a given natural material is consistently higher for

TABLE I. RELATIVE ACTIVITY OF VITAMIN B<sub>12</sub> ANALOGUES AND DEOXYRIBOSIDES FOR ASSAY ORGANISMS

Factor	<i>Ochromonas malhamensis</i>	<i>Lactobacillus leichmannii</i>
Vitamin B <sub>12</sub>	100	100
Vitamin B <sub>12</sub> III	40	50
Factor B	Inactive	Inactive
Factor A	Inactive	20
Pseudo-vitamin B <sub>12</sub>	Inactive	50
Factor C	Inactive	Inactive
Deoxyribosides	Inactive	Active

TABLE II. RECOVERY FROM SEA WATER, BY 'PHENOL PASSAGE' TECHNIQUE, OF VITAMIN B<sub>12</sub> ADDED TO IT

Sample	Vitamin B <sub>12</sub> present. Portion extracted directly and assayed (μg/l.)	Vitamin B <sub>12</sub> added to second portion (μg./l.)	Vitamin B <sub>12</sub> found in second portion (μg./l.)	Percentage recovery
3½ % saline	—	0.02	0.017	85
Unfiltered unpreserved sea water from 58° 40' N., 6° 10' W. (23 April)	0.003	0.004	0.006	75
Filtered preserved sea water from 60° N., 4° E. (1 June)	0.0003	0.004	0.0036	80
Unfiltered unpreserved sea water from 59° N., 1° E. (3 August)	0.0015	0.0006	0.002	83
Unfiltered unpreserved sea water from 61° N., 3° E. (10 August)	0.0035	0.001	0.0044	90
Unfiltered unpreserved sea water from 58° 40' N., 6° 10' W. (30 August)	0.0066	0.003	0.0094	90
Unfiltered unpreserved sea water from 59° N., 1° W. (3 October)	0.0033	0.004	0.0071	95

*Lactobacillus leichmannii* than for *Ochromonas malhamensis* and provided the higher *leichmannii* response is shown, by appropriate controls, not to be due to the presence of deoxyribosides in the material, it is reasonable to infer that vitamin B<sub>12</sub> analogues active for *Lactobacillus leichmannii* but inactive for *Ochromonas malhamensis* are present in the material. Thus the presence of vitamin B<sub>12</sub> analogues in natural materials can be detected by differential assays of this sort. Recovery tests (Table II) were performed by dividing a 4 l. sea-water sample into two 2 l. portions. One of these was extracted directly;

vitamin B<sub>12</sub> in about the concentration expected in the sample was added to the other which was then extracted. The two extracts were then assayed and the percentage of recovered vitamin B<sub>12</sub> determined.

### RESULTS

The results obtained are shown in Table III. Values for filtered preserved samples are taken as indicating the concentration of vitamin B<sub>12</sub> in the sea water; those for unfiltered unpreserved samples as an indication of the concentration which vitamin B<sub>12</sub> might reach as a result of bacterial multiplication and action on detritus and organic matter contained in the water; those for the unfiltered preserved samples indicate the total amount of vitamin B<sub>12</sub> present in the water (i.e. that present in solution and in suspended matter), they form controls to the corresponding unfiltered, unpreserved samples.

The vitamin B<sub>12</sub> content of filtered preserved samples throughout the period under investigation varied between 0.006 µg/l. in coastal water (Wee Bankie, 56° 11' N., 2° 08' W., in January) and 0.0001 µg/l. in North Atlantic water (57° 55' N., 17° W., in July); since the Seitz-filtered, bacteria-free, samples so far examined contained vitamin B<sub>12</sub> in the same concentration as paper-filtered preserved samples collected at the same time, it is not unreasonable to assume that all the 'filtered preserved' values represent vitamin B<sub>12</sub> present in solution in the sea. Droop (1954) gives a value of 0.005–0.01 µg vitamin B<sub>12</sub>/l. for West Scottish coastal waters in February and March 1954, and Lewin (1954) found 0.01 µg vitamin B<sub>12</sub>/l. in sea water of the North-west Arm (Halifax, Nova Scotia) during the winter of 1952–53; their values agree adequately with that of 0.006 µg/l. for Wee Bankie in January and 0.016 µg/l. for Loch Fyne in March. Droop goes on to state that a cobalamin concentration of this order would not be a limiting factor in the growth of diatoms and other vitamin B<sub>12</sub>-requiring protista. The cobalamin concentration of 0.0001 µg/l. recorded in North Atlantic water in July might, however, prove limiting, though vitamin B<sub>12</sub> is probably only one of several factors (e.g. nitrate and phosphate concentrations) likely to be limiting at this time of the year.

Droop (1955, private communication) has also drawn attention to the fact that certain chrysomonads, and the only two diatoms known to require vitamin B<sub>12</sub> (*Skeletonema costatum* and *Amphora perpusilla*) respond to analogues of vitamin B<sub>12</sub> as well as to cyanocobalamin itself, and states 'it is likely that the analogues of vitamin B<sub>12</sub> will have an importance equal to that of the vitamin in the ecology of the sea'. Differential assays of our sea-water extracts have shown the presence of vitamin B<sub>12</sub> analogues in sea water. Water collected at Aberdeen Bay in January contained 0.006 µg vitamin B<sub>12</sub>/l. when assayed with *Lactobacillus leichmannii*, and 0.004 µg/l. when assayed with *Ochromonas malhamensis*. Since vitamin B<sub>12</sub> analogues are less

TABLE III. VITAMIN B<sub>12</sub> CONTENT OF SEA WATER AND PLANKTON

NORTHERN NORTH SEA								
Sample	Locality and date							
	59° N., 0° 40' E., 29 May	60° 43' N., 0° 30' E., 3 June	59° N., 1° E., 3 Aug.	60° N., 00° 00', 9 Aug.	58° 45' N., 00° 00', 7 Sept.	57° 15' N., 1° 30' W., 12 Sept.	59° 00' N., 1° 00' W., 3 Oct.	57° 00' N., 1° 00' W., 10 Oct.
Sea water								
Filtered preserved (μg/l.)	0.0002	0.0002	0.00013	0.0002	0.0007	0.0006	0.0009	0.0012
Unfiltered preserved (μg/l.)	0.00035	0.0002	0.00015	0.00018	0.001	0.005	—	—
Unfiltered unpreserved (μg/l.)	0.0005	0.0006	0.0015	0.004	0.0075	0.008	0.0083	0.0042
Plankton: Hensen net haul								
Total (μg)	0.6	1.2	0.2	0.07	—	—	—	—
Wet wt. (μg/g)	0.06	0.07	0.03	0.01	—	—	—	—
Plankton: Standard net haul								
Total (μg)	0.07	0.4	0.01	0.02	—	—	—	—
Wet wt. (μg/g)	0.03	0.06	0.01	0.003	—	—	—	—
BUTT OF LEWIS								
Sample	Locality and date				NORWEGIAN DEEPS			
	58° 40' N., 6° 10' W., Feb.	58° 46' N., 6° 30' W., 4 Apr.	58° 40' N., 6° 10' W., 23 Apr.	58° 40' N., 6° 10' W., 30 Aug.	61° 01' N., 3° 30' E., 4 Apr.	60° 00' N., 4° 00' E., 1 June	61° 01' N., 3° 30' E., 10 Aug.	
Sea water								
Filtered preserved (μg/l.)	0.002	0.001	0.0004	0.0006 0.00055 (Seitz-filtered)	0.002	0.0003	0.0005	
Unfiltered preserved (μg/l.)	—	—	0.0008	0.0006	0.003	0.0002	0.0005	
Unfiltered unpreserved (μg/l.)	0.004	0.006	0.003	0.0066	0.006	0.0005	0.004	
Plankton: Hensen net haul								
Total (μg)	0.02	0.06	0.1	0.27	—	0.1	0.13	
Wet wt. (μg/g)	0.05	0.05	0.07	0.04	—	0.03	0.08	
Plankton: Standard net haul								
Total (μg)	0.005	0.02	0.5	0.05	—	0.006	0.03	
Wet wt. (μg/g)	0.02	0.02	0.06	0.06	—	0.06	0.003	
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0.0004	—	—	—	
WEE BANKIE, LOCH FYNE, FAROE CHANNEL AND NORTH ATLANTIC								
Sample	Locality and date							
	56° 11' N., 2° 08' W., Wee Bankie Jan.	Loch Fyne Mar.	60° 23' N., 7° 49' W., (F.C.), 23 Apr.	59° 56' N., 7° 27' W., (F.C.), 31 Aug.	59° 54' N., 14° 15' W., (N. Atl.), 8 July	57° 55' N., 17° 00' W., (N. Atl.), 19 July		
Sea water								
Filtered preserved (μg/l.)	0.006	0.16	0.0003	0.00073 0.0007 (Seitz-filtered)	0.00025	0.0001		
Unfiltered preserved (μg/l.)	—	—	0.0002	0.0009	0.0003	0.0002		
Unfiltered unpreserved (μg/l.)	—	—	0.008	0.005	0.005	0.003		
Plankton: Hensen net haul								
Total (μg)	0.02	—	—	0.3	0.2	0.02		
Wet wt. (μg/g)	—	—	—	0.04	0.03	0.005		
Plankton: Standard net haul								
Total (μg)	0.01	—	—	0.08	0.01	0.002		
Wet wt. (μg/g)	—	—	—	0.02	0.008	0.003		
Filter-paper: used in filtering 4 l. sea water (μg)	—	—	—	0.0007	—	—		
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0.0008	—	—		

Notes. The vitamin B<sub>12</sub> found in the filter-paper (0.0007 μg) after filtering 4 l. of sea water through it, presumably represents the vitamin B<sub>12</sub> present in suspended matter in the water. There would then be 0.0007/4, i.e. 0.00017, μg vitamin B<sub>12</sub> present in suspended matter in each litre of sea water. If this value is added to that obtained for the filtered preserved water (0.00073 μg/l.) the sum tallies well with the value obtained for the unfiltered preserved sample, from which suspended matter had not been removed. A similar agreement was obtained with the Seitz-filtered and unfiltered preserved samples.



active for *Lactobacillus leichmannii* than vitamin B<sub>12</sub> itself (e.g. factor A has only 20% of the activity of vitamin B<sub>12</sub>) they are clearly present in this instance in a concentration greater than 0.002 µg/l. In the oceanic waters I have so far examined the concentration of analogues present when compared with that of vitamin B<sub>12</sub> was very low, and a situation has not yet been encountered where the relative concentrations of vitamin B<sub>12</sub> analogues and vitamin B<sub>12</sub> were such as to limit the growth of organisms able to utilize vitamin B<sub>12</sub> only, while permitting growth of organisms responding to vitamin B<sub>12</sub> analogues and to vitamin B<sub>12</sub>. In Table III only one value for vitamin B<sub>12</sub> concentration is quoted for each sample from each station, since values obtained from *L. leichmannii* and *Ochromonas malhamensis* assays were not significantly different. When sea-water extracts used for routine assays were further concentrated and chromatographed cyanocobalamin alone could be detected. However, chromatograms of an extract of a 90 l. bulk of sea water showed that, besides vitamin B<sub>12</sub>, factor B was also present.

TABLE IV. VITAMIN B<sub>12</sub> ANALOGUES DETECTED IN MARINE ANIMALS

	Factor B	Factor A	Pseudo-vitamin B <sub>12</sub>	Vitamin B <sub>12</sub>
Haddock, gut contents	+	+	+	+
Cod, gut contents	+	o	+	+
<i>Crangon allmani</i> gut and contents	+	o	+	+

Ericson & Lewis (1953) have demonstrated that marine bacteria, isolated from sea water and from marine algae, are able to produce vitamin B<sub>12</sub> factors. Similarly, the presence of such factors has now been shown chromatographically in the contents of the alimentary tract of various marine animals (Table IV), where they doubtless arise as a result of bacterial metabolism. Excretion by marine animals must contribute to some extent to the vitamin B<sub>12</sub> analogues to be found in the sea.

The most obvious feature of the results to date is a seasonal variation in the vitamin B<sub>12</sub> content of the waters examined; it is amply demonstrated in Fig. 1 for the three areas sampled. Although lack of time has made it impossible to obtain results for all three areas over a 12-month period, the similarity in seasonal trends in these areas is already apparent. Furthermore, the concentration of vitamin B<sub>12</sub> in the few samples of North Atlantic and Faroe Channel water that have been examined was similar to the concentration in water from the other three areas at that time. From a winter maximum of about 0.002 µg vitamin B<sub>12</sub>/l. the concentration fell to a level of about 0.0002 µg/l. by July and then gradually increased again to the winter level. Though there were slight temporal differences between the sequences in the three areas, the vitamin B<sub>12</sub> content of the waters was broadly similar at any given time and none of these waters was characterized by a consistently

higher vitamin B<sub>12</sub> content than others. Thus in the biologically distinct areas so far examined a significant difference in the concentration of vitamin B<sub>12</sub> has not been established.

The vitamin B<sub>12</sub> content of the water decreased in March–April, the period during which plankton organisms were multiplying most rapidly. Figs. 2 and 3 show for the Butt of Lewis and Northern North Sea areas the total vitamin B<sub>12</sub> content of Standard net and Hensen net plankton hauls taken at the same time as water samples. Though the volume of plankton taken must depend to some extent on weather conditions at the time, it can be seen that

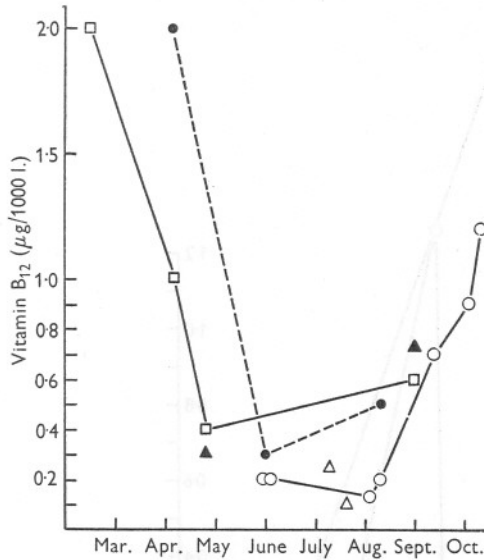


Fig. 1. Vitamin B<sub>12</sub> content of filtered, preserved sea-water samples collected during 1955. □, Butt of Lewis; ●, Norwegian Deeps; ○, Northern North Sea; ▲, Faroe Channel; △, North Atlantic.

the quantity of vitamin B<sub>12</sub> present in plankton was greatest when the vitamin B<sub>12</sub> content of the water was lowest. Whether or not this was the result of a direct uptake of vitamin B<sub>12</sub> from the sea water by diatoms is uncertain, since very little is known about the numbers of phytoplankton species that require vitamin B<sub>12</sub> and the species, if any, that synthesize vitamin B<sub>12</sub>, but the finding at least suggests the possibility. No estimate is as yet available of the relative contributions of the various factors affecting the regeneration of vitamin B<sub>12</sub> in the sea during the late summer and autumn, though supposedly excretion by marine animals, bacterial action on detritus, and possibly mixing of surface waters with lower layers are all important in this respect.

In untreated sea water (unfiltered unpreserved samples) the number of bacterial species present falls during the first 3–6 h of storage, and there then follows an increase of many hundred per cent of the surviving species

(ZoBell & Anderson, 1936). It can be seen from Table III that, although the unfiltered unpreserved samples contained more vitamin B<sub>12</sub> than the unfiltered preserved controls, the difference was never very great. Observations were not made of types and numbers of bacteria that multiplied in the unfiltered unpreserved samples and it is unlikely that similar types multiplied in each sample, but at least some of those that multiplied had a definite, if comparatively restricted, ability to produce vitamin B<sub>12</sub> under conditions which

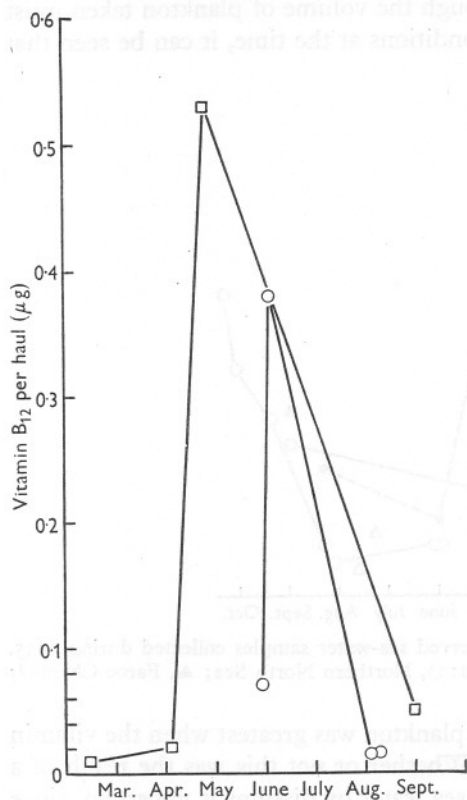


Fig. 2

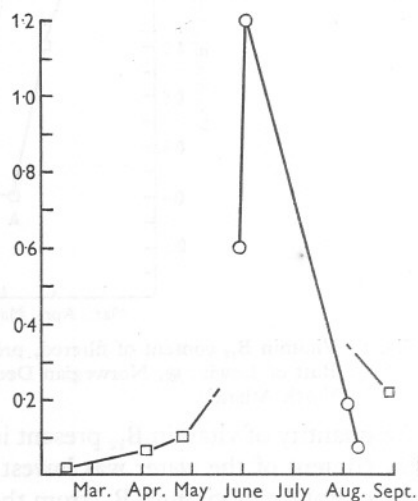


Fig. 3

Fig. 2. Vitamin B<sub>12</sub> content of plankton taken in Standard net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

Fig. 3. Vitamin B<sub>12</sub> content of plankton taken in Hensen net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

simulated the natural ones. The vitamin B<sub>12</sub> content of the unfiltered unpreserved samples showed similar trends to those of the filtered preserved samples (Fig. 4), i.e. generally more vitamin B<sub>12</sub> was produced in samples collected during autumn and winter than in samples collected during the summer.



The physical conditions (such as relationships between solid surface area and volume) likely to control bacterial multiplication in untreated sea water stored in bottles were maintained constant from sample to sample. If one can assume that the vitamin B<sub>12</sub> content of these samples was in some measure a reflexion of bacterial activity, it seems that under these conditions sea water collected during autumn and winter was more conducive to production of vitamin B<sub>12</sub> by bacteria than water collected in summer.

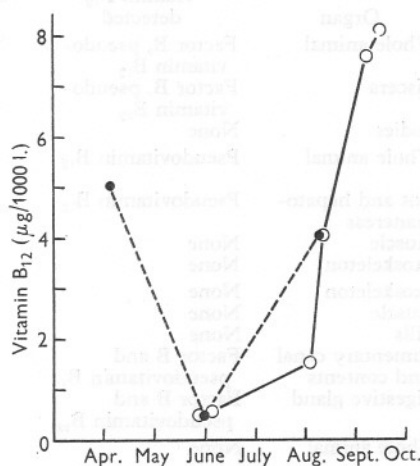


Fig. 4. Vitamin B<sub>12</sub> content of unfiltered, unpreserved, sea-water samples collected during 1955. ○, Northern North Sea; ●, Norwegian Deeps.

The large zooplankton collections contained mainly *Calanus finmarchicus*, and their vitamin B<sub>12</sub> content was 0.06–0.07 µg/g wet weight. Collections of *C. finmarchicus* alone contained 0.09 µg vitamin B<sub>12</sub>/g wet weight (Table V). Another mixture of zooplankton, including the siphonophore *Lensia*, and the copepods *Rhincalanus*, *Pleuromamma*, and *Calanus finmarchicus*, gave 0.05 µg vitamin B<sub>12</sub>/g wet weight. They could thus constitute an adequate source of vitamin B<sub>12</sub> for higher marine forms. By comparison, the richest animal sources of vitamin B<sub>12</sub> (e.g. cow liver) contain up to 1.3 µg vitamin B<sub>12</sub>/g fresh tissue (Ford, Holdsworth & Porter, 1953).

There is not yet sufficient information to show whether species of the zooplankton from different sea-water masses differ in their vitamin B<sub>12</sub> content. Indications are that they do not: thus *Meganyctiphanes norvegica* and *Euchaeta norvegica* both contained 0.05 µg vitamin B<sub>12</sub>/g wet weight no matter where they were collected.

Phytoplankton contained much less vitamin B<sub>12</sub> per unit wet weight than zooplankton. Thus samples of live phytoplankton in hauls were found to contain 0.02–0.03 µg vitamin B<sub>12</sub>/g wet weight. The comparative richness of

zooplankton may indicate a rapid rate of grazing; on the other hand, zooplankton might derive its vitamin B<sub>12</sub> largely from a bacterial flora in the gut.

TABLE V. VITAMIN B<sub>12</sub> IN MARINE INVERTEBRATES

Species	Organ	Analogues of vitamin B <sub>12</sub> detected	Activity expressed in terms of vitamin B <sub>12</sub> . Wet weight (μg/g)	
			<i>Escherichia coli</i>	<i>Ochromonas malhamensis</i>
<i>Crangon allmani</i> Kinahan	Whole animal	Factor B, pseudo-vitamin B <sub>12</sub>	0.14	0.12
	Viscera	Factor B, pseudo-vitamin B <sub>12</sub>	0.5	0.4
<i>Pandalus bonnierii</i> Caullery	Bodies	None	0.09	0.08
	Whole animal	Pseudovitamin B <sub>12</sub>	0.14	0.11
	Gut and hepato-pancreas	Pseudovitamin B <sub>12</sub>	0.52	0.45
	Muscle	None	0.07	0.06
<i>Nephrops norvegicus</i> L.	Exoskeleton	None	0.13	0.09
	Exoskeleton	None	0.03	0.02
	Muscle	None	0.03	0.03
	Gills	None	0.03	0.02
	Alimentary canal and contents	Factor B and pseudovitamin B <sub>12</sub>	0.08	0.05
	Digestive gland	Factor B and pseudovitamin B <sub>12</sub>	0.12	0.11
<i>Meganyctiphanes norvegica</i> (M. Sars)	Whole animal	None	0.06	0.05
<i>Euchaeta norvegica</i> Boeck	Whole animal	None	0.05	0.06
<i>Thysanoessa raschii</i> (M. Sars)	Whole animal	None	0.08	0.07
<i>Calanus finmarchicus</i> (Gunnerus)	Whole animal	None	0.09	0.09
<i>Aurelia</i> spp. and <i>Ctenophores</i>	Whole animal	None	0.02	0.02

## REMARKS

This work is exploratory and hence, of necessity, is only capable of giving pointers in new directions. No decisive conclusions are possible. Because of the time involved in the extraction procedure, results had to be based on single 4 l. samples from each station, i.e. there is no statistical basis for the values quoted. This drawback can best be overcome, and work of this nature generally facilitated, by acquiring marine micro-organisms that could be used for rapid direct assay of micronutrients in sea water. Furthermore, values obtained by direct assay of sea water are perhaps intrinsically more reliable than those obtained otherwise.

Besides the need for information on the concentration in sea water of vitamins, amino acids, and growth factors generally, the nutrition and synthetic powers of marine bacteria require examination, more especially of

any forms associated with diatoms *in vivo* or inhabiting the alimentary tracts of marine animals. Only then can we construct a reasonable picture of the interplay between different factors, upon which productivity in the sea is ultimately dependent.

I am grateful to the Director of the Scottish Home Department Laboratory at Aberdeen for providing facilities for the collection of sea water and plankton samples, and to Drs R. Johnston and J. H. Fraser of that Laboratory for constructive discussion in the initiation and indeed throughout the course of this work. I am especially indebted to Dr Johnston for supervising the collection of samples.

I have derived much benefit from the interest, advice and critical approach during discussions of Drs L. R. Fisher, J. E. Ford, E. S. Holdsworth and S. K. Kon.

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#### SUMMARY

The vitamin B<sub>12</sub> concentration in biologically distinct sea waters has been measured over a period of several months during 1955, a solvent-extraction technique being used to concentrate the vitamin and reduce the salt concentration in the water.

Similar seasonal changes in vitamin B<sub>12</sub> content were found in water from the Butt of Lewis, Northern North Sea, and Norwegian Deep, with a high winter level (about 0.002 µg/l.) and low summer level (about 0.0002 µg/l.). There was no evidence of a spatial variation in vitamin B<sub>12</sub> concentration of the waters examined.

The vitamin B<sub>12</sub> contents of plankton from hauls taken throughout the year (1955) at stations where water samples were collected, and of collections of individual zooplankton species were also determined and are reported on.

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## THE BACTERIAL OXIDATION OF AMMONIA IN THE SEA

By C. P. Spencer

Marine Biology Station, University College of North Wales,  
and The Plymouth Laboratory

(Text-figs. 1-3)

The final regeneration stages of the nitrogen cycle in the sea are believed to consist of the bacterial oxidation of ammonium-nitrogen to nitrite and finally nitrate-nitrogen. There have been many attempts to demonstrate the presence in the sea of marine counterparts to the terrestrial *Nitrosomonas* and *Nitrobacter*. (See Zobell, 1946, for bibliography of the earlier work.) Bacteria which can oxidize either ammonia or nitrite are easily demonstrated in samples of bottom material or water contaminated by land drainage or bottom deposits. On the other hand, there has been general failure to demonstrate the presence of such species in the upper layers of the open ocean.

Nevertheless, oceanographical evidence suggests that considerable nitrification occurs in these upper layers. There is no evidence that, under natural conditions, purely chemical catalysis occurs to any appreciable extent. The photo-oxidation of ammonia in sea water by ultra-violet light has been demonstrated (Zobell, 1933), but it is unlikely that this can be important except to a depth of about 1 m. The failure to demonstrate the presence of ammonia-oxidizing bacteria in the middle layers of open ocean water therefore leaves a serious gap in our knowledge of the nitrogen cycle in the sea. Zobell (1946) has suggested that some marine nitrifiers may exist which are of a very different physiological character from the strictly autotrophic terrestrial species, and that failure to demonstrate their presence might perhaps therefore be due to the use of unsuitable culture methods.

The present paper reports the results obtained during an investigation of the nitrifying behaviour of the surface water from the International Hydrographical Station E1. These studies were commenced whilst working at the Plymouth Laboratory and were continued and extended at the Marine Biology Station of the University College of North Wales, Menai Bridge.

### METHODS

All cultures were grown in 250 ml. Pyrex conical flasks capped with small beakers. The medium used was essentially that employed by Carey (1938). For initial enrichment cultures 10 g of acid-washed fine sand and 1 g calcium

carbonate were dispensed in each flask and sterilized by autoclaving at 10 lb. for 20 min; 40 ml. samples of sea water were added followed by aseptic additions of phosphate ( $0.1 \times 10^{-3}$  M) and ammonium chloride ( $2.0 \times 10^{-3}$  M) from sterile stock solutions of the required strength. For making sterile media for subsequent cultures, 40 ml. of aged sea water were added to each flask, together with the sand and calcium carbonate, and the whole sterilized by autoclaving at 5 lb. for 30 min. All cultures were incubated at 22° C in the dark.

Experience showed that little nitrification occurred without a solid phase in the medium. Further tests demonstrated that sand alone was not very effective as a solid phase. Carbonate was much more effective, and in the presence of calcium carbonate an addition of sand had little further effect. In the later experiments the sand was therefore omitted.

The progress of ammonia oxidation was followed by withdrawing samples at intervals and estimating for nitrite using the method of Rider & Mellon (1946). The cultures were agitated before samples were withdrawn to suspend the carbonate evenly. After development of the colour, the intensity was read by means of a Harvey absorptiometer using Ilford Filters no. 604. Nitrite was determined by means of a calibration curve prepared by assaying solutions of sodium nitrite of known strengths containing suspended calcium carbonate in the same proportions as in the cultures. The carbonate dissolves in the acid medium used for the diazotization, but this and the final coupling reaction are pH sensitive, and the calibration curve was therefore prepared using samples analogous to those obtained from cultures.

#### EXPERIMENTAL

In the preliminary experiments the nitrifying behaviour of surface water from the International Hydrographical Station E1 was investigated by setting up enrichment cultures. Surface water was collected after the development of the thermocline and dispensed in 40 ml. amounts in sterile flasks containing sand and calcium carbonate with additions of phosphate and ammonium chloride. Periodic sampling showed that no formation of nitrite could be detected in this series of cultures in up to 80 days' incubation, and if any oxidation of ammonia was occurring it must have been extremely slow. No tests were made for the formation of nitrate since most previous investigators agreed that nitrite formation became maximal before appreciable oxidation to nitrate occurred (von Brant, Rakestraw & Renn, 1937).

These preliminary results largely confirmed the much earlier findings of Harvey (1926) with surface-water samples from the same station. They further suggested that it was not solely the lack of a solid phase in the culture medium that was inhibiting ammonia oxidation as suggested by Cooper (1948).



In a further attempt to set up enrichment cultures a series of water samples were incubated as before except that an additional addendum of 1 ml. of a uni-algal (but not bacteria free) stock culture of a diatom was made to each flask. After an initial induction period of 35 days nitrite formation commenced in all cultures of this series and continued vigorously until a maximum value was reached after some 60 days. The observation that the presence of plankton material allowed nitrification to proceed was reminiscent of the results of Carey (1938).

Another series of enrichment cultures was set up to identify the origin of the ammonia-oxidizing bacteria. These cultures showed that no nitrite formation occurred if the sea-water samples were sterilized by autoclaving regardless of whether the diatom addendum was sterile or not. Further, with freshly collected unsterilized water samples, the progress of nitrite formation showed little difference whether the diatom suspension together with associated bacteria was added in a fully viable condition or after having been sterilized by autoclaving. It therefore seemed certain that ammonia-oxidizing bacteria were present in the water samples and not in the crude diatom culture. Further confirmation of this was obtained by the use of a bacteria-free culture of a marine diatom. The addition of this also allowed nitrite formation to occur in non-sterile samples of the sea water, whereas nitrite formation could not be detected after 80 days' incubation in any control culture set up with sterilized sea water or without additions of the diatom suspension.

Attempts were next made to subculture the nitrifying organism. 1 ml. samples of the initial enrichment cultures described above were transferred to sterile medium without the addition of the diatom suspension. The formation of nitrite proceeded in all the subcultures without the marked induction period observed in the original enrichment cultures but at a much reduced rate. In subsequent subcultures the rate of nitrite formation was further successively reduced. Progress curves for the production of nitrite in initial enrichment cultures and in a subsequent subculture are shown in Fig. 1. The concentration of nitrite in the enrichment cultures varied exponentially with time. This is typical of an autocatalytic reaction and is the effect that might be expected with a proliferating catalytic organism. On the other hand, the concentration of nitrite in the subsequent subcultures was directly proportional to the time of incubation.

An investigation was made of the effect of variations in the size of inocula on the kinetics of nitrite production in subcultures without the addition of diatoms. Within limits, the rate of nitrite production varied directly with the size of inocula and remained linear. With the largest inocula, however, the production of nitrite, though initially linear, tended to give way to an exponential rate. The kinetics of nitrite formation in subcultures without added diatoms and the results of variations in the inocula size in such subcultures

suggested the presence of a non-proliferating but enzymically active bacterial population.

The addition of a sterilized diatom suspension to a subculture inhibited the production of nitrite for periods of up to 30 days. Thereafter rapid exponential formation of nitrite occurred. A hot-water extract of diatoms from which the insoluble debris had been removed by centrifuging was considerably less inhibitory, the induction period in this case lasting only 2 or 3 days. Such extracts seemed equally effective as whole cells in ultimately promoting exponential nitrite formation in subcultures. In control experiments only

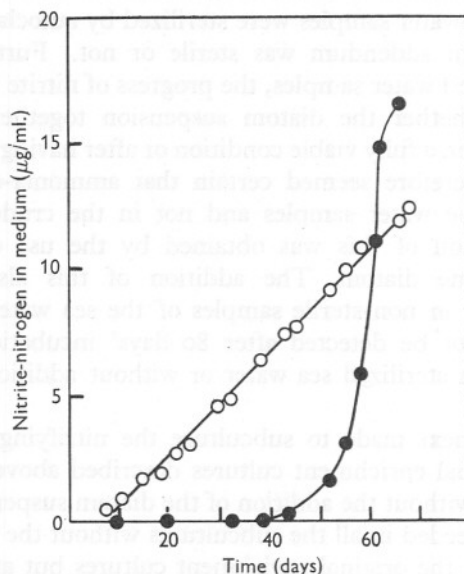


Fig. 1. Progress curve for nitrite production in medium made up with E1 water. —●—●—, Initial enrichment culture with addition of diatoms; —○—○—, subculture without addition of diatoms.

negligible production of nitrite occurred in cultures containing sterile diatom suspensions or hot-water diatom extracts without the addition of ammonium-nitrogen. It therefore seemed probable that the ammonia-oxidizing bacteria in the water samples needed for growth some factor or factors supplied by the diatoms.

An examination was made of the effects of the initial concentration of ammonia on the rate of nitrite production in non-proliferating cultures. Variable results were obtained, and it proved impossible to obtain repeatable data for a concentration-rate curve. However, the results indicated that initial concentrations of over 0.01 M ammonium-nitrogen were increasingly inhibitory to the rate of nitrite formation.

Quastel, Scholefield & Stevenson (1952) have reported the direct production of nitrite from organic nitrogen without the intermediate formation of ammonia. The results of control cultures and the recoveries of nitrogen as nitrite, which agreed well with the original addition of ammonia, made it unlikely that the nitrite could in any case be formed entirely from the organic nitrogen of the diatoms. It remained possible that appreciable nitrite might be formed directly from organic nitrogen synthesized by the heterotrophic bacterial flora present. All attempts to demonstrate the presence of any organism capable of producing nitrite from pyruvic acid oxime gave negative results.

The experiments were continued at the Marine Biology Station, Menai Bridge. An investigation was first made of the nitrifying behaviour of freshly collected water from the Menai Straits. These water samples showed vigorous formation of nitrite when incubated with calcium carbonate and suitably enriched with phosphate and ammonium-nitrogen. Furthermore, when subcultures were made from these enrichment cultures to sterile media made up from Menai Straits water without the addition of diatoms, the exponential production of nitrite persisted without diminishing throughout numerous successive subcultures. This behaviour was in marked contrast to that shown by cultures set up in E1 water. The sea water used in these and all subsequent subcultures was 'aged' water which had been filtered through Whatman no. 2 filter-paper immediately after collection and then allowed to stand for at least 1 month in the dark.

These results agreed with those reported by many previous workers, for there seems to be general agreement that nitrifying bacteria occur in coastal waters contaminated by land drainage and bottom deposits. In conjunction with the results reported above, which demonstrated the presence of ammonia-oxidizing bacteria in E1 water which apparently required for growth a factor or factors in diatoms, there were two possible interpretations of the observed experimental observations. Either the ammonia-oxidizing organisms which were present in the two waters were of two types with differing nutritional requirements or, alternatively, the Menai Straits water contained the factors supplied to E1 water by a diatom suspension. Experiments were therefore made to investigate these possibilities.

Cultures were set up in sterile media of identical composition made up with either aged Menai Straits water or a sample of aged E1 water (membrane-filtered immediately after collection). These cultures, together with the relevant controls, were inoculated from an enrichment culture set up with Menai Straits water. The results of such an experiment are shown in Fig. 2. It will be seen that the cultures in medium made up with Menai Straits water exhibited an exponential production of nitrite and therefore presumably grew well. In contrast, the cultures in medium made up with E1 water exhibited a slow linear production of nitrite. This behaviour was similar to the subcultures from the original E1 enrichment cultures. This similarity was



emphasized by the subsequent observation that the addition of a sterile diatom suspension allowed an exponential production of nitrite in cultures made up with E1 water and inoculated from Menai Straits enrichment cultures. It therefore seemed likely that there was a difference between the two water masses with respect to their ability to support the growth of the ammonia-oxidizing bacteria, there being no evidence to suggest that the ammonia-oxidizing bacteria from the two localities had different nutritional requirements.

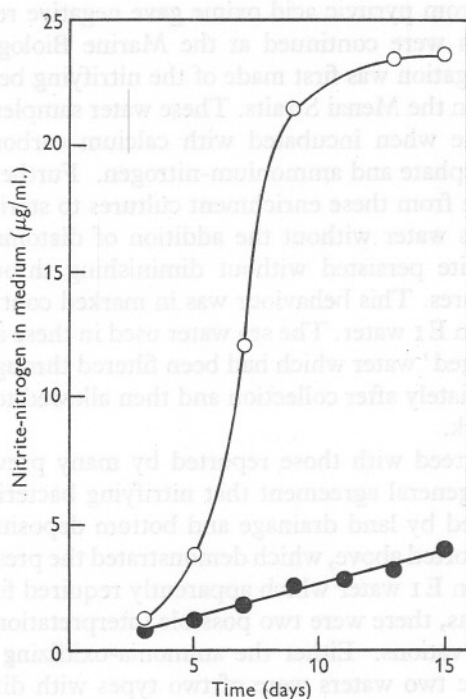


Fig. 2. Progress curve for nitrite production after subcultures from Menai Straits enrichment culture. —○—○—, Medium made up with Menai Straits water; —●—●—, medium made up with E1 water.

An examination of the two waters showed markedly different contents of iron (estimated by the method of Cooper, 1935). The sample of E1 water used contained a barely detectable amount of iron, whereas water from the Menai Straits had an iron content of the order of 20–30 µg/l. Meiklejohn has shown that the terrestrial species of *Nitrosomonas* and *Nitrobacter* require considerable amounts of iron for growth. In addition, it seemed certain that some iron was being added with the diatoms since these had been grown in iron-rich medium and are known to accumulate considerable quantities of iron. It therefore appeared possible that variations in the iron content might account

for the variations in the ability of the two waters to support the growth of the marine nitrifiers. Experiments showed (Fig. 3) that the addition of iron to subcultures in medium made up with EI water allowed an exponential production of nitrite. The concentrations of nitrite in Fig. 3 are plotted on a logarithmic scale and the slope of the graphs is presumably a measure of the growth rate of the nitrifying bacteria. It will be seen that the additions of iron to EI water allowed growth at a rate comparable to that in Menai Straits water.

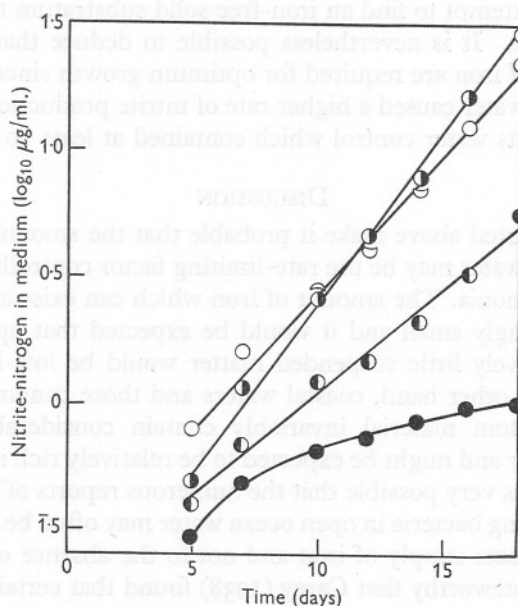


Fig. 3. Progress curves for nitrite production after subcultures from a Menai Straits enrichment culture. —●—●—, EI water medium, no additions; —○—○—, Menai Straits water medium, no additions; —●—○—, EI water medium with addition of diatoms; —●—●—, EI water medium with addition of 100 µg./l. iron.

Medium made up with EI water with the addition of iron supported growth of the ammonia-oxidizing bacteria in numerous successive subcultures and after ten subcultures there was no indication of any diminished growth rate. Subsequent successive subcultures without the addition of iron showed decreasing rates of growth and after three or four subcultures of this type the production of nitrite again became proportional to time and characteristic of a non-proliferating bacterial population. It is noticeable that several subcultures are necessary for the effect to become apparent in this case, in contrast to the linear production of nitrite in the first subcultures from Menai Straits water to EI water without the addition of a diatom suspension. This is probably due to the much lower content of iron in the latter parent cultures.

The iron content of Menai Straits water can be greatly reduced by filtration through a membrane filter. The growth of the ammonia-oxidizing bacteria in medium made up with this filtered water was similar to that observed in medium made up with E1 water.

It has not been found possible to determine the optimum concentration of iron for the growth of the marine nitrifiers since analysis showed relatively considerable quantities of iron in the calcium carbonate, it being impossible to assess how much of this would be available to the bacteria. No success was obtained in an attempt to find an iron-free solid substratum that would allow optimum growth. It is nevertheless possible to deduce that relatively high concentrations of iron are required for optimum growth since the addition of 100  $\mu\text{g/l.}$  to E1 water caused a higher rate of nitrite production than occurred in a Menai Straits water control which contained at least 30  $\mu\text{g/l.}$  (Fig. 3).

#### DISCUSSION

The results reported above make it probable that the amount of iron present in a body of sea water may be the rate-limiting factor controlling the bacterial oxidation of ammonia. The amount of iron which can exist in solution in sea water is exceedingly small and it would be expected that open ocean water containing relatively little suspended matter would be low in available iron content. On the other hand, coastal waters and those contaminated by land drainage or bottom material invariably contain considerable amounts of suspended matter and might be expected to be relatively rich in available iron. It therefore seems very possible that the numerous reports of failure to detect ammonia-oxidizing bacteria in open ocean water may often be due to failure to provide an adequate supply of iron and not to the absence of the catalysing bacteria. It is noteworthy that Carey (1938) found that certain waters naturally rich in phytoplankton (or after the addition of phytoplankton townetting) allowed the vigorous oxidation of ammonia, whereas in similar samples containing a preponderance of zooplankton little nitrification occurred. Judging by the result obtained by Cooper (1935) on the iron content of phytoplankton and zooplankton, Carey's observation could be due to the relatively higher iron content of the phytoplankton.

If this interpretation is correct, considerable bacterial oxidation of ammonia may occur in the upper layers of the open ocean as suggested by the oceanographical evidence. The actual rate of oxidation in nature would be expected to be much lower than that observed in culture, due not only to relatively low levels of iron but also to low substrate concentrations. It is obvious, however, that such waters might fail to show appreciable nitrifying activity if cultured without the addition of iron. A wide survey of the nitrifying activity of freshly collected open ocean waters using the culture techniques described in this paper is required to test this possibility.

Details of the identity and nature of the catalysing organism must await its



isolation. Several previous workers have reported the failure of attempted isolation studies. Waksman, Hotchkiss & Carey (1933) noted the formation of nitrite on silica gel plates inoculated from their enrichment cultures, but apparently no observable growth occurred. This is reminiscent of the production of nitrite without proliferation of the catalysing organism noted during the present studies. It seems probable therefore that any silica gel medium used in isolation studies with the marine nitrifiers will have to be well enriched with iron. There is, however, no evidence to suggest that the marine nitrifiers are of different physiological type to their terrestrial counterparts. The results obtained by von Brandt *et al.* (1937), by Carey (1938), and in the present studies show that the onset of the oxidation of ammonia is delayed in cultures containing organic matter. *Nitrosomonas* is a strict autotroph and its growth is inhibited by organic matter. During the induction period presumably the heterotrophic flora present is removing the inhibitory organic matter. Furthermore, the observation of von Brandt *et al.* that oxidation of nitrite does not commence until all the ammonia has been oxidized is typical of the behaviour of *Nitrobacter*, the growth of which is inhibited by quite low concentrations of ammonia. The inhibition of ammonia oxidation, with increasing ammonia concentrations of the order noted in the present studies, is typical of *Nitrosomonas*.

It is interesting that the optimum iron concentration in culture lies well above the solubility of the ferric ion. Similar results were obtained by Meiklejohn (1953). Working with the terrestrial *Nitrosomonas* she reported optimum iron concentrations of the order of 6 mg/l. At the high pH values employed for culture these iron concentrations are certainly in excess of the solubility of iron. These observations and the present studies suggest, therefore, that these organisms are capable of, and may be dependent on, utilizing particulate iron. The nitrifying bacteria show a very marked tendency to attach themselves to particles of the solid phase in the culture medium. Lees & Quastel (1946) have shown that ammonia is not oxidized in soil cultures unless it is absorbed on to the surface of the soil colloids. The adherence tendencies of these bacteria may thus be necessitated by their method of mobilization of ammonia. If, however, considerable quantities of iron are required, the bulk of which is in particulate form, it is difficult to see how this could be mobilized unless physical contact between the cells and the particles was achieved. The attachment propensities of these organisms may thus be due in part to the necessity of their mobilizing iron in particulate form.

Part of the work reported above was performed at the Plymouth Laboratory. It is a great pleasure to thank the Director and Staff for making freely available the facilities of the laboratory, especially to Dr H. W. Harvey, F.R.S., for his continual interest and encouragement, and Mr F. A. J. Armstrong for collecting water samples.

## SUMMARY

The failure of a sample of E I water to oxidize added ammonium-nitrogen to nitrite-nitrogen has been shown to be due, not to the absence of catalytic bacteria in the water, but rather to the lack of suitable nutrient conditions for proliferation of the nitrifying bacteria.

The addition of sterile diatoms or sterile hot water extracts of diatom cells to E I water allowed the proliferation of the nitrifying organisms with concurrent oxidation of ammonium-nitrogen to nitrite-nitrogen.

Water from the Menai Straits allows the proliferation of its naturally occurring nitrifying flora without additions of diatom material. These organisms, however, will not grow in E I water unless diatom material is added.

Additions of iron allow the nitrifying bacteria from Menai Straits water to grow in E I water. These additions of iron have a quantitative effect comparable to additions of diatom material.

It is suggested that the many previous failures to demonstrate the presence of nitrifying bacteria in the middle layers of open ocean waters may be due not to their absence but to failure to supply sufficient iron for appreciable growth to occur in culture. This may bring the bacteriological evidence more in line with the oceanographical evidence which suggests that considerable formation of nitrite occurs in these layers.

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## EVIDENCE OF SELF-FERTILIZATION IN CERTAIN SPECIES OF BARNACLES

By H. Barnes

The Marine Station, Millport

and D. J. Crisp

Marine Biology Station, University College of North Wales, Bangor

Isolated individuals of certain species of cirripedes are known to remain unfertilized at the time when the majority of contiguous individuals are carrying egg masses. From a very large number of observations on both *Balanus balanoides* (L.) and *Elminius modestus* Darwin (Crisp, 1950, 1956) there remains little doubt that in these two species copulation is necessary before eggs are brought into the mantle cavity where they are fertilized. Though fewer field observations have been made, *Balanus crenatus* Bruguière appears to behave similarly, isolated specimens grown on raft-exposed panels never bearing fertilized egg masses (Crisp, 1950; Barnes, unpublished observation). *B. balanus* (L.) (= *B. porcatus* da Costa) is also in all probability an obligatory cross-fertilizing hermaphrodite, though the available evidence does not exclude the possibility of self-fertilization in rare instances (Crisp, 1954; Barnes & Barnes, 1954).

It appeared possible to one of us that obligatory cross-fertilization might be the rule for all cirripedes, and some ecological consequences of this were pointed out by Crisp (1950). However, we have independently come to the conclusion that certain species, though they may normally cross-fertilize whenever this is possible, can also bear apparently viable egg masses without copulation having taken place.

### EVIDENCE FOR SELF-FERTILIZATION IN *CHTHAMALUS STELLATUS*

Under natural conditions it is exceedingly difficult to find an individual of *Elminius modestus*, *Balanus balanoides* or *B. crenatus* fertilized when separated by a distance of 5 cm or more from its neighbour. This critical distance represents the maximum extension of the penis. Since the possibility usually exists that another individual might be carried into the vicinity of an experimentally isolated barnacle on the shell of a wandering mollusc, any rare exception may be attributed to such an accident. In some circumstances such a remote possibility may be entirely excluded, as for example in the case of *Elminius modestus* which has been shown to be cross-fertilizing by carrying out experiments on individuals



introduced into an area that was not at the time populated by this species (Crisp, 1956). Nevertheless, even when the possibility of individuals approaching accidentally is not entirely excluded, the artificially isolated individuals are almost invariably found to be without fertilized egg masses in these three species. It is therefore reasonable to conclude that if in such an experiment with another species a significant proportion of isolated individuals become gravid, the majority of them, if not all, must have deposited their eggs in the mantle cavity without having copulated with another individual.

TABLE I. SELF-FERTILIZATION IN *CHTHAMALUS STELLATUS*

Date	Locality	Distance between individuals (cm)	No. of individuals in sample	Percentage fertilized	Percentage fertilized among controls
24. vi. 54	Millport	2.5- 5.0	20	45	96
		5.0- 7.5	20	40	
		7.5-10.0	20	40	
		>10.0	20	45	
12. vii. 54	Millport	2.5- 5.0	20	55	48
		5.0- 7.5	20	60	
		7.5-10.0	20	50	
		>10.0	20	32	
4. viii. 54	Millport	2.5- 5.0	20	55	55
		5.0- 7.5	20	50	
		7.5-10.0	20	60	
		>10.0	20	30	
8. vi. 52	Aberffraw	1-3	57	53	57
		3-5	20	50	52
		5	13	23	28
20. vii. 52	Aberffraw	1-3	10	80	70
		3-5	14	86	71
		>5	37	41	50
22. vi. 52	Black Rock* nr. Portmadoc	>7	14	36	43
7. vi. 53	New Brighton*	>10	50	18	74
29. vi. 53	New Brighton*	2-5	10	20	100
		>10	8	0	90
5. vii. 53	Aberffraw	>10	7	57	55

\* Naturally occurring individuals which were not experimentally isolated.

This conclusion applies with special force in the case of *Chthamalus* growing sparsely near high-water mark in areas where it is not very abundant, for *Chthamalus* is only found on mollusc shells when it is extremely common on the rocks throughout the intertidal zone.

Table I gives the percentage of individuals carrying egg masses, compared with the percentage found among a large number of controls gathered from the same piece of rock at the same level. Control individuals were in all cases in physical contact with one or more other specimens of mature size. Great care is necessary in choosing control individuals to ensure that they are drawn from the same type of environment since tide level, aspect, and access to surf can profoundly influence the fecundity. At Millport, the results refer to

naturally isolated individuals of mature age. In the North Wales experiments the individuals were in most cases isolated by removing all others within a given distance prior to April, since breeding does not start until May, although in some cases naturally isolated individuals were taken in the sample.

Table 1 clearly shows that while at some places isolated individuals appear to be just as fertile as contiguous ones, in other instances, as for example at New Brighton, there is evidence that isolated individuals have not been fertilized to the same extent as contiguous individuals.

There is some evidence to show that oviposition may be delayed in isolated as compared with contiguous specimens. The observations at Aberffraw on 9 June 1952 were made early in the breeding season at a time when the first brood was developing in the mantle cavity. Although there was only a small percentage fertilized in both isolated and contiguous individuals, the latter were at a slightly later stage of development. Thus the percentage of the population which had reached a given stage of development was always greater in the contiguous than the isolated populations. Similarly, on 29 June 1953 at New Brighton, where a very great disparity existed between isolated and contiguous individuals, the latter had just produced a second brood and the embryos were all in an early stage of development. At Millport, where conditions are less favourable for this warm water species, the percentage fertilized in June was much higher in the contiguous individuals. The evidence as a whole is consistent with the view that oviposition may take place without insemination by another individual if the ovaries remain unfertilized for some time after reaching a ripe condition. It follows, therefore, that unless these eggs develop parthenogenetically, self-fertilization must take place.

In addition to these observations, fertilized isolated individuals have also been met with from time to time. These are more obvious in areas where the species is not very abundant, as in the north of Scotland. Examples of apparently self-fertilized individuals have been seen at W. Loch Tarbert, Argyll 1 August 1953 at about M.H.W.S.T.L., at 'the spur' west of Dunnet Head, Caithness, and by Dr A. J. Southward (personal communication) at Stenness, Esha Ness, Shetland. Individuals grown in isolation on raft-exposed panels at Millport have also been found to contain ripe egg masses.

#### VIABILITY OF EGGS WHICH ARE NOT CROSS-FERTILIZED

Eggs produced without copulation appear to be less viable than normally fertilized eggs. Occasionally in egg masses of many species, groups of ova may be found which do not develop, but remain in the primitive unsegmented condition. Such eggs are probably unfertilized and are voided with the hatched nauplii when the latter are fully developed. It was noticed that a considerable proportion, sometimes as many as half, of the egg masses from isolated individuals contained unsegmented ova together with developing ova.

In other cases abortive development took place, giving rise to segmented eggs with an abnormal appearance. For example, certain of the eggs examined from isolated individuals collected at Aberffraw on 8 June 1952 were difficult to classify as a stage in normal development, and one pair of egg masses appeared to be decomposing. It is therefore possible that the earlier state of development of broods from isolated individuals may not indicate later oviposition, but be due to abnormally slow development. Further evidence supporting this hypothesis is that at Millport during the late summer, whilst all stages of embryos were found in contiguous individuals indicating the development of successive broods in the population, only late stages were found in the isolated individuals. Slow development in the latter would prevent a further generation from being developed since eggs are not brought into the mantle cavity, even when ripe, in the presence of an earlier brood (Crisp & Davies, 1955).

Nevertheless, viable nauplii can be produced by isolated individuals, and indeed in every observation at Aberffraw recorded in Table I, and in the observation at Black Rock, at least one isolated individual contained nauplii which were fully developed and able to hatch.

At Millport undeveloped and degenerate egg masses in the mantle cavities of isolated individuals appear to be common earlier in the breeding season. In the early summer most of the eggs in the mantle cavities of isolated individuals were incompletely developed and in many cases the whole egg mass had begun to degenerate *in situ*; on being touched with a needle the egg mass broke down into an oily suspension of tissue. This is perhaps related to the fact that in such regions where the species is approaching its limit of distribution, only in late summer are the environmental conditions suitable for the species to reproduce. Thus even the early broods developed in contiguous individuals, although giving apparently normal nauplii which on teasing out will swim actively, do not give rise to any shore settlement. However, the broods produced from isolated individuals in the late summer appear completely normal; the nauplii hatch readily and swim vigorously. Similar temperature conditions apply in the Isle of Man, where the settlement of *Chthamalus* is confined to a period in late summer or autumn (Southward & Crisp, 1956).

The efficiency of self-fertilization also appears to be less than that of cross-fertilization as regards the number of normal embryos produced. Counts of normal embryos in isolated and contiguous individuals of several size groups show a greater mean number (*t*-test) in the latter.

#### OVIPOSITION IN THE DRY STATE

While in the dry state *Chthamalus stellatus* remains closed and does not protrude either the cirri or the penis. Copulation is therefore impossible under these conditions. However, there appears to be no reason why ovi-



position should not occur, provided that copulation were not a necessary stimulus, just as moulting can take place with the shell closed (Darwin, 1854). A number of stones covered with *Chthamalus* were stored in a moist warm place and sampled from time to time to determine the stage of development of the embryos. The egg masses developed normally, the earlier stages disappearing progressively from the population with the passage of time. Development was completed in 15–16 days at 18–20° C and in 21–24 days at 14–15° C. The initial divisions of ova to form a blastoderm covering the yolk cell (stage 4, see Crisp, 1954) normally occupy less than 15% of the total time of development. Occasionally such stages were found long after this period had elapsed. In one individual egg masses at the eight cell stage (stage 3) were found in addition to fully developed egg masses 9 days after the eggs had been maintained at 18° C out of water. In another two, egg masses with blastoderm covering an undivided or partially divided yolk cell (stage 4–5) were found 14 days after the eggs had been maintained out of water at a temperature of 15° C. In all three instances the eggs appeared to be healthy. These occurrences were exceptional and did not invalidate conclusions on rate of development being drawn from the experiment. Unless the rate of development can be greatly reduced by some factor other than temperature (e.g. water loss or oxygen lack) the conclusion must be drawn that oviposition in the dry state may occur under such conditions.

#### EVIDENCE FOR SELF-FERTILIZATION IN OTHER SPECIES

Single specimens of *Verruca stroemia* on shells of *Modiolus* are often dredged from Beaumaris bay. These apparently isolated specimens have been found on several occasions to contain developing egg masses. The shells of *Modiolus* are very sparsely scattered over the sea bed in this area (Baird, private communication) and the possibility of two individuals on separate shells lying close together must be very small. At Millport, *Verruca stroemia* gives one brood each year; fertilization takes place in late December and early January; the nauplii are shed in late February and early March, a little before those of *Balanus balanoides* and *B. crenatus*. Isolated and contiguous individuals on stones collected at extreme low water were examined for egg masses. By mid-January all individuals contained developing egg masses. There was little trace of any degeneration in those from isolated individuals, but eggs showing delayed development were often found with normally developing embryos in the same animal. This again suggests that cross-fertilization is more efficient. By mid-February some actively swimming nauplii could be obtained from most individuals. There was no apparent difference between nauplii derived from isolated and from contiguous barnacles. Further, barnacles which had been isolated by 5 cm and maintained on a raft for many months contained ripe egg masses with normal embryos.

*Balanus perforatus* is a large intertidal species with a thick calcareous base which remains if an individual dies or is knocked off. Consequently, if an individual appears isolated it is easy to check whether another, no longer present, had formerly occupied a site sufficiently close to it for cross-fertilization. The species is rarely found on the shells of littoral molluscs. In parts of north Devon individuals isolated by a distance of 10 cm or more may be found in the same locality as groups of three or four together.

Table II gives the results of an investigation on a reef at Saunton Sands, North Devon. There is no doubt that in mid-August, when the observations were made, individuals situated more than 10 cm from each other not only can carry egg masses, but also are as fertile as contiguous specimens. However, the embryos of individuals grouped together were generally in a more advanced state of development.

TABLE II. SELF-FERTILIZATION IN *BALANUS PERFORATUS*

	Percentage with egg masses	Percentage with eggs at Stage 9 or below	Percentage with eggs at Stage 10, 11 and 12	Percentage with fully developed eggs
Contiguous individuals	66	14	15	37
Individuals separated by more than 10 cm	67	33	12	21

#### MECHANISM OF DEVELOPMENT OF EGGS OF ISOLATED INDIVIDUALS

It is not possible to decide except by a cytological investigation whether the eggs in the mantle cavity of isolated individuals are fertilized or have been developed parthenogenetically. The abnormal development of the eggs in some instances suggests the possibility of parthenogenesis. On the other hand, the testes and ovaries are ripe simultaneously and the penis is suitably placed for self-fertilization by discharging spermatozoa into the mantle cavity. Both *Chthamalus stellatus* and *Verruca stroemia*, and probably *Balanus perforatus*, are normally cross-fertilizing; *Chthamalus* has frequently been observed to copulate both in the laboratory and in the sea. However, in those species in which the development of eggs in isolated individuals occurs at all, it appears to take place with such regularity that it can hardly be considered as an unnatural or exceptional mode of reproduction. Moreover, the process is probably of some value to at least two species, for isolated individuals of *Chthamalus* are by no means uncommon towards high tide mark, while in deep water isolated *Verruca* are often encountered. If the eggs of such individuals were developing parthenogenetically, however, a further process would be required subsequently to bring about duplication of the chromosomes. The simpler hypothesis is, therefore, that self-fertilization takes place.

The possibility that water-borne spermatozoa can effect fertilization at a considerable distance from their origin must also be considered. The spermatozoa of barnacles, unlike those of most other crustacea, are flagellated. Direct

microscopic observations, as well as electron micrographs, show that there is very considerable variation morphologically within the group. Spermatozoa of *Balanus balanoides*, and probably of other species of the genus, are spindle-shaped and very elongated; under the electron microscope there appears to be no difference between the extremities (Barnes & Dainty, unpublished results). Spermatozoa of *Chthamalus* and *Verruca*, on the other hand, have a definite head and tail which are distinguishable even under the light microscope. The spermatozoa of barnacles are usually immobile in the seminal fluid, but have been observed to become extremely active, both in *Balanus balanoides* and *Elminius modestus*, either after being discharged through the penis into the mantle cavity of another individual or as a result of treatment with chelating agents such as ethylene diamine tetra-acetic acid (Crisp, unpublished observation; cf. Fujii, Utida & Mizuno, 1955). In the last two species the spermatozoa are not otherwise readily activated, though an occasional movement may sometimes be seen when they are mixed with sea water. In *Verruca* and *Chthamalus*, however, the spermatozoa more frequently show activity when squeezed from the seminal vesicles into sea water. This greater spontaneous activity of the spermatozoa in sea water of those species in which fertilization in isolated individuals has been shown to be common might suggest that water-borne spermatozoa are responsible. However, if this were so it would be expected that the incidence of fertilization in isolated individuals separated by more than 3–5 cm, the distance reached by the extended penis, would be a function of the distance of isolation. This is not the case; the incidence is sometimes equal to that of individuals lying within 3 cm of each other, and sometimes lower. There is never any indication of a gradual reduction of the incidence of fertilization with increasing distance of isolation, such as would be expected if water-borne spermatozoa were responsible. This difference in the activity and morphology of the spermatozoa may, nevertheless, be related to the capacity for self-fertilization.

Further, oviposition normally takes place in barnacles as a result of the stimulus of copulation and does not occur when embryos are developing in the mantle cavity (Crisp & Davies, 1955). Eggs awaiting fertilization are therefore rarely, if ever, found in the mantle cavity of cross-fertilizing species, nor have they been observed during the examination of many egg masses of *Chthamalus*, *Verruca* and *Balanus perforatus*. Similarly, seminal fluid in most species is emitted only as a result of stimulation during copulation; it is only rarely discharged directly into the water. When it comes in contact with sea water the seminal fluid usually coagulates and is dispersed only with difficulty. Water-borne insemination therefore appears most improbable since it requires that two processes (unstimulated oviposition and unstimulated seminal discharge), which on present evidence very seldom occur in nature, should occur simultaneously. The mechanism can be considered feasible only on the assumption that the barnacle acting as a female collects spermatozoa in sufficient



quantity to provide an oviposition stimulus. The apparent oviposition and development of eggs in the mantle cavity of individuals which have not been immersed in water for long periods shows that water-borne spermatozoa are not essential.

If in these species self-fertilization accounts for the fecundity of isolated specimens, it is pertinent to inquire what is the stimulus leading to oviposition when it is not provided by copulation. In *Chthamalus* the ovaries give rise to a succession of broods, and should oviposition be held back because the barnacle is not suitably stimulated by insemination the ovaries may become very full of ripe eggs. It is possible that under these conditions the threshold of the oviposition stimulus becomes so reduced that movements of the barnacle's own penis in the mantle cavity are sufficient to cause eggs to be released, and seminal fluid is then simultaneously discharged.

If the continued ripeness of the ovary were the stimulus leading to self-fertilization, one would expect the process to be more in evidence where the species was growing rapidly and had abundant food supplies. Individuals kept out of water for long periods, or those growing in unfavourable environments, would not be so likely to self-fertilize as isolated specimens on rocky coasts. The environment at New Brighton is sheltered, silty and unfavourable to the species, whereas it thrives on the exposed west shores of Anglesey and Caernarvonshire (Crisp & Knight-Jones, 1954). The greater readiness with which self-fertilization occurs in the latter area at Aberffraw and Black Rock compared with New Brighton might therefore be explained in terms of nutrition. Similarly, in the earlier part of the summer more planktonic food would probably be available and the lower temperatures would cause basal metabolism to make less call on food reserves; hence self-fertilization might occur more readily at this season than later on, when reserves are becoming spent.

#### SUMMARY

1. Isolated individuals of *Chthamalus stellatus*, *Verruca stroemia* and *Balanus perforatus* often carry apparently fertilized egg masses. Fertilization by water-borne spermatozoa seems improbable because spermatozoa are not normally shed into the water by barnacles, nor are unfertilized eggs usually present in the mantle cavity except during copulation. Either self-fertilization or parthenogenesis must therefore be assumed to have taken place.

2. Eggs found in isolated individuals are often less viable than normal eggs. Nevertheless, they are sometimes capable of full development, and hatch to give swimming nauplius larvae.

3. The percentage of normal embryos is significantly less in egg masses of isolated individuals, than in egg masses of contiguous individuals.

4. Oviposition can probably occur in *Chthamalus stellatus* while it is out of water.

5. There is some evidence that self-fertilization takes place more readily in *C. stellatus* when external conditions are most suitable for breeding. Self-fertilization might therefore be ascribed to a lowering of the threshold of the oviposition stimulus resulting from the continued presence of mature eggs in the ovary.

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## ABSTRACTS OF MEMOIRS

### RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

#### THE OXIDATION OF L-AMINO ACIDS BY *MYTILUS EDULIS*

By H. Blaschko and D. B. Hope

*Biochem. J.*, Vol. 62, 1956, pp. 335-9

The enzymic oxidation of amino acids by preparations of the digestive gland of *Mytilus edulis* was described some time ago. A study has now been carried out of the stereospecificity of the oxidase; it has been established that the enzyme is a L-amino acid oxidase. The enzyme acts upon a great number of L-amino acids. Methionine was the substrate most rapidly oxidized. The enzyme was found to be associated with insoluble tissue constituents; in sucrose homogenates the enzymic activity could be sedimented by centrifugation.

A similar but less active preparation was obtained from the digestive gland of *Cyprina islandica*. Earlier findings of a D-amino acid oxidase in the digestive gland of *Helix aspersa* were confirmed. H.B.

#### EGG-CAPSULE PROTEINS OF SELACHIANS AND TROUT

By C. H. Brown

*Quart. J. micr. Sci.*, Vol. 96, 1955, pp. 483-8

The material of the egg-capsules of selachians and the chorion of trout eggs has been examined by physical, chemical and histochemical methods. The material of the egg-capsules has been found to be a quinone-tanned protein. The chorion of trout eggs is not quinone tanned but its formation and chemical behaviour allies it with the invertebrate cuticular proteins rather than with the vertebrate keratins. C.H.B.

#### ON THE HORMONAL CONTROL OF WATER BALANCE IN *CARCINUS*

By D. B. Carlisle

*Pubbl. Staz. zool. Napoli*, Vol. 27, 1955, pp. 227-31

Experiments on *Carcinus* indicate that the uptake of water at moulting and the water and osmotic balance throughout the rest of the moult cycle are under the control of a hormone secreted by the X organ-sinus gland complex. This factor appears to be distinct from the hormones controlling moulting and from the gonadotrophic hormone. D.B.C.



MUSCLE MORPHOGENESIS IN PRIMITIVE GASTROPODS  
AND ITS RELATION TO TORSION

By Doris R. Crofts

*Proc. zool. Soc. Lond.*, Vol. 125, 1955, pp. 711-50

The retractor muscle of the pre-torsional larvae of the four primitive gastropods investigated is a single group of cells attached to the right side of the shell apex in *Haliotis*, *Patella* and *Patina*, but half-way along the right side in *Calliostoma*. The anterior attachments on the right side are retractors of the velum and mantle: those on the left belong to sickle-shaped cells curving over the gut and are retractors of the left side of the velum and foot rudiment.

The primary ontogenetic cause of torsion appears to be the asymmetry of this *single* pre-torsional muscle, which may have been an ancestral mutation, instead of asymmetry in *two* ancestral larval muscles, a left one related to the foot and a right one related to the head, as in Garstang's hypothesis (1929).

The first phase of torsion is rapid, but varies in time and extent in the genera. The main cause of the variation may be the position of shell attachment of the retractor, with other contributory factors. The second torsion phase is slow and is mainly due to differential growth. The pedal musculature of the right side begins development during this phase, and at the completion of 180° rotation its shell attachment on the definitive right side is roughly the same size as that of the original larval retractor, now on the left. The definitive right shell muscle, which, contrary to earlier supposition, becomes the columellar muscle of *Calliostoma* and the hypertrophied one of *Haliotis*, may be a pair to the pre-torsional retractor, delayed in development. The two muscles may represent the two ancestral retractor muscles of gastropods, whose scars were discovered by Knight (1947) in *Bellerophon* and *Simulites*.

D.R.C.

ENVIRONMENTAL FACTORS GOVERNING THE INFECTION OF MUSSELS,  
*MYTILUS EDULIS*, BY *MYTILICOLA INTESTINALIS*

By B. T. Hepper

*Fish. Invest., Lond.*, Ser. 2, Vol. 20, No. 3, 1956

The importance of *Mytilicola*, its spread to and around the shores of Britain, its method of dispersal, life history and hosts are briefly discussed.

A number of extensive field surveys produced evidence to show that mussels raised from the sea bed, or in fast moving water or at either end of an estuary are less heavily infected than bottom-dwelling mussels, those in slow moving water or those in the middle region of an estuary respectively. These conclusions are supported by the results of field trials.

It is concluded that control of the parasite is possible by using culture methods similar to those practised in France and Italy on stakes, fences or ropes. By growing mussels on selected sites in fast moving water or at the brackish-water end of an estuary, good quality mussels may be cultured on the flat. In addition to restricting the level of infection, such culture methods appear to provide conditions which enable the mussels to withstand parasitization without serious loss of condition.

B.T.H.

THE NERVOUS SYSTEM OF THE EPHYRA LARVA OF  
*AURELLIA AURITA*

By G. A. Horridge

*Quart. J. micr. Sci.*, Vol. 97, 1956, pp. 59-74

Methylene-blue preparations of living ephyra larvae show two nerve nets, of which one, of bipolar cells, overlies only the radial and circular muscle sheets and is identified with the giant fibre net (Shäfer's nerve net) of the adult. The other net, which consists of smaller, multipolar cells, spreads over both surfaces of the animal: it is identified with the physiological conducting system which co-ordinates the movement of a single arm with the directed pointing of the mouth in the feeding reaction. It is called 'the diffuse nerve net'. The 'spasm', in which the animal folds up, stops beating, and sinks through the water, is considered to be an extension of the feeding reaction round the disc, and is shown to be co-ordinated by the diffuse net. The independence of the locomotory beat from the feeding responses and from the spasm is thus explained by the dual histological arrangement. A double innervation of the muscle is also suggested. Both nerve nets connect with the marginal ganglia, where four types of nerve cell have been described. A partial explanation of the action of the ganglion is possible, with an input of excitation from the diffuse net (which affects the rate of the rhythm) and an output to the giant fibre net which initiates the beat. This scheme gives a histological foundation for Romanes's old experiments, in which he demonstrated the difference between 'excitational' and 'contractional' continuity across the bell of the adult, and in which he found an indirect action on the rhythm following stimulation at points far from the marginal ganglia.

G.A.H.

INACTIVATION ENZYMATIQUE D'UNE SUBSTANCE CHROMACTIVE  
DES INSECTES ET DES CRUSTACÉS

By Francis Knowles, David Carlisle and Marie Dupont-Raabe

*C.R. Acad. Sci., Paris*, Vol. 242, 1956, p. 825

Enzymic studies on the A substance of the eye-stalk of *Leander* suggest that this may be a polypeptide.

D.B.C.

EXPERIMENTAL FEEDING OF THE COPEPOD *CALANUS FINMARCHICUS*  
(GUNNER) ON PHYTOPLANKTON CULTURES LABELLED WITH  
RADIO-ACTIVE CARBON ( $^{14}\text{C}$ ).

By S. M. Marshall and A. P. Orr

*Pap. Mar. Biol. Oceanogr., Deep-Sea Res.*, suppl. to Vol. 3, 1955, pp. 110-14

Cultures of the diatom *Skeletonema costatum* and the flagellates *Cryptomonas* sp. and *Syracosphaera carterae* were grown using  $^{14}\text{C}$  as carbonate in the culture medium. The cultures were then used in feeding experiments on female *Calanus* in order to measure the uptake and percentage assimilated. The volume filtered varied from less than 1 to about 40 ml. in 24 h. and the amount of  $^{14}\text{C}$  assimilated from 53-78 %. The results are in agreement with those from similar experiments done with  $^{32}\text{P}$ . S.M.M., A.P.O.

COMPARISON OF THE LEVEL OF CHLORIDE REGULATION BY *NEREIS*  
*DIVERSICOLOR* IN DIFFERENT PARTS OF ITS GEOGRAPHICAL RANGE

By R. I. Smith

*Biol. Bull., Woods Hole*, Vol. 109, 1955, pp. 453-74]

This paper summarizes data obtained on the level of coelomic-fluid chloride in *Nereis diversicolor* adapted to a series of dilutions of sea water and to fresh water. The populations studied have been collected in marine-dominated habitats (Millport and Kingsbridge Estuary), regions of stable low salinity (Gulf of Finland and the Isefjord, Denmark), and from typically estuarine conditions of variable and low salinity (River Tamar). Ecological findings have been reported in the *Journal of the Marine Biological Association* and elsewhere. Despite the wide differences in salinity and its pattern of variation in the several habitats studied, populations of *N. diversicolor* from different areas were found to show no significant differences in the level of coelomic chloride regulation after adaptation to low salinities. This level is thus not diagnostic of physiologically distinct races of *N. diversicolor*, although the ability to tolerate fresh water or sudden salinity changes may, upon further study, prove to differ in populations from regions of unlike salinity. R.I.S.



## BOOK REVIEW

## THE PROTOZOA, SARCODINA

By Margaret W. Jepps

Oliver and Boyd, London and Edinburgh. 183 pp. 1956

Dr Jepps has written this excellent book in a lucid style, and she has selected and prepared a fine series of illustrations. The book is a welcome addition to the meagre list of good monographs on the Protozoa. It is designed especially for senior students in zoology, and as such it provides a valuable supplement to laboratory work. In her introduction the author gives a competent summary of protozoan cellular behaviour, especially with reference to the cytoplasm. Although the reader is referred to appropriate listings in the index, a more detailed discussion of the nucleus would have enhanced the value of the introduction. After reading the book I was left with the impression that the author had neglected some of the more recent research on problems of protozoan physiology and biochemistry. One should be reminded, however, of the unavoidable lapse of time between submission of a manuscript to a publisher and its final publication. The emphasis on the flexible and tenuous nature of systems of protozoan classification is commendable. The inclusion of the slime moulds helps to establish the author's broad concept of unity within the Protista. The general format of the book is attractive, and the well-chosen bibliography and detailed index serve to increase the book's usefulness to all students of protozoology.

E. R. NOBLE

## MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

### Report of the Council for 1955-56

The Council have great pleasure in reporting that Prof. A. V. Hill, C.H., O.B.E., F.R.S., was elected President of the Association in June in place of Prof. Sir James Gray, Kt., C.B.E., M.C., F.R.S., who had served for the preceding ten years.

The Council wish to record their deep appreciation of the many services rendered to the Association by Sir James Gray during his long term of office as President, and are glad to report that he will continue to serve on the Council as Governor representing the Royal Society. Sir James Gray has been elected a Vice-President of the Association.

The Council have to record with regret the death of Mr D. J. Matthews, a former member of the scientific staff of the Plymouth laboratory, and of Dr A. G. Lowndes who was a regular visiting research worker at Plymouth for many years.

#### 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 Plymouth. At these the average attendance was seventeen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

During the year Mr N. B. Marshall has been appointed to succeed Dr H. W. Parker as Governor representing the Zoological Society of London.

#### The Plymouth Laboratory

The passage between the north building and the constant temperature and radioactive substances laboratories has been closed in with a glass roof and door. This has greatly improved the conditions for those working in these two small laboratories.

During the year the roof of the old library building has been relaid with new asphalt.

#### New Sea-Water Reservoirs

The excavation of the new sea-water reservoirs in the yard between the north and south buildings began in October 1955, and the work is progressing steadily. It has been decided to postpone the building of the new outside circulation tanks until the winter of 1956-57.

Dr D. P. Wilson has given much time to the planning of this scheme which, when completed, will enable all tanks used for research purposes to receive a supply of pure sea water from the new reservoirs, which will together hold about 30,000 gallons. The existing reservoirs, which have up till now supplied research tanks as well as the aquarium, will then only be used for the latter.

#### The Aquarium

The Aquarium has been well attended by the public throughout the year, but the takings in the summer were lower than usual owing to the exceptionally fine weather. In January and February the aquarium was closed for four weeks to enable four tanks, including the two largest, to be emptied for repair of the rockwork.

During the spring and early summer improvements were made to the circulation in most of the tanks. Wide-bore ebonite pipes were installed in corners and other inconspicuous places to carry much of the inflowing water down to the bottom, instead of discharging it only into the surface layers. This has noticeably improved the health of some of the fishes and is undoubtedly responsible for the absence of the usual warm weather troubles during a summer in which the water reached higher temperatures than it had for several years.

A further twenty picture labels have been painted by Miss Jenefer Peter so that few of the species shown are now without such an aid to identification by the visitor.

#### Research Ships

During the year the three research vessels have been in regular and continuous service.

During her annual survey it was found that the hull of the *Sarsia* was in excellent condition.

A new trawl winch has been installed in the *Sula*. This is a more powerful winch than that originally supplied with the ship and is proving most satisfactory. Certain modifications have also been made during the year to give increased accommodation in the engine room, in the wheel house and on deck.

A new Kelvin Ricardo  $7\frac{1}{2}$  h.p. engine has been fitted in the *Gammarus* to replace the engine on the port side, which had been in service since 1929.

#### Staff

Dr D. P. Wilson has been promoted to Senior Principal Scientific Officer on grounds of individual merit as from 1 July 1955.

Dr B. C. Abbott joined the staff of the Plymouth laboratory as Principal Scientific Officer on 1 September 1955.

Dr H. G. Vevers, M.B.E., left the staff of the Plymouth laboratory on



31 August 1955 to take up his appointment as Curator of the Aquarium and Director's Assistant on the staff of the Zoological Society of London.

Mr N. A. Holme has succeeded Dr Vevers as Bursar at the Plymouth laboratory.

Dr T. I. Shaw joined the staff of the Plymouth laboratory at the beginning of November 1955 on a special temporary appointment.

Dr J. A. C. Nicol accompanied cruises of R.R.S. *Discovery II* in October and November 1955, and February and March 1956 by kind invitation of the Director of the National Institute of Oceanography, to do research on luminescence in *Pyrosoma* and other oceanic animals.

Mr F. A. J. Armstrong has been elected an Associate of the Royal Institute of Chemistry.

Mr F. S. Russell, C.B.E., F.R.S., attended the International Conference on Marine Biological Laboratories held in Rome in April 1955, under the auspices of the International Union of Biological Sciences. He also visited the Stazione Zoologica at Naples.

Dr D. P. Wilson attended a symposium on marine biology held at the Scripps Institution of Oceanography at La Jolla in March 1956.

Mr N. A. Holme attended the meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1955.

Miss D. Ballantine spent a month at the East African Fisheries Research Laboratory at Zanzibar by arrangement with the Colonial Office during February and March 1956, while on leave in Africa.

#### Occupation of Tables

The following one hundred and forty-three workers have occupied tables at the Plymouth Laboratory during the year:

- E. ADAMS, Plymouth (Library).
- Miss M. N. E. ADAMS, Southampton (Culture of phytoplankton).
- R. D. ADAMS, Cambridge (Structure of the continental shelf).
- J. ARMSTRONG, London (Nerve cells of molluscs).
- D. C. ARNOLD, St Andrews (Holothuria).
- Miss D. E. ASHHURST, Oxford (Blood groups in fish).
- Dr DAPHNE ATKINS, Plymouth (Ciliary mechanisms of brachiopods, *Phoronis*, etc.).
- A. L. BARETS, Paris (Muscle receptors of fishes).
- G. T. BARLOW, California (Calibration of underwater photometer).
- P. R. O. BARNETT, Derby (Library).
- Miss G. BEESON, Oxford (General).
- Dr L. G. E. BELL, London (Development of *Ciona*).
- Dr JOYCE A. BENTLEY, Aberdeen (Algal culture).
- Miss D. BEXON, Nottingham (Algae).
- F. BEYER, Oslo (Hydrography and zooplankton).
- E. J. BINYON, London (Library).
- J. BLAGDEN, Cambridge (Interpretation of echo-sounding records).
- Miss J. L. BLOOM, Exeter (General).

- Dr B. P. BODEN, La Jolla (Submarine illumination).  
 A. D. BONEY, Plymouth (Culture of red Algae).  
 Prof. R. BUCHSBAUM, Pittsburg (Marine ecology and photography).  
 Dr P. C. CALDWELL, Beit Memorial Fellow (Physiology of crab muscle).  
 Mrs P. A. CALDWELL, Plymouth (*Spartina*).  
 Dr J. D. CARTHY, London (Library).  
 Miss Y. M. CHAMBERLAIN, London (Marine Algae).  
 Dr P. N. J. CHIPPERFIELD, Brixham (Library).  
 J. C. CLEVERLY, Cambridge (Structure of the continental shelf).  
 Dr H. A. COLE, Burnham-on-Crouch (Lynher and Tamar oyster fishery).  
 J. W. COLES, London (Nematodes parasitic on and associated with seaweeds).  
 R. J. CONOVER, Southampton and U.S.A. (Cruise on R.V. *Sarsia*).  
 Mr and Mrs E. COOPER-WILLIS, Wales (Drawings of marine organisms).  
 K. COPELAND, London (Electronic equipment).  
 Dr E. D. S. CORNER, International Paints Research Fellow (Effects of toxic substances on marine organisms).  
 C. A. COSWAY, Torquay (Library).  
 Dr T. J. LE COSQUINO DE BUSSY, The Hague (Waterblooms).  
 Miss W. A. M. COURTNEY, London (Respiration of cirratulids).  
 Dr D. J. CRISP, Bangor (Library).  
 D. CURRY, Pinner, Middlesex (Geology of English Channel).  
 Dr R. PHILLIPS DALES, London (Fat storage and metabolism in polychaetes).  
 E. W. DAWSON, Cambridge (Behaviour of lamellibranchs).  
 A. A. DAY, Cambridge (Structure of the continental shelf).  
 R. D. DESHPANDE, Reading (Physiology of Trochidae).  
 P. S. B. DIGBY, London (Copepods).  
 Miss E. J. DIMELow, Reading (Functional morphology of *Antedon*).  
 D. A. DORSETT, London (Feeding of *Polydora ciliata*).  
 Mme M. DUPONT-RAABE, Paris (Colour change in crustaceans).  
 Dr MARIA FELINSKA, Oundle (Marine ciliates).  
 Dr L. R. FISHER, Reading (Vitamin A in plankton animals).  
 Cdr R. H. C. FRAMPTON, R.N. (Rtd.), Plymouth (Library).  
 R. F. H. FREEMAN, London (Behaviour of *Scrobicularia*).  
 Dr VERA FRETTER, Reading (Library).  
 Dr D. N. GANGULY, Calcutta (General).  
 J. B. GILPIN-BROWN, Bristol (Nervous system of *Nereis*).  
 Dr SYLVIA J. GILPIN-BROWN, Bristol (Behaviour of littoral gastropods).  
 A. GLANVILLE, London (Library).  
 Dr D. R. GLASSON, Plymouth (Library).  
 C. K. GODDARD, St Andrews (Endocrine glands of elasmobranchs).  
 R. U. GOODING, Cambridge (Parasitic crustaceans).  
 Prof. A. GRAHAM, Reading (Library).  
 P. GRAY, Plymouth (Library).  
 A. V. GRIMSTONE, Cambridge (Ciliates).  
 Dr E. R. GUILER, Tasmania (Intertidal ecology).  
 Y. HALIM, Alexandria (Chemistry of sea water).  
 Prof. A. C. HARDY, F.R.S., Oxford (Drawings of marine animals).  
 Prof. F. T. HAXO, La Jolla (Algal physiology and biochemistry).  
 B. T. HEPPER, Burnham-on-Crouch (Lynher and Tamar oyster fishery).  
 Prof. A. V. HILL, F.R.S., London (Heat production in crustacean nerve).  
 Dr M. N. HILL, Cambridge (Structure of the continental shelf).

- G. E. HOBSON, London (Annelid phosphagens).  
Prof. A. L. HODGKIN, F.R.S., Cambridge (Nerve conduction in *Loligo*).  
J. V. HOWARTH, London (Heat production in crustacean nerve).  
Dr A. F. W. HUGHES, Cambridge (Cytology of fish neurones).  
O. D. HUNT, Newton Ferrers (Library).  
D. C. INGRAM, Oxford (General).  
L. A. J. JACKMAN, Paignton (Library).  
F. J. JEFFERY, Plymouth (Library).  
H. S. JEFFRIES, Bradford-on-Avon (Library).  
Dr C. H. JELLARD, Plymouth (Library).  
Miss P. M. JENKIN, Bristol (Library).  
Dr ELIZABETH M. KAMPA, La Jolla (Submarine illumination).  
Dr G. Y. KENNEDY, Sheffield (Porphyrins and chlorophyll pigments).  
A. K. KENT, Aberystwyth (Paper electrophoresis and endocrinology).  
Dr R. D. KEYNES, Cambridge (Nervous conduction in *Loligo*).  
Prof. W. B. R. KING, F.R.S., Cambridge (Geology of the English Channel).  
M. C. KINGWELL, South Brent (Library).  
Sir FRANCES G. W. KNOWLES, Bart., Marlborough (Colour change in crustaceans).  
G. A. KNOX, New Zealand (Shore ecology; library).  
Dr P. L. KRAMP, Copenhagen (Medusae).  
C. V. KURIAN, Travancore (Larvae of decapod crustaceans).  
Dr D. LACY, London (Green gland of crustaceans).  
Dr MARIE V. LEBOUR, Plymouth (Decapod crustaceans).  
Dr J. LLEWELLYN, Birmingham (Trematode ectoparasites of fishes).  
Prof. O. E. LOWENSTEIN, F.R.S., Birmingham (Sensory physiology of elasmobranchs).  
Dr A. G. LOWNDES (the late), Plymouth (Mineral content of sea water).  
Dr J. LOWY, Belfast (Mechanical properties of *Mytilus* muscle).  
Miss A. C. MADDOCKS, Oxford (General).  
Dr A. M. A. MAGHRABY, Alexandria (Plankton).  
Dr S. B. MARKOWSKI, London (Marine Algae and zooplankton).  
A. L. MARTIN, London (Feeding of amphipod crustaceans).  
Mrs J. B. MATTHEWS, St Merryn (Library).  
Dr D. M. MAYNARD, Cambridge and U.S.A. (Histology of decapod nervous system).  
G. F. MEES, Leiden (Feeding of *Zeus faber*).  
P. L. MILLER, Cambridge (Shore Diptera).  
Miss F. M. MOLLOY, London (Digestive system of crustaceans).  
Dr J. E. MORTON, London (Biology of *Lasaea rubra*).  
Dr R. W. MURRAY, Birmingham (Ampullae of Lorenzini).  
Dr MARGARET NAYLOR, London (Algal cytology).  
Miss M. E. NEEDLER, London and Toronto (Neuromuscular physiology of sea anemones).  
D. NICHOLS, Oxford (Feeding of sea urchins).  
Prof. E. R. NOBLE, Santa Barbara (Protozoan parasites of fishes).  
Mrs E. OLDFIELD, Guildford (Embryology of *Lasaea rubra*).  
Dr C. F. A. PANTIN, F.R.S., Cambridge (Neuromuscular physiology of *Actinia*).  
L. B. PRADHAN, Madras (Mackerel fisheries).  
G. L. RAO, Madras (Fishing gear and methods).  
Dr W. J. REES, London (E. T. Browne manuscripts).  
Miss B. RICKARD, Plymouth (Library).  
Dr F. H. RIGLER, Toronto (Ion exchange by plant cells).  
Miss E. A. ROBSON, Cambridge (Neuromuscular physiology of *Actinia*).



Dr D. M. ROSS, London (Neuromuscular physiology of sea anemones).  
 M. J. S. RUDWICK, Cambridge (Brachiopods).  
 Dr W. M. S. RUSSELL, London (Humane killing of crabs).  
 A. SHARPLES, Plymouth (Library).  
 G. SMITH, Aberystwyth and Perth, Australia (Estuarine fucoids).  
 R. E. SOERIAATMADJA, Indonesia (Chemistry of sea water).  
 M. K. SOONG, Malaya (Library).  
 Dr A. J. SOUTHWARD, D.S.I.R. (Distribution and breeding of marine animals).  
 Dr EVE C. SOUTHWARD, Plymouth (Polychaetes).  
 B. W. SPARROW, Newton Ferrers (Library).  
 Miss F. A. STANBURY, Plymouth (*Cladophora*).  
 Dr F. C. STOTT, Ewell (Food canal and haemal network in holothurians).  
 Prof. K. STRØM, Oslo (Geomorphology of Devon and Cornwall).  
 O. SUDDABY, Plymouth (Library).  
 Dr OLIVE S. TATTERSALL, Hayling Island (Breeding cycles in mysids).  
 Miss A. M. TAYLOR, Manchester (Rock pool Algae; *Ascophyllum*).  
 M. UNNI, Madras (Fishing gear).  
 A. J. VINSON, Nottingham (Algae).  
 G. E. WALSTER, Plymouth (Library).  
 Prof. G. P. WELLS, F.R.S., London (*Arenicola*).  
 Dr MARY WHITEAR, London (Nervous system of tunicates).  
 Dr D. I. WILLIAMSON, Port Erin (Larvae of pagurid crustaceans).  
 Miss M. J. WOOD, London (Culture of flagellates).  
 H. V. WYATT, Plymouth (Biology of *Calyptrea*).  
 Prof. J. Z. YOUNG, F.R.S., London (Nerve cells of molluscs).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr Reynolds, U.S.A.; Dr R. W. Hiatt, Hawaii; Dr P. Dohrn, Naples; H. C. Butcher, Penang; Dr J. C. Medcof, Canada; Dr Helen I. Battle, Ontario; Dr Tham Ah Kow, Singapore; Dr T. Imai, Japan; R. G. Collins, Nigeria; G. R. Fish, Malaya; Prof. C. Smith, Nova Scotia; Sidney W. Fox, Tallahassee; Dr D. Davenport, Santa Barbara; P. Bougis, Banyuls-sur-Mer; Prof. Ricardo Jorge, Lisbon; Dr R. Revelle, La Jolla; Dr R. C. Swan, New York; Dr G. A. C. Herklots, Trinidad; J. C. Yaldwyn, New Zealand; Dr X. M. Aubert, Belgium; Dr Antoine Dohrn, Naples; R. Beary, U.S.A.; Dr D. E. Hurley, New Zealand; Dr C. Mary John, Travancore; Dr A. C. Redfield, Woods Hole; Dr W. Schmitz, Germany.

The Danish Fishery Research Vessel *Dana* visited Plymouth on her return voyage from Greenland waters in August 1955. Among those on board were Dr P. Hansen, Mr F. Hermann, Mr J. Nielsen and Dr Mario Ruivo.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty students from the following Universities and University Colleges: Oxford, Cambridge, Glasgow, London, Durham, Liverpool, Sheffield, Southampton, Exeter, Aberystwyth, Galway, Chelsea Polytechnic and Regent Street Polytechnic.

Also during the Easter Vacation Mr K. W. Wilkes brought a party of five boys from Harrow School and Mr E. C. Turner brought seven boys from Buckhurst Hill County High School.

Dr G. E. Newell and Dr J. E. Morton conducted a course in September for sixteen students from Queen Mary College.

#### Scientific Work of the Plymouth Laboratory Staff

##### *Sea Water and Plankton*

Dr L. H. N. Cooper has continued his work on the inter-relation of Arctic climate, the deep circulation of the North Atlantic and the biological productivity of the English Channel. The views presented in the Report for 1953-54 have required little change, have been developed further, and are now much more firmly based. All links in the process are now seen clearly, though some are still hypothetical. They are presented schematically in two papers which are to appear in the *Journal of Marine Research* and in the Bigelow Birthday Number of *Deep Sea Research*. Not all the data underlying the arguments have yet been written up; those from the Denmark Strait region are at present being brought together.

Associated hypotheses have been erected for the North Pacific. These are an attempt to relate fluctuations in the success of the fisheries of California and the Pacific coast of Japan with fluctuations in the strength and position of the Eastern Asiatic winter anticyclone. The facts so far unearthed have required no revision of the hypotheses.

The winter maximum for inorganic phosphate at station E1 in 1955 reached a concentration greater than in any of the preceding 24 years. In March and May two cruises to deep water on R.V. *Sarsia* were made by Dr Cooper and Mr Armstrong to find out where this enriched water came from. No clear-cut answer was obtained. Rich water was present along the continental slope between the Great Sole Bank and South-west Ireland, but no connexion of this with the Plymouth area was found. The distribution of properties, especially oxygen, over the continental slope was perplexing. Vigorous water movements were in progress in a highly heterogeneous honeycomb of water masses. With the data available one cannot separate internal wave phenomena, including possible submarine eagres, from along-slope currents. The means are now available to differentiate between the several interpretations which may be placed on the past year's work.

Dr Cooper's detailed account of the occurrence of chemical elements in sea water is being amalgamated with one prepared independently by Dr E. G. Goldberg of the Scripps Institution of Oceanography. An article on 'Oceanography in Past Ages', prepared some years ago for *The Science of Petroleum*, has been brought up to date. A joint paper with Mr J. S. Colman, of Port Erin, on 'Underwater illumination and ecology in tropical estuaries' written

in 1939, has now been revised and published in the *Bulletin* of the British Museum (Natural History).

Mr F. A. J. Armstrong has continued the monthly cruises to Station E1 and analyses for phosphate, total phosphorus and silicate. Further analyses for aluminium have shown that the amount present in solution in sea water is rarely more than about  $2\mu\text{g}$  atom Al/l. and shows little variation. Some earlier figures indicating larger amounts of aluminium have been rejected, owing to unsuspected contamination by copper from the metal sampling bottles, and the method has been amended to obviate the interference of copper. He has developed a method for the estimation of iron in sea water which appears to determine the total iron content. The results of analysis of samples from E1 and three deep-water stations show much higher values than have previously been reported, and these are being prepared for publication.

Mr Armstrong has constructed an absorptiometer for colorimetric analysis at sea, and a prototype was tested during a cruise in R.V. *Sarsia*. It appeared to be reliable, and needed little space in the laboratory.

In order to find whether any of the considerable quantity of organic nitrogen, known to be present in solution in sea water, is available as a nitrogen source for the growth of phytoplankton, a method of bioassay of available nitrogen compounds has been developed by Dr H. W. Harvey. The opportunity was taken to compare the quantities found by assay in a water collected in the summer with the quantity of inorganic nitrogen compounds found by analysis by Dr J. P. Riley, of the Department of Oceanography at Liverpool University, using a method which he has developed for the determination of nitrate nitrite and ammonium nitrogen in one operation. The results indicate that almost none of the organic nitrogen was utilized.

Preliminary experiments have been made by Dr Harvey in collaboration with Mr E. Soeriaatmadja, of Indonesia, to investigate the uptake of phosphate at low concentrations in sea water by ferric hydroxide. Treatment with fifty times more iron (as recently precipitated ferric hydroxide) than the phosphate-phosphorus present in sea water resulted in insignificant uptake at pH 8, but considerable uptake at pH 6.5. The uptake and resulting equilibrium appeared to depend upon the ratio of iron to phosphate, their initial concentration, and the pH of the sea water. Kaolin in suspension at a concentration more than a thousand times greater than that of the phosphate caused little change in the phosphate concentration of sea water, but adsorbed all the 10 mg phosphate P per  $\text{m}^3$  which had been added to a fresh water.

The first paper by Dr Mary Parke, in collaboration with Prof. Irene Manton and Mr B. Clarke, of Leeds University, on new members of the Chrysophyceae has been published in Vol. 34, No. 3, of the *Journal*. The three species described there are the first of a considerable series of related forms which have been isolated and are being maintained in culture at Plymouth. A further twenty new members of this series have been isolated during the



present year. These forms all possess a number of rather unusual characters which make their classification difficult. They have two flagella, in some equal in length and in others unequal, and an additional retractile filiform appendage used for the temporary anchorage of the cell to which the name *haptonema* has been given.

In some species pigmented and non-pigmented individuals have been observed and phagotrophic feeding has already been demonstrated in a large number of the different species. All the species in this series show a covering of sculptured scales, of varied size and shape in different forms; they may or may not bear spines. In the majority the scales are so small that they are invisible under the light microscope and can only be studied successfully under the electron microscope. Prof. Irene Manton is continuing to collaborate with Dr Parke on this side of the work. The study of another group of these new forms should be completed by the end of the year.

The collection of unialgal cultures of marine phytoplankton organisms has been maintained throughout the year, with the assistance of Miss I. M. Adams. A very large number of cultures have been distributed for research purposes to institutions in this country and abroad.

Miss D. Ballantine has continued her experiments on the toxicity of flagellates in particular on the toxin from *Gymnodinium veneficum* nom. prov. This work has been done in collaboration with Dr Abbott. The toxin has been isolated from cultures by adsorption on active charcoal followed by elution with organic solvents. It is believed that a reasonably pure extract has been obtained, and this is being used to study its physiological effects on animals and isolated tissues. It renders muscle inexcitable to either direct or indirect stimulation, and reduces both action and membrane potentials in muscle and in certain nerves. Samples of the toxin have also been sent to Dr J. M. Ritchie, of the National Institute of Medical Research.

The results of Miss Ballantine's work with Dr J. E. Morton of Queen Mary College, London, on *Lasaea rubra* were published in Vol. 35, No. 1 of the *Journal*; a filtering rate of 0.05 ml./h/animal was obtained, using acceptable organisms, but filtering can be reduced or stopped when toxic flagellates are used. The distinction between filtering and feeding has been made, and it is found that in algal cultures *Lasaea* is capable of filtering off many more organisms than it can ingest. This probably does not occur in nature. It is proposed to follow this up with an investigation of the digestive system of *Mytilus*.

Some work has been done on the sand-living dinoflagellates at Port Erin. It is hoped to continue this work, and to examine specimens from this and other areas.

Mr F. S. Russell has been examining collections of deep-sea jellyfish made on the research vessel *Sarsia*. Some interesting Scyphomedusae have been found, among which were numerous well-preserved specimens of a new species

of the extremely rare genus *Paraphyllina*. A description of this species has been published in Vol. 35, No. 1, of the *Journal*. It is hoped also to describe certain species of the genus *Nausithoë*; and two Hydromedusae which appear to be new species.

Mr P. G. Corbin's examination of the 1955 catches of the 2 m stramin ring-trawl showed that *Sagitta setosa* continues to prevail and the level of macro-plankton production to remain low.

During the 1955 breeding season of *Echinus esculentus* Dr D. P. Wilson and Mr Armstrong continued their experiments on biological differences between sea waters. Once again they were hampered by weather and other factors beyond their control, but none the less made some progress. This year the difference between Clyde and E I waters was more marked than in the immediately preceding years and was nearly as great as during the early experiments. It was again found that the *Echinus* eggs and larvae behaved differently in bottom and surface waters collected at the same time and at the same station. Several water samples were successfully filtered free from bacteria at the time of collection, and these waters showed only insignificant differences from those collected in the ordinary way. Unfortunately, the experiments were to some extent vitiated by the poor condition of the *Echinus* eggs. Indeed adult *Echinus* were unusually scarce on the usual trawling grounds, owing perhaps to severe gales earlier in the year. Other results included some with artificial sea water, using both *Echinus* larvae and larvae of *Sabellaria alveolata*. The results are being written up for publication in the *Journal*.

#### *Macro-Fauna and Flora*

Dr D. P. Wilson's experiments, made during 1954, on the settlement of *Ophelia bicornis* and referred to in the last report, have during the current year been fully considered, prepared for publication and published in Vol. 34, No. 3, of the *Journal*. The preliminary assessment already reported was found to be substantiated, and this work has therefore reached a stage where further development is not immediately possible. The next phase would appear to involve identification of the bacteria and other micro-organisms occurring on the sand grains of the natural adult habitat, their growth in pure cultures and the testing of their influence singly and mixed after transfer to acid-cleaned sands. Until this can be done progress is halted.

Collections made in the Helford River in 1949 by Mr G. M. Spooner and Mr Holme have been fully worked out, and the results have been supplemented by fresh collections made this year by Mr Spooner on the east side of the Fal Estuary, chiefly by digging and surface collecting. The seaward ends of the estuaries in this part of Cornwall contain a rich fauna, about which our knowledge has been incomplete. Interesting comparisons with the more restricted grounds at Salcombe and Noss Mayo can be made. One unexpected

find was *Myxicola infundibulum* flourishing in clean sand, and a populous bed of *Labidoplax digitata*.

Mr Spooner has also studied the three British species of the Clunioninae (an aberrant sub-family of Chironomidae), particularly with a view to determining the characters of the larvae and to obtain a clearer picture of their distribution on the shore. The larva of each species proves, happily, to possess diagnostic features by which it can be separated from the other two and from any orthocladine gnat. *Psammathiomyia pectinata*, a lusitanian species of exposed rocky shores, seldom hitherto observed, was discovered in two new localities on the north coast of Cornwall. Emergences of adults are evidently very sporadic, and the duration of adult life is found to be only 1 or 2 h. The larvae, now observed and recognized for the first time, will provide the best means of detecting the species on the shore.

The larvae of *Clunio marinus* are frequent amongst algal collections from rocks in the Plymouth area, and the species is evidently more general than would be judged by observation of adults. *Thalassomyia frauenfeldi* appears to be restricted to *Enteromorpha* patches in the upper half of the tidal zone: adults emerge throughout the winter, and can be found on sheltered rocks in the stormiest or coldest period.

Specimens of the *Gobius* sp., collected by Mr G. R. Forster while diving off Rame Head, have been examined by Mr Corbin. The species is certainly new to the British list and very probably new to the European list. Mr Corbin has also been collecting data of another *Gobius* sp. close in appearance to the Rock Goby, *G. paganellus*, but distinct in several characteristics, particularly its off-shore habitat, where it is not infrequently taken in the Agassiz trawl or fine mesh cover of the otter trawl. It is not recorded in the British list, but may possibly prove to be among the recorded European species.

Mr Corbin has recently been examining the hermit crabs taken in Agassiz trawl hauls in order to assess the relative abundance of the Plymouth species. *Anapagurus hyndmanni*—unrecorded since 1912—is, together with *A. laevis*, one of the most abundant species: 150–200 of each may be caught in a 15 min haul. Small *Eupagurus prideauxi* are about half as numerous. *E. cuanensis* is caught regularly in small numbers—under half a dozen. Small *E. bernhardus* are even less numerous off-shore in contrast to their intertidal abundance in periwinkle shells. *E. sculptimanus* is the least common of the listed species: five specimens were found in 1955. One specimen of *Catapaguroides timidus*, previously unrecorded, was taken. The foraminiferan, *Astorbhiza limicola*, was also found in the Agassiz hauls: it is also unrecorded for the area. It occurs in fair numbers and is of considerable size, up to 1.5 cm diameter, and may thus well be an important food organism for bottom feeders.

Mr N. A. Holme has continued work on the ecology of lamellibranchs, particular attention being paid to the distribution of certain species southward from the British Isles. In April 1955 a week was spent shore-collecting on



sandy beaches in S. Brittany, most of the collections being from sheltered shores in Quiberon Bay. The fauna here is little different from that in south-west England, but the abundance of the lusitanian species *Sipunculus nudus* and the relatively higher level at which low-tide species occurred on the shore were noted. In June a cruise on R.V. *Sarsia*, using the bottom-sampler, was devoted to a study of the off-shore fauna of the area. Samples were taken in the Grande Vasière and in shallow water inside Belle Ile, in Quiberon Bay, and off Lorient. All these collections will be used for a general study of the distribution of lamellibranchs, with special reference to particle size of the soil.

Further trials of the new 'vacuum' grab have been made, and the instrument has been tested to a depth of 448 m with success. Some modifications to the original grab have been made to aid vertical descent through the water. A description of the grab appeared in Vol. 34, No. 3, of the *Journal*.

Mr G. R. Forster has completed notes on the sponge *Hemimyscale columella*, and on the differences between the sublittoral rock fauna of Plymouth and Dartmouth, and these have been published in Vol. 34, Nos. 2 and 3, of the *Journal*.

In 1955 the underwater collecting from the yacht *Sunset* has been continued, thirty-nine dives having been made from July till October, including five near St Helier, Jersey, and six in Falmouth Bay. Further samples of sessile animals have been obtained, and in addition several living specimens of an unusual goby, previously referred to in this report, which is conspicuously marked by large red or brown spots.

In April Mr Forster spent a week shore-collecting from the Station Biologique de Roscoff, and much help was received in the identification of sponges. One sponge, *Stelletta grubei*, well known at Roscoff, has since been taken on the Eddystone reef.

An annual population sample of young prawns, *Palaemon* (= *Leander*) *serratus*, has been taken each year since 1949, to ascertain whether the growth of the young O group prawns is related to variations in temperature. From July to October 1955, the mean of the daily sea-surface temperatures was 2.0° C higher than that of 1954, but the mean length of the O group prawns showed only a very slight increase over the previous year.

Dr A. J. Southward, on a D.S.I.R. research grant, is studying the distribution, breeding and ecology of intertidal animals. Regular observations on the barnacle populations around Plymouth, and elsewhere in Britain, have made it clear that, following a recession up to 1951-52, the northern species, *Balanus balanoides*, is now returning in some numbers to the south-west of England. The recent fluctuations in the distribution and abundance of this barnacle, and of the southern species *Chthamalus stellatus*, are discussed in a contribution to the *Journal*, Vol. 35, No. 1, written in collaboration with Dr D. J. Crisp of the Marine Biology Station, Bangor. Dr Southward and Dr Crisp are also preparing a series of papers on the distribution of the British intertidal fauna;

the first will deal with the English Channel coasts. In collaboration with Dr J. M. Dodd of St Andrews University, Dr Southward is preparing for publication the late Prof. J. H. Orton's work on the biology and breeding of British limpets. An introductory note, and a description of the breeding of the common limpet have been contributed to the *Journal*.

With his wife, Dr Eve C. Southward, Dr Southward is investigating the breeding of the lugworms *Arenicola ecaudata* and *A. branchialis*, which live on the shore in muddy gravel under stones. Specimens containing gametes can be found all the year round, and the spawning periods appear to be longer than in the sand-living *A. marina*. So far, there seems little relation between the geographical distribution of these animals and their breeding periods.

Dr Southward and Mr Forster are studying the biology of the barnacle *Pyrgoma anglicum*, which grows on living cup-corals. The barnacle shell is intimately mingled with the coral skeleton, and is covered by a layer of coral tissue except round the aperture. It is hoped to find out the nature of the relationship between the two animals, and to work out the breeding and settlement of the barnacle.

Dr Southward visited the Isles of Scilly during his distributional work. As the marine fauna of these Islands is not fully known, some general faunistic surveys were made with Dr Eve C. Southward. It was discovered or confirmed that several intertidal animals common in Britain are absent or very rare in the Isles of Scilly. These Islands are also interesting for the occurrence there intertidally of some animals found otherwise only sublittorally. A preliminary fauna list is in preparation, and it is hoped to make further visits in collaboration with Mr Forster.

Dr Southward is also investigating the physiology and behaviour of barnacles. Papers on the frequency of cirral beat, and the effects on it of temperature and habitat have been published in Vol. 34, No. 3, of the *Journal*.

Preliminary experiments have shown that barnacles can feed on a wide range of organisms, from 1 mm down to  $2\mu$  in length, and it is proposed to compare the rate of feeding, and respiration, of the different species. The cirral mechanisms, and the reactions of the cirri to water movements are being studied cinematographically in collaboration with Dr D. J. Crisp.

#### *Physiology of Marine Organisms*

Utilizing the luminescent response as an indicator of neuro-effector activity, Dr J. A. C. Nicol has continued his researches into neural functioning among invertebrates. Some of the earlier physiological studies of luminescence were made on sea pens (Pennatulacea), and it has now become possible to secure quantitative information about the luminescent responses of these animals by the use of photo-electric instruments. Parameters of the lumi-

nescent response and conduction velocity in the nerve net have been measured, and analyses made of neuro-neural and neuro-photocyte transmission (*Renilla*, *Leioptilus* and *Pennatula*). An account of part of this work has appeared in the *Journal of Experimental Biology*, Vol. 32, p. 299 (1955). Particular attention is now being paid to investigating the responses of single zooids. Another paper, in collaboration with Dr D. Davenport, in the *Proceedings of the Royal Society*, Vol. 144, describes the luminescent responses of Hydromedusae, especially *Aequorea*. Light is emitted by patches of photogenic tissue in the marginal canal, and appears as brief flashes, *ca.* 1 sec in duration. Although no evidence was obtained for propagation of luminescent waves, it was found that the intensity of the separate flashes was influenced by the character of stimuli, in the same manner as in the neuromuscular system of other coelenterates. Dr Nicol has also contributed a review paper to a symposium on luminescence, which has appeared in *The Luminescence of Biological Systems*, p. 299, published by the American Association for the Advancement of Science, 1955.

Dr J. S. Alexandrowicz has completed his study on receptor elements in the muscles of *Leander serratus*. In the 1st to 5th abdominal segments two muscle receptor organs have been found on each side. An unusual arrangement is shown in the median receptors in the 1st and 2nd segments in which the two nerve cells are connected with one long receptor muscle running through two segments. In the thorax only two receptor muscles have been found, one of which connects with four nerve cells. It is suggested that the three supernumerary cells may be remnants of the retrograding receptor organs in the thoracic segments which are fusing together and losing their motility. Receptor elements of a different category, the N-cells, have been found, five in number, sending their dendritic expansions into one of the thoracico-abdominal muscles. It has been established that their axons join the nerve trunks of the 3rd-7th thoracic segments. The results are being published in the *Journal*.

A further part of the 'Notes on the nervous system in the Stomatopoda', dealing with the sensory elements of *Squilla mantis*, has been prepared for publication. It has been found that in these animals, besides sensory cells of the common type, i.e. with short single distal processes, there are cells with very long branching processes spreading their terminal ramifications under the epithelium of the integument. A description of these elements and a survey of various types of sensory nerve cells in stomatopods is being published in *Pubblicazioni della Stazione Zoologica di Napoli*.

Dr D. B. Carlisle has continued work on the endocrinology of crustaceans, particularly on a water-balance or antidiuretic hormone which he has isolated in *Carcinus*. This hormone, emanating from the X organ-sinus gland complex, plays a part in restraining the amount of size increase at each moult and in osmo-regulation, besides its antidiuretic function. It is not one of the two known moulting hormones. In collaboration with Dr D. Lacy of the Depart-



ment of Zoology, St Bartholomew's Medical College, an investigation has begun into the ultracytology of the 'kidney' of *Carcinus* and into the changes which it undergoes upon hormonal stimulation.

Dr Carlisle's collaboration with Mme M. Dupont-Raabe of the Sorbonne, Paris, and Sir Francis Knowles of Marlborough into the endocrinology of colour change in *Leander* and the stick-insect *Carausius*, has been most profitable and has led to the separation and partial characterization of a number of chromactivating substances: this work is continuing. A preliminary account has been published in *C.R. Acad. Sci., Paris*, and a longer paper presenting some of the results has appeared in Vol. 34, No. 3, of the *Journal*.

The physiological evidence of differences in the populations of *Leander* from Plymouth and Roscoff, reported last year, have been further confirmed, and morphological differences have been found also. An account has appeared in Vol. 34, No. 3, of the *Journal*.

Dr Carlisle, in collaboration with Dr C. G. Butler of the Bee Department, Rothamsted Agricultural Research Laboratory, has begun an investigation of the ovarian inhibiting hormone of prawns and the 'queen-substance' of bees. These substances appear, from the preliminary experiments, to be similar in nature and function, and may possibly even be identical. A preliminary account has been published in *Nature*.

In collaboration with Dr Lacy and Dr A. J. Southward, Dr Carlisle has begun an investigation into the micro-anatomy, cytology, and possible function of a presumed endocrine organ in the pedal ganglion of *Patella*.

Dr Carlisle has continued his work on the Plymouth tunicates, concentrating especially on an investigation of the locomotory powers of adult ascidians.

Dr B. C. Abbott has continued to study the uptake from the sea of certain radioactive products of uranium fission. Experiments on the radioactive yttrium have been concluded: the uptake of algae is by adsorption and is accentuated by the tendency of yttrium to form radiocolloids and to precipitate. Attention has also been concentrated on the fission product Niobium 95. In conjunction with Dr Carlisle the uptake of niobium has been compared with that of radio-vanadium (obtained by courtesy of the Physics Department of Birmingham University), both by cellophane surfaces and by several species of ascidians.

Measurements of the thermal and mechanical properties of lamellibranch muscles have been continued, partly in conjunction with Dr J. Lowy of Queen's University, Belfast. Heat production has been demonstrated during the long maintained contractions induced in the *Mytilus* anterior byssus retractor muscle by the addition of acetylcholine. Isolated muscle and nerve-muscle preparations from other lamellibranchs are under review.

Thermal measurements are also being carried out on nerve tissue under the guidance of Prof. A. V. Hill, and in conjunction with Mr J. V. Howarth

of University College, London. The initial heat production in the motor nerves of crabs, associated with brief tetanic stimulation, has been measured with thermopile and fast, sensitive recording equipment.

Dr E. D. S. Corner, International Paints Research Fellow, is making an investigation of the effects of heavy metal poisons on crustaceans.

Preliminary studies have been concerned with the changes which occur in the respiration rate and motility of *Artemia salina* larvae when these animals are placed in sea water containing copper, mercury, and other poisons. The results so far obtained indicate that observed differences between the toxicities of various compounds of mercury (e.g. mercuric chloride, mercuric iodide and ethylmercury chloride) may be attributed to differences in the ease with which the respective substances penetrate the animal to produce their effects internally. There are also indications that the mode of action of copper may differ from that of mercury.

By courtesy of Mr O. D. Hunt, experiments have also been conducted in the International Paints Research Laboratory at Newton Ferrers. In collaboration with Mr B. W. Sparrow, a study has been made of the effects of various organomercurial compounds, and of other poisons on the copepod *Acartia clausi*. The results of this investigation are consistent with the view that the mercury compounds so far studied act by penetrating the animal and inhibiting metabolic changes.

It is hoped to extend these studies by testing different compounds of mercury, and of copper, as inhibitors of isolated enzymes and enzyme systems.

Before he left the staff in September, Dr H. G. Vevers was able to continue his work on the pigmentation of marine invertebrates. In addition to work on the carotenoid pigments of certain echinoderms, he has continued his collaboration with Dr G. Y. Kennedy, of the University of Sheffield, on the occurrences of porphyrins in marine animals. They have prepared a paper on the presence of uroporphyrin I in the tectibranch mollusc *Akera bullata*, which has been published in Vol. 35, No. 1, of the *Journal*.

#### The 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.

The Library has again been much used by visiting members of the Association.

The new library extension has been in use during the year. Miss L. M. Serpell, with the assistance of Dr J. A. C. Nicol, has been much occupied in planning the reclassification and rearrangement of the books and periodicals.

## Published Memoirs

Volume 34, No. 2, of the *Journal* was published in June 1955, No. 3 in October 1955 and Volume 35, No. 1, in February 1956.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

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- BLASCHKO, H. & HOPE, D. B., 1956. The oxidation of L-amino acids by *Mytilus edulis*. *Biochem. J.*, Vol. 62, pp. 335-9.
- BRULL, L. & CUYPERS, Y., 1955. Blood perfusion of the kidney of *Lophius piscatorius* L. III. Action of CO<sub>2</sub>, cyanide and fluoride. *Arch. Int. Physiol. Biochim.*, Vol. 63, p. 138.
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- CROFTS, D. R., 1955. Muscle morphogenesis in primitive gastropods and its relation to torsion. *Proc. zool. Soc. Lond.*, Vol. 125, pp. 711-50.
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- DAVENPORT, D. & NICOL, J. A. C., 1956. Observations on luminescence in sea pens (Pennatulacea). *Proc. roy. Soc., Ser. B*, Vol. 144, pp. 480-96.
- FRANKENHAEUSER, B. & HODGKIN, A. L., 1956. The after-effects of impulses in the giant nerve fibres of *Loligo*. *J. Physiol.*, Vol. 131, pp. 341-76.
- HARVEY, H. W., 1955. *The Chemistry and Fertility of Sea Waters*, 224 pp. Cambridge.
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- SMITH, R. I., 1955. Comparison of the level of chloride regulation by *Nereis diversicolor* in different parts of its geographical range. *Biol. Bull., Woods Hole*, Vol. 109, pp. 453-74.
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#### Membership of the Association

The total number of members on 31 March 1956 was 861, being 34 more than on 31 March 1955; of these the number of life members was 98 and of annual members 763. The number of associate members is five.

## 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 received during the year:

From the Fishmongers' Company (£400), 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. 10s.), Leeds (£20), Durham (£10. 10s.), Manchester (£10. 10s.), Sheffield (£10. 10s.), Southampton (£15. 15s.), Reading (£10. 10s.), Nottingham (£10. 10s.), Hull (£10. 10s.), Exeter (£10. 10s.), Leicester (£10. 10s.), the Imperial College of Science and Technology (£10) and the Zoological Society of London (£10. 10s.).

## President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1956-57:

*President*

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

*Vice-Presidents*

The Earl of IVEAGH, K.G., C.B., C.M.G.	Admiral Sir AUBREY C. H. SMITH,
Sir NICHOLAS E. WATERHOUSE, K.B.E.	K.B.E., C.B., M.V.O.
Col. Sir EDWARD T. PEEL, K.B.E.,	A. T. A. DOBSON, C.B., C.V.O., C.B.E.
D.S.O., M.C.	Major E. G. CHRISTIE-MILLER
Vice-Admiral Sir JOHN A. EDGELL,	MORLEY H. NEALE, C.B.E.
K.B.E., C.B., F.R.S.	The Earl of VERULAM
Sir EDWARD J. SALISBURY, Kt., C.B.E.,	Prof. Sir JAMES GRAY, Kt., C.B.E., M.C.,
D.Sc., F.R.S.	Sc.D., LL.D., F.R.S.

## COUNCIL

*To retire in 1957*

Miss ANNA M. BIDDER, Ph.D.  
D. J. CRISP, Ph.D.  
Prof. J. E. HARRIS, Ph.D., F.R.S.

C. E. LUCAS, C.M.G., D.Sc.  
Prof. C. M. YONGE, C.B.E., D.Sc., F.R.S.

*To retire in 1958*

Miss VERA FRETTER, D.Sc.  
MICHAEL GRAHAM, C.M.G., O.B.E.  
N. A. MACKINTOSH, C.B.E., D.Sc.  
G. E. NEWELL, Ph.D.  
Prof. LILY NEWTON, D.Sc.

*To retire in 1959*

G. E. R. DEACON, C.B.E., D.Sc., F.R.S.  
M. N. HILL, Ph.D.  
O. D. HUNT  
Prof. R. J. PUMPHREY, Sc.D., F.R.S.  
Prof. G. P. WELLS, Sc.D., F.R.S.

*Hon. Treasurer*

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2.

*Secretary*

F. S. RUSSELL, C.B.E., D.S.C., D.F.C., F.R.S.  
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. WALL (Ministry of Agriculture, Fisheries and Food)	Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
The Worshipful Company of Fish- mongers:	S. SMITH, Ph.D. (Cambridge University)
The Prime Warden	EDWARD HINDLE, Sc.D., F.R.S. (British Association)
Major E. G. CHRISTIE-MILLER	N. B. MARSHALL (Zoological Society)
HARRISON S. EDWARDS	Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)



# BALANCE SHEET 1955-56

## THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

### BALANCE SHEET

31 MARCH 1956

	£	£
<b>CAPITAL RESERVE ACCOUNT:</b>		
As at 31st March 1955	170,972	
Add: Expenditure on fixed assets recovered	3,855	
		174,827
<b>SURPLUS ACCOUNT:</b>		
As at 31st March 1955	8,146	
Add: Excess of income over expenditure for the year	754	
	8,900	
Less: Provision for diminution in value of investment	44	
		8,856
		183,683
<b>BALANCES ON SPECIAL FUNDS (see annexed statement)</b>	9,009	
Less: Provision for diminution in value of investments	1,487	
		7,522
<b>CURRENT LIABILITIES:</b>		
Sundry creditors and accrued expenses	1,738	
Subscriptions and grants received in advance	151	
Equipment and apparatus—R.V. 'Sarsia'	—	
		1,889
<i>Note: Capital commitments outstanding amount to £10,193 (1955 £8,522) of which £7,555 will be recoverable under Development Fund grants.</i>		

D. J. CRISP }  
EDWARD HINDLE } *Members of the Council*

£193,094

	£	£
<b>FIXED ASSETS:</b>		
Boats and Equipment:		
At cost:		
R.V. 'Sarsia'	137,761	
At valuation as estimated by the Director at 31st March 1956:		
M.F.V. 'Sula'	12,500	
R.L. 'Gammarus'	200	
	150,461	
Laboratory apparatus, equipment and machinery:		
At cost	13,380	
Less: Depreciation	1,338	
	12,042	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost	21,600	
		184,103
<b>INVESTMENTS:</b>		
General Fund at book amount	232	
(Market value £188; last year £217)		
Composition Fees Fund at cost	1,330	
(Market value £1,031; last year £1,110)		
E. T. Browne Bequest Funds at cost	4,368	
(Market value £3,180; last year £3,440)		
	5,930	
Less: Provision for diminution in value of investments	1,531	
		4,399
<b>CURRENT ASSETS:</b>		
Stocks on hand as valued by the Director	1,550	
Sundry debtors	885	
Prepayments	211	
Balances at bank and cash in hand	1,946	
		4,592
		<u>£193,094</u>

### 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 1956, 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.

Norwich Union House  
2 St Andrew's Cross  
Plymouth  
17 May 1956

PRICE WATERHOUSE & CO.  
Chartered Accountants

# INCOME AND EXPENDITURE ACCOUNT

	£	£
SALARIES, NATIONAL INSURANCE AND SUPERANNUATION SCHEME CONTRIBUTIONS ... ..		27,010
LABORATORY AND BOATS' CREWS' WAGES, NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND EMPLOYERS' LIABILITY INSURANCE ... ..		26,506
UPKEEP OF LIBRARY ... ..		699
SCIENTIFIC PUBLICATIONS, <i>less</i> SALES ... ..		1,666
UPKEEP OF LABORATORIES:		
Buildings and machinery ... ..	609	
Electricity, gas, coal and water ... ..	1,317	
Chemicals and apparatus ... ..	1,457	
Depreciation of Laboratory apparatus, equipment and machinery (no depreciation is provided on the other fixed assets)	1,338	
Rents and insurance ... ..	175	
Travelling expenses ... ..	685	
Audit fee (1955 includes £63 for previous year) ... ..	126	
Stationery, postage, telephone and sundries ... ..	1,235	
Specimens ... ..	195	
Collecting expenses and upkeep of truck ... ..	298	
		7,435
MAINTENANCE OF BOATS:		
Petrol, oil, paraffin etc. ... ..	1,721	
Maintenance and repairs ... ..	3,641	
Insurance ... ..	2,366	
Hire of Decca Navigator—R.V. 'Sarsia' ... ..	395	
		8,123
ENTERTAINMENT EXPENSES ... ..		54
BALANCE being excess of income over expenditure for the year ...		754
		<u>£72,247</u>

# FOR THE YEAR ENDED 31st MARCH 1956

	£	£
GRANTS AND TABLE RENTS:		
Ministry of Agriculture, Fisheries and Food Grant from Development Fund ... ..		64,970
Fishmongers' Company ... ..		400
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. 10s., Leeds £20, Southampton £15. 15s., Durham £10. 10s., Exeter £10. 10s., Leicester £10. 10s., Manchester £10. 10s., Nottingham £10. 10s., Hull £10. 10s., Reading £10. 10s. and Sheffield £10. 10s., Imperial College £10, Zoological Society of London £10. 10s., Ministry of Works £104, Imperial Chemical Industries Ltd. £52. 10s. and International Paints Ltd. £52. 10s.) ... ..	1,230	
		66,600
SUBSCRIPTIONS (excluding those received in advance) ... ..		777
SALES:		
Specimens ... ..		2,882
Fish ... ..		705
		£
Nets, gear and hydrographical apparatus ... ..	825	
<i>Less:</i> cost of materials ... ..	587	
		238
		3,825
SUNDRY PUBLICATIONS ... ..		18
INCOME FROM INVESTMENTS ... ..		49
INTEREST ON BANK DEPOSITS, <i>less</i> CHARGES ... ..		75
AQUARIUM:		
Admission fees ... ..		1,414
Sale of guides ... ..		89
		1,503
<i>Less:</i> Maintenance, printing and advertising ... ..		600
		903
		<u>£72,247</u>

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31st MARCH 1956

	Aquarium Sinking Fund £	Com- position Fees £	E. T. Browne Bequest			A. R. T. Mombet Bequest £	Rockefeller Foundation Fund £	War Damage Com- pensation Part II £	Library Extension and Dogfish House £	'Plymouth Marine Fauna' Fund £	M.F.V. 'Sula' Winch £	Reservoir and Sea Water Tanks £	Research Funds* £	TOTAL £
BALANCE AT 31st MARCH 1955	341	1,251	1,335	2,876	(21)	1,090	518	45	1,567	—	—	—	1,212	10,214
Add: Income during year:														
Grants	—	—	—	—	—	—	2,680	—	4,375	750	2,000	1,885	2,429	14,119
Income from investments	—	—	41	83	7	—	—	—	—	—	—	—	—	131
Bank deposit interest	10	—	—	—	1	34	—	—	—	—	—	—	—	45
Other income	35	78	—	—	377	—	—	—	—	—	—	—	—	490
Transfers-Income and Expenditure account	—	—	—	—	—	—	—	—	—	—	5	—	5	10
	386	1,329	1,376	2,959	364	1,124	3,198	45	5,942	750	2,005	1,885	3,646	25,009
Deduct: Expenditure	—	—	—	60	—	—	3,207	45	4,589	753	2,005	1,885	3,456	16,000
BALANCE AT 31st MARCH 1956	<u>£386</u>	<u>£1,329</u>	<u>£1,376</u>	<u>£2,899</u>	<u>£364</u>	<u>£1,124</u>	<u>(£9)</u>	<u>—</u>	<u>£1,353</u>	<u>(£3)</u>	<u>—</u>	<u>—</u>	<u>£190</u>	<u>£9,009</u>

\* Including International Paints Ltd. Research Fellowship.



# LIST OF GOVERNORS, FOUNDERS, MEMBERS, HONORARY AND ASSOCIATE MEMBERS

1956

## GOVERNORS

- THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, Burlington House, W. 1  
 THE UNIVERSITY OF OXFORD  
 THE UNIVERSITY OF CAMBRIDGE  
 THE WORSHIPFUL COMPANY OF CLOTHWORKERS, 48 Fenchurch Street, E.C. 3  
 THE WORSHIPFUL COMPANY OF FISHMONGERS, London Bridge, E.C. 4  
 THE PRIME WARDEN. (**Council**, 1886→)  
 EDWARDS, HARRISON S., Westhumble Lacey, near Dorking, Surrey. (**Council**, 1950→; **Hon. Treasurer**, 1956→)  
 CHRISTIE-MILLER, Major E. G., 38 Hyde Park Street, W. 2. (**Council**, 1941→; **Hon. Treasurer**, 1941→56; **Vice-President**, 1951→)  
 THE ZOOLOGICAL SOCIETY OF LONDON, Regent's Park, N.W. 1  
 THE ROYAL SOCIETY, Burlington House, Piccadilly, W. 1  
 MINISTRY OF AGRICULTURE, FISHERIES & FOOD, 3 Whitehall Place, S.W. 1  
 BAYLY, ROBERT (the late). (**Council**, 1896-1901)  
 BAYLY, JOHN (the late)  
 BROWNE, E. T. (the late). (**Council**, 1913-19; 1920-37)  
 THOMASSON, J. P. (the late). (**Council**, 1896-1903)  
 BIDDER, G. P., Sc.D. (the late). (**Council**, 1899-1953; **President**, 1939-45; **Vice-President**, 1948-53)  
 THE LORD MOYNE, P.C., D.S.O. (the late). (**Vice-President**, 1929; 1939-45; **President**, 1930-39)  
 ALLEN, E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary). (**Council**, 1895-1942; **Secretary**, 1895-1936; **Hon. Governor**, 1937-42)

## FOUNDERS

- 1884 THE CORPORATION OF THE CITY OF LONDON, The Guildhall, E.C. 3  
 1884 THE WORSHIPFUL COMPANY OF MERCERS, Mercers' Hall, 4 Ironmonger Lane, E.C. 2  
 1884 THE WORSHIPFUL COMPANY OF GOLDSMITHS, Goldsmiths' Hall, Foster Lane, E.C. 2  
 1884 THE ROYAL MICROSCOPICAL SOCIETY, B.M.A. House, Tavistock Square, W.C. 1  
 1884 BULTEEL, THOS. (the late)  
 1884 BURDETT-COUTTS, W. L. A. BARTLETT (the late)  
 1884 CRISP, Sir FRANK, Bart. (the late). (**Council**, 1884-92; **Hon. Treasurer**, 1884-88)  
 1884 DAUBENY, Captain GILES A. (the late)  
 1884 EDDY, J. RAY (the late)  
 1884 GASSIOTT, JOHN P. (the late)  
 1884 LANKESTER, Sir E. RAY, K.C.B., F.R.S. (the late). (**Hon. Secretary**, 1884-90; **President**, 1891-1929)

- 1884 Lord MASHAM (the late)  
 1884 MOSELEY, Prof. H. N., F.R.S. (the late). (**Chairman of Council**, 1884-88)  
 1884 Lord AVEBURY, F.R.S. (the late). (**Vice-President**, 1884-1913)  
 1884 POULTON, Prof. Sir EDWARD B., F.R.S. (the late). (**Council**, 1888-94)  
 1884 ROMANES, Prof. G. J., LL.D., F.R.S. (the late). (**Council**, 1884-91)  
 1884 WORTHINGTON, JAMES (the late)  
 1885 The 15th EARL OF DERBY (the late)  
 1887 WELDON, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890-1901; representing British Association, 1901-5)  
 1888 BURY, HENRY, The Gate House, 17 Alumdale Road, Bournemouth West  
 1888 THE WORSHIPFUL COMPANY OF DRAPERS, Drapers' Hall, E.C. 2  
 1889 THE WORSHIPFUL COMPANY OF GROCERS, Grocers' Hall, Princes Street, E.C. 2  
 1889 THOMPSON, Sir HENRY, Bart. (the late). (**Vice-President**, 1890-1903)  
 1889 Lord REVELSTOKE (the late)  
 1890 RICHES, T. H. (the late). (**Council**, 1920-25)  
 1892 BROWNE, Mrs E. T. (the late)  
 1898 WORTH, R. HANSFORD, M.Inst.C.E. (the late)  
 1899 The EARL OF IVEAGH, K.G., C.B., C.M.G., 11 St James's Square, S.W. 1  
 (**Vice-President**, 1929→)  
 1902 GURNEY, ROBERT, D.Sc. (the late). (**Council**, 1932-5)  
 1904 SHAW, JOSEPH, K.C. (the late)  
 1909 HARDING, Colonel W. (the late)  
 1910 MURRAY, Sir JOHN, K.C.B., F.R.S. (the late). (**Council**, 1896-99; **Vice-President**, 1900-13)  
 1912 SWITHINBANK, H. (the late)  
 1913 SHEARER, Dr CRESSWELL, F.R.S. (the late)  
 1913 HERON-ALLEN, E., F.R.S. (the late)  
 1918 EVANS, GEORGE (the late). (**Hon. Treasurer**, 1915-31; **Vice-President**, 1925-33)  
 1920 McCLEAN, Capt. W. N., 39 Phillimore Gardens, W. 8  
 1920 Lord BUCKLAND OF BWLCH (the late)  
 1920 LLEWELLYN, Sir D. R. (the late)  
 1921 HARMER, F. W. (the late)  
 1924 THE MACFISHERIES, LTD., Ocean House, Pudding Lane, E.C. 3  
 1924 Lady MURRAY (the late)  
 1925 THE INSTITUTION OF CIVIL ENGINEERS, Great George Street, Westminster, S.W. 1  
 1925 DISCOVERY COMMITTEE  
 1927 BIDDER, Miss ANNA M., Ph.D., 6A Hills Avenue, Cambridge. (**Council**, 1948-51, 1954→)  
 1933 PEEL, Col. Sir EDWARD T., K.B.E., D.S.O., M.C., c/o Messrs Peel and Co., Ltd. P.O. Box 331, Alexandria, Egypt. (**Vice-President**, 1936→)  
 1938 BUCHANAN, Dr FLORENCE (the late)  
 1945 BROWN, ARTHUR W. W. (the late)

## MEMBERS

### \* Life Members

- 1949 ABBOTT, B. C., Ph.D., F.Inst.P., The Laboratory, Citadel Hill, Plymouth, Devon  
 1945 ABERDEEN UNIVERSITY LIBRARY, The University, Aberdeen  
 1934 ADAM, Mrs K. M. G., 84 Lasswade Road, Edinburgh 9

- 1951 ADAMS, E., 2 Woodford Crescent, Marsh Mills, Plympton, Devon
- \*1954 ADAMS, Miss M. N. E., 61 Ethelburt Avenue, Swaythling, Southampton.
- 1940 LORD ADRIAN, O.M., M.D., D.Sc., LL.D., F.R.S., The Master's Lodge, Trinity College, Cambridge
- 1947 AFFLECK, R. J., 1 Helmsdale Road, London, S.W. 16
- 1950 ALEXANDROWICZ, J. S., Ph.D., M.D., The Laboratory, Citadel Hill, Plymouth, Devon
- 1954 ALLEN, G. L., The Nook, 87A Bury Old Road, Sedgley Park, Prestwich, Manchester
- 1951 ALLEN, J. A., Dove Marine Laboratory, Cullercoats, Northumberland
- 1952 ALLEN, Miss J. M., Tenements Farm, Chipperfield, Herts
- 1953 ALVARINO, Señora A., Doce de Octubre 11-1 °C, Madrid, Spain
- \*1927 AMIRTHALINGAM, C., Ph.D., 2 Dickmans Path, Colombo, Ceylon
- 1956 ANSELL, A. D., 22 Mannion Road, Henley-on-Thames, Oxon
- 1950 ARNOLD, D. C., Gatty Marine Laboratory, The University, St Andrews, Fife
- 1944 ASHBY, D. G., Littlecroft, High Street, Harston, Cambs
- 1954 ASHHURST, Miss D. E., Heron Court, Alexandra Road, Epsom, Surrey
- \*1929 ATKINS, Miss D., D.Sc., c/o The Laboratory, Citadel Hill, Plymouth, Devon
- \*1939 ATKINS, W. R. G., C.B.E., O.B.E. (mil.), Sc.D., F.R.I.C., F.Inst.P., F.R.S., The Old Vicarage, Antony, Torpoint, Cornwall
- \*1910 ATKINSON, G. T., Gresham House, Esplanade, Lowestoft, Suffolk
- 1951 ATLANTIC BIOLOGICAL STATION, St Andrews, N.B., Canada
- 1948 BAAL, H. J., 3 Bel Royal Villas, Jersey, C.I.
- 1950 BAERENDS, Prof. G. P., Zoölogisch Laboratorium, Rijksstraatweg 78, Haren (Gron.), Holland
- 1949 BAGENAL, T. B., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1956 BAILEY, Miss J. A., 110 Cambridge Road, North Harrow, Middx
- \*1952 BAILY, JOSHUA L. Jr., 4435 Ampudia Street, San Diego 3, California, U.S.A.
- 1950 BAINBRIDGE, R., Ph.D., 43 Strathmore Avenue, Hull
- 1953 BAINBRIDGE, V., West African Fisheries Research Institute, Freetown, Sierra Leone
- \*1920 BAKER, J. R., D.Sc., Dept. of Zoology and Comparative Anatomy, University Museum, Oxford
- 1936 BALDWIN, Prof. E., Ph.D., Dept. of Biochemistry, University College, Gower Street, London, W.C. 1 (Council, 1946-48)
- 1950 BALLANTINE, Miss D., The Laboratory, Citadel Hill, Plymouth, Devon
- 1955 BALLANTINE, W. J., A3 Downing College, Cambridge
- 1953 BARKER, J. A., 27 Finchley Way, Finchley, London, N. 3
- 1949 BARNARD, E. E. P., 7 Webster Gardens, Ealing, London, W. 5
- 1956 BARNARD, J. LAURENS, Ph.D., Allan Hancock Foundation, University of California, Los Angeles 7, California, U.S.A.
- 1939 BARNES, H., D.Sc., F.R.I.C., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1954 BARNES, M. McC., Mandeville, Rosebank Crescent, Pennsylvania, Exeter, Devon
- 1955 BARNETT, P. R. O., Cloudshill, Eaton Avenue, Allestree, Derby
- 1953 BARNS, H. N., Spearwood, Combpyne, near Axminster, Devon
- 1939 BARRINGTON, Prof. E. J. W., D.Sc., Dept. of Zoology, The University, Nottingham
- 1951 BARRON, H., 65 Sumerton Road, Belfast, N. Ireland
- 1939 BASSINDALE, R., Dept. of Zoology, The University, Bristol



- 1946 BATHAM, Miss E. J., Ph.D., Portobello Marine Biological Station, Portobello, Otago, New Zealand
- 1950 BAUGHMAN, J. L., Las Olas Oceanographic Foundation, Rockport, Texas, U.S.A.
- 1939 BAXTER, E. W., Biology Dept., Medical School, Guy's Hospital, London, S.E. 1
- \*1929 BAYLIS, L. E., Ph.D., Dept. of Physiology, University College, Gower Street, London, W.C. 1
- 1934 BEADLE, L. C., Dept. of Biology, University College of East Africa, P.O. Box 262, Kampala, Uganda
- 1955 BEALL, I. D., 19 Beresford Street, Stoke, Plymouth, Devon
- 1928 BEER, Sir GAVIN DE, Kt., D.Sc., F.R.S., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1955 BEESON, Miss G., Redgate, 216 Unthank Road, Norwich, Norfolk
- 1954 BELCHER, J. H., 197 Risley Avenue, Tottenham, London, N. 17
- 1950 BELL, Mrs E. B., Solva, Glanford Road, Brigg, Lincs
- 1954 BERNER, L. D. Jr., Scripps Institution of Oceanography, La Jolla, California, U.S.A.
- 1947 BERRILL, Prof. N. J., F.R.S., Dept. of Zoology, McGill University, Montreal, Canada
- 1955 BERRY, Miss P. M., Fairhaven, Hanwell, Banbury, Oxon
- 1955 BERRY, R. J., 42 Liverpool Road, Penwortham, near Preston, Lancs
- 1947 BEST, A. C. G., c/o The Laboratory, Citadel Hill, Plymouth, Devon
- 1953 BHATTACHARYYA, Dr R. N., 44/B Kalighat Road, Calcutta 26, India
- 1956 BHAVANARAYANA, P. V., Zoological Laboratories, Andhra University, Waltair, S. India
- 1953 BHIMACHAR, B. S., D.Sc., F.N.I., Central Inland Fisheries Research Station, 47/1 Strand Road, Calcutta 7, India
- 1903 BIDDER, Col. H. F., The Malting House, Nettlebed, near Henley-on-Thames, Oxon
- \*1945 BINGLEY, F. J., Flatford Mill Field Centre, East Bergholt, near Colchester, Essex
- 1955 BINYON, E. J., 44 Kendall Avenue South, Sanderstead, Surrey
- 1925 BIRKBECK COLLEGE, The Library, Malet Street, London, W.C. 1
- 1951 BIRKETT, L., Fisheries Laboratory, Lowestoft, Suffolk
- 1947 BISHOP, M. W. H., Meadow Farm, Waterbeach, Cambs
- 1945 BLACK, J. A., Ash House, Caton, near Lancaster, Lancs
- 1951 BLACKBURN, Prof. M., D.Sc. Dept. of Zoology, University of Hawaii, Honolulu 10, Hawaii, U.S.A.
- 1955 BLAKE, M. L., Picket Piece, Avening, near Stroud, Glos
- 1930 BLASCHKO, Dr H., Dept. of Pharmacology, South Parks Road, Oxford
- 1952 BLAXTER, J. H. S., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1955 BLOOM, Miss J. L., 88 Pennsylvania Road, Exeter, Devon
- 1910 BLOOMER, H. H., Longdown, Sunnysdale Road, Swanage, Dorset
- 1953 BOALCH, G. T., Westfield, Beer, near Seaton, Devon
- 1951 BODEN, B. P., Ph.D., Scripps Institution of Oceanography, La Jolla, California, U.S.A.
- 1947 BOËTIUS, Dr JAN, Fysiologisk Laboratorium, Danmarks Akvarium, Charlottenlund, Denmark
- 1936 BOGUE, Prof. J. YULE, D.Sc., Heyscroft, Hartley Road, Altrincham, Cheshire
- 1932 BOLITHO, Capt. R. J. B., Gorey, Jersey, C.I.
- 1945 BONEY, A. D., 24 Ford Park Road, Mutley, Plymouth, Devon

# LIST OF GOVERNORS, FOUNDERS, AND MEMBERS 675

- 1954 BONHAM, Dr K., Applied Fisheries Laboratory, University of Washington, Seattle, Wash., U.S.A.
- \*1933 BOSCHMA, Prof. Dr H., Rijksmuseum van Natuurlijke Historie, Leiden, Holland
- 1947 BOSSANYI, J., Dove Marine Laboratory, Cullercoats, Northumberland
- 1954 BOWERS, A. B., Marine Biological Station, Port Erin, Isle of Man
- 1955 BRADLEY, D. J., 71 Linden Drive, Evington, Leicester
- \*1954 BRADSHAW, J. S., P.O. Box 891, Del Mar, California, U.S.A.
- 1940 BRAMBELL, Prof. F. W. ROGERS, D.Sc., F.R.S., Dept. of Zoology, University College of North Wales, Bangor, Caern. (Council, 1944-47, 1948-51)
- 1954 BREHAUT, R. N., La Canurie, Collings Road, St Peter Port, Guernsey, C.I.
- 1924 BRIGHTWELL, L. R., 1 Edith Avenue, Peacehaven, Sussex
- 1933 BRISTOL UNIVERSITY, Dept. of Zoology, Bristol
- \*1941 BRITISH CELANESE LTD., Celanese House, Hanover Square, London, W. 1
- 1939 BRITISH ROPES LTD., Western Avenue, Cardiff
- 1955 BRITISH SUB-AQUA CLUB, 79 Great Titchfield Street, London, W. 1
- \*1946 BROCK, Mrs C. H., Ph.D., 32 Barton Road, Cambridge
- 1953 BROCKMAN, Miss P. E., Pathology Dept., South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon
- 1946 BROUGH, Prof. JAMES, D.Sc., Dept. of Zoology and Comparative Anatomy, University College, Newport Road, Cardiff
- 1955 BROWN, C. A., Lynton, Tolcarne Road, Newquay, Cornwall
- 1928 BROWN, Miss E. M., Ph.D., 6 Effingham Lodge, Surbiton Crescent, Kingston-on-Thames, Surrey
- 1936 BROWN, HERBERT H., O.B.E., Ph.D., Fisheries Division, F.A.O.U.N., Viale delle Terme di Caracalla, Rome, Italy
- 1955 BRUCE, Dr A. J., Pembroke County War Memorial Hospital, Haverfordwest
- \*1954 BRULL, Prof. L., Institut de Clinique et de Policlinique Médicales, Hôpital de Bavière, Université de Liège, Liège, Belgium
- 1953 BUCHSBAUM, Prof. R., Ph.D., Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pa., U.S.A.
- 1953 BUCKEE, R. E., 32 Weston Drive, Stanmore, Middx
- \*1925 BULL, HERBERT O., D.Sc., Dove Marine Laboratory, Cullercoats, Northumberland
- 1955 BURCH, J. Q., 1584 West Vernon Avenue, Los Angeles 62, California, U.S.A.
- 1951 BURGESS, G. H. O., D.S.I.R., Humber Laboratory, Wassand Street, Hull
- 1954 BURLEY, Miss E. A. M., Avondale, 2 Narborough Road South, Leicester
- 1948 BURROWS, Mrs E. M., Ph.D., Hartley Botanical Laboratories, The University, Liverpool 3
- 1950 BURSA, A., c/o J. Kordowski, 206 W. 109 Street, New York 25, U.S.A.
- 1954 BURT, WAYNE V., Ph.D., Associate Prof. of Oceanography, Oregon State College, Corvallis, Oregon, U.S.A.
- 1947 BURTON, Miss J. M., Pincroft, Ockham Road North, East Horsley, Surrey
- 1930 BURTON, M., D.Sc., British Museum (Natural History), Cromwell Road, London, S.W. 7. (Council, 1936-39)
- 1947 BURTON, R. F., Pincroft, Ockham Road North, East Horsley, Surrey
- 1949 BUSH, Prof. S. F., D.Phil., Dept. of Zoology, University of Natal, Pietermaritzburg, S. Africa
- 1949 BUTCHER, A. W., Three Salmons Hotel, Usk, Mon
- 1950 BUTLER, Mrs B. R. R., c/o Trinidad Oil Company., G.P. and S. Division, Forest Reserve, Trinidad, B.W.I.

- 1955 CALDWELL, P. C., D.Phil., The Laboratory, Citadel Hill, Plymouth, Devon  
 1955 CALDWELL, Mrs P. C., c/o The Laboratory, Citadel Hill, Plymouth, Devon  
 1949 CAMERON, H. D., 5 Compton Park Road, Mannamead, Plymouth, Devon  
 \*1950 CAMERON, Prof. W. M., Institute of Oceanography, University of British Columbia, Vancouver, Canada  
 1920 CANNON, Prof. H. GRAHAM, Sc.D., F.R.S., Dept. of Zoology, Victoria University, Manchester. (**Council**, 1927-30, 1932-34, 1937-41, 1942-45, 1952-55)  
 1954 CANTERBURY MUSEUM, Rolleston Avenue, Christchurch, New Zealand  
 1950 CAPSTICK, C. K., Ph.D., The Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancs  
 1953 CARR-SAUNDERS, E. M., Bridge House, St John's Vale, Keswick, Cumb  
 1956 CARRIE, B. G. A., Carisbrooke, Milverton Road, Totton, Hants  
 1927 CARRUTHERS, J. N., D.Sc., F.Inst.P., National Institute of Oceanography, Wormley, near Godalming, Surrey. (**Council**, 1948-51, 1953-56)  
 1923 CARTER, G. S., Ph.D., Dept. of Zoology, Downing Street, Cambridge  
 \*1931 CATTELL, Dr McKEEN, Cornell University Medical College, 477 First Avenue, New York City, U.S.A.  
 \*1948 CATTLEY, J. G., Fisheries Laboratory, Lowestoft, Suffolk  
 1949 CENTRE DE RECHERCHES ET D'ETUDES OCÉANOGRAPHIQUES, 1 Quai Branly, Paris VII, France  
 1955 CEURVELS, A. RUSSELL, 1054 Hyde Park Avenue, Hyde Park 36, Mass., U.S.A.  
 \*1949 CHANG, H. W., Zoological Institute, Academia Sinica, 320 Yo Yang Road, Shanghai, China  
 \*1952 CHAPLIN, Viscount, Wadstray House, Blackawton, near Totnes, Devon  
 \*1949 CHAPMAN, GARTH, Ph.D., Nunns, Coxtie Green, Brentwood, Essex  
 1953 CHAPMAN, Mrs R. F., c/o International Red Locust Control Service, P.O. Box 37, Abercorn, N. Rhodesia.  
 1936 CHARTERHOUSE SCHOOL, Biological Dept., Godalming, Surrey  
 1947 CHENG, Prof. CHUNG, Ph.D., Dept. of Oceanography, National Amoy University, Amoy, China  
 \*1947 CHIDAMBARAM, K., Fisheries Biological Station, West Hill Post, South Malabar, India  
 1952 CHILDS, P., F.R.C.S., 8 The Crescent, Plymouth, Devon  
 1953 CHILE: Dr Parmenio Yañez Andrade, Director, Marine Biological Station of the University of Chile, Casilla 13-D, Viña del Mar, Chile.  
 1946 CHIPPERFIELD, PHILIP N. J., Ph.D., Hillside, Manor Road, Brixham, Devon  
 1951 CHRISTIE, I. G., Guy's Hospital, London, S.E. 1  
 1942 CHRISTIE-MILLER, Major E. G., 38 Hyde Park Street, London, W. 2. (**Council**, 1941-; **Hon. Treasurer**, 1941-56; **Vice-President**, 1951-)  
 1952 CLARK, J. T., 12 Hazel Avenue, Torquay, Devon  
 1952 CLARK, R. B., Dept. of Zoology, The University, Glasgow, W. 2  
 1951 CLARKE, Mrs J., 32 Boolarong Road, Pymble, Sydney, N.S.W., Australia  
 1949 CLARKE, K. U., Dept. of Zoology, The University, Nottingham  
 1944 CLARKE, ROBERT H., National Institute of Oceanography, Wormley, near Godalming, Surrey  
 1951 CLAY, Miss E., Green Hollow, Gattery Lane, Brixham, Devon  
 1936 CLOTHIER, PETER, Hill Close, Street, Somerset  
 1939 CLOWES, A. J., Division of Fisheries, Beach Road, Sea Point, Cape Town, S. Africa  
 \*1886 COATES AND CO. (Plymouth) LTD., Black Friars Distillery, Southside Street, Plymouth, Devon



- \*1945 COBHAM, Lt.-Cdr. A. J., R.N., Noel Cottage, Castle Street, Porchester, Hants
- \*1925 COCKSHOTT, Lt.-Col. A. M., R.A.S.C., Cotteswold Naturalists' Field Club, City Library, Gloucester
- 1933 COLE, H. A., D.Sc., Fisheries Laboratory, Burnham-on-Crouch, Essex
- 1955 COLES, J. W., 70 Bridgewood Road, Worcester Park, Surrey
- 1955 COLES, Miss S., 145 Shenley Lane, London Colney, Herts
- \*1948 COLLIER, ALBERT, c/o Fish and Wildlife Service, Ft. Crockett, Galveston, Texas, U.S.A.
- \*1885 COLLIER AND CO., 53 Southside Street, Plymouth, Devon
- 1952 COLLINGE, C. F., The Mount, 37 Derry Downs, Orpington, Kent
- 1954 COLLINS, D. J., 48 Belgrave Road, Mutley, Plymouth, Devon
- 1955 COLLINS, R. G., Naraguta Tin Mines Ltd., P.O. Box 24, Jos, Nigeria
- 1947 COLLIS, Miss M. M., 27 Mowbray Road, Cambridge
- 1930 COLMAN, J. S., Marine Biological Station, Port Erin, Isle of Man. (Council, 1951-54)
- 1956 CONROY, D. A., Newby, 5 Westlands Road, Copthorne, Shrewsbury
- \*1940 COOK, R. H., Moor Close, Melbourn, Cambridge
- \*1933 COOPER, L. H. N., D.Sc., F.R.I.C., The Laboratory, Citadel Hill, Plymouth, Devon
- 1955 COOPER, R., 33 Fanshawe Street, Bengoe, Hertford, Herts
- 1956 COPENHAGEN: UNIVERSITETETS ZOOFYSIOLOGISKE LABORATORIUM, Juliane Maries Vej 32, Copenhagen, Denmark
- 1937 CORBIN, P. G., The Laboratory, Citadel Hill, Plymouth, Devon
- 1937 CORBIN, Mrs P. G., Ph.D., Dostabrook, Horrabridge, Devon
- 1954 CORCORAN, E. F., Scripps Institution of Oceanography, La Jolla, California, U.S.A.
- 1946 CORLETT, JOHN, Fisheries Laboratory, Lowestoft, Suffolk
- 1937 COSWAY, C. A., 177 Windsor Road, Torquay, Devon
- 1941 COTT, H. B., D.Sc., University Museum of Zoology, Cambridge
- 1955 COTTON, M. J., 27 Hatherley Street, Cheltenham, Glos
- 1952 COWPER, T. R., C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australia
- 1936 CRAWFORD, G. I., 18 East Drive, Carshalton Beeches, Surrey
- 1952 CRAWSHAW, K. RIDGWAY, Heron Cottage, White Notley, Witham, Essex
- 1953 CREEK, Miss GWEN (Mrs J. M. Grant), 9 Caergog Terrace, Aberystwyth, Cardigan
- \*1928 CREW, Prof. F. A. E., M.D., D.Sc., F.R.S., Usher Institute, Warrenden Park Road, Edinburgh, 9
- \*1952 CRISP, D. J., Ph.D., Bryn Hydd, Holyhead Road, Bangor, Caern. (Council, 1954→)
- 1929 CROFTS, Miss D. R., D.Sc., Deerbanks, Noisey Wood, Billericay, Essex
- 1954 CROGHAN, P. C., Dept. of Zoology, Downing Street, Cambridge
- 1951 CURRIE, R. I., National Institute of Oceanography, Wormley, near Godalming, Surrey
- 1954 CURTIS, A. S. G., 24 Lingfield Road, Wimbledon, London, S.W. 19
- 1950 CUSHING, D. H., D.Phil., Fisheries Laboratory, Lowestoft, Suffolk
- 1955 CUTCLIFFE, A. S., Tranmere Hotel, St James Place, Ilfracombe, Devon
- \*1930 CUTHBERTSON, NORMAN, 101 Johnstone Avenue, Dartmouth, Nova Scotia, Canada
- 1952 DALE, E., 6 Garforth Cliff Estate, Selby Road, Garforth, Leeds
- \*1948 DALES, R. PHILLIPS, Ph.D., 67 Westmoreland Avenue, Squirrels Heath, Essex

- 1947 DALL, WILLIAM, C.S.I.R.O., Division of Fisheries, Marine Biological Laboratory, Cronulla, N.S.W., Australia
- \*1919 DAMANT, Capt. G. C. C., C.B.E., R.N., Thursford, Cambridge Road, East Cowes, I. of W. (**Council**, 1928-31, 1937-40)
- 1939 DANIELLI, Prof. J. F., D.Sc., Dept. of Zoology, King's College, Strand, London, W.C. 2. (**Council**, 1944-45)
- 1954 DARE, P. J., 30 Aylesbury Crescent, Whiteleigh, Plymouth, Devon
- 1948 DARTMOUTH: THE ROYAL NAVAL COLLEGE, Dartmouth, Devon
- 1930 DAUNTSEY'S SCHOOL, (The Headmaster), West Lavington, near Devizes, Wilts
- 1952 DAVENPORT, DEMOREST, Ph.D., Dept of Biology, University of California, Santa Barbara College, Goleta, California, U.S.A.
- 1920 DAVIDSON, Dr W. CAMERON, Avonleigh, Acadia Road, Torquay, Devon
- 1956 DAVIES, J. B., 5 Queen's Gate Place Mews, London, S.W. 7
- 1954 DAVIES, Miss P. A., Dept. of Zoology, The University, Exeter, Devon
- 1931 DAWES, B., D.Sc., Dept. of Zoology, King's College, Strand, London, W.C. 2
- 1955 DAWSON, E. W., Zoological Laboratory, Downing Street, Cambridge
- 1944 DAY, Prof. J. H., D.F.C., Dept. of Zoology, University, Rondebosch, Cape Town, S. Africa
- 1938 DEACON, G. E. R., C.B.E., D.Sc., F.R.S., Flitwick House, Milford, Surrey. (**Council**, 1946-49, 1950-53, 1956-)
- 1952 DEEVEY, Miss GEORGIANA B., Ph.D., The Bingham Oceanographic Laboratory, Yale University, New Haven, Conn., U.S.A.
- 1952 DELAWARE: DEPT. OF BIOLOGICAL SCIENCES, UNIVERSITY OF DELAWARE, Newark, Delaware, U.S.A.
- 1939 DENNELL, RALPH, D.Sc., Dept. of Zoology, The University, Manchester, 13
- 1954 DESHPANDE, R. D., Zoology Dept., The University, Reading
- \*1915 DICK, G. W., J.P., 500 Manning Road, Durban, Natal, S. Africa
- 1944 DIGBY, P. S. B., Dept. of Biology, St Thomas's Hospital Medical School, Westminster, London, S.W. 1
- 1955 DIMELow, Miss E. J., Dept. of Biology, Mount Allison University, Sackville, N.B., Canada
- 1955 DIXON, P. S., Ph.D., Dept. of Botany, The University, Liverpool
- 1948 DODD, J. M., Ph.D., Gatty Marine Laboratory, The University, St Andrews, Fife
- 1954 DOMINION MUSEUM, Wellington, New Zealand
- 1953 DORSETT, D. A., 68 Slewins Lane, Hornchurch, Essex
- 1949 DOUGLAS, LESLIE, 11 Harbour View, Seahouses, Northumberland
- 1954 DOWSON, Mrs L. R. M., 27 Pemberton Gardens, London, N. 19
- 1955 DRINNAN, R. E., Fisheries Experiment Station, Castle Bank, Conway, Caern
- 1952 DROOP, M. R., Ph.D., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1946 DULY, S. J., 68 Richmond Hill Court, Richmond, Surrey
- 1956 DUNBAR, Capt. J. T., Browsing, Salcombe, S. Devon
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- 1939 DUNDEE UNIVERSITY COLLEGE LIBRARY, Dundee, Forfar
- 1949 DUSSART, B. H., 13 Quai de Rives, Thonon (Haute-Savoie), France
- 1954 DUVAL, Miss D. M., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1
- \*1934 EALES, Miss N. B., D.Sc., Dept. of Zoology, The University, Reading
- 1945 EDGELL, Vice-Admiral Sir JOHN A., K.B.E., C.B., F.R.S., 4 Royal Avenue, Worcester Park, Surrey. (**Council**, 1945-48, **Vice-President**, 1948-)
- 1951 EDWARDS, C., Marine Station, Millport, Isle of Cumbrae, Scotland

- 1928 EGYPT: COASTGUARD AND FISHERIES SERVICE, Alexandria, Egypt  
 1948 ELGOOD, J. H., 27 Knightscroft Avenue, Rustington, Sussex  
 1950 ELLIOTT, Mrs J. H., 39 Woodhaw, Egham, Surrey  
 1953 EL MAGHRABY, A. M., Ph.D., Dept. of Oceanography, Faculty of Science, University of Alexandria, Moharrem Bey, Alexandria, Egypt  
 \*1929 ELMHIRST, L. K., Dartington Hall, Dartington, near Totnes, Devon  
 1954 ELTRINGHAM, S. K., 8 Farrington Road, Westbury-on-Trym, Bristol  
 1955 EMERSON, D. C., Will's Hall, Stoke Bishop, Bristol, 9  
 1951 ETHERINGTON, D., 1 St John's Gardens, London, W. 11  
 1951 ETHERINGTON, Mrs. D., (Miss J. Kerslake), 1 St. John's Gardens, London, W. 11  
 \*1923 EVANS, W. EDGAR, 38 Morningside Park, Edinburgh  
 1955 EVERARD, C. O. R., 24 Burton Road, Kilburn, London, N.W. 6  
 1955 EWELL COUNTY TECHNICAL COLLEGE, Reigate Road, Ewell, Surrey  
 1942 EWER, Dr D. W., Dept. of Zoology and Entomology, Rhodes University, Grahamstown, Cape Province, S. Africa  
 \*1929 FAOUZI, Dr HUSSEIN, Faculty of Science (Dept. of Zoology), Farouk I University, Moharram Bey, Alexandria, Egypt  
 1955 FARBER, Srta. E., Marine Laboratory, 439 Anastasia Avenue, Coral Gables, 34, Florida, U.S.A.  
 1956 FARNWORTH, Miss M., BM/LSW. FR., London, W.C. 1  
 1953 FARRINGTON, Major G. B. O., 4b Balmoral Place, The Hoe, Plymouth, Devon  
 1950 FELINSKA, Mrs MARIA, DrPhil., Grammar School, Lilford, near Oundle, Northants  
 \*1933 FELLOWES, Miss ROSALIND, 58 Gloucester Court, Kew, Surrey  
 1956 FENTON, L. S., 145 Geary Road, Dollis Hill, London, N.W. 10  
 1948 FIELD STUDIES COUNCIL, Dale Fort Field Centre, near Haverfordwest, Pems  
 1950 FISHER, L. R., Ph.D., Green Bower, Beech Lane, Lower Earley, near Reading, Berks  
 1954 THE FISHING NEWS, 68 Victoria Street, London, S.W. 1  
 1955 FOLLETT, Miss A. E., 80 Suttons Avenue, Hornchurch, Essex  
 1953 FORD, D. F., 71 Brooklyn Road, South Norwood, London, S.E. 25  
 1928 FORD, E., F.R.S.E., c/o Bank of Scotland, Millport, Isle of Cumbrae, Scotland. (**Council**, 1950-53)  
 1935 FORD, E. B., D.Sc., F.R.S., Dept. of Zoology and Comparative Anatomy, University Museum, Oxford  
 1952 FORD, Miss V. E., 43 Woodbourne Avenue, Brighton, Sussex  
 1939 FORREST, J. E., Ph.D., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1  
 1950 FORSTER, G. R., The Laboratory, Citadel Hill, Plymouth, Devon  
 1949 FOUNTAIN, H. C., The Store Cottage, Polbathic, Torpoint, Cornwall  
 1954 FOX, Prof. D. L., Scripps Institution of Oceanography, La Jolla, California, U.S.A.  
 1912 FOX, Prof. H. MUNRO, F.R.S., 2 Westbourne Terrace Road, London, W. 2. (**Council**, 1928-30, 1931-34, 1944-47, 1953-56)  
 1942 FOXON, G. E. H., Dept. of Biology, Guy's Hospital Medical School, London Bridge, London, S.E. 1  
 1953 FÖYN, Prof. BJØRN, Universitetets Biologiske Stasjon, Drøbak, Norway  
 1950 FRAMPTON, Cdr R. H. C. F., R.N. (Rtd.), Ministry of Agriculture, Fisheries and Food, Citadel Hill, Plymouth, Devon



- 1924 FRASER, Miss E. A., D.Sc., Orchard Cottage, Abinger Common, Dorking, Surrey
- 1935 FRASER, F. C., D.Sc., British Museum (Natural History), Cromwell Road, London, S.W. 7. (**Council**, 1950-53)
- \*1935 FRASER, JAMES H., D.Sc., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1952 FREEMAN, R. F. H., 43 Sherwood Road, Croydon, Surrey
- \*1939 FRETTER, Miss VERA, D.Sc., Dept. of Zoology, The University, Reading. (**Council**, 1955→)
- 1953 FRINGS, Prof. H., Dept. of Zoology and Entomology, The Pennsylvania State College, Pennsylvania, U.S.A.
- 1949 FULLER, A. S., 30 Staines Avenue, Cheam, Surrey
- 1948 FURNESS, W. J., Inglewood, Abbey Park Road, Grimsby, Lincs
- 1955 GAINES, R. A., Hopkins Marine Station, Pacific Grove, California, U.S.A.
- 1956 GANGULY, D. N., D.Phil., Dept. of Zoology, Calcutta University, 35 Ballyguni Circular Road, Calcutta 19, India
- 1941 GARDINER, Mrs A. C., United Nations Korean Reconstruction Agency, P.O. Box 20, Grand Central Station, New York 17, N.Y., U.S.A.
- \*1928 GATES, Prof. R. R., D.Sc., LL.D., F.R.S., Biological Laboratories, Harvard University, Cambridge 38, Mass., U.S.A.
- 1948 GATTY MARINE LABORATORY (The Principal), The University, St Andrews, Fife
- 1950 GAULD, D. T., Ph.D., Dept. of Zoology, University College, Achimota, Gold Coast
- 1956 GEDDES, D. C., 20 Northway, Sheriff Hill, Gateshead 9, Co. Durham
- 1953 GIBBON, Mrs E., Glenview, Clearbrook, Yelverton, Devon
- 1956 GIBBONS, F. B., Aquarium, Paignton Zoo, Paignton, Devon
- 1954 GILPIN-BROWN, J. B., Ph.D., Church Knowle, near Wareham, Dorset
- 1935 GILSON, H. CARY, Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside, Westmorland. (**Council**, 1940-43, 1947-50, 1951-54)
- 1951 GLAISTER, Mrs K., 12 Grey Close, London, N.W. 11
- 1954 GLANVILLE, A., Devonshire Club, St James Street, London, S.W. 1
- 1945 GLASGOW UNIVERSITY, Dept. of Zoology, Glasgow, W. 2
- 1951 GLASSON, D. R., Ph.D., 34 Craven Avenue, St Judes, Plymouth, Devon
- 1946 GLOVER, R. S., Oceanographical Laboratory, 78 Craighall Road, Edinburgh, 6
- 1951 GOKHALE, S. V., Ph.D., c/o The Directorate of Marine Products, Jamnagar (Saurashtra), India
- 1954 GOLDBERG, Dr E. D., Chemistry Division, Scripps Institution of Oceanography, La Jolla, California, U.S.A.
- 1951 GOLD COAST, UNIVERSITY COLLEGE OF, P.O. Box 4, Achimota, Gold Coast
- 1951 GOODBODY, I. M., Ph.D., University College of the West Indies, Mona, St Andrew, Jamaica, B.W.I.
- 1954 GOODIER, R., Tsetse Fly Operations Dept., P.O. Box 8100, Causeway, Salisbury, S. Rhodesia
- 1954 GOODING, R. U., c/o Dept. of Zoology, University of Washington, Seattle 5, Wash., U.S.A.
- 1950 GOODLEY, E. F. W., Ashgrove, 3 Marine Parade, Millport, Isle of Cumbrae, Scotland
- 1939 GOODRICH, HELEN PIRELL, D.Sc., 12 Park Town, Oxford
- 1939 GORDON, Miss ISABELLA, D.Sc., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1943 GOUROCK ROPEWORK CO., LTD., 92 Bay Street, Port Glasgow, Renfrew

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(**Council**, 1951-54)
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- 1931 GRAHAM, MICHAEL, C.M.G., O.B.E., Fisheries Laboratory, Lowestoft, Suffolk.  
(**Council**, 1931-32, 1933-36, 1943-46, 1949-52, 1955→)
- 1912 GRAY, Prof. Sir JAMES, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S., Dept. of  
Zoology, Downing Street, Cambridge. (**Council**, 1920-24, 1928→; repre-  
senting Cambridge University, 1928-45; representing Royal Society, 1955→;  
**President**, 1945-55; **Vice-President**, 1955→)
- 1954 GRAY, P., 47 King's Tamerton Road, King's Tamerton, Plymouth, Devon
- 1947 GRAY, Mrs P. M., 51 Runnymede Crescent, Streatham, London, S.W. 16
- 1943 GREAT GRIMSBY COAL, SALT AND TANNING CO., Fish Dock Road, Grimsby,  
Lincs
- 1955 GREEN, D. R., 58 Mulgrave Road, Sutton, Surrey
- 1949 GREEN, J., Dept. of Zoology, Bedford College, Regent's Park, London, N.W. 1
- 1950 GREENFIELD, LEONARD J., Hopkins Marine Station, Pacific Grove, California,  
U.S.A.
- \*1953 GRIFFITHS, Lt.-Col. R. J., Holly Cottage, Hunsdon, Herts
- 1954 GRIMSTONE, A. V., Pembroke College, Cambridge
- 1948 GROVE, A. V., 86 Gower Road, Sketty, Swansea, Glam.
- 1947 GUILER, E. R., Dept. of Zoology, University of Tasmania, Hobart, Tasmania
- 1947 GUNDRY, JOSEPH, AND CO., Bridport, Dorset
- \*1954 GUTHE, Miss ANN, Kepwick Hall, Thirsk, Yorkshire
- 1950 HAINES, Surg.-Lt. Cdr., D. O., R.N., 217 Cliffe Road, Strood, Rochester, Kent
- \*1952 HALL, D. N. F., Fisheries Research Station, P.O. Box 749, Singapore 1
- 1950 HALL, Major H. W., O.B.E., M.C., Downton Fields, Hordle, Lymington,  
Hants
- 1949 HALL, Miss N. F., Tyne Brand Products Ltd., North Shields, Northumber-  
land
- 1955 HALLAM, Miss M. E., 78 Manor Farm Road, Southampton
- 1952 HAMBURG: INSTITUT FÜR KÜSTEN- UND BINNENFISCHEREI, Neuerwall 72,  
Hamburg 36, Germany
- \*1946 HAMOND, RICHARD, Morston, Holt, Norfolk
- 1954 HAMPTON, Capt. T. A., Boat Cottage, Warfleet Creek, Dartmouth, Devon
- 1953 HANCOCK, D. A., Fisheries Laboratory, Burnham-on-Crouch, Essex
- 1954 HANNAFORD, Miss J. E., 17 Lorrimore Avenue, Stoke, Plymouth, Devon
- 1954 HARDING, J. P., Ph.D., British Museum (Natural History), Cromwell Road,  
London, S.W. 7
- 1923 HARDY, Prof. A. C., D.Sc., F.R.S., Dept. of Zoology and Comparative  
Anatomy, University Museum, Oxford. (**Council**, 1938-41, 1942→;  
representing Oxford University, 1946→)
- 1956 HARDY, M. G., Dept. of Zoology, The University, Reading
- 1950 HARLEY, Miss M. B., Dept. of Zoology, Queen Mary College, Mile End Road,  
London, E. 1
- 1932 HARRIS, Prof. J. E., Ph.D., F.R.S., Dept. of Zoology, The University, Bristol  
(**Council**, 1946-49, 1950-53, 1954→)
- 1939 HARRISON, Prof. R. J., D.Sc., M.R.C.S., L.R.C.P., Vinicombe, The Woodlands,  
Farnborough, Kent
- 1929 HART, T. J., D.Sc., National Institute of Oceanography, Wormley, near  
Godalming, Surrey

- 1934 HARTLEY, The Rev. P. H. T., Badingham Rectory, Woodbridge, Suffolk  
 1955 HARTMAN, Dr W. D., Peabody Museum of Natural History, Yale University, New Haven, Conn., U.S.A.  
 1924 HARVEY, H. W., Sc.D., F.R.S., The Laboratory, Citadel Hill, Plymouth, Devon  
 1933 HARVEY, Prof. L. A., Dept. of Zoology, The University, Exeter, Devon. (Council, 1940-43)  
 1951 HARVEY, Mrs M. S. J., 12 Trevarrick, St Austell, Cornwall  
 1954 HASEGAWA, YOSHIO, Hokkaido Regional Fisheries Research Laboratory, Yoichi, Hokkaido, Japan  
 1950 HASWELL, G. A., 23 Russell Avenue, Hartley, Plymouth, Devon  
 1954 HATLEY, Miss J., 1659 Pershore Road, Cotteridge, Birmingham 30  
 1952 HAWES, F. A. B., R.O.S.C.M. Ltd., Research Laboratory, 46 New Quay Road, Hamworthy, Poole, Dorset  
 1939 HAYES, Dr F. R., Dalhousie University, Halifax, N.S., Canada  
 1939 HAYES, Mrs F. R., Dalhousie University, Halifax, N.S., Canada  
 1950 HAZEVOET, A. C., Vogelenzangstraat 19-I, Amsterdam West, Holland  
 1954 HEDGPETH, Dr JOEL W., Scripps Institution of Oceanography, La Jolla, California, U.S.A.  
 1948 HEDLEY, RONALD H., Ph.D., British Museum (Natural History), Cromwell Road, London, S.W. 7  
 1955 HEEZEN, B. C., Lamont Geological Observatory, Palisades, New York, U.S.A.  
 1931 HENDERSON, G. T. D., D.S.C., Ph.D., Oceanographic Laboratory, 78 Craighall Road, Edinburgh 6  
 1953 HENDERSON, Mrs W. M., 37 Moor Lane, St Budeaux, Plymouth, Devon  
 1925 HENTSCHEL, C. C., 7 Dudley Court, Upper Berkeley Street, London, W. 1  
 1952 HEPPER, B. T., Elmfield, Combe-in-Teignhead, Newton Abbot, Devon  
 1950 HERRING INDUSTRY BOARD, 1 Glenfinlas Street, Edinburgh 3  
 1939 HEWER, H. R., Assistant Professor, Dept. of Zoology, Imperial College of Science, London, S.W. 7  
 1955 HEWITT, Miss C. E., Fiershill Farm, Trysull, near Wolverhampton, Staffs  
 1926 HICKLING, C. F., C.M.G., Sc.D., Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W. 1. (Council, 1947-50)  
 1951 HIDAKA, Prof. K., Geophysical Institute, Tokyo University, Tokyo, Japan.  
 1926 HILL, Prof. A. V., C.H., O.B.E., Sc.D., LL.D., F.R.S., 16 Bishopswood Road, Highgate, London, N. 6. (Council, 1925-29, 1930-33, 1934-37, 1938-41, 1942-; representing Royal Society, 1944-55; Vice-President, 1948-55; President, 1955-)  
 \*1949 HILL, D. K., Postgraduate Medical School of London, Ducane Road, London, W. 12  
 1947 HILL, M. N., Ph.D., 11 Chaucer Road, Cambridge. (Council, 1956-)  
 \*1921 HINDLE, E., Sc.D., F.R.S., 51 Warwick Avenue, London, W. 9. (Council, 1946-49; representing British Association, 1950-)  
 1937 HINTON, M. A. C., F.R.S., Glaister, Wroughton, near Bristol, Somerset  
 1926 HOBSON, Prof. A. D., King's College, Newcastle-upon-Tyne 1, Northumberland  
 1954 HOBSON, G. E., 3A Western Court, Western Road, Bournemouth, Hants  
 1948 HOCKLEY, A. R., Dept. of Zoology, The University, Southampton  
 1939 HODGKIN, Prof. A. L., F.R.S., Physiological Laboratory, Cambridge (Council, 1951-52, 1953-56)  
 \*1953 HOESTLANDT, Prof. H., D.Sc., 13 rue de Toul, Lille (Nord), France  
 1952 HOLDEN, M. J., W.A.F.R.I., Field Station, Birnin Kebbi, Sokoto Province, Nigeria



- 1954 HOLLAND: The Director, Koninklijk Zoologisch Genootschap, Natura Artis Magistra, Amsterdam
- 1956 HOLLIDAY, F. G. T., 43 Maple Road, Rubery, near Birmingham
- \*1948 HOLME, N. A., The Laboratory, Citadel Hill, Plymouth, Devon
- 1939 HOLMES, W., D.Phil., Dept. of Zoology and Comparative Anatomy, University Museum, Oxford
- 1948 HOLSGROVE, H. E., 121 Eden Bridge Road, Bush Hill Park, Enfield, Middx
- 1950 HONEKER, C., 59 Uplands, Alkrington, Middleton, Manchester
- \*1954 HOPE, D. B., Dept. of Pharmacology, South Parks Road, Oxford
- 1956 HOPSON, A., Marine Biological Station, Port Erin, Isle of Man
- 1951 HORNBY, Miss C. E., 53 Langley Drive, Wanstead, London, E. 11
- 1933 HORNE, F. R., National Institute of Agricultural Botany, Huntingdon Road, Cambridge
- 1950 HORRIDGE, G. A., Ph.D., Gatty Marine Laboratory, The University, St Andrews, Fife
- 1955 HOUGHTON, D. R., 7 Muriel Road, Waterlooville, Hants
- 1950 HOWARD, G. H., 14 Westland Avenue, Bargate, Grimsby, Lincs
- 1948 HOWE, Surg.-Cdr. D.C., R.N., c/o 80 Lumsden Avenue, Shirley, Southampton
- 1950 HOWELLS, D. V., 42 Radstock Road, Reading, Berks
- 1953 HOWELLS, Dr W. R., 60 James Street, Llanelly, Carmar
- 1932 HOWES, N. H., Dept. of Zoology, University College, Gower Street, London, W.C. 1
- 1952 HOYLE, G., Dept. of Zoology, The University, Glasgow, W. 2
- 1955 HUDSPITH, W. H., 1 King's Avenue, Rochester, Kent
- \*1954 HUGHES, A., Sc.D., Anatomy School, Cambridge
- 1953 HULL: THE LIBRARIAN, University College, Hull
- 1928 HUNT, O. D., Corroffell, Newton Ferrers, Devon. (Council, 1944-47, 1948-51, 1952-55, 1956-)
- 1947 HUNTER, W. RUSSELL, Dept. of Zoology, The University, Glasgow, W. 2
- \*1947 HURRELL, H. G., J.P., Moorgate, Wrangaton, Devon
- \*1952 HUTTON, ROBERT F., Ph.D., State Board of Conservation Marine Laboratory, Maritime Base, Bayboro Harbor, St Petersburg, Florida, U.S.A.
- 1946 ICELAND: ATVINNUDEILD HÁSKÓLANS (FÍSKIDEILD), Reykjavik, Iceland
- 1956 ILLG, Associate Prof. P. L., Dept. of Zoology, University of Washington, Seattle 5, Wash., U.S.A.
- 1945 IMPERIAL CHEMICAL INDUSTRIES, LTD., Nobel House, 2 Buckingham Gate, London, S.W. 1
- 1954 INDONESIA: THE PROFESSOR OF ZOOLOGY, University of Indonesia, Djalan Taman Sari 64, Bandung, Java
- 1952 INGLIS, W. G., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1953 INSTITUTE OF SEAWEED RESEARCH, Inveresk, Midlothian
- 1952 JACKMAN, L. A. J., 44 Old Torquay Road, Preston, Paignton, Devon
- 1954 JAGER, J. C., van Reesstraat 122, The Hague, Holland
- 1953 JAGGER, J. R., 5 Duckworth Street, Stoke, Devonport, Devon
- 1952 JEAN, Prof. YVES, Fisheries Research Station, P.O. Box No. 531, Colombo 3, Ceylon
- 1945 JEFFERIES, H. S., 16 Ashley Road, Bradford-on-Avon, Wilts

- 1952 JEFFERY, F. J., Plymouth College, Plymouth, Devon  
 1953 JELLARD, Dr C. H., B.M., Public Health Laboratory, South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon  
 1935 JENKIN, Miss P. M., Dept. of Zoology, The University, Bristol  
 1934 JEPPE, Miss M. W., D.Sc., 17 Manor Court, Grange Road, Cambridge  
 1937 JERSEY: CONSERVATEUR HONORAIRE DU MUSÉE DE LA SOCIÉTÉ JERSIAISE  
 \*1924 JESUS COLLEGE, Oxford  
 \*1947 JOHN, C. C., D.Sc., Dept of Marine Biology and Fisheries, Beach P.O., Trivandrum, S. India  
 \*1947 JOHNSON, D. S., 10 St John's Road, Cambridge  
 1944 JOHNSON, Dr F. R., Osu Fisheries Station, P.O. Box 630, Accra, Gold Coast Colony  
 1956 JONES, B. W., 62 Great Bushey Drive, Totteridge, London, N. 20  
 \*1948 JONES, C. BURDON, Ph.D., Dept. of Zoology, University College of N. Wales, Bangor, Caern  
 \*1950 JONES, E. IDRIS, M.D., 6 Kewferry Road, Northwood, Middx  
 \*1949 JONES, Prof. E. W. KNIGHT, Ph.D., Dept. of Zoology, University College, Swansea  
 1949 JONES, F. R. HARDEN, Ph.D., Dept. of Zoology, Downing Street, Cambridge  
 1948 JONES, J. D., Nantyci, Llysonnen Road, Carmarthen, S. Wales  
 1946 JONES, L. W. G., Cumber House, Manor Road, Brixham, Devon  
 1946 JONES, N. S., Ph.D., Marine Biological Station, Port Erin, Isle of Man  
 1953 JONES, P. G. W., Woodberry, Tolmers Road, Cuffley, Herts  
 1949 JONES, R. J., 17 Haling Park Road, South Croydon, Surrey  
 1936 JONES, RODNEY R. M., Tros-yr-Afon, Penmon, Anglesey  
 1946 JONES, Prof. R. V., C.B., C.B.E., D.Phil., F.Inst.P., Dept. of Natural Philosophy, Marischal College, Aberdeen  
 1955 JONES, S., D.Sc., Central Fisheries, West Hill P.O., Calicut 5, Malabar, India  
 1952 JONES, T. HOWELL, Mydrim House, Llewellyn Street, Tycynon, Aberdare, Glam  
 1950 JONES, W. C., Dept. of Zoology, University College of N. Wales, Bangor, Caern  
 1951 JONES, W. E., Marine Biology Station, University College of N. Wales, Menai Bridge, Anglesey  
 1947 JØRGENSEN, C. BARKER, Slettevej 8, Copenhagen Søborg, Denmark  
 1950 JUNIPER, A. J., The Polytechnic, Regent Street, London, W. 1  
  
 1952 KABATA, Z., 26 Orchard Road, Aberdeen  
 1954 KAIN, Miss J. M., 11 Acol Road, London, N.W. 6  
 1951 KAMPA, E. M., Ph.D. (Mrs B. P. Boden), Scripps Institution of Oceanography, La Jolla, California, U.S.A.  
 1954 KARNATAK UNIVERSITY: Karnatak University Library, Dharwar (State Bombay), India  
 1956 KASTURIRANGAN, L. R., Central Fisheries, Ernakulam P.O., S. India  
 1945 KATZ, MAX, Ph.D., c/o Prof. R. E. Dimick, Dept. of Fish and Game Management, Oregon State College, Corvallis, Oregon, U.S.A.  
 1951 KAY, Miss E. A., Dept. of Zoology, University of Hawaii, Honolulu, Hawaii, U.S.A.  
 1955 KEADY, P. J., 31 Seapoint Avenue, Blackrock, Dublin, Ireland  
 1940 KELLIN, Prof. D., Sc.D., F.R.S., Molteno Institute, Cambridge. (Council, 1940-43)

- 1950 KEIR, RONALD S., Newfoundland Fisheries Research Station, Water Street East, St Johns, Newfoundland
- 1955 KELLAWAY, Mrs R., 18 Thornyville Villas, Oreston, near Plymouth, Devon
- 1946 KELLEY, D. F., Pear Tree Cottage, Cheapside, Horsell, Woking, Surrey
- 1954 KELLY, A. L., 352 Nautilus, La Jolla, California, U.S.A.
- 1949 KENNEDY, G. Y., Ph.D., F.R.I.C., Dept. of Cancer Research, University Field Laboratory, Blackbrook Road, Sheffield 10
- 1946 KENYA: THE GAME WARDEN, Game Department, P.O. Box 241, Nairobi
- \*1952 KERKUT, G., Sub-Dept. of Physiology, The University, Southampton
- \*1954 KESTEVEN, Dr G. L., c/o F.A.O.U.N. Viale delle Terme di Caracalla, Rome, Italy
- 1950 KEYNES, R. D., Ph.D., Physiological Laboratory, Cambridge
- 1949 KIMMINS, B. J., P.O. Box 603, Addis Ababa, Ethiopia
- 1928 KING, Mrs A. REDMAN, Mixton House, Lerryn, Lostwithiel, Cornwall
- 1947 KINGSBRIDGE MODERN SECONDARY SCHOOL, Kingsbridge, Devon
- 1952 KINGS' COLLEGE: Dept. of Zoology, Newcastle upon Tyne 1
- 1954 KINGWELL, M. C., Beechfield, South Brent, S. Devon
- 1927 KIRTISINGHE, P., Dept. of Zoology, University of Ceylon, Colombo 3, Ceylon
- 1950 KISCH, B. S., C.I.E., 38 Boulevard Thiers, St Jean-de-Luz, B.P., France
- 1956 KITAMURA, HIROYUKI, Kobe Marine Observatory, Kobe, Japan
- 1930 KITCHING, J. A., O.B.E., Ph.D., Dept. of Zoology, The University, Bristol
- 1939 KNIGHT, Miss MARGERY, D.Sc., University Hall for Woman Students, Holly Road, Fairfield, Liverpool. (Council, 1943-46)
- 1955 KNOTT, C. C., 76 Pasley Street, Stoke, Plymouth, Devon
- 1945 KNOWLES, Sir FRANCIS G. W., Bt., D.Phil., Marlborough College, Marlborough, Wilts
- 1955 KNOX, G. A., Zoology Dept., Canterbury University College, Christchurch, New Zealand
- 1955 KOHN, A. J., Hawaii Marine Laboratory, University of Hawaii, Honolulu 14, Hawaii, U.S.A.
- 1950 KORN, M. E., 62 Aberdare Gardens, Hampstead, London, N.W. 6
- 1949 KRISTENSEN, Dr INGVAR, Zoologisch Station, den Helder, Holland
- \*1954 KROGH, ERIK, M.D., Associate Prof., Normal Anatomist Institut, Aarhus Universitet, Aarhus, Denmark
- 1955 KRUSE, P. J., Jr., Marine Laboratory, University of Miami, Coral Gables 34, Florida, U.S.A.
- 1954 LACEY, A. J., 24 The Gardens, Monkseaton, Northumberland
- 1955 LADE, Miss B. I., Southdown House, Ewhurst, near Guildford, Surrey
- 1956 LANCE, Miss J., Dept. of Zoology, The University, Southampton
- 1953 LANDA, ANTONIO, P.O. Box 3665, Balboa, Canal Zone, S. America
- 1950 LASKER, REUBEN, Scripps Institution of Oceanography, La Jolla, California, U.S.A.
- 1956 LAVERACK, M. S., Sub-Dept. of Physiology, The University, Southampton
- \*1925 LEBOUR, Miss M. V., D.Sc., Kean Hill, Cawsand, near Plymouth, Devon
- 1947 LEEHANE, J. D. B., 14 Wyndham Street East, Plymouth, Devon
- 1955 LEIVESTAD, H., Klostergt 2, Bergen, Norway
- 1935 LEMARE, D. W., Fisheries Dept., Federation of Malaya and Singapore, Penang, Malaya
- \*1954 LESTON, D., 44 Abbey Road, London, N.W. 8



- 1956 LESUEUR, R. F., The Museum, Jersey, C.I.
- 1951 LEWIS, J. B., The Bellairs Research Institute of McGill University, St James, Barbados, B.W.I.
- 1956 LEWIS, J. G. E., Hawkesden, Yew Lane, East Grinstead, Sussex
- 1952 LEWIS, J. R., Dept. of Zoology, The University, Leeds
- 1951 LIGHTOWLERS, F., 60 Harberton Park, Belfast, N. Ireland
- 1954 LILLY, S. J., Ph.D. (Mrs Gilpin-Brown), Church Knowle, near Wareham, Dorset
- 1956 LING, J. K., C.S.I.R.O., Division of Fisheries and Oceanography, Cronulla, N.S.W., Australia
- 1932 LISBON: INSTITUTO DE BIOLOGIA MARITIMA, Cais do Sodré, Lisbon, Portugal
- 1955 LLEWELLYN, J., Ph.D., Dept. of Zoology and Comparative Physiology, The University, Edgbaston, Birmingham 15
- 1950 LONGHURST, A. R., Landford Cottage, Landford, Wilts
- 1953 LONGTON, P. A., 37 Headland Park, North Hill, Plymouth, Devon
- 1954 LORRAINE, N. S. R., M.D., Ch.B., D.P.H., F.R.S.E., Public Health Department, London Road, Hadleigh, Essex
- 1948 LOVEGROVE, T., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1953 LOWE, Mrs E. K., 27A Greenbank Road, Ambleside, Westmorland
- 1950 LOWENSTEIN, Prof. O. E., D.Sc., Ph.D., F.R.S., Dept. of Zoology, The University, Birmingham 15. (**Council**, 1952-55)
- 1950 LOWY, J., Ph.D., Dept. of Zoology, Queen's University, Belfast, N. Ireland
- 1931 LUCAS, C. E., C.M.G., D.Sc., Marine Laboratory, Victoria Road, Torry, Aberdeen. (**Council**, 1949-52, 1954-)
- 1956 LUNZ: BIOLOGISCHE STATION LUNZ, Lunz am See, Niederösterreich, Austria
- 1953 LYNCH, Dr G. A. C., Beeches, Crapstone, Yelverton, Devon
- 1938 LYSAGHT, Miss A. M., Ph.D., 6 Cumberland Gardens, London, W.C. 1
- 1938 MACDONALD, R., 112 Antrim Road, Belfast, N. Ireland
- 1935 MACKENZIE, Col. W., O.B.E., c/o Messrs Peel & Co., Ltd., P.O. Box 331, Alexandria, Egypt
- 1929 MACKINNON, Prof. D. L., D.Sc., 44 St Leonard's Terrace, Chelsea, London, S.W. 3. (**Council**, 1938-42)
- 1937 MACKINTOSH, N. A., C.B.E., D.Sc., 7 Hinde House, Hinde Street, London, W. 1. (**Council**, 1946-49, 1951-54, 1955-)
- 1947 MACNAE, WILLIAM, Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa
- \*1954 MADDOCKS, Miss A. C., 28 Denison Road, Victoria Park, Manchester 14
- 1956 MADEIRA: Museu Municipal do Funchal, Madeira
- \*1925 MAGDALEN COLLEGE, Oxford
- 1955 MAHAKOSHAL MAHAVIDYALAYA (The Principal), Jabalpur, India
- 1951 MALKIN, J. J. S., 33 Elton Parade, Darlington
- 1948 MANSFIELD, A. W., Dept. of Zoology, McGill University, Montreal, Quebec, Canada
- 1952 MANSFIELD, Lt.-Col. K. F., Luckwell Bridge, Wheddon Cross, Minehead, Som
- 1955 MANSUETI, R. J., Maryland Dept. of Research and Education, Chesapeake Biological Laboratory, Solomons, Maryland, U.S.A.
- \*1928 MANTON, S. M., Sc.D., F.R.S. (Mrs J. P. Harding), 18 Ennerdale Road, Richmond, Surrey
- 1953 MANWARING, Miss R. A., Dept. of Zoology, The University, Exeter, Devon

- 1951 MARINE BIOLOGY STATION, University College of North Wales, Menai Bridge, Anglesey
- 1956 MARKOWSKI, S., D.Sc., Central Electricity Authority, The Biological Research Laboratory, Grove Road Generating Station, Lodge Road, London, N.W. 8
- 1951 MARSHALL, C. J., M.D., F.R.C.S., Penhaven, The Drive, Mevagissey, Cornwall
- 1949 MARSHALL, DOUGLAS, M.P., Lanwithan House, Lostwithiel, Cornwall
- 1951 MARSHALL, K. E., The Ferry House, Far Sawrey, Ambleside, Westmorland
- \*1947 MARSHALL, Miss S. M., D.Sc., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1953 MARSHALL, P. T., The Priory, Kingswear, Devon
- 1955 MARTIN, A. L., 22 Luttrell Avenue, Putney, London, S.W. 15
- 1951 MASON, J., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1939 MATTHEWS, L. HARRISON, Sc.D., F.R.S., Zoological Society of London, Regent's Park, London, N.W. 1. (Council, 1944-47, 1949-52)
- \*1947 MATTHEWS, Mrs J. B., Ph.D., (Miss G. C. Evans), The Rock Garden, Gerrans, Portscatho, Cornwall
- 1954 MAUCHLINE, J., 4 The University, Glasgow, W. 2
- 1937 MAYNE, CYRIL F., O.B.E., F.R.C.S., c/o Barclays Bank Ltd., Plymouth, Devon
- 1953 MAYNE, R. B., Martins Meadow, Yelverton, Devon
- 1949 MCCARTHY, Miss D. F., 70 Halton Terrace, Kensington Park, Adelaide, S. Australia
- 1951 MCCARTON, Miss L., 89 Eglantine Avenue, Malone Road, Belfast, N. Ireland
- 1910 McCLEAN, Capt. W. N., 39 Phillimore Gardens, London, W. 8
- \*1929 McEWEN, Mrs LAWRENCE, Isle of Muck, Isle of Eigg, Inverness-shire
- \*1952 MCGILL, D. A., 252 Terrace Park, Rochester 11, New York, U.S.A.
- \*1954 MCGOWAN, J. A., Trust Territory of the Pacific Islands, Office of the Staff Marine Biologist, Koror, Caroline Islands
- 1955 McINNES, Miss F. R. B., 65 Gwendoline Avenue, Upton Park, London, E. 13
- 1948 McINTYRE, A. D., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1950 McINTYRE, J. M., Greenfield, Cramer Street, Stafford, Staffs
- 1953 MENKER, DONALD F., Marine Laboratory, University of Miami, Coral Gables, Florida, U.S.A.
- \*1948 MENON, M. DEVIDAS, Parvathi Nivas, Diwans Road, Ernakulam, United States of Cochin and Travancore, S. India
- 1954 MENZIES, Dr R. J., Lamont Geological Observatory, Torrey Cliff, Palisades, New York, U.S.A.
- 1953 MERCER, B. V., Blakewell House, Painswick, Glos
- 1953 MEREDITH, Miss H. M., 7 The Lynchets, Lewes, Sussex
- 1954 MERRETT, M. G., 22 Fullerton Road, Milehouse, Plymouth, Devon
- 1950 MESSENGER, K. G., Uppingham School, Rutland
- 1951 MESSENT, R. K., 13 Sedberg Street, Preston, Lancs
- 1939 METROPOLITAN WATER BOARD, 177 Rosebery Avenue, London, E.C. 1
- 1950 MEXICO: DEPARTMENT OF PHYSIOLOGY, NATIONAL UNIVERSITY, Apartado Postal 13699, Mexico, D.F.
- 1947 MIAMI UNIVERSITY MARINE LABORATORY, Coral Gables 34, Florida, U.S.A.
- 1951 MIJ TOT EXPLOITATIE VAN KOOKSOVENGASSEN (Mekog), Ijmuiden, Holland
- 1923 MILFORD HAVEN TRAWLER OWNERS ASSOCIATION LTD., Milford Haven, Pems
- 1949 MILLAR, R. H., Ph.D., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1946 MILLER, CYRIL J., 16 Beechwood Terrace, Mutley, Plymouth, Devon

- 1956 MILLER, M. C., Marine Biological Station, Port Erin, Isle of Man  
 1955 MILLER, P. L., Downing College, Cambridge  
 1955 MILLIGAN, W., Sgts. Mess, A.P.L., Singapore Lines, Khormaksar, Aden  
 1949 MILLOTT, Prof. N., Ph.D., Dept. of Zoology, Bedford College, Regent's Park, London, N.W. 1  
 1953 MILLS, D. H., Atlantic Biological Station, St Andrews, New Brunswick, Canada  
 1949 MISTAKIDIS, M. N., Fisheries Laboratory, Burnham-on-Crouch, Essex  
 1947 MITRA, G. N., Assistant Director of Fisheries, Cuttack (Orissa), India  
 1952 MOHAMED, Prof. ABDEL FATTAH, Ph.D., Oceanography Dept., Faculty of Science, Alexandria University, Maharram Bey, Alexandria, Egypt  
 1951 MOHR, J. L., Ph.D., Dept. of Zoology, University of Southern California, Los Angeles 7, Calif., U.S.A.  
 1956 MOLLOY, Miss F. M., Dept. of Botany and Zoology, Chelsea Polytechnic, London, S.W. 3  
 1940 MOORE, HILARY B., Ph.D., Marine Laboratory, Miami University, Coral Gables, Florida, U.S.A.  
 1955 MORGAN, Miss R. B., 37 Fidas Avenue, Llanishen Cardiff  
 1949 MORTON, J. E., Ph.D., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1  
 1953 MOSS, Miss M. P., Lady Margaret Hall, Oxford  
 1931 MOUNT DESERT ISLAND BIOLOGICAL LABORATORY, Salisbury Cove, Maine, U.S.A.  
 1942 MOYNAHAN, Dr E. J., Wayside, Jordans, near Beaconsfield, Bucks.  
 1953 MULLER, R. L., 38 Brendon Street, London, W. 1  
 1952 MUNN, R. B., 11002 East Saragossa Street, Whittier, Calif., U.S.A.  
 1955 MURRAY, R. W., Ph.D., Dept. of Zoology and Comparative Physiology, The University, Edgbaston, Birmingham, 15  
 1955 NAGABHUSHANAM, R., Dept. of Zoology, Andhra University, Visakhapatnam, India  
 1952 NAYLOR, E., Officers' Mess, R.A.F., Kirkham, Lancs.  
 1955 NAYLOR, Miss MARGARET, Ph.D., Dept. of Botany, The University, Hull  
 1933 NEALE, MORLEY H., C.B.E., Chaffcombe House, Chard, Somerset. (**Council**, 1934-36, 1939-42, 1943-46, 1947-50; **Vice-President**, 1951→)  
 1939 NEEDHAM, JOSEPH, Sc.D., F.R.S., Gonville and Caius College, Cambridge  
 1953 NEWELL, B. S., I.N.F.R.O., P.O. Box 754, Zanzibar  
 1947 NEWELL, G. E., Ph.D., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1. (**Council**, 1955→)  
 1951 NEWFOUNDLAND FISHERIES RESEARCH STATION, Water Street East, St John's, Newfoundland  
 1947 NEWTON, Prof. LILY, D.Sc., Dept. of Botany, University College of Wales, Aberystwyth, Cardigan. (**Council**, 1947-50, 1955→)  
 1930 NICHOLLS, A. G., Ph.D., C.S.I.R.O., Division of Fisheries, Stowell, Hobart, Tasmania  
 1950 NICOL, J. A. C., D.Phil., Woodview, Tideford, near Saltash, Cornwall  
 1947 NISBET, R. H., 8 Lichfield Road, Woodford Green, Essex  
 1948 NOBBS, Miss G. E. D., 4 Clifton Estate, Plympton, Devon  
 1953 NORDLI, O., Statens Fiskerifagsskule, Florø, Norway  
 1949 NORRIS, E., Dept. of Biology, Ewell Technical College, Reigate Road, Ewell, Surrey



- 1951 NORTH CAROLINA, UNIVERSITY OF, Institute of Fisheries Research, Box 629, Morehead City, N. Carolina, U.S.A.
- 1950 NORTH, J., 316 Lee High Road, Lewisham, London, S.E. 13
- 1953 NORTH, L. G., 71 Orchard Close, Fetcham, Surrey
- 1948 NUNN, S. M., The Laboratory, Citadel Hill, Plymouth, Devon
- 1950 NURSE, F. R. (Mrs R. M. Allison), Dept. of Biology, Canterbury University College, Christchurch, New Zealand
- 1934 OAKLEY, Prof. C. L., 14 Cardigan Road, Headingley, Leeds 6
- 1954 O'hEOCHA, C., Chemical Laboratory, University College, Galway, Ireland
- 1947 OLDFIELD, Mrs E., County Technical College, Guildford, Surrey
- 1938 OLIVER, JAMES H., Ph.D., 16 Southwark Street, London, S.E. 1
- 1937 OMER-COOPER, J., Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa
- 1939 OMMANNEY, F. D., Ph.D., Fisheries Research Station, P.O. Box 749, Singapore
- 1949 ORKIN, P. A., Natural History Dept., Marischal College, Aberdeen
- 1947 ORR, A. P., D.Sc., Marine Station, Millport, Isle of Cumbrae, Scotland
- 1945 OTAGO UNIVERSITY LIBRARY, Dunedin, N.I., New Zealand
- \*1928 OTTER, G. W., Southwood, Broadstone, Dorset
- 1950 OWEN, G., Dept. of Zoology, The University, Glasgow, W. 2
- 1953 OWEN, Miss G., Rustington, Flat 2, St Wilfred's Road, Worthing
- 1949 OWRE, Mrs HARDING B., Marine Laboratory, University of Miami, Coral Gables 34, Florida, U.S.A.
- 1951 OXFORD: DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY, University Museum, Oxford
- 1951 PACIFIC BIOLOGICAL STATION, Nanaimo, B.C., Canada
- \*1954 PACKHAM, J. R., Rylstone, 8 Highview Avenue, South Patcham, Brighton 6
- 1953 PAMPAPATHI-RAO, K., Ph.D., Dept of Zoology, S.V. University, Tirupathi, S. India
- \*1939 PANIKKAR, N. K., D.Sc., Central Marine Fisheries Station, Mandapam Camp P.O., S. India
- \*1923 PANTIN, C. F. A., Sc.D., F.R.S., Trinity College, Cambridge. (Council, 1930-32, 1933-36, 1937-40, 1941-44, 1945-49, 1951-53; representing Cambridge University, 1946-49, 1951-53)
- 1951 PAPWORTH, A. H., 6 Ashford Avenue, Ashford, Middx
- 1941 PARKE, Miss M. W., D.Sc., The Laboratory, Citadel Hill, Plymouth, Devon
- 1954 PARKER, R. H., Scripps Institution of Oceanography, 616 Bonair Street, La Jolla, California, U.S.A.
- 1954 PARRISH, B. B., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1937 PARRY, D. A., Ph.D., Dept. of Zoology, Downing Street, Cambridge
- 1950 PARRY, Miss G., Dept. of Zoology, Bedford College, Regent's Park, London, N.W. 1
- \*1920 PASS, A. DOUGLAS, Wooton Fitzpaine, Charmouth, Dorset
- \*1946 PAUL, Mrs VIDYA VATI, Ph.D., 116-D, Kamlanagar, Delhi-6, India
- 1952 PAUL, A. R., 22 Cooper's Hill, Willingdon, Eastbourne, Sussex
- 1925 PAWLYN BROS., Mevagissey, Cornwall
- 1925 PAWLYN, T. A., Mevagissey, Cornwall
- 1933 PEEL, Col. Sir EDWARD T., K.B.E., D.S.O., M.C., c/o Messrs Peel & Co., Ltd., P.O. Box 331, Alexandria, Egypt. (Vice-President, 1936-)

- 1954 PEMBERTON, M. R., 3 Arden Gate, Lancaster, Lancs  
 1925 PENTLOW, F. T. K., Old Slade Lane, Iver, Bucks  
 1929 PERCIVAL, Prof. E., Canterbury University College, Christchurch, New Zealand  
 1947 PERKINS, E. J., Cross Keys, Post Office, near Hereford  
 1936 PERKINS, F., Red Hand Composition Co., 2 The Firs, York Road, Ripon, Yorks  
 1954 PEYER, Prof. B., Zoologisches Museum der Universitaet Zürich, 16 Kunstlergasse, Switzerland  
 1950 PHILLIPSON, J., Ph.D., Dept. of Zoology, Science Laboratories, South Road, Durham  
 1934 PICKEN, L. E. R., Ph.D., Trinity College, Cambridge  
 1940 PIKE, R. B., Ph.D., Marine Station, Millport, Isle of Cumbrae, Scotland  
 1956 PILGRIM, Miss A. M., 1 Guildown Road, Guildford, Surrey  
 1953 PITTAM, M. D., Magdalen College, Oxford  
 1906 PLYMOUTH CORPORATION (Museum Committee), Tavistock Road, Plymouth, Devon  
 1910 PLYMOUTH EDUCATION AUTHORITY, Cobourg Street, Plymouth, Devon  
 1906 PLYMOUTH, PORT OF, INCORPORATED CHAMBER OF COMMERCE, Plymouth, Devon  
 1924 PLYMOUTH PROPRIETARY LIBRARY, 1 Alton Terrace, North Hill, Plymouth, Devon  
 1929 PLYMOUTH PUBLIC LIBRARY, Tavistock Road, Plymouth, Devon  
 1948 POLLARD, D. G., 60 Alcester Street, Stoke, Plymouth, Devon  
 1950 POLYTECHNIC, The Library, Regent Street, London, W. 1  
 1947 PORTSMOUTH GRAMMAR SCHOOL, Biology Department, Portsmouth, Hants  
 1929 PORTSMOUTH MUNICIPAL COLLEGE, Portsmouth, Hants  
 1950 POSNER, GERALD S., Institute of Fisheries Research, University of North Carolina, Morehead City, N.C., U.S.A.  
 1955 POTTS, W. T. W., Ph.D., Natural History Dept., Marischal College, Aberdeen  
 1953 POUNCE, A. G., Sedgley, The Crescent, Darnley Drive, Southborough, Kent  
 1950 POWELL, H. T., Marine Station, Millport, Isle of Cumbrae, Scotland  
 1950 POWELL, R. R., 170 Streatfield Road, Kenton, Middx  
 1955 PRADHAN, L. B., M.Sc., Central Marine Fisheries Research Station, Karwar (Bombay Station), India  
 1954 PRATT, Dr IVAN, Dept. of Zoology, Oregon State College, Corvallis, Oregon, U.S.A.  
 1951 PRINGLE, I. G. St C., Preston Hall, Uppingham, Rutland  
 1939 PRINGLE, J. W. S., M.B.E., Sc.D., F.R.S., Dept. of Zoology, Downing Street, Cambridge  
 1954 PRITCHARD, Dr D. W., Chesapeake Bay Institute, The Johns Hopkins University, Box 426A, RFD 2, Annapolis, Maryland, U.S.A.  
 \*1947 PRYOR, Thomas, D.S.O., Westbourne House, Saltash, Cornwall  
 1954 PUGH, Miss D. I., 1 Curzon Crescent, Willesden, London, N.W. 10  
 1939 PUMPHREY, Prof. R. J., Sc.D., F.R.S., Dept. of Zoology, The University, Liverpool. (Council, 1956→)  
 \*1939 PURCHON, Prof. R. D., Ph.D., Dept. of Zoology, University of Malaya, Singapore 10  
 1953 QUEBEC: STATION DE BIOLOGIE MARINE, Grande Rivière, Gaspé, P.Q., Canada  
 1936 QUEEN MARY COLLEGE, Dept. of Zoology, Mile End Road, London, E. 1

- 1954 QUEENSLAND MUSEUM, Brisbane, Queensland, Australia  
 1939 QUEEN'S UNIVERSITY, Belfast, N. Ireland  
 1947 QUECKETT MICROSCOPICAL CLUB (F. F. H. Boxall, Hon. Sec.), 135 Woodland Gardens, Isleworth, Middx  
 1932 QUICK, H. E., F.R.C.S., Craythorne, Shinfield Road, Reading, Berks  
 1954 RADFORD, Miss M., 27 Thornhill Road, Mannamead, Plymouth, Devon  
 1954 RAKESTRAW, Dr N. W., Scripps Institution of Oceanography, La Jolla, California, U.S.A.  
 1932 RAMALHO, Dr A., Instituto de Biologia Maritima, Cais do Sodré, Lisbon, Portugal  
 1949 RASMUSSEN, E., The Zoological Laboratory, Landbohøjskolen, Bülowsvej, Copenhagen V, Denmark  
 1947 RAYMONT, Prof. J. E. G., Dept. of Zoology, The University, Southampton  
 \*1933 RAYNER, G. W., 265 Woolooware Road, Cronulla, N.S.W., Australia  
 1956 READ, R., 43 Holly Terrace, Hensingham, Whitehaven, Cumbria  
 1949 REES, C. B., D.Sc., Oceanographic Laboratory, 78 Craighall Road, Edinburgh 6  
 \*1936 REES, W. J., D.Sc., 37 Kings Road, Richmond, Surrey  
 1955 REEVE, P., 28 Sunnymede Avenue, Carshalton, Surrey  
 1955 REILLY, Miss S., Dept. of Biology, University College, Galway, Ireland  
 1951 REISH, DONALD, J. Allan Hancock Foundation, University of Southern California, Los Angeles, Calif., U.S.A.  
 1935 RENOUF, L. LE PAGE, D.Sc., F.R.S.E., Lough Ine, Skibbereen, Co. Cork, Eire  
 1953 RESEARCH ORGANIZATION OF SHIPS' COMPOSITIONS MANUFACTURERS, LTD., Grosvenor Gardens House, Grosvenor Gardens, London, S.W. 1  
 1955 REYNOLDSON, T. B., D.Sc., Dept. of Zoology, University College, Bangor, Caern  
 1940 RICHARDS, P. B., c/o Lloyds Bank Ltd., Truro, Cornwall  
 1951 RICKARD, Miss B., 68 Furze Hill Road, Mutley, Plymouth, Devon  
 1946 RIFAAT, AHMED, Alexandria Institute of Hydrobiology and Fisheries, Kayed Bey, Alexandria, Egypt  
 1951 RIGBY, Miss J. E., 184 Walm Lane, London, N.W. 2  
 1952 RIJAL, Z., c/o Government Boys' Secondary School, P.O. Box 873, Zanzibar  
 1953 RIKSMUSEUM (The Director), Evertebratavd., Stockholm, Sweden  
 \*1919 RITCHIE, Prof. A. D., Old College, The University, Edinburgh. (**Council**, 1929-32, 1934-37)  
 1944 RITCHIE, Prof. JAMES, C.B.E., D.Sc., 31 Mortonhall Road, Edinburgh. (**Council**, 1944-47)  
 1955 ROBERTS, Miss P. E. M., Peile Hall, Newnham College, Cambridge  
 1953 ROBERTSON, J. D., Ph.D., F.R.S.E., Dept. of Zoology, The University, Glasgow, W. 2  
 1948 ROBINSON, G. A., Oceanographic Laboratory, 78 Craighall Road, Edinburgh 6  
 1954 ROBSON, Miss E. A., Ph.D., c/o Dept. of Zoology, Downing Street, Cambridge  
 1949 RODHE, Dr W., Institute of Limnology, Uppsala, Sweden  
 1940 ROGERS, Dr T. HOWARD, Naval Research Establishment, Halifax, Nova Scotia, Canada  
 1952 ROSA, HORATIO, F.A.O.U.N., Viale delle Terme di Carracalla, Rome, Italy  
 1939 ROSS, D. M., Ph.D., Dept. of Zoology, University College, Gower Street, London, W.C. 1  
 1939 ROSS, Miss SYBIL S., 158 Craiglea Drive, Edinburgh 10



- \*1931 ROTHSCHILD, Rt Hon. Lord, G.M., Ph.D., F.R.S., Merton Hall, Cambridge. (Council, 1939-42)
- \*1932 ROTHSCHILD, The Hon. MIRIAM (The Hon. Mrs George Lane), The Cottage, Ashton Wold, Peterborough, Northants. (Council, 1942-45)
- 1951 ROWE, Rev. A. F. C., The Vicarage, Kingskerswell, Newton Abbot, Devon
- 1945 ROWETT, Miss H. G. Q., Plymouth Technical College, Tavistock Road, Plymouth, Devon
- 1952 ROYAL HOLLOWAY COLLEGE, Englefield Green, Surrey
- 1956 RUDGE, A. J. B., Court Mill, Steyning, Sussex
- 1924 RUDGE, Chas. H., Trevalgan, Feliskirk Lane, Marazion, Cornwall
- 1927 RUSSELL, F. S., C.B.E., D.S.C., D.F.C., LL.D., F.R.S., The Laboratory, Citadel Hill, Plymouth, Devon. (Council, 1945→; Secretary, 1945→)
- 1951 RUSSELL, S. M. M., c/o National Institute of Health, Bethesda, Md, U.S.A.
- 1954 RYLAND, J. S., Marine Biological Station, Menai Bridge, Anglesey
- 1946 SAGAY, A. A. E., Warri College, Warri, Nigeria
- 1953 ST AUBREY, Miss S. N. D., St Martin's Vicarage, Vicar's Road, Gospel Oak, London, N.W. 5
- 1927 ST AUBYN, Capt. The Hon. LIONEL, M.V.O., Saltram, Plympton, Devon
- \*1951 SALISBURY, A. E., Ormonde House, 51 Amersham Hills, High Wycombe, Bucks
- \*1934 SALISBURY, Sir EDWARD J., Kt., C.B.E., D.Sc., LL.D., F.R.S., Croindene, Strandway, Felpham, Bognor Regis, Sussex. (Council, 1934-37, 1940-43; Vice-President, 1948→)
- 1946 SARTORY, P. K., 107 Rivermeads Avenue, Twickenham, Mddx
- 1955 SASTRY, A. N., 4/176 Innespeta, Rajahmundry, Andhra State, India
- 1911 SAUNDERS, J. T., Christ's College, Cambridge
- 1914 SAVAGE, R. E., O.B.E., Monfa, Pentywyn Road, Deganwy, Caern
- 1946 SAVILLE, ALAN, Marine Laboratory, Victoria Road, Torry, Aberdeen
- \*1945 SAVILLE, TOM C., 31 Mawton Crescent, Wollaton Park, Nottingham
- 1953 SCOTT, A., 2 North Street, Stichen, Aberdeenshire
- \*1921 SCOTT, PETER M., C.B.E., D.S.C., New Grounds, Slimbridge, Glos
- 1953 SEAFORD COLLEGE, Larrington Park, Petworth, Sussex
- 1950 SEGROVE, F., Ph.D., Dept. of Zoology, The University, Sheffield 10
- 1928 SEN, B., Vivekanada Laboratory, Baghbazar, Calcutta, India
- 1922 SEWELL, Lt.-Col. R. B. SEYMOUR, C.I.E., Sc.D., F.R.S., Dept. of Zoology, Downing Street, Cambridge. (Council, 1935-38)
- 1955 SHARMA, R. E., 2 Kirkleus Road, Thornton Heath, Surrey
- 1950 SHARMAN, D. P., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1947 SHARMAN, Miss M., 42 Winchester Drive, Wallasey, Cheshire
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- 1953 SIMS, RUDOLPH W., Jr., Marine Laboratory, Box 488, Coral Gables 46, Florida, U.S.A.
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- \*1884 SKINNERS, THE WORSHIPFUL COMPANY OF, Skinners' Hall, Dowgate Hill, London, E.C. 4
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- \*1954 SMITH, R. I., Ph.D., Dept. of Zoology, University of California, Berkeley 4, Calif., U.S.A.
- 1939 SMITH, S., Ph.D., St Catharine's College, Cambridge. (**Council**, representing Cambridge University, 1953-)
- \*1956 SMYTH, J. C., Ph.D., Paisley Technical College, George Street, Paisley, Renfrew
- 1953 SNELL, R. J., Haven, 5 Winterbrook Lane, Wallingford, Berks
- 1929 SOUTH AFRICA, FISHERIES DIVISION OF, Beach Road, Sea Point, Cape Town, S. Africa
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- 1934 SPAUL, Prof. E. A., D.Sc., Dept. of Zoology, The University, Leeds
- 1951 SPENCER, C. P., Ph.D., Marine Biology Station, University College of N. Wales, Menai Bridge, Anglesey
- \*1930 SPOONER, G. M., M.B.E., The Laboratory, Citadel Hill, Plymouth, Devon
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- \*1939 SPROSTON, Miss NORA G., c/o The Director, Zoological Survey of India, Japakusum House, 34 Chittarajan Avenue, Calcutta 12, India
- 1939 STANBURY, Miss F. A., Biology Dept., Plymouth Technical College, Tavistock Road, Plymouth, Devon
- 1954 STANLEY, T. P. G., c/o Mrs Prosser, 2620 Lansdowne Road, Victoria, B.C., Canada
- 1953 STEELE, J. H., Marine Laboratory, Victoria Road, Torry, Aberdeen
- 1925 STEPHEN, A. C., D.Sc., Royal Scottish Museum, Edinburgh
- 1951 STEPHENSON, R. M., Somewhere, St Mary's, Isles of Scilly, Cornwall
- 1930 STEPHENSON, Prof. T. A., D.Sc., F.R.S., Dept. of Zoology, University College of Wales, Cambrian Street, Aberystwyth, Cardigan. (**Council**, 1941-44)
- \*1949 STEPHENSON, W., Ph.D., Dept. of Zoology, University of Queensland, Brisbane, Australia
- 1951 STEVEN, D. H., Willowby House, Yelverton, Devon
- 1950 STEVEN, G. A., D.Sc., F.R.S.E., Willowby House, Yelverton, Devon
- 1930 STEVEN, Mrs G. A., Willowby House, Yelverton, Devon
- 1935 STEWART-EVISON, CHAS., Manor East Lodge, Sidbury, Sidmouth, Devon
- 1947 STOBART, T. R., Countryman Films Ltd., 18 Soho Square, London, W. 1
- 1952 STOCK, W. O., 24 Hanham Road, Kingswood, near Bristol
- \*1934 STONEMAN, ERIC, Dept. of Zoology, The University, Reading
- 1954 STOTT, F. C., Ph.D., Windrush, 22 Poltimore Road, Guildford, Surrey
- 1950 STREET, P. A. R., 6 Bellfield Avenue, Harrow Weald, Middx
- 1955 STUART-JONES, W. A., Neuadd Reichel, Bangor, North Wales

- 1936 STUBBINGS, H. G., Ph.D., 13 Third Avenue, Denvilles, Havant, Hants
- 1935 STURDY, Mrs R. S., Woodpiece, Apsley Guise, Bletchley, Bucks
- 1946 SUBRAHMANYAN, R., Ph.D., 52 Choolai High Road, Madras 7, India
- 1956 SUDDABY, O., Research Dept., Acheson Colloids Ltd., Prince Rock, Plymouth, Devon
- 1955 SUNDNES, G., Havforskninginstituttet, Boks 226, Bergen, Norway
- 1952 SURTEES, S. R., Ministry of Agriculture, Infestation Control Division, 29 Rodney Street, Liverpool, 1
- 1950 SUTCLIFFE, W. H., Jr., Ph.D., Bermuda Biological Station, St George's West, Bermuda
- 1949 SUTTON, Miss M. F., Ph.D., 6 Effingham Lodge, Surbiton Crescent, Kingston-on-Thames, Surrey
- 1952 SYMONS, H. W., The Old Forge, Cowbeach, Hailsham, Sussex
- 1948 TAIT, R. V., 62 Nightingale Road, Rickmansworth, Herts
- 1953 TALBOT, F. H., East African Marine Fisheries Research Organization, P.O. Box 668, Zanzibar
- 1932 TAYLOR, Prof. Sir GEOFFREY I., D.Sc., F.R.S., Farmfield, Huntingdon Road, Cambridge. (**Council**, 1932-35)
- 1954 TEBBLE, N., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1942 TEMPLEMAN, Dr WILFRED, Director, Fisheries Research Board of Canada, Biological Station, St John's, Newfoundland
- 1948 THAM, AH KOW, c/o Fisheries Dept., 4th Floor, Fullerton Building, Singapore
- 1956 THOMAS, Miss G. J., Ph.D., Dept. of Zoology, University of Michigan, Ann Arbor, Michigan, U.S.A.
- 1953 THOMAS, H. WILSON, M.R.C.S., L.R.C.P., Furzehatt, Plymstock, near Plymouth, Devon
- 1939 THOMAS, I. M., Dept. of Zoology, The University, Adelaide, S. Australia
- 1950 THOMAS, Miss J. G., Ph.D., Royal Holloway College, Englefield Green, Surrey
- 1956 THOMAS, M. L. H., c/o T. C. Cradock, Esq., 62 Highbury, Newcastle-upon-Tyne 2
- 1948 THOMAS, Mrs M. L. H., (Miss U. M. Grigg), c/o T. C. Cradock, Esq., 62 Highbury, Newcastle-upon-Tyne 2
- 1947 THOMPSON, J. A., c/o G. & F. Henderson, Oathill Farm, Enstone, Oxon
- 1955 THOMPSON, T. E., Dept. of Zoology, University College of North Wales, Memorial Buildings, Bangor, Caern
- 1952 THORSON, GUNNAR, D.Phil., Universitetets Zoologiske Museum, Krystalgade, Copenhagen K, Denmark
- 1953 TOBIAS, Miss M. A., 76 Blockley Road, North Wembley, Middx
- 1903 TORQUAY NATURAL HISTORY SOCIETY, The Museum, Torquay, Devon
- 1939 TOTTON, A. K., M.C., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1955 TREWHELLA, C. J. M., Truro School, Truro, Cornwall
- \*1946 TRIPATHI, Y. R., c/o S. R. Tripathi, Esq., Narsingharh, C.I., India
- 1955 TROLLOPE, Miss E. S. N., The Corner Cottage, Woodhill Road, Danbury, Essex
- 1938 TROUT, GEOFFREY C., Fisheries Laboratory, Lowestoft, Suffolk
- 1936 TRUELOVE, P. H., F.R.I.C., Red Hand Compositions Co., 2 The Firs, York Road, Ripon, Yorks
- 1946 TRUMBLE, Prof. H. C., University College, Ibadan, Nigeria, Africa
- 1947 TUCK, A. W., Upriver, Potter Heigham, Norfolk



- 1943 TUCKER, DENYS W., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1954 TUCKER, H. S., Roseland, Menheniot, Liskeard, Cornwall
- 1956 TUNKS, I. D'A. A., 8 Cannonbury Avenue, Eastcote, Pinner, Middx
- 1956 TURNER, G. L., 74 Clarendon Road, Ashford, Middx
- 1939 TUTIN, Prof. T. G., Dept. of Botany, University College, Leicester
- 1955 TWIGG, G. I., Lydgate Cottages, Bamford, Sheffield
- 1950 UMPELBY, G., 1 Togo Terrace, The Hoe, Plymouth, Devon
- 1948 VANE, F. R., Oceanographic Laboratory, 78 Craighall Road, Edinburgh 6
- 1956 VARMA, R. P., Ph.D., Central Marine Fisheries, Marine Fisheries Post Office, Ramanathapuram Dist., S. India
- \*1951 VERULAM, The Earl of, J.P., Gorhambury, St Albans, Herts. (**Vice-President**, 1953→)
- \*1946 VEVERS, H. G., M.B.E., D.Phil., Zoological Society of London, Regent's Park, London, N.W. 1
- 1951 VICTORIA: PROVINCIAL DEPARTMENT OF FISHERIES, Parliament Buildings, Victoria, B.C., Canada
- 1954 VIJAYARAGHAVAN, Dr P., Central Marine Fisheries Research Station, Mandapam Camp, South India
- 1949 VINALL, H. F., 93 Lady Margaret Road, London, N. 19
- 1954 VINDEN, J. S., 79 Ryland Road, Edgbaston, Birmingham 15
- 1952 WAGSTAFF, Miss M., 31 Guildford Road, Horsham, Surrey
- \*1948 WAKE, Lieut. K.D.O., R.N., 95 Constable Road, Ipswich, Suffolk
- 1956 WALDRON, K. D., 3434 McCarriston St., Honolulu 15, Hawaii, U.S.A.
- 1924 WALES, NATIONAL MUSEUM OF, Cardiff
- 1941 WALES: DEPT. OF ZOOLOGY, UNIVERSITY COLLEGE OF WALES, Cambrian Street, Aberystwyth, Cardigan
- 1951 WALFORD, Dr LIONEL A., U.S. Fish and Wildlife Service, Washington 25, D.C., U.S.A.
- 1954 WALKDEN, R., 76 Dominion Street, Walney Island, Barrow-in-Furness, Lancs
- 1949 WALNE, P. R., Fisheries Experiment Station, Castle Bank, Conway, Caern
- 1955 WALSTER, G. E., Plymouth and Devonport Technical College, Tavistock Road, Plymouth, Devon
- 1951 WARD, D. W., Orchard Cot, Gordon Road, Whitstable, Kent
- 1952 WARDROPPER, A. L., 24 Rupert Street, Whitburn, Co. Durham
- 1948 WARREN, F. J., The Laboratory, Citadel Hill, Plymouth, Devon
- 1925 WATERHOUSE, Sir NICHOLAS, K.B.E., 3 Frederick's Place, Old Jewry, London, E.C. 2. (**Vice-President**, 1925→)
- 1945 WATERS, C. A., E. Wood Ltd., Talbot Works, Stanstead Abbots, Ware, Herts
- 1923 WATSON, Prof. D. M. S., F.R.S., 12 Preston Way, Harrow, Middx. (**Council**, 1924-28, 1929-32, 1934-37)
- 1951 WATTS, J. C. D., West African Fisheries Research Institute, Freetown, Sierra Leone
- 1952 WATTS, Dr P., 38 Thornton Hill, Exeter, Devon
- 1950 WAUGH, G. DUNCAN, Fisheries Laboratory, Burnham-on-Crouch, Essex
- \*1938 WEBB, D. A., Ph.D., Trinity College, Dublin, Eire
- 1951 WEBBER, S. T., Anchorage, Richmond Walk, Devonport, Devon

- 1951 WEBSTER, R. W., Dept. of Chemistry and Biology, The Polytechnic, Regent Street, London, W. 1
- 1956 WEIS-FOGH, T., Dr.Phil., c/o Dept. of Zoology, Downing Street, Cambridge
- 1948 WEISS, Dr R. J., rue Neuve 33, Esch-sur-Alsette, Luxembourg
- 1955 WELCOMME, R. L., 38 Stone Hall Road, Winchmore Hill, London, N. 21
- \*1919 WELLS, Prof. G. P., Sc.D., F.R.S., Dept. of Zoology, University College, Gower Street, London, W.C. 1. (**Council**, 1935-38, 1946-48, 1952-55, 1956-)
- 1934 WENT, A. E. J., D.Sc., Dept. of Agriculture, Fisheries Branch, 3 Kildare Place, Dublin, Eire
- 1950 WEST INDIES, UNIVERSITY COLLEGE OF, Mona, Liguanea, Jamaica, B.W.I.
- 1946 WHARTON, R. H., Birkrigg, Noggarth Road, Roughlee, near Burnley, Lancs
- 1954 WHEELER, Miss M. R., St Anne's College, Oxford
- 1953 WHIFFEN, S. W., O.B.E., Seaward, Fowey, Cornwall
- 1948 WHITE, K. G., The College, Cheltenham, Glos
- 1934 WHITE, Miss KATHLEEN M., The University, Reading, Berks
- 1934 WHITE, P. H. F., Science Schools, Harrow School, Middx
- 1947 WHITEAR, Miss M., Ph.D., Dept. of Zoology, University College, Gower Street, London, W.C. 1
- 1954 WHITTAKER, Miss E. M., Y.W.C.A., Lockyer Street, Plymouth, Devon
- 1954 WICKSTEAD, J., Fisheries Research Station, P.O. Box 749, Singapore 1
- 1949 WIECZOREK, K., Hertford Grammar School, Hertford, Herts
- 1950 WIESER, Dr W., Dept. of Zoology, University of Washington, Seattle 5, Wash., U.S.A.
- 1954 WILKES, K. W., Science School, Harrow School, Harrow, Middx
- 1955 WILKES, S. N., 595 Hansen Avenue, Salem, Oregon, U.S.A.
- 1951 WILKINS, G. L., British Museum (Natural History), Cromwell Road, London, S.W. 7
- 1940 WILLCOCKS, T., Beechfield, Cawsand, near Plymouth, Devon
- 1950 WILLIAMS, F., P.O. Box 754, Zanzibar, East Africa
- 1955 WILLIAMS-ELLIS, Miss S., Cefn Cyffin, Llanfrothen, Penrhyndeudraeth, N. Wales
- \*1951 WILLIAMSON, D. I., Ph.D., Marine Biological Station, Port Erin, Isle of Man
- 1948 WILLMOTT, C. C., Meralea, Merafeld Road, Plympton, Plymouth, Devon
- 1948 WILSON, ARTHUR, 92 Crescent Road, Ramsgate, Kent
- 1927 WILSON, D. P., D.Sc., F.R.P.S., The Laboratory, Citadel Hill, Plymouth, Devon
- 1930 WILSON, Mrs D. P., Ph.D., 49 Torland Road, Hartley, Plymouth, Devon
- 1928 WIMPENNY, R. S., Fisheries Laboratory, Lowestoft, Suffolk. (**Council**, 1946-49, 1952-55)
- 1949 WISEMAN, J. D. H., Ph.D., British Museum (Natural History), Cromwell Road, London, S.W. 7. (**Council**, 1949-52)
- 1949 WITHERS, R. F. J., Middlesex Hospital Medical School, Mortimer Street, London, W. 1
- 1956 WITTERICK, P. M., Chalet Sableus, Calais, St Martins, Guernsey, C.I.
- 1955 WOOD, R. J., 61 Brookdene Avenue, Oxley, Watford, Herts
- 1952 WOODHEAD, P. M. J., Greetwell Corner, Scrawby, near Brigg, Lancs
- 1952 WOODWICK, KEITH H., Allan Hancock Foundation, University of Southern California, Los Angeles 7, Calif., U.S.A.
- 1954 WOOLF, Miss T., 61 Wimborne Road, Bournemouth, Hants
- 1939 WORTHINGTON, E. B., Ph.D., The White Cottage, Grantchester, Cambs
- 1953 WORTLEY, Miss P. J., Collingham, 3 Burlescoombe Leas, Thorpe Bay, Essex

- \*1948 WYATT, H. V., Clovermead, Elburton Road, Plymstock, Devon  
 1928 WYNNE-EDWARDS, Prof. V. C., Natural History Dept., Marischal College, Aberdeen. (**Council**, 1949-52, 1953-56)
- 1956 YALDWYN, J. C., Dept. of Zoology, Victoria University, Wellington, New Zealand  
 1927 YONGE, Prof. C. M., C.B.E., D.Sc., F.R.S., Dept. of Zoology, The University, Glasgow, W. 2. (**Council**, 1935-38, 1941-44, 1945-48, 1950-53, 1954→)
- \*1948 YORKE, Col. S. A., Littlefields, Cofton Hill, Starcross, Devon  
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 1948 PARKINSON, J. L., University College, Gower Street, London, W.C. 1  
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# THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. xxvii (p. 761) and Vol. xxxi (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

## TERMS OF MEMBERSHIP

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

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.



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